2013 APS-MSA Joint Meeting Abstracts of Presentations

Abstracts submitted for presentation at the APS-MSA Joint Meeting in Austin, Texas, U.S.A., August 10–14, 2013. The abstracts are arranged alphabetically by the first author's name. Recommended format for citing joint meeting abstracts, using the first abstract below as an example, is as follows: Abad, J. A., Li, R., Fuentes, S., Kreuze, J. F., Loschinkohl, C., and Bandla, P. 2013. Interception and identification by deep sequencing of a “caulimo-like” virus in a potato germplasm accession imported from South America. (Abstr.) *Phytopathology* 103(Suppl. 2):S2.1. <http://dx.doi.org/10.1094/PHYTO-103-6-S2.1>

Interception and identification by deep sequencing of a “caulimo-like” virus in a potato germplasm accession imported from South America

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A small portion (3%) of seedlings germinated from botanical potato seed accession JCM-23 imported from South America showed severe upper leaves deformation and necrosis. The diseased plants tested negative for most of the known viruses by bioassay, electron microscopy (EM), ELISA and nucleic acid-based technology. Presence of isometric particles by EM analysis suggested an unknown virus as pathogen. The virus induced a mild but conspicuous systemic vein clearing only on *Nicotiana tabacum* cv. Samsun plants, indicating that it is difficult to transmit mechanically. However, the virus was readily transmitted to potato and tomato plants by grafting. Tubers harvested from infected plants did not show any symptoms, but plants grown from these tubers developed necrosis, leaf deformation and rugosity (‘frog’ skin) as secondary symptoms. The diseased plants were subjected to small-RNA deep-sequencing analysis. BLAST search against virtual viral sequences showed that several contigs larger than 700 bp had identities higher than 90% with *Cauliflower mosaic virus*, suggesting the presence of a “caulimo-like” virus. The viral fragment was further amplified from the diseased plants by conventional PCR with specific primers designed from the contigs. This virus is a potentially dangerous seed-transmissible pathogen infecting potatoes and the USDA-APHIS-PPQ Plant Germplasm Quarantine Program prevented the introduction of another putative unknown foreign potato pathogen into the USA.

Field evaluation of promising breeding lines and varieties of common bean for tolerance to soilborne pathogens

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This study was conducted to identify and incorporate sources of resistance to major root pathogens into adapted bean varieties being developed in a collaborative Dry Grains PULSE CRSP project, with a focus on Ecuador and Rwanda. The use of varieties resistant to root pathogens is the most effective and practical strategy for managing root diseases of common bean. Field trials of promising lines from the Michigan State U. and ARS-Puerto Rico breeding programs were evaluated in the bean root rot nursery at the Vegetable Research Farm, NYSAES near Geneva, NY over the duration of the project. This site is heavily infested with *Fusarium solani* f. sp. *phaseoli*, *Pythium ultimum*, *Rhizoctonia solani*, and *Thielaviopsis basicola*. In 2011, 33 lines and varieties were arranged in a randomized block design with 4 replications. Each plot consisted of two rows, 7 m long and 0.75 m apart. All maintenance practices were according to recommended commercial guidelines. Root rot severity, among the lines tested varied greatly, ranging from 3.4 (10IS-6567, Eldorado pinto, Zorro black) to 6.0 (CLRK) on the 1 (healthy) to 9 (late stages of decay) scale. Also, many of the tested lines exhibited excellent vigor, productivity and high tolerance to a severe incidence of common bacterial blight, including Eldorado, RR008, RR016, RR005, 10IS-6480. Similarly, 27 bean lines and varieties were evaluated in 2012 and root rot severity ratings obtained ranged from 3.2 (Medalist) to 5.8 (Pink Panther).

Analysis of 3'-terminal region of Papaya ringspot virus-W isolates from southern United States

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Papaya ringspot virus-W (PRSV-W) is one of the most common viruses infecting cucurbits in southern United States. Fifty eight PRSV-W isolates were collected from four different States including Arkansas, Florida, Oklahoma and Texas. 3'-terminal region (including NIb and CP genes) were amplified and sequenced in these fifty eight isolates. This study showed that PRSV-W isolates in southern United States shared identity ranged from 92.2-99.9% and 94.9-99.6% at nucleotide and amino acid levels respectively, in the

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3'-terminal region (CP and part of N1b genes). Comparison of these PRSV-W isolates from USA and worldwide isolates showed that PRSV-USA isolates shared the highest identity with Australian isolates (average identity about 96% at the nt level), while shared the lowest identity with isolates from Indian subcontinent (with average identity 87% at the nt level). A Neighbor Joining tree (NJ) generated from nt sequences of the CP gene of PRSV (P & W) worldwide isolates showed that two isolates from India remained in a separate group, while the other PRSV-W isolates formed a second group.

Flooding duration affects severity of soybean sudden death syndrome

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It is well known that high soil moisture and irrigation favor sudden death syndrome (SDS), but the effect of flooding on disease severity is not clear. Greenhouse studies were conducted to test the effect of flooding duration on SDS caused by *Fusarium virguliforme* (Fv). Soybean cultivar Williams 82 (sus.) and MN1606 (resist) were grown in infested soil until VC stage. Non-infested soil was used for controls. Plants were then exposed to different periods of flooding by maintaining water level one inch above the soil surface for 0, 3, 5, 7 days, and in repeated short-term (RS) applications of 8h/week for 3 weeks. Root rot and foliar severity, root dry weight, and Fv inoculum density in soil were assessed 7, 14, and 21 days after flooding (DAF). Twenty-one DAF, seedlings in the 0-day, 3-day and RS flooding treatments showed the highest root rot and foliar symptom severity and the 7-day flooding treatment showed the lowest severity ($P<0.05$). *Fusarium virguliforme* inoculum density in soil was lower ($P<0.0001$) in 7-day compared to 0-day and RS flooding treatments. Similar results were found for root rot, foliar symptom severity, and Fv inoculum density 7 and 14 DAF. Root dry weight of infested seedlings did not differ among treatments, but in non-infested seedlings, the 0-day and RS flooding treatments showed the highest dry weight 7 and 14 DAF. These results suggest that prolonged flooding (5 and 7 days) has a negative impact on Fv infection and population in soil.

A novel endornavirus infecting lima bean (*Phaseolus lunatus*) and its occurrence in lima bean genotypes

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Endornaviruses are capsid-less viruses with RNA genomes ranging approximately from 10 to 17.5 kbp. They infect plants, fungi, and oomycetes, lack cell-to-cell movement and are present in every host cell. Endornaviruses are transmitted only via gametes and, in general, do not cause visible effects in infected hosts. While screening *Phaseolus* species for endornaviruses, we identified an endornavirus-like dsRNA of approximately 15 kbp in lima bean (*P. lunatus*). Analyses of nucleotide sequences of the viral dsRNA isolated from Henderson lima bean showed that this virus, for which we propose tentative name *Phaseolus luntatus* endornavirus 1 (PIEV-1), is distinct from all currently known endornaviruses. In phylogenetic analyses performed on viral RdRp, the only domain shared by all endornaviruses, lima bean endornavirus resulted closer to bell pepper endornavirus than to two species described from common bean. Commercial cultivars and wild lima bean from the Mesoamerican and Andean regions were tested for the presence of this virus and was found in genotypes of both origins.

Use of deep sequencing for characterization geminivirus replication fidelity

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Beet curly top virus is a geminivirus with a single circular ssDNA genome that has a broad host range and causes substantial economic damage in a variety of important crops. Even though they have DNA genomes, geminiviruses are thought to have a high mutation rate similar to that of RNA viruses. Inoculation of plants with an infectious BCTV clone, followed by deep sequencing of the population of viral progeny was used to assess replication fidelity and mutation rates for BCTV to test this hypothesis. Ion torrent sequencing of the BCTV produced a dataset of ~33 million bases with an average coverage of 11,000x over the BCTV genome. The deep sequencing data suggested a high mutation rate of ~1 change per 10^3 bases in the replicated BCTV genomes as well as the *Agrobacterium* clone used to inoculate plants. Most of the putative mutations observed in the Rep gene are indels that cause lethal frame shifts in this essential gene. Sanger sequencing of independent clones isolated from *A. tumefaciens*, *E. coli*, and infected

plants showed a much lower mutation rate and far fewer indels suggesting that the error rate associated with deep sequencing technology may be too high to make it useful for analyzing mutation rates in populations of viral progeny. Further investigation will focus on the accuracy of base calling and the potential development of filters that can screen out likely sequencing errors from deep sequencing data sets.

Control of fire blight (*Erwinia amylovora*) with trunk injection of the maximum seasonally allowed doses of SAR inducers and antibiotics in apple trees

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Trunk injection is a valuable alternative for delivering protective materials which could increase sustainability of tree agriculture by target-precise pest control with no negative effects of pesticides on the environment. Due to a systemic mode of exposure, a significant control of fire blight (*Erwinia amylovora*) should be achieved through trunk injection of Systemic Acquired Resistance (SAR) inducers. After 1-2 injections of 4 candidate and known resistance inducers and 2 antibiotics, using 4 replicates per treatment, inoculated Gala apple trees were rated for blossom and shoot blight, and Kit Jonathan trees for shoot blight. Similarly to streptomycin with blossom and shoot blight incidences of 17.1 and 4%, candidate inducer potassium salts of phosphorous acid (PhosphoJet) provided significant fire blight control with incidences of 20.4 and 6.5% compared to water injected control (46.1 and 22.3%). Imidacloprid (Imajet) was ineffective with incidences of 40.1 and 18.3%. Two doses of SAR inducer acibenzolar-S-methyl with incidences of 26.9 and 20.3%, and 27.7 and 18.5%, significantly reduced only blossom blight. Inducer prohexadione-calcium did not control shoot blight implying no translocation of the active ingredient. Oxytetracycline (ArborBiotic) significantly controlled shoot blight on Kit Jonathan with time severities of 4.7-14.2% compared to water injected control (30.1-68.6%). The results show that trunk injection could be used for apple disease control.

Control of apple scab (*Venturia inaequalis*) using trunk injection of biopesticides and fungicides in apple trees

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Trunk injection of protective compounds is a target-precise pest control approach which could eliminate negative drift-driven effects of pesticides on the environment. Significant control of leaf apple scab (*Venturia inaequalis*) should be achieved through trunk injection of systemic biopesticides and fungicides. After 4 injections of 5 biopesticides and 1 fungicide, and a single injection of 1 fungicide, using 4-6 replicates per treatment, naturally infected Red Delicious and Mac Spur apple trees were rated for leaf scab. Spur and shoot scab incidences for water injected controls, propiconazole (Alamo), and spray standards, were 72.2-81.9% and 50.9-54%, 52.5% and 22.5-26.2%, and 15.5-16.7% and 20-20.6%, respectively. After 4 injections, potassium salts of phosphorous acid (PhosphoJet) showed significant reduction of spur and shoot scab with incidences of 39.3-47.7% and 14.3-18.5%, while potassium phosphite, potassium dihydrogen phosphate, potassium bicarbonate and hydrogen peroxide (OxiDate) showed overall reduced spur and shoot scab of 53.5 and 18.4%, 60.4 and 28.1%, 72.4 and 36.5%, and 61.1 and 29.1%, respectively. After a single injection of difenoconazole + cyprodinil (Inspire Super) significant control of spur and shoot scab of 25.9 and 21.2% was achieved. However, spray standard and sprayed Inspire Super were better with spur and shoot scab of 13.9 and 16.6%, and 0.07 and 3.2%. Based on the results, trunk injection could be used for apple disease control.

Building an index of pathogens of alder

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The thin-leaf alder, *Alnus tenuifolia*, has experienced widespread dieback and mortality from Alaska to the southern Rocky Mountains. Other species of alder in the region have exhibited disease of less intensity while in Europe two major species of alder have been devastated by Phytophthora root rot and secondary associated canker diseases. Identification of the major causal pathogens in the North American alder epidemic has been a complex task. National and regional disease records and the USDA host index are sparse and reflect limited investigation of alder species, historically. Our objective has

been to collect fruiting bodies of fungi associated with cankers and isolate fungi and oomycetes from diseased tissues. The putative pathogens have been identified based on DNA sequence homology and standard morphology, and species of interest have been included in inoculation trials on one to three species of alder in replicated plots in two or more field locations of pathogen origin. The pathogens that have demonstrated significant virulence include *Valsa melanodiscus*, *Melanconis alni*, and *M. stilbostoma*. Pathogens of trees generally cause disease on stressed host plants and therefore inoculation trials often do not adequately reflect the virulence of a pathogen. Our index of putative pathogens on alder will include listing of other fungi in the inoculation trials as well as numerous wood decay fungi.

Phosphonate, carboxylic acid amide, and benzamide treatments for pre- and postharvest management of citrus brown rot

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Phytopathology 103(Suppl. 2):S2.3

Brown rot of citrus fruit caused by species of *Phytophthora* is common in California during the winter season when high rainfall may occur. Copper and phosphonates are the only effective registered preharvest treatments. Recently, phosphonates were classified as exempt from tolerance in the U.S., we supported postharvest registration of phosphonates, and evaluated the efficacy of new pre- and postharvest treatments. In preharvest studies, incidence of brown rot of fruit harvested 2 weeks after application (3330 L/ha) and inoculated with zoospores of *P. citrophthora* was 44.2% for fluopicolide (37 mg/L), 2.0% for mandipropamid (38 mg/L), 6.8% for potassium phosphite (620 mg/L), 5.6% for copper-lime (670 mg mce/L), and 77.0% for the control. Incidences after 6 weeks were 75.9%, 8.3%, 62.5%, 27.5%, and 95.9% respectively. In postharvest studies, fruit were dip-treated with aqueous solutions and then inoculated. Brown rot incidence was 0.7% for fluopicolide (300 mg/L), 0% for mandipropamid (310 mg/L), 1.3% for potassium phosphite (1242 mg/L), and 85.5% for the control. When fruit were first inoculated and treated after 12 h, only potassium phosphite was effective and incidence of decay was reduced to 5.0% as compared to the control with 82.3%. International tolerances are being established for phosphonates. Fluopicolide and mandipropamid are proposed for integrated pre- and postharvest use on citrus to ensure resistance management and long-term usage of these compounds.

Eight new viruses identified in bioenergy switchgrass

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Panicum virgatum (switchgrass), a potential bioenergy crop, was investigated for virus infections that could reduce its biomass yield. In addition to Barley yellow dwarf viruses (BYDV), *Sugarcane mosaic virus* (SCMV), *Panicum mosaic virus* (PMV), and recently, *Switchgrass mosaic virus* (SwMV), were reported to infect the crop. To determine if other RNA viruses infect switchgrass, five young leaves were collected from 18 switchgrass varieties with different foliar symptoms ranging from necrotic spots to mosaic from a five-year old switchgrass plot. Pieces (about 3-cm) were cut from the tips of symptomatic leaves and pooled. To enrich for viruses, virus particles were partially purified from 5 g of the ground pooled leaf tips. Total RNA extracted from the preparation was treated with DNase I, reverse transcribed, and high-throughput sequenced. Eight new RNA viruses and one unexpected DNA virus were identified. The replicase or coat protein amino acid sequences of the eight viruses were 30% to 52% identical to the most closely related polerovirus, tenuivirus, ctyorhabdovirus, ourmavirus, foveavirus, fijivirus, cripavirus or mastrevirus in GenBank. The viruses were detected in 5 to 18 of the original 18 varieties of switchgrass and are being further characterized.

Myxomycetes on *Orbygnia* sp. (Arecaceae) from the Brazilian Cerrado

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An intensive survey of the Cerrado Myxomycetes is on the way, but in an early stage. However about 70 species has already been described for this entire Biome. On host species belonging in Arecaceae these organisms are commonly found. However, little is known about the Myxomycetes on a common *Orbygnia* species present on dense Cerrado areas close to dry forests. A first sample indicated the presence of four different species of myxomycetes, three more abundant belonging in the *Physarales* and one in *Trichiales*. However, the most notorious event was the presence of *Badhamia viridescens*, for the first time found in the Neotropica.

Glancing at host adaptation in *Ralstonia solanacearum* through comparative genomics of highly host-adapted lineages

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Ralstonia solanacearum is a vascular soil-born plant pathogen with an unusually broad host range. This globally distributed, economically destructive organism has thousands of distinct lineages within a heterogeneous and taxonomically disputed species complex. Some of those lineages can be assigned to ecotypes that include highly host-adapted strains such as the banana Moko disease-causing strains, the cold-tolerant potato brown rot strains (R3bv2) and the recently emerged NPB strains (Not Pathogenic to Banana). The polyphyletic nature of the Moko ecotype and the unexpected closeness of some its lineages to the paraphyletic brown-rot and NPB ecotypes make those highly adapted strains a robust model for study of host adaptation and speciation in general. Genomes of 10 new strains were produced to complement the 12 publicly available ones. Using a panel of bioinformatics methods, we looked for genetic or evolutionary features that discriminate between ecotypes. There were relatively few divergent features. Those related to known virulence factors were further analysed for functional clues about host adaptation and ecotype emergence mechanisms. These analyses yield no clear signal, suggesting that the large biological differences between these closely related strains result from differences in gene expression rather than from differences in gene content. Transcriptomic analyses of these strains during host infection are underway to test this hypothesis.

Identification of resistance to *Rhizoctonia* root rot in mutant and wild barley (*Hordeum vulgare* subsp. *spontaneum*)

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Direct seeding cereal crops into non-tilled fields is a practice that is gaining importance in the Pacific Northwest region of the United States. Unfortunately, *Rhizoctonia* root rot and bare-patch caused by *Rhizoctonia solani* AG-8 limits the yield of direct-seeded cereals in this region. No resistant germplasm is available, and other available control methods have not been effective in preventing yield losses. To identify potential sources of resistance, M₂ populations of two barley lines treated with sodium azide and wild barley (*Hordeum vulgare* subsp. *spontaneum*) accessions from the Wild Barley Diversity Collection (WBDC) were evaluated in controlled environments. Ten putative M₃ mutants were identified and reciprocal crosses to their wild-type progenitors were carried out for genetic analysis. The findings that not all BC₁F₁ plants evaluated were susceptible suggest that resistance to *Rhizoctonia* root rot in the mutant barley is inherited as a dominant trait. Observed segregation ratios of progeny of BC₁F₁ plants are currently being tested against several gene models of inheritance to determine the number of genes conferring resistance. Of 317 wild barley accessions that were screened for resistance, six accessions showed potential as gene donors for *R. solani* resistance with one accession, WBDC 021, showing the greatest potential by displaying moderate resistance. Research is underway to test WBDC 021 for its reaction to *R. solani* under field conditions.

Survey for Grapevine red blotch-associated virus in the Foundation Plant Services vineyards at the University of California-Davis

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In 2008, a new disease characterized by red blotches along leaf margins and red veins under the leaf surface was observed in red grape varieties in a few vineyards in Napa Valley, CA. A new DNA virus, *Grapevine red blotch-associated virus* (GRBaV) was identified in these vines. Given the apparent widespread incidence of GRBaV and the unknown nature of its origin and mode of transmission, we decided to screen a majority of the planting stock at Foundation Plant Services for GRBaV. This planting stock included all the vines in our newly established Russell Ranch vineyard in addition to more than 1,600 vines from our Classic Foundation vineyard. Comparison of the real-time PCR test results with those from conventional PCR for the 1,102 Russell Ranch vines verified that our new assay was highly sensitive and specific for GRBaV and indicated that all Russell Ranch vines were negative for GRBaV. More than 1,600 vines from the classic Foundation Vineyard are

also being tested for the virus. The infection rate in the Classic Foundation was less than 0.2%. Fourteen samples in close proximity to the three GRBaV-positive vines were also collected and tested. Of these, six were sibling vines. These vines were all negative for GRBaV. As a leading source of orchard and vineyard planting stock in the U.S., in addition to being a central component of the Grape Clean Plant Network, these negative results indicate that FPS stock is not a major source of GRBaV spread within vineyards.

Association of a DNA virus with grapevines affected by red blotch disease in northern California

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In Napa, CA, Cabernet Franc, Cabernet Sauvignon and Zinfandel grapevines in three separate vineyards exhibited foliar symptoms comprised of red blotches, marginal reddening and red veins, and reduced total soluble solids in harvested fruits. Foliar symptoms were initially diagnosed as leaf roll disease. However, RT-PCR assays for all known leafroll viruses were negative with an exception of *Grapevine leafroll-associated virus 2* in Zinfandel. Metagenomic analysis of cDNA libraries obtained from dsRNA enriched nucleic acid preparations from bark scrapings of dormant canes on an Illumina platform, revealed virus sequences having a distant relationship with geminiviruses. Sequencing of products obtained by PCR assays using overlapping primers and rolling circle amplification confirmed the presence of a single circular genome of 3206 nucleotides, nearly identical to Grapevine Cabernet franc-associated virus recently reported from New York. Primers specific to the virus amplified a product of the expected size from DNA extracts obtained from petioles of diseased source vines. Chip bud inoculations from diseased sources successfully transmitted the virus to test plants as confirmed by PCR analysis. This is the first report of a DNA virus associated with grapevines showing red blotch disease symptoms in California and we hence propose the virus name: 'Grapevine red blotch-associated virus'.

A survey of ballistosporic phylloplane yeasts in Baton Rouge, Louisiana

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A survey of phylloplane yeasts was conducted in Baton Rouge, Louisiana during 2011. Using the spore-fall method, the leaf surfaces of seven ferns were sampled at biweekly intervals for one year. Yeasts were pure-cultured and identified by sequencing of the internal transcribed spacer region and the D1/D2 domain of the large subunit of nuclear ribosomal DNA. 463 isolates were identified, representing 81 species spanning 12 orders and six classes in Basidiomycota. Phylogenetic analyses suggest that as many as 30 of these isolates are species new to science, many of which are Exobasidiomycetes. On average, more yeasts were recovered from young fern leaves than from senescing ones. The number of yeasts recovered from abaxial versus adaxial leaf surfaces did not differ significantly. More isolates were recovered from non-fertile leaves than from those with sori, but this difference was not statistically significant. A general trend was observed in the number of isolates recovered with respect to average monthly temperature and precipitation levels as more isolates were recovered during the hottest and driest months. By far the most commonly recovered species was *Sporidiobolus pararoseus* (Sporidiobolales, Microbotryomycetes) and we designed a rapid PCR assay to screen and identify isolates of this species. These and other results will be discussed.

Molecular biodiversity of tomato yellow leaf curl disease associated viruses in Saudi Arabia

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Tomato production in Saudi Arabia and anywhere is under constant threat of whitefly-transmitted begomoviruses that cause tomato yellow leaf curl disease (TYLCD) that has a large number of strains. Tomato samples with typical symptoms of TYLCD and other crops grown near tomato field such as green bean and paper were collected from open fields and greenhouses from different locations in Saudi Arabia (Eastern Province, Qassim province, Hail and Jazan). PCR was used to identify the pathogen using degenerate primers of *Geminiviruses* (AVcore and ACcore). To determine exactly the virus strain, multiplex PCR was used with sets of specific primers for *Tomato yellow leaf curl virus* Almeria strain (TYLCV-Alm), *Tomato yellow leaf curl virus* Israel strain (TYLCV-IL), *Tomato yellow leaf curl virus* mild strain (TYLCV-mld)

and *Tomato yellow leaf curl virus* Sardinia strain (TYLCSV). Most of tested tomato samples some of paper and green bean were positive with degenerate primers and negative with all other specific primers. PCR products were cloned and sequenced and the nucleotide sequences were submitted in gene bank under accession numbers KC428387, KC428388, KC432613, KC432614, KC432615, HM590558, KC479016 and KC479017. The phylogenetic tree showed that all isolates tested were found to be related to Tomato leaf curl Iran, Yemen and Sudan viruses. The highest nucleotide identities (>95%) were obtained with members of the species from Iran and Yemen.

Occurrence and distribution of soybean viruses in Oklahoma

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Soybean is the leading oilseed crop produced and consumed in the world today. Approximately 67 viruses infect soybean worldwide and some of them pose a real threat to soybean industry by affecting the quality and quantity of soybean. In USA, soybean is one of the important legume crops and a number of viruses have been reported to infect soybean crops. In Oklahoma, soybean is grown on more than 500,000 acres annually but no information exist on the occurrence and distribution of viruses infecting soybean crops. In this study, soybean crops were examined during 2012 growing season in growers fields located at 18 counties of Oklahoma. Samples were tested serologically against 15 different viruses. Thirteen out of 15 viruses were detected by dot-immunobinding assay (DIBA) in soybean samples. PeMoV was detected more often followed by SVNV, BYMV, TSV, TRSV, SMV, PSV, ToRSV, CPSMV, BPMV, AMV, SbDV and CPMV. Of the 13 viruses, 8 viruses were never reported before in soybean crops of Oklahoma. The average incidence of viruses infecting soybean ranged from 1-20% while it was much higher (50-70% of samples) in some counties. More than 50% of the symptomatic samples did not react to the antiserum of the above 15 viruses which could represent viruses other than tested in this work or contain new or unreported viruses in soybean. These results will be discussed in the context of the future threats and possible epidemics caused by these viruses to soybean crops in Oklahoma.

Functional analysis of the host target of a *Phytophthora* RXLR effector in solanaceous crops

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The oomycete plant pathogen, *Phytophthora infestans*, which causes late blight of potato and tomato, secretes hundreds of virulence effector proteins, which are essential for host infection and disease development. Key to understanding how effectors modulate plant physiology to benefit *Phytophthora* is identifying and characterizing their host targets. Using Yeast two hybrid and Bimolecular Fluorescence Complementation-based screening, we have identified several putative host targets of *Phytophthora* effectors. Using loss-of-function, biochemical and cell imaging approaches, we have functionally characterized one of these targets, a TLD domain containing protein. Physiological significance of the interaction of *Phytophthora* effectors with its host targets will be discussed in the poster. Rationally designing R proteins can be significantly advanced if we know the molecular targets of effectors, and understand how effectors modulate their targets, yet evade detection by R proteins. Results from this project are expected to assist in discovering novel R genes.

Evaluation of global spring wheat germplasm for resistance to tan spot *Pyrenophora tritici-repentis* race 1

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Tan spot, caused by the ascomycetes fungus *Pyrenophora tritici-repentis* (Ptr), is a major foliar disease of wheat worldwide. Multiple races have been identified within the Ptr population and race 1 is the most prevalent race throughout the world's major wheat growing countries. Using resistant cultivars as a disease management strategy for tan spot is generally considered economical, durable, and environmentally friendly. In this study, 414 wheat genotypes from the CIMMYT elite wheat germplasm nurseries were screened for their reaction to Ptr race 1, by inoculating them individually with spores suspensions at the two-leaf stage in the greenhouse. Of 414 genotypes tested, 69, 144, and 201 were rated as resistant, moderately susceptible and susceptible to tan spot, respectively. The resistant genotypes with good agronomic characters can be used directly as a tan spot resistant cultivars for tan spot in

countries where they show adaptability or as sources of resistance in other wheat breeding programs. In an attempt to identify sources of resistance to multiple leaf diseases, genotypes that showed resistance to Ptr race 1 are presently under further investigation for their reactions to Ptr races 3, 5, *Stagonospora nodorum* leaf blotch, *Septoria tritici* blotch, and spot blotch.

WITHDRAWN

Evidence of field selection of DMI insensitive isolates of *Sclerotinia homoeocarpa* by the SDHI fungicide, boscalid

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Dollar spot, caused by *Sclerotinia homoeocarpa*, is one of the most economically important turf diseases in the Northeastern United States and is controlled by multiple fungicide classes. Reduced sensitivity of *S. homoeocarpa* to demethylation inhibitors (DMIs), dicarboximides, and methyl benzimidazole carbamates has been reported throughout the country but no resistance to succinate dehydrogenase inhibitors (SDHI) has been reported. Two independent studies were conducted on a golf course with a bimodal population distribution of DMI sensitive and insensitive fungal isolates. We observed in 2010, 2011, and 2012 that applications of the SDHI fungicide boscalid (Emerald) selected for DMI insensitive isolates on this golf course, shifting the population to a more unimodal distribution. *In vitro* analysis of 10 *S. homoeocarpa* DMI insensitive and sensitive isolates collected from the same location previously showed a strong correlation between EC₅₀ of the DMI propiconazole and EC₉₅ of boscalid and overexpression of the multidrug resistance transporter, *ShMR1*, in those DMI insensitive isolates. In addition, boscalid induces overexpression of this gene similarly to propiconazole. The selection of DMI insensitivity by boscalid may have important implications for managing fungicide resistance and for development of new fungicide chemistries.

Mechanisms of atoxigenicity: Characterizing genetic properties of atoxigenic *A. flavus* isolates

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Aspergillus flavus is the primary causal agent of aflatoxin contamination in crops. Aflatoxins are highly toxic carcinogens that threaten agricultural systems throughout the world. Twenty-eight known genes located in a single cluster are required to produce aflatoxins. *A. flavus* isolates that are not capable of producing aflatoxins (atoxigenics) have been utilized commercially as biocontrol agents. Atoxigenicity results from the modification of genes within the aflatoxin biosynthesis cluster. Only a few mechanisms by which *A. flavus* becomes atoxigenic have previously been described in detail. Twelve atoxigenic isolates under development as biocontrol agents in the United States and Africa were subjected to several genetic analyses including multi-

plex PCR, sequencing of aflatoxin biosynthesis genes, and SSR genotyping. Deletion patterns within the aflatoxin gene cluster varied among the atoxigenics, but certain deletion patterns were shared by multiple isolates. Sequence analysis revealed either breakpoints or insertions in several isolates. Assays to interrogate large populations of *A. flavus* for specific DNA features were developed both to aid discovery efforts for elite atoxigenic vegetative compatibility groups with potential as biocontrol agents and for monitoring specific genotypes in the environment.

Detection and identification of sweetpotato viruses in North Carolina

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Sweetpotato (*Ipomoea batatas*) is prone to virus infections due to its vegetative propagation that may result in cultivar decline. The goal of this study was to identify the most prevalent viruses affecting sweetpotato through a preliminary field survey in North Carolina (NC) using biological, serological and molecular detection and identification methods. Susceptible indicator plants (*Ipomoea setosa*) were evenly distributed in sweetpotato experimental plots at two different locations in NC during the summer of 2012. Naturally infected indicator plants showing virus-like symptoms (n=129) were collected and tested by NCM-ELISA, multiplex PCR, multiplex and quantitative RT-PCR. Single and mixed viral infections were found in 34% and 66% of the samples, respectively; with potyviruses mixed infections being the most common. *Sweetpotato feathery mottle virus* (SPFMV) was the most frequent followed by *Sweetpotato virus G* (SPVG), *Sweetpotato virus C* (SPVC), *Sweetpotato leaf curl virus* (SPLCV), *Sweetpotato chlorotic stunt virus* (SPCSV) and *Sweetpotato virus 2* (SPV2). Sequence analyses of the samples at nucleotide level showed 97 to 100% similarities, suggesting the presence of different strains amongst the detected viruses. This study showed evidence of the wide occurrence of potyviruses and a begomovirus affecting sweetpotatoes in NC. This is the first report of SPVG, SPV2 and SPLCV in NC.

Diversity of marine lignicolous ascomycetes from two mangroves of Baja California Sur Mexico

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Mangroves are inhabited by an ecological group of marine ascomycetes that play an essential ecological function as degraders of lignocellulosic debris. In Mexico, their diversity remains mostly unknown, with only 31 species registered from a worldwide total of 160 species. The diversity of marine lignicolous ascomycetes was determined from the mangroves of Zacatecas Estuary and St. Jose Island, Bay of La Paz. Twenty wood baits (*Pinus* sp.) were attached in the intertidal area of the mangrove roots and collected after 6 weeks. Afterwards the wood baits were incubated in humid chambers during 4 weeks. Each sample unit was carefully examined to identify the developed ascomycetes. A total of 20 different ascomycetes were obtained. The most frequent species were *Lulworthia grandispora* (15.79%), *Lulworthia* sp. (14.47%), *Trichocladium constrictum* (14.47%) and *Lignincola* sp. (11.84%). The highest diversity was found at Zacatecas Estuary ($H' = 0.954$) and the lowest in St. Jose Island ($H' = 0.597$). Although the results of this research are preliminary, they showed a mycobiota that appears to be different for the two locations. The possible explanation for the higher fungal diversity in the Zacatecas Estuary may be due to their protected location inside an inlet within the Bay of La Paz, while in St. Jose Island, the mangrove is more exposed to the influence of the currents of the Gulf of California.

Association of a monopartite begomovirus and defective betasatellite with okra leaf curl disease in Jazan, Saudi Arabia

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Okra plants exhibiting leaf curl and vein thickening symptoms were collected from Jazan region of Saudi Arabia. Total DNA isolated from symptomatic leaves was subjected to rolling circle amplification. The genomic component was cloned and sequenced in both directions. The complete genome sequence showed the presence of near identical 2769-nt-long isolates of a begomovirus. Blasting the assembled nucleotide sequences in GenBank database revealed a sequence identity at 88-93% to a previously reported *Cotton leaf curl Gezira virus*, (CLCuGV). According to the ICTV guidelines on species demarcation, the genomic component associated with okra leaf curl disease is strain of a

malvaceous-infecting monopartite begomovirus that was tentatively named CLCuGV-Jaz. The genome of CLCuGV-Jaz encodes six open reading frames (ORFs), and was most closely related to other monopartite begomoviruses of the Eastern Hemisphere. CLCuGV-Jaz shared highest nucleotide sequence identity (93%) with strains of CLCuGV from the Nile Basin. It shared 89% with Egyptian strains of CLCuGV that were recently introduced in Jordan.

Biological control of bacterial blight of anthurium caused by *Xanthomonas axonopodis* pv. *dieffenbachiae*

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Phytopathology 103(Suppl. 2):S2.6

Bacterial blight of anthurium continues to reduce yields even though the anthurium industry has already made substantial progress with cultural control, production of disease-free planting stocks in tissue culture and resistance breeding. Strategic application of biological control agents further reduces the impact of blight disease on highly valued susceptible plants. Repeated treatments in laboratory and greenhouse studies increased plant growth and significantly reduced disease incidence and severity. Seven field experiments were carried out in Hilo, which is the center of commercial anthurium production in Hawaii. Treatments with biocontrol agents formulated with amino acid additives reduced bacterial blight on two economically valuable susceptible anthurium cultivars, Ozaki and Marian Seefurth. Disease reduction ranged from 10 to 45% depending on the type of application and the organic compounds added to the bacterial mixture. Comparisons with a common chemical control measure used by the industry showed that the microbial biocontrol treatments were equally effective in field trials and more effective when biocontrol agents were applied under optimal conditions. A microbial product consisting of four well-characterized bacterial species in optimized amounts in prescribed buffers is available for commercial production.

Development of a blight susceptibility index for anthurium cultivars evaluated in a resistance breeding program

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Phytopathology 103(Suppl. 2):S2.6

Traditional anthurium cultivars and new hybrids were evaluated for susceptibility/resistance to bacterial blight following inoculation with *Xanthomonas axonopodis* pv. *dieffenbachiae*. Flowering plant evaluations were based on successive measurements of leaf areas colonized by the pathogen over a 12-week period following inoculation. The experiment was repeated three times with six replicates per cultivar per treatment. Microplants recently deflasked from tissue culture were evaluated four weeks after inoculation using leaf blot analysis of detached leaves. Analog and digital imaging methods were used to calculate disease severity indices and rate the selected cultivars. An X-ray film assessment method was compared to a potentially more cost effective way to monitor bacterial colonization of anthurium cultivars. Experiments were designed to obtain images of luminance from diseased leaves that would match the intensity of light absorbed onto the X-ray film. Sensitivity (ISO), exposure times, aperture settings, and positions of the camera were manipulated to get a desirable depth of field and software was developed to calculate the number of photons emitted in the infected leaves. Four of nine new hybrid cultivars showed greater resistance than Kalapana (resistant control) but none of the cultivars were more susceptible than Marian Seefurth.

Ultrastructural action of essential oils on *Xanthomonas vesicatoria* and control of bacterial spot in tomato

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Bacterial spot, caused by *Xanthomonas vesicatoria*, is among the most important diseases in tomato. The control of this plant disease is a hard task. Essential oils derived from medicinal plants have also shown promising results for controlling diseases of different crops. Then, the objective of this work was to evaluate the effects of plant essential oils (EOs) on the growth of *X. vesicatoria*, on bacterial morphology and ultrastructure, and on the severity of tomato bacterial spot. EOs from citronella, clove, cinnamon, lemongrass, eucalyptus, thyme, and tea tree were evaluated *in vitro* at concentrations of 0.1, 1.0, 10, and 100% in 1.0% powdered milk. The effect of EOs, at 0.1%, on the severity of tomato bacterial spot was evaluated in tomato seedlings under greenhouse conditions. The effects of citronella, lemongrass, clove, and tea tree EOs, at 0.1%, on *X. vesicatoria* cells were evaluated by transmission electron microscopy. All EOs showed direct toxic effect on the bacteria at a

10% concentration *in vitro*. Under greenhouse conditions, the EOs of clove, citronella, tea tree, and lemongrass reduced disease severity. EOs of clove and tea tree, and streptomycin sulfate promoted loss of electron-dense material and alterations in the cytoplasm, whereas EO of tea tree promoted cytoplasm vacuolation, and those of citronella, lemongrass, clove, and tea tree caused damage to the bacterial cell wall. The EOs at a concentration of 0.1% reduced the severity of the disease.

Pathogen detection step one: Getting the DNA out of the sample

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PCR detection of low-copy-number pathogen infections can be difficult if pathogen DNA is not efficiently released from the host tissue. Using six different types of plant tissue, several common tissue-disruption techniques were compared, including pressure cycling technology (PCT) with or without disruption using a PCT Shredder. DNA "amplifiability" was determined by quantitative real-time PCR. Bead-beating of tissues provided excellent DNA yield and amplifiability with a few, but inconsistent, exceptions. The use of PCT did not consistently improve nucleic acid yields or amplifiability, over and above that provided with bead-beating. The PCT Shredder was a poor tool for tissue disruption under the conditions of our tests. The mortar and pestle provided good, low-cost results but was not as consistently effective as bead-beater disruption methods.

The effect of postharvest treatments on survival of *Xanthomonas citri* pv. *citri* on infected grapefruit leaves

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There is concern that trade of infected citrus foliage may spread serious diseases. Presence of the citrus canker pathogen, *Xanthomonas citri* pv. *citri* (*Xcc*), for example, in citrus growing regions can severely limit shipping citrus internationally. Thus, studies were conducted to evaluate possible foliage decontamination methods, with an objective to determine the effect of postharvest, hot and cold water treatments with and without disinfectants on survival of *Xcc* on infected grapefruit leaves. A split-split-split plot design was used, with disinfectant (none or Pro-San) assigned to whole-plots, temperature (0, 10, 40, 45, and 50°C) assigned to sub-plots, treatment duration (0, 2, 5, 10, and 20 min) assigned to sub-sub-plots, and assessment time (0, 2, 7, and 14 days post treatment [dpt]) assigned to sub-sub-sub-plots. Treatments were conducted in a 160-liter, temperature-controlled, re-circulating water bath. The experiment was conducted three times, and data was analyzed by fitting a linear mixed model. Reductions in *Xcc* CFUs/leaf generally increased with increasing treatment duration and temperature, and they were greater for treatments involving Pro-San. Treatment at 45°C for 20 min or 50°C for ≥ 5 min resulted in leaf tissue damage. Experiments are in progress to limit regeneration of inoculum producing potential observed in later dpt. Results from these studies should be useful in influencing future regulatory policies regarding trade of citrus foliage.

CsrA is a positive regulator of virulence factors in *Erwinia amylovora*

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The fire blight pathogen *Erwinia amylovora* requires a functional type III secretion system (T3SS) and production of the exopolysaccharide amylovan to cause disease. The GacS/GacA two-component system is widely conserved among gamma-proteobacteria and is a global regulator of virulence factors. This system positively regulates non-coding small RNA *csrB*, which in turn binds to CsrA, a translational regulator. We have previously reported that homologous GrrS/GrrA system negatively regulates motility, amylovan production and expression of T3SS genes in *E. amylovora*. To further understand the molecular mechanism as how GrrS/GrrA regulates various virulence traits, we generated and characterized both *csrB* and *csrA* mutants. Results showed that *csrB* mutant was hypermotile, produced higher amount of amylovan, and had increased expression of T3SS genes *in vitro*, which are the same as reported for *grrS/grrA* mutants. In contrast, *csrA* mutant exhibited complete opposite phenotypes, including positive regulation of motility and expression of T3SS genes. Furthermore, *csrA* mutant did not induce HR on tobacco or caused disease on immature pear fruits and apple shoots, indicating that CsrA is a positive regulator of virulence factors. These findings demonstrated that negative regulation of virulence by GrrS/GrrA acts through *csrB*, which likely binds to CsrA and neutralizes its positive effect on T3SS gene expression, flagellar formation, and amylovan production.

Effect of intermittent pre- and post-anthesis moisture patterns on Fusarium head blight, deoxynivalenol, and fungal biomass in wheat

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Fusarium head blight (FHB) of wheat is caused by the fungal pathogen *Fusarium graminearum*. Moisture is critical for infection, colonization, FHB development, and grain contamination with the mycotoxin deoxynivalenol (DON). Both the duration and amount of rainfall around anthesis are important predictors of FHB and DON. However, it is unclear how the distribution of moisture during this period affects these responses. Field experiments were conducted to quantify the effects of intermittent pre-anthesis rainfall, and mist chamber experiments to quantify the effects of post-anthesis mist patterns on FHB, DON, and fungal biomass (FBM). For both sets of experiments, four moisture treatments, one continuous and three intermittent, were applied during the 7-8 days before or after anthesis, plus an untreated check. Intermittent treatments received similar durations and amounts of moisture, but the alternation between wet and dry periods during the 7-8-day window varied among them. FHB index (IND) was rated, and a grain sample from each treatment was analyzed for DON using GC-MS and FBM using real-time PCR. FHB, DON, and FBM were highest in the continuous moisture treatment, and varied among the intermittent treatments. FBM and DON increased as FHB increased under continuous moisture, but these relationships tended to breakdown with intermittent moisture. Scenarios of relatively low FHB and high DON, and low FBM and high DON were evident when moisture was discontinuous.

Real-time PCR assay for assessing the interaction of *Sphacelotheca reiliana* with maize seedlings

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Sphacelotheca reiliana causes head smut of maize, which can have a devastating effect on maize yield. Seed treatments and host resistance are viable management tactics for head smut. Assessing seed treatment efficacy or disease resistance in the field is time-consuming and highly variable; therefore a tool to expedite the screening process would be valuable. We have developed a seedling assay for infection by *S. reiliana* and detection of infected seedlings via quantitative real-time PCR, to both increase efficacy and decrease time expenditure for evaluating management practices or resistance to *S. reiliana* infection. To artificially infect seedlings, two methods were developed; in the first method, seeds were coated with a suspension of *S. reiliana* teliospores and allowed to dry before planting; in the second method, seeds were planted in soil infested with spores. Five maize genotypes were tested, representing low, intermediate, and high susceptibility to head smut. Seeds of one genotype were planted with or without a fungicide seed treatment. After 4 weeks the plants were surface sterilized and tissue samples were taken from the seminal roots, nodal roots, and crown tissue. DNA was extracted, purified and tested for the presence of *S. reiliana* via the species-specific qPCR assay. Frequency and progression of infection were compared among genotypes and the effect of seed treatment was assessed, based on qPCR results from seedling tissues.

Genome-wide mutation dynamic within a long-lived individual of *Armillaria*

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Mutation is the ultimate source of all genetic variation in populations and yet the events themselves remain unobservable and buried in the past. Long-lived individuals of *Armillaria gallica*, a common opportunistic fungal pathogen of tree roots in temperate forests of the northern hemisphere, provide a spatial context for the mutational dynamic. Each individual of *A. gallica* arises in a single mating event between two haploid gametes and the resulting diploid of unique genotype then grows vegetatively to occupy a discrete spatial territory including many adjacent tree root systems. In effect, this leaves a spatial record of growth over time within which mutations can be pinpointed. To identify mutations, the entire genomes of three spatially separated samples of one individual of *A. gallica* approximately 200 by 60 m in size were sequenced and compared. In this comparison, mutations and regions of loss of heterozygosity (LOH) were identified and then assayed in another 22 isolates from the same individual by conventional PCR and Sanger sequencing. The genotype network of all mutations and LOH events was without internal conflict. Further, the spatial pattern of genotypes was non-random and appeared to reflect the vegetative expansion leading to the present-day

individual. I will discuss the implications of the whole-genome data in estimating mutation rates and cellular generation times.

Multiplex PCR identification of high consequence *Bemisia tabaci* biotypes and *Trialeurodes vaporariorum*

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The whitefly (Hemiptera; Aleyrodidae) species *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) are worldwide agricultural pests and virus vectors. *B. tabaci* is a cryptic species with genetically distinct but morphologically indistinguishable biotypes. Rapid identification of whitefly vector species and biotypes via molecular methods could facilitate interventions that prevent cross-border introductions of exotic whiteflies or plant viruses. Biotype-specific mtCOI PCR primer sets were designed for the exotic B and Q biotypes, the New World A-like consensus group, and *T. vaporariorum*. Customized A/T-rich overhang sequences were incorporated at the 5' terminus of each primer, as well. Resulting PCR amplicons for primer sets lacking overhang sequences were 550-, 712-, 329-, and 252-bp, respectively, and 559-, 717-, 353-, and 258-bp, respectively, for primers with overhang sequences. Endpoint PCRs using each of the eight primer sets were carried out using genomic DNA extracted from three individual adult representatives of each target, and PCR products were separated by 1% agarose gel electrophoresis. Bands were eluted and directly sequenced to confirm primer-target specificity. Primers with and primers without overhang sequences were then pooled separately and used in multiplex PCR assays. Using these primer sets in traditional or multiplex PCR facilitated the rapid discrimination and specific identification of *B. tabaci* biotypes and *T. vaporariorum*.

Investigation of the role for CHUP1 and class XI myosins during infection of *Arabidopsis thaliana* by *Cauliflower mosaic virus*

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The P6 protein of *Cauliflower mosaic virus* (CaMV) assembles in the cytoplasm into large, amorphous inclusion bodies (IBs) that associate with and move on microfilaments. The CaMV IBs are considered virion factories, as they are the site for protein expression, genome amplification, and virion assembly. To identify host proteins that might explain movement of P6 IBs on microfilaments, we utilized a yeast two-hybrid screen of an *Arabidopsis* cDNA library with CaMV P6 as the bait. One protein identified in this screen was CHUP1 (Chloroplast Unusual Positioning 1), a plant protein that is localized to the outer envelope of chloroplasts and is responsible for their movement on actin microfilaments. To investigate the contribution of CHUP1 to CaMV infections in *Arabidopsis*, we inoculated CaMV virions to an *A. thaliana* *chup1* T-DNA knockout line and found that the CaMV infection rate was similar to the wild type Col-0 plants. The class XI and VIII myosins have also been shown to contribute to the intracellular movement of plant viruses and viral proteins. To investigate whether functional redundancies might mask a putative role for CHUP1 in CaMV movement, we evaluated the capacity of CaMV to infect *Arabidopsis* T-DNA knockout lines for class XI and VIII myosins. Several independent tests have indicated that CaMV infections of the double T-DNA knockout line (*chup1*/ myosin XI-2) are significantly delayed relative to Col-0.

Effects of management systems on stem and leaf spot diseases in lowbush blueberry

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Lowbush blueberries are grown in a two year crop cycle with the first year after pruning producing vegetative growth and the crop year producing flowers and then fruit. Lowbush blueberries have a variety of diseases that often vary among different years of the crop cycle and between fields. As part of an interdisciplinary study on crop management systems with different levels of inputs in Maine lowbush blueberries, disease levels were assessed in 2 crop cycles over 4 years. The management systems consisted of organic management and low, medium and high input of fertilizer, pesticides, pollinators and other inputs. There was significantly less blueberry cover in

organic and low input fields and significantly lower yield in organic fields. In vegetative years, there were higher levels of leaf spot in organic and low input fields than other fields. The most prevalent diseases in the prune year were Septoria leaf spot, powdery mildew and leaf rust and in the crop year, mummy berry disease, powdery mildew and Septoria leaf spot. In the 2012 vegetative year, leaf loss was highest in the organic and low input fields and was significantly correlated to levels of leaf rust. Phomopsis stem blight was the only stem disease found in most fields and was significantly higher in medium and high input fields than the other fields during prune years. Disease levels were influenced by multiple factors including management inputs, site specific factors and variation in local climate.

The production of mycotoxins by fungi isolated from maple syrup

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Maple syrup processors occasionally find syrup contaminated with fungal growth. Processors have found contaminants in bulk containers of syrup and retail size bottles. We have identified 73 fungal isolates collected from 38 containers of maple syrup from the United States. The most common genera we have identified are *Penicillium*, *Aspergillus* and *Wallemia*, and in a few containers, we have identified *Cladosporium*, *Paecilomyces* and various ascomycetous yeasts. A number of the species of *Aspergillus* and *Penicillium* we have isolated are known to have the ability to produce mycotoxins. We are interested in quantifying any mycotoxins produced in maple syrup by these fungi and determining if the mycotoxins are at a level to be a concern for human health. The most common species of *Penicillium* that was isolated was *P. brevicompactum*, which is known to produce mycophenolic acid (MPA) and other mycotoxins. Three genetically distinct strains of *P. brevicompactum*, which we isolated from maple syrup, have produced up to 1 mg/ml of mycophenolic acid when grown in a medium optimized for MPA production. We are currently testing whether *P. brevicompactum* produces MPA when grown in maple syrup, and we will present results on testing for possible mycotoxin production by other *Penicillium* and *Aspergillus* species commonly isolated from maple syrup.

An Internet-served forecast system for mummy berry disease in Maine lowbush blueberry fields using weather stations with cellular telemetry

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Mummy berry disease, caused by *Monilinia vaccinii-corymbosi*, can result in significant yield loss in lowbush blueberry. Apothecia are produced at bud break and ascospore infections kill emerging leaves and flowers. The Mummy Berry Forecast System (MBFS), developed by Hildebrand and Delbridge, is a method of applying curative fungicides within 72 hours of infection periods which are identified by air temperature and leaf wetness duration. In Maine, growers historically relied on a calendar-based fungicide timing method which often resulted in poor control. In 2 years of demonstration field trials, the same level of control was obtained with two fungicide applications using the MBFS compared to three with the calendar method. In 2009 to 2011, temperature and leaf wetness sensors and pseudosclerotia plots were placed in 6 fields and observed 1 or 2 times a week to provide 1 to 2 reports per week for growers. In 2012, 10 weather stations collected leaf wetness and air temperature data every 20 minutes and data was immediately transmitted to a website. Growers monitored pseudosclerotia plots and provided information on the presence of mature apothecia. The improved MBFS allowed for reports on infection periods soon after they occurred and provided more cost-effective, accurate and timely information for growers who now widely accept the MBFS for control of this disease.

Molecular mechanisms of eIF4E-mediated resistance against potato viruses

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The viral protein VPg, bound to the 5' end of many potyviral RNA genomes is the major determinant in virus infection. The mechanism of plant susceptibility involves a physical interaction between the VPg and eukaryotic translation initiation factor eIF4E. The disruption of this interaction, conferred by mutations in eIF4E, prevents potyviral infection and is a source of resistance

in a number of crops. We generated transgenic resistant potato lines over-expressing a modified eIF4E potato gene containing point mutations homologous to the *pvr1²* pepper allele resistant to *Potato virus Y* (PVY). Our goals are to (i) test the range of resistance conferred by the over-expression across different potato viral families including strains of PVY, *Potato virus X* (*Potexvirus*), and the VPg-bearing *Potato leafroll virus* (*Polerovirus*), for which components influencing plant susceptibility have yet to be identified, and (ii) explore the link between eIF4E-mediated resistance to plant viral translation. Our first screen revealed that the transgenic potatoes retain resistance to repetitive inoculation with PVY-O. The information gained from this plant-microbe interaction can be applied to the development of plant resistance lines in the agricultural community.

Diversity and specificity of phenotypic effects of endohyphal bacteria on foliar fungal endophytes

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Endohyphal bacteria (EHB) have been documented in foliar fungal endophytes representing all major clades of non-lichenized Pezizomycotina. These bacterial endosymbionts can influence phenotypes of fungal hosts and the outcomes of plant-fungal interactions. Because the diversity of these bacteria (including Alpha-, Beta- and Gammaproteobacteria as well as some Gram positive species) does not reflect the phylogenetic relationships of their fungal endophyte hosts, EHB are predicted to be horizontally acquired; however, this mode of transmission has not been previously tested. Here we report successful resynthesis of EHB-free fungal and axenic bacterial cultures into symbiosis and demonstrate this interaction affects growth rate in a model endophyte/EHB pair in vitro. We further compare responses of infected, cured, and resynthesized fungi exposed to different stimuli as part of a larger study to evaluate the specificity, lability, and phenotypic effects of EHB on endophytes and endophyte-host associations. Complete genome sequencing of phylogenetically and ecologically diverse EHB will further characterize the genetic basis of their relationships with, and effects upon, their fungal hosts and the plants that these endophyte/EHB inhabit.

A simple and rapid method to generate full sequence reads from small qPCR amplicons using direct sequencing

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Real-time quantitative PCR (qPCR) is the most widely used molecular diagnostic technique, and has applications in numerous fields. Generally, a product size of 100-150 bp is considered ideal for qPCR; smaller products are almost impossible to sequence directly. The lack of a rapid method for direct sequencing of short qPCR fragment limits the confirmation of PCR results during detection and diagnostic procedures. The aim of this research was to develop a method for direct sequencing of short qPCR products without the need of cloning. To validate the method, primer sets for a virus (*Triticum mosaic virus*), a bacterium (*Xylella fastidiosa* sub sp. *pauca*) and a fungus (*Phymatotrichopsis omnivora*) were used to specifically amplify qPCR products of 69 bp, 82 bp and 116 bp, respectively. The amplicons were cloned and the obtained sequences compared for quality and reads with those directly sequenced. Preparing qPCR amplicons for direct sequencing takes only about 10 minutes. This rapid technique enhances sequencing quality and accuracy, and will streamline qPCR assay confirmations for applications in disease diagnostics, agricultural biosecurity, microbial forensics, forensic entomology and other potential applications.

Modification of oligo design for enhanced sensitivity of a DNA microarray for detection of fungal onion bulb rot pathogens

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Onion (*Allium cepa* L.) is an important vegetable crop in Washington State and the United States. Losses of onion bulbs to storage rots can have a significant financial impact on the onion industry. Management of bulb rots is challenging because of limited resources for rapid detection of potential bulb rot pathogens. A method for early, accurate and sensitive detection of bulb rot pathogens in onion tissues is needed. Development of a DNA microarray was initiated to detect and identify 14 fungi that can cause onion bulb rots, of which three *Botrytis* spp. are of prime importance. Genus- and species-

specific oligos were designed from the internal transcribed spacer (ITS) and L45-550 gene regions of *Botrytis* spp. Specificity of the oligos was validated *in silico* against accessions available in GenBank. Oligos were modified by adding 10-42 customized nucleotides at the 5' and/or 3' terminus to raise and match the oligos Tm (up to 65°C), to enhance cross-linking of oligos on nylon membranes. The modification enhanced hybridized spot intensity and sensitivity of detection. This macroarray for simultaneous detection of multiple bulb rot pathogens is expected to be sensitive, accurate, and easy to implement. The macroarray will be useful for the Onion ipmPIPE network, a platform integrating diagnostic tools, pest management, and marketing of *Allium* crops.

SYBR green and Taqman qRT-PCR, helicase dependent amplification, end-point RT-PCR and Razor Ex BioDetection System for detection of High plains virus

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High plains virus (HPV), causes considerable damage to wheat if the crop is infected during early stages of development. The virus occurs frequently in mixed infections with *Wheat streak mosaic virus* (WSMV) or *Triticum mosaic virus* (TriMV) and plants infected with HPV are hard to discriminate from others infected with WSMV or TriMV in the field. A method for early, accurate and sensitive detection of HPV in plant tissues is needed for management of disease outbreaks and reservoir hosts. In this study, the effectiveness of five methods for detection of HPV was evaluated. Specific PCR primer sets and probe were designed to target the HPV nucleoprotein gene. Primer set HPV6F and HPV4R, which amplify a product of 96 bp, was validated *in silico* against published sequences and *in vivo* against an inclusivity panel of infected plant samples and an exclusivity panel of viruses. PCR products were cloned to develop positive controls. SYBR and Taqman qRT-PCR, end-point RT-PCR and helicase dependent amplification methods all allowed detection of as little as 1 fg of plasmid DNA carrying the target gene sequence as well as in infected plant samples. The described laboratory and field deployable assays are accurate, rapid, sensitive and useful for pathogen detection and disease diagnosis, microbial quantification, certification and breeding programs, as well as biosecurity and microbial forensics applications.

Development of end-point multiplex RT-PCR and helicase dependent amplification for detection of CMV, HVX, INSV, TMV, and TSWV

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Nursery and ornamental plant production is the fifth largest agricultural industry in the state of Oklahoma with \$400 million in annual sales. Virus diseases cause significant economic losses to the ornamental industry in the U.S. Plant viruses are commonly transmitted through infected tissue, dormant bulbs or other vegetative organs, and symptoms frequently do not develop during production stages and may not be seen until the plants reach the retailer. Sampling and sample shipping to diagnostic laboratories, and the return of test results may not occur in time for growers to make timely sanitary decisions. To address this, a helicase dependent amplification (HDA) assay that can be implemented at ornamental farms and an *in vitro* end-point multiplex RT-PCR were developed for detection of the industry-hampering viruses *Impatiens necrosis spot virus* (INSV), *Tomato spotted wilt virus* (TSWV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), and *Hosta virus X* (HVX). Specific PCR primer sets for HDA and multiplex RT-PCR were designed targeting nucleoprotein (INSV), coat protein (CMV, HVX, TMV) and nucleocapsid protein (TSWV). The two primer sets were validated *in silico* against published sequences and *in vivo* against an inclusivity panel of infected samples and an exclusivity panel of near-neighbor viruses. The described assays are accurate, rapid, sensitive and useful for pathogen detection and disease diagnosis from the infected nursery plants.

Working with workgroups: A neighborhood approach to area wide disease management of GLRaV-3 in CA vineyards

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In response to increasing concerns about *Grapevine leafroll associated virus-3* (GLRaV-3), an epidemiological and sociological study began to kick start

area wide disease management (AWDM) programs in California. The wine grape industry consists of many individuals managing grapevines for wine-making in different ways. The tendency towards individualism, combined with a high level of spatial mixing of viticultural blocks, creates perfect conditions for the spread of an insect-vectored virus. Without exhaustive scouting, initial infections often randomly distributed in blocks are difficult to detect. Anisotropic disease foci develop as mealybugs crawl along rows and GLRaV-3 becomes spatially aggregated at low incidence. Long term financial viability of a block can quickly be impacted under these conditions. With varying intensities and efficacies of disease and vector control between growers, neighbor to neighbor spread is common. While analyzing 5 years of previous leafroll disease incidence maps, a Q-method study was performed to assess the varying opinions of leafroll among grape growers and winemakers. The results of these studies aided in the assimilation of cooperative AWDM groups in Napa who are currently trapping mealybugs to assess vector hotspots and mapping GLRaV-3 in vineyards to coordinate future control measures and prevent re-infection of newly established replacement blocks.

Practical application of UV-B radiation against powdery mildews under greenhouse conditions

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We have previously demonstrated suppression of powdery mildew in rose, cucumber, and strawberry following brief exposures to red light and UV-B radiation during night hours. Currently the technology is being adapted for use in commercial greenhouses, and we have devised a robotic system with a boom containing UV-B fluorescent lamps moving horizontally over potted plants. Strawberry plants of cv. Korona were used to test the system, and inoculation was secured by placing diseased plants with powdery mildew (*Podosphaera aphanis*) next to healthy plants. The speed at which the boom traversed the plants was adjustable, and it was equipped with a blower to move the leaves below for more uniform exposure. Total UV-B energy at the topmost leaf of each plant was 390 or 214 J m⁻² day⁻¹ at boom speeds of 25 or 50 cm min⁻¹, respectively. Relative to the untreated control, UV-B during night reduced disease severity on the adaxial and abaxial leaf sides to 0.05 and 4.2% or 0.4 and 3.0% if the speed was 25 or 50 cm min⁻¹, respectively. UV-B treatment every three days was as effective as daily treatments. We are adapting the above technology for use on horizontal and vertical booms of commercially-available spray equipment for use against powdery mildews in commercial production of asters, cucumber, tomato, and several herbs. To promote more uniform canopy exposure, additional refinements of lamp reflectors and use of reflective material beneath plant canopies are being tested.

Isolation of antagonistic fungal consortium from rhizosphere and its evaluation against *Fusarium oxysporum* f. sp. *lycopersici* in tomato cv. Pusa Ruby

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Biological control has emerged out as an effective ecofriendly tool to protect crops from harmful pests. In general most of the studies emphasized use of single biological agent against a pathogen; however this results in inconsistent performance of the agent. To get better results a mixture of various rhizosphere antagonists was screened against *Fusarium oxysporum* f. sp. *lycopersici*. The antagonistic strains were isolated from rhizosphere of *Zizyphus jujube*. Out of 28 species isolated, four (*Trichoderma harzianum*, *T. pseudokoningii*, *Penicillium cyclopium* and *Aspergillus niger*) were found effective against *F. o. l.* in dual plate culture. These species were further evaluated singly and in various combinations and found to improve the percentage germination and growth of Tomato cv Pusa Ruby. Most effective of all was *T. pseudokoningii* followed by *T. harzianum* and *P. cyclopium*. *A. niger* showed phyto-toxicity to host plant and diluted the antagonistic effect of other partners. Although single inoculations were effective, combined inoculations have shown better results. Moreover, a remarkable improvement in growth and germination has been seen over the control in these inoculations. Further

studies are needed to understand detailed mechanism of interaction between these fungi. This study signifies successful antagonist nature of rhizosphere fungal consortium which has shown potential to reduce the use of chemical pesticides.

Inoculum density of *Podosphaera aphanis*, infection efficiency, and apparent susceptibility of the upper and lower surfaces of strawberry leaves
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Strawberry leaves become more resistant to powdery mildew (*Podosphaera aphanis*) as they mature, and resistance is characterized by inhibition of the pre and post infection processes and increase in latency period. On the susceptible leaves, powdery mildew is more commonly observed on the lower than the upper leaf surface. The explanation for this is that the upper leaf surface escapes airborne inoculum before the leaves unfold and then develop ontogenic resistance after leaves unfold. In the present study, we inoculated the upper and lower surfaces of young folded leaves with 1×10^3 conidia/cm² and assessed conidial germination and infection efficiency at 24 and 48 h post-inoculation, respectively. Rates of conidial germination were similar ($P = 0.67$) on both leaf surfaces, but infection efficiency was approximately three-fold higher ($P = 0.001$) on the lower leaf surface. In addition, both surfaces of other strawberry leaves were inoculated with conidial suspensions. Disease severity was significantly higher on the lower leaf surface when using 1×10^4 conidia per ml ($P = 0.004$), but not at 4×10^4 conidia per ml. The high inoculum dose may mask the relatively higher infection efficiency on the lower leaf surface. The ultimate impact of differential infection efficiency between the leaf surfaces rests upon whether the upper leaf surface in highly-folded leaves becomes exposed before acquisition of ontogenic resistance.

WITHDRAWN

Loop-mediated isothermal amplification for the detection of *Pseudomonas fuscovaginae*
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Pseudomonas fuscovaginae causes sheath brown rot of rice, which can also lead grain discoloration, panicle sterility and in severe cases, total yield loss. The disease is cosmopolitan and *P. fuscovaginae* often cohabits in mixed populations with other pathogens in the field. The symptoms can be confused with those produced by bacterial pathogens such as *Burholderia glumae*, *B. cepacia*, *Pantoea ananatis* and *Acidovorax avenae* subsp. *avenae*. Therefore a rapid diagnostic assay is required to differentiate *P. fuscovaginae* from these

other pathogens and to help determine its distribution and importance. Loop-mediated isothermal amplification (LAMP) is a rapid and sensitive technique which has been used widely as a diagnostic tool for plant, human and animal diseases, and which can be implemented in the field. We report on the use of genomic data to design a robust LAMP assay for the detection of *P. fuscovaginae*. LAMP primer design was based on conserved genomic regions identified using whole genome data from several isolates of *P. fuscovaginae*, that represent the known extent of variability within the species. The specificity of this assay was established using *P. fuscovaginae* isolates from Africa, Asia, Australia and South America as well as a range of other common bacteria associated with rice.

Variability in *Puccinia melanocephala* pathogenicity and sugarcane cultivar resistance in Louisiana
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Brown rust, caused by *Puccinia melanocephala*, is an important disease of sugarcane. Shifts in disease response from resistant to susceptible have been repeatedly observed for sugarcane cultivars; however, information is limited concerning pathogen variability related to differential host reactions in this pathosystem. To evaluate variability in the pathogen population and resistance responses in different host genotypes, seven cultivars were inoculated with a total of four urediniospore collections from three cultivars. Greenhouse grown plants were inoculated under conditions favorable for infection. After 2 weeks, disease severity was determined by image analysis. Three cultivars that experienced a shift from resistance to high susceptibility over time exhibited differential disease severity when inoculated with spore collections from two of the respective cultivars. Three other cultivars exhibited consistent moderate to high levels of quantitative resistance against all spore collections, and one cultivar with the *Bru 1* major resistance gene was highly resistant to all spore collections. The results demonstrated pathogenic variability related to host genotype in the pathogen. In addition, quantitative resistance was detected that could be very useful in on-going resistance research to ultimately improve breeding and selection for effective, durable resistance to brown rust.

Investigation of quantitative real-time PCR as a mechanism for evaluating the efficacy of experimental fungicides against *Septoria tritici*
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The typical latent period for the wheat fungal pathogen *Septoria tritici* in greenhouse conditions is at least 20 days, leading to long cycle times when evaluating efficacy of fungicides. Quantitative real-time PCR was investigated as a technology that might significantly shorten the length of this evaluation period and reduce cycle time for testing experimental fungicides. Fluorescent oligonucleotide probes were designed that targeted the *Septoria* cytochrome *b* (*cytb*) and 14- α demethylase (*cyp51*) genes. Using these probes, fungal DNA could be detected in infected leaf tissue as early as 24 h after inoculation. DNA was extracted at 0, 5, 10, and 18 days after inoculation from leaves of wheat seedlings that eventually developed 5-100% disease severity. Overall, the *cytb* probe produced better correlations between *Septoria* DNA levels and disease severity than the *cyp51* probe. There was a strong correlation between disease severity and fungal DNA levels in leaves with fully sporulating necrotic lesions with correlation coefficients of -0.89 and -0.85 for *cytb* and *cyp51*, respectively. However, DNA quantification at early stages of *S. tritici* development in leaf tissue was not a good predictor of final disease severity. Therefore, real-time PCR has a limited applicability for evaluating the efficacy of experimental fungicides against *S. tritici* at an early stage of development inside wheat leaf tissue.

Identification of bacteria associated with decline of ironwood trees (*Casuarina equisetifolia*) in Guam
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Decline of ironwood (*Casuarina equisetifolia*) in Guam was previously attributed to termite feeding and *Ganoderma australe*. Recently, we found that bacteria are involved in the disease complex. *Ralstonia solanacearum* (*Rs*) and two *Klebsiella* species were consistently isolated from declining trees that showed no evidence of *Ganoderma* or termite damage. Discolored wet wood and bacterial ooze gave positive results with *Rs*-specific ImmunoStrips (Agdia, Inc. SK 33900/0025) and loop-mediated isothermal amplification. Presumptive *Rs* cultures isolated from host tissues produced the same positive results.

16S rDNA sequence analysis of presumptive *Rs* strains showed maximum identity (MI) values of 99% with *Rs* (strain LMG 2299; K60) and *Rs* (strain GMI 1000). *Klebsiella* strains isolated from bacterial ooze and wet wood tissues from the same trees showed 99% MI with two *Klebsiella* species. Cultures from three trees were identified as *K. variicola* (strains F2R9 and At-22); cultures from a fourth tree showed 99% MI with *K. oxytoca* (ATCC 13182). Neither *Klebsiella* nor *Rs* were detected in healthy trees. Ironwood and tomato seedlings co-inoculated with *Klebsiella* and *Rs* showed distortion, wilt and tissue discoloration. *Klebsiella* and *Rs* were reisolated from stems 20 cm above the inoculation point. Identification and pathogenicity tests indicate that the bacterial component of ironwood decline is far more significant than previously suspected.

Additional hosts for *Balansia epichloe* in tall fescue pastures

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In an effort to determine the host range of *Balansia epichloe* (synonym *B. kunzei*), an endophyte of weed grass species in pastures that might confound toxicity symptoms of cattle by producing ergot alkaloids, a study of additional hosts were observed over a 10 year-period. We report that two new weed grasses species have been discovered in pastures monitored for 20 plus years, including reseeding of pastures with tall fescue. The initial weed grass species was smut grass (*Sporobolus poiretii*) infected with *B. epichloe* that was completely replaced in time by *B. epichloe* infections on two new weed grass host species in newly established tall fescue pastures including lacegrass (*Eragrostis capillaris*), and big top lovegrass (*Eragrostis hirsuta*). Further, molecular analyses using a rep-PCR method indicated that while validated as *B. epichloe* using morphological descriptions, these fungi actually consisted of at least two cryptic species. However, they do all have the potential to produce ergot alkaloids and are grazed heavily during periods of stress and tall fescue grass reduction. These results suggest host jumping is responsible for migration of *Balansia* species from smut grasses to the new hosts.

Nitric oxide detoxification by *Fusarium verticillioides* involves flavo-hemoglobins and the denitrification pathway

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Nitric oxide (NO) is a highly mobile and potent signaling molecule, yet as a free radical it can also cause nitrosative stress to cells. To alleviate negative effects from excessive accumulation of endogenous NO or from potential exogenous sources, flavohemoglobin proteins can convert NO into nontoxic nitrate ions. We have investigated the flavohemoglobins in *Fusarium verticillioides*, a mycotoxigenic pathogen of maize. Two genes encoding putative flavohemoglobin homologs, denoted *FHB1* and *FHB2*, were identified. Microarray analysis revealed a significant induction of *FHB2* (17-fold) when the fungus was exposed to exogenous NO (1.5 mM of NO-donor DETA NONOate). *FHB1* had a 2-fold increase. Noteworthy was the 246-fold increase of the dissimilatory nitrite reductase (*NIR1*) and high induction of other genes from the denitrification pathway. Deletion mutants (*fhb1*, *fhb2*, and *nir1*) were challenged with 1.5 mM NO-donor, and *fhb1* and *fhb2* mutants had reduced growth compared to wild type, whereas the *nir1* mutant exhibited no decrease in growth. Strains with *fhb1/fhb2* double deletion were unable to grow when challenged with NO. All mutants were able to grow on nitrate medium, yet *fhb2* and *nir1* mutants were unable to grow on nitrite. Overall, the flavohemoglobins may have differential roles for converting endogenous NO (*FHB1*) versus exogenous NO (*FHB2*) to nitrate. Our results also demonstrate a significant role of the denitrification pathway in NO detoxification.

Screening sorghum germplasm for biotic and abiotic stress tolerance and potential use of selected physiological traits as disease severity predictors

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Stalk rots are major biotic stresses of sorghum [*Sorghum bicolor* (L.) Moench], while drought is the most important abiotic stress. The objectives of this study were to screen 46 'minicore' accessions to identify sources of resistance/tolerance for both stressors and to analyze the potential of using selected physiological traits in predicting resistance to stalk rot diseases. Plants were inoculated with *Macrophomina phaseolina* (MP) and *Fusarium thapsinum* (FT) at 14 days after flowering. At harvest, plants were screened for disease

severity using lesion length (LL), number of diseased nodes (NC) and 100-seed weight (SW). Three physiological traits: chlorophyll fluorescence, chlorophyll content and canopy temperature were measured at 74, 84 and 94 days after planting (DAP). No accession exhibited significantly decreased LL or NC compared to the resistant control 'SC599R', against both pathogens. However, one genotype exhibited significantly higher SW than SC599R in FT-inoculated treatment while there were four such genotypes for MP, indicating their potential use as sources of tolerance. Chlorophyll content at 74 DAP was significantly correlated with LL in both MP and FT inoculated treatments revealing its potential use in forecasting disease severity. There was no statistical evidence for the potential use of canopy temperature and chlorophyll fluorescence as predictors of disease severity though they could be used in screening sorghum germplasm for drought tolerance.

Analysis of acquisition and titer of Maize mosaic rhabdovirus in its vector, *Peregrinus maidis*

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The corn planthopper, *Peregrinus maidis*, transmits Maize mosaic rhabdovirus (MMV), an important pathogen of maize and sorghum, in a persistent propagative manner. To better understand the vectorial capacity of *P. maidis*, we determined the efficiency of MMV acquisition at nymphal and adult stages, and characterized MMV titer through development. Acquisition efficiency, i.e., proportion of insects that acquired the virus, was determined by reverse transcriptase (RT) – PCR and virus titer of individual insects was estimated by real-time quantitative RT-PCR one to two weeks post acquisition at the 3rd, 4th, and 5th nymph stages, and in adult males and females. Acquisition efficiency of MMV differed significantly ($P = 0.003$) between nymphs (76%) and adults (40%). MMV titer increased significantly ($P < 0.0001$) from nymphal to adult stage, indication of virus replication in the vector during development. There was a positive association between the vector developmental stage and virus titer ($r_s = 0.71$, $P = 0.0001$). Also, the average titer in male insects was 3-fold higher than female titers ($P = 0.0004$), and this difference persisted up to 30 days post adult eclosion ($P < 0.05$). Overall, our findings indicate that nymphs were more efficient than adults at acquiring MMV and virus accumulated in the vector over the course of nymphal development. Furthermore, sustained infection over the lifespan of *P. maidis* indicates a potentially high capacity of this vector to transmit MMV.

Oxytetracycline resistance in *Xanthomonas arboricola* pv. *pruni* (causal agent of bacterial spot of stone fruit)

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Bacterial spot of stone fruit (*Xanthomonas arboricola* pv. *pruni* [*Xap*]) is the most important bacterial disease of peach in PA where the antibiotic oxytetracycline is used for management. Populations of antibiotic resistant bacterial epiphytes in stone fruit orchards were monitored and *Xap* isolates were screened to determine current levels of oxytetracycline sensitivity. In 2012, bacterial epiphytes growing on media amended with oxytetracycline were recovered from all 7 PA peach orchards, including 2 organic orchards where no oxytetracycline had been applied. Visual comparisons made between bacteria recovered from conventional and organic orchards showed that Gram positive bacterial colonies made up the majority of those recovered from the two organic orchards while most of the bacteria recovered from conventional orchards were Gram negative. Bacterial spot samples were collected from 8 PA stone fruit orchards and *Xap* was isolated from infected tissue and screened for oxytetracycline sensitivity. The majority of *Xap* isolates tested grew at low concentrations (i.e.: 10mg/L and less) of oxytetracycline regardless of the number of oxytetracycline applications made in the field in 2011 and 2012. The number of oxytetracycline applications, however, influenced the number of *Xap* isolates that grew in media amended with higher concentrations (i.e.: 15 mg/L and above) of oxytetracycline.

Ganoderma species in the neotropics

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Ganoderma species are important white rot fungi that can cause significant damage to forest and ornamental trees, as well as oil palm crops around the world. In 1997, Monclavo and Ryvarden listed 386 names for the *Ganoder-*

mataceae, and in 2000 Seo and Kirk listed 322 species names. Several authors report that morphological characters in some *Ganoderma* species complexes are not helpful in distinguishing between phylogenetic groups, which likely has confounded the number of species reported in the past. We collected and analyzed the ITS region of over 30 *Ganoderma* samples from the Eastern and Western regions of the Andes in Ecuador, and also find many examples where species names based on morphology do not agree with ITS sequence data. *G. applanatum* found in Ecuador fit well into the neotropical clade proposed by Monclavo and Buchanan (2008), and other *Ganoderma* (*G. lucidum* and *G. cupreum*) obtained appear to show the same geographical pattern. Based on the phylogenetic evidence, we propose that the neotropical based *Ganoderma* species in this study be given new species or infraspecies names. Our results also suggest phylogenetic clades linked to geographical origin may be common in other *Ganoderma* species and perhaps other polypores. Consideration of geographical origin along with phylogenetic analyses is important for identifying *Ganoderma*.

***Edythea quitensis* infecting *Berberis* species in Ecuador**

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There has been a renewed interest in role *Berberis* species play in the genetic diversity of rust fungi since the emergence of the new race of *Puccinia graminis* Ug99 that infects wheat, and the discovery of *Berberis* as an alternate host of *P. striiformis*. Testing of *Berberis* stocks for susceptibility to rust fungi has historically been done at the Cereal Disease Laboratory, USDA-ARS, but is now a more global effort. Ecuador has 32 reported species of *Berberis* of which 16 or more are endemic. None of these have been tested for their susceptibility to cereal rust fungi. We collected rust fungal samples from at least two previously untested *Berberis* species and nearby grasses at various locations in the highlands of Ecuador. Based on ITS sequence data, we found the previously unknown aecial-pycnial stages of the macrocyclic-autoecious rust fungus *Edythea quitensis* on *Berberis hallii*. In another location, we identified a heteroecious *E. quitensis* infecting both a different *Berberis* species from *B. hallii* and the grass species *Luzula*. Future work will include inoculation tests of cereals with rust infected *Berberis* leaves.

Factors affecting proliferation of *Salmonella enterica* in tomato fruit tissues

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In tomato fruit tissues, introduced populations of *Salmonella enterica* proliferated from ca. Log 2.0 to 7.0 during a 24-h storage at 22 C. Within the next 24 h, populations of up to 8.5 Log cfu were detected. Factors influencing these increases included storage temperature, serovar, fruit ripeness, and fruit water content. Warmer and longer storage regimes led to higher populations. Repeated analyses of three cultivars ('Florida-47,' Solar Fire,' and 'Bonny Best') grown in field plots over four seasons with three irrigation regimes beginning shortly before the first harvest failed to detect consistent proliferation differences among field treatments. Serovar Newport consistently proliferated to a significantly larger population than Typhimurium, Montevideo, or Javiana. Increased tissue water content led to increased proliferation when fruit were green to inconsistent or even reduced proliferation when fruit were pink or red. Tissue water uptake was very rapid (greater than 5% increase in weight within 15 min) if unprotected pericarp or endocarp tissues were exposed to water. Three observations were consistent with bacterial migration within non-disturbed fruit tissues. First, washing wounds with deionized water prior to inoculation, did not affect proliferation. Secondly, *S. enterica* were detected several mm below inoculated tissues. Thirdly, proliferation resulting from inoculation of a standard-sized wound increased as tissue mass increased.

The first report of *Tomato apical stunt viroid* (TASVd) and *Potato spindle tuber viroid* (PSTVd) in tomatoes in Ghana

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Tomato plants (*Solanum lycopersicum*) with symptoms of stunted growth, leaf epinasty and necrosis of leaf veins and stems, were observed in Mali and

Ghana in 2011 and found to be infected with *Columnea latent viroid* (CLVd). Surveys conducted in Ghana in 2012 revealed these CLVd-associated symptoms (locally referred to as 'rasta') in numerous fields in Agogo, Akomadan, Toubodan and Tonoso at incidences ranging from 0-15%. To test for CLVd, representative symptomatic leaf samples were applied onto Agdia absorption strips, total RNA extracted and RT-PCR performed with CLVd-specific primers. Results of these tests were negative; however, a virus-like agent was transmitted to tomato seedlings following mechanical-inoculation. In RT-PCR tests, amplification of the expected size fragment was directed by primers specific for *Tomato apical stunt viroid* (TASVd; 20% of plants) and *Potato spindle tuber viroid* (PSTVd; 80% of plants). The sequences of these putative TASVd and PSTVd fragments were 96% identical with those of TASVd isolates from the USA and Senegal, and PSTVd isolates from Western Australia and the U.K., respectively. In host range studies, the Ghana TASVd and PSTVd isolates only induced symptoms in tomato plants, although symptomless infections were detected in some species. This is the first report of TASVd and PSTVd in Ghana and, similar to CLVd, they may have been introduced in association with tomato seed.

Diversity of sooty blotch and flyspeck fungi from apples in Spain

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Fungi in the sooty blotch and flyspeck (SBFS) complex blemish apple fruit and reduce market value. In 2010 apples were collected from 16 orchards in northern Spain. SBFS colonies with subtending cuticle were excised, pressed, and shipped to Ames, Iowa for isolation. A total of 215 isolates were obtained. After a portion of the rDNA was amplified with primer pair VG9/LR5, two regions were sequenced with primer pairs ITS1-F/ITS4 and LROR/LR5. Parsimony analysis of the LSU was used to place fungi into order. Putative species were delineated from the ITS sequences and morphology. Most (89%) of isolates were within the class Dothideomycetes, order Capnodiales. Within this order, 86 isolates were *Schizothyrium* spp., including *S. pomi*, *Z. cryptogama* and three previously undescribed putative species. *Microcycluspora mali* (45 isolates) and four species of *Microcycluspora* spp. (36 isolates) were also widely prevalent. Four putative species in the *Stomiopeltis*-like clade included 11 isolates. A new putative species of *Ramichloridium* was detected in 5 orchards (11 isolates). Our survey revealed that the SBFS complex in northern Spain includes at least 25 species in 4 orders and further documents the worldwide genetic diversity within the SBFS complex.

New swainsonine producing *Undifilum* species from the locoweed *Astragalus pubentissimus*

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Locoweeds are problematic rangeland legumes from the genera *Oxytropis* and *Astragalus* due to associated fungal endophytes and their production of the toxin swainsonine. Swainsonine, an alpha-mannosidase inhibitor, causes neurological problems that are responsible for significant losses to grazing animals. Toxin producing fungal endophytes isolated from *Oxytropis* spp. and *Astragalus mollissimus* and *A. lentiginosus* have been identified as *Undifilum oxytropis*, *U. cinereum*, and *U. fulvum*, respectively. However, fungi isolated from *A. pubentissimus* plants appeared to be morphologically distinct from previously identified *Undifilum* spp. Fungal endophytes from *A. pubentissimus* were analyzed for culture and spore morphology as well as sequencing of four DNA regions. Additionally, isolates were subjected to random amplification of polymorphic DNA (RAPD) for fingerprinting and compared to previously identified species. Results show distinct features that characterize fungal endophytes isolated from *A. pubentissimus* as a new species within the genus *Undifilum*.

Survey of fungicide resistance of *Botrytis cinerea* in Virginia vineyards

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Botrytis cinerea is one of the most important fungal plant pathogens of fruit, vegetable, and ornamental crops worldwide, and commonly managed with intensive fungicide spray programs. Fungicide resistance development in *B. cinerea* has been common since the 1970s with the advent of the benzimidazoles and successive other groups. In a 2011-2012 survey of fungicide resistance in Virginia vineyards, a total of 178 *B. cinerea* isolates were tested by the germination/germ tube elongation method of Weber and

Hahn, with some modifications in fungicide concentrations in the media. Resistance to anilino-pyrimidines was present in some samples, but degree and frequency were uncertain, in part because a subset of isolates grew poorly on sucrose medium; assays were supplemented by inoculation of treated and untreated table grapes. Resistance to benzimidazoles was widespread, and a low level of resistance to iprodione (residual germ tube growth on 5 mg/L iprodione) was common as well. The many boscalid-resistant isolates in the survey, most of which also had resistance to QoI, were not cross-resistant to fluopyram. Fenhexamid resistance was rare in Virginia grape isolates (4 isolates from 2 sites), but has been reported elsewhere in 45% of Florida strawberry isolates, 17% of Carolina strawberry isolates, and was detected in samples from two of three Virginia strawberry fields tested.

New rice resistance genes via targeted genome editing

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Phytopathology 103(Suppl. 2):S2.13

Rice is one of the most important food crop in the world providing the principal source of calories for nearly half of its population. Bacterial Leaf Blight (BLB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae*, depends on direct induction of host susceptibility genes by bacterial TAL (Transcription Activator-Like) effectors. So far, efficient control of this disease relies on the use of resistant varieties. Since bacterial ability to induce specific host susceptibility genes is critical for disease development, we wish to create mutations in rice susceptibility gene promoters using the TALEN (TAL Effector Nuclease) technology, in order to make them unresponsive to bacterial infection. We show that (i) designer TALENs cleave DNA target sites in a predictable manner, (ii) engineered bacteria secrete TALENs into culture supernatants, and (iii) engineered TALEs injected into rice embryogenic tissues are able to induce their target genes efficiently. Based on the DNA recognition code, designer TALENs were created to induce site-specific protective mutations and thus mimic the natural evolution of resistance genes on a much faster scale. With this approach, we hope to provide breeders with new strategies to generate broad and durable resistance to bacterial infections, thus reducing chemical use, providing yield stability, and increasing profitability of rice farming, in particular for smallholder farmers.

Defining the stages of infection of grapevine stems with the trunk disease Botryosphaeria dieback

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Detection of *Neofusicoccum parvum*, one of the causal agents of Botryosphaeria dieback of grapevine, is limited to the late stage of infection, when the wood canker is well-established. Early detection is hampered by the internal nature of the canker, which is revealed only by cutting into the wood. A detection tool for the early stage of infection, and from leaves instead of the trunk or cordon, would inform management decisions. We defined early and late stages of infection of inoculated vs. non-inoculated (both wounded and non-wounded) plants at 2-week intervals for 2 months, based on: recovery of *N. parvum* in culture, xylem occlusions as measured by light microscopy, and xylem embolism as measured by high-resolution x-ray computed tomography (HRCT). Incubation of 1.5 months was required for recovery at 2 cm from the inoculation site (late stage). In contrast, occlusions and embolized vessels, which were significantly more frequent in inoculated plants, were found at only 2 weeks post-inoculation and up to 4 cm from the inoculation site (early stage). Our findings show that these changes in xylem anatomy occur in advance, both in terms of time and position relative to the inoculation site, of colonization. The next step is to identify candidate genes, as identified by RNAseq and confirmed by qRT-PCR, the expression of which in grape leaves corresponds with the early timing of occlusions and embolisms in the woody stem.

Evaluation of fungicides and biorational products for management of Pythium and Rhizoctonia damping-off in greenhouse-produced vegetables

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Damping-off, caused by *Pythium* spp. and *Rhizoctonia solani*, can cause significant losses in transplant and micro-vegetable production in protected

environments. Experiments were performed in a greenhouse to determine the efficacy of biopesticides and fungicides against *Pythium* and *Rhizoctonia* damping-off in lettuce and pepper. Treatments applied were Rootshield Home and Garden, Rootshield PLUS WP, Rootshield WP, Prestop, Actinovate, Mycostop, Serenade Soil, Subtlex, Regalia, SoilGard and the fungicides Double Nickel LC, Previcur Flex, Banrot and Phosphite. The center 48 cells of 288-cell trays were filled with *P. ultimum* or *R. solani*-infested potting mix (0.5 g inoculum/100 ml Fafard's Superfine Germinating mix) and the remaining cells of each flat were filled with non-inoculated potting mix. Treatments were applied to the entire flat, with the exception of the Root Shield Home and Garden, Rootshield PLUS WP and Rootshield WP treatments, which were only applied to the center 48 cells of each flat. Rootshield Home and Garden, Rootshield PLUS WP, Rootshield WP and Double Nickel LC were consistently effective in reducing *Rhizoctonia* damping-off in pepper and lettuce. While no product was effective in reducing *Pythium* damping-off in pepper, Prestop, Double Nickel LC, Rootshield Home and Garden, Rootshield PLUS WP, Mycostop, Previcur Flex, Serenade Soil and Regalia effectively controlled *Pythium* damping-off in lettuce.

Mosquito midguts and the trichomycete fungi that don't live there

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Trichomycete fungi live symbiotically in the guts of many aquatic insects. They are especially common in larval Diptera such as chironomids, black flies, and mosquitoes. Two host habitats are used by these fungi: the midgut and hindgut. Each habitat has characteristic groups of trichomycetes. *Smittium* spp. are common in the hindguts of all three insect groups. Chironomids host *Stachylina* spp. in their midguts and black flies are the only known suitable midgut habitats for *Harpella* spp. Mosquitoes have never been documented to host trichomycetes in their midguts. We asked why trichomycetes have not been found in the mosquito midgut even though they are common in the hindgut. To test the suitability of mosquito midguts to fungi, we exposed them to unculturable *Harpella melusinae* trichospores (asexual) obtained from wild larval black flies. The spores were ingested by *Aedes albopictus* mosquitoes and exhibited growth. They tended to colonize larval mosquitoes *Aedes albopictus* more readily than *Culex quinquefasciatus* or *Aedes aegypti*. We suspect that environmental factors might be more important than physiology in explaining the lack of midgut trichomycetes. Mosquitoes tend to inhabit still waters, while black flies are found exclusively in running water.

Molecular and genetic basis guiding the establishment of a mutualistic relationship between Epichloë festucae and perennial ryegrass

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The relationship between *Epichloë festucae* and *Lolium perenne* is a model system for studying mutualism between endophytes and cool season grasses. During the reproductive phase of growth, the fungus exhibits a dual nature: benign endophytic growth and seed transmission, or forming external stromata and suppressing seed production. We have identified a number of genes encoding small secreted proteins (*ssp*) that are highly up-regulated in benign infected inflorescences. The preliminary data suggest that two genes, *sspB* and *sspX*, may play a role in host specificity. Although *E. festucae* is reported to be compatible with *L. perenne* and *L. pratense*, strains generated from a series of crosses and backcrosses showed a range of compatibility with *L. perenne*, but were consistently compatible with *L. pratense*. One such strain, E2368, had low compatibility with *L. perenne*, whereas a subculture (variant E4844) showed improved compatibility with this host. Genomes of E4844 and E2368 were compared, revealing the loss of subtelomeric region containing *sspB* and *sspX* in E4844. The possible roles of *sspB* and *sspX*, and of other genomic changes in the variant, are under investigation. The set of progeny strains has been screened for the establishment of stable mutualistic symbioses with perennial ryegrass, and is slated for Illumina genome sequencing and subsequent bulk segregant analysis to identify SNP markers correlated with host-specificity phenotypes.

Host specificity of fungal phyllosphere communities of tropical trees

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Tropical microorganisms in the phyllosphere such as epiphyllous yeast and endophytic filamentous fungi contribute substantially to global organismic

diversity. Because of their presence in virtually every ecosystem and the production of enzymes, alkaloids, antibiotics, phytohormones and other secondary metabolites, they markedly increase functional diversity of the inhabited vegetation. However, an accurate estimate of phyllosphere fungi of tropical trees is lacking and their ecology is almost unknown. Using next generation sequencing technology we analysed two tree species of a tropical mountain rainforest in southern Ecuador to estimate the amount of fungal spores per host species and to analyse the fungal phyllosphere communities in terms of host specificity and microclimate. Based on 12 trees from different plots we could identify more than 1000 OTUs of asco- and basidiomycetes. Each tree was characterized by a distinctive fungal community but the similarity of the fungal communities was significantly higher between the same host tree species rather than between the trees occurring on the same plot and sharing the same microclimate.

The genetic characterization and radiation of bacterial leaf scorch of oak in New Jersey

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Xylella fastidiosa subspecies *multplex* is a pervasive pathogen of *Quercus* genera in the Northeastern and Mid-Atlantic region of the United States. This subspecies is additionally characterized by plasticity in host range, presenting itself as a problematic source of potentially transmissible inoculum. Complementing our recent, regional multilocus sequence analysis (MLSA) work with oak-based bacterial leaf scorch populations, a reverse hierarchical sampling model is now being employed to describe pathogen distribution within the state of New Jersey. From our prior, broad-spectrum sampling data generated from mixed MLSA primer sets, it was thought that infection patterns would confirm distributive clonal transmission from diseased hardwood stands, but preliminary results revealed instances of secondary host passage favoring select genetic markers. In particular, it was found that the invasive weed *Fallopia japonica* appears inclined towards genotypic strains carrying specific variable number tandem repeat regions (VNTRs) and an experimentally proposed hypervirulence mutation at the *copB* and *acvB* loci respectively. In this study we phylogenetically position isolates from defined primary and secondary hosts in previously uncharacterized locales within New Jersey. The information gleaned herein provides insight into pathogen dissemination, type-strain localization, and potential disease management strategies.

A new name for an age-old fungus: Unraveling the mystery of dollar spot disease of turfgrass

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Dollar spot is one of the most economically important fungal diseases of warm- and cool-season turfgrass species. Although the pathogen was described as *Sclerotinia homoeocarpa*, the true taxonomic placement of this fungus has remained unresolved. Previous morphological and rDNA sequence data have indicated that this pathogen is more appropriately placed in the Rutstroemiaceae family rather than the Sclerotiniaceae family; however, additional data is needed to test this theory. In this study, we used molecular data to evaluate 85 isolates of the dollar spot fungus and closely-related fungi in the Rutstroemiaceae (*Ciboria*, *Lambertella*, *Lanzia*, *Poculum*, *Rutstroemia*). Partial sequences from eight regions were generated for phylogenetic analysis: actin, β -tubulin, calmodulin, the internal transcribed spacer region (ITS), the large subunit, DNA replication factor Mcm7, TEF1- α , and the small ribosomal RNA subunit. An additional ~200 bp fragment of ITS-1 was amplified from 54 fungarium specimens, including the original specimens used by Bennett to describe the species. This data confirmed that the dollar spot pathogen is not a true *Sclerotinia* species. Phylogenetic data indicated that two distinct species are responsible for dollar spot disease of turfgrass, and that these fungi are not members of any known genus in the Rutstroemiaceae family. In this presentation, we will discuss the proposed nomenclature for the two causal agents of dollar spot disease of turfgrass.

PGPR-*Viola* extracts consortia as biocontrollers of *F. oxysporum* and its effect on *Physalis peruviana* growth

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The Cape gooseberry is an economically important crop in Colombia. However, its production is constantly affected by Fusarium wilt caused by *F. oxysporum*. In a previous research, 5 rhizobacteria were selected and all the possible consortia were evaluated as *F. oxysporum* biocontrollers. Those consortia inhibited fungal radial growth up to 67% and reduced the conidial production up to 80%. Other effects of the consortia on the Cape gooseberry development were evaluated, finding an increment in size and number of the secondary root, besides an increase in the germination percentage, in relation to the control. Based on those results, the 10 best consortia were selected, based on the effect on fungus and on the plant growth. Simultaneously, a previous evaluation of ethanolic extracts from *Viola* sp. (Myristicaceae) showed a poor effect on fungus growth and conidia production. In order to evaluate the possible synergistic effect of the mixture bacteria-*Viola* sp. on its action against *F. oxysporum*, the bacterial consortia were mixed up with plant-derived ethanolic extracts. Regarding the extracts, the mixture with the bacteria *in vitro*, increased the effect up to 17%. Outcome showed that using the mixtures tested could reduce the pathogen growing and promote the Cape gooseberry development. This research shows the potential of combining methods for the management of different plant diseases with commercial importance *F. oxysporum*.

Geographic and climatic discontinuity in production of cleistothecia in *Podosphaera aphanis*

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Although *Podosphaera aphanis* occurs commonly throughout the range of strawberry production, cleistothecia are irregularly reported - particularly from relatively warm climates. Strawberry leaves with mildew colonies representing populations of *P. aphanis* were collected across a climatic temperature gradient of the continental U.S. Extracted DNA was then subjected to a PCR-based assay to detect the MAT1-1 and MAT1-2 mating types. Isolates representing each mating type were detected among nearly all samples. In parallel work, temperatures above 13C to 15C strongly suppressed ascocarp initiation when compatible isolates were paired on strawberry leaves. Absence of cleistothecia across the range of strawberry production does not appear to be due to the absence or unequal distribution of mating types of the pathogen, but to suppression of ascocarp initiation by high temperatures in warm climates, in glasshouses, or in high-tunnel production systems. Ascocarps can be expected to form rapidly in such environments if and when temperatures fall below 15C.

Host generalism in fungal pathogen and endophytes of seedlings and forest community dynamics

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Pathogen-based regulation of forest species diversity is dependent on the presence and impact of host specific fungi that limit host survival. Studies on forest pathogens, however, show that most seedling pathogens are generalist, in that they infect more than one host species from different phylogenetic groups. In this work we aim to characterize mechanisms through which potential generalist pathogens contribute to forest dynamics. Isolates recovered from seedlings of multiple host species showing disease symptoms were evaluated for cryptic specialization. Within the *Colletotrichum acutatum* species complex, most of the recovered isolates were classified as *C. fiorinae*, based on multi-gene phylogeny, regardless of host. Furthermore, the analyses of high-throughput amplicon sequencing of the fungal rDNA LSU, showed that individual fungal taxa respond differently to host species. For instance, *Colletotrichum* taxa respond less to host species identity, compared to *Diaporthe*; and both taxa respond to host health status and light availability. In addition, genome characteristics of *C. fiorinae* are similar to those to patho-

gens with broader host range, compared to host specific pathogens. Further studies of individual isolates and amplicon sequencing, using population-lever markers will help resolve pathogen host-specificity in this system, and their contribution to host species dynamics.

Successful biological control of Canada thistle (*Cirsium arvense*) with the rust fungus *Puccinia punctiformis*

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Canada thistle (*Cirsium arvense*, CT) is one of the worst weeds in temperate areas of the world. In the U.S., CT is present in 38 states and noxious in 25. The rust fungus *Puccinia punctiformis* was first proposed as a biological control agent for CT in 1893. The rust causes systemic disease, is specific to CT, and is in all states where CT is found. Systemic infections result in permanent infection of thistle roots, and all of the shoots on infected root systems eventually die. Despite a 120-year lapse, establishment of epiphytotics of the rust, and concomitant biological control of CT, have previously been unsuccessful, due to incomplete understanding of the disease cycle. We now understand the disease cycle, and the objectives of our studies were to use this knowledge to routinely establish epiphytotics of systemic rust disease, in 13 fields in four countries, and demonstrate effective biological control. In each field we inoculated newly emerged rosettes of CT in the fall with leaves bearing telia of the rust that were collected in mid-summer in each country. Rosettes were inoculated 2, 4, 6, or 8 times each with about 1 g of telia-bearing leaves. In the spring following inoculation systemically diseased shoots emerged in all inoculated fields. Proportions of systemically diseased shoots generally increased with increasing number of inoculations. Thistle density declines of 50-100% were observed, demonstrating successful biological control of CT.

Characterization of hypothetical proteins in *Cercospora* resistance to the toxin cercosporin

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A photoactivated perylenequinone toxin, cercosporin, produced by *Cercospora* species has an important role in pathogenesis of this fungus to host plants. Cercosporin has almost universal toxicity due to its production of reactive oxygen species including singlet oxygen and superoxide. Because cercosporin has broad-spectrum toxicity, we are interested in understanding toxin-resistance mechanisms in *Cercospora* species. Putative resistance genes in *Cercospora nicotianae* were identified by subtractive hybridization between a *C. nicotianae* wild type and a transcription factor mutant, *crg1*, severely attenuated in cercosporin biosynthesis and self-resistance. Cellular resistance to singlet oxygen is poorly understood, thus we chose to characterize library genes homologous to genes encoding hypothetical proteins. Quantitative RT-PCR analysis of expression in a cercosporin-sensitive *C. nicotianae* mutant under conditions of cercosporin toxicity identified increased expression of two hypothetical protein-encoding genes, *71cR* and *24cF*. Transformation and expression of *71cR* into the cercosporin-sensitive fungus *Neurospora crassa* provided increased resistance to cercosporin toxicity, whereas no significant increase was observed in *24cF*-transformed strains. Targeted gene disruption of both *71cR* and *24cF* in the wild type *C. nicotianae* are in progress.

Identification and epidemiology of *Serratia marcescens* strains associated with cucurbit yellow vine disease in Georgia

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In June 2012, squash plants in a field trial at the UGA Horticulture Farm wilted and died. The symptoms were consistent with cucurbit yellow vine disease (CYVD), caused by the squash bug (*Anasa tritris*) transmitted bacterium *Serratia marcescens*. Since first described in 1988 in Texas and Oklahoma, CYVD has been confirmed in several states but not in Georgia. A

study was undertaken to confirm CYVD in Georgia, determine disease epidemiology, and develop organic management recommendations. Vascular tissues from 21 symptomatic plants were macerated and plated. Individual bacterial colonies were compared with known strains of the CYVD bacterium using biochemical and molecular methods. The presence of CYVD-specific strains of *S. marcescens* was confirmed in seven plants. In greenhouse inoculation studies, the bacterium could be recovered up to 4 cm from the inoculation site after 4 weeks in asymptomatic squash plants. Conditions that induce symptoms in inoculated plants have yet to be determined. In the winter of 2013, hibernating squash bugs were collected from two UGA organic farm sites. Using PCR on extracted DNA, 56% and 44% of the bugs from the two sites were positive for *S. marcescens*. Squash bug dynamics and CYVD incidence will be followed during the 2013 season in field plots. The high incidence of infested overwintering squash bugs indicates that additional studies are needed to develop organic management options for CYVD.

***Fusarium mexicanum* is the main pathogen causing mango malformation in the central western region of Mexico**

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Mango malformation is one of the most important diseases of this crop worldwide. Several species of *Fusarium* have been identified as the causal agent. In Mexico, *F. mexicanum* has been described causing mango malformation in the states of Colima, Guerrero, Michoacan, and Morelos. Researchers have also reported that *F. oxysporum* and *F. subglutinans* cause mango malformation in Guerrero and Michoacan. Recently, mango orchards in the state of Jalisco, which is located north of Colima and Michoacan, have seen an increase in trees affected with malformation. Sampling of 35 malformed mango trees in four commercial orchards in Jalisco during 2007 and 2012 was carried out to identify the *Fusarium* species involved in the disease. Thirty five *Fusarium* isolates were obtained, one isolate per each sampled tree. The morphology of all the isolates was similar to species in the *Gibberella fujikuroi* species complex. PCR amplified sequences of the translation elongation factor-1 α presented 100% similarity with sequences of *F. mexicanum*. These results indicate that *F. mexicanum* is the prevalent pathogen associated with mango malformation in producing areas of the central western region of Mexico.

Differential virulence of the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*) among panzootic, novel, and hybrid genotypes

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Batrachochytrium dendrobatidis (Bd), the fungus that causes chytridiomycosis, has been globally linked to declining amphibian populations. The known genetic variation within New World Bd is distributed between three genotypes: Bd-GPL, Bd-Brazil and Bd-Hybrid, which appears to be a combination between Bd-Brazil and Bd-GPL. The discovery of new genotypes of this fungal pathogen, specifically Bd-Hybrid, raised concern that this disease may drive more frog species to extinction. We are analyzing the virulence of Bd on *Lithobates sylvaticus* by individually exposing them to the three genotypes. Bd-free larvae are exposed individually to five strains of each Bd genotype. Dekeratinization of larval mouthparts will be quantified among different genotype exposures. Infected larvae will also be raised until they metamorphose and monitored every day for survival. Seventy days after larvae metamorphose, animals will be weighed, measured and the infection intensity on each individual will be quantified by using qPCR. We hypothesize that strains of each Bd genotype will have differential rates of virulence on individuals of frogs. Moreover, we predict that the Bd-GPL strains will be more aggressive than other genotypes, consistent with their exclusive presence

in populations of declining amphibians. By estimating virulence of Bd-Hybrid genotypes we can determine the potential for sexual reproduction to lead to large changes in virulence.

WITHDRAWN

Transmission of phage by glassy-winged sharpshooter

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The glassy-winged sharpshooter (GWSS, *Homalodisca vitripennis*), a xylem-feeding leafhopper transmits the bacterium *Xylella fastidiosa* (Xf) that causes Pierce's disease (PD) of grapevines. The GWSS is prevalent throughout grape growing regions of southern California and Texas. Our laboratory has developed a phage based biocontrol system for PD. Laboratory reared Xf-free GWSSs were fed on cowpea (*Vigna unguiculata* subsp. *unguiculata*) plants harboring either Xf or virulent phage Xfas304 for 48 h in three trials to examine the uptake of Xf or phage by GWSSs. To determine the ability of GWSSs to transmit bacteria or phage to plants, GWSSs harboring bacteria or phage were fed on bacteria and phage free plants. A subset of bacteria harboring GWSSs were challenged by feeding them on plants harboring phage for 48 or 96 h. GWSSs and plants were assayed individually in all experiments to evaluate uptake, transmission or persistence of bacteria and/or phage using quantitative Real Time PCR (qRT-PCR). GWSSs were able to uptake and transfer Xf and/or phage. In GWSSs harboring Xf and challenged with phage, the titer of phage Xfas304 increased two-fold, as compared to that observed in Xf-free GWSSs. A two-fold decline in bacterial population was observed in GWSSs when challenged with phage Xfas304, as compared to non-challenged. GWSSs transmitted Xf and/or phage to plants. This to our knowledge is the first report of phage transfer by GWSSs.

Revisiting the phylogeny of Teratosphaeriaceae using established and novel markers

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The Teratosphaeriaceae (Capnodiales, Dothideomycetes) are ecologically diverse and include important phytopathogens, extremophiles living in highly acidic soils, rock dwelling fungi and even a few human pathogens (e. g. *Hortaea werneckii*). Recent multi-locus molecular studies suggest that the current definition of Teratosphaeriaceae including at least thirteen genera can be confined to additional families. This requires the application of novel informative markers to better resolve phylogenetic relationships on the generic level and to determine the boundaries of the family. Here, we present two conservative regions of translation elongation factor 3 (P6 and P7) and one more variable part (PSS7) of the ribosomal protein L10 gene (RPL10), which encodes a ribosomal protein that is a component of the 60S subunit, complementing a number of newly generated datasets of genes that are widely

in use. The new data provide a fresh opportunity to reconstruct nutritional shifts in the Teratosphaeriaceae in the light of anamorph-teleomorph relationships and morphological characters.

Patterns of fungal diversity within pitcher plant microcosms

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Carnivorous pitcher plants hold water in their cup-shaped leaves, and passively trap insects. The plants exhibit exquisite convergent evolution: pitcher plant morphology and function has evolved separately in distant lineages in different parts of the world. Fungi, bacteria, and aquatic insects live within these microcosms and putatively assist in nutrient acquisition from prey. Using Illumina sequencing, we have begun to document and compare the diversity of fungi in pitchers from plants in Southeast Asia and the United States. The main fungal sequences from *Nepenthes* and *Sarracenia* hosts belong to different groups of fungi, although community structure is similar as one or two Operational Taxonomic Unit (OTU) sequences tend to dominate each sample. Many of the Southeast Asian fungi are difficult to assign taxonomically, and likely represent new species. Further studies will examine the functional capabilities of pitcher plant fungi, and the extent to which the plant shapes its fungal community.

Genomic and biochemical characterization of cyclic lipopeptides of *Bacillus mojavensis* RRC101, an antagonist of *Fusarium verticillioides*

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Fusarium verticillioides is an ubiquitous, mycotoxigenic maize pathogen alternately persisting as an asymptomatic endophyte. Consumption of fumonisins, the main class of mycotoxins produced by *F. verticillioides* in maize kernels, causes severe livestock diseases. Additionally, human consumption of high levels of fumonisins is associated with cancer and birth defects. The complex association between maize and *F. verticillioides* makes fungicide-based control cost prohibitive and impractical. The bacterium *Bacillus mojavensis* is closely related to *B. subtilis* and has been isolated from diverse environments including desert soils and ocean water. *B. mojavensis* strain RRC 101, isolated from surface-disinfested maize kernels, demonstrates *in vitro* antifungal activity against *F. verticillioides*, with maize co-inoculation studies reporting reduced disease severity and diminished fumonisin contamination. Whole genome shotgun sequencing of RRC 101 indicates that, like other antifungal *Bacillus* species, *B. mojavensis* produces lipopeptide-class compounds, namely of the surfactin and fengycin families. In addition to genomic description of the secondary metabolite repertoire of this strain, progress on biochemical HPLC/MS based characterization of antifungal compounds isolated from culture supernatants will be reported.

Downy mildew (*Pseudoperonospora cubensis*) pathogenicity on melon and other cucurbits in Costa Rica

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Downy mildew, caused by the biotrophic oomycete *Pseudoperonospora cubensis* (Berk. y M. A. Curtis) Rostowzew (Peronosporaceae), is one of the foliar diseases with the largest economical impact in the Cucurbitaceae; which includes cucumber, melon, pumpkin, and squash. In Costa Rica, the commercial losses due to downy mildew on melon reach 50% of the crop. Since downy mildew is an obligate plant parasite a protocol was developed for the reproduction and maintenance of the pathogen. Several isolates of *P. cubensis* from two melon producing farms (located in Puntarenas and Guanacaste) were identified. A group of 12 cucumber plants with differential response (resistance or susceptibility) were used and it was possible to characterize four different isolates of *P. cubensis*. Such characterization was carried out using a numerical code proposed previously. In order to know if inoculum from other cucurbits were able to infect commercial melon varieties; pathogenicity tests were performed. Commercial melon seedlings were inoculated with isolates of *P. cubensis* obtained from *Cucumis sativus*, *C. melo* (a common weed in commercial plantations) and *Cucurbita* sp. All isolates were able to infect melon seedlings, therefore, other cucurbits can be considered capable of harboring the pathogen and become reservoirs of inoculum that will likely serve as primary inoculum for infection in the next growing season of melon plantations.

Aerobic endospore-forming bacteria strains from potato rhizosphere for biological control of *Rhizoctonia solani* on *Solanum tuberosum* gr. *Phureja* crops

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Rhizoctonia solani is a plant pathogenic fungus that cause black scurf and stem canker on potato crops, reducing yield between 30 and 50%. Current management practices are not effective, because they creates fungicide resistant strains and environmental problems, which is forcing potato growers to look for more sustainable alternatives like biological control. Sixty-four aerobic endospore-forming bacteria strains, were isolated from rizhosphere soil of potato plants with different cropping practices (chemical and organic management). Isolates were characterized by dual culture agar assays *in vitro*, in terms of their capacity to control five phytopathogenic fungi (two *Fusarium* sp. and three *R. solani*) and two bacteria (*Burkholderia glumae* and *Pectobacterium carotovora*). Twelve strains with the highest antagonistic potential were selected for further screening. In this context, the activity of thermo-resistant secondary metabolites and volatile compound production were evaluated *in vitro*, resulting in growth inhibition of *R. solani* up to 85%. Besides, inoculation of bacterial suspension on potato seed-tubers under greenhouse conditions, reduced disease severity between 50 and 80%. A further characterization of the secondary metabolites of the *B. amyloliquefasciens* strain IBUN4P03 by RP-HPLC and mass spectrum showed the presence of lipopeptides like surfactin and fengycin, suggesting a possible mode of action to explain its biopesticide activity.

Relationship between resistance to Stewart's wilt and Goss's wilt in dent corn inbreds

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Stewart's wilt, caused by *Pantoea stewartii* subsp. *stewartii* and Goss's wilt, caused by *Clavibacter michiganensis* ssp. *nebraskensis* (*Cmn*), are the two prominent bacterial leaf blight pathogens in maize in the U.S. Goss's wilt has become much more widespread in geographic range since 2008, and many popular hybrids are susceptible. In 2010 and 2011, we screened 35 dent corn inbreds for resistance to Goss's wilt infection. The pool of inbreds represented a broad range of known Stewart's wilt disease reactions. Plants were grown in inoculated field trials and individual plant disease reactions were scored during the seed filling period, using a 1 to 9 scale (1= highly resistant, 9= highly susceptible). As a group, inbreds showing good resistance to Stewart's wilt, with mean scores ranging from 1.3 to 3.0, also showed good resistance to Goss's wilt, with mean scores ranging from 1.0 to 2.6. Inbreds that were systemically infected by *P. stewartii*, with mean scores ranging from 5.2 to 8.3, were also systemically infected by *Cmn*, with mean scores ranging from 5.5 to 8.0. The Pearson's correlation coefficient, calculated from mean disease severity ratings, was 0.92 and the Spearman's rank correlation of inbred rankings was 0.88. The strong correlation between Stewart's wilt and Goss's wilt resistance may allow maize breeders to select potential Goss's wilt-resistant parent lines based on available Stewart's wilt resistance data.

Trunk applications of phosphite for the control of pecan scab

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Pecan scab (*Fusicladium effusum*) is the major disease of pecan in wet, humid climates. Management of scab can be challenging due to issues of fungicide resistance in *F. effusum* and management of the disease in tall trees due to the technical difficulties of getting sufficient spray coverage to the upper tree canopy. The feasibility of trunk application of phosphite, which is systemically transported, was investigated in six separate experiments in 2010 and 2011. Spray application of phosphite to trunks of young 3-4 year -old trees provided excellent control of scab on foliage of cultivar Desirable, but slightly poorer control on the susceptible cultivar Wichita. Neither incidence nor severity of scab was reduced on 11-12 year-old trees of cultivar Desirable by trunk spray applications in 2010, although slightly less severe disease was indicated in 2011. In two factorial cultivar*fungicide treatment experiments in 2010 and 2011 using 13-year-old trees there was little effect of trunk applications on scab incidence or severity on foliage or fruit. However canopy foliar applications of phosphite and a conventional fungicide significantly

reduced the incidence and severity of fruit scab on most cultivars, confirming previous observations of the efficacy of these foliar sprays. Different methods of trunk phosphite application, for example trunk injection, might prove more efficacious than surface application in older trees.

A comparison of culture and bioassay for detecting citrus canker

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Citrus canker (*Xanthomonas citri* subsp. *citri*, Xcc) causes serious crop losses in tropical and subtropical citrus production regions. Detecting Xcc is important for quarantine purposes, research and disease management. Although PCR methods are available for detecting and quantifying viable bacteria, in many circumstances alternative methods are required. We compared washes of lesions from fruit, leaves and shoots using a culture-based method for detection compared to a "gold-standard" bioassay of injection-infiltrated leaves of susceptible 'Duncan' grapefruit. There was good agreement in detection of active lesions identified by culture and bioassay (26.9-87.8% active by culture compared to 16.3-88.9% by bioassay). False negatives were low (0.9% to 6.5% of lesions). False positives ranged from 4.3 to 21.4%. Accuracy of culture ranged from 0.73 to 0.90%. Comparing culture positives in relation to bioassay negatives showed that the greatest proportion was obtained when lesion bacteria flux density (BFD) of Xcc was $\leq 10^2$ bacteria/mm²/min. A Receiver Operator Characteristic analysis of these data demonstrated good to excellent accuracy of culture in detecting Xcc (Area Under the Curve = 0.79-0.98). Both empirical and binormal curve fits showed in most cases sensitivity was greater using culture (i.e. at low concentrations of Xcc, culture tended to detect Xcc when the bioassay did not). Culture of lesion washes can be a reliable way to detect Xcc.

Fungicide spray coverage from ground-based sprayers in mature pecan trees

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Air-blast sprayers are widely used to control pecan scab (*Fusicladium effusum*) on pecan trees. Good spray coverage is critical to ensure disease control and to minimize risk of fungicide resistance. Spray coverage from an air-blast sprayer, typical of the sprayer used by commercial producers, was measured with water sensitive cards placed at different heights in the canopy (0, 5, 7.5, 10, 12.5, 15 m), and when suspended from helium-filled weather balloons (0, 5, 10, 15 and 20 m) to avoid the effect of foliage obstructing sprays. Spray coverage was measured as the percent card area covered, and the number of droplets per card using digital image analysis (Assess V2.0). In trees, spray coverage was generally uniform up to 10 m. On strings, set card orientation (face up or down) affected coverage, but was generally consistent up to 10 m. Less surface area was covered by spray at heights above 10 m. The numbers of droplets could not always be accurately estimated at heights <10 m due to droplet overlap. Spray coverage was greater on cards attached to the string compared to the trees, suggesting foliage reduced the effectiveness of spray coverage in the canopy. Thus, spray coverage was reduced with spray height, confirming some previously reported effects of height on spray and disease gradients in pecan. Trees in young orchards are often >10-15 m and older trees can reach >30 m in mature orchards so adequate scab control may require additional aerial application.

Twitching motility and a type VI secretion system contribute to virulence in P673, a cool virulent strain of *Ralstonia solanacearum*

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Ralstonia solanacearum is the causal agent of bacterial wilt of solanaceous plants, a devastating disease that affects more than 200 plant species. *R. solanacearum* is distributed in tropical and subtropical areas of the world because most of the strains cannot cause disease at low temperatures. *R. solanacearum* is a complex species due to its diversity and wide host range. In previous work we identified several strains that belong to Phylotype IIB/ sequevar 4 and that are able to cause disease in tomato at low temperatures. Using a proteomics approach we identified proteins that potentially can contribute to virulence of cool virulent strains at low temperature. The objective of this study is to characterize the function in virulence of two bacterial systems identified through the proteomics approach. These systems are twitching motility and a type VI secretion system. Preliminary virulence tests of a deletion mutant impaired in twitching motility (*pilQ* mutant) of

P673, a cool virulent strain classified as Ph IIB/sequevar 4 suggest that twitching motility contributes to virulence at low temperatures. A deletion mutant of a protein integral part of a putative type VI secretion in P673 contributes to virulence at both temperatures. Currently, we are testing virulence of deletion mutants of the same genes in the R3B2 strain UW551 and we are producing deletion and over expression mutants of a putative type VI effector also identified by the proteomics study.

Genome sequencing and analysis of P673, a cool virulent strain of *Ralstonia solanacearum* can reveal virulence factors at low temperatures

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Ralstonia solanacearum is the causal agent of bacterial wilt, a widespread disease that affects more than 200 plant species, including important crops such as tomato and potato. *R. solanacearum* species is well adapted to tropical regions since most of the populations are non-pathogenic below 20°C, however race 3 biovar 2 (R3B2) strains (not established in the U.S.) can cause disease at low temperatures. Strain UW551 which belongs to the R3B2 group, has the ability to infect tomato and potato plants at low temperatures, and because of the threat that poses to U.S. agriculture it has been classified as a Select Agent. In previous work we identified several strains that belong to R1B1 (Phylotype II B/sequevar 4) which are able to cause disease in tomato at low temperatures and are present in the U.S. Using a proteomics approach we identified proteins that potentially can contribute to virulence at low temperature of P673, a cool virulent strain in this group and UW551. In this approach we identified proteins that were present in all the strains compared. The objective of this study is to identify genes present in genomes of cool virulent strains and absent in strains nonpathogenic at low temperatures. We sequenced the genome of P673. Currently we are assembling the genome using a closely related strain previously sequenced and comparing with UW551 and other genomes to identify regions that might contribute to virulence at low temperatures of the cool virulent strains.

Domestication of cereal grains: Effects on root-associated fungal communities

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Root associated fungal (RAF) endophyte symbiosis have been found to be an important component of the root biological community, especially in stressful and arid environments. We plan on comparing the fungal diversity between roots of common cereal grains [*Oryza sativa* (rice), *Triticum aestivum* (wheat), and *Zea mays* subsp. *mays* (maize)] and their progenitors [*Oryza nivara* (wild rice), *Triticum monococcum* (wild wheat), and *Zea mays* subsp. *parviglumis* (teosinte)]. We want to study how domestication of cereal grains has affected the diversity and structure of RAF colonizing the host plant. Our recent data shows a decrease in the number and the diversity of the RAF endophytes in the domesticated maize when compared with its progenitor teosinte. During domestication, the number and the diversity of the RAF in maize may have decreased because of human induced reductions in environmental stresses (provision of nitrogen, weeding, watering, etc). Our study also shows that RAF community in maize and teosinte grown in New Mexican desert soil is more diverse as compared to those grown in Missouri clay soil. Also, plants grown in similar soil types shared common fungal clades. Currently, we are characterizing the RAF endophytes in the roots of the economically important rice, wheat (and their progenitors) that will provide additional data to help address our questions.

Role of fluorescent *Pseudomonas* associated with mycorrhizosphere in suppressing the root diseases and phosphorus uptake by mungbean

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Among the various rhizosphere bacteria, the bacteria belonging to the fluorescent *Pseudomonas*, which colonize roots of a wide range of crop plants have been reported to suppress soilborne plant pathogens and enhance plant growth through various mechanisms. In this study, seventy two isolates of fluorescent *Pseudomonas*, isolated from mycorrhizosphere of different plants were tested for antifungal activity against four root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum*

in vitro. Seventeen isolates caused growth inhibition of all the four test fungi. Cell free culture filtrates of fluorescent *Pseudomonas* also showed nematocidal effects by killing the second stage juveniles of *Meloidogyne javanica* at varying degrees. Out of 61 isolates tested 30 caused 100% mortality of juveniles of *M. javanica* within 48 hours. In pots and field plot experiments, application of selected bacterial isolates to soil at the time of sowing of mungbean (*Vigna radiata*) seeds alone or with *Glomus fasciculatum* suppressed infection of root rotting fungi and improved plant growth by producing taller plants with greater fresh shoot weight. Number of VAM spores were found significantly higher in soil around the roots of plants received bacterial cultures than non-bacterized plants. Moreover these plants showed better phosphorus uptake than plants not grown in bacterized soil.

WITHDRAWN

Systemic infection in chrysanthemum plants by *Puccinia horiana*, causal agent of chrysanthemum white rust

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Puccinia horiana Henn. is a quarantine-significant fungal pathogen and causal agent of chrysanthemum white rust (CWR), first discovered in the U.S. in 1977. Our purpose was to determine if *P. horiana* infects chrysanthemum systemically, acting as a means of over-wintering. Plants, cv. Vicki, were inoculated in a mist tent and placed in a greenhouse. Up to 36 days later, leaves were collected and fixed in 3% glutaraldehyde. Other plants were placed in a growth chamber simulating fall, winter, and spring temperatures in northeastern U.S. After simulated winter, the crown, root, and newly formed stem tissues displaying CWR symptoms were collected and fixed as above. Tissues were post fixed in 2% buffered OsO₄, dehydrated in ETOH, and infiltrated with Spurr's. Images were taken with an HT-7700 Hitachi microscope at 80kV. Observations showed *P. horiana* entered through the leaf cuticle and colonized inter- and intra-cellularly. The fungus adhered to the exterior of palisade and mesophyll cells. The pathogen became common in tracheid cells of the crown, roots, and newly developed stems. Penetration between tracheid cells was through pitted and non-pitted areas of walls and appeared enzymatic. *P. horiana* had an affinity for xylem cell walls, often replacing most of the wall. D-haustoria were not observed, indicating the fungus was monokaryotic. The study showed *P. horiana* can systemically infect chrysanthemum plants.

Co-evolution of *Mortierella elongata* and its endosymbiotic bacterium

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Mortierella elongata belongs to a group of basal fungi (*Mortierellomycotina*) and is commonly isolated from forest soils and healthy plant roots. Recent reports indicate that some isolates of *M. elongata* host endosymbiotic bacteria, but it is unclear whether these are lineage-specific associations. Given the geographically widespread distribution of *M. elongata* and its ubiquitous presence in forest soils and plants we chose to sequence its genome through the JGI Forest Metatranscriptome CSP. We also sought to assemble the genome of the bacterial endosymbiont. The 50 Mb genome of *M. elongata* was sequenced to 112x coverage. Of the 220,113 putative proteins identified in *M. elongata*, only ~50% have orthologs in other fungal species having sequenced genomes). The *M. elongata* genome appears to be enriched in genes related to lipid metabolism (e.g. sphingolipids, etherlipids, and glycerophospholids), tryptophan metabolism, siderophore group nonribosomal peptides, and glucan 1,4- α -glucosidases compared to genome sequences of other basal fungi. The endosymbiotic bacterium sequenced along with the *M. elongata* isolate is related to *Glomeribacter* (endosymbiont of *Gigaspora*, *Scutellospora*) within the Burkholderiaceae. The ~2.6 MB endosymbiont genome is larger than that of *Glomeribacter* but reduced compared to free-living *Burkholderia*. Although many genes have been lost, some gene families have expanded including those involved in protein metabolism and electron transport.

Characterization of *Arabidopsis* CRT1 in plant immunity and genome stability

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A genetic screen for components involved in resistance (R) protein-mediated immunity in *Arabidopsis* led to isolation of *crt1* (compromised recognition of TCV). CRT1 was shown to be a MORC ATPase/endonuclease that physically interacts with multiple immune components. While CRT1 is mainly located in endosome-like vesicles in the cytoplasm, a subpopulation resides in the nucleus, which increases after infection. The combined findings that CRT1 i) is an endonuclease, ii) physically interacts with several components of the DNA repair and recombination (R/R) pathway, iii) is localized to heterochromatin, and iv) is implicated in epigenetic regulation, including suppression of heterochromatic transposable elements (TEs), suggest that CRT1 has an important nuclear function(s). Thus, we are investigating CRT1's role in the nucleus, particularly its involvement in stress-triggered genome stability, and to assess the importance of this function in plant immunity and evolution. To assess whether stress-triggered genome stability is regulated by CRT1, Southern blot analysis and chromatin accessibility PCR are currently being performed on consecutive generations of pathogen-inoculated wild type (WT) and mutant plants lacking CRT1 and its closest homolog CRH1.

Oxidized lipids control disease development during *Aspergillus* infection of maize

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Despite advancements in mechanical and chemical technologies, ear-rotting fungi continue to threaten the maize industry from seed maceration and mycotoxin production creating economic losses and hazards for human and animal health. Oxidized lipids have gained recent attention for their roles in regulating both plant and fungal processes. These metabolites, termed oxylipins, are potent eukaryotic endogenous signals produced through dioxygenase activity especially from the lipoxygenase (LOX) and Psi producing oxygenases (Ppo) gene families in plants and fungi, respectively. Remarkably, oxylipins from both kingdoms are biochemically similar, prompting an exciting hypothesis; during plant-fungal pathogen interactions, oxylipin signals are reciprocally exchanged between host and parasite. In this study, kernel bioassays of *lox3* and *lox5*, were performed with a diverse collection of *Aspergillus flavus* oxylipin-deficient mutant strains. Three days post infection, sporulation and colonization were determined. Results indicate these LOX isoforms have specific effects determining infection outcomes. Within this pathosystem, the unique bouquet of oxylipins generated by both host and pathogen determines the outcomes of infection. This knowledge will spearhead understanding molecular mechanism behind oxylipin-mediated signal exchange during plant-fungal interactions and may allow development of novel environmentally friendly disease resistance and prevention.

Phylogeny of mitosporic Capnodiaceae and description of a new sooty mold species *Fumiglobus pierisiculus* from British Columbia, Canada

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Capnodiaceae are sooty molds, saprotrophic fungi that grow superficially on plants, usually in association with sap-sucking insects. Our goals were to identify a new sooty mold from the ornamental shrub Japanese andromeda, and to use molecular phylogenetics to analyze patterns of character evolution of the fungus and its relatives. Morphological analysis of the pycnidial state suggested the fungus was in the genus *Fumiglobus* but it did not fit in any previously described species. We illustrate and describe the mold as *Fumiglobus pierisiculus*. We also for the first time locate the phylogenetic position of *Fumiglobus* using LSU and SSU r-DNA genes. Our analysis shows that *Fumiglobus* is an early-diverging genus within Capnodiaceae with strong bootstrap support. We also provide new sequence data of the type species of the mitosporic genus *Conidiocarpus*, also in Capnodiaceae. We confirm *Conidiocarpus* as the anamorph of *Phragmocarpus*. By rules of nomenclatorial priority, the name of the holomorph genus is *Conidiocarpus*. We comment on morphological characters that help define the Capnodiaceae including the pycnidial state and mucilaginous hyphae, and analyze the distribution of these characters in a phylogeny. Our analyses help provide a comprehensive molecular and morphological definition of the Capnodiaceae.

Identifying novel bacterial disease resistance sources for rice

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Major constraints to rice yield worldwide are bacterial diseases caused by pathogens such as *Xanthomonas oryzae* pv *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv *oryzicola* (*Xoc*), causal agents of bacterial blight (BB) and bacterial leaf streak (BLS) of rice, respectively. BLS is an emergent disease, causing considerable losses in Africa and China with no known source of single gene resistance. In Africa, no effective BB resistance is available in currently used germplasm. A broad-spectrum source of resistance effective against multiple bacterial pathogens would be a powerful resource for rice breeders. We are using a second generation-mapping resource, Multi-Parent Advanced Generation Inter-Cross (MAGIC) population, to identify new sources of resistance for BB and BLS of rice. Two MAGIC populations, one from indica and one from japonica founders, were developed using eight elite cultivars with highly diverse backgrounds. The founders of each population exhibited highly differential responses to African strains of *Xoo* and *Xoc*. Screening of the populations and genome-wide association mapping using SNP markers are in progress to identify disease resistance QTL and to provide markers for rice breeders. Because the MAGIC founders are elite cultivars, ultimate use of resistance sources by breeders will be expedited, thus improving the yield of rice crops.

Yield losses in oats due to crown rust in Alabama

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Crown rust of oats, caused by *Puccinia coronata* f.sp. *avenae*, is primarily controlled with race-specific resistance. Unfortunately, increased pathogen virulence in recent years has led to yield losses in oats cultivars that had previously been designated as crown rust resistant. In 2008, 2009, and 2012, oats cultivar Coker 227 was planted in Baldwin Co., AL (southern coastal site) and treated with fungicide programs to achieve varying crown rust levels. Fungicides included pyraclostrobin (Headline 2.09EC at 6 fl. oz.), azoxystrobin (Quadris 2.08SC at 4 fl. oz.), propiconazole (Tilt 3.6EC at 4 fl. oz), and propiconazole + trifloxystrobin (Stratego 2.08EC at 7 fl. oz.). Fungicide application timings were Feekes growth stage 9, 10.5 and dual application at both growth stages. Flag leaf rust intensity ratings on non-treated plots averaged 4.4, 3.9 and 9.7 in 2008, 2009 and 2012, respectively. Regression of rust intensity ratings done in late April on yield in each year yielded significant relationships ($P < 0.0001$) with estimated losses of 8.8 (11.9%), 9.9 (11.2%) and 4.8 (7.6%) bu/A per increment of crown rust intensity in 2008, 2009, and 2012, respectively.

CANARY and LiNK technologies for rapid detection of plant pathogens

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The globalization of travel and trade has the potential to overwhelm our plant inspection system. The majority of the current detection technologies with suitable sensitivity and specificity require specialized instrumentation, specially trained personnel and lengthy turn-around-time. CANARY (Cellular Analysis and Notification of Antigen Risk and Yield) is a new serological method. The method is fast, specific (equivalent to other immunoassays) sensitive (similar to PCR), and has been used for medical diagnostics and monitoring of bio-warfare agents. We demonstrated the utilization of the technology for detection of *Ralstonia solanacearum*, *Xylella fastidiosa* CVC strain, and *Phytophthora* in 10-20 min. In addition to the CANARY technology, we examined the Massachusetts Institute of Technology Lincoln Laboratory's Lincoln Nucleic-acid Kit (LiNK) for DNA extractions from a variety of plant tissues infected with bacterial, fungal and nematode pathogens. The method provides high quality DNA for PCR reactions, is fast (~ 6 minutes) and field deployable. We propose a pathway to combine CANARY and LiNK for on-site screening tests and laboratory confirmatory tests.

Comparative analysis of the disease resistance gene space of rosaceous species

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Most plant disease resistance (R) genes encode nucleotide binding site (NB) and leucine rich repeat (LRR) domains. Cross-species comparative analyses of NB-LRR genes have been limited by large gene numbers per genome and rapid rates of diversification. The genomes of apple (*Malus x domestica*), peach (*Prunus persica*), and *Fragaria vesca*, a diploid relative of strawberry, have been sequenced. We identified 1,016 apple, 412 peach and 221 strawberry NB-LRR genes, yielding 1,790 Rosaceae NB-encoding DNA sequences. Based on sequence identity, these were assigned to 273 diversity bins referred to as RosaR80 (Rosaceae R genes at 80% identity) groups. Neighbor-joining dendrograms constructed from consensus RosaR80 protein sequences reveal broad conservation of R gene lineages between apple and peach. While all strawberry R genes have apple and peach homologs, several clades comprising apple and peach R genes are absent from strawberry. This finding is consistent with currently accepted phylogenies and implies loss of R gene lineages in subfamily Rosoideae (strawberry) and/or acquisition of R gene lineages in subfamily Spiraeoideae (apple/peach) since the time of divergence. Some R gene lineages have undergone extreme allelic expansion in particular plant species, suggesting functional significance. The RosaR80 system will be further expanded to include information about R gene context and genome location. On line community resources are being developed.

Prevalence of grapevine (*Vitis vinifera*) viruses in Georgia

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Virus diseases are major constraints to wine grape (*Vitis vinifera*) production worldwide. Since viruses are known to affect vine health and longevity as well as fruit production and quality, studies were initiated to monitor the sanitary status of vineyards in Georgia, USA. The objective of this research was to determine the prevalence and types of grapevine viruses in Georgia vineyards. Six vineyard blocks of different wine grape cultivars were visited during August and September of 2011 and 2012. Leaf samples were collected from a total of fifty grapevines showing symptoms indicative of grapevine leafroll disease (GLRD). The samples from individual grapevines were extracted and tested separately for twenty grapevine viruses listed in standard virus indexing programs. RT-PCR or PCR, depending on the nature of virus genome, was used to detect these viruses with species-specific primers. The results showed the presence of *Grapevine leafroll-associated virus 1* (GLRaV-1, 33.3%), GLRaV-2 (66.7%), GLRaV-3 (83.3%), GLRaV-4 (16.7%), GLRaV-5 (16.7%), GLRaV-6 (16.7%), *Grapevine virus A* (16.7%), *Grapevine rupestris stem pitting-associated virus* (66.7%), and Grapevine-infecting geminivirus (50.0%). Many vineyards had multiple viruses, ranging from 2-7 different viruses per vineyard, in grapevines showing typical GLRD or GLRD-like symptoms. The data from this study will be used to improve the sanitary status of vineyards in Georgia.

Phylogenetic relationships within *Phellinus sensu stricto* (Basidiomycota, Hymenochaetales) from northern North America

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Phellinus, the casual agent responsible for white trunk rot of hardwoods, is composed of several species that currently have an unresolved taxonomic status in North America. Therefore, a multilocus dataset using 55 isolates, representing eight presumed species from northern North America and Europe (*P. cf. alni*, *P. arctostaphyli*, *P. nigricans*, *P. laevigatus*, *P. lundellii*, *P. populicola*, *P. tremulae* and *P. tuberculosus*), is in development. Results from the initial phylogenetic analysis using ITS sequences reveal that isolates of *P. cf. alni* from North America form a monophyletic group, distinct from European and Asian *P. alni*. The results also demonstrate that North American *P. lundellii*, *P. nigricans* and *P. tremulae* are conspecific with their European counterparts. Using partial *tef1* sequences, isolates representing *P. laevigatus* from Europe and North America demonstrate significant differences with strong statistical support. The current dataset indicates the existence of two potentially undescribed species. The first, collected from *Populus tremuloides* in Idaho, is phylogenetically related to *P. populicola* and *P. igniarius s.s.*, both of which are unconfirmed species in North America. The second, meanwhile, was collected from *Prunus* in the southern and central U.S. and was thought to represent North American *P. tuberculosus*. While nearly all *Phellinus* species exhibit specificity for a particular host genus, *P. cf. alni* occurs on several host genera in North America.

Effect of compost tea to control gray mold of blueberry

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Gray mold, caused by *Botrytis cinerea*, is the most common disease in blueberry fruit and many other fruits. In our days, several fungicides are used to control it, however, the economic losses follow been important. Considering the increased interest in organic production, this research studied the effect of compost tea on disease control in blueberries. Three compost were studied. The compost tea was prepared, mixed 30 g of each one compost + 5 ml of catalyst per liter of water, stirred for 24 hours under aerobic conditions. Injured and noninjured berries were treated with 10 ul of compost tea. Then, the berries were inoculated with 10 ul of a suspension of 10⁶ conidia/ml. The compost tea treatments were compared with cyprodinil (Vangard 50 WG) and pyroclostrobin + boscalid (Bellis). Sterile distilled water was used as control. Isolate BcA15 was chosen from more than 30 isolates of rotted blueberries from different localities of south of Chile. This isolate presented a good sporulation and a high pathogenicity on fruits. The assay was performed in Legacy and Duke fruits. Preliminary studies on potato dextrose agar were done. Compost tea treatment showed a control rate between 40 to 50% compared with the control, but lower than the fungicide control, which reached 100%. Based on these results, compost tea applied on the fruit, reduced botrytis incidence, however, new field assay are necessary to validate this information (Fondecyt 3120168).

Fungal community investigation across a deglaciated forefront using ITS and LSU analyses reveals strong successional trajectories

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To elucidate fungal colonization and community assembly patterns in a primary successional environment we sampled along a chronosequence of recently deglaciated soils in Washington State. We sampled soils underneath four plant species with distinct mycorrhizal ecologies (*Abies lasiocarpa* [ectotrophic], *Luetkea pectinata* [arbuscular-], *Phyllodoce empetrifomis* [ericoid], and *Saxifraga ferruginea* [non-mycorrhizal]) in addition to non-vegetated soil representing ~70 years since deglaciation. Fungal LSU and ITS PCR amplicons from soil DNA were 454-sequenced to 1) investigate successional dynamics; 2) decouple it from plant-fungal interactions; and, 3) determine if the two gene regions provide congruent community information. In addition to the fungal communities we analyzed bacterial 16S communities. Our data indicate distinct successional trajectories where early successional communities differ from late successional ones. Interestingly, vegetation affected the fungal community composition only little and richness, diversity and evenness did not vary with either time since deglaciation or vegetation. Taken together, this suggests that fungal community change during succession is dominated by species replacement. Both ITS and LSU analyses support these conclusions indicating either are appropriate for elucidating community level responses. Comparisons among fungal, bacterial and plant data suggest that fungal successional trajectories are unique.

High density genotyping of *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum is one of the most important broadleaf crop pathogens in the United States. The objective of this study was to develop a genotype by sequencing (GBS) method utilizing ion torrent sequencing capable of providing high-density genotyping of *S. sclerotiorum* isolates. A dual enzyme restriction associated DNA (RAD) GBS protocol was developed to genetically characterize natural populations of *S. sclerotiorum*. Genetic characterization of phenotypic traits can be accomplished through association mapping utilizing this genotyping and the sequence marker data will allow for the rapid identification of candidate genes underlying these loci utilizing the publicly available *S. sclerotiorum* genome. Six *S. sclerotiorum* isolates from the USA were genotyped using this technology. A single ion torrent 318 microprocessor sequencing chip resulted in 5,494,082 sequences with a mean read length of 155 bases for a total of 853 Mb of sequence. The ApeKI and HhaI dual enzyme RAD mapping protocol reduced the complexity of the ~38 Mb genome such that 31,023 unique loci were sequenced. Sequence alignment identified ~60,000 single nucleotide polymorphisms (SNPs). Positioning 34 of the 31,023 GBS markers determined they were randomly spread throughout the *S. sclerotiorum* genome and BLAST analysis determined that ~86% of the unique ApeKI loci hit predicted genes. This analysis predicts that a SNP marker should be identified approximately every 4 Kb throughout the genome.

Role of deoxynivalenol production by *Fusarium graminearum* in seedling infection of soybean, wheat, and maize

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Fusarium graminearum is a fungal pathogen of cereals and other crops, causing significant losses in yield and quality. *F. graminearum* produces deoxynivalenol (DON), a mycotoxin that can act as a virulence factor for maize ear rot and head blight of wheat. In order to examine the possible role of DON as a virulence factor in seedling diseases, soybean, wheat and maize seeds were inoculated with wild-type and *Tri6* knockout mutants (no DON production) in rolled-towel experiments. Plant weights, shoot lengths and disease severity (soybean only) were measured at 7 days. Additionally, infection levels will be compared between the two fungal strains using qPCR. Results differed among the cultivars for all crop species. In soybean, only the susceptible cultivar demonstrated a significant difference between the wild-type and the DON non-producing mutant for disease severity. In wheat, the wild type, but not the mutant, caused reductions in weight and length in the susceptible cultivar. Conversely, the partially resistant cultivar was affected by the DON knockout strain. In maize, the wild type and the mutant had an impact on shoot length and plant weight. However, in one susceptible hybrid the wild-type caused greater effects than the DON non-producing mutant. Results so far indicate that DON production is not required for pathogenicity in seedlings, but the wild-type strain generally produces greater symptoms and there are interactions between host genotype and DON effects.

Sensitivity of *Cercospora beticola* from Serbia to benzimidazole and sterol demethylation inhibiting fungicides

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Cercospora beticola, causal agent of sugar beet leaf spot (CLS), is economically the most significant sugar beet leaf pathogen and is primarily controlled by fungicide applications. One hundred single-conidia isolates from 70 localities representing sugar beet production region in Serbia were tested *in vitro* for sensitivity to flutriafol and carbendazim. Resistance to flutriafol was detected in 16% and to carbendazim in 96% of tested isolates. Sensitivity of selected isolates to both fungicides was determined using CAPS markers and coincided with *in vitro* tests. The efficacy of carbendazim and flutriafol at commercially recommended rates was evaluated in field trials after inoculation with mixtures of isolates sensitive and resistant to flutriafol and carbendazim. Carbendazim and flutriafol efficacy was lacking or very low in plots inoculated with isolates of corresponding resistance. Obtained results indicated the importance of monitoring of pathogen population sensitivity for effective CLS management.

Internal colonization of lettuce leaves by *Xanthomonas campestris* pv. *vitiensis* is influenced by lettuce cultivar

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Bacterial Leaf Spot of lettuce is a widespread and economically important disease caused by *Xanthomonas campestris* pv. *vitiensis* (Xcv). Cultivars with resistance to Xcv have been identified and mechanisms for resistance are being evaluated. We previously demonstrated that when the pathogen was spray inoculated onto leaves, susceptible lettuce cultivars support higher pathogen population levels than resistant cultivars. In order to bypass factors influencing internal colonization of the leaves we evaluated the population dynamics of Xcv injected directly into the leaves of susceptible and resistant cultivars. A rifampicin-resistant strain of Xcv was injected into the abaxial sides of leaves of resistant and susceptible lettuce cultivars at Log 6 CFU/cm². Bacterial population levels were estimated by spreading dilutions on media containing rifampicin. Pathogen population levels increased in all lettuce cultivars after inoculation, and differences in populations could be detected three days after inoculation. Xcv population levels were significantly greater in susceptible cultivars compared to the resistant cultivar Little Gem, which expresses a hypersensitive reaction to Xcv. However, population levels in susceptible cultivars did not differ from levels in another resistant cultivar, Batavia Reine des Glaces, which does not express a hypersensitive reaction. The mechanisms and genetics of resistance in these two resistant cultivars are being evaluated.

Siderophore-mediated iron uptake is important for in planta growth of *Pantoea stewartii* subsp. *stewartii*

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Pantoea stewartii subsp. *stewartii* (*Pnss*) is a bacterial pathogen that colonizes the xylem tissue of the sweet corn host. Within the plant, *Pnss* likely encounters iron limitation and oxidative stress and has evolved to cope with both conditions. Because excess intracellular iron can exacerbate oxidative damage, iron uptake must be carefully regulated, particularly under conditions of oxidative stress. Iron acquisition through the use of siderophores is important for virulence in other pathogenic bacteria, but evidence is limited for the importance of siderophores in xylem-dwelling bacterial pathogens. We have identified a *Pnss* gene cluster homologous to the *iucABCDiutA* operon of *Escherichia coli* NA114, encoding proteins involved in the biosynthesis and utilization of the siderophore, aerobactin. We demonstrate that *Pnss* produces a siderophore putatively similar to aerobactin and that its production and uptake are necessary for host colonization and full virulence. *Pnss* *ΔiucA* (siderophore biosynthesis) and *ΔiutA* (siderophore receptor) mutants show reduced growth in iron-limited media, which was restored by iron supplementation. In *E. coli*, aerobactin production is regulated by the Fur repressor (ferric uptake regulator). Likewise, the *Pnss* *Δfur* mutant overproduces siderophores and has an increased sensitivity to peroxide. This demonstrates the importance of siderophore-mediated iron uptake for virulence of *Pnss* and the regulation of iron uptake in planta.

Fine-scale genetic structuring and reproductive biology of the blueberry pathogen *Monilinia vaccinii-corymbosi*

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Mummy berry disease of blueberry (*Vaccinium* spp.) is caused by the economically important pathogen *Monilinia vaccinii-corymbosi* (Mvc). The fungus causes shoot infection followed by fruit infection through the gynoeical pathway. In this study, nine microsatellite markers were used to 1) examine fine-scale genetic structuring and gene flow; 2) compare the allele frequencies and genetic diversity of Mvc isolates that originated from infected shoots and fruit; and 3) determine if Mvc exhibits signatures of outcrossing and/or selfing. A collection of 269 isolates of Mvc was generated by sampling infected shoots and fruit from a 110 m x 134 m planting of southern highbush (*V. corymbosum* x *V. darrowii*). Genetic diversity was high, with 219 unique haplotypes detected. Spatial autocorrelation analysis did not support genetic structuring within the field, suggesting unrestricted gene flow at the sampled spatial scale. Analysis of molecular variance and Shannon partition analysis suggested that samples from shoots and fruit were not significantly different ($P=0.196$ and $P=0.057$, respectively). Analysis of single ascospore progeny indicated that five individual apothecia produced multiple recombinant ascospores, while two apothecia produced ascospores with identical haplotypes. These results suggest that Mvc can outcross, but may also be able

to self-fertilize. Further research is needed to determine the scale at which genetic structuring occurs and the mating system of Mvvc.

Estimating susceptibility to *Wheat streak mosaic virus* infection in non-crop grasses

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Phytopathology 103(Suppl. 2):S2.22

One of the difficulties in managing *Wheat streak mosaic virus* (WSMV), a mite-transmitted potyvirus, is that many non-crop grasses including weeds can serve as reservoirs for the virus. Identifying which non-crop grass species are susceptible to WSMV is critical in managing this disease. Previously, susceptibility was estimated using mechanical inoculation, followed by ELISA where a threshold of twice the optical density (OD) of uninfected wheat (2xWt) was used to identify infected plants. The accuracy of the 2xWt threshold was compared to a species-specific (SS) thresholds based on the mean and variance in OD values in virus-free plants in six weed species. Purified WSMV was added to healthy tissue to create known virus-positive samples. The SS thresholds had greater than 95% accuracy. The 2xWt threshold had much lower accuracy and in some cases incorrectly classified all virus-positive samples. In a separate experiment, infection rates in five grass species were compared following mite and mechanical inoculation. The relative susceptibility among species differed between inoculation methods. These results demonstrate that previous methods used to estimate the susceptibility of non-crop grasses to WSMV are not accurate. Accurate estimates can be obtained using mite-inoculation and improved methods for calculating the viral detection thresholds in ELISA.

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Quantitative phenotyping of powdery mildew resistance in grapevine reveals differences in host resistance biology

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The recent demonstration of race-specific resistance to *Erysiphe necator* has encouraged grapevine breeders to identify and introgress quantitative resistance genes exhibiting complementary mechanisms. In 2012, we established a phenotyping center (VitisGenPM) for detailed evaluation of resistance to powdery mildew, based on quantitative microscopic analysis of single-isolate inoculations as part of a project supported by USDA-SCRI (www.vitisgen.org). By phenotyping detached leaves received from breeding programs, VitisGenPM provides replicated testing of resistance segregation among progeny for association analysis with high-density genetic markers. Thus far, the sample processing and data collection pipeline has logged over 120,000 microscope observations across seven mapping populations. Each mapping population revealed different aspects of resistance biology, likely due to different host genetics. For instance, *Vitis* hybrid 'Horizon' x *V. rupestris* 'B38' segregated independently for quantitative resistance to penetration and to microcolony formation. In contrast, *V.* hybrid 'MN1264' x *V.* hybrid 'MN1214' showed segregation of quantitative resistance to penetration, but progeny expressed no posthaustorial resistance. By selecting individuals with complementary resistance mechanisms as parents for new cross-hybridizations, we hypothesize that the next generation of progeny will express stronger and more durable resistance.

Stability and fitness of pyrimethanil-resistant phenotypes of *Penicillium expansum* from apple

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Phenotype stability, fitness and competitive ability of pyrimethanil-resistant isolates of *P. expansum* were determined. The stability of pyrimethanil resistance (PR) was assessed after consecutive transfers on potato dextrose agar (PDA) or being cycled on apple fruit. Fitness components including mycelial growth, conidial germination, pathogenicity and virulence on apple fruit, and sporulation in vivo and in vitro were evaluated. PR was retained at the levels similar to that of the initial generation after 20 and 5 transfers on PDA and 4 and 3 cycles on apple fruit at 20 and 0°C, respectively. In general, there were no significant differences in the mean values of fitness parameters among the phenotype groups, though variability in individual fitness parameters was observed among the isolates within the same phenotype groups. After 4 disease cycles on apple fruit inoculated with a pair mixture of a sensitive isolate and one of the two resistant phenotypes at 75:25, 50:50 or 25:75 ratios, the final frequency of resistant individuals was significantly decreased compared to the initial generation except that when the mixture consisted of 75% highly pyrimethanil-resistant individuals, the frequency was

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increased. The results suggest that PR was stable and that PR did not significantly impair individual fitness parameters, but resistant phenotypes exhibited some competitive disadvantage when the pyrimethanil-resistant individuals were $\leq 50\%$ in the population.

Development of a loop-mediated isothermal amplification for detection of *Burkholderia glumae*

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Bacterial panicle blight caused by *Burkholderia glumae* is among the three most limiting diseases of rice production in southern United States. Resistant varieties and chemical control are not available for this pathogen. Rapid and early detection can help to reduce devastating problems in the field. There are several techniques that exist to detect bacterial diseases, from isolation and identification using conventional methods to DNA amplification methods including conventional and real time PCR; however, these methods are time consuming and some of them require high precision instruments for amplification or elaborate methods for detection of the amplified DNA. The need for efficient, rapid and cost effective techniques has driven the development of simple rapid gene amplification tools for early detection and identification of plant pathogens. Loop-Mediated Isothermal Amplification (LAMP) is a technique that amplifies DNA under isothermal conditions (60-65°C) without the use of a thermal cycler. A set of six specially designed primers are required to recognize six regions on the target DNA. Amplification can be achieved in one hour by mixing all the reagents in a single tube. This study reports the development of a LAMP protocol for the detection of *B. glumae* using six specific primers from the 16S-23S rDNA region. Positive results were confirmed by the emission of fluorescence under UV light and corroborated in gel electrophoresis by a ladder of multiple bands.

Entomological and physiological factors predisposing beech to infection by *Neonectria* pathogens in beech bark disease aftermath forests

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Neonectria ditissima and *N. faginata* are causal agents of beech bark disease (BBD) in North America. Infection of American beech (*Fagus grandifolia*) by these fungal pathogens is believed to follow a single predisposing factor, infestation by beech scale (*Cryptococcus fagisuga*). However, recent findings do not support this model and suggest the influence of alternative or additional predisposing factors. Because BBD related research does not commonly operate at the species level, fundamental similarities and differences between the *N. ditissima* and *N. faginata* pathosystems are not known. A fundamental understanding of these pathosystems is integral to developing effective management strategies for BBD aftermath (long-affected) forests. Although several physiological factors differ between healthy, infested, and infected beech, the possible role of these factors in predisposing infection has not been examined. A case-control study was conducted to investigate possible entomological and physiological factors predisposing beech to infection by *Neonectria* pathogens, separately and together. Bark tissue samples from 200 uninfected beech were collected in 2011. Physiological chemicals were quantified in 2012 for case (infected) and control trees. Important predisposing factors were identified using generalized linear mixed models. Distinct entomological and physiological predisposing factors were identified for *N. ditissima* and *N. faginata* infections.

Identification and characterization of a monopartite begomovirus infecting *Sida* spp. in Mali, West Africa

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Whitefly-vectored begomoviruses are an emerging threat to agriculture worldwide. A begomovirus was associated with leaf upcurling and vein swelling symptoms in the tropical weed, *Sida* spp. in Mali, West Africa. PCR analysis with degenerate begomovirus and universal betasatellite primers revealed the presence of a begomovirus and betasatellite in symptomatic plants. Analysis of the sequence of the begomovirus revealed a genome organization typical of monopartite begomoviruses and highest nucleotide identity, 88.6%, to the monopartite begomovirus, *Cotton leaf curl Gezira virus*

(CLCuGV), placing it at the threshold of a strain of CLCuGV or a new species. The betasatellite sequence was $\leq 63\%$ identical to other sequences, indicating it is a distinct betasatellite species. Agroinoculation experiments in *N. benthamiana* revealed that the virus alone induced mild symptoms, whereas the virus and the betasatellite induced stunting, downward leaf curling and crumpling. Particle bombardment inoculation of *Sida* spp. plants with the virus and betasatellite DNA into *Sida* spp. resulted in upcurling and chlorosis of leaves and vein swelling, similar to symptoms observed in the field. Plants bombarded with viral DNA became infected but did not develop obvious symptoms. Host range studies will establish if the *Sida* begomovirus/betasatellite complex causes disease in malvaceous crops, and will help resolve the virus' taxonomic status.

Influence of crop rotation on diseases, nematode activity, and yield of peanut and cotton in Southeast Alabama

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A crop rotation study which was established in 1988 at the Wiregrass Research and Extension Center consists of 37 different rotation patterns. Beginning in 2009, a split plot design with rotation as the whole plot and peanut cultivar as sub-plots was used. In 2012, a similar split plot arrangement was added to cotton. Disease ratings, soil samples for nematode assay, and yield was taken from each peanut and cotton plot. When peanut cultivars were compared for disease and yield, there were no significant differences among the Georgia 06G and Tifguard for Tomato spotted wilt virus, leaf spot, stem rot, or yield. Georgia 06G showed significantly worse root knot damage to the roots and pods. In both 2011 and 2012, peanut cropping frequency impacted leaf spot intensity, stem rot, incidence, root knot nematode damage, and yield. Continuous peanut rotations had the highest stem rot incidence, leaf spot intensity, and lowest yield. In 2011 and 2012, worst root knot damage to roots and pods was seen in rotations where bahia grass was cropped one or more years before peanuts. In 2012, rotation pattern had minimal impact on disease severity although in rotations where cotton was cropped two consecutive years behind peanuts, there was slightly higher target spot intensity. Rotation pattern had no significant impact on cotton yield.

Rates of recombination and point mutation of bacterial plant pathogens compared to bacterial vertebrate pathogens

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As the utility and popularity of multi-locus sequence typing (MLST) has emerged as a method of identification and tracking of pathogenic isolates, the underlying evolutionary processes need to be better understood in the different bacterial species. MLST was initially designed for vertebrate bacterial pathogens that experience high levels of recombination. Recombination makes identification and clustering of isolates extremely difficult. The MLST process lessens the effects of recombination. When applied to bacterial plant pathogens, recombination was found, but did not appear to have the same effect. For this study the effect of recombination and point mutation were analyzed using 7 MLST schemes for plant pathogens and 30 MLST schemes for vertebrate pathogens. Rates of recombination and point mutation were calculated using multiple alignments and the program DNAsp. When controlling for number of isolates sampled and the level of sensitivity of the MLST scheme (genus, species, subspecies, pathovar), it was found that plant pathogens had significantly lower recombination and point mutations despite having more nucleotides sampled. Several reasons for these differences include the differences in the immune systems of the hosts and how the pathogens can spread in a mobile versus non-mobile host. The difference also implies that when developing an MLST scheme, plant pathologists may need to utilize genetic loci undergoing stronger selection like pathogenicity genes.

Involvement of the Halliwell-Asada pathway in the photosynthesis shutdown during the potato and *Phytophthora infestans* compatible interaction

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Computational modeling is a powerful approach to analyze complex biological data because it integrates data in a single system. It also facilitates to predict and optimize different behaviors of the system under study. We modeled and analyzed the metabolic network of the compatible interaction between *Phytophthora infestans* and *Solanum tuberosum*. Based on a previous

computational model, stLMR1, we generated a refined computational model named stLMR2, which, in comparison with the previous version, includes more reactions and three defense pathways that are reported to be important during the interaction between pathogens and their host plants. In this study, we found oscillations in the metabolic behavior at different time points after infection and a delay in the activation of the defense biosynthetic pathways of jasmonic acid, ethylene and salicylic acid. We also predicted the involvement of enzymes of the Halliwell-Asada pathway as well as enzymes preceding it. In silico predictions were assayed and validated using real-time quantitative PCR. Acidic glucanase (Glu-A), Basic glucanase (Glu-B) and Proteinase inhibitor II (PIN2) were used as indicators of the behavior of the defense biosynthetic pathways. Glutathione synthase and glutamylcysteine synthase as well as Glutathione reductase and ascorbate peroxidase were quantified for validating the predictions concerning the Halliwell-Asada pathway.

Evaluation of *Rhizoctonia solani* AG 1 - IA and *Rhizoctonia* species for resistance to QoI fungicides

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Rice sheath blight (SB) is one of the most devastating rice diseases in the southern U.S. and foliar fungicides serve as a major management tool. Resistance to QoI fungicides has previously been reported among isolates of *R. solani* AG 1 - IA recovered from Louisiana rice fields in 2012. To assess the resistance to QoI in Arkansas, a large collection of isolates of *R. solani* AG 1 - IA and *Rhizoctonia* spp. recovered from Arkansas rice fields between 2000 and 2011 was examined. The sensitivity to azoxystrobin was evaluated by qualitative and quantitative *in vivo* inoculation assays on ryegrass (*Lolium perenne*). A known resistant and susceptible isolate were included as controls. Among 268 isolates tested, most (~91%) were pathogenic on ryegrass. Among the pathogenic isolates, 20 isolates (8.3%) showed some degree of resistance to QoI. Of these, five isolates of *R. solani* AG 1-IA (isolated between 2008 and 2011) showed a level of resistance to azoxystrobin similar to a resistant control isolate, whereas 15 isolates showed an intermediate level of resistance (30 to 89% of the resistant control). In addition, sequence analysis of cytochrome b gene was conducted on resistant and intermediate resistant isolates in order to examine the presence of SNPs known to confer QoI resistance. This study is the first report of the potential presence of resistance to QoI fungicide among isolates of *R. solani* from rice in Arkansas.

QTL mapping to identify new sources of resistance to rice sheath blight in recombinant inbred lines from the cross of two elite Indica cultivars

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Rice sheath blight (SB) is one of the most prevalent diseases of rice in the southern U.S. SB resistance is a polygenic quantitative trait, and varying levels of resistance have been observed among rice cultivars. A population of 299 F_{2.5} lines derived from a cross of two elite Indica cultivars, 'Fedearroz2000' and 'Palmar' were mapped with 99 SSR markers to identify known and potentially new quantitative trait loci (QTLs) associated with resistance to SB (qSB-QTLs). Resistance phenotyping was conducted under greenhouse and field conditions. Lines were also evaluated for plant height (PH) and heading date (HD) as these traits confound detection of true SB resistance. A total of 17 significant QTLs were identified by CIM, SIM and ST-SIM analyses. Five of these QTLs were SB-QTLs, eight were PH-QTLs, and four were HD-QTLs. The SB-QTLs were found on chromosomes 5, 8, 9 and 11 and were independent of PH and DH. These QTLs explained 5.7 to 12.6 % of the phenotypic variation. qSB-9 was mapped in field and greenhouse tests, and qSB-5 coincided with a source of resistance unique to Indica germplasm previously reported. qSB-9 showed an LOD > 3.0; however, the remaining QTLs showed LOD scores ranging from 1.5 and 2.7. Fine mapping efforts are currently ongoing to improve LOD scores. These data indicate three putative new QTL (qSB-5, -9 and -11) as sources of SB disease resistance in Indica rice germplasm.

Quantification of reactive oxygen species in plants using the fluorimetric probe Amplex Red

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Reactive oxygen species (ROS) are by-products of photosynthesis and respiration in plant tissues. Abiotic and biotic stressors also induce the production and temporary accumulation of ROS in plants, whereby they can act as secondary messengers/chemical mediators in plant defense signaling and lead to programmed cell death. Despite such key roles in fundamental cellular processes, reliable quantitative methods for the determination of actual ROS levels in plant tissues are not available. In this study we quantified constitutive and/or induced levels of ROS in woody plants (pine, *Pinus nigra* and ash, *Fraxinus* spp.), crop plants (rice, *Oryza sativa*) and model plants (*Arabidopsis thaliana*) using the fluorimetric probe Amplex Red. Overall, assay sensitivity was in the nanomol/g FW range. To estimate the antioxidant potential of each plant taxon, we spiked the respective extracts with known concentrations of hydrogen peroxide and measured the resulting fluorescence. Wild type and a lesion-mimic mutant of rice had different antioxidant potentials but both were higher than ash, followed by *Arabidopsis*. Spiking of pine tissues resulted in higher signal than the reference standard, suggesting ROS generation rather than quenching. Finally, kinetic studies were conducted to calculate pseudo-first order rate constants. With appropriate modifications, this optimized method should be applicable to any plant tissue.

Control of the HrpL regulon by global regulatory systems in the gall-forming bacterium *Pantoea agglomerans* pv. *gypsophila*

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Gall formation by *Pantoea agglomerans* pv. *gypsophila* (*Pag*) is *hrp*-dependent. It was previously demonstrated that disruption of *pagR* or *pagI* genes of the quorum sensing (QS) system, significantly reduced expression of the *hrp* regulatory cascade (i.e. *hrpXY*, *hrpS* and *hrpL*) that activate the HrpL regulon. We have further characterized the genes of the global regulatory pathway Gac/Rsm (i.e., *gacA*, *gacS* *rsmB* and *csrD*) and the post transcriptional regulator *rsmA*. Results presented demonstrate that: a) Mutants in the QS genes significantly reduce expression of *gacA* and *rsmB*. b) Gel shift experiments illustrate that PagR acts as a transcriptional activator of each of the *hrp* regulatory genes and the *gacA* in a C₄-HSL-dependent manner. c) Mutants of the Gac/Rsm genes or overexpression mutant of *rsmA* significantly reduce virulence and colonization in *gypsophila*. d) Overexpression of *rsmB* (*rsmBOE*) abolishes gall formation, colonization in *gypsophila*, and hypersensitive reaction on beet (nonhost) suggesting a lack of functional type III secretion system. e) *rsmBOE* mutant eliminates the transcription of the *hrp* regulatory genes cascade. f) Expression and overexpression of *rsmB* in the presence or absence of *csrD* mutant suggest that CsrD may act as a safeguard for preventing excessive *rsmB*. Our results indicate that the *hrp* regulatory cascade is directly controlled by PagR and indirectly by RsmA activity, whereas deficiency in RsmA activity is epistatic to PagR.

Identification of loci for resistance to Sclerotinia stem rot in a perennial relative of soybean

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Sclerotinia sclerotiorum is a necrotrophic fungus that causes Sclerotinia stem rot (SSR, also known as white mold), which can cause significant yield losses in soybean (*Glycine max*) and other dicotyledonous annual crops. Quantitative trait loci (QTL) for partial resistance to SSR have been identified in soybean, but the loci have explained relatively low levels of the observed phenotypic variation. Unlike soybean, *Glycine latifolia*, a perennial wild relative of soybean in the subgenus *Glycine*, shows high levels of resistance to SSR. To generate molecular resources for gene mapping and identification in *G. latifolia*, a population of 186 *G. latifolia* F₂ individuals that segregated resistance to SSR was genotyped by high-throughput sequencing. The analysis generated more than 10,000 single nucleotide polymorphism (SNP) markers in a very cost effective manner. The markers formed 20 large linkage groups, many of which were syntenic with soybean chromosomes. The segregation of the SNP markers and phenotypic data for responses to inoculation with *S. sclerotiorum* and incubation in oxalic acid (a pathogenicity determinant for *S. sclerotiorum*) were combined to identify QTL for resistance to SSR and oxalic

acid in *G. latifolia*. *Glycine latifolia* and other perennial wild relatives of cultivated soybean represent sources of genes that could be beneficial to soybean production, especially when resistance is lacking in the *G. max* primary gene pool as with SSR.

WITHDRAWN

Organic potato variety and production trials

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Potato yields have quadrupled in the United States since the early 1900s, with a portion of the yield increase due to healthy seed and the remainder due to use of synthetic fertilizers and pesticides. The yield increases are not attributed to varieties; some of the varieties grown did not change throughout the twentieth century and are still grown today. On conventional farms, potato yields within a class across varieties are often not significantly different. In contrast, we found significant differences in potato yield among varieties grown on organic farms. Total yields correlated with tolerance to insect damage and plant vigor. The best predictors of yield during the season were ratings of hopper burn and row closure at 10 weeks after planting. Tuber defects and diseases were a significant cause of loss, with between 24 and 38% culled. The specific tuber problems that were most important varied among varieties, with common scab, silver scurf, black scurf, shape, skin set, green ends, and soft rot being the most common causes of post-harvest losses. Among those varieties that performed consistently well on organic farms were Freedom Russet, Kennebec, Langlade, Keuka Gold, Spartan Splash, Chieftain, Red Thumb, Adirondack Red, Adirondack Blue, and Caribe. The greater variation among varieties observed on organic farms than conventional farms supports the need for continued variety trialing on organic farms in the Midwest.

Oomycetes isolated from soybeans with damping-off in South Dakota

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A survey was conducted during 2011 and 2012 to identify Oomycetes associated with damping-off of soybeans in South Dakota. This effort was undertaken as part of the Oomycete - Soybean Co-ordinated Agricultural Project. Soybeans at VE to VC growth stages with typical damping-off symptoms were collected from six fields in 2011 and six fields in 2012. Sampled fields were located in the east central region of South Dakota. A total of 145 Isolates was obtained by plating surface-sterilized diseased hypocotyl tissue on either corn meal or V-8 agar amended with antibiotics. Resulting axenic cultures were purified through single hyphal tip transfer. Sequencing of ITS rDNA was used to identify isolates to species. Nineteen different species of *Pythium*, thirteen isolates of *Phytophthora sojae* and two isolates of *Phytophthora inundata* were identified. A significant proportion of *Pythium* isolates (24%), however, could not be identified to species. Other Oomycetes identified included *Saprolegnia monoica* and *Brevilegnia gracilis*. *Pythium* species identified included: *P. rostratifingens*, *P. heterothallicum*, *P. oopapillum*, *P. ultimum* var. *ultimum*, *P. perplexum*, *P. sylvaticum*, *P. irregulare*, *P.*

aff. diclinum, *P. carolinianum*, *P. coloratum*, *P. orthogonon*, *P. afertile*, *P. aphanidermatum*, *P. catenulatum*, *P. nodosum* and *P. nunn*.

Global expression patterns of *Xanthomonas axonopodis* pv. *glycines* genes within soybean leaves determined with RNA-seq

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To better understand behavior of *Xanthomonas axonopodis* pv. *glycines* (*Xag*), the causal agent of bacterial pustule of soybean within its host, its global transcriptome within soybean was compared to that in a minimal medium using deep sequencing of mRNA. Of 5062 genes predicted from a draft genome of *Xag*, 534 were up-regulated in the plant while 289 were down-regulated. Genes encoding YapH, a cell surface adhesion as well as several other cell surface proteins were down-regulated in soybean. Many genes encoding the type III secretion system, effector proteins, cell wall-degrading enzymes, and phosphate transporter proteins were strongly expressed at early stages of infection. Several genes encoding RND multidrug efflux pumps were induced in *planta* and by isoflavonoids *in vitro* and were required for virulence of *Xag* to soybean as well as resistance toward soybean phytoalexins. Genes encoding consumption of malonate, a compound abundant in soybean, were induced in *planta* and by malonate *in vitro*. Disruption of the malonate decarboxylase operon blocked growth in minimal media with malonate, but did not alter growth in soybean perhaps because genes for sucrose and fructose uptake were also induced in *planta*. Many genes involved in phosphate metabolism were induced in *planta*. While disruption of the genes encoding high-affinity phosphate transport did not alter growth in media varying in phosphate concentration, the mutants were severely attenuated for growth in soybean.

SCAR assay as a versatile diagnostic tool for detection of *Macrophomina phaseolina* in cluster bean

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Macrophomina phaseolina is an opportunistic phytopathogenic fungus infecting major food, fiber, legume crops species including cluster bean, cotton, okra, soybean, rice in Africa, Australia, Brazil, India, North and South America; parts of Europe. Incidence of Charcoal rot results in significant yield losses and decline in galactomanan gum production in an industrially important crop cluster bean. Traditional culture-based and morphometric approaches are often time consuming, laborious and require extensive knowledge of classical taxonomy. Rapid detection tool is a prerequisite to decipher the in depth understanding of pathogenesis and disease management strategies for controlling infestation. Limitations have lead to development of alternative molecular approaches with improved accuracy and reproducibility such as SCAR as a diagnostic tool. 82 isolates from cluster bean and a range of other host plants habituating wide eco-geographic locations of India were employed for RAPD. Conspicuous RAPD monomorphic fragments specific to *Macrophomina phaseolina* were sequenced in order to develop SCAR diagnostic tool. Consensus sequences from selective RAPD markers were employed for designing SCAR. Results of SCAR diagnostic tool were validated and found to be highly reproducible and the diagnostic tool was very rapid and inexpensive. This is the first report for cluster bean and work clearly demonstrates that SCAR can readily be applied for detection of *Macrophomina phaseolina*.

Expression profiling and evolution of pathogenesis related genes in maize and teosinte in response to *Ustilago maydis*

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Ustilago maydis (*U. maydis*), the causal agent of corn smut, is an important agricultural pathogen and causes significant yield losses of approximately \$1.5 billion annually in the United States. Several methods are currently used to control corn smut; however host resistance is the only practical method for managing smut. To identify genes controlling resistance to corn smut, transcriptome profiling was conducted in maize genotypes showing high levels of resistance and susceptibility against *U. maydis*. Maize, teosinte and 40 maize teosinte near Isogenic lines (NILs) were inoculated with a strain of *U. maydis*. Two teosinte lines and three NILs demonstrated a high level of resistance and a phenotypic response similar to maize. A total of 5,639 genes demonstrated significant differential expression between inoculated and uninoculated maize lines. From this data set, 529 genes were up-regulated (≥ 1.5 fold change), whereas 5,110 were down-regulated (≤ 1.5 fold change) in

inoculated resistant and susceptible maize plants, respectively. The 529 up regulated genes and 5,110 down regulated genes were grouped into 8 functional categories. These included; biotic stress, enzyme families, receptor like kinases photosynthesis, metabolism and transcription. This work represents the first report of new potential sources of resistance to *U. maydis* from the wild progenitor (teosinte) and provides novel insight into the complexity of biotrophic interactions.

Phylogenetic relationships of endophytic and endolichenic fungi reveal a new order within the class Eurotiomycetes

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Eurotiomycetes (Pezizomycotina, Ascomycota) consists of a broad array of fungi, including animal pathogens, saprobes, lichen-forming fungi, extremotolerant rock-inhabiting fungi, plant pathogens, and endophytes. In general, little is known about the phylogenetic relationships of newly discovered endophytes relative to known taxa. We used multi-locus analyses to examine relationships of eurotiomycetous endophytes and endolichenic fungi isolated in culture from diverse sites in North America. Analyses of six loci (nLSU, nSSU, mtSSU, *RPB1*, *RPB2* and *MCM7*) grouped fungal endophytes into three distinct, well-supported clades. The majority are nested within Chaetothyriales and Eurotiales. The remaining fungal endophytes are clustered into a monophyletic clade that was never reported in previous phylogenetic analyses of the Eurotiomycetes. This novel clade seems to represent a new order of fungi mainly consisting of foliar endophytic and, to a lesser degree, endolichenic fungi. A plant pathogen that was previously considered to have uncertain placement, *Dolabra nepheliae*, falls within this novel clade. This result highlights the importance of a reliable phylogenetic framework to infer the evolutionary history and ecology of fungal endophytes, and the contribution of fungal endophytes toward the reconstruction of a comprehensive fungal tree of life.

Diverse phytoplasma strains, including 16SrXII-E and two new subgroups, associated with diseased potatoes (*Solanum tuberosum*) in China

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Potato is an important crop widely cultivated in China. Recently, potato diseases with characteristic symptoms of phytoplasmal infections were found in potato fields. In 2006 and 2007, samples with symptoms of rosette and upright growth, upward rolling, yellowing and purpling of leaves, shortened and thickened internodes and formation of aerial tubers were collected from Yunnan and Inner Mongolia and analyzed for the presence of phytoplasmas. DNA was extracted from tissues of 63 symptomatic and 10 asymptomatic plants. Phytoplasma 16S rDNA was amplified by PCR with primer pair P1/P7, followed by nested PCR with P1A/P7A, P1A/16S-SR or R16F2n/R16R2. Twenty nine symptomatic plants, but no asymptomatic plants, tested positive for phytoplasmas. Nested PCR products were cloned and sequenced. Sequence analysis indicated that the phytoplasmas from diseased potatoes shared 98.1-99.8% similarity with '*Candidatus* Phytoplasma fragariae'. RFLP and phylogenetic analyses indicated that phytoplasmas of 16SrXII group were associated with diseased potatoes in China; these strains are most closely related to subgroup 16SrXII-E. Our results showed that four strains belonged to 16SrXII-E, and all others are proposed as two new subgroups in 16SrXII group. The genetic diversity of these strains was corroborated by sequence analysis of ribosomal protein genes. These results illustrate the complexity and diversity of phytoplasmas associated with potatoes in China.

Transcription factors in *Ustilago maydis* pathogenesis and meiosis

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Ustilago maydis is the model basidiomycete plant pathogen. Its infection of corn (*Zea mays*) results in tumour formation in all aerial plant tissues. The

tumour is the ultimate stage of the fungal/plant interaction and it is the location of teliospore formation. Teliospores are the dispersal agent for the fungus and the cell type where meiosis is completed. Key to understanding smut pathogenesis is determining how the fungus controls developmental changes during growth in the plant. Three *U. maydis* transcription factors have been identified that may influence these transitions (Zfp1, Mcg1 and Ztf1). Deletion of the gene for the zinc finger protein Zfp1, in SG200, reduces virulence; deletion of *mcg1*, coding an Ndt80 related protein, leads to altered teliospore development and blocks the completion of meiosis; and *ztf1*, coding an APSES domain protein, is up-regulated in the plant. Characterization of the roles of these proteins was investigated by gene deletion, seedling pathogenesis assays, creation of over-expression lines, targeted mutagenesis, bioinformatic identification of potential target genes, and cDNA subtraction library creation (in progress). The data suggested a role for Zfp1 in pathogenic development and that Mcg1 is a positive and negative regulator of transcription. These investigations, initiated in genomics and bioinformatics, provided new insight regarding the *U. maydis* developmental transitions within the host.

Efficient "vaccination" of *Nicotiana benthamiana* and tomato plants using a lab-attenuated strain of *Pepino mosaic virus*

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Cross protection, the act of stimulating plant defense to a virus by pre-inoculating plants with a mild strain (a plant virus "vaccine") is a viable but underused disease management approach. So far, all known cross protection-inducing vaccines are field isolates associated with milder symptoms which could evolve to become more virulent. Hence, the absence of a rapid and reliable procedure for developing plant virus vaccines is a major obstacle for widespread use of cross protection. The aim of our study is to develop a knowledge-based approach to produce stable vaccines. We used *Pepino mosaic virus* (PepMV), an emerging pathogen of tomato, as the model virus for our study, and chose its capsid protein (CP) gene for modifications to produce an attenuated strain. Less conserved amino acid (AA) residues within the CP of PepMV were identified by multiple alignments with other potexviruses. Next, we replaced some of these AAs with their counterparts in *Potato virus X*. Testing the resulting mutants for their ability to cause diseases in *Nicotiana benthamiana* plants revealed that mutants KD and KD/VC retained the ability to infect plants systemically yet caused mild symptoms. KD was further shown to protect both *N. benthamiana* and tomato plants against secondary infection of wild-type PepMV. KD remained stable following five passages. Our results establish a reliable method for producing stable plant virus vaccines and provide a novel virus management option for tomato.

Morphological variation of *Phytophthora infestans*-Thai isolates from infected potato

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Morphological characteristics of 132 *Phytophthora infestans*-Thai isolates, collected from infected potato leaflets in two provinces of northern Thailand were determined. The sizes of sporangia and oospores were varied within the population. The sizes of the reproductive propagules had no relation with the original source and metalaxyl sensitivity. Colonial characteristics of the isolates on rye A agar were cottony (26% of the isolates), powdery (52%) and concentric ring (22%) type. On potato tuber slices, 64% and 36% of the isolates were mycelia and sporangial type, respectively. DNA gene (RAS and β -tubulin) sequences had not been revealed on the genetic variation.

Interaction of future climate change scenarios of elevated tropospheric ozone and decreased rainfall amounts with loblolly pine decline

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Loblolly decline is the cause of premature death of *Pinus taeda*. Two fungi associated with this are *Leptographium terebrantis* and *Grossmannia huntii*. The current study was undertaken to determine how altered climate scenarios will affect loblolly decline in the future. Our hypothesis is that exposure to

increased drought and ozone will exacerbate loblolly decline, increasing susceptibility to *L. terebrantis* and *G. huntii*. Two scientific questions will be addressed: (i) Will future ozone scenarios affect the susceptibility of *P. taeda* to *L. terebrantis* and *G. huntii*? and (ii) Will future drought scenarios affect the susceptibility of *P. taeda* to *L. terebrantis* and *G. huntii*? Four loblolly families will be used. Two are resistant to decline while the others are susceptible. Seedlings will be deployed into open-top chambers. In both experiments, each family will have three inoculation treatments including no inoculation and inoculation of *L. terebrantis* and of *G. huntii*. The first year's study (2013) will use three ozone treatments. The second experiment (2014) will be conducted similarly using three simulated rainfall treatments. Host responses to infection and conditions will be measured. Data will be analyzed using ANOVA multivariate or univariate procedures. From this project, we will gain insight into future conditions and challenges in loblolly production as well as examine interactions between abiotic and biotic stresses of plants.

Identification and characterization of mating type (*MAT*) alleles in *Sclerotinia minor*

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Recently, two *MAT* alleles that differed by the presence of an inversion [inversion negative (Inv-) and inversion positive (Inv+)] have been characterized in homothallic *Sclerotinia sclerotiorum*. We identified and characterized both *MAT* alleles in *S. minor* as well. Both Inv- and Inv+ *MAT* alleles in *S. minor* were 100% identical in structure to their corresponding *MAT* allele in *S. sclerotiorum*, and both *MAT* alleles were flanked between the putative *APN2* and *SLA2* at 5' and 3' end, respectively. *MAT* genes were arranged as *MAT1-1-5*, *MAT1-1-1*, *MAT1-2-4* and *MAT1-2-1* in Inv- *MAT*. However, in Inv+ *MAT*, a 3.6-kb region is inverted in relative to Inv- *MAT*, and as a result *MAT1-1-1* is truncated at the 3' end and the orientation of *MAT1-2-4* and *MAT1-2-1* has changed. Inverted repeat motifs (250 bp) believed to be the driving force for *MAT* inversion in *S. sclerotiorum* have also been found in *S. minor* as flanks of *MAT* inverted region. However, the size was 256 bp in *S. minor*. *MAT* genes in *S. minor* were 93-96 % identical to their homologues in *S. sclerotiorum*. Among the non-coding flanks of *MAT* genes, the 5'-*MAT1-1-5* flank was the most variable between species, and it was 1377-bp shorter in *S. minor*. The expression of *MAT* genes did not differ between Inv- and Inv+ isolates. The phylogeny of *MAT* genes revealed that *MAT* inversion occurred independently in each species. Of 38 *S. minor* isolates screened, 50%, 8%, and 42% isolates were Inv-, Inv+ and heterokaryon for *MAT*, respectively.

A cultural independent method for investigating the genetic structure of the cotton root rot pathogen, *Phymatotricopsis omnivora*, in Arizona

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Cotton root rot caused by *Phymatotricopsis omnivora* is the most destructive disease of dicotyledonous plants in Arizona. Genetic diversity of the fungus in Arizona is unknown, partly due to the difficulty of isolation. We examined its genetic diversity in cotton and five other hosts using a culture independent method. Four cotton fields at two locations were sampled by collecting 20 symptomatic plants from discrete sites in each field. Twelve samples from alfalfa, grape, olive, pine, and privet also were tested. A culture independent technique was used in which 20 mycelial strand pieces from each root were handpicked using forceps, placed into sterile water in 2 ml tubes, and used immediately or stored at -20°C for DNA extraction. Pure cultures from 8 cotton plants were used to validate the culture independent technique. DNA was extracted using FastDNA kit. Four loci (18S SSU, 28S LSU, and ITS of rDNA, and EF1- α) were amplified using *P. omnivora* specific primers and sequenced. Only the ITS and LSU regions revealed variability, and phylogenetic analysis revealed two major groups in *P. omnivora*. Group-I exclusively contained cotton isolates from only one location, whereas group-II contained cotton isolates from both locations and isolates from all other hosts. Only isolates within group-II were variable. For a given tested isolate, no differences were detected between the sequences obtained from DNA of mycelial stands and DNA of pure culture.

Prevalence of inversion negative and inversion positive *MAT* alleles in *Sclerotinia sclerotiorum* from across the United States

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Sclerotinia sclerotiorum is a homothallic ascomycete which reproduces sexually by self-fertilization. Sexual reproduction in ascomycetes is regulated by mating types *MAT1-1* and *MAT1-2*, which are usually fused end to end in homothallic ascomycetes. Recently two *MAT* alleles, inversion negative (Inv-) and inversion positive (Inv+), were reported in *S. sclerotiorum*, and both were equally distributed among lettuce isolates from CA. This current study determined the distribution of *MAT* alleles in *S. sclerotiorum* from across the United States and particularly from ND. In total 171 isolates from 23 states and 17 hosts were PCR screened for both *MAT* alleles. This collection included 77 isolates from ND and 44 soybean isolates. Generally, a distinct PCR band was observed for both Inv- and Inv+ *MAT* in their respective homokaryon isolates. However, only a faint band was observed for Inv- *MAT* in most of the *MAT* heterokaryon isolates. Comparatively, Inv- isolates were more predominant than Inv+ isolates in almost all states and hosts tested, and of 171 isolates screened, 52 %, 26 %, and 22 % were Inv-, Inv+ and *MAT* heterokaryon, respectively. Both Inv- and Inv+ *MAT* alleles were observed among isolates from 15 states and from 14 hosts, and the remaining states and hosts contained only a single isolate. In ND, 52, 30, and 18 % of isolates screened were Inv-, Inv+ and *MAT* heterokaryon, respectively. Among the hosts tested, sunflower had a higher percent of Inv- isolates (86%).

Detection of '*Candidatus Phytoplasma asteris*' in canola in North Dakota

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Canola (*Brassica napus* L.) plants showing typical aster yellows symptoms, leaf purpling, stunting, and phyllody were observed in research fields in Langdon, ND during the summer of 2012. DNA extracted from symptomatic and asymptomatic plants were analyzed using a nested polymerase chain reaction (PCR) assay and virtual restriction fragment length polymorphism (RFLP). The nested PCR was performed using phytoplasma 16S rRNA universal primers P1/P7 and R16F2n/R16R2 resulting in 1.8 and 1.2 kb products respectively. Nested PCR products (1.2 kb) were sequenced and compared with public databases. Based on iPhyClassifier species assignment, all the samples had 99 to 100 % similarity to the '*Candidatus Phytoplasma asteris*' reference strain (GenBank accession # M30790). Virtual RFLP were performed using SerialCloner. Comparison of the virtual RFLP gel profiles placed the strains into 16SrI-B subgroup. To our knowledge, this is the first report of '*Candidatus Phytoplasma asteris*' related strain infecting canola in North Dakota.

Complementing T-DNA replaces original T-DNA in tagged mutants of *Phoma medicaginis*

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Phoma medicaginis causes spring black stem and leaf spot, an important disease of alfalfa and annual medics. *P. medicaginis* forms uninucleate conidia in melanized pycnidia and is genetically tractable using *Agrobacterium* mediated transformation (AMT), resulting in random integration of T-DNA that occasionally generates pycnidial mutants. One mutant, P2-65, displayed smaller and fewer pycnidia and a poly(A) RNA polymerase gene, *PmCID13*, disrupted by a T-DNA containing three genetic markers: hygromycin resistance (*HPH*), green fluorescent protein (*GFP*), and G418 resistance (*NPTII*). Another mutant, P1-A17, possessed hyaline pycnidia and a single *HPH* T-DNA disrupting a cryptic ORF (*CPO*) located between, a serine/threonine protein kinase gene (*PmRANI*), and a conserved hypothetical protein gene (*CHP*). To confirm the disrupted genes caused the observed phenotypes, the wild type genes were reintroduced by AMT using nourseothricin resistance (*nat1*) selection. Twenty-two nourseothricin resistant transformants were generated for each mutant and displayed intermediate phenotypes indicating partial complementation. In 77% of the P2-65(*PmCID13*) transformants and 36% of the P1-A17(*PmCPO*) transformants, the original T-DNA markers were replaced by the complementation T-DNA. Our results suggest homologous recombination is favored in serial AMTs and may be useful for elimination of transgenic markers.

Modeling control strategies for maize streak disease

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Maize streak disease is economically important in sub-Saharan Africa. It results in streaked chlorotic lesions on maize leaves and reduced yield, and can frequently lead to complete crop loss. The disease is caused by *Maize streak virus* (MSV), which can infect many different grass hosts, and is vectored by several *Cicadulina* spp. leafhoppers. We designed an epidemio-

logical model to test the effectiveness of different cultural and chemical control strategies. The model consisted of a set of differential equations to describe the healthy and infected populations of the host and the vector. We tested the effects of host resistance, insecticide sprays, quarantine, and spray programs that are based on insect population thresholds. We also tested the effects of incomplete resistance and reduced efficacy in the insecticide. Host resistance offered the best long term control of the disease and even partial disease resistance still gave reasonable control. Efficacious insecticide sprays were effective at controlling the spread of the disease, but insecticides that had reduced efficacy could not control the epidemic. Quarantine procedures appeared to be the least effective control strategy. These findings can help suggest long-term, regional strategies for integrated control of maize streak disease.

Assessing the genetic structure of *Phellinus noxius* and the dissemination pattern of brown root rot disease in Taiwan

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Since 1990s, brown root rot caused by *Phellinus noxius* has become a major tree disease in Taiwan. This fungal pathogen can infect >200 hardwood and softwood tree species, causing gradual to fast decline of the trees. For effective control of the disease, it is important to know how the pathogen is disseminated and how new infection center of brown root rot is established. As a first step to decipher the biology of *P. noxius*, we performed Illumina sequencing and *de novo* assembly of a single basidiospore isolate. We obtained a total of ~40 Mb comprising 15,966 contigs and 7,403 unigenes. Based on the 10,554 simple sequence repeat (SSR) regions identified throughout the genome, 32 polymorphic SSR markers were developed to analyze ~320 *P. noxius* isolates collected from ~70 tree species from urban/agricultural areas in 14 cities/counties all around Taiwan during 1989-2012. The result revealed a high level of allelic diversity and the presence of a variety of fungal clones. These clones exist as discrete patches, suggesting that the occurrence of brown root rot was most likely caused by multiple clones rather than a single predominant strain of *P. noxius*. Isolates collected from diseased trees nearby each other tend to have similar genotype(s), indicating that *P. noxius* may spread to adjacent trees through root-to-root contact. A moderately significant pattern of "isolation by distance" also suggested the involvement of basidiospore dispersal in disease dissemination.

***Cantharocybe brunneovelutina* Lodge, Ovrebo et Aime in Mexico**

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New records of *Cantharocybe brunneovelutina*, previously known from only one locality in Belize, are described and illustrated from Tabasco and Veracruz states of Mexico. Ovrebo *et al.*'s (2011) analysis of nLSU rDNA sequences related this species to *Cantharocybe gruberi* a previously monotypic genus near the base of the Hygrophoraceae. The species is characterized by the subvelutinous brown pileus, brownish gray pruina on the stipe, and by cheilocystidia that have pronglike appendages resembling basidia. Material was collected during biotic surveys and inventories of macrofungi in southern Mexican states. Morphological and ecological comparisons among the Mexican specimens and the species type description are discussed. *C. gruberi* has been rarely reported in the literature and *C. brunneovelutina* collections are rare in Mexican mycological herbaria, despite it being a very distinctive mushroom, suggesting an intriguing phenology. Despite its rarity, *C. brunneovelutina* is found in Belizian seasonally dry limestone karst forest, Mexican Tropical Rain Forest and Mexican Montane Cloud Forest, which leads us to predict a wider distribution of this taxon.

Development of a Smartphone app to increase accuracy and early detection of new or invasive diseases

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Our nation's natural resources and ecosystems are under constant pressures from encroaching invasive species. The development of Smartphone apps and field ID cards increases the possibility of early detection of new or invasive diseases helping safeguard the environment and reducing the overall costs of successful management. Nationally, diseases such as sudden oak death, thousand cankers disease and boxwood blight are causing serious problems for the environment, Green Industry and private land owners. While precise economic impact is not known, estimates range in the tens of millions of dollars. Impacts include degradation of environmental quality, loss and quarantine of nursery crops, decreased property values, monitoring and eradication costs, and losses of recreational and aesthetic value. Smartphone apps allow users to compare photos and descriptions to field conditions while still in the field without any additional tools or equipment. They also allow sending of first reports and pictures along with precise GPS location for further confirmation by regulatory officials. Apps and ID cards are readily available to Extension, Regulatory and Green Industry professionals as well as lay citizens further increasing the possibility of early accurate detection. Field ID cards also allow users to compare photos and descriptions of target species leading to fewer false reports.

Using next-generation sequencing to determine the influence of metabolic intermediates on the *Pseudomonas protegens* transcriptome

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Pseudomonas protegens Pf-5 is a rhizosphere-inhabiting bacterium that produces several antimicrobial compounds, including 2,4-diacetylphloroglucinol (2,4-DAPG) and pyoluteorin. These antibiotics are key contributors to the effectiveness of Pf-5 in the biological control of several soilborne plant pathogens. We have shown that phloroglucinol, a biosynthetic intermediate in 2,4-DAPG production, is important in mediating cross-talk between the 2,4-DAPG and pyoluteorin biosynthetic pathways. However, the broader effect of phloroglucinol on the Pf-5 transcriptome and in the regulation of pyoluteorin production remains unknown. We hypothesize that biosynthetic intermediates act as chemical messengers and have broad effects on the Pf-5 transcriptome. We conducted phloroglucinol-spiking experiments using the derivative Pf-5 strains Pf-5*phlAG* and Pf-5*phlADG* (no phloroglucinol production), grown under conditions where secondary metabolism production and cross-talk between 2,4-DAPG and pyoluteorin biosynthetic gene clusters are well-characterized. We are using RNA-Seq to evaluate the influence of phloroglucinol on the antibiotic-production profile and the Pf-5 transcriptome. This will elucidate the role of phloroglucinol on the Pf-5 transcriptome and potentially on other secondary metabolite pathways.

A sequencing approach to soybean seed microflora assessment

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A large number of fungi and bacteria are associated with soybean seed. Some, such as *Phomopsis longicolla* and *Bacillus subtilis* can reduce seed quality, while others have unknown effects on seed performance. These microflora have been identified by plating seed on various media. This approach favors microflora that are easily culturable and possibly overlooks those that are difficult to culture or viable but nonculturable. One way of gaining a more complete picture of seed microflora is with metagenomics, which has been used to assay a variety of environments to reveal a greater diversity of microorganisms than previously known. With this method, DNA is extracted from an environment, sequenced, and organisms identified based on the sequences. A novel method using PCR and 454 Jr has been developed to detect fungi and bacteria from seed lots. Approximately 170,000 sequences were generated. Initial analysis showed organisms that were not detected within a seed lot using plating methods were found using molecular techniques. These included both fungi and bacteria such as *Alternaria* sp., *Fusarium* sp., *Cercospora* sp., uncultured ascomycetes and fungal endophytes, *Pseudomonas* sp., and others. In the plating assay, a limited number of organisms were observed and identified, including *P. longicolla*, *C. kikuchii*, *F. equiseti*, *B. subtilis*, and other *Bacillus* sp. This sequencing approach appears to give a more complete enumeration of soybean seed microflora than seed plating.

Biodiversity and potential pathogenicity of field collected oomycetes from asymptomatic soybeans in southeastern PA

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Soybean agriculture in Pennsylvania is a \$260 million industry, with production occurring primarily in the southeastern portion of the state. During summer 2012, we surveyed eight soybean farms from six counties for the presence of soil-borne oomycetes. All farms were asymptomatic at the time of collection and reported no previous issues with oomycete disease. Oomycetes were isolated from soil samples using several baits (soybean seeds and pods, hemp seeds, *Rhododendron* leaf disks) and plated on selective media (cV8-PARP and cV8-PARPH); baiting experiments were also repeated after soil samples were frozen at -4 degrees C for six months to assess overwintering. DNA from each isolate was extracted for molecular identification based on the mitochondrial cytochrome c oxidase 1 (cox1) locus. Between two and nine *Pythium* species were identified from each field, with a total of nineteen species across all eight sites. Several of these species (*P. attrantheridium*, *P. irregulare*, *P. sylvaticum*, *P. tolorosum*, *P. ultimum*) have previously been associated with seedling establishment issues in the Midwest. Pathogenicity assays are currently being conducted to determine if our isolates are potentially pathogenic on corn, soybean seeds, and soybean seedlings.

History of brown rust of sugarcane in Florida

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Brown rust of sugarcane, caused by *Puccinia melanocephala*, was introduced into Caribbean region in 1978. It soon spread throughout the region, causing severe yield losses, particularly on the widely grown variety, B4263. Yield trials confirmed losses in Florida up to 15% and higher. During the 1980's, rust severity increased noticeably on varieties CP70-1133 and CP72-1210, two popular varieties previously considered resistant, suggesting pathogenic races. Replicated inoculation trials using five rust isolates on six varieties confirmed the presence of physiological variants of the pathogen. Later, French scientists identified a major resistance gene, *Brul*, for brown rust that conferred resistance to isolates from Brazil, Colombia, Guadeloupe, Reunion and Zimbabwe, including 3 isolates from Florida. This conflicted with pathogenic differences in isolates identified previously. Once the *Brul* gene was identified in clones, this conflict was resolved, since the *Brul* gene was not present in the differential clones that determined the Florida pathogenic races. In 2009, a whorl inoculation test was developed that allowed thousands of clones to be evaluated at a time. Presently, screening for both brown rust reaction and the presence of the *Brul* gene begin in Stage 2 of the Canal Point (CP) cultivar development program. This identifies brown rust resistant clones with *Brul* and without it. Efforts to identify non-*Brul* sources of resistance are currently underway.

Can constitutive phenolic biomarkers be used to predict coast live oak resistance to *Phytophthora ramorum*?

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Sudden oak death has been wreaking havoc on forests along the coast of California and Oregon since the 1990s. In 2002, *Phytophthora ramorum*, an oomycete pathogen, was officially identified as the causal agent. Coast live oak (CLO; *Quercus agrifolia* Née), a red oak endemic to California, in particular has been plagued by high infection and mortality rates. Still, apparently resistant CLO have been observed in natural populations. Putative phenolic biomarkers of resistance have been identified in CLO. To test whether constitutive phenolics can be used to predict CLO resistance, 600 trees were selected from a naïve population. Constitutive phenolics were quantified in the phloem of each tree. A subset of trees (N=154) was then artificially inoculated with *P. ramorum* to determine resistance level. A logistic regression analysis using individual phenolic compounds as predictor variables was used to predict CLO resistance; resistance was estimated based on external canker length and beetle presence assessed approximately one year after inoculation. The model correctly classified 73% of resistant trees in our training set (N=60). No other methods currently exist for identifying resistant CLO before *P. ramorum* infection. Identification of resistant CLO ahead of the disease front will be useful in CLO management, including breeding efforts aimed at preserving this endemic California oak. Finally, similar methods could be developed for other important forest pathosystems.

Increased resistance to leaf pathogens of a 9-lipoxygenase mutant is mediated by constitutive ISR-like signaling derived from roots

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Colonization of plant roots by beneficial fungi leads to induced systemic resistance (ISR) to pathogen infection. This response is reportedly mediated by long-distance signaling molecules. Previous reports show that disruption of the root-expressed 9-lipoxygenase (9-LOX) gene *ZmLOX3* resulted in increased resistance to multiple seed, leaf and stem fungal pathogens. Here we explored the potential mechanisms behind this phenomenon. The expression of selected defense marker genes in leaf response to *C. graminicola* infection revealed no obvious difference between the mutant and wild type, reminiscent of ISR responses. Supporting the hypothesis that *lox3* mutant is constitutively activated for ISR, sap collected from the mutant induced increased resistance in wild type comparable to the levels observed in the mutant. These resistance levels were also comparable to ISR caused by root colonization by *Trichoderma virens* in wild type but not in the mutant, pointing to the constitutive nature of ISR signaling in the *lox3* mutant. Non-targeted metabolome analysis of the mutant root exudates identified several candidate 9-oxylin molecules. Comparative gene expression profiling of defense genes identified candidate genes that may potentiate the *lox3*-mediated ISR. This study identified new candidate genes and additional candidate long-distance molecules involved in the root-shoot signal communication in maize.

The protist trichomycete *Enterobryus* associated with *Anadenobolus monilicornis* in Guanica Dry Forest

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Symbiosis is the association between two non-related organisms. The common yellow-banded millipede, *Anadenobolus monilicornis* (Diplopoda: Spirobolida: Rhinocricidae) and the protist *Enterobryus* sp. (Ichthyosporae: Eccriniales), a species of hair-like microorganism that inhabits its gut, forms a commensalistic relationship. *Enterobryus* was once part of a fungal class (Trichomycetes), but now it is classified as a protist. We collected millipede in Playa Jaboncillo within Guanica Dry Forest to study the prevalence of *Enterobryus*. We characterized morphologically the *Enterobryus* species through measurements of key structures and statistical analysis. Traditionally, *Enterobryus* species are difficult to identify due to high intraspecific variation. Thus, statistical analysis of character measurements is included in an attempt to investigate character stability. Millipedes were dissected; gut linings with attached *Enterobryus* were removed. The material was preserved in lactophenol cottonblue 0.05% and observed under compound microscope. Based on the observed characters we hypothesized that this is a new species of *Enterobryus*. Morphometric data of thalli, sporangiospores and holdfasts presented a normal distribution except for the basal disk width of the holdfast, which showed extreme variation. This character, although used to described *Enterobryus* species is not reliable in the new species when using the mean or range values in taxon descriptions.

Does increased fungicide use in eastern apples mean greater pesticide risk? An evaluation using PRiME

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Over the past decade, commercial apple growers in the eastern United States have faced increasing problems with development of fungicide resistance by *Venturia inaequalis*, which causes apple scab. As a result of increasingly widespread resistance to the major classes of systemic fungicides, particularly the demethylation inhibitors and quinone outside inhibitors, growers are increasingly relying on broad-spectrum protectant fungicides that have been on the market for decades, primarily captan and the ethylene bis dithiocarbamates. Because these fungicides must be applied preventatively, and because relatively larger amounts of active ingredient per acre must be used, eastern apple growers have been applying steadily increasing amounts of fungicide in terms of numbers of applications and the amount of fungicide. It is not clear whether this represents increased environmental or other toxicity risks. Fungicide use for a group of growers was analyzed using a novel approach to risk calculation based on site-specific conditions, pesticide properties and empirical field impact data, the Pesticide Risk Mitigation Engine (PRiME). Comparing use over several years shows that with increased use of protectant fungicides there has been an increase in environmental impacts.

Spread potential of binucleate *Rhizoctonia* from propagation floors to trays containing stem cuttings

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Binucleate *Rhizoctonia* spp. (BNR), the cause of web blight, are present all year on container-grown azaleas in the southern U.S. BNR can be eliminated during vegetative propagation by submerging stem cuttings in 50°C water for 21 minutes. The objective was to evaluate risk of rooting trays being contaminated from inoculum on polypropylene fabric and gravel floors in propagation houses. Three experiments were done in 2011 and repeated in 2012. In experiment one, floors of commercial propagation houses were swab sampled on a grid pattern and sponges plated on Ko and Hora agar. 1-9% of 96 samples per cultivar were positive for BNR from fabric and gravel floors. In experiment two, samples of fabric and gravel inoculated with BNR were set under 70% shade and full sun, with and without interval timed irrigation, for six weeks. BNR recovery declined 75% under shade and 86-96% under full sun. In experiment three, trays with hot water treated azalea stem cuttings stuck in peat mixtures were set on or beside inoculated pieces of fabric and gravel and maintained under a misting regime for 12 weeks. In both years, BNR was not recovered from peat in trays of rooted stem cuttings even though BNR was recovered from 60-94% of the inoculated substrates at the end of 12 weeks. BNR persists on fabric and gravel floors but declines over the 6 weeks houses are empty. If floor surfaces are clean of organic matter, the risk of rooting trays becoming contaminated appear low.

Impacts of temperature on expression of TAL effector-activated susceptibility genes in rice

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Higher temperatures are conducive to several rice diseases, including bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The increase in BB may result from temperature-induced changes in the host, pathogen, or the host-pathogen interactions. Upon infection, *Xoo* secretes Transcription Activation-Like (TAL) effectors into rice cells to activate the expression of susceptibility genes, including rice sugar transporter genes called SWEET genes. For example, in susceptible interactions, the *Xoo*-delivered TAL effector AvrXa7, which is recognized by the BB resistance gene *Xa7*, binds to the promoter of the rice *OsSWEET14* gene to activate its expression. We are interested in the impacts of temperature on expression during AvrXa7/*Xa7* interactions because previous reports showed that *Xa7*-governed resistance is more effective at high than normal temperature regimes. Here, we show that at normal temperatures, AvrXa7-activated *OsSWEET14* expression is reduced but not abolished when *Xa7* is present, supporting the hypothesis that *Xa7* R protein blocks AvrXa7 virulence activity. Interestingly, AvrXa7-activated *OsSWEET14* expression in AvrXa7/*Xa7* interactions is greater at high relative to normal temperatures, despite the observations that at high temperatures the HR phenotype is stronger and bacterial multiplication is more severely restricted. Understanding how temperature affects disease and resistance will provide insights into how plants adapt to stress.

Variation in ectomycorrhizal community composition along a soil nutrient gradient in montane forest in western Panama

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In Panama, the ectomycorrhizal (EM) tree species *Oreomunnea mexicana* (Juglandaceae) forms monodominant stands within lower montane forests that are otherwise characterized by diverse arbuscular mycorrhizal tree communities. The objective of this project was to compare the composition of EM communities associated with *Oreomunnea* seedlings and adults across sites varying in soil fertility, and to determine whether common EM fungi could connect individuals through EM networks. A total of 472 root tips were collected yielding 95 fungal taxa. A total of 65 OTUs were collected from fruiting bodies of EM Basidiomycota in the same areas. Approximately 33% of the OTUs associated to root tips matched sequences from fruiting bodies. The mushroom community was in general less diverse than the root tip-associated community. NMDS analysis demonstrated a high variation in species composition between soil types. Although species composition dif-

fered among sites, *Russula* was the most abundant genus in all sites, infecting 40% of the sampled root tips. Based on these results we hypothesize that species of *Russula* could be involved in the formation of EM networks connecting *Oreomunnea* seedlings and adult trees, thereby facilitating monodominance in local patches. More research is needed to evaluate the prevalence of EM networks in this species and the potentially interacting mechanisms associated with *Oreomunnea* dominance in the area.

A conventional PCR and qPCR assays to detect *Harpophora maydis*—The causal agent of late wilt of corn

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Harpophora maydis is a soilborne fungal pathogen that causes Late Wilt of Corn (LWC). This pathogen has a known distribution that includes Egypt, Israel, India and scattered location in Europe, but is not known to exist in the United States. LWC poses a significant threat to corn production in the United States if *H. maydis* were to become established. Yield losses of >50% have been noted on susceptible corn cultivars in Egypt, and sources of resistance have not been identified against LWC. Although *H. maydis* can be diagnosed based on morphological characteristics, this process requires considerable time and taxonomic expertise. Species-specific PCR primers capable of distinguishing *H. maydis* from other species in the *Gaeumannomyces-Harpophora* complex have been developed but have not been validated for regulatory purposes. In this work we describe conventional and qPCR (real-time) assays to specifically detect *H. maydis* based on the nucleotide sequence of the fungus ribosomal internal transcribed spacer (ITS) region. We are in the process of evaluating the performance of these tests using environmental samples.

Novel PCR-RFLP assay for genetic diversity studies of *Elsinoë australis* isolates causing scab on citrus

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Elsinoë australis, the causal agent of sweet orange scab, was recently found in the Southern United States. The disease is a concern for citrus-growing areas because the pathogen causes unsightly scab-like lesions developing mostly on fruit rinds and leaves of sweet orange and tangerine varieties, making it a significant problem for productions intended for the fresh fruit market. This fungal pathogen has been the object of few studies and limited data is available addressing its genetic diversity and population structure. In this study, a region of 1119 bp within a polyketide synthase-encoding gene (PKS) was PCR amplified, cloned and sequenced in several *E. australis* isolates collected from Argentina, Brazil, Uruguay, Korea and USA. Based on sequence data from this PKS region a PCR-RFLP assay was developed to molecularly characterize these isolates. The restriction pattern obtained from this region using a single endonuclease (*HaeIII*) provides a sufficient level of discrimination among isolates belonging to distinct pathotypes and geographical regions. This method constitutes a simple and efficient molecular tool for a rapid characterization of *E. australis* field isolates that can be additionally applied to distinguish *E. australis* from the morphologically similar citrus scab pathogen *Elsinoë fawcettii*.

Impact of nitrogen source and pH on mycelial growth of the spring dead spot pathogens, *Ophiosphaerella herpotricha* and *O. korrae*

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Spring dead spot (SDS) is the most prevalent disease of bermudagrass in regions where cold temperatures induce winter dormancy. Recent field studies implicate applications of different nitrogen sources and manipulation of soil pH may reduce SDS severity. To further investigate this effect, mycelial growth assays were utilized to assess the impact of nitrogen source and pH on the SDS pathogens *O. korrae* and *O. herpotricha*. For pH assessment, potato dextrose agar amended with antibiotics was adjusted to pH ranges 3-9 with either sodium hydroxide or lactic acid. Nitrogen source was assessed using a basal growth medium amended with either calcium nitrate (CN) or ammonium sulfate (AMS) at concentrations of 0-800 ppm. Mycelial growth was defined as the average of two perpendicular measures of the colony diameter taken 12-d post inoculation. Analysis of variance was conducted using the PROC MIXED procedure in SAS, and pairwise comparisons were generated using the LSmeans command ($P=0.05$). No mycelial growth occurred at pH 3. Mycelial growth was greatest on media with a pHs 5 and 6, growth was significantly lower at a pH 4 when compared to pHs 5-9. Growth was significantly lower for both species on AMS concentrations greater than 50

ppm, when compared to CN treatments. These results indicate nitrogen source effect on mycelial growth of these pathogens *in vitro* does not correlate with nitrogen source impact on disease severity demonstrated in field research.

Stability of *Citrus tristeza virus* populations in field and glasshouse sweet orange

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Citrus tristeza virus possesses a number of genetically distinct genotype groups that are frequently found as members of mixed populations within a single host plant. There is essentially no understanding of what factors regulate CTV populations, how the genotypes within a population interact with each other and/or the host, or how these interactions affect symptom expression or disease severity. In this study we examined Sweet Orange inoculated with FS674, an isolate containing representatives of the VT, T30, and T36 strains that are prevalent in central Florida, over the course of a year by real time qRT-PCR under field and glasshouse conditions to determine whether there were variations in the composition of the inoculated mixtures. We found that in the 27 field plants tested (most in various stages of decline) the population structure remained consistent over time, with only minor variation in the relative titres of the three genotypes. A comparison with plants grown in the glasshouse showed similar results, however a spatial study within these plants indicated that there are significant differences in the localized population structure. Given that the population structure for this isolate is stable, why do we see differences in disease severity in the field plants that were studied? Do these genotypes complement each other? What factors would change the population structure? Answers to such questions would better enable us to develop effective control measures.

The presence of the fire blight bacterium *Erwinia amylovora* in asymptomatic apple bud wood: A potential threat to new apple plantings

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The development of shoot blight in new apple plantings in NY sometimes appears to originate from infections at the site of budding. Budwood for new cultivars and those of limited availability is commonly collected from orchards where fire blight is established. A study was undertaken to investigate the presence of *Erwinia amylovora* in the budwood from asymptomatic trees used for nursery stock. Budwood was collected from two commercial nursery stock plantings of 'Gala' and 'Topaz' apples in western NY. Individual replicated collections of buds were made from budwood with differing proximities to shoot blight symptoms and evaluated for the presence of epiphytic and endophytic *E. amylovora*. On both cultivars, virulent *E. amylovora* was recovered from both the surface and the internal contents of buds from asymptomatic shoots. For 'Topaz' there were no significant ($P > 0.05$) differences in the frequency of *E. amylovora* recovery in regards to proximity to observed shoot blight symptoms. By contrast, the frequency of 'Gala' buds from which *E. amylovora* was recovered from the internal tissues was significantly ($P = 0.034$) higher for shoots within 1 m of a shoot blight strike (>80%) than for buds from shoots more than 20m from a tree with a shoot blight strike (<60%). Although it is unlikely that the majority of such cryptic infections will result in shoot blight, these observations may necessitate a reexamination of budwood collection practices.

Interaction between *Curtovirus* capsid protein and GroEL-like protein produced by leafhopper endosymbiont

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Curly top disease is caused by plant viruses of the genus *Curtovirus* (*Geminiviridae*). This disease can infect many economically important crop plants in different geographical areas, such as western United States and Mexico. The plants affected include beans, pepper, sugarbeets and tomatoes. These viruses are transmitted in a circulative manner by the beet leafhopper (LH), *Circulifer tenellus*. Studies done on begomoviruses (*Geminivirus*) and luteoviruses have shown the importance of a GroEL-like (GroEL) protein produced by the endosymbionts of the insect vector for the successful transmission of the viruses. The capsid protein (CP) of geminiviruses is the determinant of vector specificity, and it is likely that an interaction between GroEL protein and CP protects the virus from degradation in insect. This study aims to elucidate the interactions between these two proteins. Western blot assays suggested the presence of GroEL protein in LH, and a curtovirus CP-GroEL protein interaction. The location of GroEL protein and curtovirus in different parts of the LH was investigated using PCR with specific primers.

WITHDRAWN

Analysis of transporter responsible for the secretion of fusaric acid from the plant pathogen *Fusarium oxysporum* f. sp. *vasinfectum*

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Fusaric acid (FA), a phytotoxic polyketide produced by *Fusarium oxysporum* f. sp. *vasinfectum* (Fov), has been suggested to be associated with disease symptoms on cotton. In response to a potential threat on cotton production by the introduction of high FA producing strains from Australia, new sources for resistance are being pursued. A putative transporter (*fubT*), in close proximity to the cluster responsible for FA production, was sequenced in the Australian isolate 1089. Sequence analysis of *fubT* reveals a similarity to several members of the major facilitator superfamily (MFS) of integral membrane transporter proteins known to confer resistance to various antibiotics and toxins in fungi and bacteria. To explore its function in FA production and resistance, a targeted gene disruption of *fubT* was constructed. Deletion transformants were found to lack secretion of FA and have observable deficits in growth. Some secondary metabolite transporters from other fungi have a role in self-protection by maintaining low intracellular concentration of toxin. To assess if this transporter is involved in a form of self-protection to FA that can be translated into other organisms, *fubT* was transformed into the biocontrol fungus, *Trichoderma virens* Gv29-8. Understanding and identifying new forms of resistance for transgenic use provides novel resistance mechanisms that have the potential to control high FA producing Fov strains on cotton.

Characterization of the interaction between soybean cultivars and isolates of *Fusarium oxysporum* causing seedling disease

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Fusarium oxysporum is one of the most frequent species causing soybean root rot and seedling blight in the U.S. and particularly in Iowa. Information about the relative pathogenicity and phenotypic characteristics of *F. oxysporum* populations associated with soybean roots is limited. The goal of this study was to assess the reaction of soybean cultivars to seedling infection by diverse isolates of *F. oxysporum*. Pathogenicity of 14 isolates was evaluated in rolled-towel and petri-dish assays using 11 soybean cultivars. In the rolled-towel assay, seeds were individually inoculated with 100 μ l of a 1×10^6 conidia/ml suspension. At seven days, seedlings were rated using a disease severity index. In the petri-dish assay, surface-disinfested soybean seeds were placed on 2% water agar medium containing a *F. oxysporum* colony. Seedling disease symptoms were rated at seven days using an ordinal rating scale (0-3). *Fusarium oxysporum* isolates varied in pathogenicity ($P < 0.001$), ranging from non-pathogenic to some causing severe root rot or damping-off. Known soybean cultivars, such as Jack and MN1805, were among the most susceptible, and Ripley and Forrest were the most resistant. Significant ($P < 0.05$)

isolate × cultivar interactions for both assays indicate that soybean cultivars differ in susceptibility to *F. oxysporum* isolates. These results are helpful to understand the biological diversity of *F. oxysporum* populations isolated from soybean.

Identification of a *Xylella fastidiosa* *pilY1* homolog responsible for twitching motility response to calcium

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The plant pathogenic bacterium *Xylella fastidiosa* (Xf) inhabits the xylem vessels of the host, where it forms biofilms thought to be responsible for disease symptoms. Twitching motility, dependent on type IV pili, is required for movement against the transpiration stream that results in Xf basipetal migration and colonization. We previously demonstrated that increased concentrations of calcium result in increased Xf motility. Further experiments were conducted to understand the molecular mechanisms of Ca-regulated twitching motility. Expression analyses of the type IV pili genes *pilA*, *pilT*, *pilB* and the three *pilY1* homologs (PD0023, PD0052, PD1611) were performed on cells incubated in microfluidic chambers under replete and depleted Ca conditions. Up-regulation was indicated for *pilT*, encoding the retraction ATPase in charge of pili depolymerization, and one of the *pilY1* homologs (PD1611), encoding a protein presumably located at the tip of the pili. Sequence analysis identified a Ca-binding domain only in the up-regulated *pilY1* PD1611 homolog. Deletion mutagenesis of *pilY1* PD1611 results in reduced motility and a Ca-blinded phenotype, whereas twitching motility of a *pilY1* PD0023 mutant was still affected by Ca supplementation. Results indicate that the mechanism of Ca-dependent twitching motility is associated with Ca-binding by only one of the three *pilY1* homologs found in Xf.

Functional analysis of grapevine stilbene synthase genes

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Stilbene synthases (STS) mediate the biosynthesis of stilbenic compounds that are components of the chemical defenses restricting pathogen growth. The grapevine genome contains a large family of 48 preliminarily annotated STS genes, 42 of which are clustered on chromosome 16. The regulation and function of each STS gene needs to be understood to ascertain their roles in grapevine's defense against fungal pathogens. We found that eight STS genes are regulated differentially in response to the inoculation of the powdery mildew fungus (PM), *Erysiphe necator* (Schw.) Burr. Constitutive transcript levels of these STS genes also differ in two grape varieties, PM-resistant *Vitis aestivalis* 'Norton' and PM-susceptible *Vitis vinifera* 'Cabernet Sauvignon'. We then selected two STS genes, STS22 and STS7, for further study. The promoter and coding regions of STS22 and STS7 were cloned from Norton and Cabernet Sauvignon genomic DNA and were introduced into *Arabidopsis* plants. The expression of each STS transgene was directed by its native promoter or by the constitutive *Cauliflower mosaic virus* 35S promoter. We are in the process of analyzing the abundance of stilbenic compounds in transgenic *Arabidopsis* lines and investigating their activities against PM.

Genome-wide patterns of diversity in four lineages of the sudden oak death pathogen, *Phytophthora ramorum*

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Phytophthora ramorum causes leaf blight on over 100 hosts and a lethal stem canker on Japanese larch, some oak species and European beech. There are four known lineages which have been introduced and are spreading in Europe and North America from an unknown source. The recent host jump to Japanese larch, a commercially important species in Europe, and subsequent wide-scale mortality in plantations has raised concerns on the potential impact on the forest industry and international trade. We conducted a large scale genome re-sequencing effort (92 strains) to gain insight on migration and evolutionary history of *P. ramorum*. We found a high level of fixed heterozygosity within lineages reinforcing the idea that these lineages evolved from a sexually reproducing ancestor but are now mainly clonal. Despite this, we have found sufficient intra-lineage diversity ($\pi = 0.00308, 0.00216$ and 0.00223 in EU1, EU2 and NA1) for analysis of migration patterns in North

America. We also found several lineage-specific genes including genes encoding effector-like proteins and transposons, suggesting some gain/loss patterns of pathogenicity-genes during lineage evolution. Inter-lineage comparisons using genome re-sequencing should provide insight into the ancestor population from which these lineages evolved. Intra-lineage comparisons will be important in determining migration patterns and allow quarantine efforts to be focused on pathways likely to cause future pathogen introductions.

Mapping *Alternaria cucumerina* resistance in *Cucumis melo*

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Alternaria Leaf Blight caused by *Alternaria cucumerina* is a serious disease affecting worldwide production of melon (*Cucumis melo*). The resistance gene *Ac* has been reported in USDA breeding line MR-1, but molecular markers have yet to be identified. Utilizing bulked segregant analysis, we are conducting marker discovery using recombinant inbred lines (RILs) from an initial cross of MR-1 (resistant) and Ananas Yokneam (susceptible) with randomly amplified polymorphic DNA (RAPD) and High Frequency Oligonucleotides-Targeting Active Genes (HFO-TAG) primers. RAPD and HFO-TAG analysis was performed using agarose gel electrophoresis. In addition, a modified HFO-TAG analysis was performed on a genetic analysis system which provided single base pair resolution. Identified polymorphisms are being used to construct a linkage map, which will have immediate utility in marker identification of gene loci associated with *Alternaria* resistance, as well as other resistance genes found in MR-1. Additionally, in order to increase control and consistency of *Alternaria* resistance phenotyping, we are developing an improved leaf assay adapted to melon. Leaves were inoculated with a range of concentrations of conidia and incubated under varying conditions (light, temperature, humidity) to determine the optimal method for phenotyping.

***Ralstonia solanacearum* T3SS activation in planta requires nitric oxide reductase**

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In response to pathogen recognition, plants produce nitric oxide (NO), a key host defense signaling molecule with bactericidal properties. Some pathogens are susceptible to NO toxicity, but *Ralstonia solanacearum* apparently benefits from the NO in its interactions with plants. Deleting *norB*, which encodes the catalytic subunit of NO reductase, eliminated the pathogen's ability to respire on NO, reduced its virulence on a susceptible host, delayed its growth in xylem, and caused a 1000-fold decrease in its competitive fitness in planta. Surprisingly, the *norB* mutant induced no HR in the normally incompatible tobacco host and even grew to 5×10^7 CFU/cm² tobacco leaf tissue 24hpi, unlike the WT strain. Additionally, the *norB* mutant did not produce its type III secretion system (T3SS) as indicated by dramatically reduced expression of *hrpB*, the T3S transcriptional activator, and *popA*, a T3S effector. The *norB* mutant induced significantly lower expression of plant defense genes in tobacco, which normally launches defenses following recognition of a T3-secreted effector. Genetic analyses suggest that the *norB* mutant makes less NO, and microscopy with the NO-specific dye DAF-FM DA confirmed that less NO was present in *norB*-infused tobacco leaves. The T3S transcriptional activator HrpB contains a predicted S-nitrosylation site. We hypothesize that HrpB is activated by S-nitrosylation mediated by high NO levels in planta. Ongoing experiments are testing this hypothesis.

Mating of *Aspergillus flavus* x *Aspergillus minisclerotigenes* hybrids: Are they functionally mules?

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We previously demonstrated sexual reproductive ability between isolates of *A. flavus* (horses 5, 26) and *A. minisclerotigenes* (donkeys 13, 28). Ten sexual hybrid progeny (mules) from each of two crosses (5x28, 26x13) were used in this study. The 10 F1's of 5x28 were crossed with their opposite mating type parent. Two of 10 produced viable ascospore progeny (mules x horses). The 10 F1's of 26x13 were crossed with their opposite mating type parent. Four of 10 produced viable ascospore progeny (mules x horses). The 10 F1's of 5x28 were distributed 7 *Mat1-1* to 3 *Mat1-2* allowing 21 within group crosses. Four of 21 produced viable ascospore progeny (mules x mules). The 10 F1's of 26x13 were distributed 8 *Mat1-1* to 2 *Mat1-2* allowing 16 within group

crosses. Four of the 16 produced viable ascospore progeny (mules x mules). The frequency of successful matings with hybrids as parents approximates those within and between *A. flavus* and *A. minisclerotigenes*, however no successful matings occurred between the hybrid mules and *A. minisclerotigenes* donkeys.

Unraveling legume anthracnose pathogens

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Colletotrichum species cause destructive diseases of many hosts, including legumes such as common bean, soybean, lentil, red clover and alfalfa that play important roles as vegetables or pasture plants in human and animal nutrition and in soil improvement. While the taxonomic placement of *C. truncatum* was studied previously, the identity of most of these pathogens is still unclear. Reports from the literature indicate that some of them might be confused with each other, especially regarding the sexual morphs of some species involved in this complex. Multilocus molecular phylogenetic analyses (ITS, GAPDH, ACT, TUB2, CHS-1, HIS3) of strains isolated from legumes and other related strains placed most of the pathogens associated with legume anthracnose diseases into three species complexes that have not been well studied to date. *Colletotrichum lindemuthianum* and *C. trifolii* are closely related species belonging to the *C. orbiculare* complex. *Glomerella truncata* was confirmed as not being the sexual morph of *C. truncatum*. While *G. truncata* belongs to the *C. destructivum* complex along with *C. destructivum*, *G. glycines* was found to be distinct from *C. destructivum*, and to belong to another species complex. Several new taxa were revealed in each of the three species complexes studied.

Effects of quinone outside inhibitor fungicides on Fusarium head blight, deoxynivalenol, and Fusarium graminearum biomass in soft red winter wheat

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Quinone Outside Inhibitors (QoIs) are effective fungicides against foliar diseases of wheat, but are usually not recommended for Fusarium head blight (FHB) and deoxynivalenol (DON) management. This is largely because they have been shown to increase DON in the grain, but reasons such a response are largely unknown. Field and greenhouse experiments were conducted to determine whether DON response to QoIs varied with active ingredients (AIs) and application timing, and whether it was associated with an increase in grain fungal biomass (FBM). Treatments consisted of two QoIs (azoxystrobin [AZO] and pyraclostrobin [PYR]) and a Demethylation Inhibitor (tebuconazole + prothioconazole) applied at Feekes 8, 10, and 10.5.1, plus an untreated check. All plants were inoculated with a spore suspension of *F. graminearum* at anthesis. FHB index (IND) was rated three weeks after anthesis, and a sample of grain from each treatment was assayed for DON and FBM. Treatments applied at Feekes 10.5.1 resulted in significantly lower IND than the check. Depending on the AI, Feekes 8 and 10 treatments had comparable or lower IND than the check. In general, mean DON and FBM were highest in QoI treatments applied at Feekes 8 and 10, even though mean IND was relatively low in some of those same treatments. Among the QoI treatments, AZO tended to result in higher DON than the check at all growth stages. For PYR, only the Feekes 8 treatment tended to result in higher DON than the check.

CSI in a tomato disease plot: Engaging 4-H youth and educators in STEM through investigative plant pathology

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Oklahoma 4-H youth activities focus on agriculture and leadership, providing an ideal venue for STEM experiences. As an outreach component of a USDA Plant Biosecurity grant we developed a 2-day campus experience in which youth teams assumed roles as 'lead investigators' in a potential 'biocrime.' Teams received briefings on the incident, collected and processed evidence, interviewed 'suspects' and 'witnesses,' prepared their cases and presented their findings in a courtroom scene for ultimate perpetrator 'conviction.' Key elements of the program's success were (1) close and constant guidance and

mentoring by project personnel during the exercise to allow youth discovery and thoughtful analysis, (2) careful preparation of diseased tomato plants and 'physical evidence' at the 'crime scene' field; (3) pre-event optimization of the laboratory experiments at the youths' level, and (4) preparation of an image-rich, professional quality workbook containing relevant background information, step-by-step instructions and evidence-documentation hints. Following workshops offered in two successive summers, both youths and adult educators returned high ratings for the quality and value of their experiences with the fundamentals of science and the investigative process. Participants were exposed to careers in phytopathology and biosecurity. Long-range outcomes will be enhanced by current adaptation of the workshop elements and materials for use by high school teachers.

Next generation sequencing and its application as a biosecurity tool

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Biosecurity agencies require dynamic detection systems that can be rapidly updated as needed to meet current and future biological threats. Although individual pathogen assays are available, current screening methods have limited ability to detect multiple pathogens. Microbial forensics combined with next generation sequencing (NGS) allows the simultaneous detection of any or all microbes in a sample, including detection of signatures of genetic modification. Bioinformatic pipelines were created for use on mock sample databases to simulate 454 sequence runs, electronic probe (e-probe) design and conduct BLAST searches for plant disease models. Pathogen specific queries, ranging from 20 to 140nt in length, were created for detection of the bacterial select agents *Xylella fastidiosa* 9a5c, *Xanthomonas oryzae*, and *Ralstonia solanacearum* r3b2, as well as for '*Candidatus Liberibacter asiaticus*'. All four bacterial pathogens were readily detectable in silico, suggesting that MPS technology has advantages beyond those of current pathogen detection assays. All four e-probe sets were used to query a 454 Junior sequencing run derived from orange (*Citrus sinensis*) tissue infected with '*Candidatus Liberibacter africanus*'. The 454 data contained sequences from the host genome, multiple bacteria and fungi, mitochondrial DNA, and chloroplast DNA, which is characteristic for a metagenomic infected plant sample.

Investigation of a population of Phytophthora infestans in and near central New York in 2011

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In the summer of 2011, isolates of *Phytophthora infestans* from diverse locations in central and western New York were found to be different from the clonal lineages (US-22, US-23 and US-24) that were predominant in other locations of the United States. These isolated were characterized for several genotypic markers including alleles at 12 microsatellite loci, alleles at the *glucose-6-phosphate isomerase* locus, RFLP bands detected by RG57 probe and five different mitochondrial loci. Additionally, the isolates were characterized for mating type and mefenoxam sensitivity. The ratio of mating types among the several genotypes was close to 1:1. The results of these analyses identified at least 13 unique genotypes. Mefenoxam sensitivity also differed among the isolates. The collection is being assayed to determine if these individuals might be siblings of one or more recombination events(s).

Significance of soil inoculum in the epidemiology of boxwood blight caused by Calonectria pseudonaviculata

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Boxwood blight is a serious disease of boxwood caused by *Calonectria pseudonaviculata*. While tracking disease progression in fields during 2012 symptoms were primarily on lower branches coated with soil particles splashed from the ground during rain events. Based on these observations, we tested whether soil infested with *C. pseudonaviculata* can cause foliar blight using cuttings of *B. sempervirens* 'Arborescens'. Conidia were obtained by flooding plates with distilled water and dislodging with a scalpel. Microsclerotia (MS) were collected on a 150-µm sieve after homogenizing colonized PDA in a blender. Sandy loam soil from a nursery with no history of boxwood blight was used for the experiment. Soil was inoculated with

conidia at 0, 10, 100, 1000, and 10,000/g or MS at 0, 25, 50, 100 and 200/g. Soil moisture was adjusted to 30% (w/w). Twelve *B. sempervirens* cuttings per treatment were blotted against soil until evenly coated. The cuttings were incubated under high humidity at 25°C for 7 days. The experiment was repeated twice. All inoculated treatments developed leaf blight associated with sporulation of *C. pseudonaviculata* while uninoculated controls did not. A positive correlation was consistently observed between propagule density and proportion of blighted leaves. These results coupled with field observations suggest infested soil is a significant source of inoculum in the epidemiology of boxwood blight.

Therapeutic and prophylactic application of phages to control Pierce's disease

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Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa*, is a major threat to the wine industry in the USA. An effective, sustainable and environmentally safe biocontrol method for PD is needed. A phage therapy system with high specificity and non-toxicity to animals, plants or non-target bacteria could offer a novel control strategy against the PD pathogen. Virulent phage Xfas304 and a phage cocktail composed of four virulent phages (Xfas303, Xfas304, Xfas103, Xfas106) were both evaluated for the therapeutic and prophylactic efficacy in *Vitis vinifera* (variety Cabernet Sauvignon) in greenhouse experiments. Triplicate vines were segmented and individual segments were assayed using quantitative Real Time PCR (qRT-PCR) to quantify bacteria and/or phage(s). qRT-PCR results showed movement of pathogen and/or phage(s) in vines over a 12-week period. Typical PD symptoms were visible by week 8 in pathogen inoculated control vines. No PD symptoms were observed in phage-treated vines in either therapeutic or prophylactic studies. In therapeutic and prophylactic studies, the PD pathogen declined to almost non-detectable levels as compared to vines not challenged or pre-treated with phage(s), respectively. Phage(s) were able to replicate in vines in the presence of a host. Successful application of phages as biocontrol agents for *X. fastidiosa* will offer a novel alternative method to wine industry for the control of PD.

Effects of environment and host on endophyte communities of coastal dune grasses

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Endophytic fungi represent a large portion of fungal diversity, though relatively little is known about their ecology. Communities of endophytes are shaped by a number of factors such as the environment external to the host and the host species. In particular, the relative influence of different factors on endophyte assembly is not well understood. To examine how environmental and host factors contribute to endophyte assembly, we conducted an observational study of endophytes occurring in three U.S. Northwest coastal dune grasses (*Ammophila arenaria*, *A. breviligulata*, and *Elymus mollis*). Coastal dunes provide a natural environmental gradient consisting of a harsh foredune that gives way to more stable backdune habitat. We used a culture based approach to investigate how endophyte communities in the leaves and roots changed along the dune gradient. We found support for a strong environmental influence on assembly, as endophyte operational taxonomic unit (OTU) richness increased from the foredune to the backdune. We found less support that host species influences assembly, as the grass species shared several endophyte OTUs and varied less in richness. We conclude that the environment is a strong influence in shaping endophyte communities in coastal dune grasses.

Temporary ponds: Unexplored chytrid biodiversity

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Temporary ponds are unique wetland habitats that annually dry out. For many taxa, they serve as biodiversity hotspots and epicenters of evolution. Little is known about their fungal communities, except that they are dominated by aero-aquatic and aquatic hyphomycetes. Two recent molecular inventories of vernal pools hinted at the full scope of fungal diversity found in temporary ponds, including chytrid phylotypes. Temporary ponds are a novel habitat in the exploration of chytrid biodiversity. We isolated chytrids from two temporary ponds in the Oakmulgee District of the Talladega National Forest. Isolates in the morphospecies of *Chytridiomyces hyalinus* demonstrated a previously unknown method of resting spore germination. Isolates of the

morphospecies *Rhizoclosmatium aurantiacum* showed genetic divergence and possible niche differentiation. Thus, we can conclude that temporary ponds, and possibly other temporary aquatic habitats, are a source of previously unknown chytrid biodiversity (genetic, ecological, and developmental) and represent an opportunity to examine how habitat and/or substrate specificity leads to speciation in Chytridiomycota.

A new species of *Mucor* from a Cerrado reserve in Southeast Brazil

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The Cerrado (Brazilian savanna) is one of the richest biomes that present a high number of endemic and unknown species, and consequently is listed among the world's biodiversity hot spots. The "Reserva Biológica de Mogi Guaçu" (RBMG) constitutes one of the few remnants of Cerrado in São Paulo State. Recently, we surveyed the zygomycetes from soil and leaf litter from this reserve and found a distinct *Mucor* specimen from soil, which was characterized based on morphological and rDNA data (ITS1-5.8S-ITS2 and 28S). The isolate produces sporangiospores with dimensions of 9–39 x 5.5–19 µm and giant cells with 150–500 µm of diameter in large agglomerations that were not described before for *Mucor* species. Preliminary morphological and molecular data strongly supports the establishment of a new species of *Mucor*. We thank Fapesp (project 2008/53146-4) for financial support given to C.L.A. Pires-Zottarelli.

Understanding DNA methylation patterns in *Fusarium* species

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The *Fusarium oxysporum* species complex is defined by its large number of plant hosts and highly variable genomes, especially when compared to its close relatives *Fusarium graminearum* and *Fusarium verticillioides*. Acquisition of genetic material, most likely through horizontal gene transfer, has led to each sub species having a very narrow host range, but the complex as a whole having over 100 different host species. This diverse array of genetic material, combined with the fact that *Fusarium oxysporum* contains almost 30 times more transposable elements than its sister species, has prompted us to ask how the pattern of DNA methylation varies within the *Fusarium* genus, focusing on the differences between *Fusarium oxysporum* and the remaining species. Here we report on the first round of analysis from single base pair resolution bisulfite sequencing data sets from *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium graminearum*, and *Fusarium verticillioides*.

GFP-immunoelectron microscopy of endocytic vesicles of *Neurospora crassa*

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Endocytosis is a complex process that requires the orchestrated interaction of a variety of proteins. A main component of this network involves actin and some of its binding proteins, collectively known as Actin Binding Proteins (ABPs). Proteins related to membrane bending prime early endocytic vesicles; these proteins include the clathrins and the amphiphysins. Later in the maturation of the endocytic vesicles, the complex Arp2-3 (a promoter of actin polymerization) is recruited to endocytic vesicles. The appearance of ABPs coincides with the movement and internalization of the vesicles. Recent advances have shown that immunoelectron microscopy of GFP allows for the correlation of ultrastructural and fluorescence microscopy. In *Neurospora crassa*, the components of the Arp2-3 complex, ARP-2-GFP and ARP-3 label small (X µm) patches in the cell cortex. These patches, found all along the hypha, concentrate at the subapical region forming a ring or collar. We have performed the immunolocalization of GFP bound to the protein ARP-2 of *N. crassa*. We were able to image endocytic vesicles at the ultrastructural level by TEM using a secondary antibody (Ab) coupled to gold nanoparticles directed against a primary Ab against GFP. To our knowledge, this is the first time this technique, which has proved successful in the yeast *Saccharomyces cerevisiae*, has been applied to a filamentous fungus.

Characterization of genes associated with antibacterial activity of *Burkholderia contaminans* strain MS14

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Burkholderia contaminans MS14 possesses a 56-kb *ocf* gene cluster required for production of the antifungal compound occidiofungin via a nonribosomal peptide synthase (NRPS) mechanism. MS14 also exhibits a significant antibacterial activity against some pathogenic bacteria including *Erwinia amylovora*, *Xanthomonas citri* pv. *malvacearum* and *Clavibacter michiganensis* subsp. *michiganensis*. Preliminary assays showed that antibacterial activity did not result from occidiofungin production. In this study, random mutagenesis was conducted to characterize the genes dedicated to the antibacterial activity. Mutant MS14MT357 with undetectable antibacterial activity against *E. amylovora* and mutant MS14MT577 with reduced activity were identified. A draft of genome sequence of MS14 was obtained using Illumina sequencing. The gene targeted in MS14MT357 is 726 bp in size, encoding a 241-aa protein. The deduced protein shares a 37% identity with the LuxR family transcriptional regulator BphY_4277 of *Burkholderia phytofirmans* PsJN. The gene disrupted in MS14MT577 is 9.6 kb in size, encoding a 3219-aa peptide. The deduced peptide shares a 95% identity with a NRPS protein Bcep18194_A4786 of *Burkholderia* sp. 383. The data suggest that MS14 inhibits bacterial growth by synthesis of another NRPS product. Characterization of more mutants and genome sequencing are under way. This research will provide deep insights into understanding the mechanism of antibacterial activity of strain MS14.

Shotgun poplar disease diagnostic using next gen sequencing

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Current advances in Next Generation Sequencing (NGS) technologies - higher sequence output coupled with lower costs - has resulted in a large number of genomes being sequenced. A wide variety of eukaryotic genomes from equally diverse environments can be rapidly sequenced and assembled. Our interest lies in dissecting this vast amount of NGS data from environmental samples for disease diagnostics, with the in the context of *Populus*-Dothideomycete and *Populus*-Uredinales interactions used as showcase. Once established, this data analysis pipeline could be virtually used for any host-pathogen system diagnosis. In addition to utilizing the existing genomic resources, we have sequenced the genomes of 12 poplar pathogens under the purview of Genome Canada funded TAIGA project. In a preliminary study, we have sequenced diseased hybrid poplar leaves harvested at the end of the growing season using Ion Torrent technology and analyzed the resulting dataset from two different field samples. By using genome-mapping approaches, we were able to assign reads to pathogen genomes that corresponded to visible disease symptoms on the poplar leaves. Most of the reads mapped to two of the most important poplar pathogens, *Mycosphaerella populorum* and *Melampsora occidentalis*. However, a large number of reads did not map onto these known sequenced genomes, an indication that more genomes will be required to provide a proper reference database and to identify potential new pathogens.

Microbial communities associated with the suppression of tuber blight infection in soils from Chimborazo province, Ecuador

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Late blight, caused by *Phytophthora infestans*, is a threat to potato crops worldwide. In Ecuador the incidence of late blight in foliage is high; while, tuber blight infection is almost absent. This suggests that soils from the Ecuadorian Andes are naturally suppressive to tuber blight. The aim of this study was to determine the relationship between microbial community diversity and the suppressive ability of soils from Chimborazo province. Potato is a staple crop in this region of the country, where production is dominated by small-scale, low-income farmers. Suppression of tuber infection was assessed by exposure of *P. infestans* sporangia to fresh and heat-treated soil, and subsequent inoculation of potato slices at different time points. The percentage of infected tubers inoculated with fresh soil was lower compared to treated soils. Total DNA was isolated from soils of both treatments, and all

time points. Bacterial and fungal communities were assessed using T-RFLP analyses of the 16S rDNA and ITS regions respectively. Microbial communities responded to heat treatment and exposure time. Microbial diversity was correlated to suppression of infection. Specific bacterial groups associated with tuber blight suppression belong to the genera *Bacillus*, *Geobacillus*, and *Pseudomonas*. Studies of soils from this region will contribute to understand microbial populations in Andean soils and their contribution to disease suppression.

Evidence for exogenous and endogenous forms of *Rubus yellow net virus* in *Rubus*

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Rubus species are host of *Rubus yellow net virus* (RYNV), which causes chlorosis of the tissue along the leaf veins giving an unevenly distributed netted symptom. With the development of a PCR based assay for detection of RYNV it was observed that many raspberry plants that tested positive for RYNV by PCR were negative when graft- or aphid-transmitted onto the biological indicator *Rubus occidentalis*. RYNV belongs to the family *Caulimoviridae*, which has been reported to result in endogenous pararetroviruses (incorporated into the host genome) in the case of *Banana streak virus* (BSV) and *Fig badnavirus 1*, both members of the genus *Badnavirus*. Rolling circle amplification of RYNV yielded virus specific products from a graft transmissible isolate but not from nontransmissible isolates, confirming the endogenous status of RYNV in multiple plants. RYNV-specific PCR amplicons were obtained with six sets of primers in a few plants, while other plants only gave amplicons with one or a few these primer pairs. Several isolates gave different size amplicons with some primers, suggesting significant deletions in many of the endogenous RYNV sequences. This is the first evidence of RYNV as an endogenous virus in *Rubus* species. Our next steps are to characterize the virus and multiple insertion events to determine if the entire virus is inserted in the host genome, if insertion is site specific and if endogenous virus can be released from the genome as is the case for BSV.

Conidia viability and cytology in *Moniliophthora roreri*, the causal agent of frosty pod rot of cacao

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Moniliophthora roreri causes frosty pod rot disease in cacao. Yield losses of close to 100% have been recorded and in many cases entire plantations have been abandoned because of the disease. Before the 1950's frosty pod rot was confined to the north-western part of South America but recently it has spread northwards as far as Mexico and southwards into Peru. Currently frosty pod rot represents a serious threat to cacao growers in Bolivia, Brazil, and even West Africa. *M. roreri* is only known to reproduce via conidia, and therefore it is important to know how long spores may remain viable in the field, especially after a farm is abandoned. We have found that a small proportion of spores maintain viability for at least 14 months *in vitro*. Two varieties of *M. roreri* have been formally described, *M. roreri* var. *roreri* and *M. roreri* var. *gileri*. These are differentiated by host (the latter is confined to *T. gileri*, rather than *T. cacao*) and differences in spore size. However, our studies show that spore size in *M. roreri* is quite variable and is due to nuclei number, which is also quite variable. Finally, we have conducted multiple generations of single spore isolation and cytological studies as well as analyses of multiple genetic markers to infer whether this fungus is potentially outcrossing. These and other results will be discussed.

Within-field spatial and temporal analysis of *Clavibacter michiganensis* subsp. *nebraskensis* and Goss's leaf blight of corn in Iowa

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Clavibacter michiganensis subsp. *nebraskensis* (*Cmn*), the cause of Goss's wilt and leaf blight of corn, has reemerged as an economically important pathogen in the Western Corn Belt. Pathogen inoculum sources include *Cmn*-infested surface crop residue and *Cmn*-infested seed. In 2012, 10-ft X 30-ft corn plots (8 rows X 50 plants) were used to evaluate the spatial and temporal distribution of epiphytic *Cmn* and Goss's leaf blight. A randomized complete block design with 4 replications was used to evaluate 3 treatments. Inoculum point sources were established at plot centers by wounding and inoculating 4

plants with a Rif-resistant *Cmn* strain (91R), or as mesh bags of 91R-infested residue. Non-inoculated checks were included. On five dates, the presence or absence of epiphytic *Cmn* was determined on two leaves from 16 randomly chosen plants per plot, and the presence of Goss's leaf blight symptoms of each plant in each plot was recorded. Epiphytic *Cmn* was detected in all plots and incidence increased throughout the growing season. Incidence of *Cmn* was greatest on the wounded inoculated treatment, followed by the *Cmn*-infested residue. Similarly, the incidence of Goss's leaf blight increased through the season and was greatest in the wounded inoculated treatment. Spatial analyses using ordinary runs revealed clustering of Goss's leaf blight in *Cmn*-inoculated treatments, but the disease occurred randomly within non-inoculated check plots.

***Phytophthora nicotianae* as a re-emerging pathogen in Florida citrus groves**

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Phytophthora nicotianae is found in association with citrus throughout the world, where it is known to cause root, foot, and brown rot, as well as gummosis. Previously regarded as a sporadic citrus pathogen in Florida, management efforts to control *P. nicotianae* have increased statewide among growers. In extension efforts to local grove managers around Immokalee, we processed soil samples from citrus groves for the presence of *P. nicotianae*. Soils were sampled between December 2011 and December 2012 and plated onto PARPH amended V8 and Corn Meal agars. Colonies identified as *P. nicotianae* based on morphology, and ITS analysis were further characterized for sensitivity to mefenoxam and mating type. Mefenoxam sensitivity was conducted by comparing radial growth of mycelia plugs grown on V8 agar with and without 100ppm mefenoxam (Ridomil Gold EC). Mating type was done by pairing mycelia plugs with either Imk-373 (A1), or Imk-374 (A2). Isolates recovered from grove samples revealed isolates of both mating types and ranged from fully sensitive, intermediately sensitive, and resistant to mefenoxam. These data present a more interesting dynamic in grove soils than the occasional clone introduced from nursery stock, and heighten the need for additional management strategies.

Toward a better bean: Improving genetic, genomic, breeding, and disease management resources for lima bean to benefit the Mid-Atlantic region

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Lima bean (*Phaseolus lunatus*) is the cornerstone crop of the vegetable processing industry in Delaware. It is plagued with high disease pressure correlated to humid conditions found on the east coast. Pod maturation occurs later in the season when cool, wet conditions prevail, favoring several diseases; white mold, downy mildew and pod rot. The latter two are caused by the oomycetes *Phytophthora phaseoli* and *P. capsici*, and the former is caused by the fungal pathogen *Sclerotinia sclerotiorum*. Each disease issue presents a challenge: *P. phaseoli* breaks genetic host resistance via the emergence of new races; *P. capsici* is an emerging threat with a broad host range, making control via crop rotation difficult; *S. sclerotiorum* also has a very broad host range. Additionally, lima bean is afflicted by root knot nematode, whose broad host range and limited existing control options necessitate new control strategies. A team of researchers and collaborators, sponsored by a Specialty Crops Research Initiative, are working together to put new tools into growers' hands, including predictive models, risk assessment guidelines, alternative control strategies, economic analysis, and genetic resources for lima bean that will extend well past this project. Along with an overview of the entire plan, we present initial efforts to identify, via reduced representation sequencing, robust markers that will allow us to characterize *P. phaseoli* isolates from grower fields.

Efficacy of sanitizing agents to refine best management practices for the boxwood blight pathogen *Calonectria pseudonavicula*

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Boxwood blight, caused by *Calonectria pseudonaviculata*, is an emerging, destructive disease of economic significance in North America. Initially observed in the U.K. in the 1990s and now endemic in Europe, it was first identified in the U.S. in October 2011. Now reported in 10 U.S. states and 3 Canadian provinces, it has caused substantial economic loss. Green

professionals are concerned about contaminated hard surfaces as sources for new infections. Current Best Management Practices (BMPs) specify sanitizers for decontaminating containers or equipment. This study assessed mycelial growth and conidial germination for 2 CT isolates (from boxwood and pachysandra) challenged with log concentrations (1:10-1:10,000) of sanitizers (hydrogen dioxide; hydrogen peroxide and peroxyacetic acid; hydrogen peroxide, peroxyacetic acid, and octanoic acid; isopropanol and ethanol; quarternary ammonium compound; and sodium hypochlorite). Two methods (amended or flooded plates with different contact times) were used to evaluate mycelial growth on ½-PDA for 21 days. Germination was assessed by exposing conidia to sanitizers for 5-60 min. Aliquots of conidia were spread on WA and germination assessed at 24 and 48 h. Differences were observed among sanitizers and concentrations, from complete inactivation to slowed growth. Results were similar with germination, although conidia were more sensitive to lower concentrations of sanitizers than mycelia. These results will help to refine BMPs.

Critical reassessment of specificity in the evolutionary ecology of *Pterospira andromedea* and its *Rhizopogon* spp. mycobionts

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The mycoheterotroph *Pterospira andromedea* has been reported to be highly specific to its fungal hosts. This conclusion resulted from samples collected from a limited geographic area which recovered only five *P. andromedea* haplotypes and associated with only two of the four known *Rhizopogon* species: *R. salebrosus* and *R. arctostaphyli*. During this time information on the Eastern undescribed species was limited and an *R. ellenae* association was not thought possible due to a lack of symbiotic overlap with sister taxon *Sarcodes sanguinea*. Recent fieldwork conducted throughout a large portion of the distribution for *P. andromedea* and including all known mycobionts provided a framework to critically reassess specificity within this system. Paired samples of *P. andromedea*/*Rhizopogon* spp. were sequenced and co-phylogenies analyzed. Bayesian phylogenetic reconstructions detected four distinct lineages among 13 *TRN1* haplotypes recovered for *P. andromedea*. An extremely high level of specificity was recorded for one *P. andromedea* lineage, under sympatric conditions, and was almost completely restricted to associations with *R. arctostaphyli* and subsequently *Pinus ponderosa*. Alternatively, the novel association between *Pterospira andromedea* and *R. ellenae* was found to be widespread throughout the northwestern United States but was nonspecific with respect to *P. andromedea* lineages. Additional evidence for geographic patterns and autotrophic host specificity were also detected.

Virus induced gene silencing of wheat stem rust, *Puccinia graminis*, genes resulting in reduced pathogenicity

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Wheat is the leading source of vegetable protein in the human diet and is considered the second most important agronomic crop. Cereal rust fungi cause devastating diseases of wheat and their continuous prevalence has major constraints on wheat production globally. Control of rust diseases has mainly been through genetic resistance; however many of these resistances are controlled by single genes that lose effectiveness over time. The development of a more durable resistance that can survive rust adaptation and evolution would allow for the maintenance of high yields when there is a high disease pressure. Recent experiments have demonstrated that expression of double stranded RNA fragments of *Puccinia* genes in wheat can silence the corresponding genes in rust fungi haustorial cells. In this study, one conserved rust gene was identified to cause a reduction in the severity of rust infection through transient Virus Induced Gene Silencing assays. Wheat lines with potentially durable resistance are being developed by introducing the indigenous essential rust gene through *Agrobacterium* transformation. The transgenic wheat lines should elicit a silencing response to reduce pathogenicity or development. A few stable transformants of wheat have been obtained and their characterization is in process.

Susceptibility of garden phlox (*Phlox paniculata*) to *Phytophthora nicotianae*

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Phytophthora nicotianae consistently is associated with herbaceous ornamental crops in South Carolina's greenhouses and nurseries and causes

root, stem, and foliage diseases on many different plant species. On garden phlox, *Phlox paniculata*, this pathogen can cause devastating foliage blight. Therefore, a method was developed to inoculate foliage with *P. nicotianae* to best reproduce symptoms seen in the field. Foliage was inoculated with a zoospore suspension of 3×10^4 zoospores/ml, and plants were held under warm, humid conditions. Using this method, we evaluated susceptibility of popular cultivars of *P. paniculata* grown in SC nurseries to an isolate of *P. nicotianae* recovered from this host. Results among replicates in all experiments were highly variable. In an in vitro detached leaf assay, leaves of 15 cultivars were inoculated, held at 25°C in the dark for 72 h, and evaluated. Cultivars differed in susceptibility ($P=0.043$), and wounding aided infection ($P=0.010$). In an assay conducted in a growth room (81-93% RH, 23-27°C, 14-hour photoperiod) using whole plants, foliage of 13 cultivars was inoculated and plants were evaluated over a 7-day period. Differences among cultivars were subtle ($P=0.104$), and, on whole plants, wounding did not influence infection or disease development ($P=0.348$). While *P. nicotianae* does cause foliage blight on *P. paniculata*, the exact nature of the pathogenic relationship between these two organisms is not well understood.

Host range of *Penicillium* spp. (blue mold) rotting bulb crops

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Penicillium spp. rotting bulbs in North America are inadequately characterized. Replicated pathogenicity assays with three sets of controls were conducted with isolates recovered from bulbs in North America and belonging either to series Corymbifera (*P. allii*, *P. hirsutum*, *P. tulipae*, *P. venetum*), or assigned to *P. polonicum*, based on beta-tubulin sequences. Inoculated were bulbs, cloves or corms of tulip (*Tulipa*, 'Darwin Hybrid Red Oxford'), garlic (*Allium sativum*, 'Rose Du Var'), onion (*A. cepa*, 'Gold Pearl' and 'Forum'), iris (*Iris hollandica*, 'Blue Diamond'), daffodil (*Narcissus*, 'Ice Follies'), crocus (*Crocus sativus*), ornamental onion (*A. stipitatum*), grass lily (*Ornithogalum umbellatum*) and *Gladiolus* (unnamed variety). All isolates were pathogenic to tulip and 'Forum' onion ($P \leq 0.05$). All but *P. venetum* were pathogenic to 'Gold Pearl.' *P. venetum* was pathogenic to iris, but *P. hirsutum* and *P. polonicum* were pathogenic only at $P \leq 0.10$. Only *P. polonicum* was pathogenic on daffodil. *P. hirsutum*, *P. tulipae* and *P. polonicum* were pathogenic on crocus. *P. allii*, *P. tulipae* and *P. venetum* were pathogenic on grass lily. No isolates were pathogenic to *A. stipitatum*, except possibly *P. polonicum* ($P \leq 0.10$). *P. hirsutum* and *P. venetum* were pathogenic to *Gladiolus*. All were consistently pathogenic on garlic, except for *P. tulipae*, whose behavior was inconsistent, but pathogenic for combined assays ($P \leq 0.05$).

Histological characterization of wheat leaf rust resistance components in Thatcher isolines carrying race specific and race non-specific genes

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Leaf rust caused by *Puccinia triticina* is the most prevalent disease of wheat causing significant yield losses worldwide. Over 60 leaf rust resistance (*Lr*) genes have been designated but only a few are effective against the highly diverse North American *P. triticina* population. The use of broad, non-race specific resistance has been proposed as an alternative to achieve durable rust resistance. Post-haustorial resistance is generally involved in race specific resistance and is characterized by hypersensitive reaction (HR). Pre-haustorial resistance does not generally involve a HR, and is presumed to be more durable and may be involved in broad and non-race specific resistance. In this study, Thatcher near isogenic lines (NILs) carrying different *Lr* genes were characterized for pre and post-haustorial resistance components using fluorescence microscopy to races THBL, TSCD, TQBN at multiple time points during disease development. Resistance reactions to the pathogen were characterized by pathogen infection structure development, colony size, and plant and tissue responses. When challenged with avirulent races, NILs Tc-*Lr9*, -*Lr21* and -*Lr34* differed in number and time of fungal structure development compared to susceptible cv. Thatcher. The use of fluorescence microscopy provides new insights toward unraveling the complexities of the leaf rust host-pathogen interaction.

Spatial patterns of ergot in fields of Kentucky bluegrass and perennial ryegrass grown for seed in the Columbia Basin of Oregon and Washington

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Ergot, caused by *Claviceps purpurea*, is a persistent problem in Kentucky bluegrass (KBG) and perennial ryegrass (PRG) grown for seed in the Columbia Basin of Oregon (OR) and Washington (WA). The objective of this research was to quantify and describe the spatial patterns of ergot infection in commercial KBG and PRG fields in OR and WA to better understand the epidemiology of ergot in large (50 ha) first-year fields. A total of 1072 and 1433 quadrats were examined in two KBG and three PRG fields, respectively. Ten inflorescences were collected from quadrats located 10 m apart along transects following every other center pivot irrigation wheel track. The percentage of quadrats containing at least one inflorescence bearing sclerotia ranged from 0 to 29% in KBG fields and 59 to 90% in PRG fields. The mean incidence of KBG and PRG inflorescences with ergot ranged from 0 to 2% and 12 to 25%, respectively. Mean severity indices ranged from 0 to 0.7 for KBG and 1.8 to 4.1 for PRG. Significant ($P < 0.05$) clustering was observed at the overall field level in one KBG field and all three PRG fields using Moran's I and SADIE indices of aggregation. Negative binomial regression indicated that ergot severity in PRG significantly ($P < 0.01$) decreased with increasing distance from established, neighboring PRG fields. Although ergot incidence was widely distributed throughout first-year KBG and PRG fields, ergot severity was characterized by aggregated foci of intense disease severity.

Attachment and germination of *Phytophthora capsici* zoospores on roots of susceptible and resistant peppers

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Phytophthora capsici causes root and crown rot on many vegetables, including pepper. The primary propagule for secondary spread between plants is likely the zoospore, which is motile and chemotactically attracted to host plants, and can swim short distances through saturated soil before encysting on roots. Host resistance is a key management strategy in pepper, but it is not clear how tolerant peppers evade infection by *P. capsici*. To answer this question, roots of pepper plants highly susceptible to *P. capsici* ('Red Knight'), highly tolerant ('Paladin'), and of a land race used as a source of resistance in breeding programs ('CM-334') were dipped in a zoospore suspension of a *P. capsici* isolate that produces green fluorescent protein. Zoospore attachment and germination on roots was then quantified 30 minutes and 2 hours (respectively) after inoculation with the aid of a confocal laser scanning microscope. Zoospores were equally successful at attaching to roots of 'Red Knight', 'Paladin', and 'CM-334', and nearly all of the attached spores germinated within two hours of inoculation, with no significant differences among varieties. Thus, infection by *P. capsici* is not blocked by tolerant peppers at the zoospore attachment or germination stages. Quantification of *P. capsici* colonization of 'Red Knight', 'Paladin', and 'CM-334' roots within one week of inoculation is ongoing in order to determine at what stage tolerant peppers are able to halt the infection process.

Long-term survival and seed transmission of *Acidovorax citrulli* in citron melon (*Citrullus lanatus* var. *citroides*) seeds

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Long term survival of *Acidovorax citrulli*, a causal organism of bacterial fruit blotch (BFB) of cucurbits in citron melon (*Citrullus lanatus* var. *citroides*) seeds was investigated. Citron melon seedlots infested with *A. citrulli* were generated in the field by either inoculating the pistils (stigma) or the pericarp (ovary-wall) of the female blossoms. *Acidovorax citrulli* strains from 17 different haplotypes belonging to two different groups (group I and II) were used for inoculation. Seeds from the matured fruits were harvested and stored at 4°C and 50% RH for 7 years. Upon removal from storage, bacterial viability was assessed in individual seed lots (5g of seeds/lot) by plating (seed macerates) and seedling-grow out (SGO) assay for 14 days under conditions of 28°C and 80% RH. Survival efficiency was influenced by both the inoculation method and strain type. *Acidovorax citrulli* was detected from 45 and 20% of the lots that were inoculated with group II strains as compared to 28.3 and 7.2% for group I strains, as determined by plating and SGO assays, respectively. In addition, bacterial survival was greater in seedlots inoculated using the pistil inoculation protocol than in seed lots inoculated with the pericarp method ($P=0.002$). These observations indicate that *A. citrulli* can

survive/overwinter in citron melon seeds for at least 7 years and may serve as a local source of inoculum for BFB epidemic in the field.

Emergence and characterization of powdery mildew on hop cultivars with R6-based resistance

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Podosphaera macularis, causal agent of powdery mildew on hop, is endemic to the hop growing areas of Oregon, Washington and Idaho. The disease reduces cone quality and can lead to crop rejection. Powdery mildew on hop is managed largely through fungicide application and major gene resistance. In summer 2011, powdery mildew was observed in two hop yards planted to cultivars with the commonly deployed R6-based resistance. The purpose of this study was to characterize the spread and severity of powdery mildew on formerly resistant cultivars and virulent isolates of *P. macularis*. In 2012, 31 yards planted to cultivars possessing R6 were surveyed for powdery mildew. Incidence of powdery mildew varied among geographic regions in Washington, but was not observed in Oregon. Significant differences also were found in the incidence of powdery mildew among cultivars, suggesting that background resistance traits may affect susceptibility. Among 183 *P. macularis* isolates collected from Idaho, Oregon, and Washington, all were found to be of one mating type and no v6 isolates were recovered from non-R6 cultivars. Surveys will be repeated in the summer of 2013 and 2014 to more fully characterize the distribution of powdery mildew on cultivars with R6 and their susceptibility to the disease. Additional research is ongoing to assess fitness penalties associated with R6 virulence.

Importance of potato volunteers as a source of 'Candidatus Liberibacter solanacearum' in the Columbia Basin of Oregon and Washington

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Zebra chip (ZC) disease caused by 'C. Liberibacter solanacearum' (Lso) was first reported in OR and WA in 2011. The importance of Lso infected potato volunteers as pathogen sources in OR and WA is untested. Potato cultivars Umatilla and Ranger fields heavily infected with ZC in 2011 were surveyed 11 May 2012 for symptomatic volunteers. Symptomatic plants were tested by PCR to confirm the presence of Lso. Of the tested symptomatic plants, 48.6% of the Ranger and 53.3% of the Umatilla were positive. The percent plants per acre that were both ZC symptomatic and Lso positive were 4% (Umatilla) and 7% (Ranger). To determine if seed tubers from Lso infected plants could be a source of the bacterium, naturally infected tubers from 6 potato cultivars were collected fall 2011, stored in a potato storage facility and planted, with healthy seed (control), in a screen house in the spring of 2012. Symptomatic plants were tested for the presence of Lso. ZC incidence and severity in seed tubers was determined for each cultivar. Overall emergence was 53% for tubers from infected fields and 99% from healthy seed. Of the emerged test plants, 10.3% showed ZC symptoms, and 58% of these plants were positive for Lso. ZC symptomatic plants emerged faster, were smaller, and died earlier than asymptomatic and healthy controls. Although plants emerged with Lso from infected tubers, they are not likely to be a significant source of Lso in OR and WA due to mitigating factors.

Biocontrol potential of endophytic fluorescent *Pseudomonas* isolated from *Salvadora* species

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The *Salvadora* species have a number of proven medicinal applications and almost all parts of this plant have pharmacological importance. Plants are able to tolerate dry environment and high soil salinity. In the present study 62 isolates of endophytic fluorescent *Pseudomonas* were isolated from roots, shoots and leaves of two species of *Salvadora* i.e. *S. persica* and *S. oleoides*. Most of these isolates were identified as *P. aeruginosa*. *In vitro* test several isolates showed strong antifungal activity against root rotting fungi *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani* and *F. oxysporum*. Cell free culture filtrates of these isolates also showed significant nematocidal activity by killing the 2nd stage juveniles of root knot nematode (*Meloidogyne javanica*), at varying degrees. Application of potential isolates

of *P. aeruginosa* as soil drench significantly prevented the attack of root rotting fungi and root knot nematode on mungbean, sunflower and cotton plants both in screen house and field experiments by reducing the fungal root infection and nematode's penetration in roots. Endophytic fluorescent *Pseudomonas* also caused a positive impact on plant growth by producing taller plants and healthy roots with increased in fresh shoot weight. Endophytic fluorescent *Pseudomonas* offer a non-chemical means of plant diseases control.

Mutations in the *Potato leafroll virus* non-structural protein p17 impair aphid transmission but do not affect virion assembly

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Potato leafroll virus (PLRV) is a phloem-limited positive-strand RNA virus transmitted in a persistent manner (circulative, non propagative) by aphids. A non-structural, p17 protein encoded by the ORF 4, contained within CP gene in a different reading frame, was previously identified as a host-dependent movement protein of PLRV. Twelve point mutations were generated in p17 without changing the overlapping CP amino acid sequence. These p17 mutant viruses were able to replicate and move systemically in *Nicotiana benthamiana* although they accumulated at different rates in systemically infected tissues. Efficiency of transmission of these PLRV-p17 mutant viruses from *N. benthamiana* to *N. benthamiana* by aphids was null or greatly impaired compared to the wild type PLRV. Virions purified from plants infected with these PLRV mutants were found to contain the same composition of structural proteins as the wild type PLRV. The p17 protein was not found in virions of the wild type PLRV and most of the mutants. The involvement of p17 in virus transmission, thus, occurs at other stages of PLRV life cycle, distinct from virion assembly.

Operational warning for Septoria leaf blotch and leaf rust in winter wheat: Importance of fungicide dosage, formulation, and spray time

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Field experiments were conducted in 2010 to investigate the effect of fungicide commercial formulation, timing and dosages on the severity of these two diseases in winter wheat in Luxembourg. Different types of fungicides and fungicide combinations containing active ingredients such as triazoles and strobilurins were used in field trials including susceptible cultivars to Septoria leaf blotch (SLB, caused by *Septoria tritici*) and wheat leaf rust (WLR, caused by *Puccinia tritica*). The three formulations of fungicides tested were: (i) a mix of triazole and amine (Prothioconazole 250 g/l + Spiroxamine 500 g/l) associated with chlorothalonil 500 g/l, (ii) sole strobilurin (Azoxystrobin 250 g/l), and (iii) a mix of strobilurin and triazole (Epoconazole 125 g/l; Azoxystrobin 250 g/l). The optimum time of fungicide spray was assessed through the mechanistic model PROCULTURE and a stochastic model based on night favourable weather conditions conducive to WLR development. The results showed that for plots treated with fungicide formulation containing either a triazole or a strobilurin, the grain yield earned was not significantly different from the untreated plots ($P > 0.05$). Whereas single fungicide treatment involving a mixture of triazole and strobilurin at the optimum time gave an earning (on average 7 dt ha⁻¹) compare to the control and a yield similar to that obtained with the double or triple fungicide treatments.

Profitability of using warning system for foliar disease of wheat in the Grand-Duchy of Luxembourg

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Although small grain cereals (i.e. winter wheat) are routinely protected with two or three foliar treatments in the Grand-Duchy of Luxembourg (GDL), environmental concerns and changes in the cost-benefit ratio are likely to increase the demand for more accurate identification of spraying needs. A

warning system assessing in real time the risk of progression of fungal diseases on winter wheat (*Triticum aestivum* L.) was tested in the GDL over the 2009-2012 period in four-replicated field experiments located in three representative villages of the different agro-climatological zones. The fungicide treatments recommended by the warning system during this period have ensured economic profitability equivalent to or even better than double and triple treatments. In 2010 and 2011, weather conditions impeded fungal infections of wheat and no warning was issued, reducing fungicide use. The study also highlighted that multiple fungicide applications were not better than a single application. In 2009 and 2012, although the weather conditions were very favourable for fungal wheat diseases, the single recommended fungicide application resulted in an additional yield of 30% compared to untreated plots. This study shows the importance of the positioning of fungicide treatment in such a warning system and in strategies aiming at reducing the spread fungicide molecules in the environment.

The population dynamics of *Phytophthora infestans* in Egypt

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For understanding the structure and dynamics of the Egyptian *Phytophthora infestans* population, 205 *P. infestans* isolates were collected from commercial potato fields in Egypt during the 3-year period 2010-12. Mating type for each isolate was determined. The test showed that 57% of the isolates were belonged to mating type A1, 35% were belonged to mating type A2 and the rest 8% were self-fertile. Genotyping of 85 Egyptian isolates as well as 15 references isolates represented the United Kingdom population was performed using 12 highly informative microsatellite (SSR) markers. Structure analysis grouped the 84 identified genotypes into two main clonal lineages. The first clonal lineage was comprised 21 isolates belonged to A2 mating type and 8 self-fertile isolates. This clone was identified as Blue_13 or 13_A2. Within the 13_A2 clone, 3 distinct sub-clonal lineages were also identified i.e. 13_A2_5, 13_A2_43 and 13_A2_84. The second main clonal lineage was comprised 55 isolates. This clone was identified as 23_A1. Worldwide, 19 sub-clonal lineages were identified within 23_A1. Ten out of 19 were found in the Egyptian population. They are known as: 23_A1_4, 23_A1_10, 23_A1_12, 23_A1_13, 23_A1_14, 23_A1_15, 23_A1_16, 23_A1_17, 23_A1_18 and 23_A1_19. In addition, 18 Egyptian isolates were screened for the presence or absence of 5 effector genes which trigger the host susceptibility or immunity. The present gene was sequenced to determine of its function.

Armillaria root disease in peach orchards of the state of Mexico, Mexico: Characterization of *Armillaria* species and assessment of disease impact

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Armillaria root disease is causing extensive mortality of peach (*Prunus persica*) trees within orchards in the State of Mexico, Mexico. Within 15 monitored orchards, average tree mortality was 9.7, 15.3, and 20.3% and disease-impacted area was 23.2, 24.7, and 28.3% during 2009, 2010, and 2011, respectively. Both rootstocks (cultivars of 'Nemaguard' and 'Criollo de La Goleta') used in the region were susceptible to the disease. Pathogenic *Armillaria* spp. were identified by DNA sequencing of two rDNA regions (partial 5.8S-ITS2-LSU D-domains and partial 3' LSU-IGS1) and the translation elongation factor-1 α (*tef-1 α*) gene, accompanied by phylogenetic analyses. Analyses of 49 *Armillaria* isolates revealed that five isolates (10.2%) were *A. mellea*, eight isolates (16.3%) were *A. gallica*, and 36 closely related isolates (73.5%) showed no close similarity to *Armillaria* sequences in GenBank, and apparently represent an undescribed *Armillaria* species. This undescribed Mexican *Armillaria* species is phylogenetically quite distinct from other North American *Armillaria* spp., and efforts are underway to formally describe this undescribed species. Accurate identification of *Armillaria* pathogens in peach orchards is a critical first step toward developing disease management practices, such as resistant rootstock.

Morphological and molecular characterization of *Fusarium* isolates collected from date palm in Saudi Arabia

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Fusarium is one of the most destructive fungal genera and it causes many diseases for plants, animals, and humans. We have collected 71 *Fusarium* isolates from date palm (DP) trees from seven locations in Saudi Arabia. We have single-spored these isolates and kept in 15% glycerol in -80C for long term preservation. These fungal isolates have been morphologically characterized by using appropriate media, e.g. SNA and PDA media. At the molecular level, three genes (translation elongation factor 1 α , b-tubulin, and rDNA-ITS) have been amplified and sequenced to confirm the identity of these fusaria. Morphological and phylogenetic analyses showed that there are five main *Fusarium* species recovered from DP tissues in Saudi Arabia. *Fusarium proliferatum* was the most dominant species (51%), followed by *F. solani* (11%) and *F. phaseoli* (8%). Moreover, five *F. oxysporum* were identified and we are checking if they are belonging to f. sp. *albedinis*. Based on the DNA sequence, same haplotypes were recovered from different DP-growing locations suggesting that DP offshoots or other plant materials could be moved or transported among the Kingdom leading to the dispersal of *Fusarium* pathogens. Efforts should be paid to restrict the movement of diseased DP materials among different locations within the kingdom to avoid the spread of pathogenic fungi. Also, the potential toxin risks of these toxigenic *Fusarium* isolates should be evaluated.

Efficacy of foliar applications of a phosphite fungicide for control of apple scab, caused by *Venturia inaequalis*

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Phosphite fungicides have been shown to be effective in controlling diseases caused by oomycetes, as well some fungal plant pathogens. We have studied the efficacy of foliar applications of the phosphite fungicide AGRI-FOS for control of apple scab, caused by *Venturia inaequalis*. Dilute foliar applications of the product were made with a hand gun to McIntosh and Delicious apple trees at the rate of 1.89 L per 378.5 L of water. Trees were sprayed until foliage was wet. Treatments were applied in a 7-day standard protectant program, in a curative program applied at 24, 48 and 72 hours after the initiation of an infection period, and in an extended protectant program in combination with Captec fungicide at 0.95 L per 378.5 L of water. Treatments were applied from 12.7 mm (0.5 inch) green through first cover. All treatments received cover sprays of Captec at 7 L per ha on a 14-day schedule starting at second cover for the remainder of each season. All treatments and programs provided good to excellent control of apple scab compared to the non-treated control. Incidence of primary scab for the foliar application in 2008, 2009 and 2010 on McIntosh was 3, 2, and 2% for treated trees, and 96, 92 and 79% for the untreated control. Curative treatments provided a significant level of scab control when applied up to 72 hours after infection. The extended protectant program also provided excellent control of primary scab in 2 years of testing.

Genotypic and phenotypic characterization of isolates in the *Fusarium oxysporum* species complex from soybean roots

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The *Fusarium oxysporum* species complex (FOSC) includes economically important pathogens to many hosts. In Iowa, *F. oxysporum* is the most frequent *Fusarium* species isolated from soybean roots, and isolates range from aggressive seedling and root rot pathogens to non-pathogenic isolates. The objective of this research was to characterize the genotype and phenotype of isolates within the FOSC from soybean roots. Sequence analyses of the elongation factor 1- α (TEF) gene and the mitochondrial small subunit (MtSSU), identification of mating type loci, and vegetative compatibility (VC) tests were used to genotypically characterize 102 soybean isolates from Iowa. Pathogenicity was tested using a rolled towel assay in which soybean seed was inoculated with 100 μ l of 1x10⁶ conidia/ml. Phylogenetic analysis of the TEF and MtSSU identified four previously described clades, including *F. commune*. Mating type loci MAT1-1 and MAT1-2 were present in isolates from all clades. Isolates differed in aggressiveness within and among clades; one clade had many isolates that were significantly less aggressive ($P < 0.0001$) when compared to isolates from the other *F. oxysporum* clades and *F. commune*. Current data indicate that aggressiveness may correspond to VC

group. Analysis of pathogenic and non-pathogenic isolates will be done to determine if conditionally dispensable chromosomes may be responsible for pathogenicity to soybean.

PCR-RFLP fingerprinting of the intergenic spacer region to determine the lineage of fungi in the *Fusarium oxysporum* complex isolated from soybean

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The *Fusarium oxysporum* species complex (FOSC) contains morphologically indistinguishable species frequently associated with soybean diseases, including seed and seedling diseases, root rot, and vascular wilt. A previous phylogenetic analysis of the elongation factor 1- α gene and the mitochondrial small subunit separated 134 isolates, collected from soybean in Iowa, into four clades (lineages), including *F. commune*, a species previously in the FOSC. The objective of this study was to use PCR-RFLP analysis of the intergenic spacer (IGS) region as a high-throughput method to haplotype soybean isolates within the FOSC. The IGS regions for two representative isolates from each clade were sequenced and aligned to determine which restriction enzymes showed polymorphisms among clades. PCR-RFLP fingerprinting was then completed for the 134 isolates using restriction enzymes Aval, ClaI, and XhoI. Digestion with Aval or XhoI produced unique banding patterns that resolved two of the four clades, while the last two clades could be resolved using the restriction enzyme ClaI. Future work will be completed to determine if these same enzymes can be used to differentiate lineages of soybean isolates from other geographic areas. PCR-RFLP fingerprinting can be a useful tool for determining field populations, which will be important for developing and implementing proper management strategies for soybean seedling disease and root rot caused by the FOSC.

Comparison of the *Fusarium* species composition between a New England and Chinese salt marsh affected by dieback and climate change

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A dieback of *Phragmites australis* (PA) at Dongtan of Chongmong Island in the Yangtze River estuary near Shanghai, China was found in areas where the invasive *Spartina alterniflora* (SA) had made serious inroads into PA communities. In contrast, New England dieback of SA occurs in the low marsh while PA remains a vigorous colonizer of the high marsh. *Fusarium palustre* and *F. incarnatum* are fungal endophytes/pathogens of SA along the Atlantic and Gulf coasts in and comprised approximately 80 % of the species found. Both species were recovered in greater densities from salt marshes stressed by dieback. Our objectives were to assay PA and SA in dieback and healthy areas on Chongmong Island to determine what species of *Fusarium* are present. Twenty sites were sampled. Three to five plants per sites were sampled, bulked, washed, and surface-disinfested in 0.53% NaClO for 1 min and rinsed. From these plants, 40 pieces of stems and 20 pieces of roots were placed on Peptone PCNB agar and incubated for 5 days. A single spore from each colony was sub-cultured onto carnation leaf agar for 7-12 days and identified under 200 X magnification. Out of 270 colonies from China, 60% were *F. incarnatum*, 24% were *F. palustre*. Given the differences between these sites, it was surprising to find both marshes were dominated by the same two species of endophytic *Fusarium*. This suggests these species may have been introduced with SA.

WITHDRAWN

WITHDRAWN

Epidemiological study on laurel wilt

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Laurel wilt is a new disease that affects several plant species in the family Lauraceae, including avocado trees. The pathogen, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva, is transmitted by the Asian ambrosia beetle *Xyleborus glabratus* Eichhoff that carries the pathogen in its mycangia. Since the first detection of laurel wilt in the redbay forests along the Southeastern coast of the U.S., it has spread north into N Carolina, west into Mississippi and south into Miami-Dade County of Florida, where commercial avocado production is present. It is important to characterize environmental factors that affect the spread of the disease. Growth of *R. lauricola* was evaluated *in-vitro* at different temperatures. Laurel wilt incidence was surveyed in several islands along the coast of S Carolina, Georgia, and Florida in 2008 and 2009. In Archbold Biological Station, Florida, 26 plots with different numbers of susceptible hosts were surveyed regularly between 2010 and 2012. Spatially referenced incidence data and rates of disease spread were related to temperature, rainfall, host density and soil type in ArcGIS. *R. lauricola* growth was optimal around 24°C, with no growth below 4°C and above 35°C. Spatial spread was fast in S Carolina, Georgia and N Florida, but limited in mid and S Florida. Growth rates in culture and outdoor environmental variables will be related to the rate of spread in the forest to predict the potential distribution of laurel wilt.

Characterization of the citrus black spot pathogen and its potential spread in the U.S.

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Citrus black spot (CBS) was recently introduced into southern Florida. The causal agent *Guignardia citricarpa* Kiely is a quarantine organism in Europe

and the rest of the USA. It is therefore important to estimate the potential spread of CBS in the U.S. The ITS regions of ten *G. citricarpa* isolates from south Florida were compared with *Guignardia* species worldwide and temperature-growth response of several isolates were determined. Effects of temperature and relative humidity on postharvest lesion formation were also studied. The climatic requirements of *G. citricarpa* were used to estimate parameters in the CLIMEX model. Comparison of the ITS region of the isolates to those in international databases confirmed the identity and uniformity of *G. citricarpa*. Colony growth and conidia production *in vitro* were optimal at 27°C, while there was no growth below 4°C and above 37°C. On fruits, lesion growth and conidia production were observed at 4°C, though at lower rate. Variations in humidity had little effect. Input parameters in CLIMEX reflecting conditions for CBS infection in spring/summer in Florida, predicted potential establishment in Florida but not in California. Altering the parameter values to account for survival of the pathogen in leaf litter in winter predicted potential establishment in California as well. Thus, *G. citricarpa* could possibly establish beyond Florida if it is transported outside of the current quarantine zone.

Ectomycorrhizal community structure along a complex hydrologic gradient S. ERLANDSON (1), J. Savage (2), J. Cavender-Bares (3), K. G. Peay (1)

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Complex environmental gradients consistently demonstrate that water availability is a key driver of plant species distribution and productivity and is important to microbes, such as bacteria and fungi. However, reported outcomes of biotic and abiotic drivers of microbial community structure along complex gradients are varied. This study examined community structure of ectomycorrhizal fungi (EMF) in response to a complex water availability gradient and host species identity. To achieve this aim, we sampled roots from seven common *Salix* species that grow along an established water availability gradient in Minnesota and identified EMF species using the ITS DNA region. Percent organic matter was the best predictor of differences among EMF communities (pseudo-F=3.215, p=0.003). EMF species composition shifted along the gradient. *Peziza* taxa occurred only in soils with higher percent organic matter; *Tomentella* and *Russula* taxa were ubiquitous across the gradient; *Hebeloma* and *Sebacina* taxa were restricted to higher water availabilities. Host species identity weakly accounts for differences among EMF communities across the gradient (pseudo-F= 1.1474, p=0.029). In conclusion, soil organic matter most strongly influences EMF community structure, even across a strong water availability gradient and abiotic factors may outweigh any EMF specificity toward *Salix* host species in this system.

WITHDRAWN

Effect of native-Mexican strains of *Bacillus subtilis* on melon (*Cucumis melo* L.) fruit quality and diseases

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Melon is susceptible to pests and diseases. *Bacillus subtilis* protects against diseases, promote plant growth and improve fruit. The objective of this work was to determine the effect of four native-Mexican and one commercial (Kodiak®) strains of *B. subtilis* on melon fruit quality and reduce disease impact. The experiment was carried out during the Spring-Summer 2012, and a CRD was used. Data was subject to ANOVA and means were separated by Tukey's Test. Eighteen melon cv. Top Mark plants were used for each treatment. The strains were applied as Mena-Violante and Olalde-Portugal (2007) reported. Plants were cultivated inside of a greenhouse in plastic bags (11.3 L) filled with course-grade zeolite. Training, pruning, and fertigation of plants were performed according to those recommended (Nunez-Palenius et al., 2006). Hermaphrodite flowers were self-pollinated by hand. Only three fruit were kept on each plant. Six plant agronomic characteristics and nine postharvest fruit features were evaluated. No pests were detected during the entire experiment. Powdery mildew was the only disease present on all the plants from all treatments. Rally® 40WSP treatment was not able to control this disease, but Amistar 250 did. Surprisingly, no significant differences on either agronomic or postharvest variables were obtained. The *B. subtilis* tested strains neither avoided the presence of powdery mildew nor improved the melon fruit quality. This Project was supported by DAIP-UG-2011.

Where is *Phytophthora ramorum* now? An update on clonal populations in the U.S.

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Approximately 20 years after the first report of disease caused by *Phytophthora ramorum* in the United States, genetic variation in the form of novel multilocus microsatellite genotypes, continues to appear. From 2001 to 2012, a total of 732 samples were collected from nurseries in 20 states in the U.S. and Canada, and genotyped at 9 microsatellite loci to determine clonal lineage and infer relationships. Microsatellite variation was low for members of the EU1 and NA2 lineages, where 100% of the 40 NA2 and 79.8% of 119 EU1 isolates had the same genotype. The dominant EU1 genotype first reported in 2003 now represents 79.8% of isolates and has been recovered from the western U.S. (CA, OR, WA), western Canada, and in 2009 was reported for the first time in the southeastern U.S. (NC). Overall, the NA1 lineage is still the most abundant, with 573 isolates reported predominantly from eight states: 52.2% from the western U.S., and 40.5% from the southeastern U.S. (GA, SC, NC, AL, and FL). Among the 109 genotypes recovered, 19.3% were found in both western and the southeastern U.S., suggesting establishment of these genotypes. Since the last reports, seven new EU1 genotypes and 36 new NA1 genotypes have emerged. Whole-genome sequences are currently being explored to find loci better able to detect inter-lineage variation.

Local dispersal of *Puccinia striiformis* f.sp. *tritici* from single source lesions

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We examined the spread of *Puccinia striiformis* f.sp. *tritici*, causal organism of wheat stripe rust, from single uredinial lesions over the course of a single generation. In 2011, single lesions were identified and their isolation was confirmed visually by examining all plants within a 3m radius. Plants were reexamined after one generation of disease spread, noting the number, tiller location, and leaf position, of all lesions. In 2012, an individual leaf per plot was artificially inoculated, and the plot was sampled after one generation of disease spread, noting every tiller, its location, and the number of lesions present on each of the top three leaves. Fit to an inverse power law was superior to that of a negative exponential. Results indicate that the slope of the number of lesions at a given distance away from the source lesion was considerably shallower than predicted in previous field-wide dispersal studies, but fell within a small range in both years of the study. A significant directional effect corresponding to prevailing winds was observed in 2012, but not in 2011. Timing and leaf-position of the source infection affected the dispersal gradients quantitatively, but not qualitatively. Mean number of progeny produced per parent lesion within 3m radius was estimated to be 755 in 2011, and 270 in 2012.

First detection and pathogenicity of *Rhizoctinia solani* AG-1 1A on peanut in Arkansas

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In 2012, symptoms typical of Aerial blight were observed on peanut in a field in Arkansas. Leaves in the lower canopy had water soaked, grayish green lesions or tan to brown lesions. Localized mats of leaves and mycelium collected on stems and threads of hyphae spread along stems to adjacent leaves. Dark brown sclerotia (1.5 - 4 mm) were attached to the surface of infected peanut tissue. Isolations from surface-disinfected leaves consistently yielded light brown to brown colonies with sclerotia typical of *Rhizoctonia solani* AG-1 1A. The fungus was identified to be *R. solani* AG-1 1A based on hyphal anastomosis group reactions. Koch's postulate was successfully completed in a greenhouse experiment on five peanut cultivars. Only leaves, petioles, and were infected whereas stems were visually non-affected. In a detached leaflet assay, larger ($P = 0.05$) brown lesions developed on cv. Flavor Runner 458 than GA09B, FL07, FloRun107, and Red River Runner. Although *R. solani* AG-1 1A is an important pathogen on rice and soybean in the state, to our knowledge this is the first report of Aerial blight of peanut in Arkansas. Currently, there is a renewed interest in peanut production in the state and production practices include irrigation and various rotation schemes with soybean and less frequently rice, which can potentially increase inoculum for the subsequent crop. Thus, this may be an important disease of peanut in Arkansas.

Controlling soilborne pathogens using *Trichoderma*: The Integrated Pest Management Innovation Lab's work in Bangladesh, India, and Indonesia

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Microbial biopesticides are used for the biological control of insect pests, plant pathogens, and weeds. One such bioagent, *Trichoderma*, a naturally occurring fungus in agricultural and forest soils, is used by biological control programs in seed treatments, in foliar sprays, in compost, and as tricholeachates. This inexpensive, environmentally-friendly bioagent and its common species *T. harzianum* and *T. viridae* have been promoted by the Integrated Pest Management Innovation Lab (IPM IL) as part of its IPM packages to control diseases in cucurbits, cabbage, eggplant, onion, and tomato in Bangladesh, India, and Indonesia. *Trichoderma* offers an alternative to expensive and often toxic pesticides, and farmers have seen significant increases in yield and income when using it. Because of this success, the IPM IL is now scaling up *Trichoderma* adoption through collaboration with NGOs, the private sector, and host country institutions in program countries.

IPM Innovation Lab successful delivery of IPM technologies in the developing world: Capacity building through long- and short-term training

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Research and implementation of Integrated Pest Management (IPM) strategies in developing countries is limited by the lack of effective technology dissemination programs, strategies, and infrastructure. The IPM Innovation Lab (IPM IL, formerly the IPM Collaborative Research Support Program or IPM CRSP) has been very successful in delivering tailored, farmer-friendly, cost-effective, environmentally and ecologically sound, and gender-sensitive IPM technologies through long- and short-term capacity building programs. These programs are integral parts of the IPM IL mission and goals. The IPM IL trains local practitioners, project managers, and farmers on how to use and adopt IPM technologies through long-term training such as internships and undergraduate and graduate academic and professional degrees. Short-term training focuses on designing and implementing IPM packages, holistic suites of IPM recommendations for specific crops, in the pre-planning phase, during field implementation, and throughout the dissemination phase of the programs. The IPM IL recognizes the importance of bridging gaps between research scientists, technicians, field agents, extension agents, farmers, and other stakeholders in order to successfully deliver and disseminate IPM packages and promote their adoption.

Genome-wide signature of adaptation in a recently introduced pathogen

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Introduction success of a new plant pathogen involves adaptive processes to its novel habitat. Such local adaptation processes are influenced by the interplay of demographic effects and selective forces. The Eurasian poplar leaf rust *M. larici-populina* is responsible for severe damage in European poplar cultivation. Outbreak of this fungus in North-America was first reported in early 1990. Since then the pathogen appears to have become well established, infecting new host species such as balsam poplars. To obtain a comprehensive picture of the evolutionary changes involved in the adaption of this rust to its new environment, we sequenced 44 strains from the source and the introduced populations and mapped the reads onto the *M. larici-populina* reference genome. Our objectives were to reconstruct the history of the introduction and then the evolutionary history of alleles under selection. Genome-wide distribution of polymorphisms displayed the signature of a severe founder event into the North-American population. We then developed a model-based approach with a subsample of 22K SNPs to infer the null-demographic parameters. Finally, in a genomic scan of the four largest scaffolds, we found 32 regions having a strong divergence between the two populations. These regions include 14 secreted protein-coding genes that are candidate for adaptation; this hypothesis will be tested by conducting coalescent simulations based on the demographic model previously inferred.

Examination of molecular diversity of the spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae* with SSRs

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Downy mildew is the most economically important disease of spinach. This disease is caused by the obligate oomycete pathogen *Peronospora farinosa* f. sp. *spinaciae* (Pfs). More than ten new races and multiple novel strains of Pfs have been identified during the last two decades. Examination of the molecular diversity among these races or isolates of Pfs may provide insights as to the evolution and molecular identification of the pathogen. Based on the genome sequence of Pfs isolate UA0510C sequenced with Illumina technology, 48 pairs of SSR primers were designed from sequences confirmed as oomycete sequences in Genbank. These primers were tested on 6 Pfs races (Race 6, 10, 11, 12, 13 and 14), 3 novel strains (UA4711, UA1012B and UA1312) and three other oomycete pathogens, including the spinach white rust pathogen *Albugo occidentalis* (Ao), the Swiss chard downy mildew pathogen *Peronospora farinosa* f. sp. *betulae* (Pfb), and the quinoa downy mildew pathogen *Peronospora variabilis* (Pv). All 48 primers could amplify fragments from Pfs. Four primers could amplify the DNA from the other three pathogens, and a few primers could amplify DNA from Pfb and/or Ao. The majority of the primers were Pfs specific. Thirty-two of the 48 pairs of primers were polymorphic among the Pfs isolates tested. Heterozygous alleles were observed in some isolates when amplified with a few primers. A robust molecular marker system is being developed for Pfs.

A novel recombinant of *Bean common mosaic virus*

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Bean common mosaic virus (BCMV) exists as a complex of strains distinguished based on reactions towards seven resistance genes known in common beans; at least seven BCMV pathotypes have been identified. In order to understand the genetic determinants of pathogenicity for BCMV, the whole genome was cloned and sequenced for the BCMV strain US10 which belongs to pathotype VII. This sequence was compared to a recently characterized isolate of BCMV, RU1-OR, which also displays the same pathotype VII. Inspection of the nucleotide sequences for BCMV RU1-OR and US10, and two closely related sequences BCMV RU1-D and RU1-W (both pathotype VI) revealed that BCMV RU1 isolates originated through a series of recombination events between US10 and a yet unknown parental genome, resulting in changes in virus pathology. The data obtained suggest that a fragment of the US10 genome in the P1 region may be involved in its ability to overcome the BCMV resistance in beans conferred by the *bc-2²* gene.

QTL mapping of resistance to stripe rust in spring wheat PI 182103

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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is an important wheat disease worldwide. Seeking new genes is essential for developing wheat cultivars resistant the disease. PI 182103, a spring wheat originally from Pakistan, showed high stripe rust resistance for many years in the field

tests. To map the resistance gene(s) in PI 182103, 186 F₆ RILs were developed from a cross with Avocet S. The seedlings of the parents and F₁ and RIL progeny tested with PST-100 and PST-114 indicated that PI 182103 had three recessive genes for resistance. In 2011 and 2012, the parents and RILs were evaluated in randomized field experiments at Pullman and Mt. Vernon, Washington with three replications at each location. Infection type and disease severity (DS) data were recorded three times for each line. Both IT and DS-based area under the disease progress curve (AUDPC) data indicated that the PI 182103 resistance to stripe rust is controlled by quantitative trait loci (QTL). About 860 pairs of simple sequence repeat (SSR) primers were used to map the resistance QTL. Three QTL that were detected in the seedling stage were mapped on chromosome 2AS, 3AL and 4DL and two major QTL only detected in the field experiments were mapped on chromosomes 5BS and 7BL, which explained 23.2-30.1 and 23.1-58.2 of the phenotypic variation, respectively. Our results suggested that these genes in combination provide high and potentially durable resistance to stripe rust.

The coat protein of Tobacco necrosis virus acts elicits HR in *Nicotiana* species belonging to section *Alatae*

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We tested the capacity of Tobacco necrosis virus (TNV) to infect 21 *Nicotiana* species and found that virion inoculations triggered a hypersensitive response (HR) in all species except *N. benthamiana*. In the case of *N. benthamiana*, TNV moved systemically. To identify putative viral avirulence proteins responsible for eliciting HR, we agroinfiltrated TNV genes individually into *Nicotiana* species. To date, we have found that the TNV coat protein triggers HR in six members of section *Alatae*. These species include *N. langsdorffii*, *N. longiflora*, *N. bonariensis*, *N. alata*, *N. forgetiana* and *N. mutabilis*. The only member of section *Alatae* that failed to respond with HR to agroinfiltration of the TNV CP was *N. plumbaginifolia*. Mutational analysis of the TNV CP confirmed that the protein rather than the RNA sequence was responsible for HR elicitation. A previous study had shown that the TBSV CP is also an avr determinant for the same *Nicotiana* species in section *Alatae*. TNV and TBSV are related, as both are type species of their respective genera in the family Tombusviridae. An alignment of the CPs of TNV and TBSV reveals some similarities at the primary sequence level. Further research will be directed towards investigating whether sequences shared by the CPs of TNV and TBSV are responsible for recognition by a single host resistance protein or if host resistance proteins are able to distinguish between the CPs of TNV and TBSV.

Mechanisms of adaptation to host rice cells by the blast fungus

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To infect rice, the devastating blast fungus *Magnaporthe oryzae* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. How the fungus monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf, what controls the genetic reprogramming necessary to produce infectious hyphae, and how it acquires nutrient during biotrophic in planta growth is poorly understood. *M. oryzae*'s trehalose-6-phosphate synthase 1 (Tps1) enzyme integrates carbon and nitrogen metabolism in the fungal cell to regulate virulence via a novel NADPH-dependent genetic switch. Loss of Tps1 function results in Δ tps1 strains that can form functional appressoria and penetrate the rice surface but fail to grow beyond the first infected cell. Impaired invasive growth of Δ tps1 strains is due to loss of glucose sensing, inactivation of the NADPH-dependent genetic switch, and altered carbon assimilation. Moreover, NADPH-requiring antioxidant systems are shut down in Δ tps1 strains, rendering them hypersensitive to oxidative stress. Taken together, we are exploring the dynamics of this critical NADPH-dependent genetic switch to understand how *M. oryzae* controls infectious hyphal development during biotrophy.

Monitoring for resistance in *Botrytis cinerea* from strawberry to seven chemical classes of fungicides in the eastern United States

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Chemical control of gray mold of strawberry caused by *Botrytis cinerea* Pers. is essential to prevent pre- and postharvest fruit decay; however, resistance to multiple chemical classes of fungicides including anilinopyrimidines (APs), dicarboxamides (DCs), hydroxylanilides (HAs), methyl benzimidazole carba-

mates (MBCs), phenylpyrroles (PPs), quinone outside inhibitors (QoIs), or succinate dehydrogenase inhibitors (SDHIs) was found recently in *B. cinerea* from strawberry fields in the Carolinas. Resistance to DCs, HAs, MBCs, QoIs, and SDHIs, was caused by point mutations in the corresponding target genes (*Daf1*, *Erg27*, *b-tubulin*, *Cytb*, and *SdhB*); however, resistance mechanisms for APs and PPs are still unknown. A resistance-monitoring program was implemented to help growers determine location-specific resistance profiles. The analysis of samples from Arkansas, Florida, Georgia, Kansas, Maryland, North Carolina, South Carolina, and Virginia strawberry fields revealed *B. cinerea* isolates resistant to multiple chemical classes, including the PP fungicide fludioxonil, and to several SDHI fungicides not yet available for gray mold control. Our results indicate that resistance to some chemical classes including APs, HAs, MBCs, QoIs, and SDHIs is widespread and resistance DCs and PPs is emerging.

Genetic variability suggests that *Ceratocystis fimbriata* is native to Rio Grande do Sul, Brazil, where it is causing a new wilt disease on kiwifruit

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Ceratocystis fimbriata is a native, soilborne pathogen in South America with a broad host range. *Ceratocystis* wilt on kiwifruit (*Actinidia deliciosa*) was first recognized in 2010 in the state of Rio Grande do Sul, Brazil. Affected plants show wilting and death of leaves, which hang on the branches, and reddish-brown staining in a radial pattern in the xylem. The disease reduces the number of harvestable fruit, and most affected plants die. We examined genetic variation in the pathogen population to see if there was only a single genotype, suggesting that the fungus had been introduced from elsewhere in infected nursery stock, or if there was substantial genetic variation, suggesting that the fungus was soilborne and indigenous to the region. We used 14 microsatellite markers to identify 16 genotypes of *C. fimbriata* among 76 isolates from individual trees in eight kiwi plantations. There was also significant genetic variation in ITS-rDNA, *MAT1-1-2*, and *MAT1-2-1* DNA sequences, which placed the fungus among other South American populations of *C. fimbriata*. One of the genotypes was found in seven plantations, including an area where grafted plants had been produced and sold to other growers. However, other isolates showed substantial genetic variability, indicating that the pathogen is native to Rio Grande do Sul. This is the southern-most report of *C. fimbriata* in Brazil, where the disease may prove to be a major limiting factor in kiwi production.

Identification and detection of wheat pathogens through volatile organic compound analysis

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Research shows that fungi produce different VOCs in different concentrations depending on their species, developmental stage, and abiotic and biotic factors. The objective of this work was to detect and distinguish fungal wheat pathogens by VOC profile analysis. Single spore isolates from *Fusarium graminearum*, *F. culmorum*, *F. poa*, *F. avenaceum*, *Phaeosphaeria nodorum*, *Mycosphaerella graminicola*, and *Pyrenophora tritici-repentis* were grown 7 days in 5cm-PDA petri dishes. In parallel experiments, wheat plants were inoculated with *B. graminis*, *F. graminearum*, and *P. nodorum* and sampled 7, 14 and 21 days post inoculation dpi. We collected VOCs using a Super Q filter fitted on airtight glass containers, eluted with hexane, and injected the extract into the GC/MS. The GC data sets were analyzed, aligned and compared using MetAlignTM. VOC collection of each isolate and each wheat-pathogen combination was repeated 3-5 times. In contrast to the controls, *P. nodorum* isolates produced Mellein, *M. graminicola* produced 6-methyl-5-hepten-2-one, Isosativene, 2-one, and Mellein, while *F. poa* produced 1,2,3-trimethylbenzen and Sesquiterpen, possibly Germacrene D. Powdery mildew, leaf blotch and Fusarium head blight produced clearly distinguishable VOC profiles. We will discuss the application of these findings in early detection of plant diseases and in site specific disease control.

Stability of azoxystrobin resistance and fitness of fungicide-sensitive and -resistant field isolates of *Didymella bryoniae*

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Stability of azoxystrobin (AZO) resistance and fitness of fungicide-sensitive (S) and -resistant (R) isolates of *Didymella bryoniae* were investigated using

isolates with different sensitivity to AZO, boscalid (BOS), penthiopyrad (PEN), and thiophanate-methyl (TPM). To determine stability of AZO resistance, isolates were allowed to undergo four consecutive reproductive cycles on ¼-strength potato dextrose agar. Conidial germination on AZO-amended (10 µg/ml) and non-amended medium was measured after each cycle. AZO-S isolates remained sensitive and AZO-R isolates remained highly resistant to AZO after up to four cycles. Fitness components measured included mycelial growth at 14, 20, 24 and 28°C, spore germination, and virulence. No significant differences in mycelial growth were observed between AZO-S and AZO-R, BOS-S and BOS-R, or PEN-S and PEN-R isolates at any temperature. TPM-R isolates grew significantly less than TPM-S isolates at some temperatures. No significant differences in germination were observed between isolates. Virulence was determined by measuring lesion length on inoculated intact petioles of watermelon seedlings. There were no significant differences in lesion length among isolates. These results suggest that AZO resistance is stable in the absence of fungicide and fitness of AZO-R and AZO-S, BOS-R and BOS-S, and PEN-R and PEN-S isolates is similar while TPM-R isolates are less fit than TPM-S isolates in terms of growth at some temperatures.

Publicly available website for the identification of psyllid-‘*Ca. Liberibacter*’ interactors using comparative transcriptome analysis

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‘*Ca. Liberibacter solanacearum*’ is the putative bacterial causal agent of Zebra chip and vein-greening diseases. A close relative, ‘*Ca. L. asiaticus*’, is the pathogen associated with citrus greening disease. The fastidious agents are propagative and circulative in their psyllid vectors, potato psyllid (PoP) *Bactericera cockerelli* Sulc, and Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama. Neither disease complex is fully understood or efficiently controlled. To aid in the identification of molecules important in vector-mediated transmission processes, we have created transcriptome databases using Illumina sequencing. *Liberibacter*-infected and uninfected PoP and ACP adult, nymph, salivary gland, and gut transcriptomes were sequenced, yielding 45,976, 82,224, 110,937 and 83,231 transcripts, respectively. Transcripts were annotated with the Transcriptome Computational Workbench, which provides a publicly available web-applet for query and display. PoP and ACP tissue- and developmental-specific databases can be queried using keyword searches, sequence similarity (BLAST), Gene Ontology (GO) terms, and clustering analysis (OrthoMCL). Results from queries coupled with *in silico* expression analysis within and between psyllid species and life stages revealed a number of genes with predicted functions associated with host-parasite interactions that could mediate ‘*Ca. Liberibacter*’ infection, propagation, and circulation in the psyllid, as well as transmission processes.

Hyphal tip ultrastructure and cytoplasmic organization in the Zygomycota

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Despite exhibiting rapid, polarized growth, a Spitzkörper (Spk) has not been documented in a member of the zygomycetous fungi, with the exception of *Basidiobolus* sp. Past ultrastructural studies, using chemical fixation, have focused on vesicle organization in the hyphal apex of zygomycetes such as *Gibbertella persicaria* and *Mucor rouxii*. Although an accumulation of vesicles at the hyphal tip was observed, the complex arrangement of vesicles as documented in the Spk of ascomycetous and basidiomycetous fungi was lacking. We have examined the ultrastructural organization of the hyphal tip and subapical cytoplasm of several members of the zygomycetes, including *M. indicus*, *G. persicaria*, *Rhizopus oryzae*, and *Coemansia reversa* using cryofixation and freeze-substitution preparation methods, which greatly improved preservation of cellular detail. Using phase-contrast light microscopy of growing hyphae, a phase-dark, highly dynamic vesicle crescent in the hyphal apex was observed. These studies have provided further insight into zygomycetous fungal cell growth and cytoplasmic organization, particularly at the hyphal apex.

Lytic cycle genes of ‘*Ca. Liberibacter asiaticus*’ prophage were strongly induced in periwinkle but not citrus, even following heat treatment

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Huanglongbing (HLB) is caused by ‘*Ca. Liberibacter asiaticus*’ (Las), ‘*Ca. L. americanus*’ (Lam) and ‘*Ca. L. africanus*’ (Laf), a group of alpha proteobacteria that have not been cultured. Both Las and Lam each carry two very similar prophages, evidently as stable lysogens in citrus. While the lytic cycle has been documented and phage particles were readily apparent in Las infected periwinkle, phage particles have never been seen in Las-, Lam- or Laf- infected citrus, despite numerous electron micrographs. Our goal is to develop a treatment that will trigger the lytic cycle of these phages in citrus. In an effort to functionally confirm lytic cycle genes and promoter regions, we used RT-qPCR to monitor the expression of four putative phage lytic cycle genes in Las- infected periwinkle and citrus. In citrus, expression was monitored before and after heat treatment at 42°C for two days, a level demonstrated to cure plants of Las infection. The relative expression of three of the four putative lytic cycle genes examined, SC1_gp025, SC1_gp110 and SC2_gp095, were much more highly expressed in periwinkle than citrus. However, heat treatment of citrus leaves failed to increase expression of these genes, indicating that lytic cycle induction of these prophages is not tied into a presumed heat stress response of Las in citrus. The possibility of activation by biochemical inducer(s) specific to periwinkles is being examined.

Alteration of the ergot alkaloid profile through chromosome end knockoff

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The fungal endophyte *Neotyphodium coenophialum* is an obligate, mutualistic symbiont with its host, the popular forage grass *Lolium arundinaceum*, (tall fescue). The host plant’s resistance to biotic and abiotic stresses is considerably increased by the presence of the endophyte, which is rich in biologically active compounds. Among these compounds, complex ergot alkaloids such as ergovaline have deleterious effects on livestock performance and cause major losses annually. *Neotyphodium coenophialum*, is a hybrid of three parental species, two of which contributed homologous ergot alkaloid gene clusters. The genome sequence of *N. coenophialum* revealed that both clusters are telomeric, and that *EAS2* has an inactivating mutation in the late-pathway gene, *lpsB*. We devised a novel approach that eliminated *EAS1* and created a marker free line. Moreover, the genome sequence of one such mutant confirmed the elimination of the *EAS1* cluster and absence of the marker gene. This mutant, which produces precursor clavine alkaloids but no ergovaline in planta, will be compared to the wild-type strain and to strains lacking any ergot alkaloids, with respect to their effects on host plants and mammals.

Phylogenetic placement of fungi causing spring dead spot of bermudagrass

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Spring Dead Spot (SDS), the most damaging disease of bermudagrass, is caused by three species of ectotrophic root-infecting fungi, *Ophiophora herpotricha*, *O. korrae* and *O. narmari*. These species are placed in *Ophiophora* based on morphologies of rare sexual states and monophyly of ITS sequences. Recent phylogenetic studies demonstrate *Ophiophora* to be polyphyletic within *Pleosporales*, implying morphological characters insufficiently define this genus. To better understand the phylogeny of SDS-causing *Ophiophora* species, rDNA, EF1a and RPB2 gene sequences were obtained from isolates, type cultures and databases, and used to perform maximum likelihood and Bayesian analysis. Ordinal level phylogenetic trees strongly supported inclusion of a monophyletic lineage of SDS species in *Phaeosphaeriaceae* within *Pleosporineae*. In generic level trees, the three SDS species each cluster in well-supported groups that include the Australian type cultures for *O. korrae* and *O. narmari*. However, two European type cultures for *O. herpotricha*, did not group with any of the SDS species. All phylogenetic trees distinguish the three SDS species within a monophyletic genus. SDS-causing *O. herpotricha* was described prior to the other two SDS-causing species, thus has precedence. But since *O. herpotricha* type cultures are unrelated to SDS-causing fungi, the genus *Ophiophora* may be invalid for these fungi. The renaming of the SDS-causing genus seems warranted.

The identification of genes undergoing adaptive evolution in multiple subspecies of *Xylella fastidiosa*

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Adaptive evolution is an unambiguous indicator of natural selection that is found when a particular variant of a trait increases the fitness of an organism.

At the molecular level, adaptive evolution is measure by calculating the rate of nonsynonymous substitutions to synonymous substitutions. In sexually reproducing vertebrates, genes involved in the immune and reproductive systems have been found in many genomes undergoing adaptive evolution. Bacterial genes with high rates of evolution are less clear. More than 1400 genes shared by the 4 subspecies of *Xylella fastidiosa* were aligned and the rate of evolution was calculated using CodeML. While 146 were found to have evidence of selection, only 39 of these were indicative of adaptive evolution. A significant portion of these genes were classified as pathogenicity genes, outer membrane proteins and transmembrane transporters. To increase the detection power of the method, more genomes were added including the non-pathogenic strain of Pierce's disease and the recombinant strain of almond leaf scorch. Ten percent of the original 1400 genes were reanalyzed with CodeML and DataMonkey. Within this subset no new patterns of adaptive evolution emerged. This result indicates that the differences in pathogenicity may be due to differences in genes not classically included with pathogenicity genes.

Digitization of 120,000 fungal collections at the University of Michigan Herbarium as part of the Macrofungi Collection Consortium

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The Macrofungi Collection Consortium is a project to digitize herbarium collections and build a public database of approximately 1.4 million dried scientific specimens from 35 institutions in 24 states, encompassing essentially all macrofungal specimens deposited in the United States in the past 150 years. The digitization efforts include assigning unique barcode identifiers to each collection, imaging collection notes and annotation labels tied to specimens, and recording data such as collector, determination, locality, date, and collection number into a publicly accessible database. All of the collections are being georeferenced as part of the project. Ancillary data including photographs, fieldbooks, notecards, and other information tied to collections are also being digitized. These data will provide new insight into fungal species distribution and phenology. The ecological questions that can be addressed with 1.4 million databased and georeferenced collections spanning 150 years are truly spectacular and will launch fungal ecology into a new era. The University of Michigan Herbarium has one of the largest holdings of macrofungi in the United States (180,000+ collections) including the extensive collections of Alexander H. Smith (92,000+ collections) as well as collections from many other highly regarded mycologists. Our digitization efforts at Michigan began in January 2013 and we are currently generating over 4,000 database records with label images per month.

Effect of inoculum concentration on development of anthracnose fruit rot of strawberry cultivars in detached fruit and field experiments

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Florida is the largest producer of winter strawberries in the world. Anthracnose Fruit Rot (AFR), caused by *Colletotrichum acutatum*, can greatly affect production if not controlled. Cultural practices such as the use of certified pathogen-free plants and less susceptible cultivars are important tools for control AFR. The susceptibility of two strawberry cultivars ('Strawberry Festival' and 'Camarosa') was compared in a detached fruit experiment using six inoculum concentrations (0, 10², 10³, 10⁴, 10⁵ and 10⁶ conidia/ml). The experiment was arranged in a completely randomized design with six treatments (inoculum concentrations) per cultivar and four replications. Fruit were inoculated with a 5-µL droplet and kept in plastic boxes to maintain humidity. AFR incidence was assessed on fruit for 9 days and the experiment was repeated twice. There was a high correlation for the disease incidence from the detached fruit assay and field experiments conducted in the previous season. Disease incidence was lower on 'Strawberry Festival' than on 'Camarosa' independent of the inoculum concentration. Moreover, the minimum inoculum concentration for symptom development was 10⁴ for 'Camarosa' and 10³ for 'Strawberry Festival'. The detached fruit assay allows better control of the environmental conditions and is more practical to perform than field experiments.

The potential roles of WRKY transcription factors in regulating maize defense responses against *Aspergillus flavus* infection

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The infection of maize (*Zea mays* L.) by the fungal pathogen *Aspergillus flavus* results in the contamination of kernel tissues with carcinogenic mycotoxins known as aflatoxins. Resistance to *A. flavus* is mediated by numerous defense proteins, but the mechanism regulating the expression of these defenses is poorly understood. This study examined the potential roles of six WRKY transcription factors, *ZmWRKY21*, *ZmWRKY53*, *ZmWRKY53.1*, *ZmWRKY67*, *ZmWRKY68*, and a putative *ZmWRKY*, in regulating defense responses to *A. flavus* infection. The expression of these WRKY transcription factors was monitored over time in two maize lines, B73 (susceptible) and TZAR101 (resistant) using real-time PCR. In addition, the Nonexpressor of Pathogenesis-Related Protein -1 (*ZmNPR1*), Pathogenesis-Related Protein 1 (*ZmPR-1*), and Ethylene Responsive Factor 1 (*ZmERF1*), were examined to determine if salicylic acid, jasmonic acid, or ethylene-mediated pathways were induced by *A. flavus* infection. Taken together, the WRKY transcription factors and pathway genes regulated in response to *A. flavus* infection seem to function in promoting salicylic and ethylene-based defense pathways, anti-fungal defense protein expression, and antioxidant enzyme expression to sequester reactive oxygen species during pathogen infection or abiotic stress in the resistant line. These WRKY transcription factors may contribute to the association between abiotic stress tolerance and *A. flavus* resistance in maize.

Blueberry silverleaf: Morpho genetic diversity of *Chondrostereum purpureum* isolates that are affecting blueberries

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The increasing production and expansion of the blueberry in Chile has resulted in a major industry, today the country is the third producer and the second exporter in the world. The cultivated area stretched along 1,400 Km from north to south, and new diseases have been reported along this area, including ones only described in Chile. This is the case of Silver leaf, caused by the basidiomycete *Chondrostereum purpureum* (Pers.:Fr.) Pouzar, which has been detected only in this country affecting *Vaccinium corymbosum* plants. Furthermore, this is the only detection of *C. purpureum* in the *Vaccinium* genus or the Ericaceae family worldwide. The goal of this research was to characterize the *Chondrostereum purpureum* strain that causes silver leaf in the Chilean blueberry plantations. Samples from symptomatic plants were collected along the planted area, cultured in agar media and characterized by colony morphology, color and growth rate at different temperatures. Also, morphology of the mycelium, clamp connections and terminal hyphae were studied. The DNA analysis includes amplification, sequencing and restriction of the ITS PCR products (PCR-RFLPs) and RAMS markers. Morphological and molecular differences were detected in the *C. purpureum* population that is affecting blueberries, indicating a significant variability inside the fungus. This may explain the appearance of new susceptible variety per season and the variations in symptomatology as the disease expands.

Additional components involved in thaxtomin induction and regulation by *Streptomyces scabies*

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Streptomyces scabies is the major scab-causing pathogen on several important root and tuber crops and is responsible for considerable annual losses worldwide. A family of nitrated dipeptidic phytotoxins called thaxtomins have been identified as the major virulence factors. Thaxtomin A, the predominant form, acts as a cellulose synthesis inhibitor in expanding plant tissue, therefore enabling the bacterium to infect living plants, a rare trait among the commonly saprophytic streptomycetes. Toxin production is induced by cellobiose, the smallest subunit of cellulose, and also a well-known inducer of cellulases and other cell wall-degrading enzymes. In our search to elucidate the mechanisms used by the pathogen to detect a nearby growing plant and subsequently respond by inducing thaxtomin production, we identified three ABC-transporters involved in cellobiose uptake, of which one appeared to be specific for scab-causing streptomycetes. Several cell wall-degrading enzymes seemed to be upregulated by cellobiose, among which a cluster containing a gene encoding a putative expansin-like protein, normally found in plant cell walls where it loosens linkages between cellulose microfibrils during cell expansion, and a reported virulence factor of *Clavibacter michiganensis*. Furthermore, a cluster containing a metallophosphatase appears to be part of the regulatory network leading to thaxtomin production and therefore virulence.

Reclassification of bacteria causing corky root of lettuce

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The genus *Rhizamonas* (previously known as *Rhizomonas*) was created for strains of Gram-negative bacteria causing corky root of lettuce, a wide-spread and important lettuce disease. Only one species was described, *R. suberifaciens*, which was reclassified as *Sphingomonas suberifaciens* by Yabuuchi et al. based on 16S rRNA sequences and the presence of sphingoglycolipid in the cell envelope. However, *Sphingomonas* is so diverse that reclassification was deemed necessary. Twenty new *Rhizamonas*- and *Sphingomonas*-like isolates were obtained from lettuce or sowthistle roots or from soil using lettuce seedlings as bait. These and previously reported isolates were characterized in a polyphasic study including 16S rRNA gene sequencing, DNA-DNA hybridization, G+C content, whole-cell fatty acid composition, morphology, substrate oxidation, temperature and pH sensitivity, and pathogenicity to lettuce. The isolates causing lettuce corky root belong to the genera *Rhizamonas*, *Sphingobium*, *Sphingopyxis*, and a newly proposed genus *Rhizorhabdus*. Several nonpathogenic strains isolated from lettuce roots belong to the genus *Sphingomonas*, but none of the pathogenic strains belong to this genus.

Identification of phytoplasmas infecting native and introduced tree species in Bogota, Colombia

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An exotic disease was reported affecting *Fraxinus uhdei* in Bogotá the 1990s; in 2004 it was shown that they were infected with phytoplasmas of group 16SrVII. Since 2008, symptoms that change the shape and growing patterns of the crown have been observed in an increasing number of tree species, in prevalences greater than 98%. Symptoms include epicormic shoots, tufted foliage, branches with elongated internodes, abnormal apical branches, small leaves, yellowing, atypical pigmentation of crowns etc. This work summarizes the findings made since 2009 by nested PCR, RFLP and sequencing of the 16SrDNA, in trees and insects of Bogota. Phytoplasmas of groups 16SrI and 16SrVII have been confirmed in native species *Croton* sp. and *Quercus humboldtii* and in introduced *Liquidambar styraciflua*, *Fraxinus uhdei*, *Pittosporum undulatum*, *Populus nigra*, *Acacia melanoxylon*, *Eugenia* sp. and *Magnolia grandiflora*. Groups 16SrV and 16SrXII are present in *L. styraciflua*. Other symptomatic trees are under study. Phytoplasmas of 16SrI and/or 16SrVII have been detected in 7 of 12 morphotypes of cycadellids tested. The phytoplasma disease in trees of Bogota is a serious threat for the environment of the city and its surroundings. Groups 16SI and 16SVII have been found in strawberry crops in nearby locations in plants with virescence and phyllodia. Urgent action is needed to stop dissemination of the disease to other locations, tree species, ornamentals and plants of economic interest.

In silico identification and characterization of NB-LRR-encoding resistance genes in the bioenergy plant switchgrass (*Panicum virgatum* L.)

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Switchgrass has received attention for its potential use as a second generation biofuel feedstock. Sustainable switchgrass biomass production could be negatively impacted by the epidemics of various disease problems. Therefore, the molecular mechanisms underlying tolerance to various diseases must be elucidated. The majority of plant disease resistance (R) genes belong to a large family of nucleotide binding - leucine rich repeat (NB-LRR) genes that can be classified into two major groups: Toll-IL-1 receptor (TIR)-NB-LRR and coiled-coil (CC)-NB-LRR genes. In this study, we used a homology-based computational method to identify 610 putative NB-LRR-containing R genes in the newly released draft genome of switchgrass. Phylogenetic analysis of these genes revealed that they can be classified into 2 major groups. As expected, no TIR-NB-LRR genes were discovered in the switchgrass genome. Interestingly, 28 genes were identified that contain unique domains other than CC and LRR domains. Using readily available transcriptome resources, 526 of these genes were found to have transcription evidence. This suggests that most of the NB-LRR genes identified in this study are expressed. We also determined that 208 microRNA families potentially target 335 NB-LRR genes. The results of this study will aid in understanding the genetic mechanisms that control disease resistance in switchgrass and may lead to the development of switchgrass cultivars with improved disease tolerance.

Seasonal pattern of virus uptake by the grape mealybug in a leafroll-diseased vineyard

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Leafroll disease affects the profitability of the grapevine industry worldwide. Five viruses from the family *Closteroviridae* have been isolated from leafroll-affected vines. In the Finger Lakes region of New York, *Grapevine leafroll-associated virus 1* and 3 are prevalent in leafroll-diseased vineyards and low populations of the grape mealybug (*Pseudococcus maritimus*) vector are present. To address seasonal patterns of virus acquisition, mealybugs (immatures, adults and eggs) were collected from April to November over two consecutive years on selected vines in a vineyard of *Vitis vinifera* cv. Chardonnay that is naturally infected with GLRaV-1 and GLRaV-3. Collected specimens were tested for the presence of viral genetic elements by RT-PCR using specific primers. In addition, eggs collected in June were allowed to hatch on Petri dishes in the lab and crawlers were tested for transovary virus transmission. Results were consistent with a preferred virus uptake from bud break to bloom (April to June) by overwintered crawlers and at a pre-veraison stage (July-August) by summer generation crawlers. Very few overwintering crawlers were viruliferous and there was no transovary transmission of GLRaV-1 and GLRaV-3, indicating a semi-persistent transmission mode. These results suggest that leafroll management strategies based on mealybug control should target vector populations from bud break to expanding leaves.

Effects of seed type and variety on the agronomic performance of potato minitubers and the incidence of Potato virus Y

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Seed potato production begins by propagating plants in tissue culture and then transplanting them into pots or a hydroponic system in a greenhouse to produce minitubers. Minitubers are planted in the field to produce conventional seed potatoes, which are re-planted for multiplication. Seed lots must have low levels of diseases in order to be certified. The use of minitubers as the initial seed source minimizes the risk of infection by important seed potato pathogens, such as *Potato virus Y* (PVY). Expanding seed production from minitubers is potentially an effective strategy for minimizing initial PVY inoculum; however more research is needed to determine agronomic performance of minitubers. We evaluated the effects of seed type for three different varieties under overhead and drip irrigation on potato yield and PVY incidence. 4-row (20ft/row) plots were planted for each combination of seed type and variety in a randomized complete block design with 4 replications. Potato tuber weight and number of tubers was measured after harvest. PVY incidence data collection is still in process. Yield data analyses indicate that plants grown from minitubers have lower total tuber yield ($\alpha < 0.001$; $df=1$), and the effect varies for each variety. However, there is no significant effect of variety alone on potato tuber yield. This result is also supported by data collected from 1994 to 2006 at the Lelach Starks Elite Foundation Seed Potato Farm.

Effects of solarization and biocontrol on soilborne *Phytophthora* spp. in container nurseries

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Infested nursery beds are an important source of *Phytophthora* spp. in container nurseries. Soilborne inoculum of *Phytophthora* spp., shown to be located mostly in the top 10 cm, can initiate disease through movement with surface water or splashing onto foliage. We investigated soil solarization, alone or with subsequent amendment with a *Trichoderma* sp. biocontrol agent, on the survival of *Phytophthora* species. In field trials conducted with *P. ramorum* in San Rafael, CA or with *P. pini* in Corvallis, OR, infested rhododendron leaf inoculum was buried 5, 15, and 30 cm below the soil surface. Solarization for 2 or 4 weeks during summer 2012 eliminated recovery of *Phytophthora* buried at 5 cm in CA and OR, and at 15 cm in CA. Maximum soil temperatures exceeded 50°C in CA and 45°C in OR. The biocontrol treatment was added to solarized and non-solarized plots, but there was no effect on *Phytophthora* recovery 2, 4, or 8 weeks later. Populations of the introduced *Trichoderma* sp. and of indigenous *Trichoderma* spp. were significantly greater in solarized as compared to non-solarized plots. Data from lab experiments at controlled, stable temperatures will be combined with field data to establish a model to predict the minimum time period required for solarization to eliminate *P. ramorum* and *P. pini* from different soil depths in container nurseries. Soil solarization appears to be a promising technique for disinfecting the upper layer of soil in container nurseries.

WITHDRAWN

WITHDRAWN

Islands in stone: Fungal community structure within Hawaiian kipuka

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The Hawaiian archipelago represents an ideal natural laboratory in which to study the geographic, ecological and evolutionary factors that determine microbial community structure. The kipuka system found on Hawaii Island, in particular, provide excellent natural sites to study species-area relationships as well the effectiveness of specific sampling techniques in accessing species diversity and abundance. The goal of the kipuka study will be to assess fungal species abundance and diversity as a function of kipuka size. The natural isolation, variable size and ascertainable age of substrate, eliminates a number of confounding factors common in other fungal diversity studies. Analytical components of this study consist of molecular analysis of soil samples to determine presence of fungi paired with classic observation, recording and collection fruiting bodies throughout approximately one year of time. Pairing both molecular and classic sampling techniques will allow us to assess how effective classic techniques are in capturing diversity measures and abundance of specific species. Peripheral benefits of this study will likely include the collection of novel fungal species, and will advance our understanding of seasonality in Hawaiian fungal reproductive patterns within the kipuka system. Preliminary results will be presented and discussed.

Susceptibility of commercial boxwood cultivars to *Cylindrocladium buxicola*, the causal agent of box blight

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Box blight, caused by the fungal pathogen *Cylindrocladium buxicola* (= *C. pseudonaviculatum*), is characterized by circular black to brown foliar lesions with light margins and elongated black stem lesions. Many *Buxus* spp. and cultivars are susceptible, including the most widely grown cultivars English and American boxwood, *B. sempervirens* 'Suffruticosa' and 'American,' respectively. During the summer of 2012, twenty-three commercial *Buxus* cultivars were screened for resistance to *C. buxicola* in Mills River, NC. Two highly susceptible plants were inoculated with a conidial suspension of *C. buxicola* and six test cultivar plants were placed around the inoculated plants under a sprinkler irrigation system. The trial included four replications. Percent leaf area diseased was assessed over 53 days. A wide range in susceptibility of *Buxus* spp. to box blight was observed. Four cultivars were identified as partially resistant based on minimal symptom development, *B. microphylla* var. *japonica* 'Green Beauty', *B. sinica* var. *insularis* 'Nana', *B. harlandii*, and *B. microphylla* 'Golden Dream'. Appropriate cultivar selection will be an important long term strategy for managing the disease in nurseries and landscapes.

A bacterial effector targets a non-canonical signaling pathway for suppressing *Arabidopsis* defenses

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Pseudomonas syringae delivers type III effector proteins into the cytosol of host cells. HopM1 contributes to full virulence of *P. syringae* pv. *tomato* strain DC3000 (Pto) by causing degradation of several *Arabidopsis* proteins, including AtMIN7, an ADP ribosylation factor-guanine nucleotide exchange factor. *P. syringae* pv. *phaseolicola* strain NPS3121 (Pph) lacks a functional HopM1 and elicits robust defenses on *Arabidopsis* including *PR-1* expression and callose containing cell wall fortifications. We expressed HopM1_{Pto} in Pph and investigated its effect on these Pph-induced defense responses. HopM1 suppressed Pph-induced *PR-1* expression as measured by western blot and q-RT PCR. *PR-1* expression is widely considered a reflection of salicylic acid (SA) signaling. Surprisingly, HopM1_{Pto} did not affect Pph-induced SA accumulation, as measured by HPLC, and suppressed the low levels of *PR-1* expression apparent in SA-signaling deficient plants. HopM1 failed to eliminate AtMIN7 during Pph infection and suppressed Pph-induced defense responses in wild-type and *atmin7* plants, indicating that its ability to suppress Pph-induced defense responses is mediated through targets other than AtMIN7. This adds to our current understanding of *Arabidopsis*-*Pseudomonas* interactions as it links HopM1, AtMIN7, and other HopM1 targets to a pathway distinct from the canonical SA-signaling pathway contributing to *PR-1* expression and callose deposition.

Human exposure to aflatoxin in mesquite pod flour produced for personal consumption, cottage industry, and commercial markets in southeastern Arizona

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Arizona mesquite (*Prosopis velutina*) produce protein- and carbohydrate-rich pods that are staple for wildlife and have been consumed by indigenous people for generations. Native food enthusiasts in Arizona conduct public millings of wild- and landscape-collected mesquite pods to produce mesquite flour which is often consumed in the same localities where it is produced without conventional food safety inspection. Aflatoxin contamination of food and feed is a perennial concern in Arizona where aflatoxin contamination, produced mainly by *Aspergillus flavus*, has been previously reported in Fabaceae fruits including *Prosopis* pods. This study identified aflatoxin exposure risk posed by mesquite flour from southeastern Arizona and northern Sonora, Mexico. Aflatoxin was found in both commercial (imported and domestic) and non-commercial mesquite flour batches. Aflatoxin contamination above FDA action levels for human food occurred in 10% of the

sampled flour, and no flour had aflatoxin levels low enough for European export. Aflatoxin was also detected in flour imported from Peru and Argentina. In Tucson, precipitation on mature pods explained 62% of the variability in aflatoxin content. Pods harvested in Tucson before monsoonal rains produced mesquite flour with aflatoxin below FDA action limits. Immunochromatographic lateral flow assay of aflatoxin in mesquite flour proved a viable option for testing at public events.

Diversity and evolutionary relationships of bacteria affiliated with tropical seeds and seed-associated fungi

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Seed-associated fungi have variable effects on survival and germination of seeds of tropical trees, and thus play an important but little-studied role in tropical forest dynamics. Recently, bacterial endosymbionts (i.e., endohyphal bacteria, EHB) were detected in phylogenetically diverse Sordariomycetes associated with seeds of the tropical pioneer genus *Cecropia* in Panama. These EHB appear to be diverse, facultative, and horizontally transmitted, suggesting that they may be found free-living in the seeds from which their fungal hosts were isolated, or in the soil with which seeds have contact after dispersing into the forest floor. We compared EHB from seed-associated fungi with bacteria obtained directly from soil and from surface-sterilized seeds of three neotropical pioneer species involved in a large seed-burial experiment at Barro Colorado Island, Panama. The relative contribution of soil-borne and seed-borne bacteria to formation of the endohyphal symbiosis was assessed using phylogenetic congruence tests. Our results provide a first perspective on the capacity of seed-borne fungi to acquire local bacterial symbionts. Because different bacterial/fungal partnerships are likely to change fungal phenotypes and the outcome of fungus-seed interactions, our work illuminates one factor that may influence an important component of plant community dynamics in tropical forest ecosystems.

Examining ectomycorrhizal communities in *Pinus ponderosa* and *Pinus contorta* in the Deschutes National Forest

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Rise of global temperatures, decline of winter precipitation, earlier loss of snowpack, and increased summer drought are altering the range of *Pinus contorta*. As environmental conditions change, *Pinus ponderosa* may establish within the historic *Pinus contorta* range. Successful pine species migration will be constrained by the distribution or co-migration of ectomycorrhizal fungi (ECM). Knowledge of the linkages among soil fungal diversity, community structure, and environmental factors is critical to understanding the organization and stability of pine ecosystems. The aim of this study is to establish an informational foundation of ECM communities of *P. ponderosa* and *P. contorta* in the Deschutes National Forest and to examine soil characteristics associated with community composition. We examined ECM root tips of *P. ponderosa* and *P. contorta* in soil cores and conducted soil chemistry analysis for *P. ponderosa*. Results indicate that species in the genera *Rhizopogon*, *Cenococcum*, *Cortinari*, *Inocybe*, *Piloderma*, and *Suillus* are dominant in both *P. contorta* and *P. ponderosa* soil cores. *Rhizopogon* spp. were ubiquitous in *P. ponderosa* soil cores. There was no significant difference between ECM communities of the two pines at the species level. Ordination analysis suggests that pH, carbon, NO₃ and NH₄ affect are driving ECM community composition in *P. ponderosa*. We found a significant linear relationship between species richness and mineralizable nitrogen.

Root rot disease of wheat survey in South Dakota

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Common root rot (CRR), crown rot (CR), and take-all (TA), caused by *Bipolaris sorokiniana*, *Fusarium* spp., and *Gaeumannomyces graminis* var. *tritici*, respectively, are important soil-borne diseases of wheat. Under favorable conditions for disease development, either alone or in combination, these diseases can significantly impact wheat production. The objective of this study was to conduct a disease survey to obtain a complete picture of root rot pathogens and their proportion of occurrence in South Dakota. Fifty wheat fields were systematically sampled representing the wheat production region

in the state. One hundred tillers were sampled from each field (5 tillers x 20 locations). The root systems of sampled tillers were washed under tap water to remove soil and then rinsed twice in sterile distilled water prior to storage at 5°C. Sub crown internode (SCI) and crown tissues were plated onto Specific Screening Media (SSM), modified SM-GGT7 and PDA media for recovering *F. graminearum*, *G. graminis* var. *tritici*, and *B. sorokiniana*, respectively. Isolates of *F. graminearum*, *G. graminis* var. *tritici*, and *B. sorokiniana* were recovered from samples obtained from 19, 14, and 2 wheat fields. The results indicate that *F. graminearum* is the most prevalent pathogen among three root pathogens. Molecular identification of recovered isolates and more root samples are under investigation.

Evaluation of *Fusarium graminearum* isolates from wheat roots for their ability to cause crown rot

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Crown rot (CR), caused by *Fusarium graminearum* (Fg) or *F. pseudo-graminearum*, is an important root disease of wheat. One hundred and twenty-two Fg isolates were recovered from wheat roots samples collected during summer 2012 in South Dakota. Ten random isolates of 122 Fg isolates were assessed for their ability to cause CR on four hard red spring wheat cultivars; Alsen, Oxen, Wheaton and Len. Two runs of greenhouse studies were established in randomized complete block design with three replications. Twenty days old-seedlings of each cultivar were inoculated with all ten isolates individually by placing 700 µl of inoculum (10⁴ spores mL⁻¹) onto the cotton wrapped around the base of the main stem and held in place by a plastic straw of same size. The plants were rated for CR development 35 days post inoculation. Crown rot severity index was calculated as the product of the proportion of stem length discolored and the number of leaf sheath layers discolored. In combined analysis of runs, the main effect of cultivars, isolates and their interactions were highly significant for CR. Isolates also significantly varied for their ability to cause CR. When isolates were combined, cultivar Len was significantly susceptible for CR compared to rest of the three cultivars. The results indicate that Fg isolates recovered from wheat roots can cause CR and wheat cultivars varied in their reaction to crown rot.

Open Tree of Life: Challenges and progress for the fungi

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The Open Tree of Life Project aims to synthesize a comprehensive tree of life including all of the ~1.9 million described species, and eventually, all undescribed species known only from environmental sequences. Our collaboration includes practicing systematists, as well as experts in phylogenetic theory, software development, and bioinformatics. The Hibbett laboratory is tasked with synthesis of data coming from mycological studies. To date, we have examined more than 2,000 peer-reviewed articles published between 2000 and 2012. Unfortunately, we found that less than 30% of the studies have deposited their alignments or tree-files in publicly available databases. We also contacted authors of 100 studies requesting their collaboration. From this first assessment, we concluded that the majority of fungal phylogenies produced in the last decade are inaccessible, existing only as printed figures. These results highlight the importance of data deposition for downstream analyses and re-use, such as the assembly of a comprehensive tree of life. Beyond collecting data to synthesize a complete tree, the Open Tree of Life Project seeks to create new tools that will allow community-driven synthesis and annotation of phylogenies. We will present results of preliminary analyses that illustrate how the Open Tree project acquires data, as well as new approaches to representing conflict, support, and taxonomic coverage in fungal phylogenies.

WITHDRAWN

Development of diploid potato breeding lines with resistance to late blight caused by new clonal lineages of *Phytophthora infestans*

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Phytophthora infestans is the causal agent of tomato and potato late blight. In potato, late blight can cause severe losses by reducing yield, lowering quality of tubers and reducing tuber storability. The population of the pathogen is very dynamic due multiple factors, including natural mutation, genetic drift and sexual recombination. Such factors contribute to the development of new population structures with more diversity and more pathogenicity. The use of resistant cultivars is an important component of an integrated late blight disease management program. Wild potato relatives have been used as a source of resistance to late blight. Crosses were made between diploid late blight resistant *Solanum verrucosum* and the dihaploid *Solanum tuberosum* US-W4. 150 progeny were screened for resistance to late blight. A whole plant disease resistance assay was performed on 7-week-old seedlings by inoculating with a mixture of the *P. infestans* clonal lineages of US-22, -23 and -24. 8 plants progenies were selected as resistant and crossed with the tetraploid *S. tuberosum* cultivars MegaChip and White Pearl, and diploid hybrids HC (an interspecific hybrid) and XD3 (a hybrid between US-W4 and *S. chacoense*). Seed were formed only in fruits from crosses to XD3. 174 progeny were screened for resistance to late blight. Twelve plants were selected as resistant. Those breeding lines are being used to develop more advanced breeding lines for resistance to late blight.

Novel clonal lineages of *Phytophthora infestans* elicit differential disease and pathogen responses on solanaceous hosts

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Late blight caused by *Phytophthora infestans* is a recurring and serious disease in potatoes and tomatoes worldwide. In recent years, novel *P. infestans* clonal lineages have displaced lineages with well characterized virulence and control schemes. We sought to expand our knowledge of new lineages, US-22, -23, and -24, on solanaceous cultigens of commercial and research interest. The virulence of representative isolates from each of the 3 lineages was evaluated on 13 tomato and potato cultigens, and on common weeds, *Solanum dulcamara* and *S. physalifolium*. Intact plants were inoculated with a sporangial suspension and evaluated for disease after 11 days of incubation under high humidity. Late blight severity of 26-100% resulted on potato, tomato, and weed foliage of plants lacking Ph-2/Ph-3 or RB genes. Plants with Ph or RB genes were resistant. Mean disease severity values were statistically different ($P < 0.05$) among clonal lineages, with US-23 causing greatest severity and US-24 least. In our previous work, we also identified the ability of Ph and RB resistant plants to resist production of oospores when co-inoculated with opposing mating pairs of the novel clonal lineages. Our results revealed durable host resistance to novel pathogen lineages, the importance of controlling solanaceous weeds in production fields, and the need for continued characterization of *P. infestans* for long-term integrated late blight management.

The importance of fungicides for feeding the world

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The worldwide crop fungicide market is approximately \$10 billion. High yields of wheat in Europe and rice in Asia depend on fungicides for control of key diseases. The use of fungicides in Brazil prevents the loss of about 50% of the country's soybean crop to rust. Fungicide use is low in Africa and smallholder yields of groundnuts and maize are typically reduced by two-thirds due to uncontrolled diseases. In India, fungicide use could greatly

increase the production of pulse crops - a key source of protein. Current low yields of pulses are a major cause of hunger in India since poor people cannot afford them. Worldwide potato production is dependent on fungicide sprays to control late blight, and countries where fungicides are not widely-used (such as Russia and in Africa) potato losses to disease are large. Worldwide, most fruit and vegetable crop fields are regularly treated with fungicides to produce high yields and to produce crops acceptable to consumers. Worldwide export of tropical export crops (banana, coffee, mango) would collapse without regular fungicide applications. In wheat, fungicide applications have increased in China, Australia, and India due to the breakdown of resistance to rust. Timely applications of fungicides in wheat are preventing huge yield losses.

Surveying stone fruit trees for viruses in Texas: 2011-2013

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Plum pox virus (PPV) is a quarantine pathogen that was first reported in the U.S. in Pennsylvania in 1999. Since then it has been detected in other peach growing areas of the country (Michigan & New York, 2006). Texas participates in the USDA National Stone Fruit Survey to ensure and maintain our pathogen free status. Surveys for PPV have been conducted in Texas since 2011 and are still ongoing, including the testing of over 2,694 *Prunus* trees from around the state. Breeding material plots were sampled at a rate of 100% while orchards grown for commercial production were sampled at a rate of 25%, following a federally approved hierarchical sampling method. Additionally, 737 trees from the Texas A&M University stone fruit breeding plots were sampled and tested for *Peach rosette mosaic virus* (PRMV) and *Raspberry ringspot virus* (RpRSV), and 310 trees were tested for *Tomato ringspot virus* (TRSV). Leaves were tested using ELISA according to the protocols implemented by the U.S. Department of Agriculture and National Plant Diagnostic Network. To date, all samples tested negative for PPV and the Texas A&M breeding stocks tested were free of TRSV, PRMV and RpRSV.

Foliar disease incidence associated with giant miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*) cultivars in Mississippi

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The establishment of perennial grasses as biomass feedstock crops has increased the production acreage of giant miscanthus (*Miscanthus x giganteus* Greef et Deu, MXG) and switchgrass (*Panicum virgatum* L., SG). Yield loss and establishment failure associated with foliar fungal disease could be detrimental to the sustainable production of these crops, and therefore, exploitation of differentiation in cultivar response to fungal diseases could be a key management strategy. In 2010 a two year study was initiated to assess 'Freedom', 'Illinois', and 'Nagara' MXG as well as 'Alamo' and 'EG1101' SG on a monthly basis throughout the growing season for foliar disease incidence (FDI). Three separate locations in Starkville, MS with plots of 2, 3, and 10 year-old Mxg and SG were observed for FDI based on the percentage of foliar disease symptoms observed within each plot. FDI was compared for each location by repeated measures covariance analysis using PROC MIXED program of SAS (SAS v 9.2). Least square means were used to determine statistical significance among cultivars ($P = 0.05$); and regression best fit models were developed when significant cultivar variation occurred. FDI was similar for the three Mxg cultivars at the 2 year location. However, FDI was significantly different between the Mxg and SG cultivars at the 3 year and 10 year locations.

Population genomics of plant-associated model Ascomycota species

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Divergence of populations, adaptation of diverged populations, and subsequent development of reproductive barriers are the processes that create the tree of life. Published study of *Neurospora crassa* discovered recently diverged populations containing genetic "islands" of differentiation involved in adaptation to temperature. To extend this finding, and to address its universality, we are conducting a population genomic study of a species in the *Neurospora discreta* phylogenetic species complex with a latitudinal range from New Mexico to Alaska. Genomes of next-generation-sequence assembled to a reference genome have been obtained for 5 to 10 individuals from populations in New Mexico, California, Washington and Alaska. Phylogenetic analyses of single nucleotide polymorphisms in the individuals find two, nearly simultaneous divergences that define three clades: Alaska,

California-Washington and New Mexico-Washington. The phylogeography of these clades is consistent with one ancestral lineage that diverged to a Rocky Mountain lineage and a Pacific Coast lineage and another lineage that simultaneously repopulated Alaska and Europe. Comparison of the montane and coastal lineages will address existence of regions of low diversity consistent with gene flow between the lineages. Comparison of the Washington state populations will address existence of “islands” of divergence and possible reinforcement of mating barriers in this area of sympatry.

A cyclophilin binding domain present in XopAG(AvrGf2) effector determines the elicitation of HR in grapefruit

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Citrus canker, one of the most important diseases in citrus, is caused by two bacterial species, *Xanthomonas citri* (Xc-A), and *X. fuscans* pv. *aurantifolii* (Xfa). Two avirulence (avr) genes have been identified which cause a hypersensitive reaction (HR) in grapefruit (GF) and are designated as *avrGf1* from Xc-A^{av}, and *avrGf2* from Xfa-C. Infiltration of Xc-A:*avrGf2* causes a more rapid HR compared to Xc-A:*avrGf1*. Upon comparison using bioinformatic approaches of the two genes, both contain putative cyclophilin binding site ‘GPxL’ which is also present in all *xopAG* class effectors including other xanthomonads, *Acidovorax*, *Ralstonia*, and *Pseudomonas* species. Using PCR-mutagenesis, we mutated the GPLL site present in AvrGf2, to AASL and SPLL, then expressed these constructs in Xc-A, and inoculated into GF leaves. Although both mutations abolished the HR caused by the wild type (wt) AvrGf2, pathogenicity reactions caused by the mutants AASL and SPLL were attenuated in comparison to the wt Xc-A, and Xc-A:*avrGf2*. We also identified three C-terminal domains in *xopAG* family. To determine if the motifs were responsible for the difference in time for eliciting an HR, we replaced the C-terminal sequence from *avrGf2* with that of *avrGf1* and determined that the hybrid protein expressed in Xc-A was non-functional in GF. Understanding the interaction between these type III effectors and any citrus proteins will be essential for the understanding how this type of resistance reaction is elicited.

Soil microbes in organic vs. conventional vegetable production: Capturing the active players through soil RNA analysis

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Soil microbes are fundamental to the health and productivity of agricultural systems. Organic management may increase soil microbial diversity and crop production, and has the potential to reduce losses to pathogens. We evaluated total and active microbial community responses in a fertility field experiment after three cycles of a two-year tomato and Chinese cabbage rotation. The fertility treatment included low and high levels of organic or conventional fertility. Low fertility treatments in both organic and conventional managements received nutrients only through a cover crop (buckwheat). The conventional high fertility treatment used potassium nitrate, calcium nitrate, and inorganic pre-plant fertilizer, and the organic high fertility treatment fish hydrolysate and compost pre-plant fertilizer. We compared total resident fungal, bacterial, and archaeal communities using PCR-amplified DNA and the actively metabolizing microbial communities using reverse-transcribed and PCR-amplified cDNA. Our data suggest that organic management with high fertility may increase bacterial diversity. We recovered a number of bacterial genera with important agroecological roles, including *Nitrospira*, and *Rhizobium*. Our preliminary results show that cDNA and DNA provide different views of the community. For example, frequencies of fungal genera that are potentially associated with plant health—*Aspergillus*, *Glomus* and *Alternaria*—differed between the cDNA- and DNA-derived communities.

Evaluation of ‘Pera’ (*Citrus sinensis*) genotypes resistance to citrus canker on greenhouse conditions

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Citrus canker, caused by *Xanthomonas citri* subsp. *citri*, brought serious damages to crops. The disease progression, particularly in tropical countries, is been facilitated by favorable weather conditions, in the disposed managing on harvesting and fruits marketing. The use of resistant genotypes is an important tool to control pathogens in several crops, including in citrus. Researches has shown that the artificial inoculation presents typical symptoms of citrus canker, when wounds, infiltration or spraying the inoculum was used in plant tissue. Therefore this study evaluated the resistance genotypes of sweet orange (*Citrus sinensis*), ‘Pêra’ variety to *X. citri*, under controlled conditions (greenhouse). A total of 25 ‘Pêra’ orange genotypes were evaluated in two trials, being the leaves inoculated by needle punching (0.55 x 0.20 mm), with constant wetness in the inoculum. The inoculum *X. citri* was adjusted to a concentration of 10⁸ CFU/mL using a spectrophotometer at 600nm and the evaluations were made by measuring the lesions diameter. The bacterial sum was performed by counting the Colony Forming Unit (CFU) isolated from each lesion. The results showed that the genotypes ‘Pêra EEL’, ‘IAC 2000/1’ stood out with smaller lesion diameters in both assays, moreover, ‘Pêra Bianchi/CC’ and ‘IAC’ had the smallest diameter and bacterial populations in the second test, suggesting that these genotypes have pathogen resistance.

Identification, molecular characterization, and evolution of group I introns at the expansion segment D11 of 28S rDNA in *Rhizoctonia* species

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The nuclear ribosomal DNA of *Rhizoctonia* species is polymorphic in terms of the nucleotide composition and length. Insertions of 349-410 nucleotides in length with characteristics of group I introns were detected at a single insertion point at the expansion segment D11 of 28S rDNA in twelve out sixty-four isolates. Eleven corresponded to *R. solani* (teleomorph: *Thanatephorus*) and one (AG-Q) to *Rhizoctonia* spp. (teleomorph: *Ceratobasidium*). Sequence data showed that all but AG-Q contained conserved DNA catalytic core regions (P, Q, R, and S) essential for self-splicing. The predicted secondary structure revealed that base paired helices P1-P9 corresponded to subgroup IC1. Isolates from same anastomosis group and even sub-groups within *R. solani* were variable with regard to possession of introns. Phylogenetic analyses indicated that introns were vertically transmitted. Unfortunately, sequence data from the conserved region from all sixty-four isolates was not useful for delimiting species. The present study uncovered new questions on ribosomal DNA variation within this genetically diverse group of fungi.

WITHDRAWN

A new genetic clade of the pythiosis pathogen, *Pythium insidiosum*, revealed by environmental sampling in Florida

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There are few fungal pathogens known to be “trans-kingdom”, infecting both plants and animals, but these include several emerging pathogens of humans. Pythiosis is a deadly disease of horses, dogs, and other animals in tropical and subtropical regions, including the Southern United States. It infects humans in Southeast Asia and is considered a potential emerging pathogen in the United States due to its apparently expanding geographic and host range. The causal

agent of pythiosis is the oomycete *Pythium insidiosum*, the only known mammalian pathogen in the genus *Pythium*. Three genetic clades of *P. insidiosum* have been described based on isolates from clinical infections and show some geographic affiliation. Environmental sampling has been conducted only in agricultural areas in Thailand. We sampled 13 lakes and ponds in North Central Florida for *P. insidiosum*. We found the pathogen in the majority of the lakes or ponds sampled. Our environmental isolates fall into three clades, including a new clade previously represented by a single isolate from a captive bear in South Carolina. AFLP genotyping has revealed genetic variation within clades and between lakes. These populations will be used to study the population structure and ecology of *P. insidiosum* in the United States.

Cell-based high-throughput assay for evaluating the response of *Mycosphaerella graminicola* to different doses of fungicides

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Mycosphaerella graminicola, which causes Septoria leaf blotch of wheat, is one of the most economically important pathogens of wheat, particularly in Europe. This fungus is a common target for development of novel fungicide chemistries. However, disease development in whole plant-based assays can require up to three weeks and proves to be a bottleneck in rapid compound screening for fungicidal activity. To expedite this process, a cell-based assay aimed at assessing potential activity of fungicidal compounds using a high throughput setup has been developed. This method uses robotics to apply spores suspended in liquid media to 96 well plates containing fungicides at different rates. After 4 days, the plates are rated either visually using a touch screen based method, or the growth of the pathogen is determined by measuring its optical density and the minimum effective rate of the compound is determined. This strategy has proved to be effective in ranking compounds according to their relative activity against each other and market standards. The activity detected in this assay has also been found to translate to activity on whole plants.

Abolishing the nematode transmissibility of a Grapevine fanleaf virus vector engineered for functional genomics

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Current efforts to functionally characterize grapevine genes are limited by a lack of tractable means to experimentally validate gene function in grapevines. Viral vectors are useful tools for functional genomics, and *Grapevine fanleaf virus* (GFLV) is well suited for this purpose in grapevine because functional cDNA clones of the two genomic RNAs are available and no tissue tropism is known. The use of GFLV as a vector could be limited due to its transmission by the ectoparasitic dagger nematode, *Xiphinema index*. To develop a versatile GFLV vector that could be useful in minimum containment facilities and field situations, it is essential to abolish vector transmission. The determinant of nematode transmission within the GFLV coat protein gene was mutated in our viral vector. Nematode transmission assays were conducted with recombinant, reassortant, and wild-type strains of GFLV in grapevines and herbaceous plants. The results showed that transmission of our modified GFLV vector by *Xiphinema index* is abolished under all conditions tested. This research will facilitate the widespread use of a GFLV vector for grapevine functional genomics by restricting its unintended dissemination.

WITHDRAWN

Comparison of the Pennsylvania and Ontario Plum pox virus survey and eradication programs

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Plum pox virus (PPV) was first detected in North America in Pennsylvania in 1999, and in Ontario, Canada in 2000. In response to these detections, both countries implemented survey and eradication program in an effort to limit the spread of PPV. The objective of this study was to compare the two countries' PPV survey programs, with regards to their abilities to detect PPV at the leaf, scaffold, and tree scales. To address this objective, we developed a simulation model to assess how the following criteria (i) ELISA test kit, (ii) sample size (no. leaves/sample), (iii) sample design (random vs. stratified random), and (iv) number of positives leaves required for a sample to test positive, affected detection efficiency. At the tree scale, the Pennsylvania survey program was found to have a detection efficiency of 71.8%, whereas the Ontario survey program had a detection efficiency of 40.5%. ELISA test kit was found to have the largest impact on detection efficiency. Sample size, and number of positive leaves required for a sample to test positive had significant, but smaller, effects on PPV detection efficiency. Sample design did not affect detection efficiency. Our results have important implications in the development of future PPV survey programs, and provide an effective new method for evaluating eradication programs.

Street-tree incidence and severity of bacterial leaf scorch of oak in the New Jersey urban forest

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Bacterial leaf scorch (BLS) of amenity trees, caused by the xylem-limited bacterium *Xylella fastidiosa*, is widespread throughout the eastern and mid-western U.S. In New Jersey, the disease primarily affects trees in the red oak group, and since the 1980s has had a major impact in the New Jersey urban forest. A long-term study of BLS incidence and severity in two central New Jersey communities concluded in 2012. Disease incidence in a population of 700 pin, red, or scarlet oaks increased in 2002 to 2012 from 20 to 35% (disease severity ratings >20%). During that period, approximately one-quarter of these street-side oaks were removed by property owners or municipalities; of these, 15% were identified as trees severely affected by the disease within the previous 5-year period. Of the street-side trees remaining in 2012, 20% were severely affected by BLS (disease severity >50%) and an inverse relationship between disease development and tree growth was evident. This disease clearly represents a significant impact to the urban forest in these communities; with no cost-effective rescue technology, arborists are forced to remove trees to prevent liabilities that result from declining trees.

Prevalence and characterization of iprodione and fludioxonil resistance in *Botrytis cinerea* isolates from small fruits in the southeastern U.S.

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The control of gray mold in small fruits is largely dependent on the application of fungicides, including iprodione and fludioxonil. A total of 516 single-spore isolates of *Botrytis cinerea* were collected from commercial strawberry fields in Florida, North Carolina, and South Carolina and blackberry fields from the Carolinas to determine occurrence and prevalence of resistance. Based on a spore-germination assay, most isolates were either low resistant (LR) or sensitive (S) to iprodione. Five isolates (1%) highly resistant (HR) to iprodione were found in blackberry fields and isolates with moderate resistance (MR) were found at low frequencies in strawberry fields. Detached fruit assays indicated that field rates of Rovral (a.i. iprodione) controlled the S and LR but not the MR and HR isolates. Moderate resistance to iprodione was associated with both the Q369P and N373S mutations in the Class III Histidine-Kinase Bos1. The I365S and I365N mutations were present in LR and HR isolates indicating that these mutations were not responsible for the HR phenotype. Of the five HR isolates to iprodione, two were S, one was

MR, and two were HR to fludioxonil. The presence of the R632I mutation in the *mrr1* transcriptional regulator gene and overexpression of the *atrB* gene indicated MDR1 activity in the two isolates highly resistant to fludioxonil.

Field trial evaluation of resistance to *Sclerotinia sclerotiorum* in annual bedding plants

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Sclerotinia sclerotiorum causes crown rot, bulb rot, stem rot, wilt and death of a wide variety of annual bedding plants including zinnia, petunia, salvia and snap dragon. Disease is commonly observed annually in infested beds. Planting resistant species could be an effective means of disease control. In 2011, one to three varieties each of 13 genera of unknown susceptibility were planted at two locations. Each variety was planted in a bed of nine plants and replicated six times per location. Profusion White zinnia was the susceptible control. Plants were inoculated at canopy closure with sorghum seed colonized by *S. sclerotiorum*. After inoculation plants were irrigated three times per day for 10 minutes to maintain canopy moisture. Disease incidence and percent canopy death were rated biweekly for all plots. The plant genera demonstrated a range of resistance. *Scirpus* sp., *Carex* sp., *Juncus* sp., *Pennisetum glaucum*, and *Colocasia esculenta* were highly resistant to *S. sclerotiorum* and exhibited no symptoms. Localized infections were observed in *Canna* sp. but did not progress to wilt or death of stems. Death of individual stems but not entire plants was observed in *Acorus* sp., *Impatiens walleriana*, *I. hawkeri*, *Pentas* sp., *Portulaca* sp. and *Caladium* sp. Damage to *Scaevola* sp. ranged from death of individual stems to death of entire plants. In control plots 99% of zinnias were infected 30 days after inoculation resulting in 68-90% canopy death.

Stimulation of radial growth *in vitro* of *Sclerotinia homoeocarpa* by subinhibitory doses of thiophanate-methyl

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Subinhibitory doses of antifungal agents can have stimulatory effects on fungi and oomycetes (chemical hormesis). In previous studies, *Rhizoctonia zeae* displayed radial growth stimulation when grown on solid media amended with ethanol at low concentrations; while radial growth and pathogenicity of *Pythium aphanidermatum* were stimulated by subinhibitory doses of fungicides. *Sclerotinia homoeocarpa*, the causal agent of dollar spot, can readily develop resistance to certain fungicides. Given the intensive use of fungicides for management of turfgrass diseases, exposure to sublethal doses is possible. The objective of this study was to assess the effect of subinhibitory doses of Thiophanate-methyl on the radial growth *in vitro* of a resistant isolate of *S. homoeocarpa*. Ten treatments were evaluated, including 9 fungicide concentrations and a fungicide-free control, with three replicates per treatment. The experiments were repeated three times. Radial growth was measured and compared among treatments using a Brain-Cousens non-linear regression model. Several statistics were estimated to detect the presence of hormesis (NOAEL= 10.3 µg/µl; MSD= 3.7 µg/µl; EC₅₀= 83.3 µg/µl). Hormesis was detected, with radial growth stimulation of up to 12.6% at doses from 7.6 µg/µl to 0.5 µg/µl. Future research will assess the effect of hormetic responses on the pathogenicity of T-methyl resistant *S. homoeocarpa* isolates and on dollar spot severity.

Trunk and soil applications of imidacloprid, thiamethoxam and acibenzolar-S-methyl for SAR control of citrus canker on young fruiting citrus trees

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Soil applications of neo-nicotinoid insecticides provide extended systemic acquired resistance (SAR) control of citrus canker caused by *Xanthomonas citri* subsp. *citri* on non-bearing citrus trees. Currently, use of neo-nicotinoids insecticides for control of the Asian citrus psyllid (*Diaphorina citri*) vector of Huanglongbing disease and leafminer (*Phyllocnistis citrella*) is limited to trees less than 2.75 m tall in part due to the potential risk for leaching of soil applied materials into the groundwater at the higher rates required for pest control on larger size trees. An alternative approach is spray application of the chemicals on the trunk. Four soil drench or trunk applications of the neonicotinoids imidacloprid (IMID; Admire Pro, Bayer) and thiamethoxam (THIA; Platinum, Syngenta) and the commercial SAR inducer acibenzolar-S-

methyl (ASM, Actigard, Syngenta) were compared to untreated controls and 9 or 10 standard 21-day interval copper sprays for protection of foliage and fruit on 5 to 6-yr old 'Ray Ruby' grapefruit trees in an orchard in Ft. Pierce, Florida. Soil drench and trunk applications similarly reduced the incidence of canker lesions on foliage and fruit but were less effective than copper sprays. SAR inducers appeared to protect fruit by reducing incidence of foliar disease, and thereby, when integrated with 21-day interval copper sprays may improve control canker on young, fruiting trees.

A microfungus from Costa Rica: *Ticosynnema* gen. nov.

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During an ascomycete-basidiomycete workshop field course at the VII Latin-American Mycological Congress, more than 100 samples of dead plant material colonized by anamorphic fungi were collected in a dry forest at the wildlife station in Santa Rosa National Park, Guanacaste, Costa Rica. Among these samples a conspicuous synnematus new microfungus was collected on the twig of an unidentified plant. Fungus was described, illustrated and identified as *Ticosynnema carranzae* R.F. Castañeda, Granados & Mardones, anam. gen. & sp. nov. (MycoBank MB800232). It is distinguished by synnematus determinate conidiomata, monoblastic integrated determinate conidiogenous cells, and solitary, 3-4-septate, oblong, cylindrical to vermiform, brown conidia that secede rhexolytically. Differs from *Leuliusinea* by brown to black conidiomata and determinate conidiogenous cells, from *Endophragmiella* by synnematus conidiomata and determinate conidiogenous cells, and from *Kramasamuha* by synnematus conidiomata and unbranched filaments. Its etymology is *Tico*-, is an indigenous word for Costa Rica, -*synnema*, referring to the type of conidioma of this anamorphic fungus and *carranzae* (Latin), named in honor of Dr. Julieta Carranza, a Costa Rican mycologist, for her contribution to Latin-American mycological progress and development.

Influence of environmental factors on aerial concentrations of *Pseudoperonospora cubensis* sporangia and cucumber downy mildew disease severity

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Aerial concentrations of *Pseudoperonospora cubensis* sporangia (sporangia m⁻³) were monitored 0.5 meters above the soil surface from May to late Sept/early Oct 2010 and 2011 in unsprayed cucumber fields at research farms in Frankenmuth and Benton Harbor, Michigan. Cucurbit downy mildew incidence and severity were evaluated weekly within each field from Jun until Sept/Oct. The first airborne sporangium was detected within five days of trapping initiation for each site-year. The greatest airborne sporangia concentrations were detected when moderate to high disease severity (>5% foliage covered) was detected within the field, fewer airborne sporangia were detected with low disease severity (<5% foliage covered), and the lowest airborne sporangia concentrations were detected prior to planting the cucumber crop. Using the combined dataset for both sites and years, it appeared that airborne sporangia concentrations, plant age (weeks post planting), cumulative solar radiation, and average temperature were the key factors that determined whether or not infection occurred. Michigan growers currently use an aggressive, calendar-based fungicide program to manage cucumber downy mildew. By better understanding the key factors that influence cucumber downy mildew disease onset, we can work toward developing an effective fungicide program that allows for fewer fungicide applications on the developing cucumber crop.

One genome, two genomes, one thousand genomes

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Genomics enables better understanding of fungal biology, interactions with plants and each other. The first sequenced genomes of mycorrhizal symbiont *Laccaria bilolor*, poplar rust *Malampsora larici-puccinia*, and saprotrophs revealed genome-encoded potentials for interactions with live and dead plants, expansions or contractions of gene sets including small secreted proteins, carbohydrate active enzymes, secondary metabolism genes. Actual interactions with plant hosts, mutualistic or parasitic, in systems like poplar-*Laccaria bilolor* and maize-*Cochliobolus heterostrophus* can be monitored

using transcriptome analysis. Decoding the combined transcriptomes of these systems and finding correlated expression of plant and fungal genes depends on availability and quality of reference genomes for both partners. Metagenomic and metatranscriptomic analyses of fungal communities, for example, in soil samples reveal their complexity and requires larger reference genome sets as produced by the 1000 genome and other large scale fungal genomics initiative. Different genomic approaches tuned for different biological questions and systems will be presented.

Introduction of orange rust caused by *Puccinia kuehnii* into the Louisiana sugarcane industry

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The first observation of orange rust infecting sugarcane, caused by *Puccinia kuehnii*, in the Americas was in Florida in 2007. To monitor for the possible introduction of orange rust into Louisiana, visual surveys were initiated throughout the Louisiana sugarcane industry among plantings of cultivars known to be susceptible to the pathogen. Additionally, four wind-vane passive spore traps were positioned at four locations in Louisiana, and ionic spore traps were positioned at two locations. Real-time qPCR was used to analyze the weekly deposits in the spore traps. Urediniospores of *P. kuehnii* were detected in the spore traps in late-October and early-November 2010, which coincided with a period of high spore production in Florida. Symptoms of orange rust, however, were not observed in Louisiana sugarcane until June 2012. The identity of *P. kuehnii* was verified using the species-specific qPCR assays. Although extensive surveys were conducted throughout 2012, observations of orange rust were limited to one cultivar, HoCP 05-961, a recently released cultivar with limited distribution. Initially, disease incidence and severity were low, increasing gradually throughout the growing season, becoming severe in November at two locations. Because disease symptoms only increased in severity late in the harvest season, the economic impact is unknown. Studies have been designed to determine what effect orange rust has on sugarcane yield.

***Sarcodon* in the neotropics—New species and the emerging circum-Caribbean distribution**

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The ectomycorrhizal (ECM) tooth fungus genus *Sarcodon* (Bankeraceae, Thelephorales, Basidiomycota) is poorly known from the neotropics. Recently we discovered a new species, *Sarcodon pakaraimensis* ined. in the Pakaraima Mountains of Guyana, in northeastern South America. The species was collected in forests dominated by the ECM tree *Pakaraimaea dipterocarpacea*, one of only two dipterocarp species known from the New World. The finding is significant given that the majority of the ~75 described species of *Sarcodon* are north temperate in distribution and frequently associate with coniferous ECM host plants. In addition to the Guyana material, we examined undescribed collections of *Sarcodon* from Puerto Rico, Belize, and Panama. Their generic assignments in *Sarcodon* were confirmed by morphology and corroborated by blastN searches of ITS sequences. Putative ECM host plants for these *Sarcodon* collections were broad-leaf genera including *Dicymbe* (Fabaceae subfam. Caesalpinioideae) and *Pakaraimaea* (Dipterocarpaceae) in Guyana, *Quercus* (Fagaceae) in Belize, and *Coccoloba* (Polygonaceae), leguminous, or unknown hosts in Puerto Rico or Panama. Among these circum-Caribbean collections we have several new species of *Sarcodon* based on preliminary molecular sequencing results and morphological comparisons. Morphological and habitat data are provided for the new taxa, and comparisons with phenotypically allied *Sarcodon* species.

Sequential utilization of hosts from different fly families by the fungal pathogen *Entomophthora muscae*

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Dynamics of infection were described for the entomopathogenic fungus *Entomophthora muscae* growing on the plant feeding fly *Delia radicum* and the predatory fly *Coenosia tigrina*. Populations of infected flies were sampled in 2011-2012 in Durham, North Carolina, USA. Infections first appeared in the middle of March on the fly genus *Delia*, and later switched to of *Coenosia*. Optimal conditions for fungal infection were: average temperature 11-28°C, dew point 3-21°C, and rain 1-3 days before sampling. Occurrence of infected flies is correlated moderately well with average temperature, lowest temperature, and dew point ($r = 0.33, 0.36$ and 0.33 respectively) and is weakly correlated ($r = 0.14$) with relative humidity. Collected specimens were genotypically close to *E. muscae* and *E. scatophagae* but differed morphologically and karyologically. This suggests the broader range of these characteristics may occur in the still incompletely resolved *E. muscae* species complex. Genotyping of rRNA ITS region identified twelve genotypes in *E. muscae* population infecting both *Delia* and *Coenosia*. Transcriptomic analysis of mRNA from infected fly bodies reveals differences in gene expression on different stages of infection development and between host species. Results illustrate the complexity of insect-fungus relationships in this ecosystem that should be considered for development of biological control methods.

Field screening of diverse *Brassica* germplasm identifies high level resistance against white leaf spot disease

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White leaf spot (WLS) is an important fungal disease of oilseed Brassicas in many countries including Australia. The causative agent *Pseudocercospora capsellae* infects a range of wild and cultivated crucifers. In Australia, WLS became prevalent during a period associated with the development of commercial canola varieties against blackleg disease with single dominant gene-based resistance, highlighting a need to identify resistance sources. Diverse *Brassica* germplasm collections from Australia, India and China (200 genotypes), were tested in field trials conducted at two locations. After sequential inoculations with a mixed mycelial suspension of four isolates of *P. capsellae*, disease severity was assessed on a 1-10 scale and Area Under Disease Progress Curve (AUDPC) values were computed. Wide variation was observed in AUDPC values among genotypes, ranging from highly susceptible at value of 375 to completely immune at 0. When comparing *B. napus* and *B. juncea* genotypes tested, *B. juncea* from India and Australia were overall more susceptible. AUDPC values for vegetable Brassicas varied from 21 to 316 demonstrating wide variations in host resistance within the group. Twenty six genotypes from *B. carinata* (viz. 21 genotypes with AUDPC = 0 and 5 genotypes with AUDPC <5) showed high levels of resistance, indicating the tremendous potential to use such species in breeding programs for improvement of resistance in commercial *Brassica* crops against this pathogen.

Gene regulatory network reconstruction in wheat pathogen *Fusarium graminearum*

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Genus *Fusarium* contains pathogens that infect hundreds of crop plants as well as humans and thus threatens global food safety and human health. Diseases caused by this group of organisms are modulated by their Gene Regulatory Networks (GRNs). Reconstructing GRNs that dynamically control all cellular functions will enable a comprehension of the complex biological processes related to pathogenesis and have broad implications in disease controls. We will report a systems biology approach to infer GRNs of *F. graminearum*, the causal agent of head blight in wheat and barley. A robust searching algorithm combining Bayesian networks model and hierarchical clustering algorithm was developed. We tested the algorithm on a collection of 198 *F. graminearum* transcriptomic datasets including both existing data from PLEXdb and the data generated in our lab to infer the relationship between candidate regulators and their target genes. Preliminary validation of the inferred networks using prior biological knowledge proofs the effectiveness of the program. In addition, we comparatively analyzed transcriptomes of *F. graminearum*, *F. verticillioides* and *F. oxysporum* f.sp. *lycopersici* under stress conditions and mutations to study the functional conservation of *Fusarium* core genomes, judging by their gene expression under same biological conditions. The conservation of the regulatory modules will allow us to transfer the network knowledge gained from *F. graminearum* to other *Fusarium* spp.

Population genetic analyses of *Verticillium dahliae* from lettuce indicates regular introduction of novel genotypes

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Verticillium dahliae has caused serious yield losses in lettuce since 1995 when the disease was first described on this host. The causes of this sudden host range expansion of *V. dahliae* into lettuce has become an intense area of research over many years. We genotyped 13 microsatellite loci of 283 *V. dahliae* isolates sampled during surveys over the past two decades. The data showed low levels of genetic variation. Comparative analyses of older collections (1995-2005, $n=69$) and more recent collections (2006-2012, $n=214$) of *V. dahliae* populations indicated that some genotypes shared by isolates collected in 1995 are still present in current populations. More importantly, new genotypes have been introduced every year. Bayesian model-based population structure analyses designated three clusters; two of which contained isolates from both newer and older collections, whereas one cluster comprised isolates collected between 2009 and 2011. Analysis of pathogenic race structure using race-specific primers showed 66% of isolates as race 1 and 34% as race 2, and all of the isolates contained the MAT1-2 idiomorph. This study indicates that the increase in the genetic variation may be attributed to continuous inflow of novel genotypes primarily from infected spinach seeds planted extensively in coastal California and a few other sources. This study provides the contemporary population genetic structure of *V. dahliae* in coastal CA.

Quantitative PCR of *Xanthomonas albilineans* in sugarcane tissues as methodology for evaluating resistance

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Leaf scald, caused by *Xanthomonas albilineans* (Xa), is a major sugarcane disease that reduces tonnage and juice quality in susceptible cultivars. The primary control measure is host plant resistance. The current system of resistance evaluation using visual rating of disease severity can be uncertain due to erratic symptom expression. A qPCR assay was developed with demonstrated potential for resistance screening; however, only four cultivars were compared. Therefore, Xa populations were compared in multiple tissues of 30 clones at different times after inoculation over two seasons. Correlations within and between qPCR and visual ratings were determined. Differences in Xa populations among clones varying in resistance were greatest in young, emerging, systemically infected leaves compared to apical meristem and stalk tissues at 8 weeks after inoculation (wai). The highest correlation between qPCR and visual ratings occurred at 8 wai (0.62, $p<0.0001$). The intermediate correlation between the methods was due in part to the erratic symptomatology. Consistency was determined by the correlation among data obtained with the same method at different times. The qPCR was more consistent among different inoculations (0.81, $p<0.0001$) compared with the visual rating system (0.54, $p=0.002$) at 8 wai. The high sensitivity, specificity, and consistency suggest that qPCR can provide an improved method to evaluate resistance to leaf scald in sugarcane.

Differentiation of *Pseudoperonospora* species and subpopulations of *Pseudoperonospora cubensis* using microsatellite loci

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Pseudoperonospora cubensis and *P. humuli* are closely related species of downy mildews that cause devastating diseases of cucurbit crops and hops, and are distributed worldwide. There are some reports that would reduce the two species to synonymy, and classify the species as *P. cubensis*. Here we report population differentiation of *P. humuli* and *P. cubensis* using seven polymorphic microsatellite loci. Our results revealed that genetic variation among all isolates accounted for 53% of total variation indicating high levels of genetic differentiation and limited gene flow. When *P. cubensis* and *P. humuli* isolates were analyzed as one hierarchical group, AMOVA results revealed that majority of diversity is distributed among groups (62.3%) rather than among individuals within those groups. When these four groups (historical, European and American isolates of *P. cubensis*, and *P. humuli*) were divided based on species, AMOVA results showed that majority of variation is distributed among individuals within groups (40.95%) when compared to among groups. Analyses with STRUCTURE indicated four different clusters among *P. cubensis* and *P. humuli*, revealing greatly consistent individual assignment probabilities. Four clusters corresponded well to geographic regions, historical samples and species differentiation. Our study

supports maintaining *P. humuli* as a unique species and clearly indicates the existence of subpopulations (both historically and geographically) of *P. cubensis*.

Two new species of *Diphymyces* (Fungi, Laboulbeniales) from Borneo

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Laboulbeniales are ascomycetous fungi that parasitize arthropods, mostly insects. The order currently comprises about 2,050 species in 140 genera. Systematic and taxonomic contributions are available for several countries in Western Europe and Northern America. A 2012 field trip by entomologist Dr. Menno Schilthuizen in Malaysian Borneo also has revealed two new species of *Diphymyces* I.I.Tav. *Diphymyces* is a Laboulbeniales genus parasitic upon beetles of the families Leiodidae and Staphylinidae. Members can be easily separated from other genera by the neighboring cells II and VI, next to the preapical outgrowths of the perithecium and the four tiers of perithecial wall cells. In recent years, nine new species have been described: *D. urbasoli* Santam. (1993), *D. penicillifer* A.Weir & W.Rossi (1997), *D. depressus* M.B.Hughes et al., *D. leschenii* M.B.Hughes et al. (2004), *D. spelaei* W.Rossi (2006), *D. arnaudii* W.Rossi & Santam., *D. giachinoi* W.Rossi & Santam., *D. pavecicii* W.Rossi & Santam., and *D. pusillus* W.Rossi & Santam. (2010). In the present poster, two more *Diphymyces* species are added to the fourteen species known so far. The hosts of the new taxa are recently described species of *Ptomaphagus* Portevin, 1914 (Leiodidae, Cholevinae, Ptomaphagini). Reviewing all available literature of Laboulbeniales from the island of Borneo, which is known for its rich insect fauna, yielded up to 95 species of these parasites for the island, including the two new *Diphymyces*.

The Laboulbeniales of the Boston Harbor Islands

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Laboulbeniales (Fungi: Ascomycota: Laboulbeniomycetes) are obligately ectoparasitic on arthropods. Thalli persist on their hosts' exoskeleton after host death thus existing arthropod collections can be used to determine parasite ranges and host relationships. Laboulbeniales mainly infect insects, though a number of species have been reported to occur on mites and millipedes. Because of the wide range of insects preserved, All Taxa Biodiversity Inventories (ATBIs) of arthropod communities are particularly useful for determining the associated Laboulbeniales diversity at a given site. Between 2005 and 2010 76,211 specimens of arthropods, comprising about 1800 species, were collected from the Boston Harbor Islands National Recreation Area, which spans 34 islands and peninsulas in waters near Boston, MA. The islands consist of a variety of habitats - including marine and estuarine intertidal wetlands and freshwater marshes. Although surveys of the Laboulbeniales have not hitherto been conducted based on these collections, the arthropods of the Boston Harbor Islands are well documented. Here we present the first assemblage of Laboulbeniales found on beetle hosts in the families Carabidae (ground beetles) and Coccinellidae (lady beetles). Further identifications of Laboulbeniales on arthropod collections will elucidate not only the ecology of Laboulbeniales but also provide insight into the diversity and distribution of arthropod-associated fungi.

MSA Student Section 2013

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During this year's annual meeting in Austin, the Mycological Society of America will be developing its Student Section. This student-run group within the MSA will facilitate communication among all students of the Society as well as provide opportunities for students to network with other students in their own fields and beyond. It will also be valuable for student members of MSA seeking connections with those performing cutting edge research in mycology and thus has the potential to inspire future collaborative research. The Student Section is open and inclusive, welcoming the participation of all students (and faculty!) in building this group. We look forward to your participation in our mixer and hope you can also join us at our future events.

Target spot, caused by *Corynesporium cassiicola*, reduces yield of cotton in Alabama

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Impact of target spot on lint yield and quality was evaluated using a factorial design arranged in a split plot with cotton variety (Phytogen 499 and Deltapine 499), which were planted May 9, as the main plot and pyraclostrobin (Headline 2.09SC) application number as the split plot treatment. Headline 2.09SC at 9 fl oz/A applications began at first bloom (July 5) and were repeated at 14-d intervals for a total of one, two, three, four, and five fungicide applications. A non-treated control was included. While the study was rained, rainfall totals for July and August exceeded the 30 yr average. Disease was rated weekly over 6-wk beginning the 3rd week of bloom (July 31) when symptoms were first seen to September 13. Target spot intensity, which was higher on Phytogen 499 than Deltapine 1050, declined as Headline application number increased on both varieties. Yield gains obtained with increasing Headline application numbers were higher on the susceptible Phytogen 499 than the tolerant Deltapine 1050 variety. Yield sharply declined as target spot intensified on Phytogen 499 but less so on Deltapine 1050. Disease-related yield loss on Phytogen 499 may have exceeded 40% as compared with 6% for Deltapine 1050. Lint quality and loan value was not impacted by Headline application number or disease intensity but often differed by variety. Efficacy of the recommended two Headline application program was limited against target spot on the susceptible Phytogen 499 variety.

Surprising results and implications of the Florida psyllid testing project

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Huanglongbing (HLB), citrus greening disease, is one of the worst citrus diseases in the world. In Florida, it is associated with '*Candidatus Liberibacter asiaticus*' (Las) and transmitted by a psyllid, *Diaphorina citri* Kuwayama. We tested *D. citri* collected in many different venues over a period of six years for Las by molecular methods with surprising results. First, positive *D. citri* can be found long before symptoms develop on the plants at the site. Second, positive psyllids can ride on unprocessed fruit in trailers, even when there is no foliage. Third, about 10% of psyllid samples collected from plants for sale in Florida tested positive for Las. Fourth, samples from neighboring conventional and organic citrus groves showed that although the pesticide in the conventional grove reduces the numbers of psyllids, the samples that were collected in the conventional grove frequently were positive for Las. Finally, our data, and a related mathematical model, predict a form of transmission of Las that bypasses the latent period in the plant. This means that increase of infected vectors follows the growth of the insect population, independent of the latent period in the plant. The implications of this result completely change the epidemiology of HLB. It is possible to have positive *D. citri* throughout a grove before ever seeing a symptomatic plant. The mechanism for this novel transmission now is known.

Multi-drug resistance to site-specific fungicides in populations *Monilinia fruticola* in Pennsylvania orchards

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This study was initiated following grower concerns about decreased efficacy of fungicides used for control of brown rot (*Monilinia* spp.) in some Pennsylvania orchards. Single spore isolates were obtained from samples collected from 13 orchards and confirmed as *M. fruticola* with a PCR protocol targeting the *cyt b* gene. The sensitivity of 582 isolates to the sterol-demethylation inhibiting (DMI) fenbuconazole and propiconazole, and the strobilurin (QoI) azoxystrobin fungicides was assessed in vitro. Isolates were separately plated on potato dextrose agar (PDA) amended with 0.5 µg/ml of the DMI fungicides, or 0.2 µg/ml of azoxystrobin and 75 µg/ml salicylhydroxamic acid. The percentage of relative mycelial growth (RMG) on amended PDA compared with normal growth on nonamended PDA was determined and used as an indicator of sensitivity to the fungicides. There was significant variation among orchard in sensitivity to the fungicides with median RMG values ranging from 0.0-97.9% for fenbuconazole, 0.0-85.6%

for propiconazole and 3.3-71.9% for azoxystrobin. Out of the 582 isolates 22, 27, and 22% of the isolates were resistant to fenbuconazole, propiconazole and azoxystrobin, respectively. Isolates with shifted sensitivity to azoxystrobin comprised of 41% of the population, while only 21 and 29% were shifted to fenbuconazole and propiconazole, respectively. These results confirm that some Pennsylvania orchards have a severe problem with multiple fungicide resistance.

Genetic diversity in epichloae of *Bromus laevipes*

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Fungal endophytes belonging to the genus *Epichloë* and *Neotyphodium* can provide substantially greater fitness traits for their host plant. Many of these endophytes are known to produce bioactive compounds (e.g. ergot alkaloids, indole-diterpenes, lolines and peramine) that can deter grazing animals. To better understand the selective advantages and how these associations form, we isolated endophytes from *Bromus laevipes*, a cool-season bunchgrass native to California and Southern Oregon. A total of 58 isolates from 12 different populations were then analyzed for their morphological and genetic characteristics, and each endophyte was defined to the species level. PCR was used to determine the alkaloid genotype of the *PER* (peramine), *EAS* (ergot alkaloid), *LOL* (lolines), and *IDT* (indole-diterpenes) loci of individuals from each population. The PCR data revealed four distinct genotypes, all of which were positive for two or more different classes of alkaloid. PCR of the mating type genes (*MTA* and *MTB*) combined with DNA sequencing of the house-keeping genes, *tefA* and *tubB*, provided evidence of the ancestral progenitors and revealed at least three different epichloae species can be hosted by *B. laevipes*. The diverse evolutionary histories identified within *B. laevipes* endophytes may provide insight into the broader ecological implications of endophyte-plant symbioses.

Fungi of the healthy human gut

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In the past couple years, awareness of the importance of the human-associated microbiome has blossomed. The composition and role of bacteria in the gut are now well-established, but the role and composition of fungi in healthy individuals is just beginning to be characterized. We have profiled the gut fungi from 55 healthy adults - 41 on a conventional Western diet and 14 vegetarians. *Candida* yeasts are the most abundant gut fungi, with *C. tropicalis* predominant in persons consuming a Western diet but in much lower abundance in the vegetarians. Yeasts in the Dipodascaceae are second to *Candida* yeasts in overall abundance. *Cladosporium* is the most prevalent filamentous fungus. *Malassezia* is detected with moderate frequency, while a number of additional fungi are detected in one or two samples. Some of these "other" fungi include *Fusarium*, and food-associated fungi such as *Penicillium roqueforti* (blue cheese manufacture) and *Agaricus bisporus* (button mushroom, portabella).

Genetic and epigenetic profiling of *Fusarium graminearum* following serial subculture

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Fusarium isolates are notably unstable in culture and given to degradation unless certain precautions are taken. After only a few rounds of serial subculture, isolates can irreversibly lose the ability to form sporodochia, followed by conidia. The mechanism of these changes is unknown. To understand the nature of *Fusarium* morphological changes in culture, we began subjecting the sequenced strain of *F. graminearum* to serial subculture in July, 2011. Multiple lineages were begun from an initial soil stock, and each lineage is subcultured weekly, and a sample stored under glycerol at -80°C. For this study, we have performed shotgun pyrosequencing using 454 FLX-Plus on five of the lineages from the 37th subculture; we have also sequenced one lineage from the beginning of the study (when all lineages should have been identical, and not significantly different from the published *F. graminearum* genome), and compared all to the published genome. Finally, we have used Illumina for bisulfite pyrosequencing to obtain methylation profiles.

Evaluating the silencing suppressor activity of proteins encoded by *Maize rayado fino virus*

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Maize rayado fino virus (MRFV), the type member of the genus *Marafivirus*, family *Tymoviridae*, is transmitted in a persistent, circulative manner by leafhoppers of the genus *Dalbulus*. Symptoms of MRFV infection on leaves of its maize host are small chlorotic spots that coalesce into short stripes. The 6.3 kb genomic ssRNA encodes a 224 kDa polyprotein with replication protein motifs at the amino terminus, and two capsid proteins (CPs) with 3' co-terminal sequences at the carboxy terminus. A putative ORF of 43 kDa with unknown function has been proposed. Silencing suppressor proteins encoded by plant viruses are diverse; it is not known if MRFV-encoded proteins possess silencing suppressor activities. Expression of the MRFV CPs in *Nicotiana benthamiana* using a *Potato virus X* (PVX)-based vector resulted in MRFV VLP accumulation and enhanced the pathogenicity of PVX. In addition, co-infiltration of agrobacteria containing GFP and MRFV CP constructs resulted in enhanced GFP fluorescence and accumulation of GFP protein, but at levels lower than observed in the presence of the silencing suppressor p19. These observations suggest that the MRFV CP may be a weak silencing suppressor. Additional MRFV proteins did not show the same silencing suppressor activity. This is the first report suggesting that marafiviruses encode silencing suppressor proteins; however their role in viral pathogenicity is not known.

Effect of different drainage systems on soybean root rot

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Soybean root rot and seedling diseases can lead to significant stand loss and yield reduction. Several soil microorganisms are able to infect soybean plants early in the season, especially in soils with excessive soil moisture. In 2008, four drainage regimes (conventional, controlled, shallow and no drainage) were established in a field in southeastern Iowa. In 2012, soybean seedlings were sampled at V2 stage. Root rot severity was visually assessed and root pieces were plated to assess frequency of fungi infecting roots. Diseased and healthy root pieces were surface sterilized prior to plating onto water agar, V8 agar and corn meal agar. Root pieces on water agar were incubated under continuous light, and V8 and corn meal agar were incubated in darkness. Fungi were identified to genus based on morphological characteristics. Root rot severity was not significantly different among drainage regimes. Differences were observed between drainage regimes for frequency of isolation but with poor repetitiveness. The most frequently isolated fungi were *Fusarium* spp., which were isolated from 70% of roots. No *Phytophthora* or *Pythium* species were isolated. The extreme drought in Iowa in 2012 may explain the lack of drainage effects in this trial. Greenhouse work is ongoing to investigate how soils from different drainage regimes affect root rots at three moisture gradients, and field trials will be repeated in 2013.

Observations on the life cycle of *Ophiostoma vaccinii*

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Ophiostoma vaccinii causes a foliar disease of *Vaccinium arboreum* known as fly speck disease. Although the host is not considered economically important, the similarity to commercial blueberries makes it desirable to understand the biology of this disease in the event of host transfer, inasmuch as the plants often occur in close proximity. Field and laboratory observations over a period of several years have yielded previously unknown details of the life cycle of this disease. The disease cycle is initiated in the spring with the advent of warm temperatures and ample rainfall, which stimulate the maturation of ascomatal initials in overwintered leaves on the ground. Ascospores are discharged from the resulting perithecia upward onto the undersides of newly formed leaves at the base of the host plant. Ascospore germination leads to the penetration of the leaf tissues and the subsequent formation of yellowish lesions containing acervuli, which produce large numbers of filiform conidia. These conidia spread the disease throughout the plant resulting in heavy infection that continues during the summer and into autumn. In early autumn, ascomatal initials can be detected in the leaf tissues; these remain in suspended development as the leaf dehiscence and falls to the ground in late autumn. The leaf overwinters in this condition until spring,

when favorable conditions begin the cycle again. The complete life cycle thus requires one year to complete.

Fusarium stalk blight and rot in sugar beet

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Fusarium stalk blight of sugar beet can cause reductions or complete loss of seed production. The causal agent is *Fusarium oxysporum*. In addition, *Fusarium solani* can cause a rot of sugar beet seed stalk, and other species have been reported associated with sugar beet fruit, but their effect on seed production is not known. We sampled diseased seed stalks and examined isolates for their pathogenicity and virulence on sugar beet seed stalks in greenhouse tests. Isolates of *F. oxysporum* representing three different genetic groups associated with *Fusarium* yellows were examined for their effect on seed stalks. Seed stalk tissue of sugar beet germplasm that had been found to vary in response to *Fusarium* yellows, in response to stalk blight in field screening, or recombinant inbred lines were inoculated. *Fusarium oxysporum* was the most commonly isolated species from seed stalks, but three other species also were isolated from stalk lesions, and all caused damage to seed stalks when inoculated. Isolates from two of the three genetic groups of *F. oxysporum* f. sp. *betae* isolates caused similar symptoms on seed stalks. Isolates of other species caused more cortical rot. Response to different *Fusarium* species varied among the sugar beet germplasm. There was evidence of low to high susceptibility to all species tested among the beet germplasm screened, but low susceptibility to one species did not always correlate with low susceptibility to other species.

Biological control of *Phytophthora nicotianae* by heat-tolerant bacteria in irrigation water

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Bacteria in the genera *Bacillus*, *Paenibacillus*, and *Pseudomonas* survived an improved heat treatment (48°C, 24 h) of irrigation water and became abundant in this aquatic environment. The objective of this study was to examine whether such heat-tolerant bacteria had any antagonistic activity against *Phytophthora nicotianae*, one of the most destructive plant pathogens in irrigation water. Specifically, eleven selected bacterial isolates from these genera were evaluated individually and in combination using a liquid culture assay. Bacterial and *P. nicotianae* zoospores suspensions were mixed for 24 h, and the survival of zoospores was determined by plating the mixtures on selective media. Two *Paenibacillus* isolates, two *Pseudomonas* isolates, and their combinations showing inhibitory activity against zoospores of *P. nicotianae* were further assessed *in planta*. They all provided disease control on lupine seedlings. These results demonstrated that some naturally-occurring, heat tolerant bacteria could potentially contribute to the control of plant pathogens in the aquatic environment. They also indicate that the new heat treatment is compatible with other disease management strategies.

Investigating the role of motility in *Salmonella enterica* root colonization

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Salmonella enterica, the causal agent of non-typhoidal salmonellosis, is commonly vectored to humans by plants, specifically raw produce. However, genes required for plant colonization are largely unknown. A previous report has shown that swarming is involved in *S. enterica* colonization of roots. Thus, we hypothesized that flagella-mediated motility is required for *S. enterica* root colonization. Multiple single gene deletion mutants from each of the three functional categories, flagella biosynthesis, motility, and chemotaxis, were examined for root colonization competence. A *flhF* mutant has no functional flagella while a *motA* mutant is paralyzed; neither had a root attachment or colonization defect at 1, 24, 48 or 72 h post inoculation (hpi). These results suggest motility is not required for *S. enterica* root colonization. CheY is the response regulator which upon phosphorylation generates clockwise rotation. CheB is a phosphorylation-activated response regulator that resets bacterial movement upon recognition of repellents in the environment, lengthening the pauses in flagellar rotation. Both *cheY* and *cheB* mutants were defective in swimming and swarming, but only the *cheB* mutant had smaller root populations at both 24 and 48 hpi compared to the WT. The role of chemotaxis in plant colonization is still under investigation. This research will provide critical understanding of the basic biology of human pathogens in association with plants.

The root-shoot dichotomy in citrus–*Citrus tristeza virus* interactions

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The path by which *Citrus tristeza virus* (CTV) establishes an infection in susceptible species is well understood: Movement of particles from the site of inoculation to sink tissues in the roots and young flush, followed by systemic movement into more distant phloem cells. Some species however, prevent systemic infection to specific strains of the virus. Here we infected a gradient of host species ranging from susceptible *Citrus macrophylla*, and the selective hosts *C. reticulata*, and *C. sinensis* x *P. trifoliata*, with two different CTV strains, T36 and T68, and seven T36-T68 hybrids covering the 3' ORFs and the L1/L2 domains, to examine what determines host selectivity. Testing the roots, stem and young flush tissue of infected plants by ELISA at three month intervals after inoculation showed that while all isolates tested could readily infect *C. macrophylla*, the more selective hosts restricted virus infection to the roots for T36 and 6 of 7 T36-T68 hybrids. Isolate T68 and hybrid 1390 (partial p20 to 3' UTR substitution) were weakly positive in the stem tissues as well as positive in the roots of Swingle citrumelo, while T68 alone was able to move out of the roots of Carrizo citrumelo. These data suggest that the ability to establish a systemic infection involves specific sequence variants located in the 3' end of the genome and raises a more important question, of why the roots of selective hosts are differentially susceptible to infection by some isolates?

***Citrus tristeza virus* seedling yellows symptom induction changes microRNA expression levels**

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Seedling yellows (SY), the stunting and leaf chlorosis of juvenile trees, caused by select *Citrus tristeza virus* isolates, is unusual in that unlike the two other disease syndromes of CTV, stem pitting and quick decline, it is a temporary condition. To understand the expression of seedling yellows we inoculated a series of *Citrus macrophylla* with T36/FS577 strain hybrids that induce severe or mild seedling yellows on this host and compared to wild type T36 and healthy control plants. Symptom expression occurred 6 months post inoculation, at which time the small RNA population from symptomatic tissue of each host was sampled and sequenced to examine the differences in microRNA expression levels. A total of 28 miRNAs were found to have greater than three-fold difference in expression between symptomatic, asymptomatic and uninfected tissue. RT-qPCR showed that while most were downregulated relative to the healthy control, five (miR166, miR168, miR169, miR395 and miR477) involved in host cell regulatory, replication and AGO1 regulation process were differentially expressed in SY tissue compared to the controls. In addition, 10 potential novel microRNAs and their stem-loop precursor sequences were identified, of which 4 were found to be expressed by RT-qPCR in all hosts examined, and 2 were differentially expressed in SY tissue relative to the controls. Further investigation of these miRNAs and their targets will aid in the understanding of SY symptom expression.

Problems in ITS-rDNA taxonomy: Hypervariable ITS sequences among isolates and within single-ascospore strains of *Ceratocystis fimbriata sensu stricto*

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The South American species *C. fimbriata* ss is homothallic, soilborne, and depends on insects or humans for long-distance dispersal. Thus, natural populations are genetically isolated and may acquire local variations in DNA sequences, especially in ITS-rDNA. Some ITS haplotypes have been introduced from Brazil to other locations and described as new species without distinguishing phenotype. Isolates from introduced populations that appeared to be clonal based on microsatellite markers varied at up to 14 bp in ITS sequence. In four isolates, TA cloning and sequencing of PCR products from single-ascospore strains identified two or more ITS sequences representing different putative species (1 spore = 2 species). Strains of eight ITS haplotypes representing the range of ITS diversity were fully interfertile in sexual crosses. Maximum parsimony (MP) and maximum likelihood (ML) analyses of ITS sequences of 116 isolates of recently described species and Brazilian isolates gave poorly resolved trees. In contrast, analyses of mating type genes (*MAT1-1-2* and *MAT1-2-1*) resolved a single MP tree with a topology identical to that of the ML tree, with branches of high bootstrap and posterior probability support. These analyses support three pheno-

typic species (based on pathogenicity): *C. platani*, *C. cacaofunesta* and *C. colombiana*. However, five ITS species (*C. manginecans*, *C. mangicola*, *C. mangivora*, *C. acaciivora*, and *C. eucalypticola*) appear to be synonyms of *C. fimbriata* ss.

WITHDRAWN

WITHDRAWN

Screening of biocontrol agents for protection of chile peppers plants against *Phytophthora* and *Verticillium* caused wilts

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Phytophthora capsici and *Verticillium dahlia* are among the worst plant pathogens that affect chile production in the desert southwest. Chemical and cultural controls are not effective for controlling these pathogens and despite years of intensive breeding effective natural resistance has not been identified in chile for either pathogen. Bio-control is an approach that has the potential to provide sustainable control of these pathogens. We are currently performing a large scale screen to identify potential biocontrol agents for use against these pathogens. To date ~500 bacterial strains have been isolated from soil or chile roots and tested for biocontrol activity. Isolations were intentionally biased for the selection of *Bacillus* spp. which have shown promise as biocontrol agents in other systems. Results presented include efficacy testing against chile infecting strains of *P. capsici* and *V. dahlia* in both *in vitro* plate inhibition assays and *in vivo* plant protection assays. ~10% of bacterial strains tested to date have shown activity against one or both of the target pathogens and are being carried forward for further analysis including rRNA sequence based identification of the bacterial strains and additional efficacy testing.

A multilocus phylogenetic reconstruction of the *Pachyphloides-Scabropezia* lineage (Pezizaceae)

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Pachyphloides, a poorly known ectomycorrhizal truffle genus from the Northern Hemisphere temperate zone, has 13 recognized species. *Pachyphloides* is an unusual genus because species morphologies span the range of variations in Pezizaceae truffles. The variation is represented at one extreme by *P. austro-oregonensis* with open canals, amyloid cylindrical asci, and discrete paraphyses and at the other extreme by *P. virescens* with a solid gleba, subglobose inamyloid asci, and indistinguishable paraphyses. The cup fungus *Scabropezia* has been phylogenetically placed as sister to *Pachyphloides* or embedded within the genus, making *Pachyphloides* paraphyletic. This study more closely examined the relationship between *Scabropezia* and *Pachyphloides*, using phylogenetic analyses of ITS, LSU, RPB1, and RPB2 alignments, and morphology to delimit species. The multilocus analyses infer *P. austro-oregonensis* within the *Scabropezia* lineage within the *Pachyphloides* lineage. The polarity of fruitbody evolution in the *Pachyphloides-Scabropezia* lineage is unresolved. This is the only known occurrence of an epigeous form within a hypogeous lineage in the *Pezizales*. Results from phylogenetic analyses provide significant support for 35 species.

Population genomic RAD-Seq characterization of the boxwood blight fungus, *Calonectria pseudonaviculata*

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Boxwood blight was first detected in North America in October 2011 in Connecticut and North Carolina. Since then, it has been found in seven additional U.S. states and three Canadian provinces. The source of the causal fungus *Calonectria pseudonaviculata* is unknown. The pathogen may have been introduced from infections in Europe and/or New Zealand, but could have originated from endemic non-pathogenic populations. The objectives of this study were to develop a whole genome-scale suite of variable genomic markers in *C. pseudonaviculata*, estimate general world-wide genetic diversity, and determine the source of the boxwood blight fungus in North America. Ten *C. pseudonaviculata* isolates obtained from the U.S. (CT, NC, OH, OR, and VA), the United Kingdom, Italy, Belgium, and New Zealand were sequenced using paired-end RAD-Seq technology. Sequences were analyzed for polymorphic SNPs and other variable markers. Genetic variation was estimated among samples, and U.S. samples were compared to one another and those collected from outside the U.S. to address potential origins of *C. pseudonaviculata* in North America. Locating the source of outbreaks will allow targeted mitigation efforts against the spread of boxwood blight, and estimates of genetic diversity will be used to improve best management practices.

Improving recommendations for grape berry moth and fruit rot management in high brix Niagara grape production

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The production of high brix grape juice (*Vitis labrusca* 'Niagara') requires a delay in harvest increasing the risk of loss from fungal fruit rots. Our objective was to evaluate that risk and improve pesticide recommendations for high brix Niagara production by comparing a current grower standard (GS) pesticide program to an IPM strategy utilizing site specific weather and pest information. In both years of the study, fruit rot (*Botrytis cinerea*) increased with delay of harvest and was associated with grape berry moth (*Polychrosis viteana*) infestation (GBM). In 2011, there was significantly more GBM damage at 14 brix with the GS than the IPM program, but no significant difference with respect to fruit rot ($P \leq 0.05$). With delay of harvest (16 brix), the severity of GBM damage was 37% greater in the GS than the IPM program, and the IPM program significantly reduced fruit rot by 28% over the

GS. In 2012, there was no difference in rot between programs at 13 brix, but at 16 brix the IPM program had 44 % less rot than the GS though the difference was not significant. Phomopsis fruit rot (*Phomopsis viticola*) was not an issue in either year, but the IPM program significantly reduced the incidence and severity of Phomopsis shoot lesions in 2012 when compared to GS, due to an early mancozeb spray at 3-5" shoots. Better timing of insecticide sprays through the IPM treatment reduced losses to rots when harvest was delayed for higher brix accumulation.

Cantharellaceae of the Guiana Shield: New species of *Craterellus*, and new distribution records for *Cantharellus guyanensis* and *Craterellus excelsus*

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Nearly 100 species of Cantharellaceae are known from tropical regions, although a small proportion of these (ca. 15 species) are documented from the neotropics. In the present study we provide data on all known neotropical *Cantharellus* and *Craterellus* species, the majority of which occur in the Guiana Shield region of northeastern South America. *Craterellus olivaceoluteus* ined. and *Craterellus cinereofimbriatus* ined. are new to science. These fungi were collected from Guyana in association with ectomycorrhizal trees in the genera *Dicymbe* (Fabaceae subfam. Caesalpinioideae) and *Pakaraimaea* (Dipterocarpaceae). *Cantharellus guyanensis* Mont., originally described from French Guiana, is redescribed from recent collections from Guyana, with additional range extensions from French Guiana, eastern Venezuela and central and eastern Brazil, circumscribing nearly the entire Guiana Shield region. A new distribution record from French Guiana is provided for *Craterellus excelsus* T.W. Henkel & Aime. Macromorphological, micromorphological, and habitat data are provided for each species as well as DNA sequence data from the nuclear ribosomal regions of the internal transcribed spacer and 28S large subunit. The systematics of these taxa within the Cantharellaceae was evaluated using molecular phylogenetic analyses. This work brings the number of Cantharellaceae species known from the Guyana to nine.

Inoculation-based assessment of infection susceptibility to anther smut disease (*Microbotryum* spp.) among wild species of the Montiaceae

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Historically, coevolution has been considered the primary process to explain disease dynamics between hosts and their pathogens. Recent research has highlighted the relative importance of host shift events in the evolutionary divergence of pathogen species. The purpose of this project is to examine the evolutionary history of the association between smut fungi (*Microbotryum* spp.) and the plants they infect within the Montiaceae family. Spore samples from infected specimens collected as part of a large-scale herbarium survey were used in molecular analyses. Initial phylogenetic reconstructions suggest a pattern of divergence among *Microbotryum* isolates that reflect host taxonomic classification. However, geographic distribution also seems to have contributed to speciation, in particular to the potential for host shift events involving sympatric species from the Caryophyllaceae. We carried out a series of controlled inoculations on selected species of Montiaceae using ten different isolates of *Microbotryum* under greenhouse conditions. The percentage of successful infections and the severity of smut symptoms were determined and compared against the phylogenetic distance between source host and novel host species. We discuss these results in light of the potential role of host shift events on the observed patterns of diversification in these plants.

WITHDRAWN

Uncertainty and agricultural decision making under climate change: When do decision support systems fail, become more important, or require updating?

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Effective management is imperative to avoid or minimize yield losses caused by diseases, pests, or abiotic stressors. Decision support systems (DSS) and early warning systems (EWS) have the potential to support adaptation strategies for management under climate change. We developed a framework for evaluating the circumstances where DSS/EWS are effective for improving decision making at the season-level or using within-season information. Variability in weather patterns is an important factor, as is the nature of non-stationarity in weather time series under climate change. The quality (skill) of models used for constructing weather indices for yield loss is also an important factor, along with the spatial heterogeneity of the environment in which the models are applied. DSS/EWS may break down if unusual weather conditions that have not been used in model development become more frequent. In general, if a disease is too common or too rare, DSS/EWS are less useful. Under climate change, the frequency of important epidemics at a given location may shift, such that DSS/EWS become important tools. Under rapidly changing conditions, DSS/EWS may require updating, particularly when their models include more limited mechanistic components. We present a summary of the current and future climate scenarios in which DSS/EWS are most useful, and the types of ongoing modifications that may be necessary to make DSS/EWS resilient under climate change.

Networks of stored grain diseases and pests: Strategies for sampling and mitigation

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Pathogens and pests of stored grains move through complex dynamic networks linking fields, farms, and bulk storage facilities. Human transport and other forms of dispersal link the components of this network. A network model for pathogen and pest movement through stored grain systems is a first step toward new sampling and mitigation strategies that utilize information about the network structure. An understanding of network structure can be applied to identifying the key network components for pathogen or pest movement through the system. For example, it may be useful to identify a network node, such as a local grain storage facility, through which grain from a large number of fields will be accumulated and move through the network. This node may be particularly important for sampling and mitigation. In some cases more detailed information about network structure can identify key nodes that link two large sections of the network, such that management at the key nodes will greatly reduce the risk of spread between the two sections. In addition to the spread of particular species of pathogens and pests, we also evaluate the spread of problematic subpopulations, such as subpopulations with pesticide resistance. We present an analysis of stored grain pathogen and pest networks for Australia and the United States.

Diversity of lignicolous freshwater ascomycetes from an urban lentic environment of Mexico City

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The lignicolous freshwater ascomycetes play an important ecological function in the decomposition of submerged woody debris in the aquatic environments. The diversity of this group of fungi from lentic Mexican ecosystems is little-known. A sample was taken from interconnected artificial ponds located in the St. Angel Ecological Reserve to determine species diversity. Fifteen stations were established and wood baits were submerged for 8 wk, afterwards, the sample units were processed and examined in the laboratory. The fungi obtained were isolated, identified and preserved *ex situ*. The diversity of species recorded was $H = 0.801$. A total of 13 ascomycetes was recorded, but only *Naïs inornata*, *Trichocladium lignicola*, *Triadelphia uniseptata* and *Trichocladium* sp. were strictly freshwater species, with the first mentioned species being the most frequent (23.9 %). The low values of species diversity and strict freshwater species probably is due to this aquatic ecosystem being relatively new, as it was established in 2005. In addition, other elements, e.g., the quality of groundwater that feeds the ponds, the vegetation that is growing on their banks, also could be involved. The presence of *N. inornata* is interesting because it is a species commonly reported from natural lentic environments, whereas in this study, it occurred in an artificial aquatic system within a densely urbanized area of Mexico City.

Phylogenetic analyses place *Paranectria* in the Nectriaceae

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The genus *Paranectria* Sacc. (Ascomycota, Sordariomycetes, Hypocreales) includes nectrioid fungi that are characterized by their lichenicolous habit; orange, KOH– perithecia; and multiseptate to muriform ascospores. Because this genus is KOH–, it has been placed in the Bionectriaceae. The anamorph of *Paranectria* was unknown. A pure culture was isolated from a recent collection of *Paranectria oropensis* and, surprisingly, the anamorph was determined to be *Fusarium*-like. The discovery of the anamorphic state challenges its current higher classification because *Fusarium*-like fungi are only known from the Nectriaceae. A multilocus tree was constructed based on five loci (ITS, *act1*, *mcm7*, *rpb1*, and *tubB*) to determine phylogenetic placement of *Paranectria*. Our results indicate that *Paranectria oropensis* is nested within the clade of *Microcera* (Ascomycota, Sordariomycetes, Hypocreales, Nectriaceae), which have an insecticolous habit and *Fusarium*-like anamorph. The *Microcera* clade also included isolates that have been identified as *Fusarium larvarum*, but isolated from lichens. Our results suggest that the generic concept of *Microcera* needs to be expanded to accommodate fungi with a lichenicolous habit. *Paranectria* is considered a taxonomic synonym of *Microcera*, and a new name combination will be proposed to reflect this relationship.

A begomovirus infecting Gold Veined Oxalis

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Plant viruses have been shown to be the cause of the yellow vein foliage of several ornamental plants. Gold Veined Oxalis (*Oxalis corymbosa*) is an ornamental plant with attractive yellow vein foliage available through some nursery catalogs in the United States. Due to the nature of the symptoms, we suspected that a begomovirus may be the causal agent of the yellow vein. Therefore, we performed PCR amplifications with Gold Veined Oxalis DNA using degenerate primers for the genus *Begomovirus*. A 1.2 kb PCR product was obtained using primers PAL1v1978 and PAR1c496 which were reported to amplify 1.1–1.4 kb DNA fragments of several begomoviruses. Sequencing of the fragment confirmed the begomovirus nature of the amplified DNA. Successful whitefly transmissions of the putative begomovirus to healthy *O. corymbosa* were obtained with *Bemisia tabaci*. Partial sequence information and phylogenetic analyses revealed that the oxalis begomovirus is closely related to *Tomato yellow spot virus* and *Sida mottle virus*, two begomoviruses reported in Brazil. The host range of the oxalis begomovirus is not known. Nevertheless, this finding illustrates the potential spread of plant viruses to

different geographical locations through the commercialization of virus-infected ornamental plants.

Carbon source-dependent efficacy of anaerobic soil disinfestation (ASD) in suppression of *Rhizoctonia* root rot of apple

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Rhizoctonia solani AG-5 is a significant component of the pathogen complex that incites apple replant disease (ARD). A non-fumigant alternative, such as anaerobic soil disinfestation (ASD), is highly desired for control of ARD. We examined the influence of carbon input as a determinant of ASD efficacy in the suppression of apple root infection by *R. solani* AG-5. Pasteurized (P) and non-pasteurized (NP) *R. solani* infested orchard soil was amended with rice bran (RB), *Brassica juncea* seed meal (SM), ethanol (ET), steer manure (M) or grass clippings (GR). Pots were inundated to field capacity, bagged, incubated for two weeks, aerated and then planted with five 9 weeks old 'Gala' seedlings per pot. After 5 wks, plants were harvested, and biomass and *R. solani* root infection were determined. To examine activity of ASD-generated volatiles against *R. solani*, pure cultures of the pathogen were placed on the surface of similarly amended NP soil and incubated in sealed bags during the anaerobic phase. Among treatments, GR and SM yielded the highest seedling weights and, with the exception of M, all treatments significantly reduced root infection compared to the no treatment control. Disease was higher in P than NP soil. Volatiles from all C amended ASD soils significantly retarded colony growth compared to control, but volatile composition differed.

Plant-microbe relationships determine winners and losers in response to nitrogen pollution

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In response to Nitrogen (N) enrichment, *G. rossii*, a co-dominant plant in moist meadow alpine tundra at Niwot Ridge (CO), dies back while *D. cespitosa*, its principal competitor, expands. We ask whether contrasting host responses to N are mediated by shifts in plant-microbe symbioses. Using 454 pyrosequencing, we compared the effects of N on root-associated microbes (RAM) of *G. rossii* and *D. cespitosa* in experimental plots. We found root-fungal communities from a nitrophobic host were more sensitive to N than communities from a nitrophilic host. The *G. rossii* fungal taxa most (negatively) affected by N (Helotiales) are also the most abundant (>80% of sequences), and may play a role in accessing organically bound N. Conversely, *D. cespitosa* bacterial communities were more sensitive to N relative to *G. rossii*'s. The *D. cespitosa* bacteria species most (positively) affected by N has fungicidal properties, and forms symbioses with animals to improve resistance to fungal infection. Mutualistic fungi can become parasitic under nutrient enrichment, these bacteria may improve *D. cespitosa*'s immunity to fungal parasitism. Pathogens were unaffected by N, indicating loss/gain of mutualists may be underemphasized in diagnosing plant health. This evidences the potential importance of belowground microbial dynamics in determining plant response to N deposition.

The localization and dynamics of actin in *Aspergillus nidulans*

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Fungal hyphae develop exclusively by apical growth. New ways of controlling plant and animal mycoses may be discovered through the understanding of the hypha and other cellular dynamics in filamentous fungi. F-actin, a cytoskeletal component of microfilaments, plays an important role in fungal growth and development. It can be found in different forms, including cables and patches. Lifeact is a fluorescent reporter of actin dynamics in live cells. The Lifeact construct was transformed into *Aspergillus nidulans* to monitor the dynamics of actin using time lapse imaging and fluorescence microscopy. Sub-apical collars, composed of F-actin patches associated with endocytosis, were found to be necessary components for polar growth in the hyphae of *A. nidulans*. Actin cables, which form rings, are observed in the formation of septa. Actin rings were also found to predict the site of emerging, lateral branches. Similar to what has been observed in *Neurospora crassa*, apical arrays of actin were found in the apices of growing

hyphae. A new structure was documented which was termed the sub-apical actin web (SAW). The SAW consisted of a mass of F-actin cables and was found distal to the apex. The SAW is more often present in mature hyphae, whereas the apical array is typically found in younger hyphae. Further research is needed to understand the dynamics and mechanisms of the SAW and apical array. Current work documenting actin dynamics during anastomosis will be discussed.

General suppression of Fusarium wilt of watermelon via spring incorporated *Vicia villosa* and *Trifolium incarnatum* cover crops

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Fusarium wilt of watermelon (*Fusarium oxysporum* f. sp. *niveum*), is prevalent on the eastern shore of Maryland and in Delaware. A fall planted, spring incorporated cover crop, *Vicia villosa*, reduces Fusarium wilt severity of watermelon. However, the means of this suppression is unknown. The objective of this experiment was to determine if the mechanism of *V. villosa* disease suppression is general or specific. In five trials, watermelons were planted in nonamended soils and soils amended with the cover crops, *V. villosa*, *Trifolium incarnatum*, *Secale cereale*, or *Brassica juncea*. Changes in overall soil microbial activity were measured using an EGM-4 gas analyzer, which monitors soil respiration, and changes in *F. oxysporum* soil populations were monitored via soil dilutions. Fusarium wilt severity was also recorded during the growing season. In four of the five trial's there were significant elevations in CO₂ respiration in *V. villosa* and *T. incarnatum* amended plots compared to nonamended plots. These elevations were significantly negatively correlated with Fusarium wilt in *V. villosa* and *T. incarnatum* amended plots, indicating that general disease suppression occurred. *Fusarium oxysporum* also increased significantly in *V. villosa* amended plots, as much 15.54x compared to bare ground, suggesting a form of specific suppression may play a role in this pathosystem.

Sensing stomata: The circadian regulation of pathogenicity in *Cercospora zeae-maydis*

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Cercospora zeae-maydis (*Czm*) causes gray leaf spot of maize, one of the most widespread and destructive foliar diseases of maize in the world. Hyphae of *Czm* exhibit stomatal tropism and form appressoria over host stomata before penetrating leaves. Recently, the blue-light photoreceptor *CRP1* was shown to be required for stomatal tropism, appressorium formation, and lesion development. Orthologs of *CRP1* in other fungi regulate innate circadian rhythms by modulating the expression of *Frequency (FRQ)*, a core clock component. To elucidate the role of circadian rhythmicity in gray leaf spot, we deleted *FRQ* in *C. zeae-maydis* and characterized the effect on infection. Interestingly, *FRQ* deletion strains were apathogenic when inoculated on maize leaves. Histological observations revealed that *FRQ* deletion strains did not exhibit stomatal tropism, and failed to form appressoria upon encountering stomata. Because of deficiencies in pre-penetration infectious development, lesions were not observed following repeated inoculations. These findings demonstrate a unique role for a fungal circadian clock in foliar pathogenesis and illuminate a previously unrecognized level of complexity underlying the regulation of stomatal infection during gray leaf spot of maize.

Atkinson's fungi: Documenting the legacy of Cornell's preeminent mycologist, George F. Atkinson

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George F. Atkinson (1854-1918) was Cornell's 2nd professor of mycology and a world authority on mushrooms and other fungi. His herbarium now forms a subcollection of the Cornell Plant Pathology Herbarium (CUP). The Atkinson collection includes our oldest specimens (dating from the 1850s) and nearly 1,000 types: 300 of them species that Atkinson himself described. A pioneer in scientific photography, Atkinson documented many collections with photographs as well as notes. Since mushrooms are preserved in a dried state, but characters of fresh specimens are key in their identification, his photos and notes provide critical information. A three-year NSF DBI (Biological Research Collections) grant for databasing and conserving Atkinson's specimens, photos and notes has resulted in 45,000 new specimen records, 8,000 digitized photographs and 4,600 digitized notecards—tripling our existing digital specimen database and digital photo collection. In

addition, many specimens, and all photographic negatives were re-housed during the project. Outreach and education were a big part of our project; 33 students were trained in managing biological collections. Atkinson data and photographs are now accessible through MycoPortal (<http://mycoportal.org>) and ArtStor (<http://library.artstor.org>), building international infrastructure for biodiversity research. These online records are an important first step towards a mycoflora of the Finger Lakes region of upstate New York.

WITHDRAWN

The quantity and genetic diversity analysis of Grapevine vein clearing virus in four types of grapevine tissues

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Grapevine vein clearing virus (GVCV) is the first DNA virus discovered in grapevine and closely associated with a vein-clearing and vine decline disease in the Midwest region of the United States. Its genome of 7,753 bp contains three open reading frames (ORFs). ORF3 is predicted to encode domains of Zinc Finger (ZF), Reverse Transcriptase (RT) and RNase H. In this study, qPCR was conducted to measure the copy number of GVCV in young leaf, fully expanded leaf, stem phloem tissue and root tip of three individual grapevines that were infected with the GVCV type isolate. A grapevine β -actin gene was also quantified to calibrate the copy number of GVCV in these four tissues. As a result, GVCV has an average of 4.6 copy/actin in young leaf, 3.7 in fully expanded leaf, 14.4 in phloem, and 0.5 in root tip, respectively. Statistic analysis of GVCV copy number per β -actin indicated that the quantity of GVCV in the root tip is significantly different from that of GVCV in the other three tissues. A genetic diversity study has also been performed within the ZF and RT region of GVCV variants from the four tissue types. It was found that the tissue type did not impact the genetic diversity of the GVCV ZF and RT region.

WITHDRAWN

Bacterial population changes in fields treated with anaerobic soil disinfestation

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Anaerobic soil disinfestation (ASD) can control a broad spectrum of soilborne pests. During ASD treatment, soil becomes anaerobic and pH decreases. It was hypothesized that resident bacterial populations play a role in generating anaerobic conditions and pathogen control. In the present study, time course soil organism 16S rDNA samples were taken and partially amplified using the V2 and V3 primers. LH-PCR was performed using a tagged V3 primer. The greatest changes in the ratio of amplified 16S fragments over the course of the treatment were seen with amplicons found in the 445 (+/- 3bp) base pair range. In pretreatment samples from two fields, amplicons in this range were either undetectable or were found to constitute less than 20% of the population. Within 48 hours of ASD initiation, these amplicons were detected in all samples and contributed 24-78% of the total population. The increasing population of amplicon 445 is correlated with decreasing soil pH. In untreated soil, there were no shifts in pH, and minimal fluctuation of the 445 amplicon group. At the end of ASD treatment, a reduction in the 445 amplicon group was associated with oxygen returning to the system, indicating this could be composed of facultative or obligate anaerobes. The sequenced 445 amplicons were identified as either *Bacillus* or *Paenibacillus* spp. using BLASTn.

WITHDRAWN

Population biology of *Microcyclus ulei*, the causal agent of South American leaf blight of rubber trees in Latin America

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An extensive sampling was conducted to assess the genetic variability of *Microcyclus ulei*, the causal agent of South American leaf blight (SALB), in commercial plantations of rubber trees in Central and South America. Fourteen local populations from Brazil (Amazon and coastal region) and 5 from Ecuador, Guatemala and French Guiana were analyzed using 16 microsatellite loci. A total of 283 multilocus genotypes were identified among 312 isolates sampled. Isolation by distance and high gene diversity were detected even in areas distant from the Amazon region, the putative center of origin of the pathogen. Some geographically distant populations were genetically related and five clusters of local populations were detected: 3 clusters comprised of local populations from Brazil, 1 of Ecuadorian and Guatemalan isolates and 1 with isolates from the French Guiana. Isolates from

the Brazilian coastal area were introduced from three different sources: two areas located in the upper Amazon region and another from an unsampled region. Restricted gene flow was detected between populations. The spatial pattern of the genetic variation of *M. ulei* is the result of historical gene flow probably affected by anthropogenic action and genetic drift caused by the fail of connectivity between rubber tree plantations from Latin America.

Influence of hosts with partial resistance on the genetic structure of the pathogen *Microcyclus ulei* in *Hevea* spp.

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Strategies to manage South American leaf blight (SALB), the most destructive disease of rubber trees, are limited and the main resource has been planting partially resistant clones. Proper knowledge about how the deployment of resistant and susceptible *Hevea* clones affects the genetic structure of populations of the causal fungal pathogen *Microcyclus ulei* needs to be generated. We analyzed 39 isolates of *M. ulei* collected in susceptible (Fx 3864 and IAN 717) and 29 from resistant (CDC 312, FDR 5788, MDF 180 and PMB1) rubber clones of *Hevea brasiliensis* planted in the Northeast region of Brazil. All individuals were genotyped for 17 microsatellites loci and 67 multilocus genotypes were identified. Bayesian cluster analysis revealed that *M. ulei* individuals could be clustered into four genetically distinct groups that coexist locally. Groups 1 and 4 were formed by individuals collected in susceptible clone; group 2 is a mix of individuals from susceptible and partially resistant clones; and group 3 has individuals sampled from the partially resistant clones. There was strong genetic differentiation between isolates sampled from susceptible and resistant clones. The type of resistance and the genetic background influence the levels of heterozygosity of the population of *M. ulei*. Higher heterozygosity and allele richness were recorded for groups 2 and 3 compared to groups 1 and 4.

Diverse TAL effectors converge on a single host susceptibility gene in citrus canker

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Citrus canker is a devastating disease that infects many citrus varieties. The causal agents are members of a complex of distinct yet related strains of *Xanthomonas*. *X. citri* subsp. *citri* strain Xcc306 harbors four members of the type III transcription activator like (TAL) effector gene family, one of which (*pthA4*) is required for pustule formation. Transcriptional profiles of citrus leaf infected with Xcc306Δ*pthA4* complemented with either *pthA4* or *pthAw*, also from *X. citri* subsp. *citri*, included two genes that were highly up-regulated and contained predicted effector binding elements (EBE) in the respective promoters. *CsLOB1* is a member of the lateral organ boundaries (LOB) domain gene family, and *CsN3-1* is a member of the nodulin 3 (SWEET) gene family. *pthA4*, *pthAw*, *pthB*, and *pthC*, of which the latter two are from *X. fuscans* pv. *aurantifolii*, all complemented pustule formation, activated *CsLOB1* transcription in citrus and drove expression of *CsLOB1* promoter reporter gene fusions. Only *pthA4* and *pthAw* induced *CsN3-1*. Artificially designed TAL effectors (dTALEs) which target specific sequences in the *CsLOB1* promoter region, but not the *CsN3-1* promoter, restored pustule formation to Xcc306Δ*pthA4*. Transient expression of *CsLOB1* in citrus also elicited pustule-like symptoms. The results indicate that diverse citrus canker-inciting species of *Xanthomonas* converged on a single host susceptibility gene, *CsLOB1*, using different members of the TAL effector family.

Live bacterial population dynamics of ‘*Candidatus Liberibacter asiaticus*’, the bacterial agent associated with citrus huanglongbing, in two plant hosts

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Citrus Huanglongbing (HLB) is a century-old destructive disease which presents an unprecedented challenge to citrus industries worldwide. In Florida, HLB is associated with the phloem-limited bacterium ‘*Candidatus Liberibacter asiaticus*’ (Las), and is mainly transmitted by Asian citrus psyllid (*Diaphorina citri*). Quantification of the pathogen population in a host aids in investigation of virulence mechanisms and disease management. Recently a

procedure was developed to detect live bacterial populations using a novel DNA-binding dye, propidium monoazide, with real-time polymerase chain reaction (PMA-qPCR). From August 2010 to April 2012, the change of the live Las populations in psyllid-inoculated sweet orange (*Citrus sinensis* ‘Valencia’) and Chinese Box Orange (*Severinia buxifolia*) plants was monitored by PMA-qPCR. Our results showed that the live Las population was lower from August 2010 to March 2011, and significantly higher live populations developed from April 2011 in both hosts. No obvious pattern in the total bacterial population was observed in either host. This pattern may indicate a seasonal growth of Las along with the growth of both plants. These new findings should provide useful information on HLB.

Persistent effects of wildfire on foliar endophytes of *Quercus hypoleucoides* and *Juniperus deppeana* in southeastern Arizona

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Trees in fire-adapted ecosystems form intimate symbioses with endophytic fungi that inhabit healthy leaves and stems. How these symbionts respond to major wildfires has not been studied previously. Using a patchwork of burned and unburned areas of otherwise contiguous coniferous forest in southeastern Arizona, we examined endophytes in regrowth of trees that burned in a large wildfire in 2005 vs. those in neighboring, conspecific trees that never burned. Fresh foliage of 24 mature individuals of *Quercus hypoleucoides* and *Juniperus deppeana* was collected in early 2011 and 2013. The importance of burn status, microsite, host species, and relevant interaction terms was evaluated. Isolation frequency differed significantly only among microsites, and diversity differed only between host species; neither differed as a function of burn status nor varied directly with leaf water content (both species) or indices of plant health (evaluated only for *Q. hypoleucoides*). Both leaf chemistry and endophyte community structure for each species differed as a function of burn status in 2011, revealing detectable traces of fire history ca. 5 years after the wildfire; however, sampling in 2013 did not find such differences. Comparison with regional datasets revealed that burned and unburned trees are inhabited by distinct subsets of the larger mycoflora of the Sky Island biological zone, some of which may be especially suited to survival or colonization after fire.

Maize lipoxygenase LOX2 regulates pathogenesis of mycotoxin-producing *Aspergillus flavus*

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Maize is not only a major cereal consumed, but also the most important feed grain in the United States. Infection by mycotoxigenic fungi contaminates seed with hazardous metabolites which render them unfit for consumption leading to severe economic losses worldwide. Especially concerning are *Aspergillus* species that produce aflatoxin, the most potent naturally occurring carcinogen. Emerging evidence has implicated a group of oxygenated lipids, termed oxylipins, as important endogenous signals in plants and fungi. In plants, oxylipins are produced by the lipoxygenase (LOX) pathway and the best understood oxylipins are jasmonic acid and green leaf volatiles produced by the 13-LOX reaction. However, the role of 9-LOXs, especially in plant-pathogen interactions, has not been well studied. Fungal oxylipins are produced mainly by *Psi* producing oxygenases (Ppo) and involved in regulating sporogenesis and mycotoxin biosynthesis. Interestingly, oxylipins produced in plants and fungi are structurally and biochemically similar, suggesting that they may mediate cross-kingdom communication. In this study, maize kernels of wild-type and an oxylipin-deficient mutant (*lox2*) were infected with *A. flavus* strains (WT, Δ*lox*, Δ*ppoA*, Δ*ppoC*, Δ*ppoD*, and OX-LOX). Three and five days after infection, ergosterol, conidia, and aflatoxin were measured to elucidate the role of individual host and pathogen enzymes in producing the oxylipin blend that determines the outcome of the interaction.

The Hesler papers: Digitization of unpublished photographs, species descriptions, and morphology of collections in Tennessee and other herbaria

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Phytopathology 103(Suppl. 2):S2.62

Lexemuel Ray Hesler (1888–1977) was an eminent American mycologist. During his tenure at the University of Tennessee, he worked together with Alexander Smith (University of Michigan) to compile North American

Monographs of numerous genera always with emphasis on fungi of the southern Appalachians. His research notebooks contain morphological data on individual specimens in TENN and MICH, species descriptions, illustrations and keys. Type specimens and other worldwide materials are also included. As part of the Macrofungi Consortium effort to database and digitize fungal images, we undertook the task of digitizing the Hesler research notebooks. Initial tests with digitization quality using a camera, several copiers and an HP scanner indicated that a copier provided high quality images and was considerably faster than alternate procedures. We selected a Konica-Minolta BizHub 283 copier and scanned the notebooks directly to pdf files. Some photographic images did not copy well. For these, we used a Nikon camera to create a jpg of the image. Jpg files were converted to pdf files and files representing one notebook were assembled using Adobe Acrobat Pro. Images representing TENN collections were extracted from the pdf files as jpg files and are available at IdigBio as part of the TENN collections. PDF files of complete generic notebooks are available at <http://tenn.bio.utk.edu>.

Evolutionary consequences of putative intra- and interspecific hybridization in agaric fungi

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When formerly allopatric, genetically divergent populations return to sympatry, three outcomes are possible: 1. Individuals from different populations are reproductively isolated as a consequence of genetic divergence and no inter-mating occurs; 2. Individuals from different populations interbreed but there are postzygotic barriers to gene exchange between them; and 3. Individuals from different populations freely interbreed. The southern Appalachians are populated by plants and fungi that were formerly isolated in diverse glacial refugia. Four putative agaric inter-population hybrids were identified in collections from the southern Appalachian Mountains: *Armillaria mellea*, *Amanita citrina* f. *lavendula*, *Gymnopus dichrous* and the *Hygrocybe flavescens/chlorophana* complex. Within *Armillaria mellea* and *Amanita citrina* f. *lavendula*, we found evidence of interbreeding and recombination. In *G. dichrous* and *H. flavescens/chlorophana*, hybrids were identified but there is no evidence for F₂ or higher progeny in natural populations suggesting that the hybrid fruitbodies may be an evolutionary dead end. The association between levels of ITS haplotype divergence of less than 5% with the presence of putative recombinants, and greater than 5% with apparent failure of F₁ hybrids to produce F₂ or higher progeny in nature may suggest a generalized correlation between genetic distance and reproductive isolation.

De novo RNA-Seq and bioinformatic analyses uncover genetic determinants of fungicide detoxification in the turfgrass pathogen *Sclerotinia homoeocarpa*

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Cool season turf grasses on golf courses in New England require high fungicide inputs to control dollar spot, making *S. homoeocarpa* an ideal system for investigating the transcriptomic basis of reduced fungicide sensitivity. We used massively parallel sequencing, including *de novo* hybrid assembly and RNA-Seq, to investigate mechanisms of reduced sensitivity to demethylase inhibitor (DMI) fungicides, a quantitative trait in *S. homoeocarpa*. Our hybrid assembly was generated from 454 pyrosequencing and paired-end reads of sequencing by synthesis (SBS) data, yielding 12,494 contigs over 500 bp. The SBS reads for two biological replicates were mapped back in pairs to the hybrid assembly for fungicide treated and untreated samples of two isolates. Statistical analyses of digital expression values and gene ontology (GO) categories of differentially expressed transcripts identified metabolic pathways likely involved in reduced sensitivity to DMIs. We report enrichment of transcripts associated with multiple stages of xenobiotic detoxification, as well as lipid metabolism. Quantitative Real-Time PCR in field isolates causing practical field resistance, which displayed reduced sensitivity to multiple DMIs and additional fungicide classes, verified findings for select transcripts. From these data, we report parallels to genetic determinants of resistance identified in other fungal pathogens, and to those described from research into herbicide and insecticide detoxification.

Development of molecular diagnostic assays for fungicide resistance in an important turfgrass pathogen, *Sclerotinia homoeocarpa*

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The dollar spot pathogen, *Sclerotinia homoeocarpa*, has demonstrated the ability to overcome the dicarboximide, Demethylation Inhibitor (DMI) and Methyl Benzimidazole Carbamate (MBC) fungicide classes in the United States. Reduced sensitivity and resistance to these three fungicide classes, which constitute important control options for dollar spot, indicates the need for an accurate and quick diagnostic assay for fungicide resistance in the dollar spot pathogen. By mining transcriptomic data of *S. homoeocarpa*, we have targeted the resistance determinants for these three fungicides, and designed PCR-based assays for the detection of fungicide resistance alleles and gene expression of fungicide resistance determinants in field isolates. Two techniques are being optimized for these assays: high-resolution DNA melting analysis (HR-DMA) and quantitative Real-Time PCR (qRT-PCR). Preliminary data indicate that HR-DMA and qRT-PCR can be used to detect resistance to all three classes. If implemented, these assays could help turfgrass managers to minimize costs and environmental consequences from overuse and misapplication of fungicides that prove ineffective at sites with resistance.

Identification of sources of resistance to *Plasmopara halstedii* in wild annual sunflower (*Helianthus annuus*)

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Downy mildew, caused by *Plasmopara halstedii*, is an economically important disease of sunflower, and in 2009, isolates virulent on the widely-used *Pl₆* gene were identified in the United States (U.S.). The primary sources of resistance to *P. halstedii* have been wild *Helianthus* species, including wild *Helianthus annuus*. The objectives of this research are to 1) identify sources of resistance to commonly occurring *P. halstedii* races and, 2) identify resistance to isolates virulent on *Pl₆*. To identify sources of resistance to commonly occurring *P. halstedii* virulence phenotypes, 286 wild *H. annuus* from 25 U.S. states were screened against field isolates with virulence phenotypes 300, 730, and 773. To identify sources of resistance to isolates conferring virulence to the *Pl₆* gene, accessions previously identified as resistant to commonly occurring races, and an additional 215 accessions originally derived from Texas, were screened against a field collection of *P. halstedii* race 734. Of the 286 accessions screened against common races, 21 percent had less than 10 percent infection to race 300, 9 percent had less than 10 percent infection to race 730 and 3.8 percent had less than 10 percent infection to race 773. Those eleven entries had less than 10 percent infection against all three races; nine of which originated from Texas. Preliminary data suggests that sources of resistance to race 734 will be found in ongoing screening efforts.

Testing the effect of *Trichoderma* volatile organic compounds on *Arabidopsis thaliana*

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Plant and bacterial volatile organic compounds (VOCs) are known to influence plant growth but less is known about the effects of fungal VOCs. *Arabidopsis thaliana* was used to test the effects of VOCs from *Trichoderma viride* on plants. Petri plates of *T. viride* were placed in a growth chamber in a shared atmosphere with *A. thaliana* without physical contact. Compared to controls, plants grown in the presence of *T. viride* VOCs were taller, bigger, flowered earlier, had more lateral roots, increased total biomass (45%) and chlorophyll concentration (58%). GCMS analysis of *T. viride* VOCs revealed 51 compounds; several compounds were tested individually at one part per million to determine the causative agent of growth promotion. Acute exposure to these compounds produced differential physiological effects. For example, 2-ethylhexanal inhibited 100% of seeds from germinating, while 2-heptanone prevented 80% of seeds from germinating. Seeds exposed to 1-decene and octanoic acid germinated but were not able to form seedlings. We conclude that VOCs emitted by *T. viride* have growth promoting effects on *A. thaliana*; but the individual compounds tested are not responsible for this phenomenon at a concentration of one ppm with acute exposure. Further tests at lower concentrations may mimic the result in observed in *T. viride* VOC exposed

plants. This research represents a new avenue of study in the complex interactions between microbes and plants.

Species concepts in Geoglossomycetes

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The class Geoglossomycetes (Ascomycota), commonly referred to as earth tongues, is currently comprised of one family (Geoglossaceae) and six genera (*Geoglossum*, *Glutinoglossum*, *Nothomitra*, *Sabuloglossum*, *Sarcoleotia*, and *Trichoglossum*). Published estimates of species diversity have been mostly local, with global estimates approaching 60 distinct species. However, more than 200 names of species, varieties, and forms have been validly published over the course of nearly three centuries of study, with many now reduced to synonymy or regarded as inaccurate. This research presents the latest results of our modern, multi-gene, species-level phylogeny of Geoglossomycetes. Maximum likelihood and Bayesian phylogenetic analyses using multi-gene sequences (ITS, LSU, MCM7, RPB1) were conducted along with analyses of morphological characters to reconcile gene trees and species trees. Species complexes in *Geoglossum* including the *Geoglossum barlae/nigrum/umbraile* complex and the *Geoglossum cookeanum/glabrum* complex have been identified and are discussed. Biogeographic patterns of speciation are also addressed. This study reveals cryptic speciation is common in *Geoglossum* and *Trichoglossum*.

Thermophilic fungi across diverse latitudes and elevations in the western United States

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Defined as fungi that grow better at 45°C than at 25°C, thermophilic fungi were discovered more than a century ago. Yet very little is known about the natural roles and distribution of these organisms. Although common in “sun-heated soils,” they have most often been recovered from manmade composts, and one hypothesis suggests that they evolved as decomposers in naturally-occurring compost. An extension of this hypothesis suggests that propagules found outside compost have been dispersed by wind, an idea that seems nearly impossible to reconcile with their high frequency and broad distribution of occurrence. To better understand natural distributions, we have isolated thermophilic fungi over small and large spatial scales. Our survey has focused on soil, litter and herbivore droppings sampled from diverse ecosystems (deserts, grasslands and forests) across eight western states from southern deserts to alpine ecosystems in Colorado and Montana. Our results show that thermophiles can be isolated readily from all of these substrates at every latitude and elevation. As part of this survey we also discovered that several species of thermophilic fungi can survive storage in soil samples for several years at -80°C. We are currently characterizing many of the isolates from these collections to assess patterns of species distribution on local and broad scales.

Ppmd1 plays an important role in the asexual development of *Phytophthora parasitica*

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Phytophthora parasitica is a notorious oomycete pathogen that causes severe disease in a wide variety of crop species. Plant infection by this pathogen involves mainly its asexual development, whereby papillate sporangia are formed, followed by differentiation and release of biflagellate zoospores. Calcium signaling has been proposed to play a key role in these processes. This study deals with the functional characterization of *Ppmd1*, which encodes a component of a putative calcium channel. We analyzed the expression of *Ppmd1* at different life stages of this pathogen by qRT-PCR. Moreover, we generated *Ppmd1*-silenced mutants of *P. parasitica*, which showed reduced expression of *Ppmd1* and exhibited severe defects in its asexual development. Sporangia formed by the mutants lacked papilla and failed to release zoospores in response to cold shock. Instead, they tended to germinate directly. Further analysis revealed that, in response to cold shock for induction of zoospore formation, no sign of cytoplasmic cleavage was detected in the sporangia of *Ppmd1*-silenced mutants. Interestingly, when the sporangia induction solution was amended with CaCl₂, the mutants became able to form papillate sporangia, which released zoospores upon cold shock. Inoculation experiments indicated that *Ppmd1*-silenced mutants showed

reduced virulence toward *Nicotiana benthamiana*. These results indicated that *Ppmd1* plays an important role in the asexual development of *P. parasitica*.

It takes a village: New insights on the fungi that raise mycoheterotrophic plants from seedlings to adults

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Mycoheterotrophy entails plants meeting all, or a portion of their carbon and other nutrient demands via fungi. Some plants have adopted a mycoheterotrophic strategy at only the very earliest stages of their development, while others remain partially or fully dependent on fungi throughout their life cycles. While a fair number of studies have illuminated the fungi associated with adult mycoheterotrophs, the identities of seedlings' mycobionts have remained a mystery. Here we present new data on the fungal hosts for initially mycoheterotrophic plants in the tribe Pyroleae (Ericaceae). Using a molecular and phylogenetic approach, we found that unlike many mature and fully mycoheterotrophic plants, seedlings associated with a suite of fungi. These included ectomycorrhizal Basidiomycetes and taxa with a diversity of trophic functions in the order Sebaciales. We juxtapose our results with prior findings on the fungi associated with adult ericaceous and orchidaceous fully and partially mycoheterotrophic species. For the first time, we now have a more complete picture of the fungal communities that nurture these intriguing plants through all stages of their development.

WITHDRAWN

Draft genome sequence and partial annotation of *Xanthomonas arboricola* pv. *corylina*

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Xanthomonas arboricola pv. *corylina* (*Xaco*) is a leaf spot and twig blight pathogen of filbert (*Corylus* spp). We recently identified *Xaco* on Turkish filbert (*C. colurna*) in Colorado. To obtain insights into the genomic characteristics of this isolate compared to those collected from other hosts and regions, we sequenced its genome using Illumina Hi-Seq 2000 system and then assembled it using A5 pipeline and SPAdes assembler. The resulting assemblies were similar to each other, having a total length of about 5.2 Mbp and a GC content of 65.4%. A total of approximately 4,450 coding sequences representing 445 gene categories were identified using the RAST server. We used a BlastN approach to look for common genes reported in *Xaco* isolates, and also for genes coding for the Type III secretion system and Type III effector proteins, both considered important for pathogenicity and host specificity in pathogenic bacteria. We found that *avrBs3* that is present in all

other *Xaco* isolates was absent, based on computational and subsequent PCR assays, in the Colorado *Xaco* genome. However, we detected another gene with similarity to an *avr* gene described in *X. campestris* pv. *vasculorum*. The results indicate that the Colorado *Xaco* isolate is distinct from isolates collected from European filbert (*C. avellana*) in Europe and from a *C. maxima* isolate collected in the USA in 1939.

Genomics-based diagnostic marker development for *Pythium* and *Phytophthora*

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Pythium and *Phytophthora* are important pathogens of many plants, but they can be difficult to identify, even to the genus level, by morphological characteristics in routine laboratory diagnosis. Thus, it would be desirable to have a rapid and accurate method of identifying these pathogens to the genus, clade or species level. We used a genomics pipeline to compare the sequenced genomes of 7 *Pythium* spp., representing 6 phylogenetic clades within the genus, and 3 *Phytophthora* spp. in order to identify unique regions for development of diagnostic markers. A suite of sequences were identified that computationally distinguished *Phytophthora* from *Pythium*, *Pythium* clade K (*Phytophthora*) from *Phytophthora* and other *Pythium* clades, and between filamentous and globose sporangial *Pythium* species. Other sequences have been identified that exhibit species specificity in *Pythium*. Results of the computational analysis were confirmed through development and testing of oligonucleotide primers in conventional PCR. However, these markers could be adapted to other molecular diagnostic techniques.

Transcriptome analysis of the snow rot pathogen *Pythium iwayamai*

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Pythium iwayamai is a psychrophilic pathogen of turfgrasses and small grains. The mechanism by which this oomycete survives and infects plants under snow cover has not been studied in detail. We isolated RNA from *P. iwayamai* grown at 7 C and 19 C and in the presence and absence of 'Penncross' creeping bentgrass. RNA-seq was used to assemble and compare the transcriptomes under the various treatment combinations. These transcriptomes were also compared to those of the non-psychrophilic *P. irregulare* grown under the same conditions. *P. iwayamai* differentially expressed some proteins, including proteases and abc transporters at the lower temperature and in the presence of bentgrass. The proteases were not expressed at low temperatures in the absence of the bentgrass. Few glucosidases were differentially expressed and no antifreeze proteins were detected. Transcriptomes of *P. irregulare* differed from those of *P. iwayamai* at both temperatures, with more proteases and glucosidases differentially expressed at the higher temperature. Results suggest that increased protease activity may be an important component of the infection process of *P. iwayamai* at low temperatures and *P. irregulare* at higher temperatures.

Characterization of protein biomarkers linked to transmission competent and transmission refractive aphid and whitefly populations in Nigeria

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Some of the most economically important plant viruses in sub-Saharan Africa (SSA) are vectored by aphids and whiteflies. Variation in competence in transmission of circulative viruses has been demonstrated in aphids and whiteflies. This study is assessing occurrence of homology of previously identified transmission biomarkers in two species of aphids, *Aphis craccivora* (cowpea) and *Pentalonia nigronervosa* (banana), and a whitefly, *Bemisia tabaci* (cassava), and to discover additional species-specific markers in whiteflies in SSA. Genomic DNA, mRNA, and proteins were isolated from insect colonies obtained from different locations and used for mining the biomarker expressing genes. 6/15 markers are conserved across *S. graminum*, *A. craccivora*, *P. nigronervosa* and *B. tabaci*, showing that the genes involved in virus transmission may be conserved in multiple vector species and taxa. Polymorphisms at ATPD biomarker loci were observed in CACol3 (one of the colonies of cowpea), at 332/757 consensus positions. Also, PITP biomarker discriminated between cowpea aphid colonies and two other field collected cowpea aphids at 249/302 consensus positions, where the colonies had A and the field collected ones had G and A/G, respectively. Virus transmission

assays and mass spectrometry-based proteomics experiments are ongoing to determine the phenotype transmission competence and to characterize the expression of these biomarkers in aphids.

Relationship of canopy reflectance and foliar NO³-N to anthracnose severity on an annual bluegrass putting green

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Anthracnose of *Poa annua* L., caused by *Colletotrichum cereale* Manns sensu lato Crouch, Clarke and Hillman, continues to be a challenging disease on putting greens throughout the United States. Nitrogen fertility is known to influence anthracnose, although optimum rates to suppress the disease may vary annually and are often based on subjective assessments of color. A field study was conducted on a *P. annua* putting green turf in Storrs, CT during 2011 to evaluate the relationship between anthracnose severity and foliar NO³-N, chlorophyll index (CHL), and normalized difference vegetative index (NDVI). Nitrogen treatments were applied at 0.0, 2.4, 4.9, 9.8, 14.7, 19.5, 24.4, and 36.7 kg ha⁻¹ every 14 days from 30 May to 11 August. Anthracnose developed in late-June and severity declined from 76 to 4% with increasing N rate during July. No consistent relationship was observed between NO³-N measured in sap extracted from clippings and anthracnose severity. CHL and NDVI values were lower in plots where anthracnose was more severe; plots with values greater than 250 or 0.780 generally were ≤ 10% blighted, respectively. These data suggest that it may be possible to use reflectance meters to guide N fertility practices to minimize anthracnose incidence on putting green turf.

Evaluation of atoxigenic strains of *Aspergillus flavus* for aflatoxin control in corn on commercial farms in Texas

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Two products consisting of non-aflatoxigenic strains of *Aspergillus flavus*, Afla-Guard and AF 36, were evaluated for their effectiveness to reduce aflatoxin contamination in corn. The products were applied to the tops of the rows by hand at 10 lb/A at V5 or VT growth stage. Replicates were 8 rows by 100 feet long and there were four replicates per treatment. Treatments were separated by 100 feet. The grower's combine was used to obtain a steady stream sample of 5 to 10 lb., which was ground using a Romer mill and analyzed using the Vicam system. In 2011, rainfall was substantially below normal during the growing season at all three farms. In Ellis county, the atoxigenic strain treatment significantly (*P*=0.05) reduced the average aflatoxin levels to 37% of the control, which was 340 parts per billion (ppb). In Hill county, aflatoxin levels with atoxigenic treatments were 35 to 42% of the control, which was 161 ppb. However, this reduction was not uniform among replicates, nor was it statistically significant (*P*=0.05). At the Nueces county farm, aflatoxin was significantly (*P*=0.05) reduced by 93% with Afla-Guard from the control, 31 ppb. In 2012, rainfall was adequate at the Jackson county farm. Here, the control aflatoxin was 60 ppb, while all the atoxigenic treatments were 0 to 5 ppb. This experimental approach showed that atoxigenic strain treatment reduces aflatoxin and can be used to evaluate factors to improve their effectiveness.

Phenology of infection on apple fruit by sooty blotch and flyspeck fungi in Iowa apple orchards

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Sooty blotch and flyspeck (SBFS) is a fungal disease complex that can cause significant economic losses to apple growers by blemishing apple fruit with dark fungal colonies. In 2009 and 2010, we investigated the timing of infection on apple fruit by SBFS species in six commercial apple orchards in Iowa. Five 'Golden Delicious' trees in each orchard received no fungicide sprays after fruit set. Within 3 weeks after fruit set, 50 apples per tree were covered with Japanese fruit bags to exclude SBFS inoculum. Subsequently, 5 apples per tree were exposed for a single 2-week-long period and then re-bagged for the remainder of the growing season. There were seven consecutive exposure periods. Apples that were bagged for the entire season or exposed all season served as checks. Apples were stored at 2°C for 6 weeks after harvest, and SBFS colonies on the apples were identified to species using PCR-RFLP. A total of 15 species were identified. Seven species (*Schizothyrium pomi*, *Microcyclosporella mali*, *Dissoconium aciculare*, *Stomiopeltis* spp. RS1 and RS2, *Colletogloeopsis* sp. FG2, and *Peltaster* sp. P2.1) infected apples during each of the exposure periods, but higher numbers of colonies per apple were observed on fruit exposed during June than during July or

August. Our findings are the first evidence of SBFS species-specific patterns for timing of inoculum deposition and infection on apple fruit.

Phylogenetic analyses to assess the evolutionary origins of sooty blotch and flyspeck fungi on apple

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The sooty blotch and flyspeck (SBFS) complex of ascomycetes causes economically important blemishes of apple fruit worldwide. About 90% of SBFS species are in the order Capnodiales. However, evolutionary relationships of SBFS fungi with close relatives that do not cause SBFS remain unclear. We attempted to reconstruct the evolutionary history of major SBFS lineages by using ancestral state reconstruction, utilizing the 28S nuclear large subunit region (LSU) of rDNA and RPB2, which encodes the largest subunit of RNA polymerase II. The analyzed taxa encompass numerous genera of SBFS and non-SBFS fungi from seven families within the Capnodiales. The non-SBFS taxa were selected based on their distinct ecological niches, including plant parasites, animal parasites, and saprobes. Results of phylogenetic analysis of LSU sequences suggest that most SBFS species are closely related to plant parasitic fungi. A preliminary ancestral state reconstruction based on LSU data suggests that plant parasitism represents an ancestral state for most SBFS lineages. Further ancestral state reconstruction of ecological niche of SBFS fungi and their closest relatives are underway using a Bayesian approach and RPB2 sequences. Knowledge gained from this study may help us to better understand the ecology and evolution of epiphytic plant-inhabiting fungi on apple fruit.

Characterization of plant growth-promoting and disease suppressing abilities of certain actinomycetes isolated from groundnut rhizosphere

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Actinomycetes are widely used bacterial biocontrol agents in plant disease management. The present study focused on isolation and characterization of certain actinomycetes from groundnut rhizosphere for growth-promotion and pathogen suppressiveness. Dual culture studies were conducted to assess the antifungal abilities against aflatoxin producing fungi (*Aspergillus flavus*) and stem rot pathogen (*Sclerotium rolfsii*). Sixteen isolates showing high antagonism were further characterized for biocontrol and growth-promotion in groundnut. *In-vitro* antifungal assays indicated that 50% inhibition was exhibited by 10 isolates against *S. rolfsii* and more than 40% inhibition was shown by five isolates against *A. flavus*. Two superior isolates (RP1A-12 and RP1A-15) were selected based on fungal antagonism and crop growth promotion. Culture filtrates of RP1A-12 and RP1A-15 exhibited good antagonism against test pathogens, with crude extract of RP1A-15 showing complete inhibition of the mycelia at 1.5%. The HPLC chromatograms of RP1A-15 and RP1A-12 crude extracts showed eight and three peaks respectively. Separation of eight compounds in RP1A-15 was obtained with silica gel flash column chromatography. Bio-efficacy studies were conducted on the obtained fractions and results suggested one fraction significantly delayed the germination of sclerotia of *S. rolfsii*. Identification of these two isolates using 16S rRNA sequencing and bio-active fraction using LC-MS, NMR are in progress.

Microscopic interactions between butternut (*Juglans cinerea*) trees and the butternut canker fungus (*Ophiognomonia clavignenti-juglandacearum*)

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Butternut canker is a lethal disease of butternut (*Juglans cinerea*) trees caused by the fungus *Ophiognomonia clavignenti-juglandacearum* (Oc-j). The disease has decimated populations of the species in many areas and is one of the main impediments to maintaining *J. cinerea* as a component of hardwood forests throughout its native range. Small elliptical cankers form at wound sites and natural openings on all woody tissues. Coalescence of multiple cankers kills branches and trees of all ages. In addition to complications from disease, *J. cinerea* readily hybridizes with non-native Japanese walnut (*J. ailantifolia*). Recently, Oc-j was found to cause leaf lesions on butternut and its hybrid. Prior research on the histological interactions between the fungus and host focused solely on canker progression within stem tissue. Little is

known about surface interactions between the primary inoculum in the pathosystem (conidia) and leaf and stem tissues. In this study, detached leaflets and stem sections in moisture chambers were inoculated with deionized water suspensions of Oc-j conidia and interactions were analyzed by scanning electron microscopy. We report evidence that spore germination on plant surfaces readily occurs. Subsequent hyphal growth is apparently haphazard and no active penetration structures were observed. We further report the likely mode of infection through the surface of non-wounded leaflets, as well as infection development within foliar tissue.

***Ralstonia solanacearum* requires PopS, an ancient virulence effector, to suppress SA-mediated defenses during tomato wilt**

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Ralstonia solanacearum requires a Type III (T3) secretion system for bacterial wilt pathogenesis, but the biological functions of individual effectors remain unknown. During tomato wilt, *R. solanacearum* expresses *popS*, which encodes an AvrE-family T3 effector. *popS* homologs were present in all 17 sequenced *R. solanacearum* strains, and the phylogeny of *popS* parallels that of the *R. solanacearum* species complex, suggesting that PopS is an ancient effector needed for association with plants. We determined that *popS* is required for full virulence on multiple *Solanum* crop hosts (susceptible potato and susceptible and quantitatively resistant tomato), but not for virulence on a related epidemiologically relevant weed, *S. dulcamara*. The *popS* mutant was also significantly delayed in tomato stem colonization following direct inoculation through cut petioles. AvrE-type effectors in other plant pathogenic bacteria suppress plant defenses triggered by the plant signaling molecule salicylic acid (SA). The *popS* mutant induced higher expression of SA-responsive tomato *PR* genes than its wild-type parent. Further, pretreating plant roots with SA exacerbated the *popS* virulence defect. Finally, PopS was dispensable for bacterial colonization of SA-deficient NahG transgenic tomato plants. These results indicate that this conserved T3 effector suppresses SA-mediated defenses in tomato roots and stems, which are the natural infection courts of this soilborne vascular pathogen.

Temporal dynamic of *Aspergillus flavus* community structure in soils of fields treated with the atoxigenic biocontrol *A. flavus* AF36 in Arizona

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Aflatoxins, toxic and carcinogenic metabolites produced by *Aspergillus* Section *Flavi*, frequently contaminate crops. Atoxigenic members of *A. flavus* competitively displace aflatoxin producers and consequently reduce aflatoxin contamination. The atoxigenic *A. flavus* AF36 is a natural inhabitant of soils in Arizona and has been successfully used commercially to reduce contamination in cottonseed and other crops. After successful applications, the population structure of *Aspergillus* in the soil is modified benefiting subsequent crops. Populations in treated fields reach equilibrium with those in non-treated fields in aflatoxin-producing potential without additional applications. The time and factors influencing equilibrium have not been examined. The current study sought to describe the process of equilibrium after application of AF36. Population structures of *A. flavus* in soils of twelve fields with cotton crops treated with AF36 were examined several times during four years in two areas. Results indicate that over time, AF36 decreased, while the highly toxigenic strain S increased to reach equilibrium with populations across the areas, but at higher AF36 incidences than before applications. However, time required to reach equilibrium (strain S > 75% and AF36 < 10%) was differential between the Yuma Valley (2 years) and the Mohawk Valley (3 years). In both areas, soils of treated fields had *A. flavus* populations containing over 50% AF36 one year after application.

Transformation of *Liberibacter crescens* using two wide host range shuttle vectors

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Huanglongbing (HLB) is caused by '*Ca. Liberibacter asiaticus*' (Las), '*Ca. L. americanus*' (Lam) and '*Ca. L. africanus*' (Laf), a group of alpha proteobacteria that have not been cultured. At least three other *Liberibacter* species have been described, including '*Ca. L. solanacearum*' (Lso, affecting potato, tomato, and carrots), '*Ca. L. europaeus*' (Lep, a nonpathogenic endophyte of

pear trees, and *L. crescens* BT-1 (Lcr, isolated from mountain papaya). Unlike the other *Liberibacter*s, BT-1 has been cultured and sequenced (GenBank CP003789) and thus has the potential to be developed into a model system. To determine if BT-1 might be tractable for functional genomics studies, the minimum inhibitory concentrations (MICs) of several antibiotics commonly used for plasmid selection was determined. BT-1 was found to be quite sensitive to: chloramphenicol, < 4 mg/L; gentamycin, < 1 mg/L; kanamycin, <2.5 mg/L, and tetracycline, < 0.3 mg/L. Both the repW (on pUFR071) and *Bordatella* replicons (pUFJ05, derived from pBBR1MCS5) were transformed by electroporation at high frequencies into BT-1. Stability of pUFR071 was evaluated; this plasmid was >95% stable, without selection, when grown in BM7 medium for over 20 generations. pUFR071 was extracted from BT-1, retransformed into *E. coli* and appeared from restriction analysis to be unchanged. Attempts to artificially inoculate marked strains into tobacco, citrus and periwinkle are currently in progress.

Pan-genome analysis of *Xanthomonas citri* subsp. *citri* provides insights into bacterial evolution and pathogenicity

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Citrus canker is a devastating disease that affects most commercial citrus varieties. Asiatic (A) type citrus canker is the most widespread and destructive form of citrus canker and is caused by *Xanthomonas citri* subsp. *citri*. Multiple variants of XccA including XccA^w and XccA* were reported. Compared with XccA, XccA^w and XccA* are limited in host range. To investigate the genomic variation and evolution of *X. citri* subsp. *citri*, a pan-genomic analysis was performed on 21 newly sequenced *X. citri* strains and three other reference strains representing distinct geographic, temporal, and host of origin. Analysis of these genomes showed that the pan-genome of *Xanthomonas citri* subsp. *citri* strains consists of a core genome shared by all isolates, accounting for about 85% of a single genome. The chromosomes of all the XccA strains were highly homogeneous. Comparatively, greater genetic diversity was observed in XccA^w and XccA* strains, with the majority being attributed to deletion and transposition events associated with insertion sequences and plasmids. We will present our current progress in understanding the evolution and pathogenesis of XccA based on comparative genomic analyses.

The development of a phage therapy for the control of the causal agent of horse chestnut bleeding canker, *Pseudomonas syringae* pv. *aesculi*

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Bleeding canker of horse chestnut trees poses a great threat to the species in the U.K. and the rest of Europe. A large number of trees in the South East of England have the disease and as yet no control strategy has been found. Without appropriate intervention we may face the loss of an amenity species. To identify novel control strategies requires considerations of new therapies, especially since antibiotic usage is restricted. Phage therapy offers the opportunity for a natural, chemical-free biocontrol agent. A number of bacteriophages from diseased and non-diseased tree material from the South East of England were isolated. These have the ability to infect and lyse a number of isolates of the causative agent of this disease, *Pseudomonas syringae* pv. *aesculi* from the U.K. and Europe and some other *syringae* pathovars. A subset of 4 of these phage were further characterised and discovered to be tentatively belonging to the order Caudovirales and the families Myoviridae and Podoviridae, based on TEM and genetic sequencing results. These phage isolates were evolved in co-evolution experiments with the host to create new genotypes to combat bacterial resistance to the therapy. This will hopefully mean the development of a viable phage therapy strategy for the control of bacterial bleeding canker.

Wound response and ROS production in blue mold resistant wild apple germplasm from Kazakhstan and central Asia

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Blue mold caused by *Penicillium expansum* is the most destructive pathogen of stored apples in the U.S. and worldwide. It was recently shown that resistance to blue mold exists in wild apples, *Malus sieversii*, from Kazakhstan and central Asia maintained in a germplasm collection in Geneva, NY. We initiated studies to determine the mechanism of resistance to *P. expansum* in select wild apple accessions. Wound responses (up to 96 h in 24 h intervals), affecting *P. expansum* infection, and related cytological changes were observed in accessions with varying resistance levels. In general, the more resistant the accession, the quicker the wound response that prevented fungus from infecting tissue and causing decay. On moderately resistant accessions, a 24 h interval between wounding and inoculation was sufficient to avert decay. More time was needed for the wounds to develop resistance to higher inoculum concentrations of the pathogen. No decay developed on immune apple accessions, even when inoculated immediately after wounding. Reactive oxygen species (ROS) were detected at high level immediately after wounding in the immune accessions, and gradually accumulated around the wound over time in both resistant and moderately resistant accessions. Results from this study suggest an involvement of ROS in the mechanism of resistance to *P. expansum* in select wild apple accessions.

Pandemic, novel, and hybrid genotypes of the amphibian pathogen *Batrachochytrium dendrobatidis* in the southern Atlantic Rainforest of Brazil

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Chytridiomycosis of amphibians, caused by the chytrid pathogen *Batrachochytrium dendrobatidis* (*Bd*), is the emerging infectious disease implicated in the recent population declines and extinction of amphibian species worldwide. Current data suggest that *Bd* has been recently introduced to naïve host populations by the intercontinental movement of amphibians. In the decade after its discovery in 1998, all isolated strains of *Bd* were shown to belong to a single, hyper-virulent clonal genotype. Recently however, multiple groups have reported the existence of novel *Bd* genotypes, including the existence of a hybrid genotype from the Atlantic Rainforest of Southeastern Brazil. In-depth sampling is needed to define the spatial distribution of the putative endemic and hybrid genotypes. To investigate the population structure of novel, pandemic and hybrid lineages in this putative hybrid zone, pure isolates of pathogen strains were collected from *Bd* infected anurans at six field sites along a 1400 Km transect of the historical range of the Atlantic Rainforest. Isolates of *Bd* strains were genotyped by multi-locus sequence typing to assess the effect of latitude, presence of invasive amphibian species, and anthropogenic habitat fragmentation on the spatial distribution of *Bd* genotypic diversity. Insights gained from this investigation will provide a future framework to test the hypothesis that the hyper-virulence of the pandemic lineage arose from past hybridization events.

CRISPR systems in plant pathogens: A new tool for epidemiological surveillance

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Rapidly evolving genetic loci, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), are of special interest to develop new molecular typing tools. The widely distributed CRISPR loci consist of highly conserved DNA repeats that are interspersed by unique, similarly sized spacers which originate from previous attacks by viruses and/or plasmids. For efficient management of plant diseases, knowledge about the pathogen's population structure and tools for epidemiological surveillance are prerequisite. We wish to exploit CRISPR loci as a molecular typing tool of plant

pathogens, as exemplified by *Xanthomonas*, an important clade of Gram-negative bacteria infecting a plethora of plants, including rice, cereals, cassava, banana and citrus fruits. CRISPR loci of 56 *X. citri* subsp. *citri* strains of world-wide origin were sequenced, revealing a repertoire of 37 unique spacers. A dendrogram based on the presence and absence of spacers was largely congruent with previous typing using AFLP. Our results demonstrate that CRISPR-based spoligotyping can be used as an efficient and robust method to study the phylogenetic relationships among isolates of plant-pathogenic xanthomonads, such as the citrus cancer pathogen *X. citri* subsp. *citri*. Implementation of *Xanthomonas* spoligotyping will serve phytosanitary measures by assisting the epidemiological surveillance of outbreaks of citrus canker and other diseases.

Diversity of Blastocladiomycota and Chytridiomycota of the “Parque Estadual da Ilha do Cardoso” (PEIC), Cananéia, São Paulo State, Brazil
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Here, we report the results of a study to analyze and compare the richness and diversity of Blastocladiomycota and Chytridiomycota in freshwater and soil ecosystems of the “Parque Estadual da Ilha do Cardoso” (PEIC), which is a protected fragment of the Atlantic Rainforest area located in Cananéia municipality, São Paulo State. On two sampling dates (winter and spring), we collected water and soil samples at 15 different points. These samples were processed in the laboratory by using cellulosic, chitinous and keratinous substrates. Of the 60 samples analyzed, we obtained 155 isolates of 31 morphological species, 29 taxa belong to the Chytridiomycota and two to the Blastocladiomycota, 26 of them were identified to the species level. Approximately 30% of the isolates were purified in culture media and characterized on the basis of morphological and molecular data (18S, ITS and 28S regions of the rDNA). We performed the first phylogenetic analysis of *Nowakowskiella elongata*, *N. multispora* and *Cladochytrium tenue*. *Chytrium lucidus* was noted for the first time in Brazil and 94% of identified species are first records for the PEIC. Our results demonstrate the importance of biodiversity inventories, particularly in South America, where the knowledge of zoospore fungi is scarce. Financial support: CAPES (grant)/FAPESP.

Diversity of zoospore assemblages from Pirarungaua stream, São Paulo, Brazil

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The present study aimed at surveying the zoospore assemblages (Fungi and Chromista) of the Pirarungaua stream, “Parque Estadual das Fontes do Ipiranga” (PEFI), which is one of the few remnants of Atlantic Forest in urban areas of São Paulo city. At six stream sites, water and mixed leaf litter were collected monthly from June 2011 to April 2012. Samples were processed in the laboratory by a multiple baiting technique using cellulosic, keratinic and chitinous baits. A total of 676 isolates was recorded, 309 from water and 367 from leaves. The isolated taxa were morphologically identified and incorporated either into the Fungal Culture Collection or the Herbarium of the “Instituto de Botânica”. Forty-two taxa were recovered, 27 identified to the species level (15 Chytridiomycota, one Blastocladiomycota and 11 Oomycota). Of the total taxa, seven are new records for the PEFI and one for Brazil. Richness, evenness, Shannon’s and Simpson’s indices were high from both water and in the mixed leaf litter, with a high similarity based on Sorensen’s index. We thank National Council for Scientific and Technological Development (CNPq) for financial support.

Effect of quinolone alkaloids isolated from *Esenbeckia alata* and *Raputia heptaphylla* (Rutaceae) on *Botrytis cinerea*

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Botrytis cinerea is the fungus responsible for the gray mold disease, which affects an important amount of crops worldwide. In Colombia, gray mold causes 76% of the losses of fruit and ornamental crops. In previous studies, quinolone alkaloids from plants of the family Rutaceae showed biological activity against several plant and human pathogens. In this work, 9 quinolone alkaloids, isolated from *R. heptaphylla* and *E. alata*, were evaluated *in vitro*

against *B. cinerea*. Evaluation included a test was aimed to determine the effect of alkaloids on mycelial growth, and a second test to determine the effect on conidial germination. Five out of nine substances tested were found to be active against the pathogen by inhibiting its growth and reproduction. Conidial germination and mycelial growth were inhibited almost in a 100%, in few cases conidial germination was observed but sclerotia, instead of aerial mycelium, was formed. Quinolone alkaloids that showed activity against *B. cinerea* were used in an additional *in silico* study, aimed to elucidate their putative target. Active sites of enzymes, reported as target for some fungicides and acquired from PDB, were chosen for molecular docking simulation. Five alkaloids exhibited better affinity scores for cystathionine γ -lyase and cytochrome-b. This preliminary result will be used as a working hypothesis in the following experiments in order to delineate the mechanisms of action for quinolones alkaloids on *B. cinerea*.

Genetic differentiation between *Verticillium dahliae* populations from asymptomatic and symptomatic hosts

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Verticillium dahliae (Vd) causes disease on over 400 hosts. Monocotyledonous crops have been traditionally considered to be non-hosts of Vd, and cereal species have been used in rotations as a strategy to manage *Verticillium* wilts. When sampling oats planted in with potatoes in PA fields with a history of *Verticillium* wilt, we found Vd growing endophytically; the fungus was isolated from surface-disinfested internal stem tissue of asymptomatic oat plants. A population genetics approach was used to examine Vd obtained from potato and oats grown in rotation in the same fields. Microsatellite marker analysis showed that the populations were significantly different. While at least seven distinct Vd genotypes were present in the populations from potato samples, only one of those genotypes was consistently found associated with oats, in different sampling years and from different fields. These results indicate that the interaction between Vd and oats is highly specialized; not every Vd genotype seems to be able to establish an endophytic relationship with oats and possibly other monocots, but this is potentially restricted to certain Vd genotypes. The dual role of Vd, pathogenic on certain plants and endophytic on others, raises interesting questions about the biology, ecology, persistence, and spread of this fungus, and has important potential implications in the management of *Verticillium* wilts in agroecosystems.

Possible infection of above-ground plant tissue by airborne conidia of nonpathogenic *Fusarium oxysporum*

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Fusarium oxysporum (Fo) is a common soilborne fungus that causes *Fusarium* wilt in plants by infection through plant roots and colonization of the vascular system. Fo can also infect plants asymptotically as an endophyte. The extent of endophytic colonization by different nonpathogenic Fo originally isolated from diverse hosts was tested by inoculating chickpea plants and recovering the fungus from surface-disinfested roots, crown, stem, and seeds of the plant. Inoculations were performed by planting pre-germinated seeds in soil infested with a given Fo genotype. Plants were grown under controlled conditions until seed set. Fo was always recovered from roots and crowns and frequently recovered from stems and seeds. However, recovered isolates were frequently different from the genotype used to inoculate the soil initially. This mismatch increased with the height of the plant, with 31% of the isolates from the crown, 63% of isolates from the stem, and 83% of the isolates from the seeds differing from the soil-inoculated genotype. These results suggest that conidia of Fo were dispersed aerially and infected the above-ground portions of the plant independently of infection that took place through the root system, possibly through infection of the flower or other above ground tissues. Although these observations were made in a growth chamber under controlled conditions, this aspect of Fo biology may have important implications in the ecology of this important fungus.

Formulation development of nontoxicogenic biocontrol strain of *Aspergillus flavus*: Wetting agent selection and physical property comparison

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To enhance the efficacy of *Aspergillus flavus* Afla-Guard (NRRL 21882) as a biological control agent for control of aflatoxin contamination in corn, a series of laboratory evaluations were conducted to improve/optimize Water Dispersible

Granules (WDG) as a delivery system. These studies focused on improved wettability and physical properties to replace the current solid grain formulations. WDG formulations have several advantages over wettable powder, emulsifiable, oil or granular formulations. The development of WDG does not require solvents, and WDG formulations can greatly reduce the dust during application. Moreover, WDG has less long term residual impact on our environment than the oil or emulsifiable formulations. In the development of a non-toxin producing strain of *A. flavus* Afla-Guard based WDG formulations, 14 wetting agents and two prototype formulations were evaluated. The results indicated that aerial conidia of *A. flavus* Afla-Guard produced by solid fermentation were highly hydrophobic in nature. Silwett L-77 was the best among the 14 wetting agents evaluated. Two prototype formulations have been developed with improved physical properties. These findings can be employed for further commercial formulation development and field testing for efficacy.

Marram grass and dune fungi: Hunting for hidden change

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Plant host specificity amongst communities of arbuscular mycorrhizal fungi (AMF) has been demonstrated but the extent and consequences of this phenomenon are poorly understood. This project investigates AMF communities in the invasive dune grass European marram (*Ammophila arenaria*) and in the native dune grass spinifex (*Spinifex sericeus*) in coastal foredunes in New Zealand. A pilot study of the AMF in spinifex in one dune revealed 32 taxa, suggesting complex communities can occupy what appears to be a simple system. Roots were then collected from marram and spinifex across two beaches, in areas where these plants grow alongside each other and in areas where they grow apart. AMF DNA has been extracted and next generation sequencing will be performed. This will reveal whether the AMF communities in marram and spinifex are different, and whether they change when the plants are adjacent. This is the first study to catalogue AMF communities in dunes in New Zealand. If differences by host plant are found, the possibility of marram leaving changed AMF communities behind after removal could have implications for dune restoration. If AMF communities in spinifex are influenced by neighbouring marram, a mechanism by which marram outcompetes this plant will be suggested. A comparison of AMF sequences derived from marram in Europe, in New Zealand and in the United States will also be performed, shedding light on the international biogeography of AMF associated with this plant.

Citrus huanglongbing root loss is independent of phloem plugging and carbohydrate starvation

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Huanglongbing (HLB) is a systemic disease of citrus caused by the phloem-inhabiting bacterium '*Candidatus Liberibacter asiaticus*' (Las). Although disease in grove trees is initially identified by foliar symptoms, 32-46% root loss occurs before foliar symptom development. This root loss has been linked to root infection by Las. Before the discovery that fibrous root loss occurs prior to foliar symptom development, root decline was thought to result from carbohydrate starvation caused by phloem plugging in leaves and branches. However, carbohydrate content of roots was similar to healthy roots even after the early root loss. Carbohydrate status of roots is correlated with canopy infection status rather than root loss. In addition, the phloem plugging associated with foliar symptoms is not observed in root tissue. This lack of root phloem plugging may explain why Las infects roots more systemically compared to the sectorized canopy infection. These data suggest that the disease mechanisms leading to root loss are different from those causing symptoms in the tree canopy. Some evidence of seasonal root loss associated with flushes has been observed. The dynamics of root loss during alternating leaf and root flushes may provide added insight into the cause of HLB-associated root loss.

Role of a thioredoxin family protein in *Xylella fastidiosa* virulence

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Xylella fastidiosa is an economically important pathogen and the causal agent of Pierce's disease of grapevines. The *X. fastidiosa* gene PD1199 encodes a DsbA-like protein, thought to belong to the thioredoxin family of proteins, designated Frne. Deletion of the *frne* gene from *X. fastidiosa* resulted in a strain that was hypervirulent on grapevines, developing Pierce's disease symptoms approximately two weeks before wildtype. The mutant was not

affected in growth in PD2 broth, growing to OD₆₀₀ levels similar to wildtype *X. fastidiosa* and the complemented mutant strain. The *frne* deletion mutant produced biofilm which was more fragile, i.e. more easily dislodged from surfaces, than wildtype *X. fastidiosa*. Expression of the *frne* gene was reduced in sap collected from susceptible grapevines further suggesting that the Frne protein plays a role in suppression of virulence on susceptible plants.

Comparative metagenome sequencing of '*Candidatus Liberibacter solanacearum*' haplotypes A and B reveals hypervariable phage-like regions

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It has been demonstrated that Zebra Chip disease of potato is caused by two distinct haplotypes (A and B) of '*Candidatus Liberibacter solanacearum*' (Lso). Lso is an unculturable phloem restricted α -proteobacteria transmitted from plant to plant through the feeding activity of the potato/tomato psyllid (*Bactericera cockerelli*). In order to better understand the biology and genetic variability of Lso, metagenomic sequencing was performed on Lso-positive psyllids. Field collected insects were used to start colonies on eggplant and potato in greenhouse cages. QPCR was used to monitor the percentage Lso+ psyllids, the relative Lso titer of infected psyllids, and Lso haplotype of each colony. High titer Lso DNA extracted from psyllids was subjected to IonTorrent sequencing using the IonTorrent 318 chip. The previously sequenced LsoB genome was used in template based genome assemblies of the IonTorrent reads. A reference LsoA genome was developed and expanded from sequences identified by homology to LsoB. Comparisons were made between the LsoA and LsoB genomes resulting in identification of distinct hypervariable regions of DNA. BLAST analysis of these regions reveals homology to bacteriophage sequences.

Grape crown gall: Distribution in vines and detection using a Taqman real-time PCR assay

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Grape crown gall is an economically important disease caused by the bacterium *Agrobacterium vitis*. This pathogen has been found on symptomatic and non-symptomatic vines. Because the pathogen can be disseminated in non-symptomatic dormant cuttings it is important to determine if there are areas within canes where the bacterium can be preferentially found. Our laboratory developed a new sensitive real-time SYBR green assay was developed for specifically for detection of tumorigenic *A. vitis* that is ideal for use in analyzing distribution of *A. vitis* in canes. We determined that the bacteria could be found in both nodes and internodes, at the base of the cane, and also in tips of the canes. However, the bacterium was found to occur more in nodes than internodes. A new Taqman probe was developed to increase specificity of the *A. vitis* real-time PCR assay. The Taqman assay was as sensitive as the SYBR green assay using *A. vitis* strain S4 cells with a detection threshold of 10⁴ CFU/mL. The specificity of the assay was also tested using *A. vitis*, *A. tumefaciens*, and *A. rhizogenes* strains.

Ultraviolet treatment of surface irrigation water for improved plant health and food safety

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Surface water, the primary source of irrigation water for many fruit and vegetable growers, is open to many routes of contamination by both plant disease and human foodborne illness causing microorganisms. Most members of the oomycete genus *Phytophthora* are pathogenic to plants and are well-suited to be spread through surface water, hence their common name, water-molds. Many species of *Phytophthora* have been documented from surface water sources. *Escherichia coli* and *Salmonella* spp. are responsible for many foodborne illnesses associated with the consumption of fresh fruits and vegetables; both have been reported from agricultural surface water sources. A pathogen survey in surface irrigation water sources in New York found *Phytophthora* spp. in nearly 100% of samples analyzed. Some of these species include *P. capsici*, *P. citricola*, and *P. nicotianae*. *E. coli* and *Salmonella* spp. were present in 30% and 38% of water samples tested, respectively. Currently, water treatment options available for growers are limited due to the varying pH and turbidity of most surface waters. An ultraviolet treatment system engineered for turbid liquids was used to treat surface water inoculated with

the abovementioned pathogens. A 5-log or greater reduction in the number of viable bacterial cells or zoospores was achieved in all inoculated samples. This UV system could be used by both conventional and organic fruit and vegetable growers for enhanced plant health and food safety.

Sensitivity of *Erwinia amylovora* in Illinois apple orchards to streptomycin, kasugamycin, and copper

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Streptomycin and copper are widely used to manage fire blight, caused by *Erwinia amylovora*, in commercial apple orchards in Illinois. Statewide surveys were conducted in 2010, 2011, and 2012, and 117, 129, and 170, *E. amylovora* isolates were collected, respectively, from 20 counties, to determine sensitivity of *E. amylovora* to streptomycin, copper, and other antibiotics. None of the 416 *E. amylovora* isolates tested were resistant to streptomycin (Agrimycin 17WP) at 50 mg/L. Seven non-*E. amylovora* bacterial isolates were collected from blossoms and *E. amylovora*-infected shoots that contained both a strA-strB streptomycin-resistance gene and IS1133 on transposon Tn5393. Colony development of all 84 *E. amylovora* isolates tested were inhibited on Luria-Bertani medium amended with oxytetracycline (Mycoshield) at 50 mg/L and kasugamycin (Kasumin 2L) at 100 mg/L. Similarly, colony development of the 84 *E. amylovora* isolates was inhibited on the casitone-yeast extract medium amended with copper sulfate (Cuprofix Ultra 40DF) at 0.16 mM. In 2011 and 2012, field trials were conducted to evaluate efficacy of oxytetracycline (Mycoshield), kasugamycin (Kasumin 2L and ARY-0416-06), copper hydroxide (Kocide-3000 41.6DF), *Bacillus subtilis* (Serenade Max, QST713), and *Pseudomonas fluorescens* (Blight Ban A506) for management of fire blight in an apple orchard. Only kasugamycin (Kasumin 2L and ARY-4016-06) reduced blossom and shoot infection significantly.

Resistance to postharvest fungicides in the blue mold fungus from the Mid-Atlantic area

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Penicillium expansum causes blue mold and is of utmost concern for pome fruit packers and processors worldwide. In 2004, two new postharvest fungicides, Scholar® (fludioxonil) and Penbotec® (pyrimethanil) were registered for application on apple and pear fruit. Since then, resistance to pyrimethanil, but not fludioxonil, has been reported for *P. expansum* isolates obtained from Washington state. Tolerance to fludioxonil and/or pyrimethanil has not been investigated for the Mid-Atlantic apple growing region. *Penicillium* spp. isolates were obtained from dump tank water containing Scholar® and from decayed apple fruit treated with Penbotec® from cold storage from the same facility located in Pennsylvania. Isolates were propagated as monoconidial cultures and identified to species via PCR amplification of the 3' end of β -tubulin gene. Isolates were screened on a discriminatory dose of 0.5 ppm technical grade pyrimethanil- and fludioxonil-amended minimal medium. Only those that grew at the discriminatory dose were considered 'resistant' and analyzed for MIC (Minimum Inhibitory Concentration) and EC₅₀ (half maximal Estimated Concentration). Results from this study will aid the apple storage industry in the application and rotation of the most efficacious chemistries to control blue mold decay on apple and pear fruits during storage.

Phylogeography and taxonomy of *Pluteus* section *Pluteus* (Basidiomycota, Agaricales) in the Northern Hemisphere

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The diversity and biogeography of *Pluteus cervinus* (the deer mushroom) and allied species was investigated using molecular data. Over 300 specimens spanning the major areas of boreal and temperate forests of the Northern Hemisphere were studied and nrITS and tef1-alpha were obtained. A

considerable effort was made to locate, study and sequence type collections in order to stabilize the taxonomy of the group. A total of 26 species occurring in Eurasia and North America were recovered in the phylogenetic analyses, 16 of which represent new taxa. The problems for morphological delimitation of the species and the different resolution power of ITS vs. tef1-alpha will be discussed. Phylogeographic patterns are strikingly different among the species in this clade and all taxa except *Pluteus petasatus* seem to have some geographic and/or ecological limit in their distribution. Relatively restricted endemics occur throughout the area of study but Western North America and the boreal and (sub) alpine areas of Eurasia and North America seem to be particularly rich in number of species.

Etiology of aflatoxin contamination of maize in Zambia

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Aflatoxins are carcinogenic and immunosuppressive toxins that frequently contaminate maize and groundnut, important staples in Zambia. *Aspergillus flavus* and *A. parasiticus* are the fungi most frequently implicated as causal agents of aflatoxin contamination. Because of the importance of maize in Zambia, a government parastatal, the Food Reserve Agency (FRA), was established to both administer a national strategic maize reserve and facilitate marketing. Aflatoxin levels and causal agents in FRA reserves remain largely unknown. This study was undertaken to develop improved information on the prevalence of aflatoxin contamination within maize stored in Zambia and of the causal agents of any detected contamination. Maize samples of the 2012 crop were collected in a stratified pattern from a single FRA depot and aflatoxins were quantified with lateral-flow assays. Soils in corn and peanut fields were also assayed for *Aspergillus* section *Flavi* and isolated fungi were evaluated for aflatoxin-producing ability. Soil communities of aflatoxin-producing fungi were dominated by *A. parasiticus* (65.5%) and section *Flavi* members with S strain morphology (23%). The most toxigenic isolates produced over 150,000 ppb aflatoxin B₁ on maize. However, atoxigenic isolates of potential value as biocontrol agents were also identified. More extensive surveys across Zambia of aflatoxin contamination are needed to determine the extent to which increased aflatoxin management is needed.

The *poppr* R package for genetic analysis of populations with mixed (clonal/sexual) reproduction

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We developed the *poppr* R package for population genetic analysis. R is an open-source statistical programming language that has a large, well-maintained repository of statistical packages. *Poppr* provides open-source, cross-platform tools for quick analysis of population genetic data enabling focus on data analysis and interpretation. While there are a plethora of packages for population genetic analysis, few are able to offer quick and easy analysis of populations with mixed reproductive modes. *Poppr*'s main advantage is the ease of use and integration with other packages such as *adegenet* and *vegan*, including support for novel methods such as clone correction, multilocus genotype analysis, calculation of Bruvo's distance, the index of association and graphing including dendrograms with bootstrap support and minimum spanning networks. Moreover, *poppr* allows analysis of data on any level of a sampling hierarchy including batch analysis. *Poppr* thus provides novel and convenient implementations of routine and previously unavailable population genetic analyses in R.

***Arabidopsis* CRT1 dimerizes with itself and some of its family members through the C-terminal domain carrying a coiled-coil motif**

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Arabidopsis CRT1 (Compromised for Recognition of TCV), an ATPase and endonuclease, is necessary for a wide range of resistance including effector-triggered immunity mediated by resistance (R) proteins and PAMP (pathogen-

associated molecular pattern)-triggered immunity (PTI) mediated by PAMP recognition receptors (PRR). Consistent with its role in multiple levels of immunity, CRT1 interacts with 11 R proteins from different structural classes and the FLS2 PRR. Although predominantly localized in endosomes, a subpopulation of CRT1 exists in the nucleus increase in response to pathogen challenges. We found that CRT1 formed a dimer with itself and CRH6, and that CRH6 physically also associated with the HRT R protein. The MultiCoil program predicted that *Arabidopsis* CRT1 family has a coiled-coil structural motif likely mediating dimerization but not trimerization. Consistent with the prediction, our yeast two hybrid assay with CRT1 truncated variants suggested that the coiled-coil motif residing C-terminal domain of CRT1 is responsible for the intrafamily interaction. Analysis of RNA-seq database indicated that *CRT1* is expressed significantly higher than any other CRT1 family members in *Arabidopsis*. Taken together, these results may suggest that, as either a homo-dimer or a hetero-dimer with CRH6, CRT1 is an important player in the CRT1 family function in *Arabidopsis*.

Antifungal activities of *Xylogone ganodermorphthora* KACC93082P against several plant pathogens

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Xylogone ganodermorphthora is an acomycetous fungus that causes a yellow rot on cultivated *Ganoderma lucidum*. To investigate the antifungal activities of *X. ganodermorphthora* against plant pathogens, we carried out *in vitro* and *in vivo* assays against four plant pathogens: *Phytophthora capsici* (PC), *Fusarium oxysporum* (FO), *Sclerotium rolfsii* (SR), and *Podosphaera fusca* (PF), using culture extracts. The minimum inhibitory concentration of the ethyl acetate extract was 10 ppm against PC. Methanol extract inhibited the mycelial growth of FO at 1,000 ppm. The methanol extract was developed on a thin-layer chromatography (TLC) plate in a dichloromethane-methanol (9:1, v/v) mixture, with an overlay of molten potato dextrose agar (PDA). When SR was inoculated on the PDA-coated TLC plate, mycelial growth was completely inhibited at Rf values between 0 and 0.8. The control efficacy against PF on watermelon plants was tested using four vines grown in a plant growth chamber. Eighteen days after inoculation, the average diseased leaf area of the vines treated with the extract was 12.8%, whereas that of the control vine treated with 30% aqueous ethanol was 100%. These results indicate that *X. ganormorphthora* culture extract represents a potential and effective biocontrol for some plant pathogens.

Records of *Entolomataceae* species with cuboid basidiospores from Brazil based on morphological and multi-locus phylogenetic analyses

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Taxa within the *Entolomataceae* Kotlaba & Pouzar (Agaricales, Basidiomycota) are easily recognized by the pinkish spore deposit and by the shape of the basidiospore that is bumpy, ridged or angular with four to twelve angles in polar view. Around 100 species have eight-angled (cuboid) basidiospores and the majority of these species are placed in the genus *Inocephalus* (Noordel.) P.D. Orton. Despite their diversity in tropical and subtropical regions, there are very few reports documenting the diversity of cuboid species from Brazil. Recent collection efforts in the Amazonian and Atlantic regions of Brazil have yielded 24 species with cuboid basidiospores. Of these, nine are new to science, five species are new records for Brazil (*Entoloma pinnum*, *E. roseomurinum*, *E. salmoneum*, *Inocephalus luteus* and *Trichopilus luteolamellatus*) and six species are new records for the region (*E. caribaeum*, *E. lycopersicum*, *I. cervinus*, *I. dragonosporus* and *I. murrayi*). Additionally, a multilocus phylogeny of the *Entolomataceae* supports the placement of species with cuboid spores in a segregate, sister clade to *Inocephalus* species that lack cuboid spores. Analyses of the evolution of the cuboid spore type will be discussed along with an evaluation of morphological diversity of species with cuboid spores. (FAPESP 2010/10218-5)

An inordinate fondness for *Fusarium*: Phylogenetic diversity of fusaria cultivated by *Euwallacea* ambrosia beetles on avocado and other plant hosts

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Ambrosia beetle fungiculture represents one of the most ecologically and evolutionarily successful symbioses. Here we document the evolution of a clade within *Fusarium* associated with ambrosia beetles in the genus *Euwallacea* (Coleoptera: Scolytinae). Ambrosia *Fusarium* Clade (AFC) symbionts are unusual in that some are plant pathogens that cause significant damage in naïve natural and cultivated ecosystems, and currently threaten avocado production in the United States, Israel and Australia. In a four-locus phylogenetic analysis, the AFC was resolved in a strongly supported monophyletic group within the previously described Clade 3 of the *Fusarium solani* species complex (FSSC). Divergence-time estimates place the origin of the AFC in the early Miocene ~21.2 Mya, which coincides with the hypothesized adaptive radiation of the Xyleborini. Two strongly supported clades within the AFC (Clades A and B) were identified that include nine species lineages associated with ambrosia beetles, eight with *Euwallacea* spp. and one reportedly with *Xyleborus ferrugineus*, and two lineages with no known beetle association. AFC lineages consisted mostly of genetically identical individuals associated with specific insect hosts in defined geographic locations, with at least three interspecific hybridization events inferred. Overall, these data are consistent with a strong evolutionary trend toward obligate symbiosis coupled with secondary contact and interspecific hybridization.

Effect of amended media, temperature, and light on the growth and development of *Cercospora janseana*

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Cercospora janseana causes the fungal rice disease narrow brown leaf spot. In favorable conditions, this disease can cause more than 40% yield loss. Recent and frequent outbreaks of this disease are serious concerns for researchers and growers. Due to sparse information available related to this pathogen and disease, studies have been conducted to explore the factors affecting growth and development of *C. janseana*. Different kinds of basic media were tested: PDA, ½ PDA, ¼ PDA, water agar, corn meal agar, yeast agar, rice leaf extract, V8 (10%) and V8 (20%). Using V8 (20%) as a base media, further amendments were done with L-amino acids, vitamins and inorganic salts. Different temperature (10, 15, 20, 25, 28, 30, 32, 35 and 40°C) and light (12h Light & 12h dark, 14h light & 10h dark, 16h light & 8h dark, continuous dark and continuous light) regimes were tested to determine optimum temperature and light for its growth. Observations were recorded on mycelial growth, spore production and cercosporin production. Of the media amended with methionine, proline, thiamine has shown significant increased growth and sporulation of *C. janseana* as compared to unamended V8 (20%). Media amended with histidine increased the cercosporin production and significantly reduced the spore production. No growth was seen on 10, 35 and 40°C. Different light regimes had significant effect on the development of *C. janseana*.

Biological control of crown gall on grape and root colonization by nonpathogenic *Agrobacterium vitis* strain ARK-1

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A nonpathogenic strain of *Agrobacterium vitis* ARK-1 was tested as a biological control agent for crown gall of grape. When roots of grapevine were soaked in a cell suspension of strain ARK-1 before planting in the field, the number of plants with tumors was reduced in treated plants. Results from

7 field trials in 2009 to 2012 were synthesized in a meta-analysis. The integrated relative risk of treatment with ARK-1 was 0.15 (95% confidence interval: 0.07-0.29, $p < 0.001$), indicating that disease incidence was significantly reduced by ARK-1. On the other hand, results from 4 field trials in 2007 to 2009 using *A. vitis* VAR03-1, which was previously reported as a biological control agent for crown gall of grapevine, were synthesized in a meta-analysis. The integrated relative risk of treatment with VAR03-1 was 0.24 (95% confidence interval: 0.11-0.53, $p < 0.001$), indicating the superiority of inhibitory grape crown gall of ARK-1 over that of VAR03-1 in field condition. ARK-1 did not cause necrosis on grape shoot explants. ARK-1 established populations on roots of grape tree rootstock and persisted inside roots for 2 years.

Syngomycin E as an organic-compatible agrofungicide

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Syngomycin E (SRE) is a natural fungicide produced by certain strains of *Pseudomonas syringae*. It is lethal to many fungi at concentrations below 10 $\mu\text{g}\cdot\text{ml}^{-1}$. In organic farming, disease control is difficult because of exclusions of synthetic chemicals. The goals of this study were to develop and determine the ability of SRE as an organic-compatible agrofungicide. SRE activity was tested against the soil-borne pathogen *Pythium ultimum*. Fermentative production of SRE by newly isolated strain G10 yielded 50 $\text{mg}\cdot\text{l}^{-1}$ of SRE in 40 h. Organic-compatible SRE was obtained by chromatography using an AKTA avant 150 system (RPC-3 column, 6.4×100 mm) with 0.1% formic acid in water and 0.1% formic acid in isopropanol as solvents. Incubation of *P. ultimum* oospores in 31.3 $\mu\text{g}\cdot\text{ml}^{-1}$ of SRE for 4 h resulted in 3.4% ($n=170$) germination while 23% ($n=175$) of oospores successfully germinated in water. SRE was coated on organic-certified cucumber seeds (5-10 μg per seed) with 5% starch gelatinized by heat. There was no significant difference between germination rates of SRE-coated seeds (90%, $n=30$) and non-coated seeds (93%, $n=30$) on water agar. SRE-coated seeds germinated at the rate of 72% ($n=23$) on naturally infested soil while non-coated seeds had a germination rate of 0% ($n=23$). SRE was not phytotoxic for organic cucumber seeds and provided protection from infection by *P. ultimum*. This study demonstrated that SRE has potential as a novel organic agrofungicide.

Two distinct carlaviruses detected in elderberry

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Cuttings of four elderberry cultivars, 'Bob Gordon', 'Marge', 'Adams 2' and 'Wyldewood' were sent from Missouri to the USDA-ARS laboratory in Corvallis, Oregon to be tested for the presence of viruses. Double-stranded RNA (dsRNA) extracted from the four cultivars showed the same dsRNA pattern, with multiple bands of about 8000 base pairs plus several smaller bands. The dsRNA from cv. 'Bob Gordon' was used as template for sequence and further analysis. DsRNA was converted to cDNA using reverse transcription with (DOP – degenerate oligonucleotide primers), the cDNA was then amplified using PCR and subjected to High Throughput Sequencing using the Illumina format with an 80 bp, paired end kit. Approximately 75 million reads were obtained and assembled into contigs using SCRAPE.pl followed by CAP3. Two large but distinct contigs of 8521 and 8425 were obtained, which showed significant levels of sequence identity to several carlaviruses and 51% nucleotide sequence identity between each other. From the nucleotide sequence data and translated amino acid data, it is clear that there are two distinct carlaviruses infecting elderberry and it appears that one is related to *American hop latent virus* and the other related to *Poplar mosaic virus*. Examination of the serological relationships of these viruses to *Elderberry latent virus* is currently underway and will be presented along with more detailed information on the two carlaviruses.

Genotyping imazalil resistance in an international collection of *Penicillium digitatum* isolates

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Imazalil (IMZ) is the most effective fungicide against postharvest citrus green mould, caused by *Penicillium digitatum*. However, resistance development renders IMZ practically ineffective. Three IMZ resistance genotypes of *P. digitatum* have been characterised and termed R1, R2 and R3. However, the relative prevalence of these genotypes in IMZ resistant *P. digitatum* populations was largely unknown. In this study, 223 IMZ-resistant *P. digitatum* isolates were obtained from green moulded citrus fruit from South Africa, USA, Uruguay, Spain, Israel, Cyprus, Chile, Australia and Argentina. The resistance genotypes were identified using a multiplex PCR assay employing previously published primers. A total of 148 isolates yielded PCR products that could be separated and visualised using gel electrophoresis. Isolates from the USA showed the most diversity with 20% identified as R1, 14% R2 and 66% R3 type resistance. All the isolates from the other countries were classified as R3 type resistance. The R3 gene (199 bp insertion into the CYP51B gene) therefore could be used as target gene to quantify IMZ resistance in *P. digitatum* populations using real-time PCR. The results furthermore indicate that other uncharacterised resistant genotypes might be involved in IMZ resistance in *P. digitatum*, as 75 resistant isolates could not be genotyped during this assay.

Sequencing the metagenome of '*Candidatus Liberibacter psyllaurous*' associated with tomato psyllid yellows using a BAC library

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Liberibacters have been associated with important plant diseases like citrus huanglongbing, tomato psyllid yellows and potato zebra chip disease. We have sequenced the complete genome of '*Candidatus Liberibacter psyllaurous*', the bacterium associated with tomato psyllid yellows using metagenomics approaches. We have constructed a BAC library from the genomic DNA of infected tomato psyllids (*Bactericera cockerelli*) consisting of 57,600 clones arrayed in 150 plates, each with 384 wells. Pooled DNAs were used for screening. Initial identification of clones with Liberibacter genome fragments were conducted by real time PCR analysis based on 16S rDNA sequences. End sequences of these clones were used for designing new sets of primers for both screening as well as for testing infected plants and psyllids for confirmation. 245 BAC clones were characterized by pulsed field gel electrophoresis for insert size estimation. 63 bar-coded BAC clones (average size 70 kb) were sequenced by using Roche 454 technology. The genome of the bacterium was assembled *de novo* using the sequences of BAC clones. Large deletions, insertions and genome rearrangements were apparent when we compared the genome of '*Ca. L. psyllaurous*' with that of the organism associated with potato zebra chip disease, '*Ca. L. solanacearum*'. Our goal is to compare and study the genomics of related Liberibacters to understand the potential mechanisms of evolution and pathogenesis.

Development of a home detection kit for '*Candidatus Liberibacter asiaticus*' (LAS) associated with citrus huanglongbing from psyllids

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Citrus huanglongbing (HLB) has caused major crop losses. Mitigation of HLB would be effective if rapid detection tests can be conducted by citrus growers without a laboratory, trained technicians or expensive lab equipment. Testing of HLB associated bacteria ('*Candidatus Liberibacter asiaticus*' [LAS]) in psyllid vectors is desirable since it provides an early warning of HLB. Detection in psyllids often precedes finding LAS in trees by several months to up to a few years. We have utilized a loop-mediated amplification (LAMP) based method that can be performed in 40 minutes in field conditions to test the HLB vector, *Diaphorina citri*, for the presence of LAS. A compact, portable and battery-operated Smart-Dart® unit is used for the test. The unit, designed by Diagenetics, is connected by bluetooth to an Android device. DNA extraction is performed on the test psyllids in the Smart-Dart® unit for 10 minutes, followed by the LAMP reaction in the same unit for 30 minutes. An enzyme that amplifies a fragment of the Liberibacter genomic DNA by isothermal amplification method is used. We are developing methods for

multiple detection of the pathogen and the host DNA from both psyllids and plant hosts. The technique will be useful for citrus growers, homeowners, extension workers and researchers in situations where quantitative PCR testing is not feasible. If the samples test positive, the extractions can be shipped to a laboratory for confirmatory tests.

Genome data mining and diagnostic marker development of *Tilletia indica* for agri-food system detection screening

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Karnal bunt, caused by *Tilletia indica*, is an important disease of wheat, reducing grain quality and severely affecting international trade. Quarantine testing is conducted in Canada and elsewhere on wheat imports from infected regions. The primary challenge is accurate differentiation from the closely related but non-regulated ryegrass bunt pathogen, *T. walkeri*, which may be present as contaminant. Current detection systems are based on teliospore morphology and minor differences in the ITS or a short mitochondrial gene region. We are developing a diagnostic system based on multiple independent markers identified from comparative genome analyses. Both species were sequenced using the Illumina platform with paired-end reads of 100bp inserts. The genomes were assembled at 20, 25, 30 and 35Mbp sizes using 30Kmer strings for each genome size. Out of 120 genome assemblies built for each sample, we found Kmers 59 and 55 to produce largest n50 contig size for *T. indica* and *T. walkeri* respectively and both assembled well at 20Mbp genome size. Diagnostic sites were identified by comparing both genomes against each other while single and multi-copy regions were determined for *T. indica* by searching its own genome with the diagnostic sites. Identified regions were targeted and primers for 12 multi-copy, 9 single copy and 5 mitochondrial loci were designed. Initial results show one of the markers to be more variable (96.9%) than ITS (99.5%) between two species.

Heterokaryons of *Sclerotinia homoeocarpa* exhibit enhanced adaptability to multiple fungicide pressures

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Heterokaryosis, in which genetically distinct nuclei coexist within a cell, contributes to genotypic and phenotypic plasticity in multinucleate fungi. *Sclerotinia homoeocarpa*, causal organism of dollar spot of turfgrasses, is a multinucleate fungus with a history of resistance to multiple fungicide classes. We demonstrate that two homokaryons (HOKs), resistant to either a demethylation inhibitor or benzimidazole fungicide, can form heterokaryons (HEKs) with limited dual-fungicide resistance and adaptability to different fungicide pressures. HOKs were co-cultured to give rise to HEKs, which were isolated from the zone of interaction by hyphal tip isolation. HOKs and resultant HEKs were assayed on single and dual-fungicide amended media for resistance. HEKs displayed reduced fungicide sensitivity overall compared to HOKs. Reduced fungicide sensitivity and shifts in genotype indicated that HEKs could adapt to changes in fungicide pressure. Presence of both HOK nuclei in HEKs was confirmed in HEKs by detection of a SNP in the β -tubulin gene, and by the presence of both HOK microsatellite alleles. In fungicide resistance assays, survival of nuclei in HEKs was observed in a dose dependant fashion. Results from this study suggest that *S. homoeocarpa* HEKs can harbor nuclei with different fungicide resistance profiles. These data also suggest that heterokaryosis is a means by which the pathogen adapts to multiple fungicide pressures through nuclear disproportion.

What are the best ways to manage *Rhizoctonia solani* of sugar beet?

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Rhizoctonia root rot caused by *Rhizoctonia solani* Kühn is the most important sugar beet disease for producers in Minnesota and North Dakota. There is no variety completely resistant to *R. solani*, and varieties with some levels of resistance are susceptible at seedling and early growth stages. Greenhouse and field research were conducted to evaluate fungicides as seed treatments, in-furrow application at planting, and foliar application. In both greenhouse and field studies, *R. solani* AG 2-2 IIIB grown on barley was used to artificially inoculate pots or the research site. Fungicides used included penthiopyrad as a seed treatment, and azoxystrobin at 167 g a.i ha⁻¹ applied in-furrow and or in a band application. In greenhouse studies, penthiopyrad consistently provided effective control as a seed treatment at rates of 7, 14 and 28 g a.i per 100,000 seeds. Azoxystrobin provided effective control when used in-furrow or in a

band application. In field studies, penthiopyrad as a seed treatment followed by a band application of azoxystrobin provided similar effective control as azoxystrobin applied in-furrow followed by a band application. Penthiopyrad as a seed treatment was not effective at providing season-long control. The use of penthiopyrad, a succinate dehydrogenase inhibitor in a rotation program with azoxystrobin, a quinone outside inhibitor has the potential not only to provide effective control of *R. solani* but also to mitigate fungicide resistance.

Identifying host targets for *Ralstonia solanacearum* type III effectors in *Solanum lycopersicon*

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Soilborne gram negative plant pathogenic bacterium *Ralstonia solanacearum* infects more than 200 plant species including crops such as tomato, potato, tobacco, banana and eggplant. It uses Type III secretion system (T3SS) to inject virulence effectors into host plants. Not much is known about the molecular interaction of these effectors with host targets, and defects in the T3SS can result in loss of ability of *Ralstonia* to cause hypersensitive response in nonhost plants and pathogenicity in host plants. Determining targets of these effectors in host plant species is a step towards elucidating the pathways involved in the disease development process. We have screened a Y2H library made from *Solanum lycopersicon* with *Ralstonia* effectors. We have identified targets for some of these effectors using Y2H system and are conducting further experiments to confirm these interaction using Co-Immunoprecipitation and Bimolecular fluorescence complementation. To determine functional significance of the interaction of *Ralstonia* effectors with their host targets, loss-of-function approaches, such as Virus Induced Gene Silencing, are being utilized in *Nicotiana benthamiana* and *S. lycopersicon*. Results of these experiments will lead to molecular insights into how *Ralstonia* infects plant cells, and will likely assist in devising novel disease management strategies.

Association of endornaviruses of Black Turtle Soup common bean with seed germination, grain yield, and host reaction to Tobacco ringspot virus

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Two endornaviruses, *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and *Phaseolus vulgaris* endornavirus 2 (PvEV-2), have been reported infecting many common bean (*Phaseolus vulgaris*) cultivars. We have identified two lines of the cultivar Black Turtle Soup (BTS), one double infected with PvEV-1 and PvEV-2 and the other virus-free. We conducted comparative studies between the two lines to search for variations that could be associated with the endornaviruses. Virus-infected BTS plants could be differentiated from virus-free BTS plants by their purple pigmentation on the stems and petioles which was lacking in the later. Grain yield of the virus-infected line was lower than that of the virus-free line. Furthermore, seeds of the virus-infected line germinated faster and were slightly smaller than seeds of the virus-free line. Mechanical inoculations of virus-infected BTS plants with an isolate of Tobacco ringspot virus (TRSV) resulted in severe symptoms that consisted of chlorotic lesions, systemic necrosis, and stunting. In contrast, inoculations of virus-free BTS plants resulted only in necrotic local lesions without systemic symptoms. These results suggest that endornaviruses can negatively affect the grain yield of BTS common bean and that co-infection of endornaviruses with TRSV in BTS may overcome the host resistant to systemic infection by TRSV.

Cost to Soybean mosaic virus for gain of virulence on *Rsv1*-genotype soybeans

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The multigenic *Rsv1* locus in soybean PI96983 confers extreme resistance against most Soybean mosaic virus (SMV) strains, including SMV-N, but not SMV-G7 and SMV-G7d. In contrast, in susceptible soybean cultivars, such as Williams82 (*rsv1*), SMV-N induces severe disease symptoms and accumulates to a high level whereas both SMV-G7 and SMV-G7d induce mild symptoms and accumulate significantly lower. Gain of virulence by SMV-N on PI96983 (*Rsv1*) requires concurrent mutations in both HC-Pro and P3. This is because of the presence of more than one resistance (*R*) genes, likely belonging to NB-LRR class of *R* genes, within the *Rsv1* locus mediating independently recognition of HC-Pro or P3. Here we show that majority of mutational pathways evolved experimentally to disrupt the avirulence functions of SMV-N on PI96983 (*Rsv1*) also resulted in induction of mild

symptoms in Williams82 (*rsv1*) and accumulated significantly lower compared to avirulent SMV-N. Evaluation of phenotypes and accumulation rates of SMV-N-derived HC-Pro and P3 chimeras, containing homologous sequences from virulent SMV-G7 or SMV-G7d strains, as well as SMV-N-derived HC-Pro and P3 point mutants harboring gain of virulence mutation(s), revealed direct correlation between perturbation of HC-Pro and fitness penalty in Williams82 (*rsv1*). Collectively, these data demonstrate that virulence of SMV on PI96983 (*Rsv1*) is associated with fitness cost, which is a consequence of HC-Pro, and not P3 perturbations.

Genetic diversity and population structure of the Korean oak wilt fungus (*Raffaelea quercus-mongolicae*) using RAD sequencing

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Raffaelea quercus-mongolicae causes an oak wilt disease in Korea. So far, this ambrosia beetle-vectored pathogen has only been found in Korea, and it is phylogenetically distinct from *R. quercivora*, which causes a similar oak wilt disease in Japan, and other *Raffaelea* spp. Since the discovery of this pathogen on a dead Mongolian oak (*Quercus mongolica*) in 2004, the disease epidemic has centered around Seoul and Gyeonggi Province and continued to spread southwards. Despite continued expansion of the disease and associated significant impacts on forest ecosystems, information is lacking about the origin and genetic diversity of *R. quercus-mongolicae*. Restriction site-associated DNA sequencing (RAD-seq) was used to assess genetic diversity and structure among populations at nine sites in South Korea. Sequencing the RAD-tag library generated 143,696,855 reads using Illumina HiSeq. In total, 481 single nucleotide polymorphisms (SNPs) were identified among 2,639 RAD loci in the nuclear genome of 55 *R. quercus-mongolicae* isolates (0.0021 SNPs per bp) with overall low expected heterozygosity, indicating a low level of genetic diversity across these samples. Analyses of genetic diversity and population structure of *R. quercus-mongolicae* are ongoing. Understanding population structure is critical to associate differential virulence of this pathogen among geographic regions, evaluate pathways of spread, and develop more efficient disease prediction and management methods.

Molecular diagnosis of bacterial spot pathogens on pepper and tomato in Pennsylvania

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Bacterial spot of tomato and pepper is caused by four *Xanthomonas* species including *X. vesicatoria*, *X. perforans*, *X. euvesicatoria*, and *X. gardneri*. This disease has been a chronic problem in Pennsylvania (PA), causing heavy losses in tomato and pepper production. From a total of 5,945 clinical samples of tomato (5,035) and pepper (911) submitted to the PA Department of Agriculture (PDA) Diagnostic Laboratory since 1984, 178 strains of presumptive Bacterial Spot *Xanthomonads* have been isolated and achieved. Based on fingerprinting by *BOX-PCR* and sequencing of 16S ribosomal RNA (rRNA) encoding gene, 15 strains were identified as *X. euvesicatoria* (1987-2012), one as *X. vesicatoria* (1996), 28 as *X. gardneri* (1995-2012), and 89 as *X. perforans* (1994-2012). We have developed a real-time PCR primers and probe based on a unique *X. gardneri* XGA_0724 gene, encoding a putative type III effector *avrBs1* class that is possibly related to increased virulence on tomato. The primers and probe were successfully used to detect *X. gardneri* at least 10³ CFU/100 mg plant tissue from both tomato and pepper in Multiplex PCR with Cox primers and probe specific to plant cytochrome oxidase gene. We have evaluated detection and identification of *X. gardneri* using clinical samples submitted to PDA from 2010 to 2012. All PCR positive results were confirmed with *X. gardneri* culture isolation and BOX-PCR profile. All PCR negative samples produced no *X. gardneri*.

Analysis of a population of *Pyrenophora teres* f. *maculata* for virulence/avirulence factors using association mapping

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Identification of virulence factors/effectors in necrotrophic plant pathogen populations provides insight into the genetic interactions occurring in the pathosystem. Many characterized necrotrophs show quantitative virulence and

contain effectors that interact with host susceptibility targets in an inverse gene-for-gene manner. Identification of effectors with major effects on virulence in the pathogen population can aid in the identification of the host susceptibility targets so they can be removed by traditional breeding practices. A natural population of *Pyrenophora teres* f. *maculata* (*Ptm*), the causal agent of barley spot form net blotch were collected from geographically distinct regions of North Dakota. Association mapping (AM) will be used to characterize QTL contributing to virulence in this population. AM requires high-density genotyping of isolates from the populations and adequate single nucleotide polymorphism (SNP) marker coverage can be obtained by genotyping-by-sequencing (GBS). Preliminary GBS data of 38 *Ptm* isolates indicated that the isolates are highly polymorphic, and molecular analysis identified both mating types, suggesting that the population is sexual. GBS analysis of the population will be completed and utilized for AM analysis. Phenotypic analyses of the isolates will be conducted on a differential set of 30 barley lines and AM will be conducted using JMP Genomics.

Black-pigmented strain of *Bacillus* with potential biocontrol capabilities

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Strains of bacteria, in the *Bacillus subtilis* complex, have been shown to have significant antifungal properties as well as the capability to colonize plants as a biotrophic endophyte. We report here information on the cultural characteristics, results of antifungal challenges, genomic analysis, and biotrophic capability of a novel black-pigmented strain of *Bacillus*, designated HU-Biol-II. Features of this strain are similar to those of *B. mojavensis*, a species known for its antifungal and endophytic nature. Each strain has been grown on PDA or in potato dextrose broth (PDB) and incubated at temperatures of 26 /37 C. Results of antifungal effect of each strain on mycelial growth inhibition and spore germination of selected test fungi, show that the HU-Biol-II strain has stronger antifungal properties. Genomic analysis of the 16S rRNA gene reveals that this strain does not match *B. mojavensis* or other species in the *Bacillus* subgroup. Topical applications of cells of both strains to cotton and soybean seeds resulted in the colonization of seedlings. Recovery isolates from seedlings possess cultural and antifungal properties typical of stock cultures. Distinctive cultural characteristics, lack of genome sequence match, and higher antifungal potency suggest that the novel strain is different from the type of *B. mojavensis* and possesses high potential for the biocontrol of assorted fungal induced diseases of plants.

Determining the genetic structure within one population of the gasteromycete *Guyanagaster necrorhiza* in the Pakaraima Mountains of Guyana

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Recent mycological work in the Pakaraima Mountains of Guyana uncovered a new gasteromycete genus and species, *Guyanagaster necrorhiza*. Salient characteristics include its persistent, long-lasting peridium and tough, decay-resistant gleba making it unlikely this species is adapted for either wind or rain dispersal. Additionally it does not possess any human detectable odors that are apparent in mammal-dispersed gasteromycetes. The lack of obvious mechanisms for spore dispersal in conjunction with evidence of extensive tunneling by invertebrates led to the hypothesis that non-mammalian animals may be vectoring *G. necrorhiza* spores through feeding. Determining whether gene flow occurs between patches of *G. necrorhiza* will help infer the magnitude at which spore dispersal is possible. To do this, fruiting bodies were collected across an area of ten square kilometers in the Upper Potaro region of the Pakaraima Mountains in western Guyana in the summer of 2012. DNA from each fruiting body was amplified at the ITS and IGS loci as well as at *G. necrorhiza*-specific microsatellites. Preliminary results found several alleles restricted to certain nearby patches, suggesting spore dispersal for *G. necrorhiza* could be limited to 100 m. To determine the vector responsible for spore dispersal, we will need to compare the distances and patterns of gene flow between patches of *G. necrorhiza* with the foraging patterns and dispersal distances of non-mammalian animals in the region.

Are polysaccharide lyase genes critical for the infection of soybeans by *Phytophthora sojae*?

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Phytophthora sojae is one of the most important and destructive agricultural parasites known today. Despite its prevalence, important questions remain

unanswered regarding the specific mechanisms involved in host (soybean) cell penetration. While it is known that the pathogen infects hosts using specialized structures, mainly the appressorium, the force of this structure alone is not sufficient to penetrate the host's rigid cell wall. Therefore, it has been suggested that cell wall degrading enzymes (CWDE) may aid in the penetration process. One subset of these enzymes, the polysaccharide lyase (PL) superfamily, has been hypothesized to play an important role in host cell penetration by degrading polysaccharides found in the cell wall. Genomic analyses have revealed that *P. sojae* contains a large number of PL genes, most of them belonging to families 1, 3, and 4. To investigate the potential role of PL genes in pathogenicity, relative expression of genes coding for PL family 3 enzymes was quantified over a 48-hour time period after soybean inoculation with *P. sojae*. Initial results indicate that several genes within this family experience significant up-regulation during the first 24 hours of infection, displaying more than 65,000-fold increase in expression. These results provide further evidence for the role of PL genes in the initiation of infection via degradation of polysaccharides within the soybean cell wall.

Molecular characterization of apple scab populations from the Eastern and Western Cape Provinces of South Africa

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Venturia inaequalis, the causal agent of apple scab, is one of the most important diseases of apple (*Malus pumila*), in terms of economic crop losses, world-wide and in South Africa. To date, 17 different scab races have been identified, each containing a different avirulence factor that is recognised by a cognate apple R-gene product. The aim of this study is the molecular characterization of three apple scab populations from different apple growing regions in South Africa. In 2011/2012 and 2012/13, single spored isolates were collected during the summer months from lesions on leaves and fruit from the Langkloof in the Eastern Cape Province, and from Elgin and Ceres in the Western Cape Province. Species identity was confirmed using ITS and *ABC2* sequencing. A high number of synonymous changes was found in the six *ABC2* haplotypes ($TD = 0.034$; $P > 0.1$), and three ITS haplotypes were identified. Six known microsatellite markers from *V. inaequalis*, EmVi10, Vitc2/D, Vitg9/129, Vitg11/70, Itc1g and Itc1a, were used for genotyping in order to characterise the variability of the South African scab populations. Results indicate that South Africa has highly variable and sexually outcrossing scab populations ($Vo/Ve = 1.18$; $P = 0.55$). Overall SSR markers used in this study were highly variable ($H = 0.62$). In addition, each region has 'private' alleles indicating moderate differences between the populations ($F_{st} = 0.15$; $P = 0.001$).

Confirming resistance in bottle gourd germplasm by quantifying powdery mildew conidia using a cellometer

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Powdery mildew (PM) caused by *Podosphaera xanthii*, an important foliar disease affecting cucurbit crops grown in the United States, commonly occurs on foliage, petioles, and stems. We have developed two highly resistant bottle gourd (*Lagenaria siceraria*) germplasm (USVL351 and USVL482) for use in our watermelon rootstock breeding program. Seedlings of the resistant germplasm lines along with a commercial rootstock 'Emphasis' and a susceptible check (PI 381848) were spray inoculated (10^5 conidia/ml) in a greenhouse and PM development was recorded after 14 days. The hypocotyls, cotyledon and first true leaf from all plants were collected in individual Ziploc bags and weighed. Conidia present on plant tissue were dislodged in sterile water (0.02% Tween 20) and quantified using a Cellometer Auto T4 cell counter (Nexcelom Biosciences, nexcelom.com). Cellometer size specification for the conidia was set at $42 \times 15 \mu\text{m}$. PM severity on hypocotyl (8%) cotyledon (8%) and true leaves (17%) on the two resistant germplasm lines was significantly less compared to the susceptible check (all $>73\%$). The rootstock Emphasis was significantly less susceptible than PI 381848 but it was not resistant as the USVL lines. PM conidial counts on all plant parts were significantly lower on USVL resistant lines (range 10^2 - 10^4 /g) compared to PI 381848 (10^6 - 10^7 /g). The cellometer can be a useful device to quantify pathogen propagules such as PM conidia for confirming disease resistance.

WITHDRAWN

Estimation of 'Candidatus Liberibacter asiaticus' populations in Texas citrus trees

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To determine which citrus tissues have relatively high 'Ca. Liberibacter asiaticus' (CLAs) populations for reliable, early detection of Huanglongbing, we determined cell populations in various tissues collected from infected field trees in Texas using the previously described grand universal regression equation $Y = 13.82 - 0.2866X$, where Y is the log of the target copy number and X is the Ct values of the assay. Overall, significantly higher CLAs populations were recorded in grapefruit compared to sweet orange. They also varied with plant tissue, with pedicel, columella, and midribs having significantly higher titers compared to other tissues. Lowest bacterial titers were consistently recorded in young shoots, young leaves, flower buds, and leaf blades, especially leaf margins. Analysis of variance (ANOVA) showed that there is no significant difference ($p > 0.05$) in bacterial concentrations in symptomatic leaf and root samples nor was there any significant difference in test results among different distances from the trunk or quadrants where the root samples were collected. Thin roots and root intersections, specifically fibrous feeder roots, consistently showed higher bacterial concentrations compared to thicker roots and thicker intersections.

Large-scale genomic and transcriptomic analysis of mycorrhizal fungi

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Mycorrhizal fungi play critical roles in host plant health, soil community structure and chemistry, and carbon and nutrient cycling. To this end the U.S. Dept. of Energy Joint Genome Institute (JGI) is building on our earlier sequencing of the *Laccaria bicolor* genome by partnering with INRA-Nancy and the mycorrhizal research community in the MGI to sequence and analyze 2 dozen mycorrhizal genomes of all Basidiomycota and Ascomycota orders and several ecological types (ectomycorrhizal, ericoid, and orchid). JGI has developed and deployed high-throughput pipelines for genomic, transcriptomic, and re-sequencing, and platforms for assembly, annotation, and analysis. In the last 2 years we have sequenced 21 genomes of mycorrhizal fungi, and resequenced 6 additional strains of *L. bicolor*. All of this data is publicly available on JGI MycoCosm's Mycorrhizal Fungi Portal (http://jgi.doe.gov/Mycorrhizal_fungi/), which provides access to both the genome data and tools with which to analyze the data. Preliminary work with the current public mycorrhizal genomes include observations that 1) short secreted proteins potentially involved in symbiosis are more enriched in some orders than in others amongst the mycorrhizal Agaricomycetes, 2) there are as much as 70-fold differences in numbers of genes involved in signal transduction and post-translational modification, 3) massive gene family expansions or contractions distinguish mycorrhizae from their non-symbiotic relatives.

Development of SCAR markers and UP-PCR cross-hybridization method for specific detection of brown patch pathogens from infected turfgrasses

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Several species and hyphal anastomosis groups (AG) of *Rhizoctonia solani* (sensu lato) cause brown patch diseases of turfgrasses. Conventional methods of identification of *Rhizoctonia* pathogens are time consuming and often inaccurate. A rapid identification assay for *Waitea circinata* (anamorph: *Rhizoctonia* spp.) varieties *zeae* and *circinata* causing patch diseases was developed based on the universally primed PCR (UP-PCR) products cross-blot hybridization with non-radioactive probes. Isolates within a *W. circinata* variety cross-hybridized strongly while non-homologous isolates did not cross-hybridize or did so weakly. Closely related *W. circinata* varieties *zeae* and *circinata* were clearly distinguished using this assay. In addition, sequence-characterized amplified region (SCAR) markers were developed from UP-PCR products to identify turf pathogenic isolates of *Thanatephorus cucumeris* (anamorph: *R. solani*) AG 1-IB and AG 2-IIIB. The developed SCAR markers could distinguish isolates of AG 1-IB or AG 2-IIIB groups and did not amplify any product from genomic DNA of non-target isolates of *Rhizoctonia*. The specific primers were sensitive and unique enough to produce a PCR band from total DNA of diseased turfgrasses infected with either AG 1-IB or AG 2-IIIB.

Development of detection tools for 10 of Canada's "most unwanted" forest pathogens

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The TAIGA (Tree Aggressor Identification using Genomics Approaches) project aims to increase Canada's capacity in forest pathogen detection and monitoring by designing high-throughput DNA-based diagnostic assays. One objective of the project is to develop diagnostic tools to detect Canada's "most unwanted" forest pathogens. Over the last year, we worked closely with stakeholders and end-users to identify the Top 50 "most unwanted" forest pathogens in Canada, which include pathogens regulated by quarantines in Canada or in key import nations. Using PCR DNA sequences obtained from target species and close relatives, we developed TaqMan qPCR detection assays for 10 of the identified pathogens. Assays were developed using standardized conditions for single array multiplexing. They were validated using a panel of pure cultures as well as environmental and/or herbarium samples, when available. In parallel, we developed genus-specific primers to perform SYBRGreen qPCR experiments to ascertain DNA sample quality. All these assays will soon be available for technology transfer as single or multiplex tests or made available as a service offered to end-users and partners. These diagnostic tools will not only be useful in preventing the introduction of unwanted exotic and invasive pathogens but may also assist the forest industry and nurseries with phytosanitary management and certification.

Simultaneous detection and differentiation of three sweet potato potyviruses by one-step RT-PCR

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Sweet Potato Viral Disease is the most devastating disease of sweet potato worldwide. The disease is caused by mix infection of *Sweet potato chlorotic stunt virus*, a member of the genus *Crinivirus* in the family *Closteroviridae*, and several viruses including *Sweet potato latent virus* (SPLV), *Sweet potato virus G* (SPVG) and *Sweet potato mild mottle virus* (SPMMV) in the family *Potyviridae*. In this study, a one-tube multiplex reverse transcription (mRT)-PCR assay was developed for the simultaneous detection and differentiation of SPLV, SPVG and SPMMV. Three pairs of primers specific for each of the three viruses were used to amplify PCR products of different sizes that can be resolved by agarose gel electrophoresis. Amplification of a plant 18S rRNA fragment was included in the assay as an internal control. Amplicons of 176 bp (SPVG), 300 bp (SPLV), 414 bp (SPMMV) and 844 bp (18S rRNA) were obtained in the assay. The mRT-PCR assay was optimized for primer concentrations and thermal cycling conditions. The mRT-PCR sensitivities were 10^{-4} for the target viruses and the host rRNA, similar to those of the individual uniplex RT-PCR assays. The mRT-PCR was validated using our

positive controls and field samples from southwestern China. This assay is a simple, rapid, reliable and cost-effective to detect these viruses in sweet potato. It will be useful for quarantine and certification programs, as well as virus surveys, when numerous samples are tested.

Characterizing a synergistic interaction between *Glycine max*, *Soybean mosaic virus*, and soil salinity

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Due to increased irrigation and agricultural expansion into marginal lands, saline soils are an increasing problem worldwide. Reaction of soybean, *Glycine max* (L.) Merr., cultivars to salt stress can differ widely based on chloride accumulation in the leaves. To address the impact of environmental factors on virus-host relationships, we set out to characterize the interactions of *Soybean mosaic virus* (SMV), soybean cultivars that differ in chloride uptake, and salt stress. Soybeans, chloride-sensitive Clark and -tolerant Manokin, were mechanically inoculated with SMV either prior to, or following salt treatment. Three concentrations (25, 50, or 100 mM chloride) of either NaCl or CaCl₂, were applied by flooding daily. After salt-stress symptoms appeared on unifoliate leaves, tissue was collected for SMV quantification via ELISA. Soybeans infected with SMV were severely stunted and developed viral symptoms regardless of salt treatment. Infection with SMV did not alter salt tolerance in Manokin; however, infected Clark and Manokin soybeans treated with the highest levels of chloride died earlier than SMV-free soybeans. In Manokin, SMV levels were significantly lower in plants treated with 25 mM NaCl but not in 50 or 100 mM NaCl. In Clark, all CaCl₂ treatments reduced SMV levels significantly compared to the water control. Salt stress impacts SMV levels and vice versa in soybean with clear implications for growers dealing with both salt stress and SMV infections.

Detection of *Xanthomonas oryzae* by loop-mediated isothermal amplification

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Molecular diagnostics of crops and livestock diseases play an important role in enhancing food security. A recent advance in molecular diagnostics is the novel loop-mediated isothermal amplification (LAMP) method. LAMP allows for rapid, highly specific amplification of target DNA sequences at a single temperature, and is ideal for field-level analysis. Rice is a staple crop for much of the world's population, including that of sub-Saharan Africa. Two important rice diseases are caused by *Xanthomonas* species. *X. oryzae* pv. *oryzae* (Xoo) colonizes xylem vessels and causes bacterial blight (BB), while *X. oryzae* pv. *oryzicola* (Xoc) colonizes spaces between leaf parenchyma cells to cause bacterial leaf streak (BLS). Both pathogens represent a significant threat for agriculture and global food security, and are considered quarantine organisms in all rice growing countries. We adapted existing genomics-based molecular diagnostic tools for these pathogens into a reliable, sensitive LAMP assay. The specific presence of each pathogen was detected from DNA, cells, leaf and seed samples. Thresholds of detection were consistently 10^3 CFU/ml of cells and 1 fg of genomic DNA. LAMP for both BB and BLS pathogens will allow surveillance activities in rice fields as well as testing of imported materials by quarantine offices.

Evaluation of the control of black rot in cabbage following treatments to transplants in the greenhouse and field

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Black rot caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Xcc) is often a serious disease in New York State crucifer fields. This pathogen is seed borne and even rigorous seed testing can not guarantee every seed is free of bacteria. In greenhouse transplant operations conditions are ideal for the spread of Xcc with dense plant populations and overhead watering. Asymptomatic transplants can initiate field infections which are very difficult to control. To investigate the efficacy of chemical and biological products used to reduce the spread and establishment of Xcc in the greenhouse and continued control of disease in the field, treatments were applied to flats

of cabbage seedlings and half were inoculated the following day with Xcc. Sprays were continued until harvest and plots were rated for symptoms of black rot and evaluated for yield. By reducing pathogen survival and spread in the greenhouse prior to transplant to the field, both incidence and severity of black rot on cabbage can be reduced with both organic and conventional products.

***Arabidopsis* nonhost resistance genes to defeat Asian soybean rust**

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Soybean is highly susceptible to *Phakopsora pachyrhizi*, the causal agent of Asian soybean rust (SBR) disease. Since traditional breeding by now has not provided soybean varieties with stable resistance to all *Phakopsora* isolates, alternative strategies are urgently needed. Nonhost resistance (NHR) is the most durable form of plant disease resistance and is, therefore, ideally suited for the identification of effective resistance traits to SBR. In the present study we used the *Arabidopsis*-*P. pachyrhizi* nonhost interaction to identify novel *Arabidopsis* NHR genes via global transcriptome analysis. By doing so, we found several genes that are essential for *Arabidopsis*' postinvasion NHR to SBR. One of these genes encodes UDP-glycosyltransferase UGT84A2/BRT1 in the phenylpropanoid metabolism. We also provide evidence that cross species transfer of identified *Arabidopsis* NHR genes quantitatively enhanced soybean resistance to *P. pachyrhizi* in greenhouse experiments. Stable overexpression of *Arabidopsis* NHR genes in the susceptible soybean cultivar Williams 82 significantly repressed fungal proliferation as well as symptom development when compared to non-transgenic controls. Future studies will confirm the transgenic lines' potential for application in the field. Currently, we are investigating the candidate genes' mode of action in plant defense.

Towards defining plant diagnostic tool development standards: Ensuring accuracy and universal communications across plant diagnostic laboratories

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Regulatory diagnoses require specific identification that increasingly can be addressed with PCR. Several current competitive funding programs encourage the incorporation of diagnostic method development and outreach via extension service and/or state regulatory laboratories. In answer to the increasing demand for molecular identification, diagnosticians are incorporating PCR assays developed in research laboratories around the world. However, what constitutes a tool for identification in a research laboratory, and its counterpart in a diagnostic setting may be very different protocols. Primarily, a protocol must be adequately described and should be validated through testing in multiple, method-appropriate laboratories. Additionally, development, dissemination, and recommendations for use of appropriate controls should be included in a valid test. We describe a set of guidelines for developing and defining a method or tool for use in plant diagnostic laboratories.

Effective implementation of disease-suppressive crops in potato rotations

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Use of disease-suppressive rotation crops, such as *Brassica* spp. (mustards, rapeseed) and sudangrass, has shown much potential for management of soilborne diseases and enhanced yield in various crop production systems. In research conducted in Maine over the last several years, soilborne potato diseases, including black scurf, common scab, and Verticillium wilt have been consistently reduced (10 to 95%, with an average reduction of 30%) and yield significantly improved (10 to 38%) with *Brassica* rotations relative to standard rotations. Recent studies have focused on how to best utilize these crops in potato production in the Northeast, determining which crops to use and effective management practices. In a direct comparison of five different rotation crops (mustard blend, rapeseed, sudangrass, barley, and soybean) under four different management types (cover crop, harvested-not incorporated, harvested-incorporated, and green manure), crops managed as green manures produced overall lower disease (by 15-26%) and higher yields (by 6-13%). Crops harvested for seed then incorporated also provided significant benefits, but rotations managed strictly as cover crops were least effective. Mustard blend, sudangrass, and rapeseed all reduced black scurf (by 16-27%) and increased yield (by 6-11%). Overall, the combination of mustard blend managed as a green manure was most effective, reducing scurf by 54% and increasing yield by 26% relative to a soybean cover crop.

Potential treatments for disinfesting runoff water from nurseries contaminated with *Phytophthora ramorum*

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The quarantine pathogen *Phytophthora ramorum* has been detected in nurseries and associated waterways in several states. Chemical treatment of runoff water from nurseries could reduce spread of *P. ramorum* to the environment, but may be toxic to aquatic life. We assayed four pond algae treatment products and an experimental material for their effectiveness against *P. ramorum* zoospores in vitro: Algae-Off® and Biological Clarifier+® (Winston Co.) Pondcare Algaefix® (Aquarium Pharmaceuticals, Inc.), Pro-Clear® (Kent Marine), and a rhamnolipid surfactant (AGAE Technologies LLC). Microtitre wells were filled with equal volumes of a zoospore suspension (10,000 zoospore/mL) and aqueous suspensions of the commercial pond treatments at 1x and 10x the recommended rate; the rhamnolipid was tested at 10, 25, 50, and 100 mg/L. Deionized water and a sodium hypochlorite solution (2 ppm) served as controls. Treatments were randomized in each of five replicate blocks, with eight wells per replicate. After 1 hr a rhododendron leaf disk was added to each well and disease incidence was observed after 2 weeks. Pondcare Algaefix and Algae-Off at the 10x rate, Pro-Clear at the 10x and 1x rate, and the rhamnolipid at the 100 mg/L rate reduced disease incidence to 0%. None of the other treatments were effective. Some of these materials offer potential for disinfesting runoff water from *P. ramorum*-infested nurseries and warrant further testing on other *P. ramorum* propagules.

Novel regulatory genes affect thaxtomin production and pathogenesis in *Streptomyces scabies*

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Potato scab disease, caused by *Streptomyces scabies* and other streptomycetes, has a global distribution and causes significant economic losses. Production of thaxtomin A, a nitrated dipeptide phytotoxin that potentiates virulence, is regulated by the *txtR* gene. Additional regulatory genes likely play a role in the production of thaxtomin, or in the expression of other genes involved in pathogenicity; their discovery may suggest new methods of combating potato scab. A total of 21 previously unstudied regulatory genes, that are conserved among the plant pathogens *S. scabies*, *S. turgidiscabies* and *S. ipomoeae*, were deleted in *S. scabies* 87-22 and mutants were evaluated for production of thaxtomin in oat bran broth (OBB). One knockout was reduced in thaxtomin production, while two overproduced thaxtomin. RT QPCR revealed that the thaxtomin biosynthetic cluster was over expressed in all three knockouts, though other pathogenicity genes were unaffected. Two of the regulators belong to the GntR family, a member of which is a global developmental regulator. Their effect may be due to the link between development and secondary metabolism. The third regulator belongs to the ribonuclease III family, a member which is a global regulator of secondary metabolism; the homolog in *S. scabies* may have a similar role. The overexpression of thaxtomin biosynthetic genes in the knockout with reduced production indicates that the regulation of thaxtomin production is a complex process.

WITHDRAWN

Protecting plant resources while facilitating trade in North America

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The North American Plant Protection Organization (NAPPO) provides a forum for public and private sectors in Canada, the USA and Mexico to collaborate in developing science-based standards to protect agricultural, forest and other plant resources against regulated plant pests, while facilitating trade. Strong regional standards help harmonize procedures to detect pests, define acceptable quarantine measures, and define risks of introduction and spread, while taking into account costs. NAPPO depends on a range of stakeholders including regulators, scientists, academics, producers and national industry associations to achieve its mission. Involving these stakeholders throughout the preparation of NAPPO documents encourages information sharing, provides the practical experience of producers and brings in environmental concerns. This leads to the development of standards and discussion documents that are up-to-date, science-based and relevant. NAPPO has also prepared diagnostic protocols for pests such as Huanglongbing and *Citrus tristeza virus*. Numerous NAPPO standards have been presented to the International Plant Protection Convention of the Food and Agriculture Organization of the United Nations. These standards have formed the basis for International Standards for Phytosanitary Measures (ISPMs) that are now applied globally. The most widely recognized example has been ISPM 15 *Regulation of Wood Packaging Material in International Trade*.

Functional characterization of two toxin-antitoxin systems of *Xylella fastidiosa*

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Xylella fastidiosa (Xf) encodes multiple toxin-antitoxin (TA) systems, including *relE/dinJ* and *mqsR/ygiT*. Phylogenetic analyses indicate these two TA systems have distinct evolutionary histories. Comparisons among Xf subspecies/strains reveal TA systems are often embedded in prophages. Tagged proteins of both TA systems were over-expressed, purified, and evaluated for activity. Toxins MqsR and RelE are ribonucleases with distinct cleavage sites. YgiT and DinJ antitoxins inhibit ribonuclease activity of cognate toxins by direct binding. Single (toxin or antitoxin) and double (toxin and antitoxin) knock-out mutants were constructed in Xf strain Temecula. Both antitoxin mutants displayed reduced planktonic cell density relative to wild type. However, the *dinJ*-mutant produced less biofilm, whereas the *ygiT*-mutant produced more biofilm, relative to wild type. Planktonic cell density of both toxin mutants was unaffected, with biofilm reduced only slightly. Although planktonic cell density and biofilm formation of each double mutant was similar to wild type, significant differences were observed in cell viability. Viable cell counts from biofilm and plankton for the *mqsR*-/*ygiT*-double mutant were consistently two orders of magnitude greater than wild type. In contrast, cell viability of the *relE*-/*dinJ*-double mutant was variable, possibly due to culture nutritional status. Collectively, the results indicate that Xf *mqsR/ygiT* and *relE/dinJ* are functional TA systems.

Analysis of rice PDR-like ABC transporter genes in sheath blight resistance

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Sheath blight caused by *Rhizoctonia solani* is one of the most damaging diseases of rice worldwide. To understand the molecular mechanism of resistance, we identified 450 differentially expressed genes in a resistant rice cultivar Jasmine 85 after *R. solani* infection with a combination of DNA microarray, serial analysis of gene expression, and suppression subtractive hybridization. Among the up-regulated rice genes, we found that transcripts of pleiotropic drug resistance (PDR)-like ATP-binding cassette (ABC) transporter rapidly accumulated at the early phases of *R. solani* infection throughout repeated time point experiments by semi-quantitative RT-PCR, and real time PCR. In contrast, alteration of transcripts of ABC transporters was not observed after Jasmine 85 was inoculated with both avirulent and virulent races of blast fungus at the same time points. One of the ABC transporters was also mapped to the major sheath blight resistant QTL *qShB9*-

2 using a mapping population derived from the cross of a susceptible cultivar, Lemont with Jasmine 85. DNA sequences of ABC transporters from Jasmine 85 and Lemont were determined, and used to develop gene specific markers. The gene specific markers developed are being used to establish a correlation between disease reactions of 568 RILs of Jasmine85 and Lemont population and to determine percentages of contribution of each member of these ABC transporter genes in sheath blight resistance and results will be presented.

Not all *Trichoderma* is created equal: Responses in plants to volatile organic compounds (VOCs) from different species and strains

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Members of one of the most frequently isolated genera of free-living soil fungi, *Trichoderma*, are well studied for their ability to reduce plant disease and promote plant growth. The purpose of this study was to examine and evaluate the effects of VOCs from several species of *Trichoderma*: *T. atroviride*, *T. brevicompactum*, *T. harzianum*, *T. virens*, and many more (20 strains total). Plants and fungi were grown together in a split petri plate with physical separation and gas exchange. *Arabidopsis* exposed to VOCs of several species of *Trichoderma* exhibited growth promotion and developmental changes including larger leaf size, increased shoot weight, increased lateral root branching, and increased total chlorophyll concentration. Plants exposed to VOCs emitted from *T. aggressivum*, *T. asperellum*, *T. pseudokoningii*, and *T. viride* had 26%-45% increase in freshweight (control, 22mg) and 50%-82% increase in total chlorophyll concentration (control, 0.61mg chlorophyll/gram). Interestingly, different strains of *T. asperellum* induced varied responses; strain GJS02-65 induced plant growth promotion (+25% fw; +52% chlorophyll) while strain CBS433.97 inhibited plant growth (-7% fw; -14% chlorophyll). Strains such as *T. inhamatum*, *T. longibrachiatum*, and *T. stromaticum* did not induce any significant changes in plants. CGMS analysis included a wide range of compounds: hydrocarbons, sesquiterpenes, terpenes, alkenes, alcohols, aldehydes, ketones, aromatics, and heterocyclics.

WITHDRAWN

Genetic variation of *Cochliobolus sativus* isolates collected from wheat, barley, and grasses

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Cochliobolus sativus is one of the most important fungal pathogens of barley and wheat. It causes spot blotch, common root rot, and kernel blight on these important cereal crops. The fungus also infects a number of grasses. To understand genetic variation of the fungus from different hosts, we evaluated 24, 24 and 52 isolates collected from barley, wheat and grasses, respectively, using 13 SSR primers previously mapped in the genome of the fungus. These 100 isolates were also tested on three barley differentials, three wheat differentials and one *Brachypodium distachyon* line for virulence. The results indicated that isolates were generally grouped according to their host origin.

The pathogenicity tests indicated that most isolates from wheat and grasses showed low virulence on the three barley differentials, while barley isolates generally exhibited low virulence on the three wheat differentials, suggesting that the fungal population differentiated according to the hosts to some extent. Two grass isolates were grouped together with the pathotype 2 isolates and they had the same virulence pattern as the pathotype 2 isolate ND90Pr on different hosts tested. Three grass isolates were not clustered with any other isolates and were avirulent on all hosts tested, which merit further characterization to determine if they belong to *C. sativus*.

Phytophthora apocalypse: Expanding host range of a *Phytophthora* hybrid threatens Midwest wildflowers

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Phytophthora species infect a diversity of host plants. Within the ornamental industry, inter- and intra-national transport of plants potentiates the possibility of hybridization between *Phytophthora* species. Hybrid isolates of *P. cactorum* x *hedraiaandra* were recently found on *Rhododendron* and *Dicentra eximia* (wild bleeding heart). *Rhododendron* has been reported previously to be a host of this hybrid and its parents, but *Dicentra* has never been reported to be a host of *P. cactorum*, *P. hedraiaandra*, or *P. cactorum* x *hedraiaandra*, which we hypothesize could be an expansion of the host range due to species hybridization. We performed Koch's postulates to test this hypothesis using *P. cactorum* x *hedraiaandra*, and the parent species *P. cactorum* and *P. hedraiaandra*. We inoculated the following *Dicentra* species: *D. eximia*, *D. canadensis*, *D. culcullaria* and *D. 'Luxuriant'* in addition to common greenhouse and nursery ornamentals. The hybrid isolate was found to infect and was recovered from all hosts tested. *P. cactorum* and *P. hedraiaandra* was isolated from all hosts except members of the *Dicentra* genus. Three different species of *Dicentra* are native to Indiana: *D. canadensis* (squirrel corn), *D. culcullaria* (Dutchman's breeches), and *D. eximia*. Wildflowers throughout Indiana and the Midwest are challenged with increasing threats from habitat loss, invasive weeds, illegal collecting, and now possibly newly developed hybrid *Phytophthora* spp. with expanded host ranges.

Evaluation of acibenzolar-S-methyl and phosphorous acid for the control of bacterial diseases and PR-gene induction

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In Michigan and worldwide, fire blight (*Erwinia amylovora*, *Ea*) and bacterial canker/blossom blast (*Pseudomonas syringae* pv. *syringae*, *Pss*) are two of the most invasive and devastating bacterial diseases affecting pome and stone fruits, respectively. Effective management of *Ea* populations relies heavily on the use of antibiotics while only copper formulations are registered for *Pss* control. During 2008-2012, field trials were conducted on apple and cherry to evaluate a plant activator (acibenzolar-S-methyl, Actigard®, ASM) and a fungicide/resistance inducer (phosphorous acid salts, Phostrol®, PH) as candidates to supplement *Ea* and *Pss* management programs. On apple, ASM provided control of fire blight either alone or in combination with antibiotics and PH controlled *Ea* blossom blight when combined with a biological control; both products were most effective under low to moderate disease pressure. On cherry, *Pss* populations on flowers were not affected after treatment with PH, ASM, or in combination with one another. PH did not have an effect on the incidence of *Pss* leaf spot but did reduce disease severity in one field trial. Additional studies included PR-gene expression profiling on ASM and PH-treated trees using real-time quantitative PCR. Overall results indicate that ASM and PH have potential in reducing the use of antibiotics and copper for *Ea* and *Pss* control in seasons when disease pressure is low to moderate.

Advances in the detection of 'Candidatus Liberibacter solanacearum', the causative agent of potato zebra chip disease

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Zebra chip disease of potato is caused by the phloem restricted bacterium 'Candidatus Liberibacter solanacearum' (Lso), transmitted to solanaceous plants by the psyllid *Bactericera cockerelli*. Accurate pathogen detection of Lso in insects and plants is essential for monitoring the disease. Current methods rely on assays using conventional polymerase chain reaction (cPCR) or quantitative real-time polymerase chain reaction (qPCR). These methods are somewhat inconsistent due largely to variability in bacterial populations and the presence of components of host tissues that may interfere with PCR

amplification. Both factors limit the efficacy of PCR reactions and have necessitated extraction of pure DNA before amplification. In this study, a protocol was developed for rapid DNA isolation from psyllids that can be used directly with cPCR or LAMP for reliable detection of Lso. Samples prepared using the fast DNA extraction method must be diluted 1:100 in sterile water for reliable amplification, presumably to dilute PCR inhibitors in the crude extract. Both cPCR and LAMP detected Lso in infected psyllids equally well from diluted samples prepared using the fast DNA extraction method or from samples prepared using a DNA purification step. In addition to being reliable, the fast DNA extraction method required approximately 5 minutes for 10 samples and no special reagents or laboratory equipment, significantly streamlining the detection of 'Ca. Liberibacter' in psyllids.

Genetic structure of soil populations of *Aspergillus* section *Flavi* and efficacy of biocontrol of aflatoxin in corn

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Toxigenic members of *Aspergillus* section *Flavi* produce aflatoxins, which are potent carcinogens and hepatotoxins. Biocontrol, using atoxigenic strains formulated as either AF36® or AflaGuard®, offers the greatest potential to manage aflatoxin contamination. However, the impact of indigenous soil populations of members of *Aspergillus* section *Flavi* on the efficacy of these biocontrol formulations has yet to be investigated. Field tests were conducted in replicated trials in Alabama, Georgia and North Carolina where AF36® and AflaGuard® were applied at tasseling. Soil samples weighing 100 g were collected prior to and after application of the treatments and at harvest from 20 predetermined points along two diagonals in each field. Corn ears were harvested at 15 to 17% moisture content and entire grain samples from each plot were collected to determine levels of aflatoxin contamination. Preliminary characterization of *Aspergillus* section *Flavi* species in soil prior to application of treatments revealed predominantly L-strains of *A. flavus* in North Carolina. Data on soil population density and genetic composition of members *Aspergillus* section *Flavi* will be presented and discussed. The implication of these results on efficacy of biocontrol will be established by linking toxin data from corn harvested from specific biocontrol treatments to soil population densities and the genetic structure of aflatoxigenic species.

Isolation, characterization, and evaluation of *Muscodor albus* isolate SA-13 for controlling plant pathogens

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A new strain of *Muscodor albus* was isolated from honey mesquite (*Prosopis glandulosa*). Based on morphology, ITS 5.8 S sequences, and chemical analysis of volatile organic compound (VOC) profiles, a unique strain *M. albus* SA-13 was confirmed. When compared *in vitro* to *M. albus* strain CZ-620, SA-13 is more robust in the production of VOCs and showed a greater inhibitory effect with complete control of *Botrytis cinerea*, *Fusarium oxysporum* f.sp. *fragariae*, *Macrophomina phaseolina*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotinia minor*, *Sclerotium rolfsii*, and *Verticillium dahliae*. The bacterial pathogens *Clavibacter michiganensis* subsp. *michiganensis*, *Pectobacterium carotovorum*, *Pseudomonas syringae*, and *Xanthomonas vesicatoria* were unable to produce any colonies in the presence of SA-13, except *C. m.* subsp. *michiganensis*, which showed recovery after SA-13 was applied for three days then removed. The VOCs produced by SA-13 were trapped with XAD7 resin, identified via GCMS analysis and compared with their commercial standards. A reconstituted mixture based on the chemical profile of SA-13 displayed growth inhibition of *F. oxysporium* f.sp. *fragariae*, *M. phaseolina*, *P. ultimum*, *R. solani*, *S. rolfsii*, and *V. dahliae*. The ability of *M. albus* SA-13 to produce VOCs with strong efficacy against a series of pathogens tested makes this isolate an ideal mycofumigant candidate for pest management.

The role of AtBAG6 as a positive regulator of autophagy in fungal pathogen resistance

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The BAG (Bcl-2-associated athanogene) family function as co-chaperones that modulate diverse cellular processes ranging from proliferation to growth arrest and cell death in both plants and animals. Bioinformatic approaches

uncovered the *Arabidopsis* BAG gene family. Our recent studies focus on AtBAG6. AtBAG6 knockout lines resulted in a dramatic loss of non-host resistance in *Arabidopsis* to the necrotrophic fungal pathogen *Botrytis cinerea*. Expression of full length AtBAG6 had no adverse effects on host plants or in yeast two hybrid studies; however expression of the AtBAG6, BAG domain alone resulted in both yeast and plant cell death. Overexpression of AtBAG6 exhibited a lesion mimic phenotype, while localization studies indicated that AtBAG6 resides in the vacuole. We identified a predicted caspase-1 cleavage site in AtBAG6, suggesting AtBAG6 needs processing for cell death/cyto-protective activity. Human Caspase-1 cleaved this site as predicted and chitin treatment or *Botrytis cinerea* inoculation, also demonstrated cleavage of this site. Site directed mutation of this site abolished cleavage. When the processed form of AtBAG6 was transiently expressed in tobacco, autophagy was observed. Collectively, these results suggest that AtBAG6 is processed following *Botrytis* challenge; presumably via a caspase-1 like protease. This processing is necessary for defense associated autophagy, restricting fungal growth and spread.

Evaluating soybean germplasm and commercial varieties for resistance to Phomopsis seed decay

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Soybean Phomopsis seed decay (PSD) is the major cause of poor seed quality in most soybean production areas of the United States. Very few soybean cultivars currently available for planting have resistance to PSD. To identify new sources of resistance to PSD, a multistate and multiyear research project funded by the United Soybean Board with support from the USDA-ARS was initiated in 2009. A total of 135 germplasm lines collected from 28 countries were field screened by natural infection in 2009 in Arkansas, Mississippi, and Missouri. Based on the seed assay results in 2009, 42 lines with the most resistant or susceptible reactions were selected and evaluated in 2010, 2011, and 2012 with *Phomopsis* inoculated and non-inoculated treatments. Significant differences in seed infection by the causal pathogen *Phomopsis longicolla* were observed among soybean lines with some lines having no infection while others had levels as high as 85%. These differences between lines also were reflected in visual seed quality and seed germination. Soybean lines with low seed infection and high germination rate at all locations and in four years will be used to develop breeding or mapping populations. Another study funded by the Mississippi Soybean Promotion Board, evaluated commercial varieties for resistance to PSD with inoculated and non-inoculated treatments and two harvest times (normal vs. delayed). Several varieties were identified with low disease incidence and good seed quality.

Early monitoring of stripe rust and leaf rust on wheat and their causal agents based on near infrared spectroscopy

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Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* and leaf rust caused by *P. recondita* f. sp. *tritici* are two kinds of economically important wheat diseases in China. Identification and quantification of the pathogens trapped by using spore traps and early diagnosis of these two kinds of diseases are of great significance for disease forecast. In this study, the spectra of 120 pathogen samples of uniformly mixed urediospores of these two kinds of pathogens in different proportions and the spectra of 150 wheat leaves divided into five categories including healthy leaves, leaves in the incubation period and in the disease period that infected with *P. striiformis* f. sp. *tritici* and *P. recondita* f. sp. *tritici*, respectively, were collected by using near infrared spectroscopy technology. A quantitative identification model to test the spore proportion was built using quantitative partial least squares (QPLS) and a qualitative identification model to identify the diseases was built using distinguished partial least squares (DPLS). The identification rates of the two optimized models for the training sets and the testing sets were more than 90% with high stability. The results indicated the method based on near infrared spectroscopy proposed in this study is useful for early monitoring and forecasting of these two kinds of wheat diseases.

***Aspergillus flavus* in corn ears from Indiana under the drought conditions in 2012**

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In 2012, the entire state of Indiana experienced severe drought conditions. Prior to corn harvest, *Aspergillus* ear rot was observed in fields throughout the state, with the highest incidence and severity observed in the southwestern region. Ears symptomatic of *Aspergillus* ear rot were collected from fields in six geographic regions in the state. A total of 30 isolates of *Aspergillus flavus* were obtained and characterized. Six isolates produced only B-type aflatoxins on yeast extract peptone dextrose (YEPD) medium, four isolates from the southwestern and two from the southeastern region. These results indicate that non-producers of aflatoxin dominated the *A. flavus* population on diseased ears. Under the same culture conditions, 13 isolates produced the mycotoxin cyclopiazonic acid. These isolates came from all regions except the western and central regions. When grown on modified Dichloran-Rose Bengal medium, ten isolates produced large (L-class) sclerotia and two isolates produced small (S-class) sclerotia. The remaining isolates did not produce appreciable sclerotia. Polymerase chain reaction (PCR) analysis indicated that MAT1-1 and MAT1-2 mating types were approximately evenly distributed among the isolates, 43% and 57%, respectively.

Molecular characterization and detection of Mexican papita viroid

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Mexican papita viroid (MPVd), in genus *Pospiviroid* and family *Pospiviroidae*, was first isolated from wild papita (*Solanum cardiophyllum* Lindl) plants in 1996. Since 2009, several disease outbreaks on greenhouse tomatoes in Canada and Mexico were shown to be caused by MPVd infection. However, the Koch's Postulates for MPVd have not been established. In the present study, we engineered infectious cDNA clones using two select MPVd isolates (MX and Mex8). Infectivity of the engineered cDNA constructs was examined by mechanical inoculation of 'Moneymaker' tomato plants with their respective *in vitro* RNA transcripts. After four weeks post inoculation, disease symptoms of stunting and leaf chlorosis on the inoculated plants were similar to those in field observation. The presence of MPVd on the inoculated plants was confirmed with real-time reverse transcription polymerase chain reaction (RT-PCR). Additional cloning and sequencing of MPVd-MX and MPVd-Mex8 progeny revealed that MPVd existed in host plants as a mixture of various sequence variants. However, the predominant progeny sequences were identical to their respective parental cDNA clones, which accounted for 81.82% for MPVd-MX and 78.79% for MPVd-Mex8, respectively. Two species-specific and sensitive molecular detection methods including real-time RT-PCR and reverse transcription loop-mediated isothermal amplification (RT-LAMP) were developed and successfully applied for plant and seed health test.

WITHDRAWN

Evaluation of beneficial bacterial isolates from citrus roots for mitigating huanglongbing damage

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Huanglongbing (HLB) is the most devastating disease of citrus in the world. The disease is associated with the phloem-inhabiting bacterium '*Candidatus Liberibacter asiaticus*' (Las) in Florida, which is transmitted by psyllid vector *Diaphorina citri*. Management of HLB is dependent on preventing and reducing the inoculum in the field through using disease-free planting materials, controlling psyllid populations, and eradicating infected trees. However, these practices have not been able to stop decline of infected trees. Early infection of citrus roots by Las causing root decline is known to be important in the development of HLB symptoms. In our previous studies, multiple bacterial strains were isolated from roots of healthy plants in HLB-infected citrus groves in Florida and, found to have the potential to enhance plant growth. We hypothesized that applying beneficial bacteria to citrus roots could decrease root damage by promoting root growth and limiting Las infection and thus improve HLB management. To test this hypothesis, a set of six beneficial bacteria were tested for their plant growth-promoting activity. Three *Bacillus* and closely relative isolates were found to increase seedling biomass in greenhouse. Furthermore, the three strains delayed the development of HLB symptoms and Las populations in greenhouse assays. Two independent field trials were initiated and data collection is currently underway.

Seasonal and daily patterns of *Magnaporthe oryzae* conidia availability in gray leaf spot-perennial ryegrass pathosystem

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Gray leaf spot, caused by *Magnaporthe oryzae* Couch, is a serious disease of perennial ryegrass turf. Airborne conidia of *M. oryzae* serve as the inoculum for development of disease epidemics. This study was conducted to monitor the availability and the dispersal pattern of conidia in the field, and to determine the relationship between certain environmental parameters and concentration of airborne inoculum. The field monitoring of airborne conidia was conducted at each golf course in Dillsburg, PA, and Leesport, PA, in 2010 and 2011. Analyses of the air samples indicated that airborne conidia of *M. oryzae* were present at both sampling sites. Average hourly conidia counts indicated that the peak concentration was generally observed during early part of the day (0500 to 0900 h). Concurrent occurrences of both high concentration of airborne conidia (>1000 conidia/m³) and favorable environmental conditions were identified prior to development of disease epidemics. Correlation analyses between environmental parameters and conidia concentration indicate that temperature may be used to forecast initial inoculum availability. However, the humidity level may also be a critical factor influencing the hourly conidia concentration. Results of the study will be employed as a component of the gray leaf spot forecast system.

The interactome of pathogenicity factors in the rice blast fungus *Magnaporthe oryzae*

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Rice blast is a disease of significant economic impact worldwide and a model system for studying fungal-plant interactions. In *Magnaporthe oryzae*, over 100 genes have been identified to play important role in infection-related growth and pathogenicity. Some of them shared common signaling pathway and biochemical processing. However, a systematic functional characterization of the pathogenicity factors and their interactions has never been reported. Mapping protein-protein interaction networks of the identified pathogenicity factors in *M. oryzae* facilitate to reveal molecular mechanisms regulating plant infection processes and to discover novel pathogenicity factors. In this study, we characterize the interactome of over 60 identified pathogenicity factors by taking advantage of the affinity purification combined with proteomics approaches. Our network showed protein-protein interactions widely exist among the pathogenicity factors and the components of signaling pathways. A number of interactions in our network are conserved in budding yeast. Co-immunoprecipitation, BiFC, or yeast two hybridization assays will be used to verify the interactions of selected genes. Based on our protein-protein interaction maps, we also have identified some novel functional genes related to pathogenicity in *Magnaporthe oryzae*. So far, it is the first study to functionally characterize the pathogenicity factors by mapping them in protein-protein interaction networks in *M. oryzae*.

Analysis of interregional dispersal of *Puccinia striiformis* in China using a coalescent method

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Phylogenetic methods can be used to infer the gene flow and dispersal directions among plant pathogen populations. In this study, a total of 761 isolates of *Puccinia striiformis* (*Pst*) were collected from northwestern China covering five provinces (Gansu, Sichuan, Ningxia, Shaanxi and Qinghai), the main areas providing initial inoculum for interregional epidemics in China. The amplified fragment length polymorphism (AFLP) method was applied to obtain molecular dataset for these isolates. A coalescent approach using the likelihood and Bayesian (Migrate-N and BayesAss) was applied to infer the interregional dispersal rates and their directions. Furthermore, 127 representative isolates were used to identify their races. Higher race diversity, genetic diversity and highly significant asymmetric dispersal were detected in Gansu and Sichuan populations than in other three provincial populations, suggesting that Gansu and Sichuan were likely the major potential source of immigrants of *Pst* in northern and northwestern China. Migration rate estimates based on Migrate-N showed that southern Gansu and northwestern Sichuan populations were the most important contributors as main dispersal source among 9 regional populations. In addition, frequent gene flows from eastern Gansu, Ningxia and Qinghai to other regions were also detected. Sichuan Basin served as the largest destination accepting migrated inoculum from other source regions based on analysis using software BayesAss.

An oxalate decarboxylase gene functions in the early infection processes of *Sclerotinia sclerotiorum*

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The *Sclerotinia sclerotiorum* genome contains two putative oxalate decarboxylase genes, *Ss-odc1* and *Ss-odc2*, which we hypothesize are involved in the fine-tune regulation of oxalate homeostasis, pathogenesis and morphogenesis. We used northern blot hybridization to determine their expression patterns across major development stages and in vegetative hyphae exposed to pH and exogenous oxalate treatments. The *Ss-odc1* transcript was detected across all sampled life stages and showed no inductive accumulation in response to low pH (3.0) or exogenous oxalate (up to 40 mM at pH 4.8). In contrast, *Ss-odc2* transcript was specifically detected during compound appressoria differentiation but not in hyphae in liquid culture. *Ss-odc1* knock out mutant showed wild type-like growth, morphogenesis, and virulence while *Ss-odc2* knock out mutants showed less efficient compound appressorium differentiation, a three- to four-fold increase in oxalate accumulation during compound appressorium differentiation, and significantly reduced virulence on bean petioles, soybean leaves, soybean petioles and celery stems. Wounding host tissues prior to inoculation rescued all the virulence defects. We hypothesize that oxalate decarboxylase activity functions in creating a beneficial microenvironment for compound appressorium differentiation and function during the early infection processes of *S. sclerotiorum*.

WITHDRAWN

Uncovering salicylic acid-mediated defense network in two cultivated strawberries (*Fragaria x ananassa*)

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Pest damage and pesticide residue problem have always been major threats to strawberry cultivation in Taiwan. Aiming to develop novel control strategies, we used the Illumina next generation sequencing technology for high-throughput identification of genes up- and down-regulated by salicylic acid (SA) in two strawberry cultivars. The RNA samples were isolated from leaves of each cultivar collected 6, 12, 24, and 48 hrs after SA- or ddH₂O-treatment. The samples were pooled for sequencing after the effectiveness of SA-treatment was verified by investigating the expression of *FaPRI*, *FaWRKY1*, and *FaOLP2*, the hallmark genes downstream of the SA signaling pathway. We applied Trinity and Velvet/Oases as well as multiple K-mers for de novo assembly to tackle the complex octoploidy genome. The transcripts were annotated based on the NCBI nr database and a reference diploid strawberry genome database. Among over 1,130 genes differentially expressed upon SA treatment, we identified genes involved in SA biosynthesis, polyphenol biosynthesis, plant-pathogen interactions, hypersensitive response oxidative, and MAPK cascade. Several NBS-LRR resistance genes and some novel genes were also revealed. We are currently investigating the time-course expression profiling of selected candidate genes using real-time quantitative RT-PCR. A set of defense-related genes, including *NPRI*, *GRX480*, *WRKY70*, *WRKY51*, *PRI*, and *PR5* genes, have been verified to be highly induced in response to SA.

Host-specific relationships between *Suillus* and *Pinus* species

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The bolete genus *Suillus* is known to form ectomycorrhizal associations with members of the pine family (Pinaceae). To understand the genetic basis of host specificity between *Suillus* and its hosts, we used nextgen sequencing of the ITS region to identify *Suillus* communities from 25 different field sites across North America. As predicted, several *Suillus* species were associated with a single host, including *S. glandulosus* under *Picea glauca* (AL); *S. hirtellus* and *S. cothurnatus* under *Pinus taeda* (FL, MS, NC); *S. granulatus*, *S. pictus* and *S. americanus* under *P. strobus* (CT); and an unidentified *Suillus* sp. under *P. monticola* (CA). *Suillus brevipes* was the most widely distributed species associated with *P. ponderosa* (OR), *P. contorta* (CA, OR), *P. banksiana* (MN) and *P. taeda* (FL). To investigate the genetic basis of host specificity, seedlings of representative pine species were inoculated with basidiospores of different *Suillus* spp. Many *Suillus* spp., including *S. decipiens* and *S. hirtellus* were able to germinate and form mycorrhizae with several different pine species. In contrast, several species including *S. granulatus*, *S. americanus* and *S. pictus* formed mycorrhizae only with *P. strobus* and *P. monticola*, and failed to even germinate under other pine species, indicating strong host specificity. Transcriptomic analysis using Illumina HiSeq is being used to profile genes involved in host preferences of these white pine associated *Suillus* species.

Comparative genomic analysis of phenotypically and genotypically diverse isolates of *Phytophthora ramorum*

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Phytophthora ramorum causes sudden oak death and has devastated many oak populations the Pacific Northwest of the U.S. and British Columbia, Canada. Three clonal lineages (NA1, NA2, EU1) of this pathogen are present in North America, and both wild type (wt) and non wild type (nwt) forms have been identified in lineages NA1 and EU1. The nwt isolates have a stunted culture morphology and reduced virulence, proposed to be influenced by lack of transposon suppression. The objective of this study was to compare the ge-

nomes of twelve *P. ramorum* isolates, including NA1 wt and nwt, EU1 wt and nwt, and NA2 wt isolates in order to identify genomic regions associated with the nwt phenotype and loss of virulence. Sequencing was completed on a HiSeq 2000 and initial de novo assembly statistics reveal that genome size of the twelve isolates ranged from 38.8 Mb to 40.6 Mb with over 125x coverage for each strain assembled onto 7,500 contigs on average. The average genome size of wt isolates was 40.1 Mb compared to 39.5 Mb for nwt isolates. In the case of isolates in the NA1 clonal lineage, the three nwt isolates were on average 1 Mb shorter than the three wt isolates. It is unclear what genomic regions are lost in the nwt strains, but we hypothesize that genes associated with virulence may have been lost in a potential genome reduction in these strains. Further analyses will identify which genes are lacking in the nwt strains and determine their potential function in virulence.

Seasonal dynamics and correlation studies of two viroids in two citrus cultivars

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Citrus exocortis viroid (CEVd) and *Hop stunt viroid* (HSVd or CVd-II) are two known citrus pathogens (documented in 1972 and 1995, respectively) that cause severe impacts on citrus industry in Taiwan. These two viroids usually co-infect citrus plants in Taiwan and their percentages of co-infection may reach up to 50%. Understanding the ecology of CEVd and HSVd is necessary by studying seasonal multiplicative dynamics and correlation between two viroids and environmental factors. For the quantitative investigation, the TaqMan® real-time RT-PCR assay was used to detect the presence and infection percentages of viroids in plant tissues, which were periodically sampled from seventeen natural hosts including eleven blood sweet oranges (*Citrus sinensis* var.) and six Murcott tangors (*Clementine* x *Citrus sinensis* Osbeck) in the middle Taiwan (Yunlin county). The results showed that both CEVd and HSVd unevenly distributed in their citrus hosts, and relatively higher concentration of viroids was found in twig barks. Correlation analysis between viroid titers and temperatures revealed that no correlation either between CEVd or HSVd and two temperature groups. A positive correlations between two viroids are observed in two citrus cultivars. The study demonstrated that viroids could adapt to temperatures, and two viroids showed significant interaction when co-infection occurred. Further studies of long-term ecological survey and cellular level test will be conducted in the future.

Characterization of *Dickeya* spp. from South China by multi-locus sequence analysis

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Several crops and ornamental plants have suffered severe soft rot caused by *Dickeya* spp. in South China, including bananas, rice, *Philodendron* 'Con-go', orchids and french marigold. Since of the complex classification and taxonomic history of *Dickeya* genera, it is difficult to identify the exact inter-specific classification of these pathogens only with biochemical characteristics. To further differentiate these pathogens and determine their phylogenetic placement contributing to epidemiological and quarantine studies, we analyzed a multi-locus sequence with *Dickeya* isolates. Multi-locus including 16S rRNA, *dnaX*, *gryB* and *RecA* was conducted to Neighbour-joining analyses subjecting the available sequences in NCBI and these isolates. The phylogenies of 64 16S rRNA, 127 *dnaX*, 59 *gryB* and 196 *RecA* sequences, strongly supported the lineages of different species leading to exact inter-specific classification of the pathogens. MS1 from banana and EC1 from rice were determined as *D. zeae*, PC1 from *Philodendron* 'Con-go' was classified into *D. diffebachiae*, but PA1 from orchids and TP1 from french marigold were grouped with other species-unknown isolates. Nevertheless, PA1 and TP1 shared higher sequence identities to PC1 than other classified isolates, and were recommended to be determined as *D. diffebachiae*. These data indicated great genetic differentiation of *Dickeya* pathogens from South China, especially *D. diffebachiae*, which is preferred by indoor flower plants.

The peptide originated from a defense-related protein of *Lilium* has antimicrobial potential

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The defense-related *LsGRP1* gene is differentially expressed in the leaves of salicylic acid-, probenazole-, and *Botrytis elliptica*-induced lily (*Lilium* cv.

Star Gazer) plants. The antimicrobial ability of LsGRP1 was analyzed by computational sequence analysis. Based on the putative amino acid sequences of LsGRP1, peptide-1 (N-terminal region without the signal peptide), peptide-2 (glycine-rich region) and peptide-3 (C-terminal cysteine-rich region) were synthesized for antimicrobial assay. The growths of bacterial strains, including Gram (+) and Gram (–) species, were significantly inhibited by peptide-1 and peptide-3 but only partially inhibited by peptide-2. However, peptide-3, but not peptide-1 or peptide-2, inhibited spore germination of the fungi tested. Thus, peptide-3 with broader target spectrum was considered a potent antimicrobial peptide. Furthermore, SYTOX Green staining were applied to examine cell morphology and membrane permeability as affected by the peptide originated from LsGRP1 and the result indicated that peptide-3 affected the integrity of fungal cell membrane. In addition, as shown by H₂DCFDA, DAPI and TUNEL assays, peptide-3 induced programmed cell death in *B. elliptica*.

Microbial diversity associated with Saharan dust storms: A developing tale of emerging pathogens

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The Sahara desert is the biggest source of atmospheric mineral dust around the globe, contributing 200-300 tg of dust deposition annually over the Atlantic Ocean. Among the effects attributed to this phenomena are reduced air quality levels and to transport of diverse pathogens from Africa to the Americas. This project explores the microbial diversity of Saharan dust events via DNA extraction from dust and filter samples. Viability of pathogens is examined by exposure of wheat and barley leaves to dust suspensions. Species detection was achieved by analyzing 18s amplicons via 454 pyrosequencing, and metagenomic Illumina shotgun sequences. Some of the fungal pathogens detected are *Humicola fuscoatra* (tomato plant root pathogen), *Botryosphaeria viticola* (pathogen of grapevines) and *Madurella mycetomatis* (human skin pathogen). The most abundant species were *Trichoderma* spp., and *Botryosphaeria rhodina*. Illumina sequences were dominated by *Bacillus* spp. although signal detection from Archaea and cyanobacteria was possible. Pathogenicity experiments onto detached leaves yielded lesions within 24 hrs of inoculation for wheat and barley plants. Results from this study have important implications for agricultural crops and human health, in the lights of a changing climate and the colonization of new territories by pathogenic species.

Impact of plant age on development of bacterial wilt on muskmelon

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Bacterial wilt of cucurbits, caused by *Erwinia tracheiphila*, is transmitted by striped (*Acalymma vittatum*) and spotted (*Diabrotica undecimpunctata*) cucumber beetles. Although the highest risk of economic loss occurs during the early part of the growing season, the impact of plant age on bacterial wilt development in muskmelon is currently unknown. To determine how plant age affects disease development, 2-, 4-, and 6-week-old muskmelon seedlings (cv. Athena) were puncture inoculated with a suspension (2.5×10^6 CFU/ml) of a rifampicin-resistant strain of *E. tracheiphila* at the base of the newest fully expanded leaf. Symptoms were observed for 3 weeks. The inoculated leaf wilted between day 4 and 6 in all treatments. By day 11 plants that were 2 weeks old at inoculation collapsed; whereas 4- and 6-week-old plants only partially wilted, about 60% and 40% of leaves, respectively. Our results suggest that muskmelon plants become more tolerant to bacterial wilt as plant age increases. In particular, plants that were 4 or 6 weeks old at inoculation were more tolerant than plants that were 2 weeks old at inoculation.

WITHDRAWN

Quantification of *Paecilomyces lilacinus* YES-2-14 by RT-PCR in corn straw bio-reactor established in greenhouse vegetable field

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Straw bio-reactor is a novel technique to reuse crop straw as the carrier of biocontrol agents of soilborne diseases in greenhouse vegetable production in China. To study interaction of biocontrol agent *Paecilomyces lilacinus* YES-2-14 and *Meloidogyne incognita* in the reactor, we designed a pair of SCAR primers and developed a real-time PCR system of YES-2-14. A straw bio-reactor was established in solar greenhouse infested with the nematode, and cucumber seedlings were transplanted 2d later. Soil samples were taken periodically and numbers of the bio-agent and nematodes were detected. Results showed that YES-2-14 population was much greater than that in treatment with only the fungus (without straw) after 4w ($P < 0.05$), indicating that *P. lilacinus* was able to utilize the straw as nutritional resource and proliferate rapidly in the straw bio-reactor system. Suppression on nematodes was intensified as the rapid population increase of the biocontrol agent, although nematode density was higher in early days in the system. Growth determination indicated that the bio-reactor system promoted cucumber height and stem diameter by 14.0% and 33.0%, respectively, and yield increased by 63.3%. This test showed that the straw bio-reactor might be a highly useful way for increasing efficacy of biocontrol of soilborne diseases in greenhouse vegetable production.

Microbial and nematode communities in soils from Nebraska soybean farms

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Soil microbial and nematode community analysis is essential for understanding pathogen ecology and the impact of farming systems on pathogen communities. Soil microbial communities and soybean cyst nematode were assessed in fields with conventional tillage, no-tillage, irrigation, and no-irrigation using dilution plating, denaturing gradient gel electrophoresis (DGGE) and DNA sequence analysis. Soybean cyst nematode (SCN) population density, expressed as number of eggs per 100 cc of soil, was determined with cyst nematode standard sieving techniques. *Fusarium*, *Pythium*, *Rhizoctonia* and *Trichoderma* were the predominant genera of microbes found in the studied sites. Population abundance of these microbes varied across sites with an undefined trend and association neither with tillage, or irrigation nor with the density of SCN. Major *Fusarium* found in NE soybean fields were *F. oxysporum*, *F. solani*, *F. equiseti*, *F. graminearum*, *F. verticillioides*, and *F. culmorum* based on morphology and DGGE. Canonical correspondence analysis showed that the microbial populations were more related to soil physical properties such as soil structure and water content. Moreover, there is no direct correlation between soil microbial and SCN populations.

Characterization and quantification of *Fusarium* spp. on wheat roots from Nebraska farms

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Fusarium crown rot is an important disease of wheat causing stand reductions and rotting of roots, crowns and lower stems. Characterization and quantification of the pathogens on roots is crucial for understanding pathogen ecology and management of root diseases. Wheat roots and soil samples were assessed in July after the harvest of 2012. Based on dilution plating, *Fusarium*

spp. colony numbers on roots was higher from the farm sites in Furnas, Frontier and Chase Counties and lower from farms at Hitchcock and Keith Counties. *Fusarium* populations in soils were highest from Chase County (2.2×10^3 average CFU/gds from 5 soil samples) and lowest from Hitchcock County (2.2×10^3 average CFU/gds from 5 soil samples). The major *Fusarium* spp. on roots were *F. oxysporum*, *F. solani*, *F. graminearum*, *F. verticillioides*, *F. culmorum*, *F. acuminatum*, *F. equiseti*, *F. pseudograminearum* and *F. semitectum* based on both morphology and DNA sequence and DGGE analysis. Pathogenicity tests for the *Fusarium* spp. is in progress. Information about the soilborne pathogen ecology, incidence, and pathogenicity will lead to the informed root disease management for farmers.

Soilborne pathogen and beneficial microbial populations were affected by herbicide treatment

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Herbicides provide effective weed control. However, some broad-spectrum systematic herbicides can have unintended effects on disease severity and soil microbial community. Soils were treated with herbicide glyphosate, 2,4-D, dicamba, and flumioxazin at the recommended rates. Soil populations of *Fusarium*, *Pythium*, *Rhizoctonia*, and *Trichoderma* were monitored using dilution plating after the treatment at 1, 4, 8, 12, 16, 20 and 30 days. The populations of *Fusarium* reach to the peak at 8 days with glyphosate (1.1×10^4 CFU/gds), 2,4-D (1.45×10^4 CFU/gds), dicamba (1.35×10^4 CFU/gds) and flumioxazin (2.4×10^4 CFU/gds) and then decreased. The populations of *Pythium* reach to the peak at 4 days with glyphosate (120 CFU/gds), 8 days with 2,4-D (100 CFU/gds) and flumioxazin (360 CFU/gds). There is no influence to *Pythium* populations from dicamba. The populations of *Rhizoctonia* reach to the peak at 12 days with glyphosate (1.4×10^4 CFU/gds) and 2,4-D (1.38×10^4 CFU/gds), and 8 days with dicamba (1.8×10^4 CFU/gds) and flumioxazin (2.2×10^4 CFU/gds). The populations of *Trichoderma* reach to the peak at 4 days with glyphosate (700 CFU/gds), dicamba (670 CFU/gds) and flumioxazin (650 CFU/gds), and 8 days with 2,4-D (920 CFU/gds). Herbicide influenced soilborne pathogen and beneficial microbial populations, which might affect soilborne diseases in field condition.

Collimonas sp. ZLZ261—A novel antagonistic strain against *Monilinia fructicola*

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Monilinia fructicola was one of the main pathogens of peaches and caused a great economic loss. The control of the disease primarily depended on chemical pesticides. However, with the increasing of awareness for food safety and environment precaution, biocontrol of plant disease has been increasingly paid attention in China. Strain ZLZ261 was isolated from a tundra at the altitude of 4530 meters in Tibet, China. Antifungal activity test showed that the strain presented a distinct antagonism against *M. fructicola* *in vitro*. But other pathogens including *Fusarium oxysporum* f. sp. *conglutinans*, *Botrytis cinerea* and *Alternaria alternata*, tested in the study, were not inhibited. The 16S rRNA gene sequences analysis indicated that ZLZ261 had a 99% identity to *C. pratensis* strain CTO291 or CTO90 or CTO91 or *C. fungivorans* CTE118, suggesting that ZLZ261 belonged to the genus *Collimonas*. Up to now, only three formally recognized species were reported in *Collimonas*. Most strains in the genus had chitinolytic activity to help themselves to obtain nutrients from other organisms including living fungal hyphae, and individual strains of them were somewhat selective in antifungal spectrum. We found that strain ZLZ261 did not produce chitinase, but inhibited hyphae growth of *M. fructicola*. This was different from other *Collimonas* strains, and therefore, *Collimonas* sp. ZLZ261 may be used as a novel antagonistic strain to control peach brown rot caused by *M. fructicola*.

Impact of essential oils on spore germination and plant colonization by *Beauveria bassiana*

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Seed application of *Beauveria bassiana* and soil amendment with bioactive monarda herbages are sustainable approaches for suppression of damping-off diseases of tomato seedlings. The objectives of this research were to determine compatibility of monarda essential oils and *B. bassiana*. Five essential oils (cymene, carvacrol, thymol, borneol, and geraniol) active against

damping-off pathogens were tested. When 10- μ L of a 500-mM cymene, 50- or 500-mM geraniol, or 500-mM carvacrol solution were allowed to volatilize for 24 h in a 100 x 15-mm Petri plate, no spores germinated on water agar blocks. Germination of spores was reduced by ca. 35% in treatments with 500-mM thymol or geraniol. Germ tube length was reduced in all oils for treatments with 5-mM or greater; only thymol reduced germ tube length in 0.5-mM treatments. Ability of *B. bassiana* to colonize tomato seedlings when grown in the presence of essential oils was tested with five monarda herbages ('Trinity Purple', 'Rose-scented', 'Violet Queen', 'Cerise', and *Monarda clinopodia*), representing five chemotypes (cymene, geraniol, carvacrol, borneol, and thymol, respectively). Herbage from three monarda cultivars ('Trinity Purple', 'Rose-scented', 'Violet Queen') had no negative impact on seed germination or *Beauveria* colonization of tomato. Effects of monarda herbage on *B. bassiana* were less than predicted in laboratory assays so future research will further investigate strategic 'stacking' of these biocontrol agents.

Population divergence in the wheat leaf rust fungus *Puccinia triticina* is correlated with wheat evolution

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As an obligate biotrophic parasite, *Puccinia triticina*, the wheat leaf rust fungus likely co-evolved with different forms and ancestors of wheat. To test this hypothesis, we generated *P. triticina* DNA sequence data from fifteen loci with different levels of polymorphism, and conducted phylogenetic (parsimony, Bayesian, maximum likelihood) and coalescence-based analyses. Four forms of *P. triticina* were determined that showed correlation with wheat type and geographic locations: 1. *P. triticina* from common hexaploid wheat found worldwide; 2. *P. triticina* from cultivated tetraploid durum wheat found worldwide; 3. *P. triticina* from tetraploid wheat from Ethiopia and 4. *P. triticina* from diploid *Aegilops speltoides* that has been found only in Israel. Phylogenetic and coalescence analyses indicated that *P. triticina* on diploid *A. speltoides*, the probable B genome donor of hexaploid wheat, diverged initially, followed by the Ethiopian durum wheat form and then by the common wheat form. *P. triticina* on worldwide cultivated durum wheat was the most recently coalesced form, which formed a clade nested within the common wheat form. By a relative time scale, the divergence of *P. triticina* forms as delimited by host specificity appears very recent. Significant reciprocal gene flow between the common wheat form and the durum wheat form, and from the common wheat form to Ethiopian durum form was detected.

Development of loop-mediated isothermal amplification for detection of *Leifsonia xyli* subsp. *xyli* in sugarcane

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Ratoon stunt, caused by the xylem-limited coryneform bacterium *Leifsonia xyli* subsp. *xyli* (*Lxx*), is prevalent in most sugarcane-producing countries. Because the disease does not cause characteristic external symptoms, a laboratory-based technique is needed for accurate diagnosis. We developed a diagnostic protocol to detect *Lxx* in host tissue based on loop-mediated isothermal amplification (LAMP). The LAMP assay is a highly specific, rapid, and sensitive method for the diagnosis of ratoon stunt caused by *Lxx* in sugarcane. This is a simple and feasible diagnostic tool in which the reaction takes place in a single tube incubated in a heat block at a constant temperature for 62 min compared to conventional PCR that takes about 2 h to detect *Lxx*. Amplified products are detected by a visual color change. The LAMP assay also does not require a thermocycler or an electrophoresis system, costly instruments required for conventional PCR. In the present study, positive and negative samples detected by the LAMP method were clearly distinguishable. When total DNA extracted from internode juice was used as the template, the sensitivity of LAMP was 10 times higher than that of the conventional PCR analysis.

Construction and expression of roGFP in *Pantoea agglomerans* as a bioreporter for host surface redox potential in plant-microbe interactions

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The redox environment of the plant-microbe interface can profoundly influence the outcome of the interaction. Nondestructive methods for examining this redox environment have use in comparative studies of host surface chemistry as it affects the activity and germination of pathogen propagules

during infection. The redox-responsive green fluorescent protein (roGFP) was developed as a tool for the detection of changes in redox potential in living organisms. In this study, roGFP was cloned and expressed under the constitutive SP6 and pfrur promoters in the epiphytic bacterium, *Pantoea agglomerans* 299R (E299R/roGFP), for use as a bioreporter to prospect redox climate of the fructoplane and phylloplane in peach and tomato. *In vitro* ratiometric analysis by fluorometry detected different concentrations of redox active chemicals when added to suspensions of E299R/roGFP, including hydrogen peroxide, dithiothreitol, menadione and plant phenolic compounds. Redox potential changes were detected within 5 minutes of addition of the chemical, with a detection limit of 100 μ M for oxidation and reduction by hydrogen peroxide and dithiothreitol, respectively. The dynamic range of E299R/roGFP was 5.3 as derived from 20 mM hydrogen peroxide (full oxidation) and 20 mM dithiothreitol (full reduction). Experiments with E299R/roGFP to examine redox changes on leaf and fruit surfaces before and after inoculation are in progress and results will be presented.

Microbial suppression of soilborne diseases in strawberries

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Management of soilborne pathogens in strawberry production has long relied on pre-plant fumigation to maintain pathogen inoculum below damaging levels, but tightening restrictions on use of the most effective fumigants requires the development of alternative approaches. Various soil amendments have been tested to achieve suppression of pathogens such as *Verticillium dahliae*, *Pythium* spp. and *Phytophthora* spp., which would otherwise have significant impacts on yield. One such amendment is compost, which is commonly used by strawberry growers in California. However, there is presently no information on the type or quantity of compost that would optimize disease suppressive effects. To redress this deficiency, four locally produced commercial composts were evaluated: steer manure-based compost, spent mushroom compost, composted municipal waste and vermicompost. Each type of compost was characterized based on nutrient composition, microbial characteristics as revealed by fluorescein diacetate hydrolysis and plate counts, and tests for disease suppression under both controlled and field conditions. Microbial activity and abundance varied significantly between composts. Under controlled conditions, plant growth and symptoms induced by *V. dahliae* did not differ significantly between treatments and controls. Field trials are in progress to evaluate compost effects on strawberry production in non-fumigated fields where yield loss averages 30-40% due to root pathogens.

The tropical tree pathogen, *Rhizomorpha coryneophora* (Agaricales, Marasmiaceae), is transported by birds in Belize

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Rhizomorpha coryneophora Kunze is an unusual pathogen of various trees and shrubs (including species of citrus) that was originally described from its vegetative state in wet forests of South America. The pathogenic phase has been observed in Surinam as a web blight that kills new shoots. The vegetative fungus usually appears as knobby-branched rhizomorphic structures of up to 50 cm in length that dangle from tree branches and initiate spread to neighboring plants. Recently, we found evidence that birds incorporate *R. coryneophora* rhizomorphs into their nests, effecting longer-range transport. Reports of this pathogen as far north as Belize (in the Yucatan Peninsula) and evidence of avian transport are of potential concern for the southern USA. The sexual state has never been found, but DNA sequencing indicates *R. coryneophora* is closely related to the genus *Brunneocorticium* in the Marasmiaceae (Agaricales). Vinnere et al showed in 2005 that a related species in the *Campanella* clade of the Marasmiaceae (named "sterile white basidiomycete 3034") was pathogenic on grass in Australia. Other arboreal species in this clade should be investigated for pathogenicity, and *R. coryneophora* should be monitored for evidence of northward movement.

Increasing the mobility and stability of nematicides using plant viral nanoparticles

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Nanotechnology offers the potential to reformulate chemical compounds to make them more effective in agricultural applications. *Red clover necrotic mosaic virus* is a robust, soil-borne plant virus that is being evaluated for use

as a plant viral nanoparticle (PVN). The uniformly sized icosahedral capsid can be loaded with significant amounts of small molecules. A potential application of this PVN platform is crop protection from nematode infections. A number of compounds are effective against nematodes but are ineffective in agricultural applications due to poor soil mobility and/or bioavailability. Preliminary testing with abamectin found that it could be loaded into PVNs and when dosed directly to nematodes, this formulation proved active against both *Caenorhabditis elegans* and *Meloidogyne* spp. in culture. When applied to columns of various soil types, the PVN abamectin formulations demonstrated increased mobility as compared to the free abamectin. However, enhanced mobility alone is insufficient to afford lasting protection. To impart controlled release, PVNs were integrated within a tunable polymeric matrix to provide an optimal abamectin release profile in the soil. It is proposed that formulating generally insoluble nematicides and other small compounds into PVNs with subsequent incorporation into polymeric matrices results in a crop protection system platform with tunable release profiles for highly efficacious and optimized seed treatments.

Investigations of ectomycorrhizal communities of *Chrysopsis chrysophylla* & *Pinus ponderosa* along a soil moisture gradient

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Ectomycorrhizae (ECM) are essential to temperate forest ecosystems for their involvement in host plant water and nutrient access, yet the ECM of some dominant understory species remain poorly studied. Little previous research on the ECM community of *Chrysopsis chrysophylla* (golden chinquapin) spurred the need for this novel investigation of the ECM communities of co-occurring *Chrysopsis chrysophylla* and *Pinus ponderosa* (ponderosa pine) along a naturally occurring soil moisture gradient in the Deschutes National Forest in central Oregon. Soil cores were taken from experimental units that were part of a larger block design, and sampling sites were based on a stratified random selection of units containing *C. chrysophylla*. Live ECM root tips were randomly selected from both host species. Preliminary morphotyping reveals high ECM species richness associated with *C. chrysophylla*. On-going investigation with molecular DNA methods will show if *C. chrysophylla* and *P. ponderosa* have the potential for common mycorrhizal networks by sharing common ECM species and indicate whether *C. chrysophylla* and *P. ponderosa* share greater richness or differing ECM species composition at high versus low soil moisture concentrations. Findings will provide knowledge useful to forest management practices in changing moisture regimes due to climate change.

Exploring a mefenoxam sensitivity screening service for floricultural crop producers

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Mefenoxam resistance in *Pythium* populations is widespread throughout the North Carolina greenhouse (GH) industry. Treating resistant strains with mefenoxam increases the cost of production without the benefit of reducing disease. In a recent study, 189 of 361 (52.4%) *Pythium* isolates obtained from GH floricultural crops in NC were resistant to mefenoxam. Additionally, resistant and sensitive strains were present within the same GH. Once resistance is present in a GH, continued use of mefenoxam is discouraged. As part of the study, we developed a rapid mefenoxam sensitivity assay (RMSA) that could be adapted by the Plant Disease and Insect Clinic (PDIC) at NCSU to provide a screening service for growers. The assay employs mefenoxam-amended media dispensed in microwell plates and costs approximately \$46.54 per eight isolates for materials and labor. Treating 1,000 6-in pots with a mefenoxam drench costs growers approximately \$6.73 per application for labor and materials. We currently are determining break-even costs for growers under various scenarios, and the PDIC is conducting grower surveys to determine the potential demand for RMSA services. The RMSA would provide growers with timely fungicide application recommendations based on their specific *Pythium* populations.

Phillospheric yeasts as potential biocontrol agents of *Botrytis cinerea* on cut roses

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Botrytis cinerea, the causative agent of grey mold is one of the most important phytosanitary constraints for cut roses crops. Its control is based on chemical botrycides, which are toxic to growers and the environment, and promotes fungicide resistance. Therefore there is a general interest to create an integrated disease management, including the development of biological control strategies. In this study, 25 isolates of phyllosphere yeasts, were selected by their capacity to antagonize *B. cinerea* under *in vitro* conditions and on artificially inoculated petals and flowers of *Rosa* spp. var. *vendela*, a variety highly susceptible to *B. cinerea*. *Candida hawaiiensis* LvGr2 and LvGr26, *Rhodotorula* sp. LvGr1, *Cryptococcus magnus* LvGr10 and *Cryptococcus* sp. LvGr3, were the most effective microorganisms against *B. cinerea* reducing disease incidence from 35.8 to 47.2 %, when were tested in complete flowers. Besides in 7 out of the 10 strains, the culture filtrates showed a significant difference to inhibit spore germination of *B. cinerea* in liquid media, after 16 hours of incubation and some of them were good proteases producers and grew on chitin as the only carbon source. Moreover, 8 and 6 out of the ten yeast strains were compatible with Switch® and Altima® respectively, which are two of the most used fungicides for the control of *B. cinerea* in cut roses crops in Colombia, suggesting that those strains can be mixed for the control of *B. cinerea* under commercial conditions.

WITHDRAWN

Composition, diversity, and resilience of fungal communities colonizing the roots of native and exotic hosts in an urban environment

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The number of humans in cities is increasing and exceeds half of the global population. Urbanization reconstructs natural ecosystems into fragmented, novel environments that are unique in organismal composition and characterized by lower biodiversity. The urban communities are in part attributable to land management choices. In this study, we elucidated the consequences of choosing native (*Quercus macrocarpa*) or non-native (*Pinus nigra*) ornamentals for the ectomycorrhizal (ECM) fungi that are necessary for tree growth. We use the conceptual framework of "ecological memory" and predict that non-native trees support ECM fungi communities lower in biomass and richness plus structurally different compared to native trees. We counted total and ECM root tips, extracted total DNA, estimated fungal biomass by quantitative PCR (qPCR), and examined communities by 25S pyrosequences. Our results show that ECM fungi colonize native and non-native trees, but pines had lower root ($F_{3,27}=6.718; P=0.0152$) and ECM densities ($F_{3,26}=5.9943; P=0.214$). In contrast, the fungal biomass did not differ, suggesting similar inoculum loads. Our analyses also indicate that ECM communities of the oaks and pines do not differ in richness or diversity, but are structurally distinct in ordination ($F_{1,8}=17.56; P=0.003$). Our findings highlight the need for land management using native ornamentals to conserve endemic fungi.

Genetic transformation of tomato (*Lycopersicon esculentum* cv. Micro-Tom) with a calcium signal modifier gene (CSM-1)

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Calcium signal modifier gene (CSM-1) is a citrus gene that has been overexpressed in several citrus species; overexpression seems to increase calcium signals in plants. Preliminary results show that CSM-1 transgenic citrus plants are resistant to a list of diseases. We expect to develop broad spectrum disease resistant tomatoes by inserting CSM-1 gene into Micro-Tom (MT) tomato. The objectives of this study are to develop transgenic tomato plants, study morphological and physiological changes, observe how CSM-1 segregates and screen for resistance to various pathogens. If CSM-1 holds true, then this can become a great benefit to tomato growers. MT cotyledons were inoculated in agrobacterium culture with an optical density of 0.2-0.6 and transgenic plants were selected in kanamycin containing medium and screened by GUS. Real-time PCR was used to verify CSM-1 expression levels compared to control. Eighteen transgenic lines were produced and no variation, compared to wild type, was observed in transgenic plants or in the fruits produced. First generation of transgenic plants (T1 plants) were screened by GUS and only one plant was negative. T1 plants were challenged with '*Ca. Liberibacter solanacearum*' through tomato/potato psyllid (*Bactericera cockerelli*) feeding, and results are being evaluated and it will be presented.

Transcriptome analysis of 'Valencia' sweet orange response to citrus huanglongbing (HLB) infection

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The pathogen, '*Ca. Liberibacter asiaticus*' was detected in Florida in 2005 and it has been causing enormous economic losses to that citrus industry. Currently, the bacterium has been introduced to all major U.S. citrus producing areas and it has the potential to cause huge economic turmoil. The bacterium does not have uniform distribution in the plant and it has a long latency period before visual symptom development in the leaves. It is very important to understand how the plant counterattacks the bacterium in an attempt to survive. To address this issue we performed transcriptome analysis by RNA-Seq to compare the response of HLB-infected 'Valencia' sweet orange with control non-infected plants. Out of 25 million reads, over 17 million were mapped to the citrus genome with over 12 million perfect matches. There were 6606 differential expressed genes with 11% being involved in plant-pathogen-interaction. Many of the new transcripts expressed in the diseased plant was the result of alternative splicing as several splicing factors were up-regulated and the alternative splicing events were increased drastically in diseased plants. Another group of genes that were highly affected were those involved in calcium signals, which are known to be in the front line of defense against biotic stresses. Real-time PCR confirmed the results of RNA-Seq. Additional results will be presented.

Ralstonia solanacearum degrades hydroxycinnamic acids, a class of plant defense molecules

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The xylem-colonizing plant pathogen *R. solanacearum* causes bacterial wilt disease. Plant defense involves production of hydroxycinnamic acids (HCA), which are directly antimicrobial and also precursors to lignin. RNAseq analysis revealed that infected tomato plants up-regulate genes involved in HCA biosynthesis. This mirrors our previous finding that the host plant environment induces expression of a pathway encoding HCA degradation in *R. solanacearum*. Biochemical analysis of xylem sap corroborates the pathogen's expression profile; total phenolic concentrations were 37% lower in infected tomatoes than in control plants. To determine if HCA degradation is important during disease, we constructed a Δfcs mutant, which lacks the first enzyme in HCA degradation. When inoculated via a naturalistic soil soak method, the Δfcs mutant caused delayed symptom development on tomatoes. We investigated several hypotheses why HCA degradation is important for virulence: (1) carbon acquisition, (2) breakdown of antimicrobial compounds, and (3) removal of lignin barriers that restrict pathogen movement. Δfcs did not have a growth defect in xylem sap, indicating that HCAs are not a significant carbon source. However, the Δfcs mutant had a 14-fold higher sensitivity to the HCA *p*-coumaric acid *in vitro*, suggesting that HCA degradation contributes to *R. solanacearum* virulence by purging antimicrobials. We are investigating differences in xylem spread between wildtype and Δfcs strains.

A hands-on project to help students understand Koch's postulates

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Disease diagnosis, pathogen isolation and inoculation are essential contents of the course Introductory Plant Pathology. When students examine a disease sample or isolate a pathogen, multiple microorganisms are likely obtained due to secondary infection and/or contamination. Koch's postulates are used to establish a causal role of a pathogenic microorganism in a disease system. A hands-on project using the apple-bitter rot disease system was developed for teaching. Students start to work on apples with bitter rot disease caused by *Glomerella cingulata*. In addition to the pathogen, some fungi such as *Aspergillus niger* could be obtained on contaminated culture plates. All of them are used for further inoculation. Symptoms and signs of inoculated apples are recorded and compared with those of the original ones. The pathogen is re-isolated from the inoculated apples. Morphology of microorganisms recovered from diseased apples is examined and recorded during the whole procedure. Analyses of symptoms, signs and microbial morphology are conducted to determine the casual role of each fungus. Four steps of the postulates can be integrated into three laboratory exercises and each student is expected to write a project report. Experimental materials are available all year round, procedures are simple, and disease symptoms and signs are characteristic. The project has extensively helped students understand basic principles, concepts and skills of plant pathology.

Application of *Clonostachys rosea* ACM941 to control soil-borne fungal diseases on *Asparagus officinalis*

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Asparagus officinalis Linne is an important high nutrition vegetable worldwide. Fungal diseases were the one of the severe limiting factors for *Asparagus officinalis* Linne production. *Clonostachys rosea* has been widely used to control a number of soil-borne or seed-borne disease of plants. In this research, the strain ACM941 (ATCC74447) of *C. rosea* was tested for its efficacy to control the soil-borne fungal diseases of *Asparagus officinalis* seedlings under greenhouse conditions. The tray experiments were prepared as following: 2 *Asparagus officinalis* seeds were planted into the tray containing 500 g soil collected from the diseased asparagus fields; Grown in greenhouse for 20 days, the root of the seedlings were treated with 100 ml of the fermentation of the strain ACM941 containing 4×10^6 or 2×10^6 CFU/mL; when the seedlings were grown for 90 days, the disease incidence and index of the seedlings were investigated. The seedlings treated with 50% carbendazol wettable powder were used as chemical control and the seedlings treated with water served as blank control. Each treatment had four replications and the experiment was repeated twice. The results showed that the efficacy of ACM941 at 4×10^6 and at 2×10^6 CFU/mL in controlling the fungal diseases of asparagus were 85% and 68%, respectively. The efficacy of the chemical fungicide was 53%. Our study indicated that the *C. rosea* strain ACM941 had a great potential to control soil-borne fungal disease of asparagus.

CHP-7, a putative serine protease effector from *Clavibacter michiganensis* subsp. *sepedonicus*, acts in the tobacco leaf apoplast

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Clavibacter michiganensis subsp. *sepedonicus* (*Cms*), a Gram-positive bacterial species in the phylum of *Actinobacteria*, is the causal pathogen of bacterial ring rot of potato. Previous studies have revealed that *Cms* triggers a hypersensitive response (HR) on its incompatible non-host plant *Nicotiana tabacum* and that the HR requires the *chp-7* gene (CMS_2989). *Chp-7* is also required for the full virulence of *Cms* and symptom development on compatible hosts, such as potato and eggplant. However, the molecular mechanisms of how *chp-7* functions in both incompatible and compatible interactions were not known. In this study, we found that purified CHP-7 is sufficient for inducing the HR, indicating that CHP-7 itself is the HR elicitor. The HR-eliciting activity of CHP-7 is dependent on its Ser-His-Asp catalytic triad structure conserved in trypsin family serine proteases, as a missense mutation from Ser to Thr at the amino acid position 232 abolished the HR-eliciting phenotype. Since Gram-positive bacteria do not have a type III secretion system to translocate effectors into host cells, we hypothesize that CHP-7 functions in the apoplast of plant tissue. This hypothesis is supported by the results of several experiments, including inoculation with purified CHP-7 protein, behavior of CHP-7-adenylate cyclase fusions, and ectopic

expression of CHP-7 in *N. tabacum* leaves. Further investigations will focus on identifying the substrates of CHP-7 in both host and non-host plants.

New marafivirus identified in yellow vein disease-affected blackberries

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In the course of a study on viruses associated with the blackberry yellow vein disease, an emerging problem in the Southern USA, shotgun sequencing from a symptomatic plant collected in Southern Mississippi revealed the presence of a putative marafivirus. Analyses of partial sequences suggested that the virus is distinct from the two marafiviruses reported from blackberries, *Blackberry virus S* and *Grapevine Syrah virus 1*, therefore justifying its further characterization. Pairwise comparisons of the genome-encoded proteins with the orthologs in related viruses showed limited levels of amino acid identities indicating that this virus is likely a new species in the taxon, which was supported by phylogenetic analyses. The virus was found in several other locations in Southern United State, suggesting that it could be rather widespread in cultivated and wild blackberries.

Development of a protocol to optimize the isolation of *Geomyces* from soil samples

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Geomyces destructans is the fungus responsible for White Nose Syndrome (WNS), which has already killed millions of bats and may be driving some species towards extinction. Different *Geomyces* species are commonly found in soils and caves, but it is not clear why *G. destructans* shows greater pathogenicity. *Geomyces* species can be difficult to isolate from cave soils because other fungi will colonize the plate at a much faster rate. The goal was to determine optimal conditions to culture *Geomyces* from soil samples. A collection of more than 20 *Geomyces* isolates was established and it will be tested to determine if incubation of soil samples at different temperatures and the use of Rose Bengal and keratin baits will facilitate the isolation of *Geomyces*. All of the isolates are psychrotolerant, while only *G. destructans* is a true psychrophile. We have had high success isolating *Geomyces* strains using hair baiting and in situ inoculations. None of the current *Geomyces* isolates show the curved conidia shown by *G. destructans* or 100% similarity to *G. destructans* for the ITS rDNA region. *Geomyces* strains survived incubations for more than two weeks at -4C, 20C and, -80C. Survival rates were greatly reduced at -80C. The large number of *Geomyces* strains represent an unique opportunity to gain a better understanding of the ecology and diversity of this genus.

Novel species of *Enterobacteriaceae* isolated from Russian wheat aphid (*Diuraphis noxia*)

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To defend against pathogens, plants are able to detect pathogen associated molecular patterns (PAMPs). These molecular signals trigger innate plant immunity during pathogen attack. Proteins present in the aphid saliva play this role during aphid feeding. Since identification of these PAMPs could lead to the understanding of plant resistance, proteins of the Russian wheat aphid (*Diuraphis noxia*) saliva were analyzed. This aphid is a pest of small grains, including wheat and barley, and is found in most wheat-growing countries. Strikingly, among the 1700 proteins identified in *D. noxia* saliva, almost 70% have *Enterobacteriaceae* origins. We isolated *Enterobacteriaceae*-like bacteria (gram-negative, rod-shaped, non-pigmented) from whole body extracts of surface sterilized aphids, symptomatic wheat leaves fed on by *D. noxia*, and artificial sucrose diets from which the aphids fed. To identify these bacteria, housekeeping genes were sequenced (*rrs*, *rri*, *gyrB*, *atpD* and *infB*) and compared in the databases. These experiments confirmed that the isolates were *Enterobacteriaceae* but did not determine a species level identification. Furthermore, biochemical analyses (Biolog, API 20E strips) showed that isolates do not match with any known species. Our data are consistent with the hypothesis that non-described *Enterobacteriaceae* species living in *D. noxia* could provide PAMPs triggering plant defense against the aphid.

Subcellular localization of *Panicum mosaic virus* proteins reflects the altered host transcriptional profiles in *Brachypodium distachyon*

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Panicum mosaic virus (PMV) alone, or mixed infection with its satellite virus (SPMV), induces chlorotic-to-necrotic symptoms in several species of grasses (*Poaceae*), including St. Augustinegrass, switchgrass, and millets. To determine the molecular and cell-biological features of PMV:host interactions, we established *Brachypodium distachyon* (*Brachypodium*) as a genetic model for PMV/SPMV infections. PMV- and PMV+SPMV-infection in *Brachypodium* alter expression of host genes in diverse cellular pathways, such as carbon metabolism, metabolite transport, photosynthesis, and defense hormone and long-chain fatty-acid biosynthesis/signaling. Here, we determined the sub-cellular localization of GFP-tagged PMV- and SPMV-encoded proteins. PMV movement-associated proteins (P8, P6.6, and P15) primarily localized to the cell-wall/plasma membrane; additionally, P6.6 appeared to also accumulate in the nucleus. PMV replicase (P112) accumulated as distinct foci within the cytoplasm, which may be viral replication complexes tethered either to membranes or organelles such as ER and peroxisomes. PMV capsid protein (CP) and SPMV CP, both localized primarily in the nucleus and/or nucleolus. Since multiple PMV-encoded proteins and SPMV CP trafficked to the nucleus, this suggests that these proteins could modulate host nuclear processes, such as cellular transcription, to facilitate virus infection.

Molecular characterization of rice blast resistance genes using a recombinant inbred population derived from a cross of Nipponbare and 93-11

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Blast disease of rice caused by the fungus *Magnaporthe oryzae* has been managed by the use of resistance genes. In the present study, a recombinant inbred line (RIL) population consisting of 259 individuals derived from a cross between reference cultivars, Nipponbare and 93-11, was used to identify blast resistance genes. A total of 131 polymorphic simple sequence repeat (SSR) markers were identified, and used to generate a linkage map. Four quantitative trait loci (QTL) for resistance to six isolates of *M. oryzae*, *qPi93-1*, *qPi93-2*, *qPi93-3* and *qPiN-1* were identified on chromosomes 1, 11 and 12. A RIL F8171 carrying a heterozygous *qPi93-2* allele was used for fine mapping. A total of 400 progeny derived from F8171 were evaluated with a field isolate (race) ARB82 (IA1) of *M. oryzae* under greenhouse conditions. Resistance to blast segregated in a 3 resistant: 1 susceptible ratio indicating a single major R gene was conferring resistance to this isolate. Five SSR markers, RM6998, RM7003, RM3246, MRG7102 and RM519 spanning *qPi93-2* were used to analyze all progeny. Consequently, *qPi93-2* was delimited to 4.2 megabases between RM3246 and MRG7102. An additional 2000 progeny of F8171 have been used for disease evaluation and genotyping for fine mapping. Results and implication for blast disease management will be presented.

***Geastrum polystigmatidis*: An important member of the sooty blotch and flyspeck disease complex in the northeastern United States**

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An ongoing investigation of sooty blotch fungi on apples in the Northeast U.S. revealed that *Geastrum polystigmatidis* is the most common cause of sooty blotch in the region. Little is known about the biology and epidemiology of this fungus. The objectives of our research were: 1.) Determine relationships among isolates obtained from apples and reservoir hosts (blackberry, sassafras, grape, and bittersweet) through genetic studies; 2.) Determine the timing of the appearance of *G. polystigmatidis* colonies on fruit and reservoir hosts' new growth; and 3.) Investigate the timing of the appearance of conidia in overwintered colonies on reservoir hosts in the field. Reservoir hosts adjacent to apple orchards and fruit in the orchards were observed at two-week intervals from mid-June to late September for signs of sooty blotch. Cultures obtained from colonies were subject to PCR analysis to determine their identity. *G. polystigmatidis* was the taxon most frequently identified on apples. Phylogenetic relationships among isolates from different hosts were analyzed. Colonies first appeared on reservoir hosts on July 3, and on apples on July 31. Conidia were first observed on reservoir hosts in May. This study is the first to investigate epidemiological aspects of this important agent of sooty blotch on apples.

Characterization of host transcription in response to infection by the rice pathogen *Xanthomonas oryzae*

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Xanthomonas oryzae pv. *oryzae* (Xoo) and the related pathogen *Xanthomonas oryzae* pv. *oryzicola* (Xoc) cause bacterial leaf blight and leaf streak diseases of rice, respectively. Both pathovars depend on a type 3 secretion system (T3SS) for virulence and harbor multiple genes of transcription activator-like effectors family. RNA microarray analysis after bacterial infection showed that genes with log₂³ and higher expression were more than the genes with the same level of suppression. The pathogens modulated 4645 common genes in rice cultivar IR24, where 2046 genes were induced and 2527 were suppressed. PXO99 was a unique strain that modulated near twice number of host genes than PXO86, T7174 and BLS303. PXO99 induced 354 genes compared to 221 for PXO86 and 166 for T7174 at 4-fold or greater induction level. Though belonging to the same pathovar, these strains induced just 26 host genes in common, indicating their uniqueness in virulence strategies. PXO99 shared 64 host genes in common with Xoc strain BLS303, indicating that though from different pathovars, these strains may share some TAL effector-mediated susceptibility pathways. SWEET11, SWEET13, OsTFX1, HEN1, flavinol synthase, pectic lyase and copper binding proteins were among the highest induced genes during susceptibility, and were validated by real-time RT-PCR. Results suggest that TAL effectors-mediated host susceptibility is highly dependent on the high induction than suppression of the host genes.

Are foliar fungicides a viable integrated pest management option for hybrid corn?

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Foliar fungicide use on hybrid corn has increased within the last decade, yet there are several questions pertaining to the value and sustainability of this practice. Field trials were conducted in Iowa, Illinois, Ohio, and Wisconsin from 2010 to 2012, using a split-split-plot experimental design. Main plots were hybrids varying in resistance to gray leaf spot and northern corn leaf blight. Subplots were four single applications of a quinone outside inhibitor fungicide at different timings. Sub-subplots consisted of inoculations with either *Cercospora zeae-maydis*, *Exserohilum turcicum* or both at two growth stages. Data were analyzed using PROC GLIMMIX in SAS. Mean disease severity was low (< 5% of leaf area affected) in 5 of 8 site-years. GLS-susceptible hybrids had significantly higher total disease severity (TDS), than resistant hybrids in Illinois (p<0.001) in 2010, Wisconsin (p=0.02) and Ohio (p=0.002) in 2010. Fungicide application significantly reduced TDS relative to the control in Illinois (p≤0.001) in 2010, Wisconsin (p=0.001) in 2011, and Ohio (p=0.02) in 2012. The susceptible hybrid had significantly lower grain yield than the resistant hybrid (p≤0.05), and the untreated control significantly lower yield (p≤0.05) than the fungicide-treated in three site-years. These results suggest that hybrid genetics (susceptibility to disease) and the prevailing environment are the biggest drivers of observed differences in disease severity and yield.

WITHDRAWN

WITHDRAWN

Pathogenicity of *Phomopsis* and *Diaporthe* isolates from Minnesota and susceptibility of soybean varieties

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Stem canker, top dieback, and early-dying of soybean associated with infection by *Phomopsis* and *Diaporthe* spp. are common across Minnesota (MN) and can cause significant damage. The objectives of this study were to determine: (i) if isolates of *Phomopsis* and *Diaporthe* from MN vary in virulence to soybean, (ii) if soybean varieties differ in susceptibility to isolates, and (iii) if growth rate and virulence of isolates are influenced by temperature. Fourteen isolates of *Phomopsis longicolla* and ten of *Diaporthe phaseolorum* var. *caulivora* from MN were inoculated individually onto six common soybean varieties using a cut stem inoculation method in a greenhouse at 24°C. Sunken, dark brown cankers developed following inoculation with all isolates on at least one variety. Isolates varied significantly ($p < 0.05$) in virulence and the varieties differed significantly in susceptibility. Radial growth rates of the isolates were measured on 0.5X PDA at 15, 25, and 32°C for 14 days. Growth rates were slowest and differed significantly among isolates at 32°C. *Phomopsis* ($n=2$) and *Diaporthe* ($n=2$) isolates were inoculated on two of the varieties at 15 and 27°C. Temperature did not affect virulence of isolates or susceptibility of varieties. In conclusion, isolates of *Phomopsis* and *Diaporthe* from MN varied in virulence to soybean, soybean varieties differed in susceptibility, and temperature influenced pathogen growth rate but not virulence.

A taxonomic and phylogenetic study of the plant pathogenic genus *Bipolaris*

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The genus *Bipolaris* includes saprobic, endophytic and plant pathogenic species. Important species such as *Bipolaris maydis*, *B. oryzae* and *B. sorokiniana* have caused devastating epidemics of important food crops such as rice, wheat and maize, influencing the course of human history. Identification of species of *Bipolaris* using morphology alone is challenging. Most species have a wide range of overlapping conidial and conidiophore dimensions and the sexual state is rarely found in nature. *Bipolaris* shares many similarities with its sister genus *Curvularia*. Isolates of *Bipolaris* were obtained from northern Thailand and USA from grasses and cultivated cereals, while available ex-type cultures of known *Bipolaris* species were acquired from culture collections. Single and combined phylogenetic analyses

were performed using ITS rDNA (internal transcribed spacer), GPDH (glyceraldehyde 3-phosphate dehydrogenase) and EF1- α (translation elongation factor 1- α) sequence data. Combined ITS rDNA and GPDH data resolve most of the species in *Bipolaris*. We determined that *Bipolaris nicotiae*, *B. papendorfii*, *B. prieskaensis* and *B. subpapendorfii* belong to the sister genus *Curvularia*, broadening the host range for that genus. Important plant pathogenic species of *Bipolaris* that lack ex-type cultures will be epitypified in order to accurately define species, provide a reliable basis for identification and determine phylogenetic relationships for those taxa.

WITHDRAWN

Rice phenylalanine ammonia lyase 4 gene (*OsPAL4*) is associated with broad spectrum disease resistance

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Most agronomically important traits, including resistance against pathogens, are governed by complex genetic loci. Determining the genes, gene combinations, or genomic regions controlling these traits would contribute to the breeding of cultivars containing durable resistance because it would provide information needed to develop molecular markers. In the last decade, several defense response (DR) genes were successfully used as good predictors and contributors to quantitative trait loci (QTL)-based resistance against several devastating rice diseases. We identified a rice mutant with a 750 bp deletion in the second exon of a member (*OsPAL4*) of the *phenylalanine ammonia lyase* gene family. *OsPAL4* clusters with three other *OsPAL* genes that co-localized with a disease resistance QTL on rice chromosome 2. The *ospal4* mutant lines exhibited increased susceptibility to bacterial blight and sheath blight diseases of rice. Within the mutant line, expression differences were detected for other *OsPAL* gene family members, including up-regulation of *OsPAL2*, which is in the same gene cluster as *OsPAL4*. These results suggest that *OsPAL4* is an important contributor to broad-spectrum resistance in rice and argue its role in the disease resistance governed by the chromosome 2 QTL. Our results unveil *OsPAL4* as a target for improving broad-spectrum disease resistance in rice.

Occurrence and distribution of pathogenic and indicator bacteria on conventionally and organically grown tomatoes and leafy greens in the mid-Atlantic

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Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* are often implicated in foodborne outbreaks associated with fresh produce. Mitigating

potential sources of pre-harvest microbial contamination is important, but the role of farming system and cultivation scale on food safety is unclear. Conventionally and organically grown tomatoes and leafy greens from small- and medium-sized farms in the mid-Atlantic region were surveyed in summer and fall 2012. Tomatoes (T), leafy greens (LG), irrigation water, compost, field soil and pond sediment samples were collected three times per farm. STEC and *Salmonella* were analyzed by enrichment and PCR; indicator bacteria (aerobic plate counts, generic *E. coli* and total coliforms (TC)) were quantified using 3M petrifilms. Of 719 total samples, 0.4% (1/259) of T, 1.0% (2/192) of LG and 1.2% (2/168) of water were positive for Shiga toxin genes, and 4.2% (8/192) of LG were presumptively positive for *Salmonella*. Sample type was a significant factor for all indicator bacteria ($p < 0.001$), but farming system was only a significant factor for TC ($p < 0.001$). Produce and water samples from organic farms, fewer of which irrigated from surface water sources, had a lower prevalence of *E. coli* (4.6% and 12.4%) than similar samples from conventional farms (7.6% and 29.1%). This data suggests that sample type and farming system are important food safety factors.

Investigation of biocontrol and plant response to reduce interactions of enteric pathogens on leafy greens

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The Centers for Disease Control (CDC) recently reported that leafy greens are the number one food commodity (43 %) associated with foodborne illness. The means by which crops become contaminated by mammalian pathogens in the field is an extremely complex issue and whether plants respond to these atypical pathogens remains questionable. The purpose of this study was to investigate the use of potential biocontrol agent *Bacillus subtilis* FB17 to increase plant defenses toward these pathogens. Microtiter assays were performed to determine the level of inhibition of FB17 on enteric pathogens. Strains in this study included 3 outbreak strains of *L. monocytogenes*, *E. coli* O157:H7, *E. coli* O104:H4 and *Salmonella* Agona. Results indicated that FB17 was able to significantly inhibit all *Listeria* isolates, one *E. coli* isolate and none of the *Salmonella* isolates ($p < 0.05$). FB17 was also inoculated directly onto the roots of spinach plants and leaf samples were taken at 1 h and 3 h post inoculation and observed by SEM to determine the affects of FB17 on spinach stomates. Direct visual observations and measurements of the stomatal aperture opening of SEM samples showed that FB17 was able to induce stomatal closing at 1 h ($p = 0.03$) and 3 h ($p = 0.0008$) post inoculation. These results indicate the potential of FB17 to be used as a biocontrol agent in leafy greens to inhibit the growth foodborne pathogens as well as increase plant defense responses via stomatal closure.

RNA editing of *Didymium iridis* mitochondrial genes

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Regions in the mitochondrial genome of the myxomycete *Didymium iridis* were identified that were similar to typical mitochondrial genes: four small subunit ribosomal genes *rpS2*, *rpS4*, *rpS11*, and *rpS16*, and one respiratory gene that showed similarity to the NADH dehydrogenase subunit 9 (*nad9*) gene. In all but one of these genes, stop codons interrupted the reading frame for encoding a functional protein. Previous studies have identified an RNA editing mechanism in the mitochondria of *D. iridis* that primarily inserts "C" nucleotides. The insertions change the reading frame, eliminating stop codons and producing a functional transcript. RT-PCR was used to amplify these regions from total RNA for cloning and sequencing to compare the transcribed sequences to the unedited genomic sequences. The *rpS11* gene does not require editing to create and open reading frame capable of directing protein synthesis. It is only the second mitochondrial gene identified in *D. iridis* that does not require RNA editing. We now have evidence of RNA editing in the other four genes above, and we are analyzing the patterns for features previously observed in RNA editing. These features may help identify the signals for the RNA editing machinery. In addition, we are comparing the editing events in *D. iridis*, to the editing patterns in the same genes in the related myxomycete *Physarum polycephalum* to determine if the sites are conserved.

Molecular diagnostics for the boxwood blight fungus, *Calonectria pseudonaviculata*: Strategies for early detection

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Since its first North American detection in October 2011, *Calonectria pseudonaviculata*, the causal agent of boxwood blight, has been found in eight additional U.S. states and three Canadian provinces. Because it has the potential to kill plants in as little as two weeks, this destructive disease has already caused severe economic losses in the green industry. Rapid, accurate, and early detection is therefore a critical component of best management practices, and the objective of this study. To that end, we have developed a suite of species-specific molecular diagnostic assays making use of real-time PCR, end-point genotyping, and loop-mediated isothermal amplification (LAMP) that allow sensitive, low-level detection of the pathogen in pre-symptomatic foliage and soil. These assays will provide a critical tool for monitoring the incidence and distribution of the boxwood blight pathogen, and contribute to a sound management plan.

Characterizing GATA factor control of nitrogen scavenging by the rice blast fungus *Magnaporthe oryzae* and its implications for hemibiotrophy

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Magnaporthe oryzae is the most destructive rice pathogen worldwide and one of the most important hemibiotrophic microorganisms. Nitrogen utilization in the biotrophic and necrotrophic stages of rice infection might be under control of different regulators. Describing how and when control switches from one regulator to another, would be pivotal in understanding the infection process. The nitrogen regulator Nut1 is dispensable for biotrophic growth but is essential for nitrogen scavenging during necrotrophy. Here we report the characterization of Nut2, a GATA factor involved in nitrogen metabolism. Gene functional analysis of Nut2, using the split marker strategy, demonstrates that Nut2 disruption abolishes pathogenicity and $\Delta nut2$ mutant strains are attenuated for *in planta* growth. Phenotypic analysis demonstrated that Nut2 is required for growth on L-asparagine, a common nitrogen source that is likely found in host cells during biotrophy. L-asparagine is assimilated via the action of L-asparaginase (AIP) to yield aspartate. We found the expression of AIP was significantly reduced in $\Delta nut2$ strains compared to wild type. This transcription data demonstrates that in *M. oryzae*, Nut2 undertakes roles in nitrogen metabolism not performed by Nut1 in biotrophy. This work gives new insights into nutrient regulatory systems operating during plant infection that will be applicable to a wide range of fungal pathogens.

Exploring the basidiomycetous endophytic community of natural and planted rubber tree populations (*Hevea brasiliensis*)

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Recent research has examined the endophyte community of the economically important rubber tree, *Hevea brasiliensis*, but the focus has been on Ascomycetes. The purpose of this study was to characterize Basidiomycete endophytes from leaves and sapwood of *H. brasiliensis* using molecular and morphological methods. Sequence data were obtained from 295 isolates collected from 190 rubber trees representing populations distributed in wild habitats (Peru and Brazil) and plantations (Peru, Mexico, and Cameroon). Community diversity and composition were compared between wild and plantation ecosystems and between leaves and sapwood tissue types. Isolate identification was conducted through phylogenetic placement using three loci: ITS, LSU, and RPB1. Phylogenies were constructed under maximum likelihood and Bayesian inference, with the inclusion of curated sequence data obtained from previously published studies. Ancestral state reconstruction was conducted under parsimony and maximum likelihood models to infer the most plausible ecological role of the most recent common ancestor containing the endophytic strains in this study. The transition between saprobic, endophytic, and parasitic habits was investigated. Results from this study suggest that even though the endophytic community of cultivable endophytes is dominated by ascomycetous strains, basidiomycetes also represent an important part of the endophytic community of rubber trees, and potentially of other tropical plants.

Virus outbreak in several Nova Scotia strawberry nurseries affects fruit growers in the United States

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Many strawberry plants grown in eastern Canada are destined for fruit growers in the United States. For early season production, strawberry plants

are grown in Canada to get early chilling required for flower bud initiation and then the plants are dug and shipped to the southeastern U.S. Some of these plants are also used for mid- to late-season production. In the autumn of 2012, it was observed that some of the new plantings in Florida, North and South Carolina, and Louisiana established and grew very poorly. Initial tests showed that plants with poor growth in several Florida fields were positive for *Strawberry mild yellow edge virus* and *Strawberry mottle virus*. Testing of strawberry plants from five nurseries in Nova Scotia, Canada showed that two of the nurseries had very high levels of these two viruses (greater than 95%), while the other three nurseries had very low levels of virus infection. Subsequent testing of plants from these two nurseries showed that these two viruses were also present in newly planted fields in Maryland, Virginia, and Kentucky. In all cases, plants infected with these two viruses exhibited severe stunting, leaf chlorosis, poor fruit set and quality. Some growers reported 100% losses in fields planted from these two sources. All plants tested from the nurseries or fruiting fields were negative for *Strawberry crinkle*, *Strawberry vein banding*, *Strawberry pallidosis*, *Strawberry necrotic shock* and *Beet pseudo yellows viruses*.

Evaluating the correlation between mitochondrial haplotype and nuclear genotype of *Phytophthora cinnamomi*

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While *Phytophthora cinnamomi* is heterothallic, analysis of field populations in many studies is consistent with clonal reproduction. In the absence of sexual recombination the ability to monitor mitochondrial haplotypes may provide an additional tool for identification of clonal isolates and analysis of population structure. To determine haplotypes for this species seven mitochondrial loci spanning 6,961 bp were sequenced for 62 isolates representing a geographically diverse collection of isolates with A1 and A2 mating type. 45 haplotypes were identified with difference due to SNPs (totaling 152 bp) and length mutations (17 indels greater than 2 bp representing 910 bp). Network analysis of the haplotypes identified 8 primary clades with the most divergent clade representing A1 isolates from Papua New Guinea. With three exceptions isolate grouping in the network corresponded to mating type and previously published isozyme classifications. In an effort to broaden the geographic distribution of isolates examined, an additional 60 isolates were more recently characterized for mitochondrial haplotype. Two genotyping methods (SSR and AFLP) have recently been used as well to elucidate the potential correlation between nuclear and mitochondrial haplotypes.

Identification of fungi associated with maize (*Zea mays* L.) growing in different planting dates at northern Tamaulipas

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Maize is one of the most important crops for Mexico, but shows significant losses by several factors where outstand fungi which produces a broad range of mycotoxins secondary metabolites with toxic and lethal effects on living organisms exposed to contaminated food and they are mainly produced by the genus *Aspergillus* (*A. flavus* and *A. parasiticus*). Here we identified fungal pathogens associated to grain produced by eight maize hybrids planted in four planting dates during 2010 in Rio Bravo, Tamaulipas, Mexico and also we detected and quantified aflatoxins produced by *Aspergilli*. The fungi isolates were identified based on macro and microscopic traits in PDA where the primers ITS (internal transcribed spacers) and PFPRIMF3 and PFPRIMR4 primers and PCR were used. The amplified sequences were analyzed at NCBI database (National Center for Biotechnology Information). We identified based on morphology and molecular methods the genera *Aspergillus*, *Fusarium* and *Penicillium* infecting maize grains and the former genus showed the highest incidences. The 98% of the maize samples were contaminated by aflatoxins which ranged from 7.19 to 75.72% of total aflatoxins. higher than the maximum permissible values according the Mexican Government regulations (NOM-247-SSA1-2008) (≤ 20 ppb). The maize hybrid G-8285 showed the highest fungal incidences while the lowest damaged hybrids were Bisonte, H-437, and H-439.

***Bacillus subtilis* ameliorate common bean production under web blight conducive and unfavorable temperature conditions**

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The Amazon region combines high temperatures and humidity, conducive conditions for web blight (WB) caused by *Rhizoctonia solani*, and unfavorable for common bean development. Plant growth-promotion rhizobacteria (PGPR) may alleviate biotic and abiotic stresses and increase yield. We evaluated seed treatment with *Bacillus subtilis* ALB629 and UFLA285 on the WB control and yield for two cultivars (Perola and Agreste) in the Amazon region (Boa Vista). Treatments consisted of each PGPR (UFLA285 or ALB629) or a nutrient-based product (initiate soy) combined or not with fungicide and water control. Treated seeds of each cultivar were sown in the field on a previously *R. solani*-inoculated area and evaluated for the disease incidence and severity as well as final productivity. The response was dependent on the considered cultivar. For Perola, WB incidence was reduced by up to 600% and severity was always lower than 14% for all treatments, while the control was 58%. For Agreste, disease severity was lower (1.7%) than the control (25%) up to 40 days after sowing for all PGPR treatments, combined or not with fungicide. Production was only obtained for the cultivar Perola was 489% higher than the control for ALB629 used alone. *B. subtilis* ALB629 potentially reduced web blight and increased yield but the cultivar choice will also play an important role in achieving sustainable common bean production in the Amazon region.

Plant-growth promoting rhizobacteria attenuates *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* defence suppression-like in common bean

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Bacterial wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* - Cff) is among the most important menaces for common bean production and is difficult to control with the available tools. Plant growth-promoting rhizobacteria (PGPR) controls the disease but little is yet known about the underlying induced resistance events. We investigated defense- and growth-related variables. *Bacillus subtilis* strains UFLA285 and ALB629 were grown, the concentration adjusted to 2×10^8 cells/g, and used to treat Cff-inoculated common bean seeds. Treated seeds were sown and plants evaluated for seedling emergence (SE), speed emergence index (SEI), relative growth index (RGI), root dry weight (RDW), shoot dry weight (SDW), as well as biochemical plant responses in the presence or absence of Cff. The disease control was up to 76% for ALB629. PGPRs promoted increase in RGI, SDW, and RDW. For biochemical responses on non-inoculated plants, PGPR induced phenylalanine ammonium lyase (PAL) activity, total phenolics content and lignin (only for UFLA285). Upon inoculation, although PAL activity decreased, phenolics' content (for UFLA285) and lignin accumulation (for ALB629) were sustained compared to the control. We demonstrated that *B. subtilis* UFLA285 and ALB629 sustain plant growth promotion in the presence of the pathogen and apparently induce compensatory artifices to overcome Cff-resistance suppression.

Comparison of inoculation methods for characterizing aggressiveness of *Phomopsis* stem canker pathogens of sunflower

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Phytopathology 103(Suppl. 2):S2.91

Phomopsis stem canker is a serious disease of sunflowers worldwide, while in the United States (U.S.), it has been of minor importance until recently. While *Phomopsis helianthi* was presumed to be the sole incitant of the disease, researchers have hypothesized that more species might be implicated. Recently, a pathogen originally characterized in Australia, *P. gulyae*, was found to be widespread in the U.S. Northern Great Plains, which coincided with an increase in PSC incidence in the region. The objectives of this study were to develop an effective technique for accurately evaluate disease severity and aggressiveness of *P. helianthi* and *P. gulyae* on sunflowers. Four screening methods, namely; wound-inoculation, straw test, stem-wound, and petiole wound were compared on three-week-old sunflower plants. In general, all methods produced significant disease 14-d after inoculation. The stem-wound caused more rapid development of disease symptoms (7-d) than other methods, and had the highest re-isolation frequency of pathogens from the diseased plant tissues. Our results also indicate that significant variation in aggressiveness among isolates within species (p less than or equal to 0.05). In general, *P. gulyae* isolates were more aggressive than *P. helianthi*. The stem-

wound method will be further validated by comparing the greenhouse and field reactions.

Improved biomass and altered transcriptome in *Medicago truncatula* as a result of intra/intercellular colonization by the mycorrhiza *Sebacina vermifera*

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Sebacina vermifera is a mycorrhiza with a demonstrated wide host range and positive effects on plant biomass. However, very little is known about the root colonization mechanism and how the plants respond to the fungus. To answer these questions, we investigated whether *S. vermifera* colonized *Medicago truncatula* and whether it was a beneficial interaction by using confocal microscopy and gene expression analyses. Roots of *M. truncatula* were inoculated *in vitro* with *S. vermifera*. Then, seedlings were transferred to the greenhouse and biomass was measured after 21 days. Plant shoot and root samples were collected from additional *in vitro* experiments at 6 and 20 days-post initial contact for microscopy and whole genome transcriptional profiling. Indeed, *S. vermifera* successfully colonized *M. truncatula* roots and plant biomass was increased by 23% and 21% for shoot fresh and dry weight, respectively, and 23% for root fresh weight. Changes in root dry weight were not significant. Root epidermal cells were colonized intracellularly whereas the fungus grew mostly intercellularly among the root cortical cells. Microarray results were validated by qRT-PCR, and showed more transcriptional changes in root than in shoot tissues. The number of differentially expressed genes in root tissue at 6 and 20 dpi was 170 and 909, respectively. Among them were genes coding for sugar and phosphate transporters, signal transduction, nodulation and pathogenesis-related proteins.

Pathogenicity of *Pythium* species affecting corn and soybean in Iowa at three temperatures using two assay methods

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Several *Pythium* species are known to cause economically important seedling diseases of corn and soybean in the United States. These diseases are predominantly managed with fungicide seed treatments; however, seedling disease continues to be problematic, especially when seed is planted in cold, wet soils with high clay content. There is little known regarding the pathogenicity of *Pythium* spp. in Iowa, so effectively managing corn and soybean seedling diseases can be difficult. The objective of this study was to determine the pathogenicity of the most prevalent *Pythium* spp. in Iowa affecting corn and soybean at three temperatures. Furthermore, two methods that are commonly used to assay pathogenicity were compared. A plate assay was used to evaluate the pathogenicity of *P. torulosum*, *P. oopapillum*, *P. lutarium*, and *P. sylvaticum* on corn and soybean at 23C, 18C, and 13C. *Pythium torulosum* was pathogenic at 13C and non-pathogenic at 23C and 18C. Conversely, *P. sylvaticum* was pathogenic at 23C and 18C, but non-pathogenic at 13C. Both *P. oopapillum* and *P. lutarium* were pathogenic at all three temperatures. In general, all *Pythium* spp. were more pathogenic on soybean than corn in the plate assay. All four species of *Pythium* are currently being screened at the above three temperatures for pathogenicity on corn and soybean in a growth chamber assay.

A pipeline for automated diagnostic primer design based on genomic sequence alignment of target and non-target genomes

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Comparative genomics strategies are highly effective for rapidly designing diagnostic primers, and will become more routine as sequencing technology improves. However, existing methods for genome-based primer design require manual steps, proficiency in bioinformatics, completed genomes, or powerful computing resources. We designed a computational pipeline to perform one-step primer design from draft genomic sequence, incorporating the open-source programs MUMmer, Primer3, and Primersearch to identify sequences specific to a group of target genomes but absent from non-target genomes. Based on complete and draft genomes downloaded from public databases or newly generated for this study, the program was used to design novel diagnostic primers specific to the species *Pseudomonas fuscovaginae*, *Burkholderia andropogonis*, and *Xanthomonas translucens*, the pathovar

Acidovorax avenae subsp. *avenae*, and geographical variants of *X. oryzae*. Five to ten primers for each test set were tested on collections of target and non-target strains. Nearly all primers amplified the sequenced strains and were specific to the target group of species; however, the proportion of primers inclusive to all strains varied between 10 and 90% depending on the number of genomes available and the genetic diversity of the group targeted. The primer pipeline will be made available in a user-friendly internet format through the RiceGalaxy server.

Classification of *Rathayibacter agropyri* sp. nov. based on analysis of the 16S rRNA and housekeeping genes

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Bacteria in the genus *Rathayibacter* can survive in dried ooze on infected seed heads of grass hosts for years. In 1982, a Gram-positive yellow-pigmented bacterium was isolated from ooze present on 30-to 40-year-old plant samples from the Washington State University Mycological Herbarium. A similar bacterium was isolated from fresh plant samples collected in Montana in 1986. In 2011, viable bacteria were again isolated from these samples. Morphological and physiological tests completed on the isolates in the 1980s and in 2011 confirmed that they were identical. Based on the morphological and physiological data, the isolates were hypothesized to represent a new species in the genus *Rathayibacter*. The 16S rRNA gene and four housekeeping genes (*rpoB*, *gyrB*, *recA*, and *ppk*) were amplified and sequenced from the isolates. PAUP* analysis of the 16S rRNA sequences indicate that the isolates are identical to each other and form a clade distinct from the six known *Rathayibacter* species. PAUP* analysis of the sequences obtained from the housekeeping genes *rpoB*, *recA*, and *gyrB* also demonstrate that the isolates form a clade distinct from the six *Rathayibacter* species. Analysis for congruence between the housekeeping genes is in progress and sequences from congruent trees will be used in an analysis of the concatenation of all four housekeeping genes. These data support the hypothesis that these isolates are a new species of *Rathayibacter*, which we propose to be named *R. agropyri*.

Acid protease production by fungal root endophytes

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Fungal endophytes are ubiquitous in healthy root tissue, but little is known about their nutritional requirements, including their protein utilization. Fungi secrete proteases in order to access the carbon and mineral nutrients contained in proteins and may also be involved in cell wall penetration and the autolysis of fungal hyphae. We compared the protein utilization patterns of several isolates of the root endophytes *Phialocephala fortinii* s.l., *Meliniomyces variabilis* and *Umbelopsis isabellina* with those of two ectomycorrhizal fungi (*Hebeloma* and *Laccaria*) and the wood decay fungus *Irpex* at pH 2-9. Protein utilization was assessed by the Bradford assay, protease activity was measured with fluorescently labeled casein, and specific inhibitors used to determine protease type. Although all fungi tested produced aspartic proteases, there was considerable variation in the pH range over which they were capable of utilizing protein. Utilization by *M. variabilis* was restricted to pH 2-3, *P. fortinii* to 3-4, *U. isabellina* to 3-5, *Hebeloma* to 4-5 and *Irpex* to 3-6. These differences may be related to the preferred habitats and ecological functions of the fungi, as *M. variabilis* also forms ericoid mycorrhizal associations in acidic habitats, *P. fortinii* is ubiquitous in moderately acidic conifer forests and *U. isabellina* occurs in a broad range of soil types where it may be involved in root decomposition.

Changes in the apple rhizosphere microbiome associated with orchard system resilience conferred by *Brassicaceae* seed meal amendment

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Pyrosequencing analysis of the apple rhizosphere microbiome was conducted two years post-planting at an orchard replant trial which included a no treatment control, 1,3-dichloropropene-C17 pre-plant soil fumigation, and pre-plant soil incorporation of a *Brassicaceae* seed meal (SM) formulation. SM treated soils exhibited resilience to re-infestation by *Pratylenchus penetrans* and *Pythium* spp., which was correlated with superior tree growth and yield in SM treated soil relative to that observed in fumigated and control soils. After two years, the rhizosphere microbiome in fumigated soils was similar to that detected in the no-treatment control, while the rhizosphere of trees established in SM treated soil possessed unique bacterial and fungal profiles. Overall diversity of the rhizosphere microbiome was demonstrably reduced in the SM treatment compared to the control or fumigation treatments, suggesting that

enhanced “biodiversity” was not instrumental in achieving enhanced system resilience and/or pathogen suppression. Relative resistance of the orchard soil system to pathogen re-infestation was, in part, rootstock genotype-dependent and was associated with differences in bacterial phyla detected in the rhizosphere. In addition, abundance and diversity of potential foliar and fruit pathogens was significantly lower in SM amended soils demonstrating that an understanding of below ground/above ground interactions may enhance orchard sustainability.

Differences in corn hybrid structural responses to infection by *Clavibacter michiganensis* subsp. *nebraskensis*

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Clavibacter michiganensis subsp. *nebraskensis* (Cmn) is a Gram positive bacterium that causes Goss' wilt and leaf blight on corn. Yield loss is dependent on the percent leaf area blighted during the grain fill period. To improve our understanding of the mechanism of resistance of corn to infection by Cmn, leaves of a susceptible and a tolerant hybrid at the V4 growth stage were wound-inoculated. Leaf blight progress was monitored over time as lesion length along the leaf blade, while movement of Cmn from the point of inoculation was enumerated using dilution plating. Structural changes in the mesophyll and vasculature of inoculated leaves were observed using scanning electron microscopy. Lesions on the susceptible hybrid were more than 60 cm in length compared to 40 cm on the tolerant hybrid ($P=0.05$) at 23 days post inoculation (DPI). Progress of lesions towards the proximal end of leaves was less than 9 cm in the tolerant and more than 18 cm in the susceptible hybrid. Bacteria colonization of healthy tissues preceded lesion development in both hybrids. Tolerance to Cmn infection occurred approximately 14DPI and a possible mechanism may be the production of a dense matrix that restricts movement of bacteria cells. The nature of this matrix is currently being investigated. Understanding the mechanisms of resistance of corn to Cmn is key as the use of resistant hybrids underlies management.

Ontogenesis of conidiation in the grapevine powdery mildew (*Erysiphe necator*)

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Spread of *Erysiphe necator* amidst grapevines is dependent upon synchronized production and dispersal of airborne conidia. However, the time course of growth events, and interactions with light and circadian rhythms were not precisely understood. We detached, inoculated, and incubated expanding leaves of *Vitis vinifera* 'Chardonnay' in double Petri plates at 22 C, with 24 h illumination, or a 6 h/6 h or 12 h/12 h day/night cycle. As colonies neared sporulation competency (5 da post-inoculation), leaves were kept either in their respective day/night cycles or in constant darkness. During darkness, colonies formed conidiophores, but not conidia. After light exposure began, single physiologically mature (detachable) conidia were produced within 5 h, and then conidiation ceased until the next day. Conidiation was not increased by 6 h day/night cycles. Conidiation in continuous light stopped after the first sporulation cycle. Thus, conidiophore production was light-independent, but conidiation was light-dependent. Under vineyard conditions, airborne doses of conidia would depend to a large degree on the time of sunrise. This study suggests several ways in which light could be used as a tool for studying genetics of sporulation. It also provides an alternative explanation for correlations of airborne conidial dose and various environmental factors observed in previous reports.

A next generation sequencing approach for the identification and annotation of the mating locus in the wheat bunt pathogen *Tilletia caries*

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The genus *Tilletia* includes common, dwarf and Karnal bunt pathogens of wheat. The role of the mating system is of fundamental importance in the bunt life cycle and to pathogenicity because mating controls the ability of the fungus to infect plants. Prior studies identified a bipolar mating system in

wheat bunt species; two mating type alleles were identified in common bunt species *T. caries* and *T. laevis* and multiple alleles in dwarf and Karnal bunt species *T. contraversa* and *T. indica*. Mating loci, however, have not been characterized in *Tilletia* and mechanisms contributing to pathogenicity are not well understood. The objectives of this study were identification and characterization of the mating locus in *T. caries* utilizing next generation sequencing methods and homology based genome comparisons with related smut fungi including *Ustilago maydis*. The whole genome of a haploid strain of *T. caries* was sequenced on Roche 454 and PacBio RS platforms (26x coverage). Using the PacBio assembler, Allora, a merged, quality-filtered assembly of 52.6 Mb and 8,592 contigs was generated. Annotation utilized FGENESH HMM algorithms trained on the *U. maydis* genome as well as BLAST and the NCBI conserved domain search tools. Putative homeodomain transcription factors bE and bW and pheromone receptor homologues have been identified in the sequence data. Next steps include development of specific primers and Sanger sequencing to verify mating locus gene annotation.

Managing Phytophthora blight with biofumigation

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Biofumigation with spring-planted mustard cover crop for managing Phytophthora blight in cucurbit crops was examined in fields naturally infested with *Phytophthora capsici* on Long Island, NY. Two replicated experiments were conducted. Mustard variety Caliente 199 was seeded at 10 lb/A on 8 April 2009 and 25 March 2010 with 0, 50, or 100 lb/A nitrogen. Mustard was flail chopped then immediately incorporated on 29 June 2009 and 14 June 2010, which were 2-4 wks after first bloom. Next the soil surface was sealed with a cultipacker, then irrigated. Acorn squash was direct-seeded or transplanted 2 wks later. Biofumigated plots had fewer symptomatic fruit, yield was increased and fruit had higher Brix levels. With increasing rate of fertilizer there was a trend toward greater mustard biomass and less blight. Three observational studies were also conducted. In the 2008 study, when blight was first observed, symptoms were found on most zucchini plants in the non-fumigated strip whereas only end plants were affected in the adjacent biofumigated strip. In 2011 there were several atypical intensive rain events creating very favorable conditions for blight, which was severe in all sections of this study as well as in other cucurbit plantings where fungicides were used. In 2012 an integrated management program was implemented that began with biofumigation in a field where blight was severe in pumpkin the previous season. It was successful: fewer than 9% of pumpkins rotted.

Efficacy of fungicides with resistance risk for cucurbit powdery mildew and fungicide sensitivity of *Podosphaera xanthii* in New York

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Fungicides at risk for resistance development are key to effectively managing cucurbit powdery mildew because their mobility enables movement to lower leaf surface. Such fungicides were tested by applying them alone with a tractor-sprayer every week to field-grown pumpkin in replicated experiments. In 2011, powdery mildew was controlled on upper leaf surfaces by products in all 3 recommended fungicide classes: FRAC code 13 (Quintec), 7 (Pristine), and 3 (Procure). Severity was lower on plants treated with new fungicides (7, U6 and U8). Control on lower surfaces was compromised by early onset before treatment. In 2012, Pristine at highest label rate was ineffective as was Fontelis (7), Quintec was very effective, and Procure at highest label rate was intermediate. A leaf disk bioassay used to determine fungicide sensitivity of pathogen isolates obtained late in the 2010 growing season revealed that 97% and 98% were resistant to FRAC code 1 and 11 fungicides, respectively, 43% were resistant to boscalid (7). One tolerated 80 ppm myclobutanil (3), 40 ppm quinoxyfen (13) and was resistant to boscalid and code 1 and 11 fungicides. Among 55 isolates collected in Sep 2011 when Pristine was effective, 79% tested were resistant to code 11 chemistry, only 6% were resistant to boscalid (Pristine contains both ingredients), 4% of isolates tested tolerated 80 ppm myclobutanil, 33% tolerated 40 ppm myclobutanil, 4% tolerated 80 ppm quinoxyfen, and 24% tolerated 40 ppm quinoxyfen.

Evolution of zygomycetous spindle pole bodies: Evidence from mitosis in *Coemansia reversa*

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One of the hypotheses being tested in AFTOL2 is that multiple losses of the flagellum led to the transition to spindle pole bodies (SPB) lacking centrioles in derived fungi. Zygomycetous fungi are critical in testing this hypothesis, but nuclear division and SPB cycles are incompletely known in these fungi. Mitosis and the SPB cycle were studied in *Coemansia reversa* (Kickxellomycotina) using 2- to 3-day-old germinated spores prepared for electron microscopy by chemical fixation and freeze substitution. At interphase the SPB consisted of two components: a cytoplasmic, electron-dense sphere containing a cylindrical structure with microtubules oriented perpendicular to the nucleus and an intranuclear component appressed to the nuclear envelope. Markham's rotation was used to reinforce the image of the cylindrical structure and determine the probable number of microtubules. The SPB duplicated early in mitosis and separated on the intact nuclear envelope. Nuclear division appears to be intranuclear with spindle and kinetochore microtubules interspersed with the condensed chromatin. The nucleolus retained a peripheral position within the nucleus or a pocket on the nucleus in the stages observed. The results suggest that this is a fourth type of zygomycetous SPB and the third type that suggests a centriolar component.

A torradovirus complex in Sinaloa, Mexico

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The whitefly-transmitted torradoviruses have a bipartite RNA genome with an RNA1 of about 7kb and an RNA2 of about 5kb in length. They infect various solanaceous plants and cause serious problems in tomato and tomatillo productions. During the investigation of a tomato viral disease outbreak in Sinaloa, Mexico, several torradoviruses were found to exist as a complex in cultivated tomato and tomatillo and wild datura. RNA samples were collected from plants in several locations near Los Mochis, Sinaloa. Two pairs of highly conserved, degenerate primers were designed to amplify an 800 bp region of RNA1 and a 600bp region of RNA2 of torradoviruses. Sequencing analyses reveals three distinct genotypes of torradovirus RNA1: two respectively with 98% and 84% nucleotide identities to *Tomato apex necrosis virus* (ToANV) RNA1, and one with an 84% nucleotide identity to *Tomato marchitez virus* (ToMarV) RNA1. Three distinct genotypes of torradovirus RNA2 were also identified in the same samples: two respectively with 98% and 90% nucleotide identities to ToANV RNA2, and one with an 83% nucleotide identity to ToMarV RNA2. These data suggest the presence of the previously reported ToANV and at least two additional torradoviruses. Various combinations of RNA1 and RNA2 and the presence of more than one type of RNA1 and RNA2 sequences in individual samples indicate an entangled torradovirus complex in the region.

The systematics of *Endoraecium* in Australia

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Endoraecium (Raveneliaceae, Pucciniales) is a genus of rust that infects several species of *Acacia* (Fabaceae) in Australia, south-east Asia and Hawaii. Thirteen species of *Endoraecium* have been described, including seven species that are endemic to Australia, one species to south-east Asia and five to Hawaii. This study investigated the systematics of *Endoraecium* from 50 specimens in Australia and south-east Asia with a combined morphological and molecular approach. Phylogenetic analyses were conducted on combined datasets of the SSU, ITS and LSU regions of rDNA. The recovered phylogeny (i) supported a recent division of *Endoraecium digitatum* into five separate species based on morphology and host specificity and (ii) found lineages that did not correspond with known species.

Unraveling *Bacillus subtilis* induced tolerance to damping-off in cotton

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Rhizobacteria confer disease resistance in many agricultural crops and transcriptomics profiling is an important way to explain the underlying mode

of action. *B. subtilis* UFLA285 induce disease resistance against damping-off caused by *Rhizoctonia solani* it in vitro inhibits the pathogen but the plant gene regulation upon exposure to the biocontrol agent is unknown. The aim of this study was to identify gene transcriptional events altered with exposure to the UFLA285 in *R. solani* infected plants. Global gene transcription was profiled using a commercially-available cotton gene chip; cotton plants with and without UFLA285-seed treatment were infected with *R. solani* 9-days after planting, harvested on day 14 for RNA extraction. Microarray data of stem tissue revealed 247 genes differentially regulated in infected plants, seed treated versus untreated with UFLA285. Transcripts encoding disease resistance proteins via jasmonate/ethylene signaling as well as osmotic regulation via proline synthesis genes were up-regulated. Consistent with transcriptional regulation, UFLA285 increased plant-proline accumulation, dry weight and photosynthesis. This study has identified transcriptional changes in cotton, induced by the beneficial soil bacterium UFLA285 and associated with disease control.

Bacterial blight in *Pelargonium*: Infections unravelled

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Xanthomonas hortorum pv. *perlargonii* (X.h.pel.) causes bacterial blight in *Pelargonium*. Growers know the disease as very destructive and contagious: in general plants will die within 2-3 weeks after infection. In the 2009-2010 growing season, the disease developed slower, symptoms appeared shortly before flowering. In first instance, it was not recognized as a X.h.pel. infection. As a result the disease was dispersed to many different growers before measures were taken. When it became clear that it was X.h.pel, a new strain was suspected. Naktuinbouw has over 100 X.h.pel isolates in its collection starting from 1990 and onwards. New isolates were compared to isolates already in the collection using DNA finger-printing, BOX-ERIC PCR and GyrB analysis. In the 2009-2010 season the disease was caused by isolates representing three different genetic profiles. Isolates with similar profiles were already in the collection. Infection experiments were carried out with a selection of isolates during summer 2012 and winter 2012/2013. Plants were sampled destructively shortly after inoculation to follow bacterial distribution in the plant. Symptom development varied between isolates and season. The genetic profiles were compared to symptom development and consequences for detection and sampling are discussed.

Use of quantitative pyrosequencing to dissect complex pathogen-pathogen interactions

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Structures of pathogen populations influence incidences and severities of both disease and mycotoxin contamination. For example, interactions among *Aspergillus flavus* genotypes during crop infection influence the extent to which crops become contaminated with aflatoxins. Quantification of pathogen population dynamics is complicated by sampling error and lack of both morphological distinction and assay precision. Quantitative pyrosequencing, distinct from 454 pyrosequencing, precisely measures frequencies of single nucleotide polymorphisms (SNPs) within pools of DNA resulting in population analyses with much less sampling error than culture-based methods. To test the utility of pyrosequencing for analyzing complex pathogen-pathogen interactions, pyrosequencing assays were designed to distinguish among seven *A. flavus* genotypes. Maize kernels were inoculated with mixtures of the seven genotypes, and frequencies of genotype-specific SNPs within pools of DNA from kernel-infecting mycelia or conidia produced during infection were successfully monitored with quantitative pyrosequencing. Frequencies of the seven genotypes changed during kernel infection with some genotypes displaying either superior invasion of host tissues or greater reproduction during coinfection. Results demonstrate the utility of quantitative pyrosequencing in elucidating complex interactions among closely related pathogen genotypes during host infection.

Effect of corn residue management practices on Goss's wilt of corn

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Goss's wilt of corn, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*), is an emerging disease in Illinois that has caused yield reductions in affected fields. Because *Cmn* survives in corn residue, Goss's wilt is more severe in fields with more residue. With current economics favoring corn

production, a trend in Illinois has been to plant corn in consecutive years in the same field, which increases the residue in the field and the risk of Goss's wilt. A field trial was conducted near Urbana, IL in 2012 to determine the effect of corn residue management on Goss's wilt. Different tillage treatments (no-till, chisel plow, or moldboard plow) were used on a field that had severe Goss's wilt the previous season. Within the different tillage regimes, 10 different corn hybrids susceptible to *Cmm* were planted, Goss's wilt incidence was evaluated, and grain yields were determined. Tillage had a significant ($P < 0.0001$) effect on Goss's wilt incidence, but not on grain yield. Plots that were not tilled (no-till) had the greatest amount of corn residue and the greatest Goss's wilt incidence (11.2%), while plots that were chisel or moldboard plowed had the least residue and the least Goss's wilt incidence (1.1% and 0.04%, respectively). These results indicate that tillage can be an effective component of managing Goss's wilt; however, corn growers must weigh the benefit of disease management vs. the risk of soil erosion when making a residue management decision.

Effect of levels of wheat residue on the severity of *Stagonospora nodorum* blotch in winter wheat

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Stagonospora nodorum blotch (SNB), caused by *Stagonospora nodorum*, is an important disease of wheat worldwide. Wheat residue can be an important source of inoculum for SNB, but the effect of different densities of infected debris on disease severity has not been previously determined. Field experiments were conducted in Raleigh and Salisbury, North Carolina, in the 2011/2012 season using the susceptible winter wheat cultivar 'DG-Shirley'. Four residue treatments (0, 30, 60, and 90% coverage of the ground with residue) were applied in a randomized complete block design with five replications. Disease was assessed three or four times during the season as overall SNB severity on a whole-canopy, per-plot basis. Area under the disease progress curve (AUDPC) was calculated and subjected to analysis of variance. Final severity of SNB ranged from 15 to 55% across the two locations. In Raleigh, AUDPC for the 30 to 90% coverage levels were significantly greater than AUDPC for the 0% coverage level, but there were no significant differences among the 30, 60, and 90% coverage levels ($P < 0.05$). In Salisbury, AUDPC for the 0% level was also significantly lower than AUDPC for the 30 to 90% levels; however, significant differences in AUDPC were observed among the 30, 60 and 90% levels ($P < 0.05$). These results indicate that wheat debris covering $\geq 30\%$ of the field surface is likely to elevate the severity of SNB in a susceptible wheat variety under favorable weather conditions.

***Salmonella* colonization activates the plant immune system and benefits from association with plant-pathogenic bacteria**

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Despite increasing incidences of human salmonellosis caused by consumption of contaminated vegetables, relatively little is known about how the plant immune system responds to and may inhibit *Salmonella* colonization. Here we show that *Salmonella* Typhimurium activates the plant immune system primarily due to its recognition of the flg22 region in *Salmonella* flagellin. Several previously identified plant genes that play a role in immunity were found to affect the host response to *Salmonella*. The *Salmonella* flg22 (Seflg22) peptide induced the immune response in leaves which effectively restricted the growth of *Salmonella* as well as the plant pathogenic bacterium, *Pseudomonas syringae* pv. *tomato*. Induction of immune responses by Seflg22 was dependent on the plant FLS2 receptor. *Salmonella* multiplied poorly on plant tissues similar to other bacteria which are non-pathogenic to plants. However, *Salmonella* populations increased significantly when co-inoculated with *P. syringae* pv. *tomato* but not when co-inoculated with a type III secretion system mutant of this pathogen. Our results suggest that *Salmonella* benefits from the immune-suppressing effects of plant pathogenic bacteria, and this growth enhancement may increase the risk of salmonellosis.

Spatial assessment of oxidative and enzymatic reactions in brown rotted wood

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Brown rot fungi are theorized to use free radical oxidations and enzymatic reactions to consume wood. Though likely incompatible *in vitro*, these reactions are proposed to occur concurrently in wood. We mapped and compared fungal growth, non-enzymatic wood modification, and cellulase activity in spruce degraded by *Postia placenta* to investigate spatial coincidence of these reactions. Nearly coincident reaction fronts were observed behind the most advanced hyphal tips and curiously behind apparent depolymerization of carbohydrates. To investigate a possible role of enzymatic reactions in the primary depolymerization of lignocellulose, we have initiated a study to assess the depth of penetration of an endoglucanase into wood cells during this process. Previous studies have shown insulin (5.7 kDa) and myoglobin (17.6 kDa), but not ovalbumin (44.4 kDa), can infiltrate the cell walls of rotted wood. The *P. placenta* endoglucanase PpCel5B (34.6 kDa) has a theoretical molecular weight considerably lower than ovalbumin. A polyclonal antibody raised against PpCel5B heterologously expressed in *Pichia pastoris* will be used to assess the extent to which a native brown rot endoglucanase is able to penetrate *Pinus resinosa* cells. Given the common supposition that pore size prevents brown rot fungal endoglucanases from accessing wood secondary walls, even in late decay stages, this work will provide a direct assessment of enzyme ingress.

Tank mix and alternation of acibenzolar-S-methyl with reduced rates of mandipropamid for control of downy mildew on basil in the greenhouse

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Previous studies have reported effective control of downy mildew on greenhouse grown basil with acibenzolar-S-methyl (ASM, Actigard® 50WG) when applied at optimal rates and timing. Disease resurgence could be a potential setback when the plants were pre-treated with ASM prior to pathogen inoculation and maintained in the greenhouse for extended periods. In this study, ASM was alternated or tank mixed at five rates (0, 12.5, 25.0, 50.0 and 100.0 mg/l) with mandipropamid (Revus® 2.08SC) at four rates (0, 1/2, 1/4, or full label rate) in a repeated greenhouse experiment. The full label rate of mandipropamid was 2.37 ml/l and the spray volume of water per hectare was 561.24 liters. Alternations were scheduled on a weekly basis but the tank mixes were tested at 7- and 14-day spray intervals. All ASM-mandipropamid treatments significantly ($P < 0.05$) reduced disease severity and incidence and increased marketable yield compared to the non-treated control (CK). Protection achieved from ASM - mandipropamid alternations ranged between 87.5 and 98.5% when compared to CK, and yield loss was reduced by as much as 97.6%. Average number of unaffected leaves per plant from the best treatment was 16.5, whereas only 0.8 from CK. Number of healthy leaves was highly correlated ($R^2 = 0.92$, $P < 0.001$) with marketable yield in all experiments. In addition, tank mix sprays at 7-day intervals provided significantly greater ($P < 0.05$) protection than sprays at 14-day intervals.

Fungi associated with diseased roots of strawberry runner plants after transplanting

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In Florida, strawberries are planted in October and grown as an annual winter crop. Transplants are obtained from nurseries in Canada and the U.S. To secure high market prices, transplants must root quickly and fruit early. Root-infecting fungi that impede this process often come from the nursery. To determine putative root pathogens, 'Strawberry Festival' transplants were obtained from multiple nurseries and planted in a fumigated field near Wimauma, FL. One month after planting, 4 diseased segments of structural roots from each of 10 to 12 plants per nursery were surface disinfested, rinsed, and plated out on 1/2 PDA plus antibiotics. Plants from 6 nurseries were tested in 2010. *Fusarium* spp. were consistently isolated from plants from all 6 nurseries at frequencies ranging from 17 to 80% of the root segments plated. Other frequently isolated fungi included *Alternaria* (two nurseries, 25 and 35%), *Rhizoctonia* (32, 35, 57%), and *Pestalotiopsis* (52%). In 2012, *Fusarium* spp. were isolated from 8 to 50% of root segments from the 7 nurseries tested. Other frequently isolated fungi were *Alternaria* (23, 33%), *Cylindrocarpum* (17, 25%), *Pestalotiopsis* (16, 23%), *Phoma radicina* (20%), and *Rhizoctonia* (33%). In some cases, nurseries with high levels of *Fusarium*, *Alternaria*, *Pestalotiopsis*, or *Rhizoctonia* in 2010 were also high in 2012.

Changes in the population makeup over time and effects of root-associated fungi on plant establishment are being investigated.

WITHDRAWN

Implications of improved taxonomic resolution in polypores—More species, more specialists, longer red lists?

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Polypores or bracket fungi make up a conspicuous and relatively well-known group of macrofungi. Many species in this group are ecologically specialized and commonly used in nature conservation inventories in Europe. Understanding of the species diversity in this group has moved forward during the last ten years due to DNA-aided taxonomy. New sequence data are accumulating not only from taxonomic research but also from ecological and forest pathological studies. For the most part new data have demonstrated the presence of more species than were previously recorded based only on morphology (i.e., cryptic species). Using examples from three species rich polypore genera (*Antrodia*, *Physiporus* and *Skeletocutis*) that we have studied extensively in Europe and North America, we show how taxonomically accurate interpretation may shape results of ecological and community studies. With increased taxonomic resolution we see a higher number of specialist species and species with a restricted range. This has direct implications for red list assessments and may also provide insight into niche-breadth in species of polypores.

Bioluminescence among North American *Armillaria* species in response to biotic and abiotic stimuli

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Bioluminescence has previously been documented for at least 60 species of white-spored members of the Basidiomycota including four species of the wood-decay genus *Armillaria* (i.e., *A. gallica*, *A. mellea*, *A. ostoyae* and *A. tabescens*). Using a Sens-Tech photomultiplier to measure mycelial luminescence, light production is reported for the first time for *A. calveolens*, *A. cepistipes*, *A. gemina*, *A. nabsnona*, and *A. sinapina*. Using series of 1000 consecutive luminescence measurements for the nine *Armillaria* species, luminescence dynamics were found to have quantifiable structure and were not simply 'white noise' processes (i.e., significant Bartlett's Kolmogorov-Smirnov statistics in all cases). Mycelia of eight of nine *Armillaria* species exhibited transient and/or statistically significant stable changes in luminescence magnitude in response to a series of mechanical shocks of 200 N applied by a chiropractic adjustment tool. Preliminary data suggest a statistically significant shift in mycelial luminescence after exposure to a con-specific or con-generic luminescent neighbor. Ongoing studies are focused on changes in directional growth of *Armillaria* mycelia in response to a neighboring con-specific or con-generic luminescent mycelium.

Spring morel fruit body emergence is primarily conditioned by soil temperature

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Fruit bodies of morels (*Morchella* spp.) are highly prized edible mushrooms which emerge in early spring. Many Missouri landowners and mushroom hunters are interested in augmenting the number of emerging fruit bodies through an understanding of the biotic and abiotic factors which condition emergence as well as through focused habitat manipulation. Previous research in a central Missouri woodland (i.e., Howard Co., Missouri) demonstrated morel fruiting in association with *Carya* spp., *Tilia americana* and *Ulmus americana* at a greater frequency than predicted by host frequency on the site. Analysis of a 9 yr sequence of daily precipitation records as well as hourly air and soil temperature records (i.e., 2001-2009), in concert with records of fruiting seasons, summarized conditions during the 10, 20 or 30 d prior to first fruit body appearance. Of the environmental variables examined, the air or soil temperature degree days (0 C basis) 20 or 30 d prior to first fruiting were most consistent over the 9 yr (i.e., minimum coefficients of variation). These metrics successfully predicted first fruiting during 2010-2012. The putative relationship between morel emergence and accumulated temperature will be further tested with data from the original field location as well as two locations in the region (i.e., Boone Co., Missouri).

Characterization of *Rhizoctonia solani* anastomosis groups on potato in the Pacific Northwest

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Rhizoctonia solani is an important pathogen of potato causing black scurf, elephant hide and stem canker. *R. solani* isolates are classified into several anastomosis groups (AGs), of which AG3 is most commonly associated with potato disease. Knowledge of the AG present is important as AGs can differ in aggressiveness to potato, host range, symptoms and fungicide sensitivity. In 2011, isolates of *R. solani* were collected from diseased potato plants throughout Idaho. The majority of isolates were identified as AG3, but sequencing confirmed that three were AG2-2IIIB or AG4 HG-II. In spring 2012, 102 seed lot tubers were collected throughout the Pacific Northwest. Direct qPCR of tubers showed 46% were positive for AG3. Using conventional isolation, 192 *R. solani* isolates were recovered and confirmed to be AG3 using qPCR. In summer 2012, stem canker samples were collected from potatoes and soil samples were collected from 7 different crops grown in rotation with potatoes throughout Idaho. Among the soil samples, oats had the highest percentage of *R. solani* AG3. Seventy-one stem canker isolates were screened for their AG using real-time PCR and 63 isolates tested positive for AG3. The remaining 8 isolates were identified as AG2-2IIIB, AG5 or AG-A. The pathogenicity of these atypical AG groups was confirmed on potato in greenhouse studies. Further results from this study could show the importance of AG screening, and could have implications on the crop rotation practices.

Foliar fungal endophytes associated with *Cornus* (dogwood) species in Japan and North America

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Fungal endophytes are ubiquitous in nature, yet their diversity and ecology remain poorly understood. *Cornus* species (dogwoods) are common ornamental trees and are important components of natural forest ecosystems. In this study we analyzed the year-to-year diversity between fungal endophytes associated with *Cornus* species in North America and Japan. Samples were collected in 2010 and 2012 from the same areas. In 2010, 258 out of 940 surface-disinfected leaf tissue segments produced mycelium. A total of 121 distinct OTUs were revealed based on the DNA sequence data from the ITS and partial LSU rDNA. Ascomycota represented the majority of taxa discovered, but Basidiomycota was also observed. The dominant orders for N. America and Japan in 2010 were Diaporthales, Xylariales, and Eurotiales. Nonmetric multidimensional scaling (NMDS) indicated that N. American and Japan fungal communities are similar in composition. In 2012, a total of 234 of 930 surface-disinfected leaf tissue segments produced mycelium which represents 107 OTUs. Diaporthales and Glomerellales were found to be the dominant orders in 2012.

Fungal digitization projects

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Recent funding initiatives through the Andrew W. Mellon Foundation and the National Science Foundation (NSF) have provided mycologists with opportunities to digitize and database large numbers of fungal specimens housed in fungaria throughout the United States. While the Mellon Foundation is focused on digitizing type specimens with data offered through the JSTOR website, the NSF Advancing Digitization of Biodiversity Collections (ADBC) program seeks to expand digitized data of all North American voucher specimens with fungal data offered through one of several Symbiota website portals. The Lichens, Bryophytes and Climate Change (LBCC) project aims to digitize over 900,000 lichens, while the Macrofungi Collections Consortium (MaCC) plans to digitize over 700,000 macrofungal specimens. A third project, the Microfungi Collections Consortium (MiCC), is being proposed to digitize microfungal specimens. Workflows, participants, progress to date, and opportunities to participate in these projects will be discussed.

Effects of nitrogen fertilization on risks and impacts of wheat streak mosaic disease

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Wheat streak mosaic virus (WSMV) is the most common cereal virus in the Great Plains and is transmitted by the wheat curl mite (WCM). Fertilization is known to alter disease and pest dynamics in other systems but has not been tested in mite-transmitted cereal viruses. A series of field and growth chamber experiments were conducted near Bozeman, MT, to examine the effects of nitrogen (N) fertilization on WSMV risk, spread, and impacts on yields in wheat. In field trials, increasing N-fertilization resulted in increased susceptibility to WSMV and increased yield losses due to WSMV in winter wheat. In a growth chamber experiment in which the effects of soil-N and WSMV infection on vector population growth rates were tested, the effects N-fertilization depended on if WCM were infected with WSMV. Without WSMV, vector populations grew faster on nutrient stressed plants. With WSMV, WCM populations grew at a higher rate on fertilized wheat plants. These results predict that N-fertilization will increase the rate of disease spread. A field experiment using spring wheat confirmed this prediction. Risk of disease spread off of mite-inoculated plants was over two times higher in high N treatments compared to low N. In general, these results demonstrate that altering rates and perhaps the timing of fertilizer application can have an impact on disease risk in winter wheat.

Screening strawberry (*Fragaria × ananassa*) germplasm for anthracnose disease resistance using traditional techniques and molecular markers

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Anthracnose of strawberry may be caused by any of three *Colletotrichum* species: *C. acutatum*, *C. gloeosporioides* or *C. fragariae*. These destructive pathogens may infect the fruit, leaves, petioles, crowns or roots and may cause plant death. Traditional and molecular approaches were used to identify anthracnose resistant strawberry germplasm in a collection of 31 cultivars and 46 selections. Following inoculation of whole plants with conidial suspensions of two *C. acutatum*, one *C. gloeosporioides*, and two *C. fragariae* isolates, 83% of the selections and 3% of the cultivars received a resistant score when averaged across the five isolates. The whole plant screening method was compared to a detached leaf assay in which leaves were removed from the 77 strawberry clones and inoculated in the laboratory with conidial suspensions of one *C. gloeosporioides* and two *C. fragariae* isolates, resulting in 78% of the selections and 3% of the cultivars rated resistant. The 77 strawberry clones were screened for the presence/absence of two molecular markers that have been reported linked to a *C. acutatum* resistant gene (*Rca2*) in strawberry germplasm. Among 39 clones rated resistant in the whole plant screen, 21 were positive for both and 14 were positive for one of the molecular markers. Correlation of the whole plant to the detached leaf assays and the presence or absence of the *Rca2* gene could significantly reduce the time it takes to identify anthracnose-resistant genotypes.

Evolution of virulence in *Puccinia striiformis* f. sp. *tritici* (Pst) on genes for adult-plant resistance in soft and hard winter wheat cultivars

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Since Stakman first described races of cereal rusts in 1914, races were based on reactions of seedlings with all-stage resistance (ASR). However, most soft and hard red winter wheats in the U.S. were susceptible as seedlings to stripe rust caused by Pst; sources of adult-plant resistance (APR) were race specific; and races based on seedling differentials did not explain stripe rust differences in the field. A susceptible check and 9 cultivars with APR were inoculated at boot stage with 17 isolates representing the pathogen population in south central U.S. from 1990-2012, and reactions on flag leaves were recorded. Five races were identified. Isolates from the 1990s (Race 1) were virulent on 7 differentials including 26R22 and Mason that was resistant to other races. Isolates from the new aggressive strain in 2000 with virulence on ASR gene *Yr9* (Race 2) were virulent on 2 differentials. Isolates from 2010 and 2012 with virulence on ASR gene *Yr17* (Race 3) were virulent on 1 differential (the susceptible check). Isolates from 2012 (Races 3,4,5) were virulent on 1 to 8 differentials. The population before 2000 was virulent on at least 6 sources of APR that provided resistance to 2 new introduced populations. In 2012, the population evolved virulence to at least 3 combinations of APR genes. This is the first report of races based on reactions of adult plants with APR genes and explains the reactions of cultivars in the field more accurately than races based on ASR genes.

Effect of rain and simulated rain events on deoxynivalenol levels in grain from winter wheat plants affected by Fusarium head blight

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Wheat affected by *Fusarium* head blight (FHB) has grain contaminated with the mycotoxin deoxynivalenol (DON), and the DON threshold in the U.S. for human consumption is 2 ppm. Developing cultivars with resistance to FHB and DON accumulation is a priority for managing the disease and DON. However, DON levels for wheat lines are complicated by variable rankings of wheat lines across environments and by variable associations with percentage of *Fusarium*-damaged kernels (FDK). DON is known to be leached from spikes in rain, and our objective was to determine if late-season rain or misting (as in FHB nurseries) leaches DON from grain and distorts the ranking of lines for DON and the association of DON with FDK. For each cultivar, spikes with similar size and FHB severity were divided into groups of 20, placed vertically into containers that allowed water passing through the spikes to be collected, and exposed to different rain/simulated rain treatments. The amounts of DON in water, chaff and grain were determined. In preliminary experiments, 5-46% of DON was leached from spikes. A field experiment to determine the effects of rain and simulated rain events on DON levels in grain will be conducted in 2013 with four cultivars ranging from susceptible to moderately resistant and with rain shelters to protect a portion of each plot from late-season rain.

Genetic relationship among *Fusarium oxysporum* f.sp. *melonis* VCGs and their relatedness to other *F. oxysporum* formae speciales

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Isolates of *Fusarium oxysporum* f.sp. *melonis* obtained from *Cucumis melo* cultivars showing yellowing and wilting from different melon producing provinces in Iran were characterized based on pathogenicity on differential melon cultivars and vegetative compatibility groups, identified as race 1,2 belonged to VCG 0134. The evolutionary relationships between isolates of different formae speciales of *F. oxysporum* were examined, with a special emphasis on *F. oxysporum* f.sp. *melonis*. Bootstrapped maximum likelihood analysis of the elongation factor-1alpha (EF-1alpha) gene was conducted on 16 Iranian and 11 foreign isolates of *F. oxysporum* f.sp. *melonis* that include different vegetative compatibility groups (VCGs 0130-0136). The tree inferred from the dataset resolved five evolutionary lineages which were correlated with the *F. oxysporum* f.sp. *melonis* VCGs with the exception of VCGs 0130 and 0131 which could not be differentiated with EF-1alpha sequences. Furthermore, based on EF-1alpha sequences, specific associations were found between *F. oxysporum* f.sp. *melonis* VCGs and the other formae speciales whose sequences were obtained from GenBank. These results support a polyphyletic origin for *Fusarium oxysporum* f.sp. *melonis* which has emerged multiple times.

Comparative genomic analyses of *Rhizoctonia solani*: Insights on evolution and pathogenesis

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The Basidiomycete fungus *Rhizoctonia solani* (telomorph: *Thanatephorus cucumeris*) is a species complex spanning over 100 members. These soilborne fungi cause blights, wilts, and damping off of more than 188 plant species covering major cultivated plants. Members of *R. solani* are divided into 14 anastomosis groups (AG), some of which are further divided into intraspecific groups. In order to better understand the biology, evolution, and pathology of this dynamic species we have utilized next-generation sequencing and comparative genomics to generate six draft genomes from members of multiple AGs spanning diverse host ranges and pathologies. The genomes range in size from 37-60 MB and show a wide variety of intraspecific variations. Gene content was generated for each of the six draft genomes, along with four additional isolates, using RNAseq and bioinformatic tools. We compared genes across seven AGs, 10 isolates, in order to identify gene families, genes involved in pathogenesis, gene family duplication and loss events, and horizontal gene transfer events. Additionally, gene expression analysis of all 10 isolates was conducted on samples infecting cucumber (*Cucumis sativus*) seedlings to assess gene regulation and pathogenicity while infecting a common host. This study represents the most complete analyses of *R. solani* to date and highlights key events in the evolution and pathogenic nature of this important plant pathogenic fungus.

Using SSR markers to understand the mechanism of powdery mildew disease resistance in *Cornus florida*

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Powdery mildew is one of the most devastating diseases in nursery production of flowering dogwoods *C. florida* across the Southern U.S. Effective fungicides for powdery mildew control have been identified; however, routine applications required for effective control have increased production costs and forced small growers out of dogwood production. While efforts in powdery mildew resistance breeding are being taken, only a few cultivars have displayed an acceptable level of powdery mildew resistance; understanding the underlying mechanism of powdery mildew resistance will facilitate breeding strategies. The aim of this research project was to study the inheritance of the powdery mildew resistance in previously selected powdery mildew resistant plant (R14) that has consistently exhibited resistance at multiple locations. Controlled crosses from hand pollination were carried out between susceptible (Cherokee Princess) and resistant selection (R14). Progeny seedlings were exposed to powdery mildew and rated for disease reaction, but only a few plants out of hundreds displayed powdery mildew resistance. Genome wide analysis of the parents and the progeny was done using Simple Sequence Repeat markers (SSR's). The polymorphism pattern was studied in an effort to identify markers that are linked with powdery mildew resistance. Results from this study will facilitate marker assisted breeding (MAB) in flowering dogwood.

Surfactin and biofilm production by *Bacillus subtilis* IN937b, a biological control agent for suppressing Phytophthora blight on squash

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Bacillus subtilis IN937b, a PGPR bacterium, is effective in control of Phytophthora blight (*Phytophthora capsici*) on squash. It has been proved by PCR that *B. subtilis* IN937b possesses a *sfp* gene which is responsible for biosynthesis of surfactin. Surfactin was extracted from a culture of IN937b and dissolved in methanol. After 3 days, mycelial growth was significantly reduced by 52% in surfactin treatment (0.4 mg/mL) compared to the non-treated control, and swollen tips of mycelia were observed under a microscope. Zoospore germination rate decreased by 50% in surfactin treatment compared to the non-treated control. *B. subtilis* IN937b was also evaluated for its capacity to form biofilm in a minimum medium (MSgg). Biofilm production was found to be strongly dependent on the bacterial

density. Thick biofilm developed only 2 days after stationary culture when the initial density of IN937b was 10^6 CFU/mL. However, biofilm appeared 4 days after when the initial density was reduced to 10^4 CFU/mL. In comparison with IN937b, *B. pumilus* SE52, a PGPR strain ineffective in suppression of Phytophthora blight on squash, failed to form biofilm in MSgg. PCR screening showed that SE52 does not possess the *sfp* gene. Results from this study indicate that surfactin produced by IN937b could play a role in biocontrol of Phytophthora blight on squash, and the biocontrol effect could be partially as a result of improved colonization by IN937b on root surfaces due to biofilm formation.

Interspecific hybrids between *Fusarium fujikuroi* and *Fusarium proliferatum*

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Interspecific hybrids offer unusual opportunities to study speciation and the segregation of traits that differ between species but that are often fixed within a species. We have recovered strains that appear to be hybrids between *F. fujikuroi* and *F. proliferatum* from rice fields in Southeast Asia and from a native tallgrass prairie in the United States. These hybrids can cross with standard mating type testers of both species and have DNA sequence profiles that are consistent with their putative hybrid conditions. The existence of these hybrids may indicate that reticulate evolution is occurring or that these two species have yet to completely finish the speciation process. We also have created such hybrids under laboratory conditions by crossing strains of opposite mating type on carrot agar. Based on the segregation of AFLPs, there are some fingerprint patterns that reoccur independently multiple times amongst the progeny, suggesting non-random segregation of at least portions of the genome. The parental strains differ in numerous traits, including secondary metabolite production and pathogenicity towards apples, onions and rice. Evaluation of recombinant progeny is providing the opportunity to identify portions of the genome involved in the speciation process and to identify regulatory and structural genes of importance in pathogenicity and secondary metabolite production.

Effect of green manure crops on *Verticillium dahliae*'s propagules in soil, potato early dying, and potato yield

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Potato Early Dying (PED) causes plants to senesce prior to full maturity, thus robbing yield. The major causal agent is the fungus *Verticillium dahliae*. Green manure crops can provide nutrients and improve soil conditions for crop growth and suppression of the pathogen. The objective of the study was to examine the effectiveness of green manures to reduce *V. dahliae* densities in soil, incidence of PED and improved yield. A 3-year study with 12 treatments was established in Manitoba. Sorghum-sudan grass and alfalfa were planted in 2006 with other treatments planted to wheat. In 2007, the sorghum and alfalfa were soil incorporated. Six green manures (oat/pea, oriental and yellow mustard, sorghum, Canada milk vetch, and rye) were grown and soil incorporated in 2007. Composted cattle manure (CCM) was applied in 2007 and 2008, and mustard seed meal in 2008. A soil fumigant-vapam was applied in the fall 2007; and wheat was grown as a control treatment. In 2008, potato was planted to all treatments. Only CCM increased yield. Mustard meal had the greatest reduction in *V. dahliae* propagules, whereas green manures were ineffective in killing the pathogen. CCM and seed-meal treatment reduced disease incidence by 30 and 40% compared to the control (60%), respectively. *V. dahliae* propagules, K, P, and soil pH accounted for 54% of the variability in the PED incidence. CCM and mustard meal showed promise to reduce PED with CCM being the only treatment that increased yield.

Effect of composted cattle manure and separated hog slurry solids on potato early dying and potato yield and tuber quality in Manitoba

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Potato early dying (PED) is a major constraint to potato production in North America. The major causal agent to this disease is the fungus *Verticillium dahliae*. Our previous studies showed composted cattle manure in Manitoba, Canada, have resulted in reduction in soil inoculum densities, delayed wilt of the crop and improved late tuber bulking and yield. The objective of this

research was to evaluate the effect of different rates (20, 40 and 80 Mg ha⁻¹) of composted cattle manure (CCM) and separated hog slurry solids (CSHSS) on *V. dahliae* densities in soil, disease severity and incidence, tuber yield, and soil nutrients. The effect of the composted materials was compared with those of different rates (40 and 60 gal acre⁻¹) of the soil fumigant-Vapam, inorganic fertilizer and an untreated control, in two experiments planted to Russet Burbank and Norland, respectively. Each experiment was replicated three times in different potato commercial fields. In the russet trials, disease was lower with composted, fumigated and fertilized treatments compared to the control. Incidence was reduced with fumigation (40 and 60 gal acre⁻¹) and CSHSS 80 Mg ha⁻¹, in russets and Norland by 2 and 3 fold, respectively. Compared to the control, only Vapam 40 gal acre⁻¹ increased Russet and Norland yields 15 and 10%, respectively. Based on initial findings, application of Vapam (40 gal) increased tuber yield, and reduced disease severity and incidence, and inoculum of *V. dahliae*.

Specific discrimination of *Fusarium proliferatum* using inter-simple sequence repeats (ISSRs) and simple sequence repeats (SSRs)

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Fusarium proliferatum (Matsushima) Nirenberg has a wide host range including both wild and cultivated plants. In the late 2000s, *F. proliferatum* was isolated from diseased white onions in Yotvata, Israel. Symptoms, which include salmon-colored blotches on the outer scales, are visible in the field on mature onion bulbs of white cultivars. But, the fungus can be isolated from internal tissues of yellow and red onion cultivars, as well as from onion sets and other nonsymptomatic plant species. Little is known about the diversity and distribution of *F. proliferatum* strains involved. We are testing the use of ISSRs and SSRs (microsatellite or repetitive genomic region based methods), to characterize populations and discriminate isolates. Seven *F. proliferatum* isolates from Israel, Germany, and North America, from cucumber, onion, garlic, maize, asparagus, and salt cedar, were screened with five ISSR primers published in the literature. Electrophoretic fingerprints of the PCR products were compared, and ISSR primers 808 (AGA)_n, 827 (ACA)_n, and 817 (CAC)_n were the most informative, demonstrating high variability among seven isolates. Primer 817 amplified a band of around 1,300 bp, but with minor size variations, from six isolates. ISSR results were used to develop specific SSRs to discriminate among *F. proliferatum* populations from multiple countries and hosts. This approach will be useful for diagnostic, epidemiological and forensic applications.

WITHDRAWN

Potential use of *G. intraradices* to control Botryosphaeria dieback in grapevine

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Botryosphaeria dieback in grapevine is caused by several Botryosphaeriaceae fungi, among them, *Diplodia seriata* has been found as the most prevalent species in Baja California. To evaluate the effect of the mycorrhizal fungus *Glomus intraradices* in grapevines inoculated with *D. seriata* under water stress conditions, one year old cuttings of the Cabernet Sauvignon/1003P cultivar were used. Two gallon pots filled with a mixture of pit moss/sand (2:1) were inoculated with *G. intraradices*. Two months later, using 100 µl of a conidia suspension of 20,000 /ml, *D. seriata* strain BY06 was inoculated into wounds on the stem. The vines were subjected to hydric stress cycles. At the end of the experiment, biomass, lesion size, and mycorrhizal colonization were measured, and reisolation of the pathogen was attempted. *D. seriata* was reisolated from inoculated vines showing low wilting and no apparent injury, indicating that the *D. seriata* isolate behaves as an endophyte. *G. intraradices* contaminated controls, and no differences in water stress, stomatal conductance, lesion size or CO₂ efflux among any of the treatments were found. However, the presence of *G. intraradices* reduced the presence of *D. seriata* in the double inoculation treatment without hydric stress, but not in the double inoculation treatment with stress. This suggests activation of the grapevine systemic defense system by mycorrhizae, however, this defense system was compromised under hydric stress.

Characterization of a novel protein from *Trichoderma virens* with chitinolytic activity and a role in mycoparasitism

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Trichoderma species attack pathogen propagules through the activity of a chemical barrage, including cell wall degrading enzymes (e.g. chitinases, glucanases). Many of these enzymes (members of the glycoside hydrolase family) that utilize insoluble substrates are modular, with catalytic modules attached to one or more non-catalytic carbohydrate-binding modules (CBMs). These CBMs play an import role in the enzymatic degradation of insoluble polysaccharides such as chitin. Protein analysis (SDS-PAGE) of culture filtrate of *T. virens* growing in minimal medium revealed an unknown 77 kDa protein (TvGH77). The protein was isolated and subjected to N-terminal sequencing. Analysis of the sequence showed the presence of five CBMs. Enzymatic and polysaccharide binding assays were performed to determine the biochemical activity of the protein. Both tests revealed that TvGH77 binds to colloidal chitin and has greater chitinolytic activity than commercial chitinase. Silenced mutants were obtained to determine the physiological and ecological role of this enzyme in *T. virens* 29-8. Positive mutants showed the absence of TvGH77 in SDS-PAGE gels. Phenotypic characterization of mutants demonstrated greatly reduced mycoparasitism capacity than wild type in a dual confrontation assay with three pathogens.

Molds isolated from New Jersey residential buildings damaged during Superstorm Sandy

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Superstorm Sandy made landfall in the United States on October 29, 2012 near Atlantic City, NJ. One and two months later, we visited eight storm-damaged homes on the border of Ocean and Monmouth Counties, NJ. Direct swabs made from floorboards, gypsum board, insulation, upright building studs and furniture were used to inoculate Petri plates of malt extract agar. After incubation at 25°C for 3-7 days, colonies of fungal growth were examined using light microscopy and subcultured for future study. The single most common genus isolated from all sites was *Penicillium*. In addition, other molds that were commonly observed at the majority of sites included *Aspergillus* sp., *Cladosporium* sp., *Phoma* sp. and *Verticillium* sp. Additionally, *Rhizopus stolonifer* was isolated from a condemned home and flooded trailer on the first sampling date, but not the second. *Stachybotrys* was not recovered from any of the swab cultures made during either trip, even when areas of blackened gypsum board were selectively sampled. Parallel air sampling by colleagues showed elevated levels of mold spores in all homes

tested. Cold temperatures are likely to be constraining mold growth, and it can be predicted that as the temperature rises in the coming months, there will be order-of-magnitude increases in the mold burden of storm-damaged, unremediated homes. In future research, we will use the “Sandy molds” for analysis of mycotoxins and volatile organic compounds.

WITHDRAWN

Determination of 2,4-diacetylphloroglucinol (2,4-DAPG) and phenazine-producing *Pseudomonas* spp. in wheat crops in southern Chile

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Take-all disease, caused by *Gaeumannomyces graminis* var. *tritici* (Ggt), is a perennial problem in wheat crops in southern Chile. Natural suppressiveness of Chilean soils to take-all disease has been described. However, this phenomenon has not been associated with presence of fluorescent *Pseudomonas* spp. that produce the antibiotics 2,4-diacetylphloroglucinol (2,4-DAPG) and phenazine-1-carboxylic acid (PCA). Presence of 2,4-DAPG and PCA-producing *Pseudomonas* was studied through a survey of 48 commercial wheat fields between Araucanía and Los Lagos Regions from December 2011 to February 2012. Roots of 20 plants per field were separated to isolate populations of bacteria of the genus *Pseudomonas* in King B broth (KMB). Bacteria isolated from each field were grown for 48 h in 1/3KMB plus antibiotics in microplate wells. Populations grown in each well were tested by PCR for the presence of loci *phlD* and *PhzCD*, which are associated with the biosynthesis of 2,4-DAPG and PCA, respectively. Individual strains from fields with *phlD*+ and *PhzCD*+ bacterial populations were obtained and their ability to reduce mycelium growth of Ggt was assessed *in vitro*. Thirteen fields had populations with the presence of the loci *phlD*+, two for loci of *PhzCD*+, and three for both genes. A subset of 12 strains with loci *phlD*+ and two strains *PhzCD*+ inhibited Ggt. These results suggest that bacteria producing 2,4-DAPG and PCA in Chilean soils could suppress the take-all disease of wheat.

Assessing genetic diversity of *Anisogramma anomala* isolates found throughout North America

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Anisogramma anomala (Peck) E. Müller is a biotrophic ascomycete endemic to the eastern United States that causes eastern filbert blight (EFB) of hazelnuts (*Corylus* spp.). EFB infection is nonlethal to *C. Americana* (the American hazelnut), but is deadly to the commercially important European hazelnut (*C. avellana*) grown in Oregon. *Anisogramma anomala* causes large cankers, girdling and susceptible trees will ultimately die within 5-12 years of

infection. Currently, little is known of the genetic diversity and population structure of *A. anomala*, although field studies with hazelnut plants known to contain the ‘Gasaway’ resistance gene have shown disease expression in New Jersey. A partial genome sequence of *A. anomala* was recently completed on an Illumina GA IIX platform to 11x coverage and was used to generate simple sequence repeat (SSR) markers. In this study, over 100 isolates of *A. anomala* were obtained from throughout the United States and Canada, with 15 polymorphic SSR loci amplified in each isolate. The resulting data was used to assess allele frequency and heterozygosity values for the isolate collection. Cluster analysis was performed to resolve genetic relationships of the fungal isolates within and among collection sites. By understanding the genetic diversity and population structure of *A. anomala*, its life history can be better explained and breeders can make more informed decisions on developing hazelnuts expressing durable resistance to EFB.

WITHDRAWN

Tracking internode stunting in *Cucumber mosaic virus* infected of bell pepper plants

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Bell pepper (*Capsicum annuum* L.) plants infected with *Cucumber mosaic virus* (CMV) developed different systemic symptoms as virus invaded newly emerging leaves. The first systemic symptom was a chlorosis over the basal portion of emerging leaves that sometimes progressed into mosaic. The next set of emerging leaves developed a chlorotic mosaic symptom throughout the entire leaf. Symptoms on subsequent leaves emerging above the branch of the main stem consisted of a dull green, non-waxy appearance of the leaf surface, some leaves had collapsed interveinal lamina and some leaves had varied degrees of strap-leaf. At the chlorosis/mosaic symptom phases, plant height and internode lengths were negatively impacted relative to the healthy control plants. The internode between leaves 2 to 3 (leaf 1 being the oldest leaf on the main stem) and all subsequent internodes along the main stem were significantly shorter than comparable internodes of healthy plants. Chlorotic symptoms first occurred on leaves 6 and 7. At the onset of flowering, average internode lengths for leaves 2 through 12 were 0.81 and 2.23 for CMV infected and healthy plants, respectively. Thus, CMV impacted internode extension 3 internodes below the first leaves to express systemic symptoms and each internode above that point.

Salinity impacts on the intensity of wilt caused by *Ceratocystis fimbriata* on mango seedlings of different rootstock-graft combinations

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The effects of salinity on plant growth are well studied, but the effects on disease intensity are not well known. This holds for wilt, an important mango disease in Oman and Brazil, where trees are commonly grown in salinized soils. We studied the effect of salinity on disease intensity on seedlings of different rootstock-graft combinations. In two experiments (E1: ‘Espada’, ‘Imbu’, or ‘Uba’ grafted on ‘Haden’; E2: ‘Uba’ grafted on ‘Palmer’, ‘Haden’,

or 'Tommy Atkins') seedlings, growing in an aerated hydroponic system with 0, 15, 30, 45, or 60 mmol NaCl.L⁻¹, were either uninoculated, inoculated in the rootstock or inoculated with *C. fimbriata* in the graft. From the grafting point, *C. fimbriata* was inoculated either 10 cm above (graft inoculation) or below (rootstock inoculation). We assessed disease intensity weekly and studied the disease progress. In all inoculated plants. The values of area under disease progress curve were higher when the fungus was inoculated on the rootstock, lower when the plants were grafted on 'Ubá', and lower when 'Tommy Atkins' was the graft. Disease symptoms (vascular discoloration, wilt, and death) were observed in all inoculated plants, disregard NaCl level. Death was delayed depending on the combination graft-rootstock-NaCl concentration, but strategies are needed to successfully manage the disease. Sponsor: Vale, Brazil.

Using next-gen sequencing to identify genes in *Macrophomina phaseolina* involved in the biosynthesis of phaseolinone

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Macrophomina phaseolina is a fungal pathogen that causes charcoal rot, a devastating disease on soybean. *M. phaseolina* has a very wide host range including economically important crops such as maize (*Zea mays*), rice (*Oryza sativa*) and soybean (*Glycine max*). Phaseolinone, a phytotoxin produced by *M. phaseolina*, is thought to play a role in the development of charcoal rot on soybean. Next-Gen sequencing was used to determine gene expression profiles in *M. phaseolina* cultures grown under conditions conducive or non-conducive to the production of the phytotoxin. The use of RNA-seq methods for transcriptomic analysis alleviates cross-hybridization problems and other forms of bias encountered with the use of some of the other gene expression profiling methodologies. Several tools were used to process the generated sequences including the CLC workbench, DSeq, and Limma. The differentially expressed and co-expressed genes will be inferred by using R statistical language. RT-PCR was further used to confirm the expression pattern of a subset of the *M. phaseolina* genes potentially involved in the biosynthesis of phaseolinone.

Combined control of late blight, early blight, and Septoria leaf spot in fresh market tomato through genetic control and supplemental sprays

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Tomatoes grown in the cool/humid conditions of NE U.S. are affected by three diseases that defoliate and cause loss of fruit/fruit quality: late blight (LB), early blight (EB) and Septoria leaf spot (SLS). The breeding program rapidly combined the *Ph2* and *Ph3* genes for LB resistance, a strong tolerance to EB, and a strong resistance to SLS in fresh market tomato lines that produced moderate to large fruit. To facilitate breeding, robust PCR-based markers were created for selecting for the *Ph2* and *Ph3* genes. SLS resistance and EB tolerance were selected based on disease screens. Hybrids using the best resulting lines were tested in 4 states, demonstrating their fruit quality and disease control. The homozygous *Ph2* and *Ph3* genotype of these hybrids fully controlled LB; plants had no symptoms in fields where susceptible cultivars were severely defoliated. The homozygous EB tolerance does not provide complete control; tolerance supplemented with 1 - 2 sprays of cupric hydroxide (Nu-Cop) provided good control for organic tomatoes, or of azoxystrobin + difenoconazole (Quadris Top) provided excellent control for conventional tomatoes. The SLS resistance strongly suppressed pycnidia development, disease spread and epidemic development. The SLS suppression is optimized in plants grown separately from SLS susceptible tomatoes. These hybrids form an integrated control system for these 3 diseases, allowing for substantial reduction in the reliance on fungicides.

Phytophthora fruit rot resistance, population structure, and genetic diversity in a diverse pepper (*Capsicum* spp.) collection

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Pepper is extensively grown throughout Europe, Asia, North and South America. *Phytophthora capsici* Leonian, a major disease of pepper, is distributed worldwide and can cause fruit, crown, and root rot in addition to foliar blight. The mechanisms of control between diseases have minimal overlap and current resistant cultivars only provide root and crown rot control. Immature, green fruit of 120 pepper (*Capsicum* spp.) germplasm lines representing 5 continents and 45 countries were evaluated for fruit rot

resistance. Fruit were inoculated with two virulent isolates of *P. capsici*. For each isolate, fifteen fruit were evaluated; lesion diameter and density of pathogen growth were evaluated 3 and 5 days post inoculation (dpi). Significant isolate-specific interactions and line differences were detected at 3 and 5 dpi. Most lines in this study exhibited a high degree of susceptibility (>70% incidence at 5 dpi) to fruit rot. No line was completely resistant to both isolates tested. However, partial resistance was detected in the population. Population structure and genetic diversity were estimated using 27 polymorphic simple sequence repeat markers on the pepper genotypes. Population structure associations with country of origin, disease resistance and continent of origin were evaluated. The partially resistant material identified in this study offer useful sources for incorporating fruit rot resistance into commercial varieties.

QTL mapping of fruit rot resistance to the plant pathogen *Phytophthora capsici* L. in a recombinant inbred line *Capsicum annuum* L. population

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Phytophthora capsici is an important pepper (*Capsicum annuum* L.) pathogen causing fruit, crown, root rot, and foliar blight in both field and greenhouse production systems. Previously, an F6 recombinant inbred line population was evaluated for fruit rot susceptibility; partial and isolate-specific resistance was found among lines. In our study, fruit rot resistance was mapped in the same F6 population between Criollo del Morelos 334, a landrace from Mexico, and 'Early Jalapeno' using a high-density genetic map. Isolate-specific resistance was mapped independently in 66 of the lines evaluated. Heritability of the resistance for each isolate at 3 and 5 days post inoculation (dpi) was moderately high ($h^2=0.48$ to 0.63 and $h^2=0.74$ to 0.83 , respectively). Significant additive and epistatic QTL were identified for resistance to isolates OP97 and 13709 (3 and 5 dpi) and 12889 (3 dpi only). QTL for fruit phenotypic traits showed potential linkage with disease resistance QTL. The partial fruit rot resistance from CM334 suggests that this may not be an ideal source for fruit rot resistance in pepper.

Effect of endophyte on tall fescue host plant metabolite levels and gene expression under drought stress

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Tall fescue is the most widely planted forage grass, often infected with endophytic symbiont *Neotyphodium coenophialum* (E+). The endophyte enhances adaptation to drought stress compared to asymbiotic tall fescue (E-). To understand the basis for enhanced drought tolerance, we conducted time course studies in which water was withheld from 0 to 5 days, and examined the differences in the metabolic profiles and gene expression of E+ and E- clone pairs. Recovery experiments showed greater survival and recovery of E+ plants than E- plants. Endophyte induced changes in plant free sugars (glucose, fructose and trehalose), sugar alcohols (arabitol) and certain amino acids (proline) during early days of drought stress. Drought stress also increased the endophyte metabolites such as mannitol and lolines. Thus, endophyte helps plants in recovery from drought stress, through endophyte-induced changes in accumulation of the plant metabolites involved in osmotic adjustments and reactive oxygen scavenging. Quantification of phytohormones like jasmonic acid, abscisic acid and salicylic acid of E+ and E- clones in response to drought stress is in progress, and will further aid in understanding the endophyte effects on drought stress tolerance. Results from Illumina mRNA sequencing of E+ and E- clones subjected to drought stress, revealing differential expression of host plant genes in presence of endophyte, will also be presented.

A critical look at comparative genomic approaches: What and how we can learn from a 1000 fungal genomes

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Large-scale genome sequencing projects generate unprecedented amounts of data with a great premise to understand genome evolution and the genetic bases of phenotypes. However, comparisons of multiple whole genomes pose

significant analytical challenges, most importantly because of the phenomenon known as phylogenetic non-independence, which makes most traditional statistics inappropriate. Here, we present a new analytical strategy, COMPARE, which evades this problem and makes it possible to infer broad patterns of genome evolution, identify the genetic bases of phenotypic traits and to formulate functional hypotheses for gene families with unknown function. In brief, COMPARE relates gene duplication and loss history in multiple whole genomes to the evolution of the focal trait and filters genes that evolve in correlation fashion with the trait. COMPARE can be used on any collection of gene families and corresponding gene trees from whole genomes. The analytical strategy incorporates theoretical aspects of phylogenetic comparative methods, such as ancestral state reconstructions, Markovian models and parsimony mapping. COMPARE can produce hypotheses about the genetic toolbox of the trait and functional hypotheses for gene families even in the absence of transcriptomic data or a priori knowledge about function. In general, COMPARE is suitable to characterize patterns of genome evolution and evolutionary innovations using whole genomes and phylogenetic information.

Zinc regulates biofilm and exopolysaccharide production in *Xylella fastidiosa*

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Biofilms are multicellular aggregates of microorganisms attached to solid surfaces where cells are in close contact with each other, surrounded by a secreted extracellular matrix. This matrix is rich in exopolysaccharides (EPS), the production of which correlates with virulence of many plant pathogens. The bacterium *Xylella fastidiosa* (Xf) is the causal agent of Pierce's Disease of grapevine and multiple diseases in other crops. Xf biofilms accumulate in the host xylem, affecting disease development and bacterial acquisition by its insect vector. Biofilm production is modulated by the chemical composition of the environment, and previous results from our group indicate that zinc added to the growth medium reduces Xf biofilm production under static conditions. Here, the medium was supplemented with Zn to obtain levels similar to those found in grape xylem sap. The effect of Zn-supplementation on growth, biofilm, and EPS production in static and flow conditions was evaluated. Zinc reduces the growth of Xf, inverts the ratio of biofilm to planktonic cells, up-regulates EPS biosynthesis genes, and increases EPS production. Xf grown in microfluidic chambers with constant bacterial supplementation showed a dramatic increase in biofilm-like aggregates in Zn-supplemented media. Mutants altered in Zn homeostasis are being characterized. Findings will help identify factors underlying biofilm development and will be useful in developing disease management strategies.

Removal of divalent cations disrupts biofilm formation by the bacterial plant pathogen *Xylella fastidiosa*

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Biofilms (BFs) form when microorganisms aggregate on solid surfaces and secrete a surrounding matrix, which protects them against environmental stresses. BFs formed in plants can affect the host's physiology depending on the tissue colonized and the nature of the microbial community. The bacterium *Xylella fastidiosa* (Xf) is the causal agent of Pierce's disease of grapevines among others. In the host xylem, Xf forms BFs that are essential for disease development. To evaluate factors important for BF stability and possible eradication methods, we screened compounds for their ability to disrupt or prevent further growth of already established biofilms *in vitro*. Several heavy metals, chelators, enzymes, and antibiotics were tested. Xf was grown in grape sap or PD2 in 96-well plates or slide-containing tubes that were incubated for 5 days. Compounds were added and cultures further incubated. BFs were quantified by the crystal violet assay and observed via light microscope. EDTA had the greatest effect, completely preventing further BF growth, followed by EGTA, a calcium-specific chelator. Both were more effective in sap. Other compounds produced a small to moderate effect. Biofilms treated with EDTA had a reticulate pattern when observed under the microscope. The results highlight the importance of divalent cations for the maintenance of biofilm structure and could be used as part of a chemical control strategy.

Importance of soil moisture and isolate origin on disease severity of three *Rhizoctonia solani* AG 2-2 IIB isolates

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Excessive irrigation can increase the potential for soil-borne diseases. The widespread use of sprinkler or surface irrigation in Idaho in combination with irrigation mismanagement can potentially predispose local growers to disease problems including *Rhizoctonia* root rot (RRR, *Rhizoctonia solani*). Differences in irrigation practices, environmental conditions and disease severity in two sugar beet growing regions in Idaho raised questions about potential interactions between soil moisture and isolate origin. A study established at the University of Idaho Kimberly R&E Center was conducted to investigate the interaction between irrigation (irrigation levels: 40%, 70%, 100% and 130% evapotranspiration (ET)) and *R. solani* AG 2-2 IIB isolates (F521 – related to *R. solani* isolate R9, F551 – found in the southern production area of Idaho and F517 – from the western production area). The study was conducted using drip irrigation to ensure consistent and precise water delivery. Statistical analysis for RRR disease index (DI) based on a 1-9 rating scale (1 = no disease on beet root, 9 = root completely dead) showed significant differences for different ET levels ($P > F$ 0.0025), *R. solani* isolates ($P > F$ <0.0001) and for the interaction between ET and *R. solani* isolates ($P > F$ 0.0201). Comparing *Rhizoctonia* isolates across ET levels showed a 6.4-fold increase in DI for F521, a 3-fold and 2-fold for F551 and F517, respectively, when compared to the non-inoculated control.

Molecular phylogenetic diversity and distribution of *Mycodiplosis* larvae on Pucciniales

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There are more than 7800 species of Pucciniales (rust fungi) described. Aeciospores and urediniospores of rust fungi are a food source for the larval stage of members of the fly genus *Mycodiplosis*, hence these could be of interest as potential biological control agents. Currently, *Mycodiplosis* contains 49 described species. A survey of 1,350 rust-infected plants from 22 countries was recently conducted to assess the occurrence of *Mycodiplosis* fly larvae across a broad spectrum of Pucciniales. Larvae were found on 258 collections. Statistical analyses explored the distribution of larvae in relation to host species. Five of 127 rust species in the survey data were found infested at a greater frequency than expected using binomial probability analysis of presence/absence. DNA was extracted from individual larvae and 28S nuclear ribosomal RNA and mitochondrial cytochrome c oxidase subunit I (COI) genes were amplified and sequences were concatenated for maximum likelihood analyses. The 207 larval specimens analyzed were resolved into 33 clades, 17 of which received significant support. The distribution of larvae within clades could be explained partially by geographic origin but not by rust host at any taxonomic level; thus even though there is evidence for preferential feeding by larvae on some rust species, there is no evidence of host-specificity between larvae and their hosts.

WITHDRAWN

Long term evaluation of the susceptibility of 16 *Musa* genotypes to banana bunchy top disease in Cameroon, Central Africa

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Banana bunchy top disease (BBTD) is a serious threat to banana and plantain production where it occurs. This disease is caused by the *Banana bunchy top virus* (BBTV) which spreads by infected plant propagules and/or through its aphid vectors, *Pentalonia nigronervosa*. Resistance to BBTD has not been yet discovered, but there is a wide range of susceptibility among *Musa* genotypes. We have been following response of 16 *Musa* genotypes to BBTV in the South region of Cameroon for 29 months in two replicated field experiments. Disease expression varied without any specific patterns related to their A and B genomic groupings. In the high disease pressure field, percent BBTD-symptomatic plants at 29 months of age were as follows: 8.87-100% for the AAA group, 33.3-92.9% AAB group; 66.7-92.9% for hybrid plantains (AAB and AAAB), 6.67-60% for AAB and ABB cooking banana; and 33.3-64.3% for plantain landraces (AAB). In the low-pressure field, disease incidence varied between 0 and 60%, with plantain landraces continuing to be asymptomatic. In contrast, AAA group had 55.6%, while hybrid plantains and cooking banana had 13.3 to 60% and 6.67 to 53.3% infection respectively. Aphids were present on all genotypes in both fields, with highest and lowest abundance respectively on plantain landraces and dessert bananas. The results of PCR detection of BBTV in plants and aphids will be discussed.

A new species of *Paratritirachium* isolated from flare pit soils and the addition of a teleomorph to the generic concept

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Paratritirachium is a basidiomycete genus in the class *Tritirachiomycetes*. Only one strain of one anamorph species was previously described (*P. cylindroconium*), which was isolated from coal spoil tips in Staffordshire, England. In mining, a coal spoil tip is a pile of dirt composed of soil, rock and low-grade coal. Three strains of a new species of *Paratritirachium* were isolated from flare pit soils in Alberta, Canada using a heat treatment method. In the oil and gas industry, a flare pit is an earthen containment area where waste gases and liquids are combusted. The strains in our study are heat resistant, xerotolerant and produce both the anamorph and teleomorph. The sexual state produce two-celled curved auricularioid basidia with dark brown, thick-walled, oval basidiospores assumed to be the heat-resistant structures; while the asexual stage produced hyaline, unbranched or sparingly branched conidiophores, sympodial and slightly vesicular conidiogenous cells with inconspicuous denticles, and dry aseptate conidia. Phylogenetic analysis using rDNA loci (SSU and LSU) and the ITS barcode region suggest these isolates are an unidentified species of *Paratritirachium*. Because *Paratritirachium* species are isolated from hydrocarbon rich environments, the bioremediation potential of these fungi will be discussed.

Use of *Trichoderma* spp. and *Glomus intraradices* to control *Botryosphaeria* dieback caused by *L. theobromae* in grapevine

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Botryosphaeria dieback caused by several members of the *Botryosphaeriaceae* fungi is a very important grapevine disease. *L. theobromae* is considered the most virulent species of this family. To evaluate the use of arbuscular mycorrhizas and native isolates of *Trichoderma* to control *L. theobromae*, several experiments were conducted. Using dual competition assays, native *Trichoderma* spp. were evaluated against three isolates of *L. theobromae*. Results exhibited similar antagonistic effects towards the pathogen displayed for all the isolates of *Trichoderma* spp. Through the analysis of their morphological characteristics and their EF1 α sequences, seven *Trichoderma* isolates were identified as members of the species: *T. atroviride*, *T. asperellum*, *T. gamsii* and *T. harzianum*. The *in vitro* sensibility to fungicides

of five *Trichoderma* strains was evaluated; the results showed that methyl thiophanate and benomyl have potential to be used in combination with these antagonists for the control of *L. theobromae*. Finally, the effect of *T. gamsii* T6, *G. intraradices* and their combination was analyzed on grapevine cv. Cabernet Sauvignon infected with *L. theobromae*. The treatment inoculated with the combination of *T. gamsii* T6-*G. intraradices* exhibited the lowest incidence of the pathogen, although, *T. gamsii* T6 alone showed some control. In conclusion, the *T. gamsii* T6 and its combination with *G. intraradices*, has potential for use in the biological control of *L. theobromae*.

Cyst nematodes in golf course greens in the western United States

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Juvenile cyst nematodes (*Heterodera* sp. and *Punctodera* sp.) were discovered in turf samples from golf course greens in the western United States. In the western USA, cyst nematodes are not common in golf course greens, but their numbers could increase due to the absence of efficient nematicides. In the past, cyst nematodes in golf course greens in the United States have been identified based on morphology, especially that of the mature cyst. When cysts are not recovered, identification must be based on the infective juveniles (J2). Due to morphological variation among J2 within a species, identification with molecular tools is necessary. Juvenile cyst nematodes were extracted from soil using a mist extractor and individual J2s were lysed. PCR was conducted with primers specific for the ITS region of nematodes. The resulting PCR products were sequenced. We have identified two species, *Heterodera ustinoi* and *Punctodera punctata*. This is the first record of *H. ustinoi* in golf course greens in the western USA.

Ceratobasidium root rot, a new disease of watermelon in Arizona

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Since 2002, young, wilting watermelon plants with numerous brown lesions on the roots have been observed in Arizona. Isolations from necrotic lesions consistently yielded a *Rhizoctonia* sp. Nuclear staining and molecular analyses identified the *Rhizoctonia* isolates as belonging to binucleate *Ceratobasidium* sp. Sequencing the ITS region showed that isolates closely matched AG- F and AG-Fa sequences (99% homology in Genbank) isolated from strawberry. To fulfill Koch's postulates, watermelon seedlings were inoculated with the *Ceratobasidium* isolates. Brown lesions on the roots matching lesions observed in the field developed on inoculated plants but not on the non-inoculated control plants. *Rhizoctonia* sp. identical to original isolates were re-isolated. A similar disease has been reported recently in Italy, but this is the first report of *Ceratobasidium* root rot of watermelon in the United States. Watermelon is a high value crop in United States, and the disease should be recognized as a potential new problem.

WITHDRAWN

Effect of mutations to *FST1* in *Fusarium verticillioides* on functionality and the regulation of gene expression

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Fusarium verticillioides is a fungal pathogen that causes seedling, stalk, and kernel diseases of maize. The pathogen also produces the mycotoxin fumonisin in infected kernels. This study focuses on *FST1*, a gene that impacts fumonisin B1 (FB1) biosynthesis, resistance to reactive oxygen, pathogenicity, and macroconidia production. *FST1* encodes a protein with 12 putative transmembrane domains and has a central loop and a C-terminus that are predicted to project into the cytoplasm. We have hypothesized that the central loop and C-terminus structures are involved in the functionality of *Fst1*. Constructs of *FST1* containing deletions in the coding regions of the central loop and C-terminus were transformed into a strain lacking a functional *FST1*. All the constructed failed to complement the strain, suggesting that the structure of both regions of the protein is critical for function. We also analyzed the impact of *FST1* on the secretome during colonization of kernels. A comparison of the transcriptome of the wild type and the $\Delta fst1$ mutant six days after inoculation of maize kernels revealed that expression of 2227 genes in the mutant were significantly ($P < 0.05$) different than wild type. Of the 1412 genes that are predicted to encode secreted proteins, expression of 256 were significantly altered (≥ 2 fold) in the mutant strain. Among these genes was the reduced expression of an extracellular peroxidase, likely involve in resistance to reactive oxygen.

Aflatoxin B₁ contamination of groundnut *Arachis hypogaea* L. in eastern Zambia

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Groundnut is a rich source of protein and it is the second most widely grown crop in eastern Zambia. A survey was conducted in 2012 to determine the incidence of aflatoxin B₁ contamination in groundnuts from several districts important in groundnut production in eastern Zambia. Nyimba and Mambwe districts are in the Luangwa Valley and are comparatively hotter and drier than Chipata and Petauke districts which lie in the cooler plateau. 399, 1 kg unshelled groundnut samples were collected from farmers' fields and also from homesteads upto 1 month after harvest. Aflatoxin B₁ was assayed in each shelled sample by weighing and comminuting 100 g subsample and extracting aflatoxin from six 20 g aliquots. ELISA was used to estimate aflatoxin levels and the six values averaged to give the sample contamination. 34 and 27% of samples from Nyimba (maximum=4,980 ppb and arithmetic mean [AM]=1.1) and Mambwe (maximum=69 ppb and AM=0.7), respectively, had aflatoxin levels greater than 20 ppb. In comparison, 38 and 28% of samples from Petauke (maximum=3,258 ppb and AM=0.9) and Chipata (maximum=1,077, and AM=0.8), respectively, had aflatoxin levels greater than 20 ppb. At the beginning of the season, priority is given to planting maize and cotton, while planting groundnut is delayed, increasing the risk of the crop being exposed to end-of-season drought. The aflatoxin contamination levels we report are a barrier to trade and the well being of consumers.

Effect of temperature and wetness period on guava styler end-rot monocycle

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The guava (*Psidium guajava*) styler end-rot, which *Fusicoccum aesculi* is one of the causal agent, can reaches 40% of incidence in post-harvest in Brazil. This work studied the favorable environmental conditions for the development of disease. Isolates of *F. aesculi* were collected on symptomatic fruits and the specie was confirmed by morphological characterization and phylogenetic analysis of the ITS and β -tubulin regions. Germination of conidia was evaluated at temperatures of 10, 15, 20, 25, 30, 35, and 40°C in combination with 4, 6, 12, 24, and 48 h of wetness period. Guava fruits cv. Kumagai were inoculated with conidial suspensions of *F. aesculi* and maintained at temperatures of 15, 20, 25, 30, and 35°C and wetness periods of 6, 12, 24, and 48 h. We evaluated the incubation period, the diameter of the lesion seven days after inoculation and the growth rate of the lesion. The optimum temperature for the conidia germination was 30°C and the maximum germi-

nation occurred after 24 h wetness period. The optimum temperature for growth of the lesion was 30°C and wetness period of 48 h increased the disease severity. The lowest incubation period (3 days) occurred at temperature of 30°C and wetness period of 48 h. The growth rate of lesion estimated by the monomolecular model was 0.3/day. The favorable environment conditions for development disease is high temperatures and prolonged wetness period.

Phylogenetic overview of the Boletineae

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Boletes are a morphologically diverse group of fungi that are mainly ectomycorrhizal and have successfully colonized all the continents, except for Antarctica. The wide diversity found in the boletes makes it an excellent group to test evolutionary rates and trends of morphological and ecological changes. The relationships of selected genera, representing the largest and most common genera of the Boletineae [Boletales], were studied using *nuc-18s*, *tef1*, and *RPB1*, producing the most comprehensive phylogeny to date. With the exception of *Hydnomerulius pinastri*, the Boletineae, Paxillaceae and Boletaceae were strongly supported. The majority of traditional, morphologically described genera were not recovered as monophyletic unless using the most narrow description available (i.e. using the description of the section of a genus that contains the type species). Specifically, *Boletus* species were found throughout the phylogeny, but Singer's *Boletus* section *Boletus* was monophyletic. Further sampling is required to identify lineages of *Boletus* species that are not members of Singer's section *Boletus* and test if subgeneric groups described previously may be described as genera. The phylogeny implies nearly every genus is in need of reorganization. Additionally, the phylogeny indicates multiple, independent evolutions of secotoid and gasteroid forms (including sequestrate forms) and lineages that may be transitions from an ectomycorrhizal nutritional strategy.

Transcriptomic, proteomic, and nutritional analyses of potato tissues infected with 'Candidatus Liberibacter solanacearum'

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Zebra chip (ZC) is an emerging destructive disease of potato and is associated with the phloem-limited α -proteobacterium, 'Candidatus Liberibacter solanacearum' (Lso). In this study, RNA-Seq, 2-DE, mass spectrometry and qRT-PCR analyses identified over 100 differentially produced gene transcripts and proteins in above-ground (AG) and below-ground (BG) potato tissues upon Lso infection. Interestingly, in spite of an Lso-mediated down-regulation of photosynthesis-related genes/proteins, over 80% of the differentially produced gene transcripts/proteins were up-regulated in AG tissues. This was accompanied by an increase in nutrient concentrations of K, Mn, Fe and Cu in both AG and BG tissues in response to Lso infection. Furthermore, there was a strong induction of proteinase inhibitors in AG tissues upon Lso infection. In contrast, the expression of proteinase inhibitors was markedly suppressed in Lso-infected BG tissues. In general, results suggest that ZC disease development involves an Lso-mediated down-regulation of photosynthesis accompanied by an ineffective and potentially inefficient up-regulation of stress response-, metabolism-, and housekeeping-related gene transcripts/proteins with concomitant increases in nutrient accumulation. This study presents a first approach of a holistic investigation of the global transcriptomic, proteomic and nutritional response of potato plants to Lso infection.

Management of *Meloidogyne incognita* with tall fescue grass rotations prior to peach orchard establishment

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Root-knot nematodes (*Meloidogyne* spp.) are important pests of peach in the USA. Preplant fumigant nematicides have been used to control *Meloidogyne* spp. associated with Southeastern peach production. Unfortunately, growers have increasingly faced economic challenges, making it difficult for them to afford application costs of preplant nematicides. Finding an alternative to control root-knot nematode is warranted. Previous studies indicated that Jesup (Max-Q) tall fescue grass is a nonhost for *M. incognita* and *M. hapla*. In 2005, the effects of 1- and 2-yr fescue rotations for the management of *M. incognita* were initiated in Georgia. Prior to orchard establishment in 2009, both fescue rotations (2005-2008) and preplant Telone II (1,3-dichloropropene) fumiga-

tion (2008) suppressed ($P \leq 0.05$) populations of *M. incognita* J2 in soil compared with nonfumigated plots. No differences in *M. incognita* J2 populations were detected among the two fescue rotations and fumigated plots. In 2010, tree growth was greatest ($P \leq 0.05$) in fumigated and 2-yr fescue rotation plots, intermediate in 1-yr fescue rotation plots and lowest in nonfumigated plots. In 2012 and 2013, tree growth was similar among the fumigated and both fescue rotation plots and lowest ($P \leq 0.05$) in non-fumigated plots. Results provide insights into the potential use of fescue as an alternative to chemical control of *M. incognita* in peach.

Antibacterial activity of mycelial extracts from submerged cultures of shiitake (*Lentinula edodes*) strains

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Bacterial spot of tomato (*Xanthomonas campestris* pv *vesicatoria*) can cause severe losses to greenhouse and field grown crops. The use of synthetic antibiotics to control bacterial diseases is generally not effective and detrimental to human health and the environment. Furthermore, organic production systems restrict synthetic compound use for pest management and crop production. The goal of this study was to develop biopesticides for managing important bacterial diseases in organic vegetable production. Our objective was to evaluate shiitake mycelial culture fluids from different strains for their antibacterial properties against several plant pathogenic bacteria including *Xanthomonas campestris* pv *vesicatoria*, *Ralstonia solanacearum* and *Erwinia amylovora*. Antibacterial studies were also conducted in tomato cultivar AgriSet 761 and effects on plant growth and disease severity quantified *in vitro* and *in vivo*. Mycelial culture filtrates of different shiitake strains inhibited growth of *Xanthomonas campestris* by 98-100% and *Erwinia amylovora* by 53-100% in pure culture. The extracts also had no deleterious effect on seed germination and plant growth. Treatment of *Xanthomonas campestris* pv *vesicatoria* infected plants with the filtrate suppressed bacterial spot symptoms by 48% as compared to control. Oxalic acid in the mycelial culture fluid and other metabolites contributed to the antimicrobial activity of the culture filtrates.

Elucidating endophyte communities of *Echinacea purpurea* to resolve plant medicinal efficacy

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Echinacea purpurea is a native North American plant, whose use for medicinal effects dates back to ancient indigenous people of several tribes. Despite its traditional use for treatment of infection for its anti-inflammatory properties, medical trials on the efficacy of extracts remain ambiguous. Alkylamides, the active compounds in the extract, appear to be neutralized by a plant immuno-stimulatory response triggered *in vitro* by lipopolysaccharides (LPS) or lipoproteins, which occur in cell walls of microorganisms. Recently, LPS presence in *E. purpurea* was linked to endophytic microorganisms rather than to contaminations during the extraction process. In this interdisciplinary study, teams of chemists, immunologists and ecologists investigate above and belowground relations between the plant and its endophytes. Microbial diversity of *E. purpurea* was assessed using tissue isolations, morphological and molecular identification tools and phylogeographic analyses of root and seed samples originating in three different locations in the U.S. Isolations yielded more than 24 fungal and over 200 bacterial strains. Seed isolations harbored mainly fungal endophytes, contrasting root endophytes that predominantly consisted of bacteria suggesting antifungal activity. Understanding endophyte communities associated to *E. purpurea* and their functional traits will provide novel insights in determining key organisms responsible for the pro-inflammatory effects of *E. purpurea*.

Do JAZs restrict/assist pathogen entry via stomata?

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Previous studies have shown that stomatal pores in the leaf epidermis close as a part of the plant innate immune response against bacterial invasion of plant tissues. Counteracting this response, the plant pathogenic bacteria *Pseudomonas syringae* pv. *tomato* strain (Pst) DC3000 has evolved the virulence factor coronatine as an important strategy contributing to pathogenesis. Two components of the coronatine receptor complex, namely COI1 (the F-box subunit of E3 ligase) and JAZ (a repressor of jasmonic acid pathway) proteins, are known to regulate the jasmonic acid (JA) pathway, influencing the disease

outcome. In this study we first focused on determining the tissue specific expression of JAZ3/4/9 transcripts. Our results indicate that not only JAZ4 and JAZ9 are more abundant in guard cells as compared to whole leaves; these genes are also induced by coronatine in guard cells in a dose dependant manner. For a robust insight of the modulation of these proteins by coronatine, bacterial inoculation experiments with Pst DC3000 and DC3118 were performed on the T-DNA insertion and transgenic lines of different JAZ4 and JAZ9 constructs. Our findings suggest that the N- terminus of these proteins is essential to develop resistance against Pst DC3000 in *Arabidopsis*. This study highlights the role of JAZ proteins in stomatal and apoplastic immunity against bacterial diseases.

RNA-hairpin mediated targeting of AGO2 in *Nicotiana benthamiana* compromises antiviral silencing of a tobamovirus

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One or more ARGONAUTE proteins (AGOs) in plants are thought to be key for antiviral RNA silencing by forming a core "slicing" component of the RNA-induced silencing complex that cleaves viral RNA in a sequence-specific manner. While a few AGOs have been identified in *Arabidopsis* to contribute to antiviral silencing, very little is known about AGOs that may function in this antiviral defense in other plants. We recently reported that *Tobacco rattle virus* (TRV) vector mediated gene silencing of AGO2 expression in *Nicotiana benthamiana* compromises the ability of these plants to mount an effective silencing response upon subsequent inoculation with *Tomato bushy stunt virus* (TBSV). To eliminate the possibility that unpredictable TBSV-TRV interactive effects might have influenced the results, the present study employed dsRNA hairpin mediated knock-down of AGO2 expression in *N. benthamiana*. These plants were inoculated with a TBSV derivative inactivated for expression of the P19-suppressor and in which the coat protein gene was replaced with *GFP*. Based on levels of GFP expression the results indicated that this viral construct was not effectively silenced compared to the rapid silencing in control plants not expressing the hairpin. These findings firmly support the notion that AGO2 in *N. benthamiana* is a crucial component of the antiviral silencing response towards TBSV.

WITHDRAWN

An atoxigenic vegetative compatibility group of *Aspergillus flavus* widely adapted to maize production in Africa and North America

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Aflatoxins are carcinogenic mycotoxins that frequently contaminate crops including maize, peanuts, cottonseed, chilies, and pistachios. Aflatoxin contamination, caused by several *Aspergillus* species, causes both economic and health-related loss. Biocontrol with atoxigenic strains of *A. flavus* is a successful strategy for preventing dangerous aflatoxin levels. Atoxigenic strains competitively exclude aflatoxin producers and atoxigenics endemic to target cropping systems are preferred due to reduced environmental risk and local adaptation. A global sampling of atoxigenic *A. flavus* isolates (n=175)

were placed into vegetative compatibility groups (VCGs). One VCG (ICA001) was associated with maize production in both Africa and North America. Over 1,000 isolates were screened at simple sequence repeat (SSR) loci to identify additional isolates belonging to this VCG. To date, 17 isolates have been detected in Kenya, Zambia, and USA. ICA001 isolates had similar SSR patterns, similar deletions in the aflatoxin biosynthesis gene cluster, and complemented VCG-defining nitrate auxotrophs. ICA001 isolates from Kenya were similar to US ICA001 isolates in ability to interfere with aflatoxin contamination of viable maize kernels. Overall, the results suggest ICA001 is broadly adapted to maize production in regions with significant aflatoxin contamination problems and, as such, has potential to be broadly useful in mitigating aflatoxin contamination through biocontrol.

Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi

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The class Dothideomycetes is one of the largest groups of fungi with a high level of ecological diversity including many plant pathogens infecting a broad range of hosts. Here, we compare genome features of 18 members of this class, including 6 necrotrophs, 9 (hemi)biotrophs and 3 saprotrophs, to analyze genome structure, evolution, and the diverse strategies of pathogenesis. The Dothideomycetes most likely evolved from a common ancestor more than 280 million years ago. The 18 genome sequences differ dramatically in size due to variation in repetitive content, but show much less variation in number of genes. Gene order appears to have been rearranged mostly within chromosomal boundaries by multiple inversions, in extant genomes frequently demarcated by adjacent simple repeats. Several Dothideomycetes contain one or more gene-poor, transposable element (TE)-rich putatively dispensable chromosomes of unknown function. The 18 Dothideomycetes offer an extensive catalogue of genes involved in cellulose degradation, proteolysis, secondary metabolism, and cysteine-rich small secreted proteins. Ancestors of the two major orders of plant pathogens in the Dothideomycetes, the Capnodiales and Pleosporales, may have had different modes of pathogenesis, with the former having fewer of these genes than the latter. Many of these genes are enriched in proximity to transposable elements, suggesting faster evolution because of the effects of repeat induced point (RIP) mutations.

Population structure of *Aspergillus flavus* before and after biocontrol treatment

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Aspergillus flavus is a fungal pathogen of many important crops worldwide. We sampled *A. flavus* strains from a cornfield in Rocky Mount, North Carolina, over a period of two years. Plots were inoculated at tasselling with either AF36 or NRRL 21882 (=Afla-Guard) biocontrol strains, both of which are mating type *MAT1-2*. Subsequently, toxigenic strain NRRL 3357 (*MAT1-1*) was applied to all plots. Sclerotia were collected from infected corn ears at harvest and ninety single-ascospore isolates were obtained from ascocarps. In addition, eighty *A. flavus* isolates were collected from soil one month after planting (before biocontrol application) and one and two years after biocontrol application. PCR revealed grouping of isolates into three distinct mating-type classes: *MAT1-1*, *MAT1-2* and *MAT1-1/MAT1-2*. A significant proportion (54%) of isolates sampled prior to biocontrol treatments and 39% of isolates obtained from ascospores were heterokaryotic for mating type (*MAT1-1/MAT1-2*). The population genetic structure before and after the application of biocontrol treatments will be discussed. The potential for the biocontrol strain to undergo sexual reproduction and the degree of relatedness of the biocontrol strain to the predominant indigenous lineage may influence the long-term success of a biocontrol strain. These findings will be instrumental in the selection of strains for use in next-generation biocontrol strategies.

WITHDRAWN

Azoxystrobin (QoI) resistance monitoring of *Rhizoctonia solani* isolates causing rice sheath blight in Louisiana

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Rhizoctonia solani AG-1 IA isolates resistant to QoI fungicides were detected in 2011 in rice fields located in Acadia Parish, Louisiana. The sequencing of the cytochrome *b* gene revealed that QoI resistant isolates had phenylalanine to leucine substitutions at codon 129 in the cytochrome *b* gene (F129L mutation). Resistance monitoring programs in rice and soybeans fields have been conducted in 2011 and 2012 for the QoI fungicide azoxystrobin. A total of 457 isolates were collected in 2011 from 23 fields located near the problem area and their sensitivity was determined using a Perennial ryegrass (*Lolium perenne*) bioassay. In 8 fields no resistant isolates were detected. In 15 fields the frequency of resistant isolates varied from 7 to 100% indicating that there is still an azoxystrobin dose response. In 2012, 237 isolates were collected from 12 fields. Isolates from only one field were all sensitive to azoxystrobin. Resistant isolates were detected in the remaining 11 fields and the frequency of resistant isolates ranged from 39 to 100%. In 2011 and 2012, resistant isolates were detected in fields located less than 40 km away from the first detection field. In both years, isolates collected from fields located more than 41 km away from the first detection site were all sensitive to azoxystrobin. Results of these resistance monitoring studies are helping in the recommendation of the best managing practices to control of *R. solani* on rice in Louisiana.

Multilocus sequence analysis of xanthomonads causing common bacterial blight disease of common bean

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Common bacterial blight (CBB) is a globally important seed-transmitted disease of common bean (*Phaseolus vulgaris*) caused by *Xanthomonas campestris* (axonopodis) pv. *phaseoli* (Xcp) and *X. fuscans* subsp. *fuscans* (Xff). These species cause identical disease symptoms, but are distinguishable at the molecular genetic level and by production of brown pigment by Xff strains. To assess genetic diversity among strains of Xcp and Xff, a multilocus sequence analysis (MLSA) was conducted, using partial sequences from the housekeeping genes *dnaK*, *gyrB*, *fyuA*, *rpoD*, *atpD*, *fusA*, and the chromosomal replication initiator protein encoding gene *dnaA*. Eight strains representative of known global diversity among xanthomonads causing CBB were selected for analysis, including two New World Xcp strains, two New World Xff strains, two East African Xcp strains, and one East African Xff strain. Px, a strain representing a hybrid phenotype that produces small amounts of brown pigment was also included. MLSA results revealed two major clades (lineages) of xanthomonads causing CBB. The first contains all New World Xcp strains and Px, whereas the second contains all Xff strains and the East African Xcp strains. Xff clade members were more closely related to *X. axonopodis* pv. *citri* than New World Xcp strains, whereas New World Xcp clade members were more closely related to *X. campestris* pv. *vesicatoria* and *X. axonopodis* pv. *citrumelo* than either Xff or East African Xcp strains.

Genetic variability of *Ceratocystis fimbriata* isolates from mango in Brazil, Oman, and Pakistan

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Ceratocystis wilt caused by *Ceratocystis fimbriata* is one of the most important diseases of mango in Brazil. The pathogen is soil-borne, believed to be native to South America, and has a wide host range. Ceratocystis wilt in mango had been known only in Brazil, mainly in the states of São Paulo (SP) and Rio de Janeiro (RJ), but it was recently reported in Oman and Pakistan. Surveys across Brazil resulted in 190 isolates of *C. fimbriata* from mango trees in 10 different states. Analyses of sequences of the internal transcribed spacer (ITS) region of rDNA found 24 different haplotypes among the Brazilian isolates. The highest variability was found in SP, mainly in the region of Limeira, where much of the nursery industry is located. Only two ITS haplotypes were identified in Oman and Pakistan, and one of these haplotypes was common in SP. One of the mating-type genes (*MAT1-I-2*) was also sequenced, and four different haplotypes were identified in Brazil. Oman and Pakistan isolates had two *MAT1-I-2* haplotypes, one common throughout Brazil and the other found in the state of Alagoas. Most mango isolates of *C. fimbriata* probably originated in SP and were moved in infected nursery stock, but not necessarily in mango plants. Earlier studies had shown that most *C. fimbriata* isolates from Brazil are not host specialized, and inoculations of six-month-old mango plants with Brazilian mango isolates showed a wide range of aggressiveness.

The role of nursery plants as a potential source of inoculum for *Botrytis cinerea* and its impact on fungicide sensitivity

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Strawberry transplants produced in nurseries across Canada, northern U.S., and California are shipped annually to other strawberry growing regions such as Florida. Plants carrying latent infections of *Botrytis cinerea* from the nurseries has been suggested as one potential source for the primary inoculum in the strawberry production regions. In this study, we investigated plants from five nurseries from Canada and North Carolina in 2011 and 2012 for the presence of *B. cinerea*. Ten to twenty plants from each nursery were disinfested upon arrival to Florida and incubated in sealed plastic bags for 7 days at 23°C before examination under a stereomicroscope. Overall, 20, 44, and 37% of plants from two nurseries in Nova Scotia and one in Quebec, respectively, were infected with *B. cinerea* in 2011. The proportion of plants infected from five nurseries varied between 20 and 55% in 2012. In total, 30 and 55 *B. cinerea* colonies were single-spored in 2011 and 2012, respectively, and tested for their sensitivity to boscalid, penthiopyrad, pyraclostrobin, fenhexamid and fludioxonil using discriminatory doses determined previously. Respective resistance frequencies were 86, 33, 93, 17 and 0% in 2011 and 90, 36, 96, 27, and 0% in 2012. These findings warrant improvement of existing management practices for gray mold as well cautious use of high risk fungicides in nurseries.

Ectomycorrhizal community responses to recurring prescribed fires on yellow pine forests: Effects of fire intervals and season

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Prescribed fire is a common management tool to reduce fuel loads in forested ecosystems. However, the long-term prescribed fire effects on ectomycorrhizal (ECM) communities remain largely unknown – particularly so in southeastern United States. Using next generation sequencing, this study takes advantage of a long-term experiment established in 1988 in the Hitchiti national forest in Georgia to elucidate the ECM community responses to recurring fire and season of the burn in southern yellow pine stands. The experimental includes unburned control treatment as well as 2 year, 3 year, and 6 year burn regimes with 3 year burns occurring either during the winter or summer in four replicate 0.8 ha plots. Our data show that, while community richness and diversity are relatively stable, short fire intervals maintain ECM communities different from those in unburned controls. This is largely a result of a limited number of taxa correlating strongly with the long fire intervals. Our data provides the basis for optimizing the fire frequencies to sustain ECM communities in the long term in this southern coniferous forest ecosystem.

Comparing the effects of southeastern U.S. strains of *Xylella fastidiosa* subsp. *fastidiosa* and *multiplex* on blueberry and tobacco

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In the Southeastern U.S., *Xylella fastidiosa* (Xf) causes disease on numerous plant hosts, and has recently emerged as a threat to blueberry. Though similar to water deficits and nutrient deficiencies, the exact causes of Xf symptoms have not been fully elucidated. In order to better understand the relative host effects of Xf isolates from blueberry versus those from other hosts, the model host *Nicotiana tabacum* cv. SR1 (Petite Havana) was used to allow for side-by-side comparisons between seven Xf isolates of Southeastern U.S. origin including three Xf subsp. *multiplex* (Xfm) isolates from blueberry, three Xf subsp. *fastidiosa* (Xff) isolates from grape, and one Xff isolate from elderberry. Infected tobacco showed differential symptoms as well as differential effects on mineral nutrient allocation and colonization between isolates. Relative to the Xfm isolates from blueberry, Xff isolates colonized tobacco in higher numbers and showed a wider range and greater severity of symptoms. To further understand the host effects of blueberry Xfm versus Xff isolates, these seven isolates were subsequently examined in the greenhouse using the blueberry variety 'Rebel'. Following inoculation, scorch and matchstick symptoms developed with evident differences in symptom severity between some isolates, and additional parameters including bacterial quantities and host mineral nutrient contents were analyzed. Contrasting results from blueberries and tobacco will be discussed.

New occurrences of rapid blight (*Labyrinthula terrestris*) and other labyrinthulids associated with turfgrasses

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Until the description of *Labyrinthula terrestris*, Labyrinthulids were believed to be associated only with marine plants. *L. terrestris* is a terrestrial pathogen that causes rapid blight on cool season turfgrasses in conditions of elevated salinity. Based on a previous study, two distinct pathogenic lineages and two non-pathogenic lineages of *Labyrinthula* were identified that revealed unexpected species diversity within the terrestrial environment. Surveys were conducted between 2010-2012 in which *Labyrinthula* spp. were isolated from golf course turfgrasses in AZ, southern CA, NM and El Paso, TX (N ~ 300). Representative isolates were used to conduct pathogenicity tests. Isolates were identified morphologically based on cell and colony morphology and phylogenetically by the analysis of two rDNA loci (partial SSU and ITS/LSU). In contrast to our previous study, three phylogenetically distinct lineages of pathogenic *Labyrinthula* spp. were detected as well as more non-pathogenic lineages which could also be differentiated from pathogenic isolates based on cell size and colony morphology. The frequent isolation and genetic diversity of *Labyrinthula* spp. from turfgrasses in this study not only emphasize the threat of rapid blight in new locations but also show a higher than anticipated diversity of Labyrinthulids associated with land plants. Current studies are underway to determine population and phylogeographic structure of the sampled populations.

Increasing awareness of soybean cyst nematode in North Dakota

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Planted soybean acreage in North Dakota has increased from less than one million acres in 1996 to over four million acres annually since 2010. Soybean cyst nematode (*Heterodera glycines*; SCN) is one of the most important diseases of soybean in the United States, but was only first identified in North Dakota in 2003. Since then, SCN has been reported in twelve additional counties. The objectives of this Extension project is to increase awareness and measure the impact of Extension efforts directed at grower-based audiences and advisor-based (consultant, Extension agent, etc.) audiences. Two 2-day invitation-only SCN workshops for advisor-only audiences were held in Fargo in 2011 and 2013, and a grower-based field-day was held in 2012. A 2-day, invitation-only, advisor-based SCN workshop for North Dakota advisors will

be held in Ames, IA. Three grower-based field days will be held in Southeastern North Dakota in 2013. The impacts of each event were based on pre and post-tests and from end of season surveys. As a result of the 2011 advisor-based workshop, advisors reported an 81% increase in conversations with growers about SCN, a 68% increase in soil sampling recommendations, an increase in management recommendations and an economic value of this workshop was estimated by advisors to be in excess of \$1 million in 2011. The economic value of the grower-based field day in 2012 was estimated in excess of \$10,000.

***Puccinia* on wheat and other Poaceae in the Ecuadorian highlands**

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Wheat, together with rice and barley, are the most important cereals in Ecuador. However, the country imports 98% of its wheat requirements. In order to assure food security of this crop the government is interested in increasing the wheat acreage in Ecuador. *Puccinia striiformis* f.sp. *tritici* is the most important disease on wheat in Ecuador. Very little is known of the genetic makeup of the stripe rust population in the country, or its alternative hosts. To investigate this, we collected rust fungi from the largest wheat farm in Ecuador and from wild Poaceae hosts in the Ecuadorian highlands. *P. striiformis* f.sp. *tritici* samples were tested for virulence on 18 single resistant gene differentials, and with stripe rust specific SSR markers, while the rust from Poaceae were identified through ITS sequencing. No virulence for Yr5 or Yr15 was found, while some samples were virulent on most of the remaining 16 differentials tested. The SSR marker analysis is in progress. On Poaceae we identified *P. holcina*, *P. coronata*, *P. graminis*, *P. striiformis*, and *Puccinia* sp. that most closely resemble *Puccinia* species infecting berberis in Sweden. The potential of these rusts species to infect commercial cereals remains unknown. Our preliminary results show a diverse group of species and genotypes of rust from Poaceae in the Ecuadorian highlands. Future work involves additional virulence and molecular characterization of the samples.

Phylogeny and haplotype diversity of three DNA barcodes in *Puccinia emaculata* causing switchgrass rust

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Switchgrass rust caused by *Puccinia emaculata* can significantly reduce biomass yield and feedstock quality. Three other *Puccinia* species have been reported causing switchgrass rust, but are now considered synonyms of *P. emaculata*. This study used three "DNA barcodes", ITS, TEF1a, and Btub, to assess the monophyly, genetic diversity and haplotype distribution of *P. emaculata* urediniospores collected from cultivated switchgrass grown in Iowa, Mississippi, Oklahoma, South Dakota, and Virginia. Barcodes were amplified and the PCR products subcloned and sequenced. At least 5 clones of each barcode were sequenced per spore collection. Phylogenetic analyses with each barcode strongly supported the monophyletic status of *P. emaculata*. Intraspecific variation among and within populations was observed. Barcodes differed in the number of haplotypes represented (ITS = 13; Btub = 24; TEF1a = 27) and their geographic distribution. Btub and TEF1a haplotypes displayed mostly local distributions; while ITS haplotypes were distributed either in multiple states or locally. Also, barcode haplotype diversity and distribution suggest prolonged propagation of urediniospores under growth chamber conditions may reduce the variability. Future studies will examine the genetic diversity, phylogeography, population structure, and pathogenicity variation within *P. emaculata*.

Variability in the aflatoxin biosynthesis gene cluster among members of the atoxigenic *Aspergillus flavus* VCG YV36 endemic to Mexico

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Aflatoxins are carcinogenic mycotoxins that frequently contaminate crops in warm areas. *Aspergillus flavus* is the most common causal agent of contamination. In commercially produced maize, peanut, cottonseed, and pistachio, aflatoxin accumulation is effectively reduced through application of atoxigenic *A. flavus* to competitively displace aflatoxin-producing fungi. *A. flavus* AF36, initially isolated from Yuma, Arizona, is an atoxigenic isolate registered as a biopesticide with the USEPA. AF36 belongs to vegetative compatibility group (VCG) YV36. Each member of VCG YV36 bears a single

nucleotide polymorphism (SNP) that introduces an early stop codon in the polyketide synthase gene (*pksA*). The *pksA* gene is essential for aflatoxin biosynthesis and this SNP is sufficient to explain atoxigenicity among isolates of YV36. VCG YV36 was found to be endemic to Mexico. Three of the 62 YV36 isolates collected in Mexico possess both a previously undetected deletion in *pksA* located 361 bp downstream from the *pksA* early stop codon SNP and a 32 bp insert. The deletion/insertion observed is evidence that *pksA* continues to deteriorate in YV36. Other mutations in the YV36 aflatoxin gene cluster may also be expected to increase as VCG YV36 continues to evolve. Implications of these observations on our understanding of the evolution and adaptations of VCG YV36 will be discussed.

Phylogeny and species delimitation in the genus *Antrodia* (Polyporales, Basidiomycota)

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Antrodia is considered one of the larger genera of brown-rot fungi with a total of approximately 60 species (ca. 30 from Europe, 13 from Asia, 11 from North America, four from South America and two from Africa). Molecular phylogenies have demonstrated that *Antrodia* is not monophyletic and *Antrodia* s.l. has been divided into three genera: *Antrodia* s.s., *Amyloporia* and *Fibroporia*. Preliminary phylogenetic studies (as part of the PolyPEET project) using molecular data from two nuclear ribosomal DNA regions (LSU and ITS) confirmed that *Fibroporia* most likely represents an independent genus, but the segregation of *Amyloporia* spp. from *Antrodia* s.s. was not strongly supported. In these preliminary studies, 33 species of *Antrodia*/*Amyloporia* and 7 of *Fibroporia* were included. To better resolve these groups we are currently generating data from protein-coding genes (RPB1, TEF1-alpha) to assess the relationships among *Antrodia* species and determine the species delimitation within the genus. Results of the ongoing research will be presented.

The chitin synthase gene in oomycete genomes: Sequence and expression analyses

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Chitin synthase (CS) belongs to family 2 of the glycosyl transferase superfamily, a group of enzymes that catalyze the transfer of a monosaccharide moiety from a glycosyl donor to a glycosyl acceptor molecule, forming glycosidic bonds. Chitin, the product of CS activity is typically found in fungi, but not in oomycetes, as a major component of their cell wall. However, using comparative genomic analyses, we have identified one putative CS homolog in *P. infestans*, at least three in *P. sojae*, and two in *P. ramorum*. We cloned the *P. infestans* gene and one of the *P. sojae* homologs, and their full-length cDNAs, and conducted structural analyses on their sequences. We also performed a phylogenetic analysis to determine the evolutionary relationships between oomycete and fungal CS genes. Finally, we conducted reverse transcription and real-time PCR analyses to determine whether the CS genes are expressed in mycelial cultures grown in vitro. Our investigations indicate that the CS gene is approximately 2,700-nucleotide long and appears to be expressed in mycelial cultures although at relatively low levels. Phylogenetic analyses revealed that the *P. infestans* homolog is more closely related to *P. ramorum*'s than to *P. sojae*'s. In turn, all putative oomycete CS genes are clearly distinct from CS genes of fungal origin.

Histopathology of bronze leaf disease of *Populus*

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Bronze leaf is a lethal disease of poplar hybrids in the section *Populus* caused by the unculturable fungus *Apioplagiostoma populi*. Infected leaves turn yellow in midsummer and reddish-brown/bronze in the fall. Observations suggest the dark coloration may be due to colonization by the co-occurring endophyte *Epicoccum nigrum*. Ascospores released from perithecia within fallen leaves in the spring infect developing leaves and later systemic infection of branches cause branch and tree death after several years of disease. We report the results of histological analysis of leaf disease development on systemically infected *P. alba* x *sieboldii* throughout a growing season in Minnesota. Leaf samples were fixed in FAA, dehydrated

and embedded in paraffin using standard methods. Sections were prepared for light microscopy using a rotary microtome and stained with safranin O-fast green or periodic acid-Schiff. Asymptomatic leaves from May and June had evidence of fungi only in vascular cells. In mid-July palisade cells were infected and by mid-August they were largely disrupted. Samples from mid-August also revealed extensive mesophyll cell death and heavily stained upper and lower epidermal cells. Spermatogonia formed between the upper epidermis and cuticle mid-August into September and perithecia developed within the mesophyll and lower epidermis September-October with ascospore maturation the following April. These observations clarify the systemic development of this disease.

***Physalospora vaccinii*: Endophyte, commensal, inquiline, or incidental pathogen?**

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Physalospora vaccinii is an ascomycete commonly isolated from stems, leaves and fruit of the cranberry plant. Our results from studies on the life cycle of this organism show that it overwinters on leaves and produces ascospores one or two years later; conidia have not been seen in this species. Ascospores are ejected onto developing leaves from perithecia formed on senescent leaves. The ascospore germinates and forms an appressorium after which it remains latent until the leaves senesce. This fungus is widely distributed in most cranberry growing regions and is very common in native habitats. In some regions, such as New Jersey and Massachusetts, *P. vaccinii* is considered one of the most important fruit-rotting species although infected fruit have never been observed to support sporulation. In other regions, such as Wisconsin, the fungus is present; however, it usually does not cause a fruit rot. Spore-trapping studies show that the majority of spore production occurs from July through October. Fungicide applications during bloom in June and early July offset the expression of fruit rot. The fungus has little or no effect on the growth of the host and only causes a fruit rot after seeds have matured. Thus, this fungus is likely an incidental pathogen that does not cause fruit rot as an integral part of its life-cycle.

Development and validation of a multiplex one-step qRT-PCR for the detection of three sweet potato potyviruses infecting imported germplasm

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Foreign plant germplasm provides a source of diverse genetic characteristics not present in the U.S. Potyvirus infecting sweet potatoes can cause serious economic damage to agricultural systems. Often sweet potato germplasm has mixed infections, requiring multiple tests. Quantitative PCR protocols had been previously developed for single virus detection with an internal control. To save time and cost, a single multiplex One-Step RT-qPCR assay using four different probes (FAM, TET, TexasRed, CY5) was developed to detect and differentiate three sweet potato potyviruses and an internal control. For this assay, the sweet potato Potyviruses: *Sweet potato latent virus* (SPLV), *Sweet potato virus 2* (SPV2), *Sweet potato mild speckling virus* (SPMSV) were multiplexed in a One-step RT qPCR Assay. Both previously sequenced positive controls and field samples were used in the validation process for this assay. Primer and probe concentration were adjusted to ensure ideal detection conditions. This multiplex one-step RT-qPCR assay is rapid, cost effective and ideal for identifying germplasm with mixed Potyvirus infections.

Comparative transcriptomics gives insights into mechanisms underlying mycoparasitism in the genus *Elaphogordyceps*

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The order Hypocreales is characterized by a dynamic evolutionary history of interkingdom host jumping, with members that parasitize animals, plants, and other fungi. The monophyly of taxa attacking members of the same kingdom has not been supported by molecular phylogenetics, however. For example, *Trichoderma* spp. and *Elaphogordyceps* spp. are both mycoparasitic, but are members of two different families within the Hypocreales, the Hypocreaceae and Ophiocordycipitaceae, respectively. The genus *Elaphogordyceps* is a group within this clade that parasitizes ectomycorrhizal truffles in the genus *Elaphomyces*. To compare *E. ophioglossoides* transcriptomic response to varying nutritional conditions, we conducted an RNA-Seq experiment. Here,

we present differential expression data for *E. ophioglossoides* when grown on media containing its host, *Elaphomyces granulatus*, as compared to growth on media infused with insect cuticle, and on rich media (Yeast Malt Extract). We focus on differences in chitinase expression, secondary metabolite cluster expression, and signal reception (e.g. G-protein coupled receptors). Our work represents the first transcriptome data from a fungus grown on truffle-infused media, and will provide insights into the mechanisms underlying mycoparasitism and interkingdom host jumping.

Development of switchgrass extractives as a value-added biopesticide against bacterial spot (*Xanthomonas perforans*) of tomato

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Fermentation of switchgrass monosaccharides to biofuels is inhibited by extractives, a biomass fraction (5-25%) with phenolics and other antimicrobials. If extractives are removed before fermentation and developed as a biopesticide, switchgrass would be a more competitive biofuel feedstock. Our aim was to evaluate switchgrass extractives against *Xanthomonas perforans*, which causes bacterial spot of tomato. Preventative copper sprays, a primary control, are marginally effective and select for copper-resistant pathogen strains. Switchgrass 'Alamo' samples from three fields were ethanol-extracted, filter-sterilized, concentrated, diluted with deionized water (DI), mixed 1:1 with 2×10^8 CFU *X. perforans*/ml DI (final ethanol <10%) and incubated for 1, 4, 8, or 12 h on a shaker at 28°C. After 8 and 12 h, *X. perforans* was not detected in any extractives treatment. Small populations were found in sample 3, after 1 and 4 h (1.3-log), and after 1 h for sample 2 (0.6-log). For sample 1, *X. perforans* was not detected after any incubation time. The control contained *X. perforans* at 5-log after 1 h, decreasing to 4-log for longer incubations. Effects of incubation time and the interaction of extractives and incubation time were not significant. Extractives treatments had lower counts of *X. perforans* than control at all incubation times ($P=0.0001$). Development of switchgrass extractives as a biopesticide will improve biofuel sustainability and provide a new crop protection tool.

Impatiens downy mildew: Management options for the greenhouse, nursery, and landscape industries

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Florida's commercial producers and landscapers experienced widespread devastation caused by downy mildew on impatiens over the past two winters. This recent introduction of *Plasmopara obducens* into Florida has created much concern for the future of growing impatiens, as the state and the nation's most economically important bedding plant is in jeopardy. Wintertime conditions in South Florida provide highly favorable weather for the pathogen and the disease is currently devastating impatiens in the greenhouse, nursery and landscape. Data collected from fungicide efficacy trials conducted at the University of Florida's Tropical Research and Education Center over the past two years indicate promising results that apply to impatiens grown under commercial production and in the landscape. The presentation will provide an overview of these trials including results on effective fungicide chemistries for containerized and landscape grown impatiens, fungicide application methods and fungicide application timing. Further, cost, benefits and economic feasibility of downy mildew control strategies will be addressed.

Alkaloids produced by a defensive symbiont may have evolved from a plant stress metabolite

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Grasses endophyte (*Epichloë* and *Neotyphodium* spp.) contributes to host defense through the production of a suite of alkaloids. The loline alkaloids are broad-spectrum insecticidal agents that accumulate abundantly in the symbiotic host. Their biosynthesis is directed by *LOL* cluster of up to 11 genes. A proposed biosynthetic pathway starts from condensation of proline and homoserine. After decarboxylation, PLP-enzymes, LolD and LolT, catalyze ring closure reactions to give the pyrrolizidine ring of lolines. Elimination of the *lolD* gene supported this hypothesis. The mutant failed to produce any detectable lolines when it was introduced back into the host. When the grass harboring the mutant endophyte was fed with 1,5-diazabicyclo[4.3.0]nonane (DABN), a small amount of *N*-formyllooline, the typical pathway end product, was consistently observed. This indicated that the endophyte could take up

DABN imbibed by the grass and the other loline biosynthetic enzymes could accomplish the subsequent steps. Interestingly, DABN is also a plant metabolite produced by the action of an apoplastic polyamine oxidase known only in grasses. This interesting coincidence and the observation that DABN chemically complements the fungal *lolD* mutant suggested an intriguing hypothesis: lolines may have originated by fungal transformation of a grass apoplast metabolite into potent plant-defense compounds. This hypothesis also has great implications for the evolution of the *LOL* cluster.

Role of humidity and light in the initial stage of *Arabidopsis-Pseudomonas* interaction

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Environmental conditions play a major role in determining the frequency of disease occurrence as well as disease progression in plants. For instance, many bacterial plant disease outbreaks occur after periods of high humidity and rain. Here, we found that high humidity leads to down-regulation of salicylic acid marker genes, important for plant defense, and up-regulation of the jasmonic acid biosynthesis and signaling in *Arabidopsis* leaves. A critical step in bacterial infection is entry into the plant interior through wounds or natural openings, such as stomata. Recent studies have shown that stomatal closure is an integral part of the plant immune response to reduce pathogen invasion. We found that high humidity can effectively compromise stomatal immunity in both common bean and *Arabidopsis*. In *Arabidopsis*, this occurs in a CO₂-independent manner. Conversely, periods of darkness are effective in decreasing pathogen penetration into leaves as most stomata are closed. However, we found that coronatine biosynthesis is activated on the leaf surface irrespective of light conditions, thus facilitating infection. We conclude that a well-known disease-promoting environmental condition, high humidity, acts in part by suppressing immunity and virulence factors, such as coronatine, appear to provide epidemiological advantages to ensure bacterial penetration through stomata even when environmental conditions (darkness and insufficient humidity) favor stomatal immunity.

Production of cell wall degrading enzymes and melanin in response to changes in temperature by *Lasiodiplodia theobromae*

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Lasiodiplodia theobromae is a pathogenic fungus of many different plant species. In grapevine, *L. theobromae* has been reported as the most aggressive pathogen among the Botryosphaeriaceae. It shows preference to warmest regions, and it has been suggested that it does not present an endophytic stage. The aim of this work was to study the production of cell wall degrading enzymes (CWDEs) and melanin production in *L. theobromae* grown under temperature stress. Fungal mycelial discs were inoculated on Vogel's salts supplemented with 1% of grapevine wood and incubated at 28 °C. After 2 days, some flasks were exposed to 42 °C or 4 °C for one hour and returned back to 28°C. At 0, 2, 3 and 4 days, samples were filtered and cell-free extracts as well as mycelia were collected. Biomass, protein, CWDEs (xylanase, pectinase and cellulase) and melanin were quantified. In comparison to cultures kept always at 28 °C, in cultures heat shocked, the amount of proteins increased independently of fungal growth, and a higher activity of CWDEs was observed. At 4 °C only cellulase activity was higher. The amount of melanin in mycelia decreased in both stress treatments, while the melanin content in the extracts increased. There seems to be a correlation between melanin and CWDEs secretion, which requires further elucidation. These results suggest that higher temperatures induce the secretion of CWDEs in *L. theobromae*, which might be related to its higher impact in warmest areas.

Interactions between *Potato virus S* and *Potato virus Y* in different genetic backgrounds of potato

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Mixed virus infections are common in potato, an important staple in the world. Among several viruses infecting potato, *Potato virus S* (PVS) and *Potato virus Y* (PVY) are widespread and are commonly found together in potato plants but the interaction between these two viruses has not been investigated. In this study, we hypothesized that PVS and PVY may interact resulting in different disease phenotypes. To test this, plants of three potato cultivars, Defender, Desiree and Russet Burbank were inoculated with PVS and PVY in single and mixed inoculations. Symptoms started to develop two

weeks post-inoculation. Plants inoculated with PVY only produced more severe mosaic and mottling compared to those infected with PVS and PVY. In contrast, PVS symptoms were similar in single versus mixed infections with PVY and were inconspicuous in Desiree and Russet Burbank, whereas Defender produced necrotic spots. ELISA was used to determine the relative levels of PVS and PVY in single and double infections. PVY levels were higher in plants infected with PVY only, whereas in mixed infections with PVS, the levels of PVY were lower. The levels of PVS were similar in both single and mixed infections with PVY. The trend was unaffected by the genetic background of the potato cultivar. Results suggest that PVS acts antagonistically on PVY levels in potato in dually infected plants. The system provides further opportunities to better understand the mechanism behind the observed phenomenon.

Fine structure mapping of the silencing suppressor activity of a tospovirus (*Bunyaviridae*, *Tospovirus*)

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It is generally accepted that most plant viruses code for a silencing suppressor gene to overcome plant defense response. Tospoviruses (genus *Tospovirus*, family *Bunyaviridae*) cause significant crop losses worldwide. *Tomato spotted wilt virus* (TSWV), the most studied tospovirus was used in our study to better understand the silencing suppressor gene as little is known about the structure-function relationships of the tospovirus-coded gene silencing suppressors. To identify regions/sequences critical in suppressor activity, NSs protein sequences of known tospoviruses were compared. In silico analysis showed two conserved regions: GKT at positions 191-193 and YL at positions 429-430. Site-directed, point mutations were made to change K192 to A192 (NSs-1), and L430 to A430 (NSs-2). The effects of mutations were evaluated using the agro-infiltration and the GFP silencing system of *Nicotiana benthamiana* line 16c. Our results indicate that the silenced GFP signal in line 16c can be partially restored by the wild-type NSs, while both NSs-1 and NSs-2 lost the ability to restore GFP expression. These results suggest that the conserved GKT and YL regions are essential for the RNA silencing suppressor function of TSWV NSs. Identification and characterization of specific sequences of NSs that are critical for gene activity would provide targets for engineering virus resistance that is potentially both broad spectrum and durable.

WITHDRAWN

WITHDRAWN

Diversity of *Rhizoctonia solani* associated with canola, wheat, and pea in Alberta, Manitoba, and Saskatchewan

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Rhizoctonia solani is a damaging soilborne pathogen, which affects both canola and wheat in the Canadian provinces of Alberta, Manitoba, and Saskatchewan. The objective of this study was to conduct a phylogenetic comparison of isolates of *R. solani* collected from a previous survey in the major canola, wheat and pea growing regions of western Canada. A total of 128 multinucleate isolates from this previous survey were identified by ITS sequence and compared to anastomosis group (AG) results and pathogenicity. The multinucleate isolates of *R. solani* were grouped into 8 distinct clades. Two distinct clades were observed for isolates classified as AG2-1 by anastomosis testing, and isolates in a closely related clade to AG2-1 did not successfully fuse with any of the tester strains. While most isolates of AG-5 clustered together according to ITS sequences, three isolates classified by anastomosis grouping as AG-5 grouped with AG2-1, AG-4, and binucleate *Rhizoctonia* in the phylogenetic analysis. There was no genetic diversity among isolates classified as AG-4. While in most instances the results from AG tests were consistent with ITS sequence, there were several cases where isolates were incorrectly classified or failed to undergo anastomosis with any of the tester strains. In addition, a previously undocumented AG group was identified, and the AG-5 tester strain was found to form hyphal fusions with unrelated groups of multinucleate and binucleate species of *Rhizoctonia*.

Factors affecting seed infection by *Colletotrichum lindemuthianum* in dry bean

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The objective of this research was to further identify conditions that favor anthracnose symptoms on dry bean seeds under controlled environments. Greenhouse inoculations for *C. lindemuthianum* commonly are conducted on dry bean seedlings. Although these methods are effective for screening for host resistance and race identification, susceptible plants generally die prior to seed production. Initial greenhouse trials conducted by inoculating pinto bean plants at five stages ranging from flowering to pod elongation with conidia of *C. lindemuthianum* race 73 and incubating at nearly 100% humidity for 4 days, resulted in pod infections ranging from 85 to 100%. However, the incidence of visual symptoms of anthracnose on seeds collected from these pods ranged from 3% to 13%. These results indicate that initial humidity treatments were conducive for pod infection, but the subsequent lack of humidity did not favor seed infection. Further greenhouse trials were conducted by applying inoculum to bean pods at three stages, ranging from 2 cm in length to pod fill. Plants were incubated in humid chambers for 2, 3 or 4 days immediately after inoculation and again for another 2 to 4 days at 10 days after inoculation. The level of disease severity and incidence on pods and seeds, seed number and weight was evaluated. Results from these experiments provide an important step in further identifying conditions favorable for dry bean seed infection by *C. lindemuthianum*.

WITHDRAWN

Effect of plant age on downy mildew of basil

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Downy mildew of basil, caused by *Peronospora belbahrii*, became an economically important disease and has rapidly spread to more than 25 states in the United States since it was first detected in 2007 in Homestead, Florida. Greenhouse experiment was conducted to determine the effect of plant age on downy mildew. Seeds of basil var. 'Genovese' were planted weekly for a total of six weeks in 10-cm diameter plastic pots containing the soilless substrate. One week after the last planting, acibenzolar-S-methyl (ASM, Actigard 50WG) at 50 mg L⁻¹ was sprayed uniformly on seedlings in nine pots for each planting and water was sprayed on plants in other nine pots as untreated control. A week after ASM treatment, *Peronospora belbahrii* was inoculated at 2 x 10³ sporangia mL⁻¹ by spraying onto both ASM treated and untreated basil plants. Inoculated plants were maintained in a humidity chamber for at least 24 h before they were moved in the greenhouse. At inoculation, the age of the basil plants ranged from 2 to 7 weeks. Disease severity was rated 9 days after inoculation (DAI). Three to seven week old plants treated with ASM had significantly lower disease compared to the untreated plants at the same age. In each group of ASM treated or untreated plants, disease severity was significantly greater in 2-week old plants compared to 4-7 week old basil. This study suggests that younger basil seedlings are more susceptible to downy mildew and should be protected by effective chemicals.

Management of downy mildew of basil by seed treatment with oxathiapiprolin

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Downy mildew of basil, caused by *Peronospora belbahrii*, was first detected in Homestead, FL in 2007 and it has spread to more than 25 U.S. states. The choices for effective management of this disease are very limited. A study was conducted to investigate the efficacy of a new fungicide oxathiapiprolin applied as seed treatment for control of basil downy mildew under greenhouse conditions. Seeds of basil var. 'Genovese' were treated with oxathiapiprolin (QGU42, DuPont) at six rates (0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 oz/A) and with azoxystrobin (Quadris, Syngenta) at the rates of 9 and 18 oz/A. The treated and non-treated seeds were planted in 10-cm diameter pots containing the soilless substrate. After emergence, azoxystrobin (9 oz/A) was sprayed weekly on seedlings as a chemical control. All plants were maintained in a greenhouse where abundant sporangia of *P. belbahrii* were available as a source of inoculum for natural infection. Seedlings from seeds treated with oxathiapiprolin did not develop any disease symptoms 15 days after planting (DAP), whereas symptoms were observed on leaves of seedlings from azoxystrobin seed and foliar treatments. Disease severity on seedlings from seeds treated with oxathiapiprolin at 0.5, 1.0 and 2.0 oz/A was significantly ($P < 0.05$) lower compared to other treatments 20 and 25 DAP. Results from this study indicate that oxathiapiprolin applied as seed treatment can effectively control downy mildew of basil in the greenhouse.

***Ralstonia solanacearum*, a new pathogen of highbush blueberry**

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During the summer of 2012 mature highbush blueberry plants c.v. Bluetta exhibited symptoms of wilting and rapid cane death. Although the symptoms superficially resembled stem blight, the pattern of leaf discoloration was unique. Also, entire bushes over 20 years old exhibited symptoms and rapidly died. The vascular tissue of the infected stems exhibited a watery, gray discoloration and significant bacterial streaming was observed when symptomatic wood chips were placed in water. Isolation of morphologically identical bacteria from streaming wood was consistent for all symptomatic bushes tested (n=18). The bacterium was identified by sequencing the 16S rRNA gene and compared with sequences deposited in GenBank. The bacterium was identified as *Ralstonia solanacearum* of an unknown biotype based on sequence of the 16S rRNA gene. Inoculation of potted blueberry plants (c.v. Duke) resulted in symptom development within 2 weeks and plant death within three weeks. In all cases the pathogen was re-isolated from the symptomatic stems. This is the first report of *Ralstonia solanacearum* as a pathogen of any species of *Vaccinium*.

Evaluation of fertility and fungicide programs on foliar diseases of bermudagrass (*Cynodon dactylon*)

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Leaf spot (LS), caused by *Bipolaris* spp., and dollar spot (DS), caused by *Sclerotinia homoeocarpa*, are of major concern to the turfgrass industry. One method of managing LS and DS is with fungicides; however, little is known about the influence of fertility inputs on these diseases. Five rates of nitrogen (N), 4 rates of potassium (K), and 3 disease control programs (conventional fungicide (CP), plant activated resistance mineral oil (PAR), and non-treated control) were evaluated across 2 cultivars (Patriot and U-3) in a split-split-split experimental design. Three and 12 applications were made for the CP and PAR programs, respectively. Nitrogen was applied every 14-d and K was applied every 28-d, both beginning in May. Disease severity and visual quality were assessed every 14-d from green-up. Disease severity was low during the study period. On 11 May 2012, LS was significantly ($P<0.05$) higher for treatments of N at 0.0 and 73.2 kg ha⁻¹ compared to N at 146.5, 220.0, and 293.0 kg ha⁻¹. The CP fungicide program resulted in less disease than PAR, which had significantly ($P<0.05$) less disease than non-treated control programs. Potassium at 0.0, 48.8, and 97.7 kg ha⁻¹ resulted in significantly ($P<0.05$) less disease than K at 146.5 kg ha⁻¹. For DS, CP and PAR programs were not different from each other and resulted in significantly ($P<0.05$) less disease than the control. These results imply fertility rates may influence foliar diseases of bermudagrass.

First record of a *Cyanodiscus* (Saccardiaceae) species in Central Brazil

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A *Cyanodiscus* species was found associated with leaves of *Vochysia rufa* in the Brazilian Cerrado. The fungus (Access number UB16523- Mycol. Collection) showed: colonies hypophyllous, irregular; ascomata 123 (176) 204.5 x 121 (175) 214 µm, discoidal, scudate, flat, black, superficial; asci 39 (49) 60.5 x 7 (10) 11 µm, bitunicate, cylindrical, pedicelate, 8-spored; ascospores 6 (14) 15 x 2 (10) 4.5 µm, 1 to 3-septate, clavate, hyaline. The genus *Cyanodiscus* (Saccardiaceae) presently accommodates just two known species: *C. occidentalis* (on *Chamaecyparis nootkatensis*) from USA, and *C. glabrescens* (on an Euphorbiaceae) from Cuba. This is a first record of an apparently new *Cyanodiscus* species, now on a Vochysiaceae, an endemic family from the Brazilian Cerrado.

Multi-year evaluation of standard- and model-timed fungicide applications for the control of *Botrytis* and anthracnose in commercial strawberry fields

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Botrytis (BFR) and anthracnose (AFR) fruit rots, caused by *Botrytis cinerea* and *Colletotrichum acutatum*, respectively, are the two major diseases of

strawberry. In this study, we report on the efficacy of model-timed fungicide applications compared to standard weekly applications in five commercial strawberry fields in Florida between 2010 and 2013. Fungicide applications based on models developed and tested previously were recommended only when the predicted proportion of flowers or fruits infected was $\geq 15\%$ and $\geq 50\%$ for AFR and BFR, respectively. Fruit were harvested weekly from December to March each year and BFR and AFR incidences and yield were estimated. The use of the predictive models reduced the number of sprays by 39 to 55%, 30 to 59%, and 30 to 50% in 2010-11, 2011-12, and 2012-13, respectively. Differences in BFR and AFR incidences were not significant between the two spray programs within the same field either in 2010-11 or in 2011-12. Preliminary data in 2012-13 confirmed results observed in the previous years for AFR whereas BFR incidence was significantly higher for the predictive model in two fields where a highly susceptible cultivar was grown. Overall, the model-timed fungicide applications did not have lower yields than standard weekly applications within the same field. These results show the value of using predictive models on reducing fungicide input without decreasing control.

Density and in vitro viability of *Sclerotium cepivorum* Berk. sclerotia are not correlated in soil samples of Guanajuato state, Mexico

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A field survey was conducted to determine the sclerotia-inoculum density and in vitro viability of *S. cepivorum* in two garlic producers' counties from the Guanajuato state, Mexico. Soil samples were taken from two locations in the Irapuato and Abasolo Counties, Gto. during the August 2009 to February 2010 period. The Abasolo's field did not have a history of *S. cepivorum* presence; on the other hand, the Irapuato's land had recently been planted with garlic and disease damage had arisen in different sowing cycles earlier. The in vitro-fungus viability was obtained from the average of viable sclerotia. The fungal growth was also evaluated on the basis of the scale proposed by the Horticultural Research International in Wellesbourne, U.K. The evaluation dates were 4, 7, 14 and 21 days after the sclerotia inoculation. The information analysis was made with a comparison of independent samples. The sclerotia average number, extracted from soil samples, was statistically equal to both locations; 11.8A for Irapuato and 9.2a in Abasolo, yet, significant differences were observed in terms of the sclerotia viability; with 25% in Irapuato and 0% in Abasolo. The results obtained in this research show that; even though sclerotia-average densities were similar in the two analyzed localities, sclerotia-viability differences might contribute to initiate the presence of this disease in the field.

Presence and relative incidence of viruses infecting *Cichorium intybus* in Guanajuato, Mexico

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A survey was conducted to determine the incidence of *Tomato spotted wilt virus* (TSWV), *Beet western yellows virus* (BWYV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Lettuce mosaic virus* (LMV) on *Cichorium intybus* of Guanajuato State. A total of 200 leaf samples were collected in two counties of Guanajuato State, Mexico and tested by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific polyclonal antibodies. The greatest virus incidence, among the surveyed municipalities, was recorded in Salamanca, followed by San Miguel de Allende. Incidence of viruses in decreasing order was BWYV (7.0, 8.0 and 6.5%), CMV (11.5, 6.0 and 3.5%), LMV (0.0, 1.0 and 3.0%), TSWV (0.0, 0.5 and 0.5%), and AMV (0.0, 1.0 and 0.0%), respectively, at 45, 60 and 75 days after planting. BWYV and CMV were detected in the two municipalities tested and both BWYV (21.5%) and CMV (21.0%) were predominant. TSWV, AMV and LMV were detected only in San Miguel de Allende. Although predominantly infected plants with one virus (92.0%) were detected, multiple (BWYV-CMV) viruses were also found by plant (8.0%). Up to our knowledge, this is the first report of viral occurrence on radicchio plants in Guanajuato State, Mexico.

Presence and relative incidence of viruses infecting *Lactuca sativa* in Queretaro, Mexico

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A survey was conducted to determine the incidence of *Tomato spotted wilt virus* (TSWV), *Beet western yellows virus* (BWYV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), and *Lettuce mosaic virus* (LMV) on *Lactuca sativa* in Queretaro State. A total of 225 leaf samples were collected in tree counties of Queretaro State, Mexico and tested by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific polyclonal antibodies. The greatest virus incidence, among the surveyed municipalities, was recorded in Pedro Escobedo, followed by Huimilpan, and Corregidora. The incidence of viruses, in decreasing order, was CMV (16.9%), BWYV (8.0%), TSWV (5.8%), AMV (0.9%) and LMV (0.5%). CMV and BWYV were detected in the tree municipalities tested but CMV was the predominant. TSWV, AMV and LMV were detected in two municipalities. Although predominantly infected plants with one virus (74.1%) were detected, multiple viruses were also found in each plant (25.9%). This is the first report of viral occurrence on lettuce plants in Queretaro State, Mexico.

***Sparassis* (Basidiomycota): Transatlantic disjunction and paraphyly**

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Sparassis can be conveniently divided into two complexes: the *S. crispa* complex, in which the ultimate branches of basidiomata are curled or crisped. Included species are *S. crispa* (typus generis), *S. radicata*, *S. latifolia* and a few others. The second complex, in which the branches are erect, stiff, blade-like and without curled or crisped margins, includes *S. spathulata*, *S. brevipes*, *S. laminosa* and some other names. Phylogenies based on ITS and LSU sequences show that the *Sparassis crispa* complex comprises several monophyletic clades, in some cases corresponding to named taxa (i.e. European *S. crispa*, American *S. radicata*), but in others lacking names (i.e. eastern North American *S. "crispa"*, southwestern American *S. "radicata"*). Morphological examination of numerous collections also distinguished subtle differences correlated with geographic distribution. Extensive sexual compatibility experiments indicate that monokaryon, haploid isolates of collections from North America and Europe are sexually intercompatible. Inherent in the study, three "species concepts" were tested, with the "biological species concept," based on sexual compatibility, being the least restrictive.

A multilocus database for the identification of *Aspergillus* and *Penicillium* species

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Identification of *Aspergillus* and *Penicillium* isolates using phenotypic methods is increasingly complex and difficult but genetic tools allow recognition and description of species formerly unrecognized or cryptic. We constructed a web-based taxonomic database using BIGSdb for the identification of *Aspergillus* and *Penicillium* species using DNA sequences from one or more of 7 genetic loci, including nuclear ITS, beta tubulin (BT2), calmodulin, minichromosome maintenance factor 7 (Mcm7), DNA dependent RNA polymerase (RPB1 and RPB2) and pre-rRNA-processing protein Tsr1. Nuclear ITS is the "barcode" locus for fungal identification, but sometimes fails to provide an unequivocal identification in the Trichocomaceae, thus the need for additional loci. Species designations are based on genealogical concordance analysis. Coverage of the species within *Aspergillus* is greater than 90%, but coverage with multilocus data is more limited for *Penicillium* species. The database will be carefully curated to reduce confusion caused by errors in strain identification or low quality data. The availability and use of this database will promote rapid and accurate identification of isolates from the Trichocomaceae.

Ramulosis of cotton is caused by a distinct phylogenetic lineage within the *Colletotrichum gloeosporioides* species complex

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Ramulosis is an important cotton disease in Brazil with its etiologic agent known as *Colletotrichum gossypii* var. *cephalosporioides* (CGC). *Colletotrichum gossypii* (CG) is associated with anthracnose of cotton. Despite the importance of the pathogens, their identities and relationships have not been assessed according to methods currently used in *Colletotrichum* taxonomy. The objective of this study was to test the hypothesis that ramulosis and anthracnose of cotton are caused by distinct pathogens belonging to the *Colletotrichum gloeosporioides* species complex using phylogenetic methods. Twenty-one strains of CGC and five strains of CG were analyzed using DNA sequences of ITS rDNA, TUB2 and GAPDH. Strains of CGC and CG formed

two distinct phylogenetic lineages within the *Colletotrichum theobromicola* clade. Representative strains of each pathogen were used in pathogenicity tests. CGC strains induced foliar necrosis beginning seven days after inoculation, followed by the death of apical meristems, production of shortened internodes, and abnormal lateral branching. Plants inoculated with strains of CG exhibited foliar necrotic spots beginning two months after inoculation, with no further symptoms. These results support the separation of CGC and CG and their placement in the *C. gloeosporioides* species complex. The identity of the cotton anthracnose pathogen in Brazil needs further investigation including comparisons with reference material of *C. gossypii* from the USA.

***Fusarium solani* f. sp. *piperis* the causal agent of fusariosis of black pepper in Brazil is a distinct phylogenetic and biological species in the FSSC**

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The main disease of *Piper nigrum* in Brazil is caused by *Fusarium solani* f. sp. *piperis* (FSP). Disease symptoms include leaf chlorosis, blight of stems, root and foot decay. The objectives of this study were to investigate whether this form corresponds to a biological and phylogenetic species within the *Fusarium solani* Species Complex - FSSC. One hundred and four isolates with characteristics of *F. solani* were obtained from diseased plants collected in the main producing areas and characterized by means of laboratory crosses, phylogenetic analyses, and pathogenicity tests. Tests for homothallism and crossings of isolates of opposite mating types identified 10 homothallic isolates and a set of 25 heterothallic intercrossing strains. In phylogenetic analyses conducted with 44 isolates using partial sequences of the genes TEF, RPB2 and ITS-LSU rDNA, the sexually compatible isolates formed a single clade (100% bootstrap support) together with a reference strain of FSP, and distinct from other species and forms in the FSSC. Remaining isolates were grouped in 10 different lineages in a phylogenetic tree based on TEF sequences. In pathogenicity tests repeated twice with 35 isolates, only isolates of FSP induced disease symptoms on inoculated plants. According to our results, the pathogen represents a phylogenetic and biological species in the FSSC. Other heterothallic and homothallic isolates of *F. solani* occur in association with black pepper, but do not cause disease.

Phylogenetic affiliations of marine fungi detected with pyrosequencing and ion semiconductor targeted amplicon sequencing

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The systematic relationships of zoospore fungi (Blastocladiomycota, Chytridiomycota, Cryptomycota, Neocallimastigomycota, and *Olpidium*) are known almost exclusively from taxa isolated in culturing surveys of terrestrial and freshwater habitats. Recent culture-independent molecular cloning studies of extreme marine habitats, such as deep-sea hydrothermal vents and methane seeps, have revealed novel fungal phylotypes belonging to the zoospore lineages. However, as environmental cloning studies frequently fail to capture all of the diversity within a given sample, our understanding of both the breadth of zoospore fungal diversity and the phylogenetic placement of marine taxa remains poor. Short (200-600bp) 454 reads from nrSSU were generated from water samples from the English Channel using general eukaryotic primers. From marine and estuarine sediments from coastal North Carolina, 330bp nrLSU reads were generated on the Ion Torrent sequencing platform using fungal-affinity primers. Using maximum likelihood and Bayesian methods, the phylogenetic affinities of marine fungi, represented by these nuclear ribosomal RNA-coding sequences, were inferred. Extensive kingdom-wide reference alignments and multiple backbone constraint trees for both loci were used in our analyses to ensure accurate placement of short reads for OTU estimation. These results will be compared to inferred phylogenies based on long (Sanger) reads (1.2 kb) from cloned amplicon fragments of the nrLSU.

Development and validation of a multiplex one-step RT-PCR for the improved detection of four nepoviruses infecting imported germplasm

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Foreign plant germplasm is a valuable source of novel genes not present in the U.S. plant gene pool. Nepoviruses infecting imported plant germplasm can

cause serious problems in agricultural, rural and native plant systems. Previously developed nepovirus primer pairs primarily focused on the detection of individual species or groups of nepoviruses. To save time and cost, a single multiplex one-step RT-PCR assay was developed using primer pairs specific to *Blueberry leaf mottle virus* (BBLMV), *Tomato ringspot virus* (ToRSV), *Black currant reversion virus* (BRV or BCRV) and *Cherry leaf roll virus* (CLRV) combined with an internal plant RNA control (Nad5). This assay was validated using sixteen different isolates representing the four viruses species (1 BRV, 1 BBLMV, 7 ToRSV and 7 CLRV) in 8 different plant species. The one-step multiplex RT-PCR assay produces expected amplicons of 450, 390, 348 and 282 bp for ToRSV, BRV, BBLMV and CLRV respectively as well as the host RNA internal quality control amplicon of 181 bp from all tested samples. PCR-amplified DNA fragments produced using this assay were cloned and sequenced to verify the specificity. This multiplex one-step RT-PCR assay is well suited for the rapid detection of ToRSV, BBLMV, BRV and CLRV.

Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles

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Ambrosia beetles (Coleoptera: Curculionidae: Platypodinae and Scolytinae) subsist on symbiotic fungi they cultivate in host tree xylem. Typically, their symbionts are saprobes and their host trees are dead or stressed. However, there are uncommon examples of phytopathogenic symbionts and ambrosia beetles that transmit these pathogens to healthy host trees. Notably, *Xyleborus glabratus* (Scolytinae: Xyleborini) carries a symbiont, *Raffaelea lauricola*, that causes laurel wilt, a lethal disease of members of the Lauraceae plant family, including redbay (*Persea borbonia*), swampbay (*P. palustris*) and avocado (*P. americana*). *X. glabratus* and *R. lauricola* are natives of Asia that recently invaded the coastal plain of the southeastern USA. In this study, lateral transfer was examined for *R. lauricola* to other scolytine species of ambrosia beetle that were recovered from laurel wilt-affected swampbay. In addition to *X. glabratus*, the pathogen was recovered from *Xyleborus affinis*, *Xyleborus volvulus*, *Xyleborus ferrugineus*, *Xyleborinus gracilis*, *Xyleborinus saxeseni* and *Xylosandrus crassiusculus*. Controlled infestations with cohorts of the seven species revealed that each could transmit *R. lauricola* to healthy trees of redbay and two could transmit it to healthy avocado; laurel wilt developed in five and one of the respective beetle x host interactions.

Physiological impacts of laurel wilt on avocado

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Laurel wilt (LW), caused by *Raffaelea lauricola*, is a systemic vascular wilt of members of the Lauraceae, including avocado (*Persea americana*). LW affects commercial production in Florida and poses a grave threat in other producing areas. Avocado is unusually sensitive to *R. lauricola*. As few as 100 conidia of the pathogen kill plants, and artificially inoculated plants can die within 2 wks. Yet, there is scant microscopic evidence of the pathogen in such plants. Xylem function is impaired 3 days after inoculation (dai), and tylose formation is associated with reduced hydraulic conductivity. In the present study, avocado cultivars that vary in susceptibility were artificially inoculated with *R. lauricola*. Significant LW developed 15 dai on 'Russell' which coincided with significant reductions in transpiration ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$). In 'Russell,' reductions in the ratio of variable to maximum chlorophyll fluorescence (F_v/F_m , an indicator of disruption of the photosynthetic apparatus), stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$), and chlorophyll concentrations (SPAD values) occurred, respectively, 16, 20 and 20 dai, suggesting that xylem dysfunction caused by LW was the principal factor in these responses. In general, the same trends were observed in 'Brogdon' and 'Marcus Pumpkin.'

Fusarium* symbionts of an ambrosia beetle (*Euwallacea* sp.) in southern Florida are pathogens of avocado, *Persea americana

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Fusarium dieback, a destructive disease of avocado (*Persea americana*), was reported in California and Israel in 2012. It is associated with an ambrosia beetle, *Euwallacea* sp., and damage caused by an unnamed symbiont of the

beetle in Clade 3 of the *Fusarium solani* species complex (FSSC) designated phylogenetic species AF-2. In 2012, a beetle that resembled *Euwallacea fornicatus* morphologically was recovered in southern Florida from infested avocado trees, and Lindgren funnel and Tanglefoot traps. Adult female beetles were assayed for fungi by macerating and dilution plating different body parts on half-strength PDA+streptomycin sulfate. A novel phylogenetic species within the FSSC designated AF-8 predominated in heads but was almost completely absent in the rest of the body (thoraxes+abdomens). When heads were surface disinfested prior to assay, AF-8 was often the only fungus isolated from the insect. Thus, it is presumed that AF-8 is a symbiont of the beetle that resides in its mandibular mycangia. AF-8 was recovered from 23 of 33 individuals, often to the exclusion of other fungi (20 of 23 individuals). Colony forming units (CFUs) of AF-8 ranged from 0 to 8600 per individual, and significantly more CFUs were isolated from living vs dead beetles. Isolates of AF-8, and two other FSSC species that associated less frequently with the beetle, caused slowly developing lesions in avocado sapwood in greenhouse and field experiments.

A next-generation sequencing approach to identifying the causal agent of funky flower in cranberry

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Previously undescribed disorders frequently appear in cultivated plants, especially when the natural range is expanded or new cultivars are planted. A novel disorder of American cranberry (*Vaccinium macrocarpon*), termed funky flower, was discovered in Massachusetts and in New Jersey in 1999. The disorder is characterized by the appearance of deformed flowers on otherwise seemingly healthy plants. Samples of affected cranberry from individual commercial fields were DNA fingerprinted and found to represent several distinct genotypes, suggesting that the disorder was not caused by a genetic mutation found in a single cranberry variety. Traditional methods for isolation and identification of a causal agent, such as culturing, electron microscopy and PCR did not reveal the presence of any candidate fungus, virus or phytoplasma. Assuming a previously uncharacterized virus might be the cause, the next-generation sequencing approach was selected to probe for virus-associated sequences. The transcriptomes of normal and affected flowers were sequenced and over 71,000 gene fragments were assembled from each data set. Three genes were identified in symptomatic plants that encode virus proteins with similarity to those in the Caulimoviridae. The closest database match was with blueberry red ringspot virus from Europe (76-85% identity). We are now at the step of confirming that this virus is in fact the causal agent.

Characterization of fruit rot resistance and inheritance in American cranberry

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Fruit rot is the primary threat to American cranberry (*Vaccinium macrocarpon*) production in the northeast and an increasing problem in Wisconsin and other growing regions. Even with a well-timed fungicide regime, losses to fruit rot can reach 25% in New Jersey. Fruit rot of cranberry in the field is caused by a complex of pathogenic fungi from several genera. Despite the variation in pathogens, we have selected genotypes from our germplasm collection that exhibit resistance to fruit rot. The selected accessions were DNA fingerprinted using SCAR and SSR markers to estimate genetic relatedness. The analysis suggests that there are four diverse genotypic sources of resistance in the collection. These were used in crosses with elite, commercially viable, varieties in an effort to introgress resistance into horticulturally superior varieties. Progeny were planted in the field and evaluated over two years for fruit rot incidence. Heritability estimates based on mid-parent offspring regression were good and the genetic diversity of the parental types suggests that there may be different loci associated with resistance which offers the possibility of 'pyramiding' genes to develop highly rot-resistant cultivars. The mechanism(s) of resistance are being explored.

Next-generation sequencing of grapevines showing redleaf symptoms implicates a leafhopper-transmissible DNA virus in the family Geminiviridae

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A graft-transmissible disease showing red veins, red blotches and total reddening of leaves, designated as grapevine redleaf disease (GRD), was observed in wine grape (*Vitis vinifera*) cultivars Merlot and Cabernet Franc. GRD significantly reduced fruit yield and affected berry quality attributes such as total soluble solids (sugars) and total extractable anthocyanins in both cultivars demonstrating negative impacts of the disease. Next-generation sequencing of high quality total RNA obtained from symptomatic and non-symptomatic leaves revealed a single-stranded DNA virus, tentatively named as Grapevine-infecting geminivirus (GiGV), and *Grapevine fanleaf virus* only in grapevines showing GRD symptoms. In contrast, *Grapevine rupestris stem pitting-associated virus*, *Hop stunt viroid*, *Grapevine yellow speckle viroid* 1, *Citrus exocortis viroid* and *Citrus exocortis Yucatan viroid* were detected in both symptomatic and non-symptomatic grapevines. GiGV was transmitted by the Virginia creeper leafhopper (*Erythroneura ziczac* Walsh). To the best of our knowledge, this is the first report of transmission of a grapevine-infecting ssDNA virus by a leafhopper species in the genus *Erythroneura*. Molecular and phylogenetic analyses indicated that GiGV represents an evolutionarily distinct lineage in the family *Geminiviridae* with genome characteristics distinct from other leafhopper-transmitted geminiviruses.

Root mycobiomes: Diversity and plant-host interactions in extreme environments

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Plant roots are colonized by complex and largely unexplored mycobiomes. In extreme environments, these associations could be vital for survival and adaptation under factors such as changing climatic conditions and increase in N deposition. We have been characterizing root mycobiomes with the main objective to identify core functional groups. Plant mycobiomes were sequenced from environmental samples and fungi were isolated in culture from a semi-arid ecosystem in NM and an alpine tundra in Colorado. Dominant taxa were evaluated with model plants (corn and *Arabidopsis*) to determine their effects on plant growth and gene expression. Root mycobiomes were dominated by Ascomycota taxa. Arid systems endophytes were dominated by the order Pleosporales. While the alpine tundra samples were dominated by Helotiales. Germination experiments showed that dark septate fungi (DSF) from different orders can have similar functional effects on plant growth. Microarray analysis of DSF showed up-regulation of plant growth and photosynthetic genes. Plant defense pathways were also up-regulated. Potential roles of these complex communities and the impact of changing environmental conditions on their diversity will be discussed.

Characterization of LSU and ITS rDNA for automated fungal classification

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Characterization of fungal communities using next generation sequencing technologies is becoming a common practice. However, optimal regions for automated fungal classification and primer design are still not well defined specially when short reads are produced. We created curated databases to validate the fungal Ribosomal Database Classifier in RDP and tested different sections of the ITS and LSU rDNA regions for taxonomic accuracy, effect of bootstrap support, and fragment size. Both regions perform well at the order and family level with fragments above 100 bp. The ITS 1 region shows the highest taxonomic accuracy but this region is highly dependent on the use of bootstrap cut-off due to the presence of introns and sequences with conflicting taxonomy. ITS 2 shows similar accuracy than ITS 1 with less number of sequences rejected after bootstrap cut-off was applied. ITS 2 seems to be the best candidate for NGS applications due to its proximity to LSU, its barcode properties, and good classification accuracy at the genus level even for small fragments.

A new aquatic cellulose-degrading chytrid in the Chytridiales

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In a survey of chytrid diversity in Lake Lurleen (Tuscaloosa County, AL), we cultured from cellulose bait a new chytrid that exhibited both monocentric and polycentric thallus forms. Its thallus development, zoospore ultrastructure, and ribosomal gene sequences were analyzed. On onion epidermal cells and nutrient agar culture, the chytrid thallus grew both endobiotically and epibiotically. Sporangia were spherical and produced numerous long, wavy discharge tubes through which zoospores were released. Typical of members of the Chytridiales, zoospore ultrastructural features included a cell coat and paracrystalline inclusion. However the constellation of character states was unique: the kinetosome-associated structure was globular; an electron-opaque plug filled the flagellar transition region; there were multiple paracrystalline inclusions (rather than single); tubes of the fenestrated cisterna (a component of the microbody-lipid globule complex) radiated outwardly (rather than parallel) from the curved surface of a lipid globule. Molecular phylogenetic analyses placed this chytrid within the Chytridiales clade, but outside of its two families, Chytriomycetaceae and Chytridiaceae. Based on its phylogenetic placement, distinctive thallus development, and unique zoospore ultrastructure, this chytrid is described as a new genus and family in the Chytridiales.

Structure and function of the blue grama grass rhizosphere microbiome under global environmental change scenarios in an American aridland ecosystem

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Our metatranscriptomic inventory of the blue-grama grass (*Bouteloua gracilis*) rhizosphere (RHZ) point to taxonomic hyperdiversity and an array of metabolic functions. Comparison of samples from field-manipulation plots examining key global environmental change factors reveals differences across treatments. At the domain level, the Eubacteria predominate (~80%). Among the Eukaryota (~20% of all sequences), fungi were the most highly represented (~48%), followed by the Streptophyta (~15%), Chlorophyta (~3%) and Metazoa (~22%), with ~10% of reads unclassified. The fungal component of our dataset indicates that the Basidiomycota account for a higher percentage of fungi (> 50% in some samples) than has been revealed in rRNA-based surveys. In the Ascomycota, our total sequencing results are consistent with rRNA-based surveys. Both indicate that sequences from the Dothidiomycetes are most abundant (~30% of Ascomycota). Other major Ascomycota members include the Saccharomycetales (21%), Sordariomycetes (~18%) and Eurotiomycetes (15%). Major Basidiomycota groups include the Agaricomycetes (94% of Basidiomycete sequences) and Tremellomycetes (3%). The 50 largest Pfam clusters reveal protein families involved in central carbon metabolism and stress responses. The observed Basidiomycota diversity in an aridland ecosystem is novel, as is the panoply of microbial metabolic functions, demonstrating the utility of the total sequencing approach for studying the RHZ.

Genetic polymorphism of *Puccinia arachidis*

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Puccinia arachidis Speg is the causal agent of peanut rust, an important foliar disease of peanut (*Arachis hypogaea*) in mainly low input peanut producing countries with warm, tropical climates. Management of this disease in these countries is best realized through host resistance. Knowledge on the variability of the *P. arachidis* populations is useful to effectively breed for stable resistance and thus effective management of the peanut rust disease on the long run, however, little is known about the population structure of *P. arachidis*. Our objective was to determine the genetic diversity of *P. arachidis*. We evaluated sequences of the 5.8S-ITS2-28S region of *P. arachidis* isolates, collected from different regions in North America, South America, Central America and Asia, from 2010 to 2012. Preliminary results from the sequenced ITS region of the *P. arachidis* isolates indicate high genetic homogeneity among the populations studied as there was no clustering of isolates by geographic origin. Analysis of more isolates from different geographic areas and of more loci is needed to determine the population structure of *P. arachidis*. These results will be presented and discussed.

Fungicide resistance in *Cercospora kikuchii*, a major pathogen of Louisiana soybean

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To determine if resistance to fungicides has occurred in *C. kikuchii*, evaluations were conducted using Louisiana populations from 2000, 2011, and 2012. Baseline fungicide sensitivities, derived from EC₅₀ values from radial growth assays, were determined in isolates from 2000 for quinone outside inhibitor (QoI) and demethylation inhibitor (DMI) fungicides. Sensitivity shifts were subsequently monitored in 2011 and 2012 populations. Because isolates were either sensitive or resistant to MBC fungicides, a discriminatory dose of thiophanate-methyl (5 µg/ml) was used to determine the percentages of resistant isolates for 2000, 2011, and 2012. Baseline sensitivities to the QoI fungicides: azoxystrobin, pyraclostrobin, and trifloxystrobin averaged 0.10, 0.02, and 0.02 µg/ml, respectively. Respective mean EC₅₀ values for the 2011 (32.60, 10.98, and 22.03 µg/ml) and 2012 (52.66, 13.83, and 29.23 µg/ml) populations were significantly higher. Baseline sensitivities to the DMI fungicides: flutriafol, propiconazole, and tetraconazole averaged 0.45, 1.66, and 0.18 µg/ml, respectively. Respective mean EC₅₀ values for the 2011 (0.40, 0.96, and 0.41 µg/ml) and 2012 (0.83, 0.44, and 1.33 µg/ml) populations were essentially unchanged. For thiophanate-methyl, respective frequencies of resistant isolates from the years 2000, 2011, and 2012 were 23.3, 44.8, and 35.7 percent. Based on these results, fungicide resistance has occurred in *C. kikuchii* since 2000.

WITHDRAWN

Molecular characterization of six new Asian prunus virus isolates: Evidences of their recombination and high genetic diversity

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Asian prunus viruses (APV) are a group of three closely related viruses (APV1, APV2 and APV3) of the genus *Foveavirus* in the family *Betaflexiviridae*. During a virus survey six peach germplasm accessions of Asian origins were found to be infected with these viruses. Sequence analysis of the 749-bp RT-PCR amplicons showed that sequence divergences of these isolates were at least 8% with the known APV viruses. To identify them at species level, sequences of the coat protein (CP) gene were obtained from five of these isolates, and phylogenetic analysis showed that isolates D9 and D11 were grouped with two known APV 1 variants, Q65 and Q86 with APV 2, and Q35 with APV 3, respectively. The complete genomic sequences of D9, D11 and Q86 were then determined to be 9,400, 9,472 and 9,400 nucleotides, respectively. Their genomes are typical of foveaviruses, containing five putative open reading frames. Pairwise comparisons of the genomic sequences show that D9 and D11 are 99% identical to one another and 84% identical to the type species of APV 1. Q86 shares 92% identity with the known APV 2 isolate at the 3' partial sequence. Phylogenetic analysis of their genomic sequences agreed with that of the CP analysis. However, different phylogeny was obtained with 3' non-coding region sequences, suggesting the possibility

of recombination events during their evaluation. The sequence data confirms the high genetic diversity among different APV viruses/variants.

Signatures of global dispersal and population structure in *Sclerotinia homoeocarpa*

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Sclerotinia homoeocarpa incites dollar spot, a disease that causes significant economic damage to turfgrass worldwide, but its population biology is poorly understood. The objective of this research is to infer the population structure of *S. homoeocarpa* on a global scale. To date we have genotyped 1,049 *S. homoeocarpa* isolates, obtained from 76 locations on five continents and Oceania, at 14 microsatellite loci and for mating type. Beyond the expected structure delimiting the C3 and C4 host type isolate clades, within each clade principal component and Bayesian clustering analyses identified limited admixture and substructure that was associated with mating type and continent of origin. In addition, analyses revealed a small group of 38 isolates that were marked by admixture and could not be classified with isolates from either C3 or C4 grasses. Of the 618 C3 and 393 C4 host type isolates, 71% and 6%, respectively, were assigned to one of several multilocus haplotypes that were identified in isolates originating from two to six continents. Within all but one of 136 multilocus haplotypes, all isolates were of the same mating type. When grouped by clade and continent, clone correction showed few deviations from an equal number of *MAT1-1* and *MAT1-2* mating types. Initial results from this study suggest that *Sclerotinia homoeocarpa* has undergone long distance dispersal followed by clonal amplification, with a possible history of sexual reproduction.

Prediction of long-term field resistance of hybrid poplars to *Septoria musiva* using a greenhouse screening protocol without wounding

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Populus species and their hybrids are short-rotation woody crops (SRWC) supplying fiber and biomass to a diversity of industries in North America. Hybrid poplar as a SRWC has been limited in many parts of North America by the fungal pathogen *Septoria musiva* Peck, the cause of leaf spot and stem canker diseases of *Populus*. An inoculation protocol that does not rely on stem wounding to achieve infection was recently developed to screen for resistance to *Septoria* canker. The relationship between this inoculation protocol and long-term field resistance is unknown. Young ramets of 14 poplar genotypes with known levels of field resistance to *Septoria* canker (Low, Intermediate, and High) were inoculated with a spore suspension of three isolates of *S. musiva* in a greenhouse. Three weeks after inoculation the following parameters were recorded: lesion number, lesions/cm, lesion area, and proportion necrotic area (PNA). The logistic regression model with lesion number and proportion necrotic area correctly predicted field resistance categories for 11/14 genotypes tested, including the most resistant (NM6, DN164, DN177, DN34, and DN74) and the most susceptible (NC11505) genotypes. These results demonstrate that both canker number and disease severity (PNA) are important for accurate prediction of long-term field resistance. This screening protocol, using young ramets, can be used to focus resources for subsequent long-term field testing on the most resistant genotypes.

An usefull antagonistic strain of *Aspergillus niger*-Y61 for control of root knot nematode

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Tomato disease caused by root knot nematode *Meloidogyne incognita* developed fast recently in the protected filed of Beijing and *Aspergillus niger*-Y61 have been displayed an antagonistic activity to inhibit the root knot nematode efficiently. The fermented broth of *A. niger*-Y61 were played a killing function with the root knot nematode when using it treat the soil and the potted test with fermented broth of Y61 presented an excellent killing ability on the nematode of *Meloidogyne incognita* J₂ and the highest exterminate mortality reached 99.75%; the inhibition rate of the hatching amount of egg masses by the method of immersion could be reached to 100% in 12 h; the Y61 broth treatment have no difference with pesticide of 33µg/ml 10% Fosthiazate tested on the protected filed of tomato, and the tomato roots treated with different concentration of broth of Y61 after seedling planting

showed both low disease index and low infection rate. At the spring season the inhibition rate were 52.5-94% from 5 times of broth, 71.3-88.1% from 10 times, and 92.3-99.6% from 20 times respectively; At the fall season the inhibition rate were 55.5-74.6% from 5 times of broth, 69.3-78.1% from 10 times, and 89.7-96.7% from 20 times respectively. The nematocidal substance produced by Y61 displayed a higher inhibit-ability to root knot nematode of *Meloidogyne incognita*.

Genetic variation and aggressiveness of *Sclerotinia sclerotiorum* in the United States

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The genetic variation and aggressiveness of *S. sclerotiorum* from crops in the United States was examined. Sixty nine isolates from 17 hosts were obtained from 14 states outside of the north central region. Isolates were evaluated for mycelial compatibility group and microsatellite haplotype. Fifty four mycelial compatibility groups (MCG's) were identified; 44 MCG's contained one isolate, eight contained two isolates, one had three isolates and one contained five isolates. These isolates were paired with 33 previously identified MCG's from the north central region and six of the previously identified MCG's were identified within the isolates. The isolates were genotyped with 11 microsatellite markers and 74 polymorphic loci were detected. Sixty six haplotypes were identified and specific haplotypes were associated with MCG's. Although most MCG's consisted of one haplotype, seven MCG's had multiple haplotypes varying from two to five. Aggressiveness of 65 isolates was tested on four crops, canola, dry bean, sunflower and soybean. A cut stem inoculation technique was used on five week old plants growing in the greenhouse. Canola and soybean were in one set of experiments and dry bean and sunflower were in another set of experiments and experiments were repeated. All isolates were pathogenic on the four crops. In the canola-soybean and dry bean-sunflower experiments there were significant differences ($P=0.05$) among isolates when averaged over crops.

Susceptibility of corn to stalk rot caused by *Fusarium graminearum* and mycotoxin mutants

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Fusarium graminearum is a destructive pathogen that infects corn and causes stalk rot. Stalk rot results in yield losses due to lodging, which limits production and harvesting of ears. In addition, high levels of mycotoxins in ethanol distillation byproducts makes infected tissues unfit for food or feed. Our objectives were to determine whether different corn varieties exhibit resistance to stalk rot, to establish if deoxynivalenol (DON) and zearalenone (ZON) mutants are pathogenic on a panel of diverse corn lines, and to quantify DON accumulation in infected stalks using targeted metabolomics. Wild-type *F. graminearum* and mycotoxin mutants (DON and ZON) were used to separately inoculate 9-week old plants of 20 corn varieties. Plants were evaluated for lesion area at 0, 2, 14 and 28 days post inoculation, then stalks were sampled to determine DON content. Regardless of their ability to produce DON or ZON, all tested *F. graminearum* strains caused stalk rot in plants with no significant differences observed in lesion size. Among the tested corn varieties, Mp717 was resistant to all three *F. graminearum* strains while Mp317 and HP301 were only partially resistant. Accumulation of DON was significantly lower in infected stalks of the resistant and partially resistant varieties than the susceptible ones. These findings are a first step towards breeding corn varieties suitable for planting in fields infested with *F. graminearum*.

WITHDRAWN

A new Raspberry bushy dwarf virus isolate from Ecuador exhibits an aberrant genetic feature

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Raspberry bushy dwarf virus (RBDV) is found naturally in *Rubus* spp. and recently in grapevine in Slovenia. The RBDV genome consists of two positive-sense single-stranded RNAs of about 5.4kb and 2.2 kb, respectively. The larger RNA encodes a putative polymerase protein, and the smaller RNA (RNA2) encodes the putative movement protein (MP) and the coat protein (CP); the CP is translated via a subgenomic RNA of approximately 0.9 kb. RBDV was reported recently from Andean blackberry in Ecuador. To obtain sequence information for this new isolate (RBDV-Ec), double-stranded RNA was extracted from infected plants and used as template for cDNA synthesis, cloning and sequencing. Partial nucleotide (nt) sequence of the replicase showed 95% similarity to the resistance breaking R15 strain. The MP and CP nt sequences were ~98% similar to a Slovenian RBDV isolate. An inverted fragment spanning 28 nt of the MP open reading frame (ORF), the intergenic region and approximately one third of the CP ORF was found concatenated to the 5' end of RNA2. Primers were designed to amplify a 725 nt fragment spanning across the normal 5' terminus and the atypical antisense region. Cloning and sequencing of PCR products of the expected size reconfirmed the existence of the sequence concatenation. This aberrant concatenation has been detected in RBDV-Ec from only one blackberry production area in Ecuador. A detailed examination of this unusual genetic feature will be presented.

Genome expression of soybean roots and leaves in response to *Fusarium virguliforme* toxins

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Sudden death syndrome (SDS) is caused by *Fusarium virguliforme* (Fv), a fungus that infects soybean roots and releases toxins that cause leaf scorching symptoms and defoliation. An RNA-sequencing technique was employed to study the genome expression of soybean roots and leaves in response to Fv toxins. Data from the susceptible genotype (Essex) was compared with those from the partial resistant genotype (PI 567.374) to identify differentially expressed genes. We identified 921 and 423 transcripts uniquely expressed in leaves and roots, respectively. Functional annotations based on sequence homology suggested that some of these unique genes likely encode proteins involved in signal transduction, cell-wall modification, detoxification, defense responses, primary metabolism, scavenging the oxidative stress and membrane transport. This complexity of different pathways suggests multiple mechanisms might be employed by soybean tissues to reduce damage caused by Fv toxins. Susceptible leaves, however, appear to respond to Fv toxins in a manner similar to an incompatible interaction to a biotrophic pathogen, where fungal toxins might induce the hypersensitive response pathways in the absence of the pathogen in these tissues. Genes identified in this study can be used to develop molecular markers that would be integrated into soybean breeding programs to accelerate breeding for resistance to both root rot and Fv toxins.

Induction of plant defense response by salicylic acid in perennial ryegrass turf against gray leaf spot caused by *Magnaporthe oryzae*

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A study was conducted to determine the salicylic acid (SA)-mediated defense response against gray leaf spot disease in perennial ryegrass turf. High performance liquid chromatography analysis of perennial ryegrass (cultivar Legacy II) leaf extracts showed that the basal level of endogenous salicylic acid is significantly low (41.12 ± 5.4 ng/g fresh weight) compared to the high amount reported in rice (5000-30,000 ng/g fresh weight). Following the inoculation of perennial ryegrass plants with *Magnaporthe oryzae*, the concentration of endogenous SA in perennial ryegrass increased over fivefold (>230 ng/g fw) within 72 hours. Exogenous application of 1.5 mM SA to perennial ryegrass reduced gray leaf spot incidence and severity by 31 and 35%, respectively. Similarly, 5 mM BTH (benzo(1,2,3) thiadiazole-7-carboxylic acid S-methyl ester), a chemical analogue of SA, also reduced gray leaf spot incidence by 50% and disease severity by 70%. This study indicates that SA-mediated defense response plays a role in protecting perennial ryegrass against gray leaf spot disease. The levels of expression of different pathogenesis-related (PR) genes associated with SA mediated suppression of gray leaf spot disease in perennial ryegrass will be further discussed.

Tomato early blight management by organically acceptable products and resistant varieties in West Virginia

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Early blight of tomato causes significant yield losses in West Virginia almost every year. Many small growers in the state do not want to use harsh chemicals. This study determined the efficacy of some OMRI approved products together with early blight (EB) resistant varieties, Mountain Fresh Plus F1 (MF+) and Juliet F1 at the WVU organic farm in a RCB design with four replicates. Plots having EB susceptible variety, Brandywine were treated with following products 1) Kocide-started at pre-bloom on a seven-day schedule; 2) Regalia + Kocide alternated by week starting at pre-bloom; 3) Kocide at first symptom appearance (seven-day interval afterwards); 4) Serenade and 5) Actinovate on a seven-day schedule starting at pre-bloom; and 6) Non-treated control. Early blight appeared later than normal due to a dry early summer and disease pressure was moderate. Later in the season, late blight appeared on MF+ and disease progressed very fast on this variety as well as on Juliet and non-treated Brandywine. So, late blight was also assessed on a 1-5 disease severity scale. All treatments except Actinovate and Kocide (on symptom appearance) significantly ($P \leq 0.04$) reduced EB severity compared to the non-treated control. Two EB resistant varieties did not get any EB but was severely affected by late blight. All treatments had lower late blight severity compared to the non-treated control and two EB resistant varieties.

WITHDRAWN

A field trial to evaluate HLB tolerance and resistance in *Citrus* and citrus relatives

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Citrus huanglongbing (HLB) is associated with the bacterial pathogen *Candidatus Liberibacter asiaticus* (Las) and spread by the psyllid vector *Diaphorina citri*. We conducted a field trial in a HLB-endemic area in Florida for four years using seedlings of 100 accessions of *Citrus* and relatives in the family Rutaceae (mainly sub-family Aurantioideae). Seeds were from the citrus variety collection, University of California, Riverside, CA and seedlings were planted in the field. Plants were naturally infected by Las through psyllid feeding. At 6 to 12 month intervals, leaf samples were tested for Las by qPCR targeting the 16 S rDNA and prophage regions of the Las genome. Disease status based on symptom expression was recorded for four years. Seven categories were identified with no. 1 being the most susceptible and no. 7 being the most resistant. Various levels of tolerance were observed. While all accessions belonging to the genus *Citrus* were susceptible to HLB, the Las bacterium was not detectable in 20 accessions of related genera for four years. These include many accessions of *Poncirus trifoliata* and some of its hybrids, some species of *Bergera*, *Casimiroa*, *Clausena*, *Eremocitrus*, *Glycosmis*, *Microcitrus*, *Murraya*, *Naringi*, and *Zanthoxylum*. Resistance to HLB in the citrus gene pool may prove invaluable in intragenic transformation and/or conventional breeding. The potential resistance mechanisms are under investigation.

Cell wall changes in an endocytosis mutant of *Neurospora crassa*

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Morphogenesis in filamentous fungi depends principally on the establishment and maintenance of polarized growth, which is accomplished by the migration and discharge of exocytic vesicles carrying cell wall components. We have been searching for evidence that endocytosis, an opposite process, could play a role in morphogenesis. Previously, we found that coronin deletion (*Neurospora crassa* mutant, *Acrrn-1*) causes a decrease in endocytosis, leads to interspersed periods of polarized and isotropic growth of the hyphae and irregularities in cell wall thickness. We used CRIB fused to GFP as an exocytic reporter. By confocal microscopy, we found that CRIB-GFP was present in wild-type hyphae as a thin hemispherical cap under the apical dome. In the *Acrrn-1* mutant, CRIB-GFP migrated to the subapical region and appeared as localized patches. Significantly, cell growth occurred in the places where CRIB-GFP was accumulated, thus the erratic location of the reporter in the *Acrrn-1* mutant correlated with the morphological irregularity of the hyphae. We found that the *Acrrn-1* mutant had a higher proportion of chitin than the WT strain (14.1% and 9.1% respectively). We compared the relative cell wall area (TEM images) and we found a different ratio wall/cytoplasm between the *Acrrn-1* mutant and the WT strain. In conclusion, we have found that the mutant affected in endocytosis has an altered pattern of exocytosis as evidenced by its distorted morphology and displaced exocytic markers.

Characterizing DAMP induced innate immune responses in rice

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Xanthomonas oryzae pv. *oryzae* (Xoo), the bacterial blight pathogen of rice, secretes several cell wall degrading enzymes including a Lipase/esterase (LipA). It has been shown that prior treatment of rice tissues with purified LipA enhances resistance against subsequent infection. LipA activity releases cell wall degradation products which serve as DAMPs (damage associated molecular patterns) and induce rice innate immune responses. In order to characterize the rice genes involved in LipA induced innate immune responses, transcriptional profiling using Affymetrix GeneChip arrays was performed with RNA isolated from leaves at 30min, 2h & 12h following enzyme treatment. 40 and 1555 genes were differentially expressed (≥ 1.5 fold) at 2h and 12h time points respectively, with no change at 30 min. A number of defense and stress response genes are up regulated while genes involved in metabolism and photosynthesis are down regulated. The data suggests up regulation of Jasmonic acid and down regulation of Salicylic acid biosynthesis and responsive genes. Some of the upregulated genes at the 2h time point are predicted to encode a Wall Associated Receptor Kinase like 21 (*OSWAKL21*), Cysteine Rich Receptor kinase (*OsCRRK*), a U3 Ubiquitin ligase (*OsUbox*) & a WRKY transcription factor (*OsWRKY*). Transient over expression of these genes results in enhanced resistance against Xoo infection.

This study provides new insights into rice functions involved in DAMP induced innate immunity.

The role of the bacterial cell surface lipopolysaccharide in grapevine colonization and insect acquisition of *Xylella fastidiosa*

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Xylella fastidiosa (*Xf*) is a gram-negative, xylem-limited bacterium that causes serious diseases in economically important crops, such as Pierce's Disease (PD) of grapevine. Bacterial cell surface lipopolysaccharides (LPSs) comprise approximately 75% of the bacterial outer membrane and mediate interactions between the bacterial cell and its surrounding environment. LPS is composed of a conserved lipid A-core oligosaccharide component and a variable O-antigen. We are investigating the role of the terminal O-antigen of *Xf* LPS in colonization of the grapevine host and bacterial acquisition by insect vectors. By targeting a key O-antigen biosynthetic gene, we demonstrate the contribution of the O-antigen to surface attachment, cell-cell aggregation, and biofilm maturation; all are critical steps for successful infection of the host xylem tissue. We also show that LPS is a major virulence factor for this bacterium. In addition, depletion of the O-antigen compromises the ability of *Xf* to be acquired by its insect vector.

Nutritional capability and substrate suitability of psychrophilic species of *Geomyces* from the United States

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Geomyces destructans, the causal agent of Bat White Nose Syndrome, was first discovered in New York State in 2006 and has been implicated in the deaths of nearly six million bats as its distribution continues to spread throughout the United States and Canada. Recently, numerous *Geomyces* species have been isolated directly from bats and from cave soil. The objective of this research is to understand the nutritional capabilities (carbons and nitrogen) and substrate suitability (water potential) for the various *Geomyces* species to ascertain biological and reservoir requirements. Currently thirty-three *Geomyces* isolates, including three *Geomyces destructans* isolates, are being assayed for various enzymatic activities including lipases, esterases, proteinase, chitinase, keratinase, and cellulases. Preliminary data indicate that only 36% of the *Geomyces* isolates demonstrated chitinase activity within four weeks, and several isolates displayed keratinase activity at pH 10.8. All isolates demonstrated varying degrees of lipase, esterase, gelatinase, and β -glucosidase activity as well as Class 2 nitrogen utilization with increased growth on all sole nitrogen sources (urea, nitrate, nitrite, L-alanine, and ammonium) at pH 7 to pH 8. The majority of *Geomyces* isolates (60%) appeared to be intolerant to matrix-induced water stress with maximum growth between -1.0 MPa and -1.75 MPa, which is typical of most soil fungi.

A method for detecting *Xanthomonas cucurbitae* in pumpkin seed

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A method was developed to detect *Xanthomonas cucurbitae*, the causal agent of bacterial spot of cucurbit, in pumpkin seed. Pumpkin seeds were collected from symptomatic fruit from 20 pumpkin fields in Illinois in 2010 and 2011. From each field, 3,000 seeds were tested for presence of *X. cucurbitae* on seeds. For each test, 600 seeds were added to a flask containing 500 ml of 0.75% NaCl and 0.02% Tween 20, and incubated at 5°C on a shaker (120 rpm) for 12 hr. The seed wash was centrifuged (16,000 x g) for 5 min. The pellet was re-suspended in 1 ml sterilized distilled water, diluted 10^{-2} to 10^{-4} , and 100 μ l of each dilution was streaked onto Kasugamycin-cephalexin agar medium in each Petri plate. Developing bacterial colonies were examined and single-cell cultures were prepared by streaking colonies onto Lauria-Bertani agar medium in Petri plates. In another experiment, 3,000 seeds were surface disinfested by soaking in 0.05% sodium hypochlorite for 1 min. Seeds were dried and their shells were removed in a flow hood. The kernels were tested for the presence of *X. cucurbitae* using the method described above. Collected bacterial isolates were identified as *X. cucurbitae* based on the morphological characteristics on yeast extract-dextrose agar and PCR, followed by a

pathogenicity test on pumpkin cultivar Howden. *X. cucurbitae* was detected in 12 of 20 seed lots tested. Also, *X. cucurbitae* was detected in the kernels of all three seed lots tested.

Pathogenic and genetic variations among isolates of *Xanthomonas cucurbitae* from Illinois

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To assess pathogenic and genetic variation among isolates of *Xanthomonas cucurbitae*, 994 *X. cucurbitae* isolates were collected from symptomatic leaves and fruit in commercial fields in Illinois during 2009-2011. Sixty-seven isolates, including 12, 26, and 29 isolates from 2009, 2010, and 2011, respectively, were tested for their pathogenicity. Each *X. cucurbitae* isolate was grown on Luria-Bertani agar medium, and an inoculum (5×10^7 CFU/ml) in 0.1 M phosphate buffer (PBS) was infiltrated into leaves of the pumpkin cultivar Howden at 3- to 4-leaf growth stages. Development of symptoms (water-soaked chlorotic and necrotic lesions) was assessed 3 to 5 days after inoculation. Virulence of the isolates was assessed based on the period for appearance of the symptoms after inoculation. The isolates were different significantly ($P = 0.05$) in virulence, and five isolates from five different locations in Henry, Jefferson, Kankakee, and Putnam Counties were the most virulent. Genetic variation among 82 isolates was evaluated using restriction associated DNA sequencing (RADSeq) markers. Whole genomic DNA of each isolate was extracted and digested with *SacI*/*MspI* restriction enzymes. Resulting fragments were ligated to two double stranded oligonucleotide adapters. A pool of the 200 to 500bp DNA fragments from each isolate was prepared and 100bp of the pooled DNA was sequenced from one end by 100nt. Analysis of the sequenced reads showed genetic difference among the isolates.

Complete genome sequencing and comparative analysis of *Pseudomonas syringae* pv. *syringae* strains B301D and HS191

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Complete genomes of *Pseudomonas syringae* pv. *syringae* strains B301D and HS191 were generated by 454 pyrosequencing and Illumina technology. Strain B301D was isolated in 1959 from a pear tree in England, and strain HS191 was isolated in 1962 from proso millet in Australia. The genomes of these strains are closely related to a U.S. bean strain, B728a, previously shown to have a 6.09 Mb genome. Newbler 2.0 software was used for *de novo* assembly of 454 sequences reads into contigs and scaffolds. Illumina sequences were assembled (CLC Genomics Workbench 4.9) and the contigs were aligned to 454 scaffolds to close the gaps. Optical maps (OpGen technologies) of the *KpnI* restriction sites in the genomes of B301D and HS191 were used to align the scaffolds. PCR amplification and sequencing were used to close the gaps between scaffolds. After genome assembly, the IMG/ER system (DOE Joint Genome Institute [JGI]) was used for annotation. The JGI GenePRIMP pipeline was used to identify misannotated genes, which were corrected using Artemis. The finished B301D genome is 6.09 Mb in size, whereas the HS191 genome is 5.94 Mb in size and contains a 52 kb plasmid. Multilocus sequence analysis of 10 housekeeping genes demonstrated that the B301D genome is more closely related to B728a than to HS191. Comparative genome analysis was used to identify differences in genes associated with plant pathogenesis, including genes for type III effectors and toxin synthesis.

Fungi and oomycete pathogens causing stem blight and root rots on blueberry in central Mexico

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Blueberry (*Vaccinium* spp.) is becoming and important crop in central Mexico, mainly in the state of Jalisco and Michoacan. In the last 5 years, blueberry cultivated area has increased from 200 to nearly 2000 ha. Although this crop is relatively recent, fungal and oomycete pathogens are becoming a problem. In order to identify the causal agents of stem cankers, stem blight and root rots, symptomatic samples from both states were collected during 2010, 2011 and 2012 seasons and the associated fungus and oomycetes isolated on PDA and V8 media. Isolated fungi and oomycete were identified by their morphological features and confirmed by PCR, amplifying the complete ITS region of the rDNA with ITS5/ITS4 set of primers. Sequences

were compared with those deposited in GenBank. Pathogenicity of fungus and oomycetes were confirmed by direct inoculation on root, crown and stems of blueberry plants cv Biloxi in the greenhouse. Results indicated that stem cankers and cane blights were caused by *Neofusicoccum parvum*, *Lasiodiplodia pseudotheobromae*, *Pestalotiopsis photiniae*, *Pestalotiopsis microspora*, and root rot and crown was caused by *Phytophthora cinnamomi*. This information will be useful to design better disease management programs from nursery to the field.

WITHDRAWN

Fungi isolated from four ambrosia beetle species emerged from stressed black walnut

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Attacks of black walnut by walnut twig beetles (*Pityophthorus juglandis*) can introduce *Geosmithia morbida* into entry tunnels and egg galleries in the inner bark. Thousand Cankers Disease forms when numerous bark cankers caused by *G. morbida* coalesce. It is not known what other bark and ambrosia beetles may vector *G. morbida* or other deleterious fungi. Consequently, we collected bark and ambrosia beetles from stressed black walnut trees and characterized their fungal associates. More than 100 trees in Indiana and Missouri were girdled and treated with glyphosate. Stem and branch sections were placed in insect emergence buckets. Over 16,000 beetles representing 15 ambrosia and 4 bark beetle species were collected upon emergence. The most abundant beetle species collected were *Xyleborinus saxesenii*, *Xylosandrus crassiusculus*, *Xylosandrus germanus*, and *Xyleborus affinis*. No *P. juglandis* was detected. Fungi carried by these beetle species were isolated by serial dilution plating of macerated beetle suspensions. Between 44 and 56 fungal taxa were associated with each assayed beetle species, and included ascomycetes, basidiomycetes and zygomycetes. Putative tree pathogens associated with > 10% of each beetle species included *Fusarium solani* and *Phaeoacremonium* spp. The blue-stain fungus, *Graphium penicilloides* and a red stain fungus, *Scytalidium cuboideum* were also commonly isolated. Two beetle species yielded *Geosmithia pallida*, a close relative of *G. morbida*.

Survey tools for begomovirus and curtovirus detection: A flexible and sensitive approach to virus screening

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Whitefly-transmitted begomoviruses and leafhopper-transmitted curtoviruses (family *Geminiviridae*) have threatened production yields of tomato, bean, cucurbit, and cotton crops for decades. The emergence of geminivirus recombinants and re-emergence of known viruses due to changes in vector

upsurges and distribution have necessitated the development of broader tools for detection. Begomovirus-specific PCR primers were developed based on a conserved region of the coat protein gene (final product, 533-536 bases). Curtovirus-specific PCR primer pairs were developed based on a unique region of the *Beet curly top*, *Spinach curly top Arizona*, and *Spinach severe curly top* viral genome sequences, that were also conserved in other known curtovirus genomes. Begomovirus- and curtovirus-specific chemiluminescent dot blot hybridization assays were developed using PCR amplified products from sets of phylogenetically representative virus species into which was incorporated digoxigenin-dUTP, making a cocktail of labeled probes specific for members of each genus, respectively. PCR amplification and the hybridization assays were used to screen collections of geminivirus-free (healthy) plant leaf tissue and the cloned viral species, homologous and heterologous to each probe, to establish probe specificity and the range of detection. The high-throughput hybridization and sequencing-capable PCR assays are useful tools in an integrated approach to geminivirus disease management.

Field screening of soybean lines for Cercospora leaf blight resistance

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Cercospora leaf blight (CLB) and purple seed stain caused by the fungus *Cercospora kikuchii* cause substantial yield loss in the United States. Identification of soybean varieties with resistance to CLB is crucial for the control of this disease. In this study, twenty different soybean varieties were screened for their level of CLB resistance under field conditions in Louisiana in 2011 using complete randomized block design. In 2012, fourteen varieties which showed the most and least susceptibility to CLB in the 2011 were replanted using the same field design. Soybean leaf samples were collected biweekly for the first four weeks after planting, then weekly until late R6. Twenty leaves were collected randomly from each plot, visually inspected for disease symptoms, and examined for the presence of *C. kikuchii* and its level using quantitative real-time PCR (qPCR). Positive *C. kikuchii* growth was detected in some varieties two weeks after planting, suggesting the seed is likely one of the sources of inoculum. Visible CLB symptoms were first observed at the late R5 and early R6 stages. Although both qPCR and visual ratings identified several soybean lines as the most and least susceptible to CLB, there is a significant difference in CLB development between the two years, and a high variation of fungal growth between collection dates. This suggests that fungal growth and CLB development in soybean is influenced by variety, environment and possibly other unknown factors.

Identifying genes involved in asexual reproduction in *Fusarium graminearum* using the developmental mutant, 8B5

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Fusarium graminearum causes Gibberella ear and stalk rot of maize and *Fusarium* head blight of wheat and barley. In addition to reducing yields, *F. graminearum* contaminates infected grain with mycotoxins. In *F. graminearum*, multicellular macroconidia play important roles in infection and dissemination. We recently identified a tagged, insertional mutant of *F. graminearum*, designated 8B5, that fails to produce macroconidia in most conditions. In culture, 8B5 forms morphologically abnormal colonies with increased aerial hyphae and a distinctive fluffy phenotype. Additionally, virulence was significantly reduced on wheat heads and maize silks. We determined 8B5 contains an insertion within a putative bi-directional promoter, resulting in the up-regulation of both genes. To understand the impact of the insertion on gene regulation, genome-wide microarray analyses were performed under conditions that promoted or suppressed asexual development. A total of 39 genes, including genes with putative transcription factor, transporter, or catalytic activities, were differentially expressed in 8B5 versus the wild-type strain under conditions favorable for asexual reproduction. Together, these results suggest that the mutation in 8B5 significantly impairs asexual and infectious development in *F. graminearum*, and thus the 8B5 strain represents a valuable tool to further unravel the genetic regulation of pathogenesis.

Effects of fumigation and bactericide application on the decontamination of *Salmonella enterica*

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Several outbreaks of salmonellosis have been traced back to contaminated tomatoes. Pre-harvest has been proposed to be one of the main stages of original contamination; however, little is known about the effects of production practices on *Salmonella* decontamination. In this study, impacts of fumigant and bactericide applications on *Salmonella* reduction were evaluated. To assess the fumigation effect, sandy loam soils were inoculated with *Salmonella* Newport strain J1892 or Typhimurium strain ATCC 14028 to reach a population density of 10^6 CFU/g, and treated with fumigants (chloropicrin, metam sodium, dimethyl disulfide, or 1,3-dichloropropene). To investigate pesticide effect, eight-week tomato plants were inoculated with the same *Salmonella* strains by dipping tomato leaves in bacterial solution of 10^8 CFU/ml. Inoculated plants were treated with four pesticides with different action mode (Kocide 3000, Actigard 50WG, Firewall 22.4WP, and Oxidate 27L). Fumigants and bactericides were applied at equivalent maximum application levels in fields. Sterile water was used as control. *Salmonella* population was measured by plate counting method. Fumigation using 1,3-dichloropropene or dimethyl disulfide can benefit the decontamination of *Salmonella* in soils. Oxidate and Firewall reduced *Salmonella* population on the leaf surface, while Kocide reduced *Salmonella* population in inoculated tomato leaves. These results would bring clues to reduce *Salmonella* contamination on tomatoes.

Comparative analysis of 35 basidiomycete genomes

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Fungi of the phylum Basidiomycota (basidiomycetes), make up some 37% of the described fungi, and are important in forestry, agriculture, medicine, and bioenergy. This diverse phylum includes symbionts, pathogens, and saprobes including wood decaying fungi. To better understand the diversity of this phylum we compared the genomes of 35 basidiomycete fungi including 6 newly sequenced genomes. The genomes of basidiomycetes span extremes of genome size, gene number, and repeat content. Analysis of core genes reveals that some 48% of basidiomycete proteins are unique to the phylum with nearly half of those (22%) comprising proteins found in only one organism. Phylogenetic patterns of plant biomass-degrading genes suggest a continuum rather than a dichotomy between the white rot and brown rot modes of wood decay among the members of the Agaricomycotina subphylum. There is a correlation of the profile of certain gene families to nutritional mode in Agaricomycotina. Based on phylogenetically-informed PCA analysis of such profiles, we predict that that *Botryobasidium botryosum* and *Jaapia argillacea* have properties similar to white rot species, although neither has ligninolytic class II fungal peroxidases. Furthermore, we find that both fungi exhibit wood decay with white rot-like characteristics in growth assays. Analysis of the rate of discovery of proteins with no or few homologs suggests the high value of continued sequencing of basidiomycete fungi.

Expression of germin-like protein genes in response to *Sclerotinia homoeocarpa* infection

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Dollar spot, caused by fungal pathogen *Sclerotinia homoeocarpa*, is one of the most economically important diseases of amenity turfgrasses worldwide. In spite of this, very little is known about the interactions between *S. homoeocarpa* and its hosts at the molecular level. In the present research, germin-like protein (GLP) family genes were evaluated for their role in defense against *S. homoeocarpa*. Specific GLP genes of rice and barley have demonstrated defense roles against other phytopathogenic fungi. A growth chamber assay was conducted on three creeping bentgrass (*Agrostis stolonifera* L.) cultivars with varying levels of susceptibility to dollar spot. Pots of each cultivar were inoculated with either four day-old plugs of *S. homoeocarpa* or plugs of PDA and arranged in a 2x3 factorial within an RCBD with four replications. Tissue for RNA extraction and RT-qPCR analysis was harvested from the site of inoculation before and at 24 and 96 hours after inoculation. Results from RT-qPCR analysis indicated that all GLP genes investigated were significantly up-regulated in inoculated versus mock-inoculated plants at 96 hpi, regardless of host cultivar, but

were not up-regulated at 24 hpi. Gene silencing techniques are now being employed to investigate the specific role of GLP genes in defense against *S. homoeocarpa*. This research will be useful for understanding molecular aspects of host defense against *S. homoeocarpa* and breeding for dollar spot resistance.

Seasonality and prevalence of *Asellaria jatibonicua* in terrestrial isopods

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The trichomycetes are microorganisms associated with the gut of arthropods. They are an ecological group composed of protists (Ichthyosporae) and fungi (Kickxellomycotina) that converged in a similar life style evolutionary solution with key adaptations to their gut environment. We are conducting a prevalence study of the endemic species *Asellaria jatibonicua* (Kickxellomycotina: Asellariales) infection in the terrestrial isopod *Lithorhopilosia culebrae*. The goal of the study is to determine seasonality and prevalence of *A. jatibonicua* in this specific host since no information is available for any of the Asellariales. Isopods were collected at the University of Puerto Rico, Mayagüez campus and dissected in the laboratory the same day. Slides were mounted in ddH₂O and then fixed with 0.05% lactophenol-cotton blue. Current data indicate a prevalence of 20% and 36% in *L. culebrae* for the summer (Aug 12 – Sep 20) and fall months (Sep 21 – Dec 20), respectively. In addition, during this study period we found another trichomycete in the same host, *Parataeniella* sp. (Ichthyosporae: Eccriniales), which represents a first record for Puerto Rico and possibly a new species.

Response of *Medicago truncatula* accessions with differing levels of triterpene saponins to infection by the necrotrophic fungus, *Phoma medicaginis*

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The necrotroph *Phoma medicaginis* causes disease on some legumes, such as alfalfa (*Medicago sativa*), and the barrel medic (*M. truncatula*). Plant secondary metabolites, e.g. triterpene saponins, can have suppressive effects on some fungal pathogens. The objective of this study is to determine the susceptibility of *M. truncatula* accessions with varying concentrations of foliar saponins to *P. medicaginis* infection. Four *M. truncatula* accessions with varying saponin profiles were inoculated with *P. medicaginis*. Ratings were assigned to plants according to a 1 to 4 rating scale. Accession A17 has high saponin levels in foliar tissue, and initial experiments indicate A17 is susceptible to *P. medicaginis*. However, accession ESP105, which has relatively low levels of foliar saponins, is equally susceptible to *P. medicaginis*. Accessions PRT178 and GRC43 have high foliar saponins levels and both accessions are significantly more resistant to *P. medicaginis* infection than the other two accessions. Isolates of *P. medicaginis* constitutively expressing GFP are being used to track fungal growth on leaves and reveals the presence of hyphal growth on all accessions tested. With the exception of line A17, which is derived from a commercial cultivar, there is a trend for accessions with high concentrations of saponins to be more resistant to *P. medicaginis*. However, the accessions tested vary in many genetic attributes and saponin concentrations are just one area in which they differ.

A *vfr* homologue of *Pseudomonas protegens* Pf-5 regulates antibiotic production and traits important to root colonization

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We have been using a mutational approach to determine how beneficial bacteria, such as *Pseudomonas protegens* Pf-5, colonize plants and suppress disease. Vfr, a cyclic-AMP binding protein in *Pseudomonas aeruginosa*, regulates traits potentially important to plant-associated activities. Mutant Vfr-B6, a derivative of Pf-5 containing a mutation in *vfr*, was constructed to test the role of Vfr in regulation of traits important to plant-beneficial

activities by *P. protegens*. Vfr-B6 was impacted in the production of a number of antibiotics. Vfr-B6 produced 100-fold more diacetylphloroglucinol than the wild-type, Pf-5, and was reduced in production of rhizoxin. Vfr-B6 was also differentially impacted by nutrient source with regard to production of orfamide and pyrrolnitrin. Vfr-B6 was substantially reduced in colonization of cucumber rhizosphere relative to Pf-5 in three soils and slightly reduced in colonization in potting mix. Preliminary proteomic analysis of Vfr-B6 grown on synthetic cucumber root exudates showed a role for Vfr in polyamine uptake and transport of glycine-betain, compounds associated with survival and colonization of the rhizosphere. Synthetic cucumber root exudate consists of a complex mixture of reduced carbon compounds supplied in the same relative proportions as detected in cucumber root exudate. These studies suggest a role for the global regulator Vfr in disparate traits important to colonization and disease suppression by *P. protegens*.

Impact of antimicrobial compounds on etiolation of creeping bentgrass putting green turf

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Etiolation of turfgrass is a widespread problem with bacteria playing a significant role. Chemical control of bacteria in turfgrass systems is limited and little is known on how labeled turf products may influence etiolation. In 2012, research was initiated on creeping bentgrass (*Agrostis stolonifera* L. cv. 'Dominant') in Raleigh, NC to examine the impact of the antimicrobials oxytetracycline, streptomycin sulfate, chlorothalonil+acibenzolar S-methyl (CA), potassium phosphite, hydrogen dioxide, fosetyl-Al, mancozeb+copper hydroxide (MCH), and non-treated. Treatments were arranged in a randomized complete block design on turf maintained as a putting green. Etiolation developed naturally and *Xanthomonas translucens* was isolated multiple times throughout the season. Overall, oxytetracycline provided the greatest reduction in etiolation, but was significantly less than the non-treated on only three dates. Streptomycin sulfate and MCH did not reduce etiolation compared to non-treated. Both antibiotics caused significant phytotoxicity that recovered quickly whereas phytotoxicity from use of MCH continued to reduce turf quality. Applications of CA resulted in more etiolation, but were significantly higher than the non-treated on only one date. Additionally, CA applied alone and with potassium phosphite often resulted in the best turf quality. All other treatments were often statistically similar for etiolation and turf quality.

Eicosapolyenoic fatty acids induce resistance in tomato to root and crown infection by *Phytophthora capsici*

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Arachidonic (AA) and eicosapentaenoic acids are common fatty acids in oomycetes that can function as elicitors, or PAMPs, to trigger immune responses in plants. These eicosapolyenoic acids (EP) are released from *Phytophthora* species during infection of plants, which normally do not contain them. Pre-treatment of tomato roots with EP induces resistance to *Phytophthora capsici*, and primes the roots and crowns to respond with rapid lignification while reducing crown rot and shoot collapse. AA also induces callose deposition in lateral root tips. These responses are not induced by water, linoleic acid (LA) or α -linolenic acid. Research has indicated the importance of lipoxygenase(s) with specific regiospecificity and oxylipin metabolism in signal-response coupling during EP action. EP may elicit increased activity of 9-oxylipin pathway enzymes in tomato roots, including 9-lipoxygenase, 9-divinyl ether synthase (9-DES) and 9-allene oxide synthase. 9-DES gene expression is strongly induced in tomato roots treated with AA but not with LA or water. Further characterization of immune responses induced by EP in tomato and alterations in oxylipin metabolism in EP-treated roots will be presented.

Cauliflower mosaic virus P6 inclusion bodies at the door: Their association with plasmodesmata during expression in *Nicotiana benthamiana*

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The P6 protein of *Cauliflower mosaic virus* (CaMV) is responsible for the formation of inclusion bodies (IBs), which are thought to be the site for viral gene expression, replication and particle assembly. Moreover, recent evidence indicates that P6 IBs move in association with actin microfilaments. Since CaMV virions accumulate preferentially in P6 IBs, we hypothesized that P6 IBs have a role in delivering CaMV virions to the plasmodesmata. We recently discovered that the P6 protein interacted with a C2 Calcium-Dependent Membrane Targeting protein (C2CDMT) in a yeast two-hybrid screen and we confirmed this interaction through transient expression assays in the CaMV host, *Nicotiana benthamiana*. A C2CDMT-RFP fusion localized to the plasma membrane and specifically associated with plasmodesmata. The C2CDMT-RFP fusion also co-localized with two proteins previously shown to associate with plasmodesmata: the host protein PDL1 and the CaMV movement protein (MP). To investigate whether P6 IBs were located at plasmodesmata, we examined the co-localization of P6-GFP IBs with PDL1, the CaMV MP, and aniline blue, a chemical stain for callose, and found that P6-GFP IBs were associated with each of these markers. Our evidence that P6-GFP IBs associate with C2CDMT-RFP at plasmodesmata provides further support for our model in which P6 IBs function to transfer CaMV virions to plasmodesmata.

In vitro evaluation of endophytic fungi from *Alnus acuminata* as antagonists of *Fusarium oxysporum* and *Botrytis cinerea*

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Endophytic organisms live in plant tissues without causing any apparent damage to the host, some of these organisms benefit the plant by producing secondary metabolites, which prevents the growth or activity of pathogenic microorganisms and insects such as aphids. Based on this information, endophytic fungi were isolated from leaves of *Alnus acuminata*, and were evaluated *in vitro* against *Fusarium oxysporum* and *Botrytis cinerea*. Six endophytic fungi were isolated, all of them belonging to the genus *Trichoderma*. All morphospecies showed antagonistic activity between 38.3% - 55.9% for *F. oxysporum* and 55.4% - 62.7% for *B. cinerea*. Also the four isolates, that showed the highest levels of antagonistic activity, were used in an experiment which aim was to explore the basis of the antagonistic activity. After cultured them in Czapeck broth for 15 days, the cultures were centrifuged, and extraction procedures using ethyl acetate were performed. Two different extraction were completed, one from the supernatants and another from the pellets. Antifungal activity of extracts was evaluated *in vitro* against *Fusarium oxysporum*. Dose-response curve was obtained for all the extracts, resulting that only one from pellets showed antifungal activity against *F. oxysporum* while those extracts obtained from the supernatants were all active against the same fungus. These results point to considerate that the factor responsible for the antifungal activity is a diffusible product.

***Fusarium decemcellulare* associated with cushion gall and dieback of tropical trees in Brazil and Mexico**

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Fusarium decemcellulare is reported as a saprophyte associated with soil and plant material, but also as a pathogen of tropical fruit trees, causing cushion galls or stem dieback. The objectives of this study were to characterize isolates of *F. decemcellulare* from different hosts and substrates by means of laboratory crosses, phylogenetic analyses, and pathogenicity tests. Among 67 isolates evaluated, nine were homothallic and produced four ascospores per ascus. The mating types of the remaining isolates were determined and those of opposite types were crossed using each isolate as a female and male parent. Approximately 1590 crossings were performed, and 54 were fertile yielding eight ascospores per ascus. Two isolates from Mexico formed fertile perithecia when crossed with three of the Brazilian isolates, showing that there is no reproductive barrier between these populations. Phylogenetic analyses performed with partial sequences of four genes (*tef-1a*, *rpb2*, *act1* and ITS+28S rDNA) grouped heterothallic isolates obtained from cocoa, mango and *Paullinia cupana* in one distinct clade. Homothallic isolates formed four distinct phylogenetic lineages. Selected heterothallic strains, obtained from cocoa and mango, induced gall symptoms in inoculated cocoa plants. Our findings show that isolates associated with galls belong to a distinct phylogenetic and biological species. There is evidence that *F. decemcellulare* represents a species complex. (Funding: CNPq ED 15/2009)

Diversity of oomycetes associated with soybean seedling diseases in the U.S.

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Oomycete pathogens of soybean result in low stand establishment and root rot often reducing yield. There is limited information regarding abundance and diversity of oomycetes species across most soybean producing states. Two-year survey was conducted across 11 states to determine diversity of oomycetes associated with seedling diseases. In 2011, a total of 2400 isolates were collected on the semi-selective medium (CMA-PARP). In 2012, a second semi-selective medium (V8-RPBH) was included, but due to drought and limited symptomatic fields the number of recovered isolates was 1100. Isolates were identified using the ITS region of rDNA and GIS data was used to find association between species and environmental data. In 2011, preliminary sequence results distinguished a total of 52 *Pythium*, 2 *Phytophthora* and 3 *Phytophthora* spp., with *Py. sylvaticum* (16%) and *Py. oopapillum* (12%) being the most frequently recovered. In 2012, a total of 57 *Pythium*, 7 *Phytophthora*, and 4 *Phytophthora* spp. were found, with *Py. sylvaticum* (15%) and *Py. heterothallicum* (13%) species being most abundant. Oomycete species composition frequency was noted to fluctuate greatly; the resulting species diversity was correlated with latitude and temperature gradients. Seed and seedling rot assays were conducted to determine the pathogenicity/virulence of the different species. This study will serve as the foundation for the development of diagnostic tools and the improvement of soybean root health.

Characterization of *Stenocarpella maydis* mutants

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Stenocarpella maydis is a major fungal pathogen of Midwest corn, causing both ear and stalk rot diseases. To better understand the molecular mechanisms that regulate development and metabolism in this fungus, we screened 1,000 insertion mutants generated by *Agrobacterium tumefaciens*-mediated transformation (ATMT). The screen examined pycnidia production, growth, and secondary metabolism, especially the production of diploidiatoxin. Here we describe the characterization of a mutant (strain 174) that secretes an unidentified green metabolite into droplets on aerial mycelia. Production of the metabolite is under circadian rhythm control, requiring a light-dark cycle. Strain 174 produces normal pycnidia, is pathogenic to developing corn ears, and produces diploidiatoxin. Southern analysis indicated that strain 174 contains a single copy of insertion T-DNA. DNA sequence at the border of the insertion site was obtained by thermal asymmetric interlaced PCR (TAIL-PCR). DNA sequence alignment of the TAIL-PCR product to a whole genome assembly database of *S. maydis* identified Contig 3148, which is 12 kb. Subsequent PCR analysis verified the site of the T-DNA insertion at the locus corresponding to this contig. Furthermore, the T-DNA landed 5' to a putative gene encoding a histidine kinase. Our current goal is to determine if this histidine kinase regulates the production of the green metabolite.

Characterization and field detection of *Cryptococcus flavescentis* strains, biocontrol agents against *Fusarium* head blight of wheat

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Cryptococcus flavescentis strain OH182.9_3C (3C) previously exhibited remarkable biological control efficacy against *Fusarium* Head Blight (FHB), a globally important disease of wheat. Multilocus sequence typing was performed on 3C and 11 other *Cryptococcus flavescentis* strains using loci within the genes of β -tubulin, Chitin Synthase 1, Elongation Factor 1, Heat

Shock Protein 70 kDa and Internal transcribed Spacer regions. Two genotypes, 3C type (5 strains) and non-3C type (7 strains), were revealed by all the four protein-encoding genes with high bootstrap support. Phenotypic assays using Biolog showed all the strains of non-3C type as one distinct group and slow assimilation of several carbon sources by two strains of 3C type. 3C-type strains showed higher biocontrol efficacy than non-3C type in a green-house bioassay against FHB. To assess the environmental risk of 3C as a biopesticide, a quantitative PCR (qPCR) assay of SYBR® Green chemistry targeting a Heat Shock Protein 70 kDa gene was developed and applied to monitor the population dynamics of 3C-like *C. flavescentis* in wheat fields and on harvested grains. qPCR results demonstrated that 3C was able to colonize inoculated wheat heads in field at the magnitude of $10^{4.5}$ to $10^{6.5}$ target gene copies per gram wheat, which was comparable to the inoculation rates. 3C population dispersed from inoculated areas to non-inoculated areas in a stochastic fashion during the growing season.

Metagenomic approaches for surveying forest soil microbial communities on permanent plots

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Soil microbes are primary drivers of forest ecosystem processes and play important roles in nutrient cycling, soil structure formation, decomposition, detoxification, disease suppression, and regulating plant productivity and diversity. Metagenomics allow us to study forest soil microbial communities in full via direct DNA sequencing and subsequent analysis of the metagenome. The value of metagenomic information relies on associated metadata that provide a context for interpretation. The Forest Inventory and Analysis (FIA) permanent plot network provides an ideal setting for conducting metagenomic studies because plots cover diverse geographic areas and widely ranging ecosystems and each is associated with long-term biological, physical, and historical data. Our objective is to evaluate the utility of metagenomics to characterize soil microbial communities within a small subset of FIA plots established in the Priest River Experimental Forest, Idaho, USA. DNA isolated from soil cores taken from replicates of contrasting habitat types will be analyzed via shotgun metagenomic sequencing on the Illumina HiSeq 2000 using 100 bp paired-end processing. Results will reveal if and how forest soil microbial communities differ among habitat types, both compositionally and functionally. Keying in on differentially represented microbial taxa, functions, and metabolic pathways is a first step toward understanding how these communities regulate ecological processes.

Tracking the distribution of the pandemic *Puccinia psidii* genotypes

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Puccinia psidii is a rust pathogen hypothesized to be of neotropical origin with a wide host range within the family Myrtaceae. Microsatellite markers were used to infer genetic relationships among pathogen samples from South America (Brazil, Paraguay, and Uruguay), Central America (Costa Rica), Mexico, the Caribbean (Puerto Rico), USA (California, Florida, and Hawaii), Australia, and Japan. Allelic patterns revealed several multilocus genotypes (MGs) among these isolates. Isolates sampled from different host species in South America had distinct, host-associated MGs. Conversely, isolates sampled from similarly diverse hosts in Costa Rica, Mexico, Puerto Rico, USA (California, Florida, and Hawaii), Australia, and Japan had closely related MGs that differed markedly from the South American MGs. These results indicate that a single set of closely related, recently introduced MGs is causing widespread disease of several myrtaceous species outside South America. Further, our data appear to exclude eastern South America as the source of these pandemic MGs. Concurrently, bioclimatic modeling is being used to predict global areas at risk from these MGs and identify putative source populations of the pathogen.

Characterizing virulence phenotypes among U.S. isolates of *Pyricularia oryzae* using IRR1 NILs, U.S. germplasm, and NERICA lines

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Rice blast disease, caused by *Pyricularia oryzae*, is a major constraint to rice production in most rice production areas, including the Southeast U.S. In continued efforts to evaluate the effectiveness of resistance genes, a collection of U.S. isolates of *P. oryzae* were evaluated for virulence using 25 to 40 U.S. rice differentials and two sets of differentials developed by IRR1. The first set comprised 31 monogenic lines with 24 resistance genes and, the second set had 20 lines with 14 resistance genes. In addition, four NERICA lines were evaluated. A total of 12 different U.S. reference races and a collection of field isolates representative of the genetic diversity in the U.S. population were evaluated on the rice lines. The sets of IRR1 differential lines discriminated the isolates into groups that differed from groupings based on the U.S. differentials. Two NERICA lines were resistant to all the isolates that were evaluated. NERICA lines did not help in virulence discrimination among the isolates evaluated. However, the resistance genes in these lines could be exploited as potential new sources of resistance to rice blast in the U.S. Future efforts continue to focus on phenotyping additional field isolates to fully characterize isolates which can overcome specific resistance genes.

Genomic sequence comparisons between isolates of *Globodera rostochiensis*

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The potato cyst nematode, *Globodera rostochiensis*, also known as the golden nematode, is considered a quarantine pest of potato worldwide and has been confirmed in 65 countries including the USA and Canada. Left untreated, yield losses of up to 80% can be observed. In Canada, the golden nematode has been detected in three independent locations across the country, Saanich on Vancouver Island in British Columbia, the Saint-Amable region in Quebec and in Newfoundland. All three isolates are considered to be of the Ro1 pathotype. Studies are underway to complete the genomic sequences of these isolates, as well as additional isolates representing pathotypes Ro1 to Ro5 for comparison. Ultimately, we expect to identify unique markers that can be used for molecular pathotype determination. Preliminary data will be presented on the genome assemblies of these nematode isolates.

Characterization of tree fruit and grapevine viruses by next generation sequencing

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As part of an interdepartmental federal government of Canadian funded project on "Protection of Canadian biodiversity and trade from the impacts of global change through improved ability to monitor invasive alien and quarantine species", our group has focused on the characterization of viruses infecting tree fruits, small berries and grapevine. Approximately 650 virus infected specimens from collections contained at the Canadian Food Inspection Agency (CFIA) Sidney laboratory, B.C., and the Agriculture Agri-Foods (AAFC) research facility in Summerland, B.C. will be processed for dsRNA extraction and Illumina HiSeq sequencing. The purpose is to gain a

better insight into plant virus diversity across species, discover previously uncharacterized viruses and develop new methods for the rapid detection and identification of viruses at quarantine facilities. So far about half the samples have been processed for dsRNA. Initial sequence data has been obtained and will be presented.

Interaction of root stress, chemical management, and ramorum blight development from soilborne inoculum in potted rhododendron plants

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Phytophthora ramorum causes foliar blight and dieback of many ornamental species. A major concern for nurseries is the potential for *P. ramorum* to colonize roots without inducing above ground symptoms in plants that can then serve as cryptic reservoirs of inoculum. Episodic abiotic stresses that reduce plant water potential can compromise disease management and host resistance to trigger systemic disease development from soilborne infections in many *Phytophthora*-plant interactions. Three trials were conducted in an outdoor experimental nursery to assess root stress, as simulated by a brief exposure to 0.2 M NaCl, and fungicide treatment on ramorum blight development from soilborne inoculum. Rhododendrons in 15 or 21 cm pots were inoculated by addition of a *P. ramorum*-infested V-8 broth/vermiculite-mixture to the potting soil. After 14 days, plants were treated by soil drench with Aliette or Subdue Maxx and subsequently exposed to overnight salt stress. *P. ramorum* readily colonized roots in all inoculated treatments. Foliar blight and dieback was apparent in two of the three trials, but many plants with colonized roots were asymptomatic even after 4-6 months. A single fungicide application reduced but did not mitigate root infection, and salt stress partially offset the benefit of Subdue Maxx. Rhododendrons can sustain extensive root colonization by *P. ramorum* without systemic disease development and symptom expression.

What determines *Alnus*-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects

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Host specificity of ectomycorrhizal (EcM) fungi may have evolved from local adaptation to a host without reciprocal selection. On another hand, host specificity may also arise from geographic isolation and habitat filtering. The high degree of specificity found in the *Alnus*-EcM fungi symbiosis has been partly interpreted as selection by coevolution, using comparative phylogenies of the host and selected fungal taxa. In order to test how far host and habitat may select specific communities, the molecular diversity of the fungi associated with the two *Alnus* subgenera present in Europe was investigated in a broad range of habitats in France. A total of 1178 internal transcribed spacer sequences were obtained from ectomycorrhizae, representing five native *Alnus* species and subspecies, 165 trees, and 39 sites. The species richness was low but still variable, and the community evenness appeared lower on organic soils and in Corsica. Similarly, between communities was influenced both by host, soil parameters, altitude and longitude, but not climate and distance. A large majority of "specific" fungi were shared between host species within a subgenus, and showed habitat preferences within the subgenus distribution range. However, a large part of alpha and beta diversity variations remained unexplained, suggesting that competition or neutral processes should also be considered to explain species distribution and specificity in this plant-fungal symbiosis.

Complete genome sequence of nuclear citrus leprosis utilizing small RNA deep sequencing

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Citrus leprosis virus cytoplasmic type (CiLV-C) was reported from Mexico, 2005-06. Various *Citrus* spp. affected with leprosis were sent to the USDA-APHIS-PPQ-CPHST, Beltsville, MD for testing. Leprosis samples from the state of Queretaro failed to react with CiLV-C and CiLV-C type 2 (CiLV-C2) specific antisera in ELISA. Furthermore, in RT-PCR CiLV-C and -C2 specific

primers also failed to produce amplicons. However, TEM of the infected leaves showed bullet shaped virions in the nuclei and cytoplasm similar to that reported for CiLV nuclear type (CiLV-N). To determine the viral genome sequence, total RNA from leprosis samples were sent for small RNA (sRNA) sequencing using the Illumina GA IIX platform. After subtracting the citrus genome and known virus sequences, the remaining sequence pools were assembled by tiling using the Velvet and Oases sequence programs. After eliminating the overlapping sequences, the assembled sRNA (20-25 nt) contigs were recognized utilizing the NCBI non-redundant protein database with blastx. The complete genome sequence and structure of CiLV-N was determined. The viral genome is composed of RNA1 (~6.4 Kb) and RNA2 (~6.0 Kb) and each ORF showed a 92-95% nucleotide sequence identity with *Orchid fleck virus* (OFV) infecting *Cymbidium* species. However, RNA-1 and -2 sequences of the virus showed 96-98% amino acid identities with OFV and shared the same clade with OFV in the phylogenetic tree, suggesting that the virus may be a strain of OFV.

Differential interaction of human pathogens with plants

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Each year roughly 48 million cases of human food poisoning or hospitalization occur due to consumption of fresh produce contaminated with human pathogens. The understanding of the early events of human pathogen and plant interaction is necessary to prevent this. In this study, we assessed plant defense responses induced by the fully pathogenic bacteria *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium SL1344 in both *Arabidopsis thaliana* and lettuce (*Lactuca sativa*). Stomatal assay of plants were performed under normal and higher humid conditions to examine the effect of relative humidity on plant defense against those human pathogens. Further, to test the post invasion behavior of those pathogens, survival of the pathogens inside apoplast was tested as well under differential humid condition. Lastly, to assess whether human pathogens can also regulate *Arabidopsis* defense responses in the whole leaves, we evaluated the expression level of the *Arabidopsis* marker gene *PR1* that is associated with immunity against bacteria. Unlike SL1344, O157:H7 induced strong plant immunity at both pre-invasion and post-invasion steps of infection. *PR1* gene expression was also found to be higher in O157:H7 infected *Arabidopsis* leaf. These results suggest plant might recognize and respond to some human pathogens more effectively than others. This knowledge can be useful in developing good agricultural practice and minimizing outbreaks related to fresh produce.

Functional characterization of virulence genes of 'Candidatus Liberibacter solanacearum', bacterium associated with potato zebra chip (ZC) disease

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Zebra chip (ZC), an economically important disease of potato (*Solanum tuberosum* L.), is caused by 'Candidatus Liberibacter solanacearum' (Lso). The disease is transmitted by a psyllid (*Bactericera cockerelli*). While the causal agent has been identified, factors associated with the virulence of the disease are largely unknown. With the availability of the genome sequence of the bacterium, putative virulence genes were identified. Based on the sequence analysis, a putative zinc transport system (*ZnuABC*), iron transport and accumulation system (ITA) and salicylate hydroxylase were selected for functional characterization. To help identify the function of these gene clusters, a Gateway vector was used for RNA interference (RNAi) of these genes which can be used for *Agrobacterium* transformation. A similar vector was also used for transient suppression of gene function. These vectors can provide some insights into the functionality of the predicted genes. Since all potato cultivars are susceptible to ZC and no transgenic potatoes with *R* genes showed promising resistance against the disease, RNAi targeting Lso pathogenicity genes provides a novel approach to controlling ZC disease.

Cryptic viruses in the flora of the Great Smoky Mountains National Park

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During the study of viruses infecting flora in the Great Smoky Mountains National Park (GSMNP) we identified and characterized a number of

phytoviruses belonging to different extant taxa of plant and/or mycoviruses. Among them, the most numerous were cryptic viruses. Novel species were characterized from *Angelica* sp., *Ranunculus* sp., *Vitis* sp., *Viburnum* sp., and other plant hosts. All of them were characterized by the genome consisting of two molecules of monocistronic double stranded RNAs, approximately 1.4-1.6 kbp in size, coding for RNA dependent RNA polymerase and viral coat protein. Phylogenetic analyses confirmed their mutual relationships as well as close evolutionary history with the known members of the fam. *Partitiviridae*.

Molecular evaluation of resistibility/susceptibility of Saudi date palm germplasm against Bayoud disease caused by *Fusarium oxysporum* f. sp. *albedinis*

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Fusarium oxysporum f. sp. *albedinis* (FOA) is a seed- and soil-borne vascular wilt pathogen causing a very serious and destructive disease to date palm, Bayoud disease (BD). Fortunately, FOA has not been recorded in Saudi Arabia (SA), but according to the literature, the disease is moving eastward from its origin (Morocco and Algeria). The main objective of this study was to develop prophylactic measures to protect Saudi date palm plantations from this destructive disease. Consequently, we have evaluated Bayoud resistibility/susceptibility of 208 trees representing 34 date palm varieties using molecular markers. We have also sequenced 43 out of 208 resistibility/susceptibility diagnostic PCR amplicons. Most of the date palm trees (185 out of 208, representing 29 varieties) were identified as Bayoud-resistant. The 23 left trees were shown to be susceptible to Bayoud disease. The DNA sequence analysis of diagnostic PCR amplicons of resistibility from Saudi date palm trees showed 100% homology with DNA sequences from GenBank database. Interestingly, the DNA sequence homology between diagnostic PCR amplicons of susceptibility and GenBank's ones was 97.6%. In conclusion, the genetic assessment of the resistibility/susceptibility of date palm germplasm in SA will enable the authorities to take a rapid response to BD outbreaks. This work is also very important to national security and economy because the date palm industry is very important socially and economically to SA.

Molecular phylogeny of fungi in the genus *Thelonectria* (Hypocreales, Nectriaceae): Are they really monophyletic?

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Thelonectria is an emblematic genus of nectriaceous fungi adapted to a wide range of woody hosts and found in many tropical and temperate environments. About 20 species have been described to date, involving morphologically similar species formerly assigned to *Neonectria*. In general, species in this group have a perithecial wall comprised of thick walled cells, a knobby or prominent perithecial apex, spinulose or tuberculate ascospores, microconidia only produced in some species, and chlamydospores absent. Although *Thelonectria* sensu lato species share morphological characters, they seem to be symplesiomorphic, shared by an assemblage of genetically very divergent species, as previous studies have found that the majority of species in this genus comprise species complexes. Based on the results obtained from multilocus phylogenetic analyses using six nuclear markers, a polyphyletic nature for *Thelonectria* species is revealed. Here it is proposed that in order to preserve monophyly, species relationships in the genus *Thelonectria* should be reconsidered. Based on these results, the generic limits of *Thelonectria* should be reduced, and new genera erected. However, as many generic limits can be applied, i.e. more than one genus can be created, segregated genera should be based on the relationship among morphological, molecular and geographical characters.

Preliminary studies of biogeography, genetic diversity and host range of the causal agents of beech bark disease (BBD) and related species

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Neonectria coccinea and *N. faginata* are the causal agents of Beech Bark Disease (BBD) in North America and Europe, respectively. Two species, *N. microconidia* and *N. punicea* have been described as closely related to the BBD causal agents. To clarify the relationships of these important pathogens and related species, we conducted phylogenetic analysis using isolates from Asia, Europe, and North America and contrasted these results with information about biogeography, host range and genetic diversity. The phylogenetic analysis indicates that the four species are conspecific, being *N. microconidia* the most basal. *N. microconidia* and *N. punicea* occur on a wide range of hardwood species. *N. coccinea* and *N. faginata*, only occur on *F. sylvatica* and *F. grandifolia*. *N. punicea* has a broad geographical and host distribution range, being encountered in Asia and Europe. One isolate from Japan was identified as *N. coccinea*, similar to isolates from Europe, however it is still not clear if this is a recent introduction and if it represents a threat for Japanese beech. DNA polymorphisms revealed low genetic divergence among these species. This could indicate that giving the appropriate conditions *N. microconidia* and *N. punicea* may have the potential to become a pathogen of beech or related hardwood species. Efforts to prevent the introduction of the BBD vector (*Cryptococcus fagisuga*) to the Asian and Japanese range of *N. coccinea*, *N. microconidia* and *N. punicea* should be accentuated.

Pierce's disease in three susceptible grape cultivars grafted on hybrid rootstocks or own-rooted

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Pierce's disease (PD) of grape caused by *Xylella fastidiosa* subsp. *fastidiosa* causes vine death in warmer areas of North America. Does grape rootstock affect PD? Symptoms and ELISA tracked PD at two sites 2009 to 2012 for three *Vitis vinifera* scions on three hybrid rootstocks (Freedom check), or own-rooted (check). There were five vines per plot in a factorial arrangement of 12 treatments in five replications. At Stonewall, definitive symptoms never developed and the few vines with ELISA-positive petiole samples did not develop chronic PD. At Uvalde, ELISA positives progressed to 100% of vines and chronic symptoms including dieback and death. Dieback scion main effect after pruning in February 2013 was less for Chardonnay (C.71%) and Merlot (M.73%) than for Cabernet Sauvignon (CS.85%); and all rootstock mean comparisons were significant among Paulsen 1103 (P3. 43%), Dog Ridge (DR.73%), Freedom (F.89%) and own-rooted (100%). Vine mortality scion main effect was similar among scions but all rootstock means were similar among P3 (2%), DR (18%), F (71%), and own-rooted (100%). Scion*rootstock interaction was significant only for vine dieback. C on P3 (28%) had less vine dieback than M on P3 (42%); which in turn had less than CS on P3 (60%), M on DR (62%), and C on DR (67%). PD management strategies should include partially resistant rootstocks. Partially resistant hybrid scions are currently being evaluated on selected rootstocks.

Host affiliations and geographic distributions of fungal symbionts of aquatic plants

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General themes in endophyte biology have been inferred from studies of terrestrial plants, with few studies evaluating endophytes in aquatic systems. We examined the diversity, composition, host- and tissue affiliations, and geographic structure of fungal endophytes associated with aquatic plants. In 2011 and 2012, the three most abundant species of submergent and emergent macrophytes were collected from three microsites in each of six reservoirs and lakes in northern Arizona. Endophytes were isolated in culture from roots and photosynthetic tissues, and the internal transcribed spacers, 5.8S gene, and partial nuclear ribosomal large subunit were sequenced for all strains. A total of 226 isolates representing 60 putative species was recovered from 9,600 plant tissue segments. Isolation frequency and diversity did not differ significantly between years, among species, or among lakes, but were greater in roots than in photosynthetic tissues. Communities differed significantly in composition among lakes, reflecting composition of local sediment and waterborne fungi. Together these analyses provide a first quantitative estimation of endophytic associations in ecologically important and economically relevant aquatic plants; reveal the relationship of endophytic fungi to environmentally available fungi in water and sediment; and provide a basis for evaluating general trends in endophyte biology that have been based mostly on plants in terrestrial ecosystems.

Fungi in a drought: The diversity of thermophilic fungi in corn

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The Midwest United States has been under extreme drought conditions for the past two years which could cause changes in the composition of microbial communities in corn. The high temperatures and low humidity conditions are favoring the colonization of grain by thermophilic fungi in corn storage facilities and very little is known about the diversity of thermophilic/thermotolerant fungi or the health risk they may represent for farmers and consumers. The objective of this project was to isolate and identify thermophilic and thermotolerant fungi from corn grain stored in bins during drought season and determine potential interactions between thermophiles and known mycotoxin producing fungi. Corn samples were collected from local farmers during the summer and winter season. The corn was plated and incubated at 50°C and the fungi were isolated and identified using ITS rDNA primers. The number of spores in the corn silos was very high, more than 90% of grains show colonization by thermophilic fungi. Multiple species of thermophilic fungi were isolated and identified including: *Thermomyces lanuginosus*, *Aspergillus fumigatus*, *Thermoascus crustaceus*, and *Rhizomucor pusillus*. Many of the species isolated were true thermophiles with optimal growth temperature at 50°C. This study raises new concerns of high abundance of previously undocumented actively growing fungi in corn which could represent a new food safety risk.

Investigating genetic mechanisms of decreased sensitivity to iprodione in field isolates of *Sclerotinia homoeocarpa*

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The dicarboximide fungicide class is commonly used to control dollar spot, caused by *Sclerotinia homoeocarpa*, on golf courses in North America. Field resistance to iprodione (dicarboximide) in *S. homoeocarpa* was reported in 1983, however genetic resistance mechanisms have not been elucidated. A putative osmosensing histidine kinase gene (*Shos1*) in *S. homoeocarpa* was mined from RNA-Seq data. Partial *Shos1* gene sequences were analyzed for one iprodione field resistant isolate from Michigan, six isolates with highest EC₅₀ values for iprodione collected during a New England monitoring study, and five sensitive and three moderate insensitive isolates. The iprodione field resistant isolate and New England isolates displayed non-synonymous polymorphism in one base pair in the *Shos1* gene. The polymorphism of *S. homoeocarpa* resistant isolates was located in the second unit of tandem amino acid repeats of *Shos1* and was present in the same position as *Bos1* in dicarboximide field resistant isolates of *Botrytis cinerea*. *S. homoeocarpa* isolates that were sensitive and moderately insensitive to iprodione did not exhibit polymorphism in *Shos1*. However, moderately insensitive isolates showed constitutive and induced overexpression of the putative multidrug resistance transporter (*ShMR1*). Results indicate that polymorphisms in the *Shos1* gene confer iprodione resistance and overexpression of *ShMR1* is associated with reduced sensitivity to iprodione in *S. homoeocarpa* field isolates.

Genetic variability of the population of *Ralstonia solanacearum* in Brazil

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Understanding the genetic variability of the population of *Ralstonia solanacearum* (RS) is useful to the implementation of breeding programs aimed at developing resistant varieties. A sample of 337 isolates of RS were collected from 22 states in Brazil, from 14 host species and characterized for biovar, phylotype, sequevar and BOXPCR in order to assess the amount and distribution of genetic variability. Biovar was determined using physiological tests. Phylotype was determined by phylotype-specific primers. PCR amplification of a 750 bp fragment of the endoglucanase gene (*egl*) was performed using the primer pair Endo-F and Endo-R. Brazilian isolates were classified into biovars 1 (N=175), 2 (N=146) and 3 (N=16); and phylotypes I (10 isolates) and II (327 isolates). Phylotype I isolates were found in the Northern and Northeastern regions. Based on the *egl* sequences isolates could be grouped in phylotype I and phylotype II subclusters IIA and IIB and sequevars 1, 2, 4, 5, 7, 26, 28, 41 and 50 could be identified. We identified 281 haplotypes and the largest clonal fraction was observed for potato isolates. Recombination seems to occur among isolates. Nineteen heterogeneous

groups were formed when analyzing BOX-PCR data using discriminant analysis of main components. All groups had isolates from the northern region. Isolates did not group according to host or geographic region of origin. There is high genetic variability in the Brazilian population of RS.

Two new *Ophiostoma* species on two host genera of the Myrtaceae from the Brazilian Cerrado

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Two *Ophiostoma* (Phyllachoraceae) species are now being registered as causing large amphigenous tar-spots on *Campomanesia adamantium* and *Myrcia tomentosa*, both forming originally intradermal then subepidermal pseudostromata that become trans-mesophilic and amphigenous, housing their ascogenous and the anamorphic phases. This phyllachoraceus genus is mainly characterized by its filiform ascospores which fill cylindric paraphysate asci in helical fashion. Species are essentially biotrophic in tropical and subtropical regions but not usual among myrtaceous hosts, except for a report from Australia, and another from India. The main and definitive difference between the two species lies on the ascospore shape. Thus, on the specimen on *C. adamantium* the spores ($59 - 85 \times 3 - 6 \mu\text{m}$), are typically appendiculate at the base, multiguttulate; while the species on *M. tomentosa* shows are larger ($84 - 139 \times 2 - 2.5 \mu\text{m}$) ascospores, with more acute apices, and a clear median guttule. Both species differ from the 36 listed in *Indexfungorum* as accessed on April, 14, 2013.

Evaluation of wheat, barley, and triticale lines for resistance to *Xanthomonas translucens* pv. *undulosa*

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Wheat bacterial leaf streak (BLS), caused by *Xanthomonas translucens* pv. *undulosa* (XTU), has become increasingly important in the northern Great Plains of the U.S. where it is a major region for hard red spring and durum wheat production. Since chemical method and other cultural practices are either ineffective or impractical to control this disease, utilization of host resistance appears to be the only option. However, breeding for resistant varieties is difficult due to lack of a reliable resistant source and poor understanding of the disease system. Our goal is to identify sources of resistance and investigate the genetic and molecular basis of host-pathogen interaction. Using a disease screening protocol recently developed in our lab, we have evaluated a number of wheat, barley and triticales lines for resistance to a local virulent strain. Several triticales were found to be highly resistant without the development of water-soaking symptom at the inoculated area. The difference was also observed among the wheat and barley lines, but most of them tended to be susceptible. Additionally, we identified another XTU strain that showed significantly low virulence toward all the genotypes evaluated. More wheat materials are being evaluated in the greenhouse, including landraces, related species and wheat-alien species derivatives. This work provides an important tool for the breeding programs as well as investigating the genetic and molecular basis of the pathosystem.

Transgenic approaches to control aflatoxins in maize

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Aflatoxins produced by the fungus *Aspergillus flavus* pose a chronic contamination risk in maize and other susceptible food crops worldwide. Two novel antifungal proteins offer the potential to provide resistance to infection and prevent aflatoxin synthesis in a way that can be incorporated into elite breeding lines without the yield drag of resistant breeding lines. 1) A novel seed lectin from *Lablab purpurea* (hyacinth bean) was previously shown to be a potent inhibitor of the α -amylase of *A. flavus* and of spore germination. We have constitutively expressed this α -amylase inhibitor in maize and monitored expression levels of the transprotein throughout the development of homozygous inbred lines. *In vitro* spore growth inhibition using leaf extracts of these lines and their segregation controls has shown that this α -amylase inhibitor may reduce spore germ tube growth of *A. flavus*. 2) A designed antimicrobial peptide AGM182 was shown to be effective at inhibiting the germination of *A. flavus* spores at concentrations as low as $5 \mu\text{M}$ *in vitro*. Transgenic maize calli expressing AGM182 are currently being regenerated.

Transgene expression levels will be monitored using an antibody specific to AGM182. The ability of the expression construct for AGM 182 to produce the peptide was first validated *in planta* by transiently expressing the construct in tobacco. The latest results from transgenic approaches using both the gene constructs will be presented.

Blueberry shock virus (BShV) sampling efficiency and cold hardiness studies in blueberry

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In 2009, *Blueberry shock virus* (BShV), a pollenborne virus endemic to the Pacific Northwest, was discovered in a Michigan blueberry field (cv. Rubel). A study was conducted with the following objectives: 1) assess the rate of spread and sampling efficiency for BShV, and 2) determine the effect of virus infection on cold hardiness of blueberry buds. Leaf samples from all bushes were tested by ELISA in August 2009. Dormant flower buds were tested in March and April 2010. More flower buds tested positive for the virus in April than in March. The virus could also be detected in leaf buds, but flower buds were easier to sample. The virus was detected in 41/240 bushes, a 41% increase over 2009. The distribution of the virus within the plant was mostly uniform, but in some bushes it was uneven. For the cold-hardiness studies, dormant twigs were taken from five BShV-infected and five non-infected blueberry 'Rubel' plants on three dates in early spring. Twigs were frozen in a programmable freezer with temperatures dropping from -18°C to -30°C at 2- to 3-degree intervals. No differences were found in the LT50 (temperature at which 50% of the buds were killed) between infected and non-infected bushes. Based on this research, we recommend sampling dormant flower buds on five random twigs per bush in late spring, testing composite samples of five to ten buds to increase testing efficiency.

The effects of environmental factors on infection of blueberry fruit by *Colletotrichum acutatum*

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Anthrachnose fruit rot of blueberries caused by *Colletotrichum acutatum* is a serious problem in humid blueberry-growing regions. Environmental factors that affect mycelial growth, conidial germination appressorium formation, and fruit infection by *C. acutatum* were investigated. Variables included temperature, wetness duration, wetness interruption, and relative humidity. The optimal temperature for mycelial growth was 26°C and little or no growth was observed at 5 and 35°C . The development of melanized appressoria was studied on parafilm-covered glass slides, and fruit infection was evaluated in immature and mature blueberry fruits. The optimal temperature for infection was 25°C , and infections increased with increasing wetness duration up to 48 h. Three-dimensional Gaussian equations were fit to the effect of temperature and wetness duration on the development of melanized appressoria ($R^2 = 0.89$) on glass slides and infection incidence in immature ($R^2 = 0.86$) and mature ($R^2 = 0.90$) fruit. Furthermore, the development of melanized appressoria and fruit infection incidence were modeled in response to interrupted wetness periods and relative humidity. The results were used for the development of a disease prediction model for anthrachnose fruit rot in blueberries.

Antagonist *Cryptococcus flavesens* OH 182.9 3C colonization of wheat heads when applied with triazole fungicides and the effect on scab

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Integrated pest management (IPM) is the best available approach for reducing Fusarium head blight (FHB; caused by *Fusarium graminearum*) and the mycotoxin deoxynivalenol (DON) in wheat grain. Utilizing FHB biological control agent *Cryptococcus flavesens* OH 182.9 (NRRL Y-30216) as part of an IPM approach against FHB is understudied. Triazole fungicides such as prothioconazole (PTC) used alone or in combination with tebuconazole are effective against FHB, but minimum pre-harvest intervals for fungicide use can restrict applications after wheat flowering. A PTC-tolerant variant, OH 182.9 3C (NRRL Y-50378), applied in a tank mix with a fungicide or after flowering, could reduce DON by establishing populations that reduce post-flowering DON-producing infections by *F. graminearum*. In a multi-year study, the colonization of glume and lemma tissues by variant 3C was determined when the agent was applied alone or in combination with a fungicide at

wheat flowering or seven days after. For all treatment combinations, the population of 3C represented 50-95% of the total microbial population recovered from both glume and lemma tissues from 8 to 11 days after flowering, demonstrating the competitive success of the strain. While the application of 3C at times reduced ($P < 0.05$, FPLSD) FHB and/or DON, combinations of fungicide and antagonist were rarely significantly more effective than either component used alone for the doses tested.

Casuarina equisetifolia decline in Guam linked to colonization of woody tissues by bacteria

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Ironwood trees (*Casuarina equisetifolia*) on the island of Guam are in decline due to a combination of biotic and abiotic factors. Bacteria associated with wet-wood and vascular wilt are emerging as significant biotic factors in addition to those previously established, which include the wood-rotting fungus, *Ganoderma australe* species complex, and termites. Symptoms include thinning of foliage and dark discoloration of the tree's central core, which are associated with the onset of ironwood tree decline. *Ralstonia solanacearum* and two other bacterial species were consistently recovered in mixed culture when initial isolations were made from discolored wood tissue and from droplets of bacterial ooze, which often form on stem cross-sections of declined trees. *R. solanacearum* and one of the unidentified bacterial species were translocated through xylem vessels of young tomato and *C. equisetifolia* plants following wound inoculation with the bacterial mixture that oozed from infected wood. Confirmation of *R. solanacearum* was based on cultural characteristics, Agdia immunostrip SK 33900/0025 and loop-mediated isothermal amplification data. Healthy tissues were negative for both the immunodiagnostic and the LAMP assays. This study presents the first evidence that bacteria are involved in the ironwood decline disease complex.

Puccinia mariae-wilsoniae and Claytonia virginica: A pathogen's tale

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The purpose of this study was to examine the life history of *Puccinia mariae-wilsoniae*, a rust on *Claytonia virginica* (spring beauty), and to observe responses in leaf anatomy to its fungal pathogen. Spring beauty is an ephemeral woodland plant that lasts three to four weeks, and *P. mariae-wilsoniae* infects *C. virginica* almost as soon as the plant emerges from dormancy in spring. Population studies in March and April, 2012, as well as March and April, 2013, were used to study the abundance and spread of infection within several populations. Infected leaves, inflorescences, and corms with attached roots were collected, fixed in FAA, stored in 70% ethanol, embedded in paraffin, sectioned, and mounted on slides for anatomical study. While the presence of aecia and aeciospores has been reported in other studies, this project has demonstrated the presence of telia and teliospores on *C. virginica* leaves and inflorescences. The population and anatomical studies will allow us to understand where the teliospores overwinter and how *P. mariae-wilsoniae* infects spring beauty so rapidly in the spring. DNA sequencing was utilized to assess the phylogenetic history and nomenclature of *P. mariae-wilsoniae* and will allow us to better understand its life history.

Characterization of Pythium species associated with Prunus replant disease

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Growth and yield of replanted almond and stone fruit orchards can be suppressed by a poorly understood soilborne complex known as Prunus replant disease (PRD). We detected elevated incidences of *Pythium* spp. in roots from PRD-affected trees in non-fumigated plots of orchard replant trials, compared to the incidences from healthy trees in adjacent pre-plant fumigated plots. Similarly, in greenhouse trials with Prunus replant soil, we found higher incidences of *Pythium* spp. in roots from PRD-affected peach seedlings grown in non-treated soil portions, compared to the incidences in roots from healthy seedlings grown in fumigated or pasteurized soil portions. *Pythium* species from the PRD sources were identified by rDNA (ITS1 and 2) sequence analysis. Isolates included *P. intermedium* (1 isolate), *P. irregulare* (5), *P. pareocandrum* (7), *P. spinosum* (5), *P. ultimum* (9), *P. vexans* (4), and two unknown ribotypes of *Pythium* sp. (4 and 8 isolates). All of the isolates were tested for pathogenicity in two greenhouse trials; Nemaguard peach seedlings were planted in pots of soil treated with isolate inoculum or sterile inoculum

substrate and grown for 3 months. *Pythium irregulare*, *P. spinosum*, and *P. ultimum* significantly reduced plant fresh weight and caused varying levels of root cortex necrosis in both experiments, while *P. intermedium* was pathogenic in one experiment. These results suggest that at least in some orchard replant sites, *Pythium* species contribute to PRD.

Identification of Cercospora-resistant sugar beet lines using multi-sensory and molecular detection systems

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The fungal pathogen *Cercospora beticola* causes the economically most destructing foliar disease of sugar beet worldwide. Introduction of resistant sugar beets into breeding programs is a crucial objective of integrated plant protection. We developed a non-invasive hyperspectral imaging system that allows the simultaneous mapping of reflectance, transmittance, and absorbance to support selection of candidate genotypes via disease scoring. *Cercospora* symptoms cause specific reflectance and transmittance reduction in the green and near-infrared region, which can be used to identify infection before visual symptoms occur. In a second non-invasive approach Magnetic Resonance Imaging (MRI) of the taproot gave insight into taproot development and sugar accumulation during above-ground leaf disease progression. MRI showed developmental reduction in taproot volume and cambial growth rings of infected plants compared to healthy control. Before visible symptoms appeared we detected differences of fungal biomass in leaf tissue by amplifying the *Cercospora* calmodulin gene with real-time PCR. Lower detected amounts of fungal biomass indicated genotypes with higher resistance levels. Hence, combining these three methods hyperspectral imaging, MRI, and molecular analyses, comprehensively characterizes resistance levels of beet genotypes, leading to improved breeding and phytopathological knowledge of *Cercospora* Leaf Spot disease.

Fungicide-induced mutagenesis in Monilinia fructicola and implications for resistance management

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Azoxystrobin is one of the most widely used fungicides in agriculture with broad spectrum activity against many diseases on many edible crops and ornamentals. It inhibits growth of the target fungus by binding to the Qo site of the cytochrome bc1 complex and thereby interrupting mitochondrial electron transport. In this study, the effect of exposure to sublethal doses of azoxystrobin was investigated in *M. fructicola* isolates from different geographical locations. Isolates were exposed in weekly transfers of mycelia and spores to a dose gradient of demethylation inhibitor fungicide SYP-Z048 and quinone outside inhibitor fungicide azoxystrobin in solo or mixture treatments for a total of three months. Transposition of a 1500 bp mobile element was observed in the majority of azoxystrobin-treated cultures, but never in the non-fungicide-treated controls and rarely in mycelium treated with demethylation inhibitor fungicides. Variation in multiple microsatellite loci was detected in 2 of 4 isolates subjected to azoxystrobin, indicating mutagenesis on the nucleotide sequence level. Fungicide-induced mutagenesis is a new phenomenon and may enable a pathogen population to quicker adapt to environmental stresses.

Improving the veracity of sequence data in GenBank

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As part of the International Nucleotide Sequence Database Collaboration (INSDC) GenBank has always fulfilled its responsibilities to provide a stable archive for user submitted data. Within these constraints efforts to improve veracity of sequence information and update taxonomic identification continue. New ways to highlight ex-type and authoritative sequences, improve links to other databases and utilize the BLAST interface will be discussed. In addition, approaches to improving data submission for users will be reviewed. Finally, changes prompted by the International Code of Nomenclature and how it will affect the taxonomy database will be discussed.

Characterization of Phytophthora cinnamomi from ornamental crops in South Carolina

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Phytophthora cinnamomi is a devastating pathogen that can infect over 900 hosts. It is the most common species of *Phytophthora* isolated from woody ornamental crops in South Carolina but little is known about variability among isolates of *P. cinnamomi* that attack these plants. Therefore, 142 isolates of *P. cinnamomi* recovered from diseased plant samples submitted to the Clemson University Plant Problem Clinic between 1996 and 2011 were characterized for growth rate, mycelium growth habit, mefenoxam sensitivity, and mating type. Average growth on PARPH-V8 selective medium was 60 mm in 72 h at 25°C in the dark. Mycelium growth habit on PARPH-V8 was classified as aerial, sparse, dwarf, or appressed, and 85% of isolates had aerial mycelium. All isolates were sensitive to the fungicide mefenoxam at 100 ppm. The population was composed of 129 A2 and 13 A1 isolates with six A1 isolates recovered from camellia. The ITS 1 and 2 loci were sequenced, and this region had low diversity with only two genotypes that were different from the majority of the population. One of these genotypes consisted of an isolate matching *P. cinnamomi* var. *parvispora*, and the other genotype included four morphologically diverse isolates. Consequently, there was a high degree of genetic uniformity in the ITS region among these 142 isolates. Host-pathogen relationships for this population were compared to reports in the literature, and 33 new associations were found.

WITHDRAWN

Steaming is a sustainable method to eradicate the quarantine pathogen *Phytophthora ramorum* from infested nursery soil

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The long-distance spread of *Phytophthora ramorum*, causal agent of Sudden Oak Death, through infected nursery plants is a serious threat for environments not yet affected by the pathogen. Nurseries tested positive for the presence of *P. ramorum* are required by federal and state regulations to eradicate infested plants and to disinfest soil and water. At NORS-DUC we study environmental friendly methods to eradicate *P. ramorum* from infested soils, among them biological control, solarization and steaming. Research beds (surface 9.1 x 3.7 m, depth 30 cm) filled with high clay content soils were treated with hot steam at a target temperature of 50°C for 30 minutes. As controls, teabag sachets containing *Rhododendron* leaf disks colonized by *P. ramorum* were buried at various depths in the soil. Leaf disks collected before and after steaming were assessed for *P. ramorum* by plating on PARPH-V8 medium. All leaf disks were *P. ramorum*-positive pre-steam and negative post-steam. Seasonal effects of environmental temperature on the steaming process and temperature gradient were measured by steaming at different times of the year. Additionally, soil at a commercial nursery tested positive for *P. ramorum* was steam-treated and -after eradication of the pathogen- released from quarantine. Soil texture and water content play an important role for the dynamics of the steaming process. Our results confirm that steaming can be used to eradicate quarantine pathogens from nursery soils.

Host-derived RNA interference targeted to the root-knot nematode parasitism gene 16D10 in tobacco

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The four major species of root-knot nematode (RKN), *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne javanica*, and *Meloidogyne hapla* have many host plant species, including tobacco, and are a global menace in agriculture. RKNs are sedentary endoparasites that transform plant cells into complex feeding sites called giant-cells via effector proteins secreted by the nematode through the stylet. Huang et al demonstrated that one secreted effector called 16D10 interacts with a SCARECROW-like transcription factor and that *M. incognita* grown on transgenic *Arabidopsis* engineered to produce 16D10RNAi produced 69-93% less eggs than controls. In this study 2 cultivars of tobacco (TN90 and Hicks) were transformed with Huang's 16D10RNAi constructs. Infection assays of the transformants with *M. arenaria*, for which there is no resistance in tobacco, showed reductions in egg counts for 3 out of the 4 lines of TN90 and 2 out of the 4 lines of Hicks tested. One of these lines, TN90 I-8, showed a 56% reduction in egg counts and was tested further with all 4 major species. TN90 I-8 was found to reduce the egg production of 3 of the 4 major species; *M. incognita* was reduced by 42%, *M. javanica* by 56%, and *M. arenaria* by 49%. To correlate the resistance with the siRNA levels, siRNA sequencing of infection assay root tissue is underway. New RNAi constructs created to improve the siRNA production are currently being transformed into both *Arabidopsis* and tobacco.

WITHDRAWN

Late blight resistance in heirloom and hybrid tomato cultivars against the US-22, US-23, and US-24 clonal lineages of *Phytophthora infestans*

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Phytophthora infestans causes late blight, an important disease of tomato worldwide. Resistance genes are known, but few cultivars with effective resistance are commercially available. Using detached leaves, we tested 11 tomato cultivars for resistance to 3 current clonal lineages, US-22, US-23, and US-24. Lesion length and percent mycelial cover were plotted separately against days post inoculation and area under each curve was analyzed. Pooling the lineages, 3 heirloom cultivars with no known resistance genes, Matt's Wild Cherry, Wapsipinicon Peach, and Pruden's Purple exhibited lesion lengths not significantly different (NSD) than Mountain Magic (contains *Ph-2* and *Ph-3* resistance genes). Analysis of mycelial cover gave similar results and indicates the utility of these cultivars for mitigating disease impact and secondary inoculum production. 'Plum Regal' (*Ph-3*) had lesion lengths NSD than 'Mountain Magic' when inoculated with US-23, but lesion lengths NSD than the most susceptible cultivar when inoculated with US-22 and US-24.

Differential disease responses of cultivars to novel clonal lineages indicates a continued need to evaluate the effectiveness of tomato cultivars against current *P. infestans* populations. Identification of resistance in heirloom cultivars to 3 common lineages of *P. infestans* indicates that there are available and perhaps underutilized sources of resistance for breeding and that these cultivars can be used to lessen reliance on fungicides.

Efficacy of methyl bromide alternatives against *Macrophomia phaseolina*, causal agent of charcoal rot of strawberry

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Charcoal Rot of strawberry, caused by *Macrophomia phaseolina*, has become more prevalent as methyl bromide fumigation is eliminated from commercial production fields. *M. phaseolina* is a soil borne pathogen which infects the crown causing wilt followed by plant mortality. To test the efficacy of methyl bromide and alternatives, infected strawberry crowns or infested corn-cob litter were buried prior to drip applications (K-pam, Vapam or DMDS+Chloropicrin+Telone) or immediately following shank application during bed formation (methyl bromide, Telone, DMDS+Chloropicrin, DMDS+Chloropicrin+Telone, TeloneC35, or Chloropicrin). The amount of surviving microsclerotia of *M. phaseolina* was quantified by counting colonies on semi-selective media and compared to infested controls buried in untreated beds. K-Pam, Vapam, all DMDS treatments and Telone C35 used in conjunction with VIF (virtually impermeable film) were as effective as methyl bromide. Telone C35 applied under LDPE (low density polyethylene) was less effective than application under VIF. Chloropicrin applied alone was ineffective against *M. phaseolina*.

A multigene phylogeny of Chytridiales (Chytridiomycetes)

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Chytridiales is one of the larger orders in the fungal phylum Chytridiomycota. Members of the order are common in aquatic habitats and are associated with freshwater algae, insect exuviae and cellulosic substrates. They are morphologically diverse, most with monocentric thalli but a polycentric genus is also present. Inoperculate and operculate sporangia are represented. Phylogenetic hypotheses based primarily on ribosomal RNA (SSU and LSU) genes have left the branching order of deep nodes of the tree poorly understood with at least 16 sub-clades. Some of these sub-clades then formed two well-supported larger clades, recognized as the families Chytridiaceae and Chytriomycetaceae. Greater taxon sampling and inclusion of protein-coding genes are believed to increase resolution of backbone phylogenies. To increase understanding of the order Chytridiales, we included a wide range of isolates and sequenced five genes (EF1, EF2, RPB1, SSU and LSU). The resulting phylogeny had basically the same tree topology as the rRNA gene tree and bootstrap support values did not significantly improve. Analysis including protein-coding genes, however, was effective in resolving some problematic lineages with long branches in the rRNA gene tree. This result differed from our preliminary multigene analyses of other chytrid orders and might indicate different evolutionary processes in Chytridiales compared to other chytrid orders.

'*Candidatus Liberibacter solanacearum*' titer over time in the potato psyllid, *Bactericera cockerelli*, following acquisition from infected plants

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The potato psyllid, *Bactericera cockerelli*, is a serious pest of potato and other solanaceous crops. The association of psyllid with zebra chip disease pathogen, '*Candidatus Liberibacter solanacearum*' (Lso) was established in several vegetable crops. The biology of Lso transmission by potato psyllid is largely unknown. The present study determined Lso acquisition by adult psyllids following different acquisition access periods (AAP) on potato and tomato, quantified Lso titer over time in post-acquisition, determined Lso-acquisition rate in psyllids at each AAP on each source of inoculum, and determined influence of host plant Lso titer on Lso acquisition rates and post-

acquisition titers in psyllids over time. Results showed that Lso detection rates and titer increased over time in psyllids following AAPs of 8, 24 and 72 h on tomato and potato and Lso titer was highest when psyllids acquired Lso from tomato vs. potato. Lso titer ranged from 200 - 400-fold higher in tomato leaves, petioles, and stems than those of potato. The increase of Lso titer in the insects reached a plateau after an average of 15 d following 24 and 72 h AAP on potato or tomato and was comparable to that of Lso-infected laboratory psyllid colony. Lso-acquisition rate in psyllids fed on potato and tomato increased up to 5 and 20, 15 and 35, 35 and 75, and 80 and 100%, respectively, when the insects were allowed access to plants for 4, 8, 24, and 72 h, respectively.

Identification of the fungal pathogen complex causing fruit rot of rambutan (*Nephelium lappaceum* L.) in Puerto Rico

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Worldwide, fruit rot of rambutan is an important disease caused by a fungal pathogen complex. During a fruit disease survey (2008-2013), fruit rot was observed at eight orchards in Puerto Rico. Diseased fruit pieces were disinfested and plated onto acidified potato dextrose agar. Seven fungal genera were identified morphologically and by sequencing of the ITS1-5.8S-ITS2 region of the rDNA, partial region of the large ribosomal subunit, fragments of β -tubulin, histone H3, and elongation factor. *Colletotrichum hongkongensis*, *Colletotrichum* sp., *Gliocephalotrichum bulbilium*, *G. simplex*, *Lasmenia* sp., *Lasiodiplodia* sp., *Pestalotiopsis* sp. and *Phomopsis* sp. were identified. In pathogenicity tests, all genera produced fruit rot eight days after inoculation. Mycelial growth was observed on the fruit with isolates of *C. hongkongensis*, *G. bulbilium*, *G. simplex* and *Lasiodiplodia* sp. Acervuli were observed on spinterns with isolates of *Colletotrichum* sp., *Lasmenia* sp., *Pestalotiopsis* sp., while pycnidia were easily observed with isolates of *Phomopsis* sp. For all isolates, fruit turned from red to brown. Water-soaked lesions on the fruit surface and aril (flesh) rot were observed with isolates of *Colletotrichum* sp., *Lasmenia* sp., and *Pestalotiopsis* sp. but the remaining fungal species only produced aril rot. Future studies will speciate the remaining genera with the same or more gene sequences.

Greenhouse evaluation of commercial soybean varieties adapted to the North Central U.S. for resistance to charcoal rot

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The fungal pathogen, *Macrophomina phaseolina* (Mp), causes the disease charcoal rot (CR), which greatly impacts soybean production. Host resistance to CR is the primary means of managing this disease, yet resistance in commercial soybean varieties in Maturity Groups (MG) I-III is currently unknown. Thirty and 67 entries to the 2012 Wisconsin (MG I-II) and Indiana (MG II-III) Soybean Variety Trials, respectively, were evaluated for resistance to CR using a cut-stem assay. Resistance was characterized based on area under the disease progress curve (AUDPC) measurements of lesion development. In each experimental run, the cut-stem method consistently differentiated between the standard CR-resistant (DT97-4290) and susceptible (Pharaoh) checks. Likewise, disease development in each of the selected varieties was comparable across all experiments based on experiment $\times$ variety interactions (WI: $p=0.263$, IN: $p=0.185$). The reaction of both the WI and IN varieties to Mp ranged from susceptible to moderately resistant. Although no variety was immune to CR development, three MG I-II and 16 MG II-III varieties performed similar to or better than DT97-4290 and had significantly lower AUDPC than Pharaoh. Results of this study indicate there are commercial varieties available to manage CR in the upper Midwest.

Phylogenetic relationships and diversity of endohyphal bacteria of plant-associated Pezizomycotina

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Newly discovered bacterial endosymbionts in diverse mycorrhizal, pathogenic, and endophytic fungi significantly alter fungal phenotypes, thus influencing the ecology and evolution of fungal symbioses. In tropical forests, seed-associated Ascomycota are important drivers of forest dynamics, with members of the same taxa sometimes enhancing or inhibiting seed survival and germination after soil contact. In the first study to date examining seed-

associated fungi for endohyphal bacteria, we used PCR to detect bacterial 16S rRNA in genomic DNA extracts of fungi previously isolated from surface-sterilized seeds of a focal genus of neotropical pioneer trees (*Cecropia*). We found that phylogenetically diverse bacteria, including species of *Rhizobium*, *Pandoraea*, *Burkholderia*, *Ralstonia*, *Pseudomonas*, *Xanthomonas*, *Luteibacter*, *Streptomyces*, and *Streptococcus* are abundant among seed-associated fungi representing two focal orders of Sordariomycetes from multiple geographic locations. Strains generally are related to, but distinct from, endohyphal symbionts of rhizosphere fungi and foliar endophytes in the same site. Incongruence between bacterial and fungal phylogenies suggests a facultative association influenced in part by spatial and temporal factors. Ongoing work will assess effects of these bacteria on fungal phenotypes, plant-fungus interactions, and the evolution of bacterial endosymbiosis in the ecologically diverse Ascomycota, the most species-rich fungal phylum.

Corn disease risk perceptions from the 2009 Midwestern crop management survey

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Corn grower (CG) and certified crop advisor (CCA) perceptions of disease risk in corn production are likely to impact their management choices. We undertook a survey of corn management practices to measure disease risk perceptions held by these two stakeholder groups. The self-administered survey was mailed to a random sample of CCAs and CGs across IA, IL, OH and WI in spring 2010. The average response rate was 47%. Missing response data were estimated by multiple imputation. The survey was analyzed as a dual-frame design with stratification by state. CGs had a 1.9 times greater odds than CCAs of classifying diseases as “Extremely important”, and a 1.7 times greater odds than CCAs of classifying disease resistance as “Extremely important” in successful corn production. CGs and CCAs rating their disease identification ability as “Excellent” had a 46 times greater odds of actively scouting corn fields than those who viewed their disease identification skill as less than Excellent. CGs generally perceived diseases as being more risky to corn production compared to the risk perceptions of CCAs. There were also regional differences in the perceived importance of different diseases; for example, in WI foliar blights had 61% lesser odds than in IL of being viewed as “Extremely risky” to corn production. Results will be used to inform the creation of educational materials and protocols on best practices in corn disease management.

DNA based diagnostic markers for detection and differentiation of North American *Heterobasidion* spp.

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Heterobasidion annosum (Fr.) Bref. *sensu lato* (s.l.) is one of the most destructive pathogen causing damage to conifers in the Northern Hemisphere. *H. annosum* consists of five species: three European (*H. annosum sensu stricto*, *H. parviporum* and *H. abietinum*) and two North American (*H. irregulare* and *H. occidentale*); all with different but partially overlapping host preferences. There is an increasing need by the regulatory agencies for rapid and accurate detection and differentiation of *Heterobasidion* species for the import/export trade of commercial wood products and green logs. Three sets of primers were designed to be used in one test tube: universal plant primers as a positive control of PCR reaction, *H. irregulare* genome specific primers, and *H. occidentale* genome specific primers. DNA was extracted from healthy and infected by *Heterobasidion* species wood samples of red alder, western hemlock, red pine, Douglas fir, and from pure fungal cultures using a standard kit. PCR followed by electrophoresis were performed and amplicons were visualized on 1% agarose gel. These 3 sets of primers proved to work with all samples checked in all combination and showed band sizes: 664 bp for plant DNA, 365 bp for *H. occidentale* DNA, and 160 bp for *H. irregulare* DNA. This research provides the framework for a robust detection and differentiation tool of *Heterobasidion* spp.

Deciphering specificities of TAL effectors in *Xanthomonas citri* and prospects in citrus

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Citrus bacterial canker is a major problem in many citrus producing regions. The citrus canker bacterium, *Xanthomonas citri*, has been grouped into canker “A”, “B”, and “C” as a result of genetic differences. Strains within the three canker groups possess a set of transcriptional activator like effectors (TALEs), some of which have been shown to be important in canker development. The number of TALEs varies within a given strain in the canker group. TALEs among different strains are also distinct in repeat variable diresidues (RVDs), which confer DNA binding specificities and predictions might not be accurate. Based on the binding specificities of TALEs, combinations of different effector binding elements (EBEs) in *Bs3* promoter background, *ProBs3₁EBE* and *ProBs3₄EBE*, were tested for their interactions against different TALEs from diverse *X. citri* strains using GUS expression studies. Our studies indicate that TALEs from different *X. citri* strains have differential binding specificities. We have developed a system by constructing engineered EBEs and an avirulence gene as an executor gene, thereby achieving elicitation of resistance pathway upon activation of EBEs by TALEs. Future prospects in ecofriendly management of citrus canker can be achieved by utilizing engineered EBE constructs in minimal promoter concept, wherein TALEs can efficiently bind and execute resistance-imparting genes.

***Rhizoctonia* spp. dynamics and optimal timing of glyphosate application to cereal cover crops to manage onion stunting in Washington and Oregon**

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Onion stunting or bare patch caused by *Rhizoctonia* spp. is an economically important disease in sandy soils of the Columbia Basin of Oregon and Washington. Patches of stunted onions develop where cover crops of wheat or barley are killed with a herbicide spray prior to spring planting of onion seed. Onion bulb yield and concentration of DNA of *R. solani* AG groups in soil were compared within patches vs. adjacent healthy areas. Onion stunting reduced bulb yields 25 to 77% within patches. Severe stunting reduced total and marketable bulb weights. DNA of *R. solani* AG8 was detected at medium to high risk levels from a majority (57%) of patched areas compared to AG4 (21%), AG3 (10%), and AG2-1 (7%). To determine the optimum timing of cover crop kill with herbicide to reduce disease pressure, the winter wheat cover crop was sprayed with glyphosate 3, 17, or 27 days prior to onion seeding in a large-scale field trial. Glyphosate sprayed 17 and 27 days prior to onion seeding reduced the number of patches, total patched area, severity of stunting, and patch severity index (severity of stunting x patch area) compared to plots sprayed 3 days prior to planting. Less DNA of *R. solani* AG8 was detected in plots with the earlier sprays, and AG8 DNA level was positively correlated with patch severity index. Although different *R. solani* AG groups were detected from stunted patches, *R. solani* AG8 was associated most consistently with stunting.

Method for detecting treatment effects on root growth and nematode densities in turf infected with *Belonolaimus longicaudatus* or *Trichodorus obtusus*

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The effectiveness of new non-fumigant nematicides for nematode management in turfgrasses has been difficult to establish with traditional field assay methods. High variability of the spatial distribution of nematode densities in the field contributes to this difficulty. An improved method for field testing was developed based on trials conducted in South Carolina. The treatments included two nematicides, a fungicide, and a nematicide and fungicide combination applied at four locations blocked by estimated initial nematode density. Treatments were assigned at random to plots within the blocks. Nematode population densities and root weight estimates were based on three or four soil cores per plot (5 cm diameter, 20 cm depth) taken at three or four sampling dates. A statistical power analysis was conducted to determine the number of cores per plot needed to detect effect of treatments on root weights and nematode population densities. In most cases four soil

cores per plot resulted in power ≥ 0.8 , an 80% or higher probability of detecting a treatment effect. We present an improved method for field evaluation of nematicides and discuss potential sources of variability other than spatial distribution of nematodes.

Assessing the reactive oxygen species scavenging activity of *Diplodia pinea*

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Water-stressed Austrian pine trees are more susceptible to the fungal pathogen *Diplodia pinea*, but the molecular mechanisms of drought-induced susceptibility are unknown. Droughted trees produce greater amounts of reactive oxygen species (ROS) in their shoots, but following infection with *D. pinea* ROS levels decrease significantly. This suggests that *D. pinea* actively degrades ROS during infection, but the ROS scavenging mechanisms of *D. pinea* have never been investigated. Therefore, we challenged the fungus with various H₂O₂ (a potent ROS) concentrations in vitro and correlated H₂O₂ levels in the medium with fungal growth and the activity of the ROS scavenging enzymes peroxidase and catalase. The fungus exhibited reduced growth, mycelia mass and protein content with increasing concentrations of H₂O₂. The amount of H₂O₂ detected in the medium was only about 5% of that applied, yet fungal growth was still significantly reduced by increasing amounts of H₂O₂, indicating that the fungus is quite sensitive to minor changes in H₂O₂. *D. pinea* possess robust ROS scavenging ability, as both catalase and peroxidase activity increased with increasing H₂O₂ concentration, resulting in nearly a complete removal of H₂O₂ from the media by 5 days post inoculation at all concentrations tested. The role of these enzymes in pathogenicity have yet to be confirmed, but clearly *D. pinea* is capable of reducing H₂O₂ levels which may help prevent detection by the plant during infection.

Characterization of *Xylella fastidiosa* *popP* gene required for pathogenicity

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Xylella fastidiosa (*Xf*) possesses the two component regulatory system (TCS) *popP/Q* which differentially regulates genes in response to environmental stimuli. To elucidate the role of *popP* in Pierce's disease of grapes, a site-directed deletion method and chromosome-based genetic complementation strategy were employed to create the *XfΔpopP* mutant and complementary *XfΔpopP*-C strains. Greenhouse-grown Cabernet Sauvignon grapes were mechanically inoculated with *XfΔpopP*, *XfΔpopP*-C and wild-type *Xf* Temecula. Three months after inoculation, no symptoms were observed in grapes inoculated with *XfΔpopP*. However, grapevines inoculated with wild type *Xf* and *XfΔpopP*-C showed a typical PD symptom. *Xf* titers in the grapevines inoculated with *XfΔpopP* were significantly lower than that of *XfΔpopP*-C and wild-type as estimated by quantitative PCR. *In vitro* studies showed that while the mutants and wild types had similar growth curves, *XfΔpopP* appeared to have significantly reduced abilities to aggregate and adhere to the wall of culture tubes. Biofilm production of *XfΔpopP* was 42% less than that of wild type and *XfΔpopP*-C. *In vitro* gene expression profile analyses of *XfΔpopP*, *XfΔpopP*-C, and wild type have been carried out to further investigate regulatory pathways of TCS *popP/Q* in response to *Xf* infection.

Flg22 derived from *Xanthomonas citri* subsp. *citri* and 'Candidatus Liberibacter asiaticus' trigger similar defense responses in mandarin and grapefruit

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Citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), and huanglongbing (HLB), caused by 'Candidatus Liberibacter asiaticus' (CLAs), are two economically important bacterial diseases of citrus worldwide. However, these two pathogens have very different life styles. Our previous research showed that flg22 from *Xcc* (Xflg22) was a potent PTI elicitor in different citrus genotypes. However, little was known about CLAs flagellin as a PAMP. CLAs has a flagellin gene but flagella have never been observed. The effect of Xlflg22 and Lflg22 peptides on different citrus genotypes was analyzed and compared by quantifying the expression of 16 defense genes using real time PCR. EDS1, NDR1, SGT1, RAR1, PAL1, NPR2 and NPR3 were upregulated by both Xflg22 and Lflg22 in 'Sun Chu Sha' mandarin, a genotype that is resistant to canker and moderately tolerant to HLB. In contrast, only PR1 was upregulated by these two PAMPs in the susceptible

'Duncan' grapefruit. Although Lflg22 has 11 amino acid differences with Xflg22, its capacity of inducing PTI was confirmed. Moreover, the differences in the induction of defense genes by these two PAMPs seem to be associated with the level of resistance/tolerance in the citrus genotypes studied.

WITHDRAWN

Simple sequence repeat DNA markers linked with broad-spectrum rust resistance in common bean PI 310762

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The rust disease of common bean (*Phaseolus vulgaris*) is caused by *Uromyces appendiculatus*, a pathogen known for its extensive virulence diversity. The accession PI 310762 has broad rust resistance to 89 of 90 rust races. PI 310762 is resistant to all races that render susceptible all ten common bean rust resistance genes. The inheritance of rust resistance in PI 310762 was determined on 157 F₂ plants from the cross of Pinto 114 (S) x PI 310762 (R). Rust resistance based on the infection type observed fit a 3:1 R vs. S ratio, suggesting that resistance is conferred by a single dominant gene. DNA was isolated from the parents and F₂ plants and three susceptible bulk DNA samples were created. The DNA of the parents and bulks was screened with 5399 SNP DNA markers. The resulting SNP genotypes were analyzed to identify SNPs at which the susceptible and resistant plants had alternative alleles and the bulks were homozygous for the susceptible parent allele. The SNP-containing fragments were aligned to the common bean genome DNA sequence at Phytozome, DOE, JGI (www.Phytozome.net) to determine the genome positions of the SNPs. Simple sequence repeats (SSRs) in proximity to the identified region were selected and SSR markers designed. The SSR markers polymorphic between Pinto 114 and PI 310762 were used to analyze the 157 F₂ plant DNAs. SSR markers were identified that distinguished homozygous resistant, homozygous susceptible and heterozygous F₂ plants.

The concentration of sporangia or zoospores of *Phytophthora ramorum* required for infection of host roots

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Propagules of *P. ramorum* have been found in nursery irrigation runoff and in streams adjacent to infected forests and nurseries. These facts lead one to ask what amount of inoculum in water is necessary to cause infection of host roots. Roots of *Viburnum* and *Rhododendron* cuttings were inoculated using sporangium or zoospore suspensions at 500, 50, 5 or 0 sporangia/mL and allowed to incubate for a month. Plant roots were then plated on selective

media and the percent root infection determined. A total of 4 isolates (from 3 lineages) were tested in 53 experiments. For both hosts, all isolates and both inoculum types, infection was observed at 5 propagules/mL, although higher levels of infection (expressed either as incidence or percent infection) were seen at 50 and 500 propagules/mL. When roots of *Viburnum* cuttings were inoculated at 500, 50, 5 or 0 sporangia/mL, production of inoculum from those roots (measured as colonies/pot collected from 20 mL samples of water poured through each pot weekly for 8 wk) was high in plants inoculated at 500 sporangia/mL (as high as 44 CFU/mL throughput), lower at 50 sporangia/mL (14 CFU/mL), and observed in only 1 of 3 experiments from plants inoculated at 5 sporangia/mL (at a maximum of 8 CFU/mL).

Race-specific PCRs for *Verticillium dahliae* reveal a high frequency of race 2 strains in spinach seed

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Verticillium dahliae causes an economically important wilt disease on a range of crops including lettuce. Two pathogenic races of *V. dahliae* have been described. Sources of resistance to race 1 exist in a few crops, due to a plant immune receptor *Ve1*, which recognizes the race 1-specific fungal effector *Ave1*. However, only partial resistance to race 2 exists in lettuce. PCR assays are available to identify race 1, but no complementary test exists to positively identify race 2. Here, we compared the genome sequences of race 1 and 2 *V. dahliae* to identify sites useful as markers to distinguish the two races. We designed primers based on polymorphisms in a hypothetical protein, which we hypothesize represent fixed nucleotide substitutions between these two races. PCRs with these primers produced an amplicon of 255 bp with DNA from race 2 isolates but failed to amplify with race 1 DNA. We screened over 400 *V. dahliae* isolates from spinach seed that previously did not amplify with a race 1-specific PCR. All of them amplified with primers designed from race 2 sequence. To validate the PCR result, 50 of these isolates from spinach seed were used to inoculate two differential lines of lettuce, Salinas (*ve1*) and La Brillante (*Ve1*). The disease reactions strongly supported the race 2-specific PCR data. These race 2 primers will complement existing race 1 PCR assays and are useful for the purposes of resistance breeding and disease surveillance.

Histology and transcriptional changes of maize seed infected by *Aspergillus flavus* and *Fusarium verticillioides*

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Maize ear rots caused by *Aspergillus flavus* and *Fusarium verticillioides* are major concerns for human and animal health. Infection and colonization by these two fungi remain poorly understood, and there is limited knowledge of how maize kernels respond to these fungi. We followed colonization, transcriptome changes and tissue-specific gene expression of developing B73 maize seeds inoculated with each of these fungi. Within three days, both fungi colonized in the aleurone, endosperm and embryo tissue, but the pattern for colonization by the two fungi differed. Transcriptome analysis using RNA-seq and qRT-PCR revealed a set of genes that was similarly expressed during infection by these two fungi. RNA in situ hybridization showed that two maize defense genes, *PRms* (Pathogenesis related protein, maize seeds) and *UGT* (UDP-glucosyltransferases), were induced in the aleurone and scutellum of seeds inoculated with either *A. flavus* or *F. verticillioides*. By comparing histological and in situ hybridization results in adjacent serial sections, we found that these two genes were expressed in the tissue before fungal colonization. These results show that these two fungi colonize three major tissue types of the seed and induce defense gene expression in a tissue-specific fashion in advance of hyphal invasion.

***Neotyphodium* endophyte infections in a native grass, *Poa alsodes*, across latitudinal range**

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Poa alsodes is a perennial, cool-season native grass found in woodlands in Eastern North America and known to harbor the *Neotyphodium* sp. fungal endophyte. Twelve *Poa alsodes* populations of 50 individual plant samples were collected spanning the latitudinal range from North Carolina to New York. *Neotyphodium* sp. infection was determined by phytoscreen immunoblotting method and ranged from 26% to 100%. Pure *Neotyphodium* cultures were grown from 20 plants per population, and variation in fungal

morphotypes was observed. Genetic and chemotypic structure of the endophyte was determined from samples originated from 18 locations. At least two different endophyte strains were identified that displayed variation with presence and absence of the PCR markers. According to their genetic profiles, one is of a hybrid origin and predicted to produce peramine, chanoclavine, and N-acetyl norlooline, representing 3 of 4 possible alkaloid types. The other strain is likely of nonhybrid origin and predicted to produce only peramine. One population sampled harbored both endophyte types. Future work will 1) reveal phylogenetic relationship of two strains, 2) determine what factors affect their distribution and frequency, 3) measure predicted alkaloids by LS-MS and correlate their levels with insect herbivory among populations, and 4) assess ecological impacts of the two strains on the host. This research would help to improve management practices of native woodland grasses.

***Phaciidiopycnis washingtonensis*: Inoculum availability, persistence and seasonal host susceptibility in Washington apple orchards**

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P. washingtonensis infects apple fruit in the orchard but decay symptoms develop during storage. Pycnidia on diseased shoots are believed to be the inoculum source for fruit infection in the orchard. However, the period of twig susceptibility and availability of viable inoculum in the field are unknown. Two separate studies were conducted to assess availability of viable inoculum during the fruit growing season and characterize the seasonal nature of twig susceptibility to infection in the orchard. In a Red Delicious orchard, 2-year-old twigs were wounded, cold-injured and inoculated with the fungus in spring 2011. Over the next 10 months, 1 cold-injured twig from each of 10 inoculated trees was harvested monthly and viability of pycnidia assessed. Viable pycnidia were detected on all twigs at each sampling time. In a second study, twigs were wounded, with or without cold injury, and inoculated every other month over 2 years. Canker development and the presence of pycnidia on each twig were monitored over 6 months. Twigs were most susceptible to infection during spring to early summer. A cold injury treatment facilitated infection establishment and production of pycnidia. Canker development also was observed on non-cold-injured twigs but the production of viable pycnidia was less. Thus, viable inoculum of *P. washingtonensis* was available during all sampling months, suggesting that viable inoculum is likely not a limiting factor for fruit infection in the orchard.

Transmissibility of *Colletotrichum lindemuthianum* by seed from the field with different disease intensity

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The beans anthracnose (*Colletotrichum lindemuthianum*), is considered one of the most important diseases of crop that can be transmitted by seeds. It was tested infection levels in the fields: 0, 0.25, 0.5, 1.0, 2.0 and 4.0% seed artificially infected with *C. lindemuthianum* race 65, with and without crop residues. After harvest, it was evaluate the pathogen transmissibility from seeds. It was deposited at 100 grains germitest paper moistened with sterile distilled water (method paper roll) and incubated at 21 ± 1 °C in a completely randomized design with six replications/infection level. After seven days, it was proceeded to assess the disease incidence in the cotyledons. According to statistical analysis, there was significant difference between the different levels of inoculum and different planting areas. For the variable level of infection, it was adjusted the model described by the equation $y = -0.001589x^2 + 0.008663x + 0.011539$ with $R^2 = 77\%$. In the Tukey test at 5% probability, it was found that the planting areas differ, with the highest average transmissibility observed in the area with crop residues.

Effects of mineral nutrition on soybean rust and yield

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Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, is one of the most serious diseases of this crop. Because there are no resistant varieties, management of this disease typically involves the use of fungicides. However, there is precedent for the use of foliar applied micronutrients in ameliorating disease severity in numerous host: pathogen systems. The objective of this work was to evaluate selected commercially available minor element formu-

lations for their effects on disease severity. Commercial products were obtained from Brandt Consolidated, Inc. that contained Fe, Mo, Mn, B, Zn and Al, and these products were applied at two rates. Disease severity was significantly reduced with the high rates of B, B plus Mo, and Mn. There were strong correlations between disease severity and leaf tissue concentrations of B, Mn and S. Although other elements, such as Cu, Mg and Ni were not applied as foliar sprays, there were significant correlations between disease severity and tissue concentrations of these micronutrients. Relationships among tissue analyses, disease severity and yield components will be provided.

Enhance resistance against *Colletotrichum gloeosporioides* in strawberry by overexpressing the *Arabidopsis NPR1* gene

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Colletotrichum gloeosporioides is the causal pathogen Anthracnose Crown Rot (ACR), a serious disease in strawberry production areas in the southeastern United States. Since chemical control is often ineffective, resistant cultivars are needed for managing this disease. We have generated Hawaii-4 (H4) accession (*Fragaria vesca* L.) plants expressing the *Arabidopsis NPR1* gene, a key positive regulator of systemic acquired resistance, and evaluated their resistance to *C. gloeosporioides*. Transgenic lines expressing different levels (high, medium, and low) of *NPR1* and control (non-transformed) plants were spray-treated with a suspension of 10^6 conidia ml⁻¹ of *C. gloeosporioides*. Leaf tissues were collected at 0, 12, 24, 48 and 72 hr after inoculation for total RNA isolation and real-time qPCR analysis for *NPR1* expression. The housekeeping gene *eIF1a* was used as an internal control. The experiment was repeated 3 times with 5 plants per treatment. The expression of *NPR1* was induced in the transgenic plants over time after pathogen infection, even though the 35S constitutive promoter was used. All control plants died within two-week period, however the transgenic plants took longer to wilt and die, indicating that they are more tolerant to *C. gloeosporioides*. Our data demonstrate that overexpressing the heterologous *NPR1* gene is able to increase disease resistance in strawberry, and holds great potential to develop strawberry cultivars resistant to *C. gloeosporioides*.

***Fusarium kyuyense*, *F. andiyazi*, and *F. nelsonii*, three new species associated to sugarcane wilt in Mexico**

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Sugarcane (*Saccharum* spp. hybrids) is the most important industrial crop in Mexico with almost 750,000 ha cultivated. In recent years, the increase in the incidence of sugarcane plants with wilt symptoms has been observed in the states of Michoacan and Morelos. Considering the importance of this disease a research was proposed in order to identify the causal agent of wilt in the sugarcane cultivars using a phylogenetic approach. Sugarcane plants showing typical symptoms were collected from Zacatepec, Santa Clara and Pedernales regions located in the central and pacific sugarcane production area. Small portions of tissue from the basal, middle and apical of the inner stem were selected, disinfested and placed on PDA medium. After five days fungal colonies developed, later they were transferred to Petri plates for obtaining pure cultures. For molecular analysis DNA extraction was performed with conventional protocols and the entire region of the ITS rDNA and Translation Elongation Factor a-1 were successfully amplified. Results showed that beside *Gibberella fujikuroi* species complex, *Fusarium kyuyense*, *F. andiyazi*, and *F. nelsonii*, were also found. These species had not previously been reported associated to sugarcane in Mexico. This information will serve to evaluate the potential risk of infection of these species and the possibility to be considered during the selection process sugarcane varieties with resistant to diseases.

Evaluating bacterial wilt resistance of tomato rootstocks in North Carolina

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Bacterial wilt, caused by *Ralstonia solanacearum*, is indigenous to the southeastern United States and threatens tomato production in many humid

temperate regions around the world. Few management options are available for controlling bacterial wilt and include soil fumigation, host resistance and grafting with disease resistant rootstocks. On-farm trials in Jackson county NC in a field with high disease pressure provided insight on the utility of nine tomato rootstocks grafted to the commercial variety 'FL47' to manage bacterial wilt compared to a self- and non-grafted 'FL47'. Rootstock selection dramatically impacted disease incidence. Seminis rootstock 'Cheong gang' had the least wilt incidence of 25% 75 days after transplanting (DAP). BHN 1054, RST-04-106, CRA66 and BHN998 rootstocks had slightly higher disease incidence of 33%, 35%, 43%, and 45% respectively. Susceptible non-grafted and self-grafted controls reached 100% disease incidence by 75 DAP. Grafting coupled with host resistant rootstock can be used to manage bacterial wilt for tomato production in western NC. Future research should include breeding for enhanced resistance and resistance that is durable to the known diversity of the pathogen.

Comparative analysis of *Fusarium graminearum* on two hosts using next generation sequencing

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Fusarium graminearum, the causative agent of Fusarium head blight, is an important fungal pathogen of wheat, barley and maize, which has recently emerged as an important pathogen on soybean. The pathogen not only reduces grain yields and quality, but also produces mycotoxins that have serious impacts on both human and animal health. The mechanism behind its emergence on soybean is not understood, but may be the result of environmental changes and/or pathogen population shifts (host-switching or a quantitative increase in virulence). In order to unravel the genetic underpinnings of this recent emergence we undertook next generation (Illumina) sequencing of seven cultures of *F. graminearum* collected from infected maize and soybean crops in Iowa. Three were isolated from maize in the 1990's, three were isolated from soybean in 2007 and the last was isolated from maize in 2007. These data were aligned to the genome available from the Broad Institute using the alignment program GSNAP (Genomic Short-read Nucleotide Alignment Program), and variants were called using VCFtools. Analysis of the single polymorphism (SNP) data has revealed a significantly lower level of genetic diversity among the samples collected from maize compared to those collected from soybean (75% reduction), indicating the possibility that a host-switching event may have occurred. Further work to elucidate the function of these SNP's is currently underway.

Delimitation of tropical endophytic *Diaporthe* species from three euphorbiaceous hosts: *Hevea brasiliensis*, *H. guianensis*, and *Micandra* sp.

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Diaporthe (Diaporthaceae, Diaporthales, Ascomycota) includes a wide array of species that are endophytes, saprobes, opportunistic pathogens, as well as aggressive pathogens of economically important crops. Unfortunately, species identification in *Diaporthe* remains problematic due to the lack of meaningful morphological variation and past reliance on host species or genus in describing species. Sixty endophytic *Diaporthe* samples were collected and isolated from asymptomatic leaves and bark of three different wild hosts (*Hevea brasiliensis*, *H. guianensis* and *Micandra* spp., Euphorbiaceae) in Peru, as well as cultivated *H. brasiliensis* trees in Cameroon and Mexico. The objective of this study was to determine the identity of the species associated with these trees in the wild and in plantation. In this study we used ecological, morphological and multigene (ITS nrDNA, tef1, MCM7, btub and SSU) phylogenetic approaches to delimit *Diaporthe* species. Preliminary phylogenetic analyses of the ITS nrDNA regions resulted in ca. 30 "phylogenetic species." However, when using a multigene approach many more species were detected. Several of the species are most likely new. Results of this study also show that *Diaporthe* is more abundant in plantations than in wild trees. This research will aid in the understanding the biology of endophytic *Diaporthe* and its relationship with pathogenic species, as well as its distribution and ecological function.

Evaluation of the blight decision support system for the integrated management of potato and tomato late blight

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We compared late blight suppression using standard grower practice to late blight suppression achieved using a web-based decision support system (DSS) in a series of naturally inoculated field experiments in 2010, 2011 and 2012. The Blight DSS links several models into a system that predicts disease dynamics based on weather, host resistance, and fungicide. The DSS contains two late blight forecasts, Blitecast and Simcast, and uses both historical weather data as well as site-specific weather forecasts up to seven days into the future. Simcast was used to guide DSS-based fungicide applications. Two different potato cultivars were used each year – one more resistant than the other. A late blight epidemic occurred in each year, and disease severity at the end of the season in the unsprayed more susceptible cultivar ranged from 39–98%. Disease suppression in the DSS-scheduled applications (no late blight) was equal to that in the standard grower practice (weekly application), but fewer fungicide applications were scheduled by the DSS on the more resistant cultivar. A major benefit of the DSS was to enable the manager to take advantage of host resistance. In each year, there was at least a 38% reduction in fungicide recommended for the moderately resistant cultivar, while maintaining the same high level of disease suppression as in the weekly application schedule.

An update on the biology of the pecan truffle (*Tuber lyonii*) in the southeastern USA

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Truffles are traditional European foods but interest and enthusiasm for truffles has grown throughout the world. In the Western USA, truffle connoisseurs have turned to local truffles. In the Southeastern USA there is an abundant and delicious local species, *Tuber lyonii*. This truffle is a spiny-spored member of the rufum lineage referred to as the Pecan Truffle. It is collected in commercial orchards with pecans (*Carya illinoensis*, *Juglandaceae*) but it has not been widely commercialized. The Pecan Truffle shows great promise as a local crop in the Southeastern USA and we have begun studying the biology of *Tuber lyonii* to explore how it might be cultivated with pecans. Here we provide an overview of our recent research on *Tuber lyonii* and clarify what is known about its distribution, fruiting phenology, and host associations. Preliminary phylogenetic analyses based on ITS rDNA indicate that *Tuber lyonii* is a species complex across its range in Eastern North America. Our recent studies using trained truffle dogs and 454 sequencing directly from ectomycorrhizal roots of pecans suggest that *Tuber lyonii* is widespread within many pecan orchards in the Southeastern USA. Preliminary results suggest that orchard management (particularly pH and weed control) may have a strong impact on the distribution and production of *Tuber lyonii* with pecans.

Spatial structure of ectomycorrhizal fungi within individual trees and across forest stands of the neotropical tree *Dicymbe corymbosa*

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Recent studies of ectomycorrhizal (ECM) fungi in the Guiana Shield region of South America have revealed high fungal diversity and widespread host sharing among plants in the genera *Dicymbe*, *Aldina*, and *Pakaraimaea*. However, little is known about spatial structure of fungal communities in monodominant stands of *D. corymbosa* that are known hotspots of fungal biodiversity. *D. corymbosa* is unique among Neotropical trees because it has indeterminate shoot and root growth, resulting in large, stand-dominating trees with aerial roots, fused pseudotrunk, and basal root mounds. The unusual growth form of these long-lived trees provides unique rooting habitats: 1) aerial litter caches in the upper canopy, 2) decayed wood in the lower canopy, 3) thick leaf litter and humic accumulations on root mounds, and 4) mineral soil that is low in organic matter. We hypothesized that the different rooting zones within *D. corymbosa* trees provide unique habitats that host distinct ECM fungal communities. We also hypothesized that spatial separation of trees creates an island biogeography effect whereby individual trees host distinct ECM communities. To examine the spatial patterns of ECM fungal diversity in *Dicymbe corymbosa* stands we sampled ECM roots from each of the four rooting zones in 20 large trees across four plots in Guyana. Here we

present our results that compare the influence of the four unique rooting zones and the spatial arrays of trees on ECM fungal community structure.

Bacteriophage ecological niches and potential role in coevolution of *Xanthomonas arboricola* pv. *pruni* on peach trees

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Bacteriophage are ubiquitous, yet there is limited research assessing the coevolutionary role with their bacterial hosts in nature. *Xanthomonas arboricola* pv. *pruni* (Xap) causes bacterial spot on *Prunus* spp., including peach. Peach trees as perennials allow monitoring of phage-bacterial interaction throughout the year as Xap survives on this host and causes epidemics during the growing season. The initial objective of our research was isolation and characterization of phages from five diseased peach trees in two orchards from spring through the following dormancy. Monthly during the growing season, five diseased leaves were sampled from each tree. Phages producing at least three plaque types were detected from cankers, diseased leaves (including individual lesions) and fruit, defoliated leaves in fall and winter, and from soil. Phages were most consistently isolated from attached diseased leaves, with 87.5% of samples containing phage. Selected phages were single-plaque purified and used for bacterial host range assays. These assays included Xap strains isolated concurrently with the phages, as well as Xap isolates collected since late 1970s from varied geographic locations and host plants. The most commonly isolated plaque-type phage produced clear lysis zones on more than 90% of Xap isolates. The Xap population apparently remains sensitive to an increasing seasonal phage population although both microbes coexist in common niches.

Development and validation of quantitative polymerase chain reaction protocols for detection of *Soybean vein necrosis virus*

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Soybean vein necrosis virus (SVNV) was first reported in Wisconsin in 2012. SVNV is a new member of the *Tospovirus* family and is becoming more frequent in occurrence in the north central region USA. New real-time reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) protocols were developed for detection and quantification of SVNV particles in plants. Primer sets were developed to target each of the ribonucleic (RNA) segments of the tripartite genome of SVNV and optimized for use in RT-qPCR. Primers were developed for the 'L' RNA and the 'M' RNA using GenBank accessions HQ728385 and HQ728386. While primers targeting the nucleoprotein coding region of the 'S' RNA were based on unique sequences of SVNV isolates obtained in Wisconsin. We established the sensitivity of SVNV detection using three methods: PCR, RT-qPCR, and nested PCR. Our findings indicate that nested PCR is required to reliably detect SVNV 'S' RNA present at less than 10 copies per sample. These qPCR methods will be used for future SVNV epidemiological studies.

Fungicide applications affect fruit diseases and quality of muscadine grape (*Vitis rotundifolia* Michx.)

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Fungicides can significantly reduce losses due to disease in the yield and quality of muscadine grapes. In three studies fungicides were applied individually or as part of a full season schedule from early bloom until harvest to three muscadine grape cultivars. The objective was to compare the effect of a full season fungicide treatment of 9–12 applications applied on a 10-day interval to fewer applications of individual fungicides on disease incidence, yield, and berry quality. Foliar and berry diseases were rated on visual scales. Sugars, acids, ellagic acid, and resveratrol content were determined by HPLC. In study 1, the highest berry yields were from the myclobutanil, azoxystrobin, and full season treatments. The lowest bitter rot and Macrophoma rot scores and lowest resveratrol levels were from the azoxystrobin and full season treatments. In study 2, four applications of the individual azoxystrobin, myclobutanil, or cyprodinil + fludioxonil mixture treatments applied at 30-day intervals were as effective in reducing total berry disease scores as the full season treatment. In study 3, the cyprodinil + fludioxonil mixture alternated with azoxystrobin resulted in the lowest total berry disease score. These data indicate that the number of fungicide applications can be reduced to as few as four without an increase in berry rot scores; however, when fruit diseases

of muscadine grapes are controlled, levels of resveratrol, a beneficial phytoalexin, decrease.

Initial detection of *Phytophthora ramorum* at two New York nurseries through sampling of water in retention ponds

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The Cornell University Plant Disease Diagnostic Clinic (PDDC) has provided suspect sample processing for the New York State Department of Agriculture & Markets (NYS DAM), the United States Forest Service (USFS), USDA-APHIS-PPQ-Eastern and Western Regional Laboratories and fellow NPDN laboratories. Cornell PDDC laboratory staff has provided testing during numerous trace forward and trace back events, national surveys, observational surveys, and for Farm Bill projects and has been certified by USDA to process *Phytophthora ramorum* samples since 2006. To date, the Cornell PDDC has processed 3,056 suspect *P. ramorum* samples. In 2004, a questionable positive was detected from a mature red oak in the Tiffany Creek Preserve but that area was later deemed free of *P. ramorum* after two years of extensive sample collection and testing provided no additional positive samples. Prior to 2010, *P. ramorum* was not detected during any of the surveys of nursery stock or surrounding natural areas. However, since then the pathogen has been detected several times, using various testing methods in multiple substrates. In 2010, the pathogen was detected in retention pond water and in 2012 was detected in retention pond water, soil and leaf tissue samples from two locations. As a result, the NYS DAM and Cornell have worked together to provide the nursery industry with best management practices for diseases caused by *P. ramorum* and NYS DAM has expanded water sampling efforts.

Fluorescent *Pseudomonas* associated with cranberry (*Vaccinium macrocarpon* Ait.) roots and soils

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Native and commercial cranberry bogs represent an ideal system for comparing differences in microbial population structures which may have arisen due to cultivation. Wild and domesticated cranberry bogs in southeastern Massachusetts are located in close geographical proximity, they share a common climate, and because many cultivated plantings are essentially wild accessions, native and cultivated plants are genotypically nearly identical. Thus differences in microbial populations can confidently be attributed to agricultural practices. As an initial part of a project to characterize wild and cultivated cranberry-associated microbial populations, soil and roots of native and commercial cranberry plants were aseptically sampled and examined for the presence of fluorescent pseudomonads. Isolates were characterized for a range of phenotypic and genotypic characters using standard and novel identification methods for plant-associated microbes, including fatty acid analysis, 16S rDNA gene sequencing, REP-PCR and MALDI-TOF MS. The forty-four characterized isolates were genetically and phenotypically diverse, but localized to three broad but distinct phylogenetic clades: *P. fluorescens sensu lato*, *P. putida sensu lato*, and an unknown clade distantly related to *P. syringae*.

Assessment of soil nematode biodiversity in preserved soil samples nematodes

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Accurate identification of individual nematodes and characterization of communities is a challenging problem. The complexity of nematode communities and the limited availability of morphological taxonomic expertise are some of the obstacles that nematode community analysis faces. Several molecular biology based methods for nematode identification have been described which have the potential to assist in routine monitoring of quarantine pests and in studies of nematode diversity. We previously developed a highly efficient DNA extraction method from individual nematodes based on sonication, proteinase K treatment and quick freeze-thaw cycles. This extraction method

was used to characterize several samples of DESS-preserved nematodes isolated from Israel. Successful PCR amplification of a ~1.2 Kb partial sequence of the 18S rDNA gene was achieved from 61 of the 82 samples processed. Sequences of PCR products from 47 individual nematodes were obtained and matched 17 different genera from the GenBank database with average pairwise identity values over 98%. The nematode samples studied included members from 3 orders and 13 different nematode families. The genera identified included bacterial, hyphal, and plant feeders as well as animal predators and omnivore nematodes.

Pathogenicity and host range of *Phytophthora infestans* population isolated from potato in Thailand

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The population of *Phytophthora infestans* was recovered from late blight infected potato leaflets collecting from northern Thailand. A total of 132 isolates was used for pathogenicity test on potato (cv. Atlantic and Spunta) leaflets and tuber slices, and tomato (cv. Delta and Seeda) leaflets using detached leaflets or tuber technique. Three parameters of pathogenicity; infection frequency (IF), lesion area (LA) and spore capacity, were measured. The mean of IF and SC of population was higher on both cultivars of potato tubers than potato and tomato leaflets. The LA on potato leaflets cv. Atlantic (87% lesion area coverage) larger than another host. One representative isolate was selected from the largest lesion producing isolate on both potato cultivars using for host range test by artificial inoculation. Three cultivated solanaceous plants; eggplant, chili pepper and petunia leaves, were expressed the soft watery and turned brown. However, the natural infection of *P. infestans* on the alternative host has not been found and reported in Thailand.

***Fusarium lactis* and *F. mexicanum* associated with galls of *Swietenia* in Mexico**

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Swietenia is a native plant of neotropical areas. *Swietenia* trees showing gall symptoms were observed in La Huacana and Nuevo Urecho, Michoacán, Mexico, in 2011. The affected trees were detected in the vicinity of commercial mango orchards with floral and vegetative malformation. Gall symptoms on *Swietenia* occurred in the branches of mature trees. The infected branches showed lateral buds that produced abundant small leaves, which initially were green but later turned dark brown as they die. The galls remained attached to the trees. The external tissues of the galls were necrotic, with some galls presenting nonnecrotic internal tissues. Occasionally, green, small galls were observed growing from dark brown galls. Severely affected branches presented few leaves. Fungal isolates associated with active gall tissues were morphologically characterized as *Fusarium*. PCR amplification of translation elongation factor (*EF-1a*) gene sequences and Blast (NCBI) analysis allowed the identification of *F. lactis* and *F. mexicanum*. *F. lactis* has been reported causing fruit rot of sweet peppers and endosepsis of cultivated figs in California. *F. mexicanum* has been shown to cause mango malformation in several mango producing areas of Mexico, including Michoacan. This is the first report of *F. lactis* and *F. mexicanum* associated coinfecting gall tissues of *Swietenia*. Gall diseased *Swietenia* trees could be acting as a natural reservoir of *F. mexicanum*.

Detection of *Fusarium mexicanum* causing malformation in mango nursery plants

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Mango is one of the most important fruit crops in Mexico. However, productivity is severely affected by the disease mango malformation, mainly in the central western region of the country. Infected trees show floral and/or vegetative malformed tissues. Several *Fusarium* species are responsible for this disease in mango producing areas around the world. In Mexico, *F. mexicanum* has been reported causing the disease in the states of Colima, Guerrero, Michoacan and Morelos. The objective of this work was to

determine if the disease was present in commercial nurseries in two states, Guerrero and Michoacan, which are among the main producers of mango in Mexico. Eighteen nurseries were inspected for mango malformation during 2011 and 2012. One to three year old plants showing the disease were observed in ten nurseries, with incidence of the disease varying from 0.003 to 25%. Five cultivars, Ataulfo, Criollo, Haden, Keitt, and Kent presented plants with vegetative and/or floral malformation. Criollo plants with malformation symptoms were observed in all of the ten nurseries. Isolates were obtained from malformed mango tissues from nurseries. Morphology and PCR amplification of sequences of the elongation factor gene (EF-1 α) of 33 isolates, identified *Fusarium mexicanum* associated with diseased plants. This result suggests that *F. mexicanum* is being disseminated through infected nursery mango plants, to commercial orchards in the central western region of Mexico.

DNA barcoding of rust fungi (Pucciniales) from the Brazilian Cerrado

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The Pucciniales represent around 30% of all Basidiomycetes, with over 7500 plant parasitic species, 400 just in Central Brazil. However, data on phylogeny, distribution, and classification are incomplete for the Neotropica. To study mainly diversity and phylogenetic relationships, freshly made collections were performed among native Cerrado hosts, and also from some cultivated and weed species members of the Annonaceae, Apocynaceae, Arecaceae, Asteraceae, Calophyllaceae, Caryocaraceae, Erythroxylaceae, Euphorbiaceae, Fabaceae, Lorantheae, Malpighiaceae, Myrtaceae, Oxalidaceae, Rosaceae, Rubiaceae, Sapindaceae, Smilacaceae, Tropaeolaceae. Identified rust fungi comprised mostly Cerrado fungi members of the genera *Batistopsisora*, *Catenulopsisora*, *Cerotelium*, *Cerradoa*, *Chaconia*, *Coleosporium*, *Crossopsisora*, *Dasyssora*, *Diorchidium*, *Ezalquia*, *Kweilingia*, *Mimema*, *Phakopsora*, *Puccinia*, *Ravenelia*, *Skierka* and *Uromyces*. PCR amplifications were conducted on CTAB-extracted genomic DNA with fungal-specific, rust-specific and Pucciniales-specific primer combinations for sequence analysis of the nuclear rDNA internal transcribed spacer (ITS) region. As a result, primer combinations comprised by ITS5/Rust2; ITS5/ITS4rust; ITS5/ITS4-u; ITS5-u/ITS4rust; ITS5-u/ITS4-u; Rust2inv/ITS4rust and Rust2inv/ITS4-u yielded reliable sequence data both for DNA barcoding markers for several taxa, and are contributing towards resolving taxonomic and phylogenetic relationships among our rust fungi.

Salinity and wilt caused by *Ceratocystis fimbriata* impacts on growth of mango seedlings of different rootstock-graft combinations

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In the last years, the wilt became a limiting factor to mango production in Brazil and Oman. In these countries, mango plants are commonly grown in salinized soils. We studied the effect of salinity and wilt on seedlings of different rootstock-graft combinations. In two experiments (E1: 'Espada', 'Imbu', or 'Uba' grafted on 'Haden'; E2: 'Uba' grafted on 'Palmer', 'Haden', or 'Tommy Atkins'), mango seedlings, growing in Hoagland solution in an aerated hydroponic system with 0, 15, 30, 45, or 60 mmol NaCl.L⁻¹, were either uninoculated, inoculated in the rootstock or inoculated with *C. fimbriata* in the graft. From the grafting point, *C. fimbriata* was inoculated either 10 cm above (graft inoculation) or below (rootstock inoculation). After 90 days of plant growing, we evaluated variables related to plant growth and nutrient absorption. As the NaCl concentration increased, plant dry matter, stomatic conductance, and the levels of N, P, K, Ca, and Mg on plants decreased, whereas Na and Cl levels increased. The effect of pathogen inoculation on dry matter and stomatic conductance was not conclusive. Given that all cultivars are wilt susceptible, apparently plant growth is more impacted by the increase on NaCl concentration. Sponsor: Vale, Brazil.

Echinodella species (Asterinaceae, Ascomycota) on *Mauritia flexuosa* (Arecaceae) from the Brazilian Cerrado

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Epiphyllous dark brown coalescent spots on leaves of *Mauritia flexuosa* (Arecaceae) were found on specimens collected from the Brazilian Cerrado (Access number UB: 9529. The fungus was an inedited *Echinodella* species (Asterinaceae) showing ascomata, 501 (677) 1409 x 163 (164) 259 μ m,

elongate, navicular, lireliform to curved sometimes branched with 317 (567,5) 1317 x 17 (45) 60 μ m longitudinal opening, encircled by a non-hyphopodiate mycelium showing 2 (3) 4 μ m diam. septate brown hyphae; asci 23 (40) 45 x 14 (20) 28 μ m, bitunicate, ovoid to globose, parallel along the internal wall of the ascomata, paraphysate, 8-spored; ascospores 15 (21) 24 x 6 (8) 10 μ m, clavate, 1-septate, with constriction at septa, hyaline becoming brown at maturity; paraphyses simple with a widened apex. The genus was reported in Brazil on *Vismia* sp. (*E. reticulata*), *Lacuma grandifolia* (*E. lacumae*), *Miconia* sp. e *Baccharis* sp., however besides being a new species, this is a first record of the genus on *Mauritia flexuosa*.

1000 Fungal Genomes project

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The 1000 Fungal Genomes (1000F) is a large collaborative research project between the Joint Genome Institute (JGI) and an international research consortium of mycologists and fungal biologists. The goal of 1000F is to facilitate the sequencing of fungal genomes across the Kingdom Fungi. Although the sequencing of fungal genomes has progressed at a rapid pace over the past several years, representative genomes for the majority of lineages of Kingdom Fungi have not been sequenced. To insure the broadest coverage of phylogenetic diversity, the sampling strategy/guideline of 1000F is to sequence two species of fungi for every family-level clade of fungi. The end product will be an encyclopedia of reference genome data that will inform all fields of mycology/fungal biology by providing a more accurate assessment of the pan-fungal genome and the metabolic potential of the kingdom. 1000F is open to participation by the global research community, and researchers, including students and postdocs, can nominate species for sequencing from undersampled family-level clades through the JGI Fungi Portal (<http://genome.jgi.doe.gov/programs/fungi/index.jsf>). A part of this presentation, a current update of 1000F, including sampling progress and phylogenomic analyses will be presented.

Logical areas of collection: A precision concept for management of *Rhizoctonia solani* AG1-IA

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Rhizoctonia solani AG1-IA causes sheath blight in rice and aerial blight in soybean. In Arkansas, rice and soybean rotations facilitate a continuous source of *R. solani* AG1-IA inoculum from one year to the next. Aerial blight is a two stage disease where colonization of the plant occurs during the early vegetative growth stages and aerial blight symptoms occur after canopy closure. The objective of this work was to determine the distribution of early season soil inoculum potential and plant colonization by *R. solani* AG1-IA. Samples were collected from GPS positions placed intermittent of the rice levee system from the previous year. Soil was assayed using a toothpick baiting procedure to assess the inoculum potential and soybeans were sampled at growth stage V3 at each position. Spatial analyses were used to determine the spatial aggregation and dependency of soil inoculum potential and plant colonization. Models for inoculum potential and plant colonization indicated an agreement with the levee system that was utilized from the prior year's rice crop. The soil inoculum potential and plant recovery of *R. solani* AG1-IA indicates that distribution is controlled by the levee system from the previous year. Therefore, disease scouting at or near points in a soybean field that correspond to "logical areas of collection" from the levee system utilized the year before should result in a more efficient scouting methodology to manage aerial blight.

Implications of host plant resistance against whitefly-transmitted *Tomato yellow leaf curl virus* in tomato for virus epidemics and management

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Whitefly-transmitted *Tomato yellow leaf curl virus* (TYLCV) severely impacts tomato production in southeastern USA. Growers typically spray insecticides against whiteflies and plant TYLCV-resistant genotypes. Semi-dominant genes such as *Ty-1* and *Ty-2* confer resistance to TYLCV. Resistant genotypes are not immune to TYLCV, display less-severe symptoms, and support TYLCV replication. Currently, less than one-third of the tomato acreage is planted with resistant genotypes. These genotypes are often planted

in a mosaic with susceptible genotypes in farmscapes. Our objectives were to evaluate how resistant genotypes influence TYLCV acquisition and transmission, and assess their impact on TYLCV epidemics. Experiments were conducted to qualitatively and qualitatively characterize the interactions of resistant genotypes with whiteflies and TYLCV. Our hypothesis was that resistant genotypes would serve as whitefly reservoirs and TYLCV inoculum sources. Detection by PCR indicated similar incidences of TYLCV infection in resistant and susceptible genotypes. However, whiteflies more readily acquired TYLCV from susceptible than resistant genotypes. Despite differences in acquisition, whiteflies transmitted TYLCV from resistant genotypes as efficiently as from susceptible genotypes. Quantitative PCR data suggested that TYLCV acquisition and transmission was dependent on TYLCV copy numbers in the genotype and also whitefly densities, this relationship varied temporally.

Metabolomic analysis of non-host pathogen induced resistance in chile pepper (*Capsicum annuum*)

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Induced resistance is a well-characterized response in plants following exposure to chemical activators, pathogen associated molecular patterns (PAMPs), or rhizosphere colonizing microbes. Induced resistance is associated with a "primed" response of the innate immune system to subsequent pathogen infection and includes callose deposition, phenolic accumulation, hypersensitive response, reactive oxygen species and free radical production. Inoculation of chile plants with non-host *Phytophthora nicotianae* zoospore solutions was previously shown to induce a systemic response, which significantly inhibited *P. capsici* foliar blight and root rot. In this study, GCMS was used to analyze metabolomic shifts associated with non-host pathogen induced resistance in chile. Chile seedlings were treated with water, *P. nicotianae* zoospore solution or Beta amino butyric acid. At 48 h. post treatment, seedlings were inoculated with a *P. capsici* zoospore solution or water. At 48 h. post inoculation, primary metabolites were extracted. Analysis of normalized metabolite data shows that *P. nicotianae* induced plants had a significant metabolic shift upon inoculation with *P. capsici*, whereas no significant response was observed in control plants. *P. nicotianae* induced plants had reduced concentrations of sucrose, TCA cycle intermediates and components of the shikimic acid and lysine degradation pathways and increases of specific hexose-phosphates, hexose-disaccharides, and amino acids.

Regulatory effect of soil matric water potentials on a unique tripartite (*Cucumis-Monosporascus-Olpidium*) host-specific rhizosphere interaction

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Colonization of cantaloupe roots by zoospores of *Olpidium bornovanus* as well as the germination and attachment of ascospore germings of *Monosporascus cannonballus* to cantaloupe roots were highest at a soil matric potential of -0.001 MPa but significantly inhibited at matric potentials of -0.005 MPa and -0.01 MPa. Matric water potentials of -0.01MPa or drier are characteristically inhibitory to the motility of zoosporic microbes but not hyphal growth of filamentous fungi. Hyphal growth of *M. cannonballus* is optimal at water potentials between -0.6 to -0.8 MPa. These results suggest that the effect of such slight reductions in soil moisture levels are most likely due to restriction on the motility of *O. bornovanus* zoospores. Lack of motility would limit root colonization by this fungus, which in turn would limit germination of ascospores of *M. cannonballus*. These studies support our previous conclusion that germination of ascospores of *M. cannonballus*, a destructive root pathogen of cantaloupe, is mediated by *O. bornovanus*, an obligate, zoosporic fungus. These results indicate for the first time that high soil moisture levels are an important environmental factor influencing root colonization by *M. cannonballus*, albeit indirectly as we currently understand the biology of this unique tripartite, host-specific rhizosphere interaction (i.e., *Cucumis-Monosporascus-Olpidium*).

***Pythium brassicum*: A novel host family-specific root pathogen**

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In October of 2011, roots of stunted broccoli plants (*Brassica oleracea*) grown in the Palo Verde Valley, CA, were observed at various stages of decay. A

species of *Pythium* with large spiny oogonia was microscopically observed and consistently isolated from decayed roots plated onto 1.5% water agar. Hyphae emerging from the decayed roots were transferred to 10% V8 juice liquid medium for identification. Broccoli isolates produced spherical and intercalary sporangia (avg. 33 µm in diam) and aplerotic oospores (avg. 36 µm in diam) in oogonia (avg. 45.5 µm in diam) bearing numerous conical spines (avg. 8.65 µm in length and 5.73 µm basal width) with blunt apices. Broccoli isolate P1 has a 99% ITS sequence similarity with *P. jasmonium* (nom. inval., GenBank AF216654.1), a species which has not been formally described. A BLAST search indicated that three other accessions in GenBank (FJ415959.1, AB512782.1, and AB512739.1) carry the specific epithet of *P. jasmonium*. All accessions of *P. jasmonium* were originally isolated from diseased plants in the Brassicaceae, and were demonstrated in our study to be pathogenic on broccoli and morphologically similar to P1. P1 was pathogenic to 10 cultivated and 12 wild plants in the Brassicaceae but not to 18 species of cultivated plants belonging to 9 other plant families. Based on their apparent family-specific pathogenicity, we propose that isolates previously designated as *P. jasmonium* (nom. inval.) be renamed as *P. brassicum*.

Characterization of *Sclerotinia sclerotiorum* isolates from USA and South America soybean fields for aggressiveness and mycelial compatibility grouping

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Sclerotinia stem rot (SSR) on soybean, caused by *Sclerotinia sclerotiorum*, is an important yield-reducing disease in the USA and South America. The use of disease resistance is the lowest cost control for growers. To screen for SSR resistance, pathogen isolates need to be determined. However, pathogen variation for soybean isolates is not understood. Thus, variability of 55 *S. sclerotiorum* isolates from USA/Nebraska, Argentina and Brazil soybean fields was analyzed using clonality (mycelial compatibility groups or MCGs) and a detached leaf test. All isolates were paired in all possible combinations and formation of a barrage line indicated incompatibility. From 55 isolates, 23 MCGs were identified with 9 MCGs composed of a single unique isolate. Of the 14 MCGs with more than one isolate, 7 MCGs were composed of isolates within a single field site, while 7 MCGs had isolates collected from multiple field locations. For aggressiveness, leaves of a resistant soybean line, NKS 19-90, were inoculated with mycelial plugs and SSR diseased leaf area was measured. In the aggressiveness test with Brazilian isolates diseased leaf area at 48 hours was correlated with Area Under Disease Progress Curve (AUDPC). Most isolates were similar in aggressiveness, but one had a significantly lower disease area on leaves. The information on isolate relatedness and aggressiveness will be used to select relevant isolates to screen for broad resistance to Sclerotinia stem rot.

***Panicum mosaic virus*—A potential threat to biofuel switchgrass production**

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New switchgrass (*Panicum virgatum* L.) cultivars are being developed for use as feedstock for biofuel production via pyrolysis. Viral pathogens have been reported in wild and forage types, but their importance in biofuel cultivars are not well known. In 2012 surveys of five switchgrass breeding nurseries in Nebraska, plants with mottling and stunting—characteristics of virus infection—were found to be widespread in distribution, with the incidence of symptomatic plants within fields as high as 59%. Leaves from 120 symptomatic plants were analyzed by ELISA for *Panicum mosaic virus* (PMV), *Sugarcane mosaic virus*, *Wheat streak mosaic virus*, *Barley yellow dwarf virus* serotypes MAV and PAV, and *Cereal yellow dwarf virus*-RPV. Most of the samples (87%) were positive for PMV, and fewer than 8% for the remaining viruses. Among 52 PMV-positive samples, 36% tested positive for the presence of *Satellite panicum mosaic virus* (SPMV) by immunoblotting. Although mottling and stunting of switchgrass by PMV was reported over 50 years ago, its occurrence on switchgrass in the field has not been recognized since. Mixed infection by PMV and SPMV can result in heightened symptom severity in other graminaceous hosts, but this combination of viruses has not been investigated sufficiently in switchgrass. The results of this survey suggest disease caused by PMV, possibly in combination with SPMV, is an important factor to consider in the development and deployment of new biofuel cultivars.

Isolation and detection of *Phytophthora rubi* in raspberry (*Rubus idaeus*) production in the western United States

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Red stele of raspberry (*Rubus idaeus*), caused by *Phytophthora rubi*, is an economically important root rot disease in the western U.S. where cool, wet conditions can be favorable for disease development. The objectives of this study were to examine the recovery rate of *P. rubi* from raspberry using different isolation methods (cane, roots, and root/soil baiting with young raspberry plants) and PCR detection, and to identify additional *Phytophthora* species associated with raspberry in this region. A total of 745 samples were collected from 10 raspberry fields, 4 in Oregon, 3 in Washington, and 3 in California. *Phytophthora* spp. were recovered from all sites. The rate of recovery in CA sites ranged from 70-80%, in OR sites ranged from 15-35%, and ranged from 5-48% in WA sites. Isolating directly from cane tissue resulted in more isolates, whereas baiting yielded the least. In all cases, the PCR method detected higher rates of *P. rubi* presence in root samples compared to the isolation methods ranging from 53 to 90%. A total of 293 of the total 745 collected *Phytophthora* isolates were sequenced at the internal transcribed region for species identification. All but one isolate (*P. bisheria*) were identified as *P. rubi*. These results show that *P. rubi* is prevalent in the western U.S. Direct cane isolation, not previously reported for *P. rubi* proved to be the most effective isolation method.

A bioinformatic pipeline for use in metagenomic virus discovery

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In the past decade, several efforts to survey the biodiversity of viruses have been made. In these surveys many previously uncharacterized virus species have been identified, showing that we have little knowledge of the number of virus species on this planet. While differing in their focus, these efforts included animal, plant, and bacterial viruses, and used next generation sequencing, leading to the common problem of overwhelming data. The analyses of such large amounts of sequencing data are both lengthy and computationally intensive. Previous work has shown that next generation sequencing can be used as a diagnostic tool, using the pipeline E-probe Diagnostic Nucleic acid Assay (EDNA). EDNA uses pathogen specific probes (e-probes) to query a metagenomic dataset for determining the presence or absence of specific pathogens. These e-probe sets can be switched to more general probes, changing the focus of the assay from species identification to family identification. Sequencing datasets of four different suspected virus-infected plants were analyzed using the EDNA pipeline using general e-probes. Three of the four samples were negative, while one sample was positive for a virus belonging to the Alphaflexiviridae family. Using *Potato virus X* (the type member) as a reference genome, 92% of a *Potexvirus* genome was obtained from the sequencing data. The contigs obtained had between 94 and 97% identity to *Potato virus X*.

Microbial disease complex of sweetpotato (*Ipomoea batatas* L. Lam.) tip/end rot

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A two year study was conducted to determine the causal agent(s) responsible for a severe outbreak of sweetpotato tip/end rot in north Mississippi. Microbes were sampled from plant tissues over eight production stages. Identifications were confirmed for all isolated bacteria and fungi using 16S and ITS sequence data, respectively. Three bacteria, *Bacillus* spp., *Lysobacter enzymogenes*, and *Paenibacillus lentimorbus*, occurred across the eight production stages, but 30 additional taxa were also identified. None were found to be pathogenic when screened. However, a number of fungi, *Macrophomina phaseolina*, *Aspergillus flavus*, *A. niger*, *A. tubingens*, *A. japonicas*, and six species of *Fusarium*, were pathogenic in trials. In addition, *F. oxysporum* and *F. solani* consistently produced necrotic lesions in root tissue. These two species accounted for nearly 70% of the overall isolates from early season seed stock and bedding plant samples. Microbial populations in post-harvest tissues differed in relative abundance from prior sampling dates. *Macrophomina phaseolina* increased to 6.5% occurrence and *F. oxysporum* and *F. solani* decreased to 27% isolation frequencies between 60 and 90 days post-harvest.

To overcome selective media biases, Illumina whole-community sequence data is being generated from tissue samples used in microbial isolation. Fungal occurrence and population data will be evaluated within and across production stages.

Biodiversity of the fungal community present in sweetpotato (*Ipomoea batatas* L. Lam.)

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Agricultural ecosystems have diverse microbial communities that are ever changing due to variable management practices, rotations and cover cropping systems. The biodiversity in sweetpotato fields was investigated due to a severe outbreak of tip/end rot disease in production fields in northern Mississippi. Sweetpotato tissues samples, above and below ground, were collected across eight distinct sampling periods each of two years. Using two selective media, fungal taxa were initially placed into morphological groupings. From these macro and microscopic taxonomic groupings, 20% of the isolates within each group were confirmed by ITS sequencing. Several anamorphic Ascomycota were the primary taxa inhabiting the tissues regardless of sampling period and tissue location. They included *Macrophomina phaseolina*, *Aspergillus* spp., *Fusarium* spp., *Trichoderma* spp., and *Penicillium* spp., with *Trichoderma* spp. and *Fusarium* spp. most frequently identified during all periods. Species richness, diversity and evenness data were compared within and across sampling dates and years based on isolation data. In addition, Illumina environmental sequence data from genomic DNA subsampled tissue pieces (used for media isolation) were compared as in isolation data and across identification methods.

WITHDRAWN

Management of curly top in sugar beet with seed and foliar insecticides

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Curly top in sugar beet can result in severe yield losses and is caused by *Beet severe curly top virus* (BSCTV) and other closely related *Curtovirus* spp. which are vectored by the beet leafhopper. Neonicotinoid seed treatments (Cruiser, NipsIt, and Poncho) have been shown to be an effective supplement to host resistance, but measures to extend control beyond the duration of seed treatment efficacy needs to be investigated. In 2012, a field study was arranged in a randomized complete block design with 8 replications and planted with the cultivar B-42. The 16 treatments included untreated and Poncho Beta treated seed with and without 6 foliar insecticides (applied 7 days before and 6 days after release of viruliferous beet leafhopper) and just Poncho and Poncho Votivo treated seed. On 22 Jun (59 days after planting), 6 beet leafhoppers per plant were released to ensure good disease pressure. Visual foliar ratings on the 15 Aug and 15 Sep indicated some seed and foliar treatments reduced ($P < 0.0001$) symptoms by 13 - 60% compared to the

check. Root yields were increased ($P < 0.0001$) by 8 - 18% compared to the check. Estimated recoverable sucrose was increased ($P < 0.0001$) by 12 - 21% compared to the check. After the seed treatments lose efficacy, it would appear that the pyrethroid insecticides Asana and Mustang may be applied to help reduce curly top and aid in the control of other pests and insecticide resistance management.

Impact of biological amendments on *Agrobacterium tumefaciens* soil survival

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The primary walnut rootstock used in California is susceptible to *Agrobacterium tumefaciens*, which causes crown gall. Prior to planting, walnut nurseries currently fumigate the soil to control weeds, *A. tumefaciens*, and other phytopathogenic agents. While *A. tumefaciens* is susceptible to the commonly used fumigants, these fumigants also significantly modify the indigenous soil microbial community which impacts their ability to inhibit seed-borne *A. tumefaciens* populations introduced into these soils. Increasing the microbial diversity and activity surrounding the walnut seeds planted in fumigated soil may provide greater competition for *A. tumefaciens*, thereby reducing its abundance and limiting crown gall incidence. Three soil amendments were tested in native and Telone-C35 fumigated soils: commercially available vermicompost and two microbial fermentation mixtures. MeBr-fumigated and a non-fumigated soil were used as controls. Each treatment was infested with a rif-resistant mutant of *A. tumefaciens* whose abundance was determined by dilution plating and quantitative PCR over the course of four weeks. The two commercial fermentation amendments had no effect on *A. tumefaciens* population dynamics. After a four-week exposure to 50% (w/w), or greater, vermicompost, *A. tumefaciens* populations declined below detection limits. Heat-treated vermicompost (12 hrs at 85°C) had no impact on *A. tumefaciens* populations.

Improvement of current detection and identification methods for select agent strains of *Ralstonia solanacearum* via multiplex PCR and qPCR

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Ralstonia solanacearum causes bacterial wilt on many economically important crops. *R. solanacearum* is a species complex that is pathogenic to a wide range of plant species in many regions throughout the world, but subgroups have more limited host ranges in different regions. Specifically detecting one subgroup of strains adapted to temperate climates (race 3 biovar 2, r3b2), the ones listed as select agents due to their potential threat to U.S. agriculture, is of utmost importance. By performing genome comparisons of the publically available *R. solanacearum* genomes, non-phage related regions conserved among all *R. solanacearum* or unique to r3b2 were identified and used to design *R. solanacearum* species- and r3b2-specific primers and probes in multiplex PCR and qPCR assays, allowing for simultaneous detection and differentiation of r3b2 from non-r3b2 strains of *R. solanacearum* in a single reaction. An internal plant DNA control primer pair targeting the cytochrome oxidase, subunit I (*cox1*) gene was also developed and included in the multiplex assays to improve the confidence and reliability of r3b2 detection in plant extracts, by validating the plant extracts and excluding false negative results. Rapid, accurate, sensitive, and reliable detection and identification of r3b2 are critical for state and government officials to make timely and appropriate recommendations in safeguarding the movement of agricultural and horticultural products into the United States.

Living in the city: Arbuscular mycorrhizal fungi in Phoenix and the surrounding desert

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As part of an extensive Central Arizona/Phoenix LTER field survey, arbuscular mycorrhizal fungal (AMF) communities were characterized at 58 sites in urban/suburban areas of the Phoenix metropolitan area and in the surrounding Sonoran desert. Urban/suburban sites differed in land use history (developed by converting desert or agricultural land) and in time since development (ranging from 100 years to 1 year). AMF species richness was significantly lower in urban/suburban sites compared to desert sites, regardless of land use history. We found no significant relationship between species richness and time since development at urban/suburban sites. We detected 26 species of AMF with many occurring in both urban/suburban and desert sites. Variation among sites was characterized by soil nutrient content and landscape habitat factors with urban sites having greater soil nutrients and

more impervious surface with tree cover. There were AMF species-specific differences to habitat factors. The abundance or occurrence of some fungal species, such as *Glomus luteum* and *Entrophospora infrequens*, had negative associations with habitat factors associated with urbanization; whereas, other species including *Paraglomus occultum* and *G. eburneum* had positive associations with urbanization. Overall it appears that urbanization alters the fungal community and there are species-specific responses to how urbanization alters nutrient and vegetation cover.

Grapevine red blotch-associated virus is widespread in California and U.S. vineyards

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In fall 2011, *Grapevine red blotch-associated virus* (GRBaV), a circular ssDNA virus, was detected in grapevines exhibiting leaves with red blotch symptoms in Napa, CA. Extensive sampling of symptomatic grapevines in California vineyards and analysis of the nucleic acid fractions by SYBR®Green qPCR assay indicated that this virus is widely distributed in California vineyards. In several vineyards, disease incidence was >90% and GRBaV was detected in ~95% (n=327) of grapevines showing red blotch symptoms and in ~2.5% (n=757) of asymptomatic grapevines. Virus was detected in asymptomatic first-leaf plantings and in symptomatic grapevines in vineyards up to 25 years of age. Fruit on GRBaV positive grapevines had reduced total soluble solids and increased titratable acidity. Among tested grapevine samples with red leaf symptoms, a higher proportion was positive for GRBaV when compared with grapevine leafroll-associated viruses. Red leaf grapevines in at least seven California counties and from grape growing regions across the U.S. have been confirmed to be infected with GRBaV. It appears that grapevine red blotch disease is endemic in California and has been overlooked because of its leafroll-like symptoms. Because of its adverse effect on fruit quality, red blotch disease poses a significant threat to California vineyards.

Application of next generation sequencing technologies for developing diagnostic tools for seed borne pathogens

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Development of reliable DNA-based detection methods requires characterization of genetically and geographically diverse group of isolates within a taxon. To develop this resource, a bacterial genome sequencing project was initiated using the Illumina Next Generation Sequencing platform. Isolates of several key pathogens in Monsanto collections were sequenced and analyzed to identify stable and unique genomic targets for PCR assay development. A total of 860 isolates were sequenced, representing tomato canker pathogen (*Clavibacter michiganensis* subsp. *michiganensis*), bacterial spot pathogens (*Xanthomonas vesicatoria*, *X. euvesicatoria*, *X. gardneri* and *X. perforans*), black rot pathogen (*X. campestris* pv. *campestris*), bacterial fruit blotch pathogen (*Acidovorax avenae* subsp. *citrulli*) and carrot blight pathogen (*X. hortorum* pv. *carotae*). Sequences were assembled and contigs generated using a novel, proprietary bioinformatic pipeline. The contigs were further compared using published reference genomes. Genome sequences from pathogen isolates and closely related saprophytic and non-target bacteria were compared to identify pathogen specific sequences and primers were developed. Conventional and realtime PCR assays were designed and validated against panels of pathogen and saprophytic isolates. NGS technology is a powerful tool for primer development, and we present the first large scale sequencing project to aid the detection of seedborne plant pathogens.

Evaluation of seed wash DNA extraction method for the detection of seed-borne plant pathogens

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Implementation of PCR-based methods for the detection of seedborne pathogens requires DNA extraction from seeds. Poor quality DNA and presence of PCR inhibitors can lower the sensitivity of detection and result in

false negatives. We have optimized simple and rapid DNA extraction methods from seed wash samples for sensitive detection of tomato canker, bacterial spot and black rot pathogens. Bacterial cells are pelleted by differential centrifugation and DNA extracted using the PowerFood Microbial DNA kit from MoBio with modifications, followed by a realtime PCR. Pathogen detection sensitivities were evaluated using both naturally infected and pathogen-spiked seed samples. This DNA extraction method when combined with realtime PCR is capable of consistently detecting *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) spiked at 200 CFU/10,000 seeds. The method was validated using seed lots with different countries of origin, sanitation treatments and varying levels of saprophytic background. The newly optimized DNA extraction method in combination with realtime PCR improved pathogen detection sensitivity, specificity and reduced the assay lead time when compared to DNA extracted by standard phenol-chloroform and culture based methods currently used in seed testing.

Peanut mini core collection at ICRISAT: A reality in identifying multiple disease resistance sources

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Susceptibility to several biotic constraints, including fungal and viral diseases, is one of the major reasons for low productivity in peanut. This situation is common in several developing countries in Asia and Africa, where resource poor farmers cannot afford expensive chemical pesticides for disease management. With an objective to identify sources of multiple disease resistance in peanut, ICRISAT's peanut mini core collection (10% of core or 1% of entire collection) consisting 184 accessions was evaluated independently for two important fungal foliar diseases such as late leaf spot (LLS) and rust under field conditions during 2012 rainy season. The same set was evaluated for peanut bud necrosis disease (PBNB) under late planting in the field during 2012 rainy season. Results indicated that 8 accessions showed less than 1% PBNB incidence, and two accessions (ICG 2019, ICG 13858) were completely immune, compared to the average disease incidence of 25% in the trial and 40% in the susceptible control. In the rust screening trial, 3 accessions (ICG 6022, ICG 11088 and ICG 11426) were highly resistant (<3.0 rating on a 1-9 disease rating scale) and coupled with superior yields of more than 3.0 t/ha. In the LLS screening trial, ICG 11426 was highly resistant with a rating of 3.0 on a 1-9 disease rating scale, with a yield of 3.8 t/ha. These accessions can be used further in peanut breeding programs for developing multiple disease resistant high yielding cultivars.

Fungal and oomycete pathogen detection in the rhizosphere of organic tomatoes grown in cover crop treated soil

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Soil management practices, including cover crop application, affect soil and plant health through various mechanisms. Impacts on microbial communities are known to be important, but are not well understood. This field study examined the impacts of a single-season application of cover crops on pathogen populations in the tomato crop rhizosphere. The hypothesis tested was that cover crops could rapidly enhance microbial communities suppressive to pathogen growth. The study took place in MD, NY and OH in the summers of 2010 and 2011, with a total of 260 plots tested using both macroarray and T-RFLP analyses. The macroarray used in this study specifically was designed to detect over 30 pathogens of solanaceous crops, but had not previously been used for such a field study. Macroarray was able to detect certain pathogens with much greater sensitivity than T-RFLP. Results suggest that a single-season cover crop application does not significantly impact pathogen populations in the crop rhizosphere.

Assessment of citrus huanglongbing (HLB) in Dominica

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In a citrus huanglongbing (HLB, citrus greening) mission sponsored by FAVACA-International Volunteer Corp and facilitated by the Caribbean Agricultural Research and Development Institute and Dominican Plant

Protection and Quarantine Unit, HLB outbreak sites were visited, disease incidence and severity evaluated, and psyllid population and distribution investigated. Inspection results indicate that HLB might have been introduced by humans through the illegal movements of HLB-infected citrus to the island and had been in Dominica for some time before Asian citrus psyllid (ACP), was detected in 2007. The disease was further spread and distributed to other residential areas via propagation of infected Mexican limes and both HLB and ACP were not detected in the commercial groves during this mission. Due to a unique island climate, geographic conditions and locations of commercial orchards, and limited spread of HLB, Dominica may still have a golden opportunity to eliminate this devastating disease from the island. Eradication efforts require an outreach for the cooperation and support from the public, a nationwide survey for any HLB on dooryard Mexican limes, removal of all infected citrus from residential areas, removal of all ACP alternate host plants, certification of the state-owned citrus nursery through advanced diagnostic methods, utilization of certified citrus stocks only and release of ACP parasitoids frequently to reduce the vector population.

Distinct SNPs present in the ITS2 region of *Elsinoë australis* organism detected from citrus in Florida

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After the USDA confirmed the detection of *Elsinoë australis*, causal agent of sweet orange scab (SOS), in Texas in July 2010, similar SOS symptoms were found on fruit of many citrus species in Florida except that symptoms were always associated with injuries such as wind scars, thorn punctures/scratches and bird-pecking wounds. An *Elsinoë* fungus was isolated and inoculated onto lemon fruit with abrading the fruit surface. Although a SOS-like syndrome developed in four months, lesions did not appear on unwounded tissues nor expand beyond the wounded areas. DNA extracted from 95 symptomatic fruit was amplified and 32 produced an expected 0.4 kb DNA band for *E. australis*-specific primers. The ITS2 region (166 bp) from 10 PCR products was sequenced and found to display distinct single nucleotide polymorphisms (SNPs). Only one SNP was found to differentiate the SOS pathotype ('T') of South America and Australia from the Natsudaikai pathotype of South Korea ('C') (base position 135). All Florida specimens displayed a distinct 'C' at this base position. Additional SNPs were detected from Florida at three other base positions; 4 display 'A' for 'C' (position 15), 3 others display 'T' for 'C' (position 42), and 3 others display a deletion for 'C' (position 46). One produced a 100% match to Natsudaikai pathotype. A sequence identical to the SOS has not yet been detected in Florida, suggesting Florida *Elsinoë* isolates are not the typical *E. australis* that causes SOS.

***Pseudomonas* sp. found on *Loropetalum* stem canker in Florida**

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A *Pseudomonas* sp. was isolated on over 40 retail nursery samples of *Loropetalum chinense* with rough stem cankers. The recovered strains fell into the *Pseudomonas syringae* group or *P. savastanoi* and *P. viridiflava* by LOPAT testing. Inoculation showed a hypersensitive reaction on tomato and pepper and reproduced identical canker symptoms on loropetalum stems, but not on oleander. Wound-aided inoculation caused affected stem tissue to swell and eventually rupture to form callus tissue around cankers, producing a symptom different from the galls induced by *P. savastanoi* pathovars. Both 16S rRNA and rpoD gene sequence profiles of this *Pseudomonas* sp. rendered a 99% similarity match with many *Pseudomonas* species including *P. syringae* pv. *erobotryae*, *P. amygdali*, *P. savastanoi* while the nuclear *iaa-L* gene sequence of the *Pseudomonas* sp. had only 96% match to other *P. savastanoi* pathovars. A sensitive qPCR assay that was designed to detect the plasmid-borne *iaaL* gene carried by all gall-producing *P. savastanoi* pathovars was negative, suggesting that this *Pseudomonas* sp. does not have a plasmid copy of the *iaa-L* gene. Although the initial observation of stem symptoms might have suggested *P. savastanoi* as the causal bacterium, further investigation on canker formation and gene sequence confirmed otherwise. Information on host range determination and multilocus sequence typing will aid in naming this new loropetalum stem canker bacterial pathogen.

Distribution and host range of *Colletotrichum acutatum* on Salicaceae in San Francisco's North Bay area

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Weeping willow trees (*Salix babylonica* L.) are widely planted in riparian landscapes in Marin County, CA, USA. Following prolonged spring rains and cool summer weather in 2010, mature weeping willows showed symptoms of a disease subsequently identified as twig canker caused by *Colletotrichum acutatum*. Small branches developed dark brown to black, sunken cankers, 3 to 20 cm in length, similar in appearance to those produced by other willow canker pathogens, such as *Colletotrichum gloeosporioides* (anamorph: *Glomerella miyabeana*) (black canker) and *Venturia saliciperda* (willow scab). Badly infected willows declined as a result of repeated defoliation and twig loss. Disease severity on afflicted trees was markedly lower in 2011 and 2012, presumably due to warmer, drier weather. Since willow cuttings are frequently used for habitat restoration projects, understanding the extent to which the disease may have migrated to native willows was key to assessing the need to prevent its transmission into unfested areas. To do this, we needed to determine its distribution and whether other pathogens or hosts were involved. Symptomatic *Populus nigra* (Lombardy poplar) grow within a few miles of infected willows, and tested as highly susceptible to infection by willow isolates of *C. acutatum*. Brief surveys have been conducted to find infected poplar, and differentiate symptoms from those produced by pathogens such as *Cryptodiaporthe salicella*.

Effect of viruses and water-stress on storage root initiation in sweetpotatoes

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Anecdotal observations suggested viruses interact with drought stress during transplanting to reduce sweetpotato yield. Greenhouse studies examined adventitious root (AR) development to determine if viruses, wilted transplants and delayed watering after transplanting alter storage root initiation in Evangeline (Ev) and Beauregard (Bx). AR architecture was measured for virus-tested (VT) and virus-infected (VI) 20-day-old plants, which were watered or wilted two weeks before transplanting. A second experiment measured AR architecture for VT and VI 20-day-old plants which were watered immediately or 8 days after transplanting. Increased lateral root (LR) density, no. LR, total LR length and surface area, and decreased ratio (AR)/LR length indicate a higher probability of storage root initiation by ARs. Wilted transplants did not reduce storage root initiation for Bx and Ev. Although, wilted-VI Ev plants initiated more storage roots, which may explain negligible yield loss when wilted Ev beds are showing virus symptoms. Late watering resulted in shorter roots. VI-Ev plants initiated fewer storage roots with normal or late watering after transplanting. Short Ev roots due to late watering and lack of storage root initiation due to viruses may explain the greater yield losses. Late watering of Bx-VI plants resulted in greater LR length and

density, but less surface area. Bx yield loss caused by viruses is not affected by storage root initiation, but probably by lack of bulking.

The ADiv project: Analyzing rates of diversification in the Agaricales

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The order Agaricales is the most species-rich group of the Basidiomycota, numbering ca. 475 genera and 14,000 described species, yet, the driving forces of evolutionary diversification in the order are hardly known. Understanding why and how certain lineages became extremely species-rich, while others are only represented by a few species, what the timing of major lineage expansions is, or the impact of fruiting body morphologies on speciation rates may have an effect on fungal conservation and taxonomy, however, theoretical and experimental evidence so far remained spurious. The ADiv is a recently launched 4-year initiative to understand patterns of speciation, extinction and variations in the rate of evolution in the Agaricales. To address these questions, we will use statistical models of lineage diversification in a phylogenetic framework. Modeling of diversification will rely on a new two-gene dataset (referred to as diversity dataset) for ca. 3000 species accepted in the Agaricales. In addition to the diversity dataset, a phylogenomic dataset is also being produced, which will provide robust support for the backbone of the Agaricales to reinforce phylogenetic inference from the diversity dataset. These two resources will allow us to address general patterns of speciation and extinction, the frequency of shifts in diversification rates, and whether transitions between different fruiting body types influence rates of speciation or extinction in the Agaricales.

Onion thrips (*Thrips tabaci*) and *Iris yellow spot virus* survival throughout Colorado winters

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Iris yellow spot virus (IYSV) remains a concern in Colorado onion production. Several weed species have been described as alternate hosts and likely green bridges for IYSV survival, however, there is little work regarding the overwintering habits of the insect vector, *Thrips tabaci*, and its potential to act as a source of inoculum during the following season. Sticky traps indicated that thrips were active until mid-December (2012 and 2013) when the average temperature fell below 0°C. In 2012, activity resumed in early March. IYSV was detected by RT-PCR in live adult and larval thrips recovered from several field sources during the winters of 2010-2011, 2011-2012, and 2012-2013. Few live thrips were found after the onset of decay in onion cull piles. Live thrips were easily recovered from winter annual species. Five of these weed species have been grown from seed in the greenhouse and exposed to viruliferous thrips to further elucidate their role as green bridges. Of the five, IYSV has been detected in *Tragopogon dubius* (western salsify) and thrips larvae reared on this plant. Results indicate winter annuals play a role in onion thrips and IYSV over-winter survival, providing inoculum the next growing season. Thrips and IYSV monitoring will continue into spring 2013 to determine pest activity in nearby fields of new crop onions.

Tall fescue endophytes: Utilization, quality assurance, and characterization

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Tall fescue (*Lolium arundinaceum*) is a valuable forage grass adapted to a broad range of growing regions and occupies approximately 14 million ha across the USA. Much of the success of tall fescue can be attributed to the seed borne *Neotyphodium coenophialum* symbiont that produces a range of bioprotective compounds. Unfortunately, most tall fescue grown within the U.S. harbors an *N. coenophialum* strain that causes toxicity to grazing livestock due to the production of ergot alkaloids. To overcome livestock toxicity, naturally occurring *N. coenophialum* strains unable to produce ergot alkaloids have been deployed in elite tall fescue cultivars. Cultivars infected with these strains have good agronomic performance and animal productivity. As part of our grass breeding efforts, we have developed a PCR-based quality assurance pipeline to assess all grass lines under evaluation. Individual seeds or tillers can be tested by PCR for endophyte presence and strain identification to ensure consistency of seed stocks, plots and pastures. In addition, new tall

fescue germplasm can be rapidly evaluated for endophyte incidence and predicted chemotype in order to identify candidate strains for future agronomic use.

Tomato fruit colonization of *Clavibacter michiganensis* subsp. *michiganensis* via external and internal routes

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Seed-disseminated phytopathogens exemplify the adaptive nature of parasites by successfully gaining access to seed, surviving seed treatment processes, colonizing emergent seedlings, and attaining global distribution. *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), the causal agent of bacterial wilt and canker of tomato, continues to induce epidemics throughout all major tomato-producing nations, and insuring healthy seed stock remains a top priority. Understanding the movement of *Cmm* and its ability to produce systemic infections will help elucidate the mechanisms involved in seed infection. In order to investigate the mode of seed infection, New York *Cmm* field isolates were stably transformed with eGFP and were used to characterize routes of infection. Preliminary experiments were designed to test the ability of *Cmm* to access the developing seeds either i) systemically through the xylem and/or ii) externally by entering fruit through lesions on the pericarp. The use of laser scanning confocal microscopy and *in planta* colonization studies confirmed that the transformants continued to express eGFP while infecting tomato plants; and *Cmm* was able to successfully invade the fruit tissue, thereby, highlighting the multifaceted infective nature of *Cmm*. These studies will increase our understanding of the adaptive nature of Gram-positive bacteria and the mechanisms utilized during seed infection.

The origin of a new race of *Cronartium ribicola*, virulent on previously immune blackcurrant cultivars, and rapidly spreading in eastern North America

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The accidentally introduced fungal pathogen *Cronartium ribicola*, which is responsible for causing the disease called white pine blister rust (WPBR), has devastated American susceptible five-needle pines throughout their distribution ranges. The fungus must complete part of its life cycle on *Ribes* species, before infecting and killing five-needle pines. One single dominant R-gene, originating from *Ribes ussuriense*, was introgressed in European blackcurrant and these WPBR-immune cultivars were commercialized as a control method for the disease. Resistance breakdown of blackcurrant immunity was first observed in 2008 in Connecticut. The new virulent *C. ribicola* race was observed and sampled in 2011 and 2012 in fields belonging to commercial blackcurrant growers across eastern North America (Quebec, Connecticut, New Hampshire, New York, Nova Scotia, and Prince Edward Island). Inoculations of leaves from immune *Ribes* cultivars were performed with spores collected from single aecidia. Results showed that all the cankers observed in the heavily infected white pine natural forest located close to a currant field originated from the new virulent race. Genetic analyses suggest that this new race of *C. ribicola* does not come from a new introduction but rather resulted from either a novel mutation or a DNA recombination event that occurred in a North American strain. These findings will have an impact on the regulation and management of WPBR.

Effect of watering regime and *Fusarium virguliforme* (Fv) infection on location of soybean cyst nematode (SCN) syncytia in soybean roots

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The interaction of Fv with SCN results in earlier onset and increased SDS severity. The mechanism of this interaction is unclear. Studies were conducted to assess the effect of Fv infection and watering on formation of SCN feeding sites, called syncytia, in roots. Soybeans were planted in SCN-infested or SCN-free soil and grown at 27°C for 8 days prior to transplant into Fv-free or Fv-infested soil. Plants were subjected to two watering regimes: normal (watered daily) and reduced (watered on alternate days). Foliar SDS severity was assessed over 14 days. Root rot severity and root weight were evaluated after 14 days, and SCN syncytia were quantified and their location noted using microscopy. Foliar SDS severity was greater ($p=0.0015$) in co-inoculated plants than in Fv-inoculated plants in both watering regimes. However, root rot was greater in Fv-inoculated than in co-inoculated plants ($p=0.04$). The number of root tips was greater ($p=0.02$) in plants grown in SCN-

infested compared to SCN-free soil. SCN syncytia were primarily located (89%) in the stele in plants inoculated with SCN alone, whereas the percent of syncytia in the stele was only 49% in co-inoculated plants ($p=0.0068$). The percentage of syncytia in the stele was 57% under normal watering and increased ($p=0.05$) to 82% under reduced watering. These data suggest that both pathogens respond to each other's presence and that the interaction is affected by water availability.

Deletions in the coat protein cistron of *Wheat streak mosaic virus* induced more severe symptoms than the wild-type virus

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Wheat streak mosaic virus (WSMV), an economically important virus in the Great Plains region, is the type member of *Tritimovirus* genus of the family *Potyviridae*. The role of coat protein (CP) in WSMV biology was examined by introducing a series of deletions covering SGSGS motifs (aa 36-40, 43-47 and 53-57), aa 58-100 and N- and C-terminal regions and their infectivity was tested on wheat. Deletion of SGSGS motifs either individually or all three motifs together elicited systemic symptoms similar to wild-type virus. However, deletions comprising downstream of SGSGS motifs, aa 58-84, 49-83 and 36-84 elicited more severe symptoms than wild-type virus with increased cell-to-cell movement and virus accumulation. In contrast, deletions comprising aa 85-100 with deletion of aa 85-100, 48-100 and 36-100 infected wheat at reduced levels with mild symptoms. CP with deletion of aa 6-27 at the N-terminal region, and 14 and 17 aa at the C-terminal region infected wheat systemically at reduced rates with mild to moderate symptoms. Deletions comprising aa 85-100 and aa 6-27 severely affected virion formation, while C-terminal 14 and 17 aa deletions did not, demonstrating that the C-terminal aa are required for cell-to-cell movement. These data suggest that CP of WSMV is able to tolerate extensive deletions without affecting virion assembly and systemic infection of wheat, which will facilitate expression and display of specialty epitopes/peptides embedded into virions.

Incidence of *Wheat streak mosaic virus*, *Triticum mosaic virus*, and *High Plains virus* in wheat curl mites in maturing wheat heads

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Wheat curl mites (WCMs) transmit *Wheat streak mosaic virus* (WSMV), *Triticum mosaic virus* (TriMV), and *High Plains virus* (HPV) to wheat in the Great Plains region of the United States. These viruses can be detected in wheat singly, doubly, or in triple combinations. To determine the frequency of occurrence of the three viruses in WCM populations at the end of the growing season, maturing wheat heads were collected from 90 fields across Nebraska in 2011 and 2012 and placed in proximity with 4-leaf stage wheat seedlings in WCM-proof cages in a growth chamber. WCMs moved off the drying wheat heads and infested the wheat seedlings. Twenty eight days after infestation, the wheat plants were tested for the presence of WSMV, TriMV or HPV using ELISA. WSMV was the most predominant virus detected. Double (TriMV+WSMV or HPV+WSMV) or triple (TriMV+HPV+WSMV) virus detections were more frequent (47%) than single detections (5%) of TriMV and HPV. Overall, 81% of the samples infested with mites were positive for at least one virus. These results indicate a high potential for double or triple virus infections of fall-sown winter wheat, and therefore a greater risk for yield loss.

Characterization and targeted deletion of Bin1 in *Magnaporthe oryzae* and its effect on fungal development and plant infection process

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The Bar domain superfamily of proteins are central regulators of membrane dynamics; they play important roles in membrane curvatures and exo and endocytosis. *Magnaporthe oryzae*, the causal agent of the rice blast disease, has two genes coding for proteins with Bar domains, called Bin1 and Bin3. The role of these proteins during fungal penetration and disease development is still unknown. Thus, the goals of this study were to knock out the Bin1 gene and check its effect on fungal growth and pathogenicity. Bin1 gene was deleted in the *M. oryzae* 7015 strain, which is the reference, sequenced strain. Mycelial growth rate was measured in minimum and complete media, and pathogenicity assays were performed on susceptible barley. Three true knockouts from independent transformations were obtained and studied further. Bin1 mutants showed decreased growth rates in both minimum and

complete media when compared to the wild-type. The pathogenicity assays showed that the Bin1 mutant produced fewer lesions than the wild-type. The size of lesions produced by the mutant and wild-type were not significantly different. Overall, it is likely that the deletion of Bin1 had an effect in spore germination and/or appressorium formation, which is currently being further investigated.

Plant growth promoting characteristics of *Bacillus* species associated with *Chenopodium quinoa*

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As demand for organic quinoa increases and production expands, organic methods, such as the use of plant growth promoting bacteria, are needed to sustain quinoa production. To better understand the potential of *Bacillus* species to promote growth and reduce disease in quinoa (*Chenopodium quinoa*), a survey of three *Bacillus* populations from Bolivian, Ecuadorean and domestic *Chenopodium* spp. was performed. Four *Bacillus* species groups common to all populations were tested, including the *B. subtilis*, *B. megaterium*, *B. simplex*, and *B. cereus* species groups. Nearly 500 isolates were assayed for tricalcium phosphate solubilization, phytase production, IAA production, chitinase production and fungal antagonism. Patterns of specific plant growth promoting phenotypes were observed in different *Bacillus* species groups across all populations. For example, the tricalcium phosphate phenotype was universal in the *B. subtilis* and *B. megaterium* species groups, whereas IAA production was only observed in the *B. simplex* and *B. megaterium* species groups. Chitinase production was observed in the *B. cereus* species group and rarely in other species groups, whereas phytase production was common to all species groups. Isolates with potential for improving quinoa sustainability were identified in this study and isolates with multiple characteristics or consortia of single character *Bacilli* will be examined in greenhouse and field studies.

Effectiveness of chemicals and biocontrol agents for management of bacterial spot (*Xanthomonas cucurbitae*) in pumpkin

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Selected chemicals and biocontrol agents were evaluated for management of bacterial spot (*Xanthomonas cucurbitae*) of pumpkin in a laboratory and a commercial field. The chemicals were acibenzolar (Actigard 50WG), copper compounds (Agion-E, L, Badge X2 DF, Cuprofix Ultra 40DF, Kocide-3000 46.1DF, Nordox 75WG, and Phyton-016B L), famoxadone plus cymoxanil (Tanos 50DWG), kasugamycin (Kasumin 2L), mancozeb (Dithane 75DF), oxytetracycline (Mycoshield 17WP), quinolin (Quintec 2.08SC), and streptomycin (Agrimycin 17WP). Biocontrol agents were a natural fungicide (Sporatec), *Bacillus amyloliquefaciens* (CX-9030), *B. pumilus* (Sonata), *B. subtilis* (Serenade), *Streptomyces lydicus* (Actinovate AG), and the extract of *Reynoutria sachalinensis* (Regalia SC). Laboratory studies were conducted by growing bacterial isolates in the casitone yeast extract broth for 24 hr at 28°C. The bacterial cell density was measured with a spectrophotometer at OD 600. The EC₅₀ values of the chemicals ranged from 0.3 ppm (Mycoshield) to 15 ppm (Badge X2 DF). Effectiveness of biocontrol agents was evaluated on Luria-Bertani agar medium (LB). The inhibition zones on LB ranged from 0.38 cm (Sonata) to 1.4 cm (Sporatec). Incidence of fruit infection with *X. cucurbitae* ranged from 11.1 % (Mycoshield plots) to 88.9% (control plots). Rates of infected fruit in plots sprayed with Phyton-016B, Badge X2 DF, Tanos plus Kocide, and Actigard plus Kocide were significantly less than control plots.

The Macrofungi Collection Consortium (MaCC) project: Unlocking a biodiversity resource for research and conservation

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A project is underway to unite digitized data derived from established and nascent herbarium collections of macrofungi (i.e., fungi with conspicuous spore-bearing structures) through the MycoPortal (www.mycportal.org). The Macrofungi Collections Consortium (MaCC) consists of 35 institutions, including two botanical gardens, two natural history museums and 31 large and small universities from 24 states who will share digitized collection information from approximately 1.6 million specimens and about 600,000 ancillary items such as photographs, field notes and fieldbook pages. At the end of the first year of this three year project (June 2013), the MycoPortal will contain of about 1 million specimen records and 50,000 images. Even at this

early stage we can detect certain trends in the data that we anticipate will persist when the project is completed, namely that the majority of specimens were collected in the continental U.S. (average collection density is predicted to be about 1 specimen for every eight kilometers); that the greatest collection density is found in those states with the longest history of research and teaching programs in macrofungi (e.g., California, Illinois, Michigan, New York, North Carolina, Oregon and Washington), and that the most active period for macrofungal collecting was 1950–1980.

Response of African horned cucumber (*Cucumis metulifer*) to southern root-knot nematode, *Meloidogyne incognita*

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The southern root-knot nematode (RKN), *Meloidogyne incognita* (Mi), significantly reduces melon yields (*Cucumis melo*) worldwide. Since methyl bromide was banned as a pre-plant soil fumigant, the cucurbit industry has sought alternative methods for managing RKN. Grafting of melon on RKN-resistant rootstocks such as African horned cucumber (*Cucumis metulifer*) may be an alternative control. Our goal was to identify genotypes of *C. metulifer* (Cm) that could be useful in development of RKN-resistant rootstocks for melon. In a greenhouse test, all 41 accessions of Cm in the U.S. *Cucumis* Plant Introduction Collection were evaluated for reaction to Mi. The Cm accessions varied ($P < 0.05$) for percent root galling (range: 35% to 72%) and all had less ($P < 0.05$) root galling than 'Athena' melon; 32 accessions had less ($P < 0.05$) root galling than 'Strong Tosa' squash hybrid rootstock. Sixteen selected Cm accessions and breeding lines were evaluated against Mi in a RKN-infested field at the U.S. Vegetable Laboratory, Charleston, SC in 2012. The Cm genotypes varied ($P < 0.05$) for percent root galling (range: 17% to 59%) and percent roots covered with egg masses (range: 1% to 26%). 'Athena' melon and 'Strong Tosa' were extremely susceptible with root galling = 94% and 99%, respectively, and percent roots with egg masses = 91% and 87%, respectively. The most resistant Cm genotypes identified in these studies may be useful for developing RKN-resistant rootstocks for melon.

Real-time detection of airborne *Erysiphe necator* (grape powdery mildew) inoculum with loop-mediated isothermal amplification (LAMP)

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Turbidimetric loop-mediated isothermal amplification (LAMP) detection of *Erysiphe necator* (grape powdery mildew) inoculum has been shown to be a useful tool in managing spray programs in the Willamette Valley of Oregon; however, the visual inspection of turbidity introduces a high risk for subjectivity and misinterpretation in amplification results. Using an assimilating probe in a real-time LAMP reaction provides more objective, consistent results than turbidity observations, and allows for quantification. In 2012, impaction spore traps were placed in a research vineyard at the Oregon State University Botany and Plant Pathology Farm, and samples were collected daily and biweekly pre-bud break until véraison. The samples were processed using real-time LAMP and quantitative PCR (qPCR). Compared to qPCR, real-time LAMP was 91.8% accurate and had 98.6% sensitivity. Spore traps were also distributed to 16 commercial vineyards to assess the viability of using real-time LAMP for the detection of grape powdery mildew as a management tool. Samples were collected from 23 impaction spore traps biweekly from bud break until July 3, 2012. Samples were analyzed using real-time LAMP, which proved to be semi-quantitative. Initial pathogen detection occurred on May 21, 2012. Most spore detections observed in the commercial vineyard trial were below 100 spore quantities. Disease pressure in 2012 was extremely low, and visual field scouting data agreed with the real-time LAMP data.

Transcriptional profiling of sclerotia formation in the soil fungus *Rhizoctonia solani*

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In this study transcriptome-based methods were deployed to better understand sclerotia formation in the soil fungus *Rhizoctonia solani*. Our experimental approach involved the use of a wild type sclerotia producing strain of *R. solani* anastomosis group 3 and an isogenic derived strain that does not form

sclerotia. Mycelium from each strain was harvested from a cellophane membrane at 12, 48, 144, and 196 h after transfer onto water agar. These sampling times represent critical stages during sclerotia formation associated with aggregation and fusion of hyphae, production of sclerotial initials (monilioid cells) and melanization. At each sampling time, total RNA was extracted from vegetative tissue of each strain and enriched for mRNA using oligo(dT) tags. The mRNA was fragmented into smaller units and used as a template to prepare cDNA. cDNA was sequenced with Illumina technology and levels of mRNA expression were compared based on Reads Per Kilobase exon Model per million mapped reads using ± 2.0 as the cutoff for log2 ratios. The sclerotia plus and minus strains had 856 and 704 unique expressed genes, respectively, with 182 expressed genes that were shared between the strains. Twenty-six of the 182 shared genes exhibited differential patterns of up or down regulation. Significantly greater differences in expression were recorded for inositol monophosphatase, manganese and iron superoxide dismutase, metalloprotease, TAP family, and transposase family Tnp2 genes.

Estimating the effectiveness of management strategies to reduce seed degeneration in vegetatively-propagated crops: A theoretical framework

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Re-use of planting material (seed) in vegetatively-propagated crops over multiple generations can result in pathogen build up and consequent reduction in yield. This seed degeneration is a problem especially among smallholder farmers in developing countries where clean seed is scarce and/or expensive. We develop here a theoretical model that integrates the various management options available to minimize seed degeneration including host resistance, acquisition of clean seed, and on-farm management strategies such as positive selection. With a focus on seed degeneration caused by viruses in potato, sweetpotato, cassava, yam, and banana, the model builds on existing models of crop virus ecology and evolution. We incorporate variability in biological and environmental system elements through stochastic model components, to study the likelihood of a range of potential outcomes. We also incorporate spatial structure in the model to evaluate the effects of decision making on multiple neighboring farms. The role of environmental factors that influence the choice and success of these management options is also tested. This model is then used to evaluate crop-specific management strategies and develop a decision support system to predict the effectiveness of different management strategies in reducing seed degeneration.

Occurrence of the A2 mating type of *Pseudoperonospora cubensis* in the United States

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Downy mildew caused by the oomycete *Pseudoperonospora cubensis* is one of the most destructive foliar diseases of cucurbits worldwide. *P. cubensis* is known to propagate asexually via production of airborne sporangia. Sexual reproduction is rare and requires both A1 and A2 opposite mating types to form oospores. Although the occurrence of oospores has been reported in parts of Europe and Asia, oospore production has previously never been reported in the United States either in the field or under laboratory conditions. Here, we report on the first occurrence of the A2 mating type of *P. cubensis* and the formation of oospores in the United States under laboratory conditions. About 80 oospores per cm² of leaf tissue were formed when single lesion field isolates from butternut squash were crossed with single lesion field isolates from cucumber in equal proportions on detached melon leaves incubated in the growth chamber at 18 to 21°C under 12/12 h dark/light cycle. Oospores were spherical, hyaline to golden yellow in color and measured between 28 and 56 µm in diameter. Data on the frequency and distribution of the two mating types and the profile of current pathotypes of *P. cubensis* in the United States will be presented. The implication of the occurrence of the A2 mating type and formation of oospores on the epidemiology of downy mildew and the population biology of *P. cubensis* will be discussed.

A routine crop-specific diagnostic macroarray for profiling viral infections in grapevine

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Control of virus infection and spread in grapevine is accomplished principally by means of healthy planting stock with requiring reliable diagnostic techniques; currently ELISA and RT-PCR are the methods of choice. However, as the number of grapevine-associated viruses increases – there are at least 58 described to-date – so does the requirement for alternative methods that facilitate multiplex detection. To this end, we have developed a macroarray containing a total of 1578 virus-specific 60-70mer oligonucleotide probes pertaining to 38 of the most important grapevine viruses. The array is printed onto a reusable, 18 x 7 cm nylon membrane. In a survey of ~200 *Vitis vinifera*, hybrid and wild grapevines from the U.S. and Europe representatives of the four main virus families Betaflexiviridae, Closteroviridae, Secoviridae and Tymoviridae were all found alone and in combination. Specifically, the array identified an established presence in New York grapevines of the nematode transmissible *Arabidopsis mosaic virus*, and the mealybug transmissible *Grapevine virus E*. The latter, a potentially damaging pathogen new to North America, being found predominantly in hybrid Concord vines co-infected with *Grapevine leafroll associated virus-3*. As a whole this work demonstrates the utility of the macroarray platform for the routine multiplex detection of viruses in a single crop and its potential for characterizing grapevine-virus associations.

Evolutionary trends and inferences for viruses of the Secoviridae: Evidence of an ancient modular evolution

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The *Secoviridae* family of viruses is a member of the Picornavirales order, an important group of non-enveloped viruses that infect vertebrates, arthropods, plants and algae. The plant infecting *Secoviridae* are vectored by a range of arthropods, including nematodes, beetles and aphids. In this work we set out to gain an understanding of secovirid evolution by analyzing full-length and partial genome sequences using a variety of computational approaches to reconstruct time-measured and co-evolutionary phylogenies, determine rates of nucleotide substitution and find evidence for interspecies recombination. The selection pressures (d_N/d_S) and phylogenies of the six principal genes 1N(ProCo), RdRp, HEL, Pro, 2N(MP) and CP were highly variable and implied contrasting evolutionary scenarios. Position-Specific Scoring Matrices generated from representatives of the principal monophyletic groups and analyzed by position-specific iterative basic alignment search tool (PSI-BLAST) found a distant though significant homology between the CP and Pro genes and those of animal infecting members of the Picornavirales, while other genes had strikingly alternative affinities both within and outside the *Secoviridae*. From these findings we propose a general model for secovirid evolution that aims at explaining both the modular composition of the genome and the process of genome segmentation.

WITHDRAWN

***Xanthomonas perforans* strains from Florida tomatoes exhibit widespread copper tolerance, changing race structure and sensitivity to other bactericides**

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Bacterial leaf spot, caused by *Xanthomonas perforans*, is a serious problem for tomato production in Florida. The tomato industry relies on repeated applications of copper mixed with mancozeb for disease management, which has led to the development of copper tolerance among field isolates. From 2011 to 2012, 176 *Xanthomonas* strains were collected from transplant and field locations throughout Florida and evaluated for pathogenicity, race, and sensitivity to copper, streptomycin, and Kasumin 2L (commercial formulation containing 2.3% kasugamycin; Arysta LifeScience, Cary, NC) using nutrient agar (NA) amended media. All strains were characterized as race 4 and grew on NA containing 200 ppm CuSO₄, which is similar to a survey conducted in 2006 when a mixture of 77% race 4 to 23% race 3 existed. Of the 176 strains, 56 were insensitive to streptomycin at 200 ppm, and 135 and 18 strains were insensitive to Kasumin applied at a rate equivalent to 25 and 50 ppm kasugamycin, respectively. None of the strains grew on media amended with Kasumin at 100 ppm kasugamycin equivalent rate. The fungicide quinoxyfen (Dow Agrosciences LLC, Indianapolis, IN) was also tested for bactericidal activity against a subset of 17 strains. No growth inhibition was observed on NA amended with 200 and 400 ppm quinoxyfen; however, only 1 out of 17 strains was limited in growth on media amended with CuSO₄ (200 ppm) and quinoxyfen (200 and 400 ppm) and significantly reducing growth of the other 16.

Seed endophytes associated with dominant plants in the alpine tundra in Colorado

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Plant associated fungi can colonized seeds via vertical or horizontal transmission. These symbionts can provide the hosts with defense mechanisms and nutrients, while also giving the plants an advantage at germination. We examined seed-associated fungi isolated from dominant plants in the alpine tundra. Fungi associated with seeds were also compared with fungi found in roots that were sequenced using high throughput sequencing and culturing techniques. Samples were collected from a moist meadow in the alpine tundra at the Long Term Ecological Research Site (LTER) in Niwot, Colorado. Fungal cultures were sequenced using Internal Transcribed Spacer rDNA and identified using Blast and phylogenetic analysis. A total of 54 fungal cultures were isolated from seeds. All fungal isolates in seeds belong to the phylum Ascomycota. Dominant orders in seeds included Capnodiales (23% of the isolates), Pleosporales (23%), and Eurotiales (16%). We isolated at least 15 unique genera from the seeds and 53% of these genera were also found in the roots of two of the plants. Germination experiments using commercial seeds will be conducted for unique isolates to determine potential roles in symbioses. Microbial interactions with plants are known to play an important role in maintaining the biodiversity in the alpine tundra. We expect this study will help increase our knowledge of endophytic abundance, as well as their taxonomic and functional relationships with dominant plants.

Control of fungal plant pathogens by *Bacillus* sp. F727 and production of novel metabolites

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Many of the most detrimental plant diseases result from infection by fungal pathogens including members of the genera *Botrytis*, *Phytophthora*, *Fusarium*, *Alternaria*, *Pythium* and *Verticillium*. Marrone Bio Innovations has isolated a novel *Bacillus* sp. from a soil sample. We have demonstrated that *Bacillus* sp. F727 controls a spectrum of plant pathogens *in vitro* and consistently controls *Botrytis cinerea* on tomato and pepper and downy mildew on lettuce *in planta*. Three active metabolites have been identified as being produced by *Bacillus* sp. F727, including a novel fungicidal peptide. *Bacillus* sp. F727 is different from other *Bacillus*-based biofungicide products on the market in terms of its metabolite production and genetic make-up and offers an effective alternative for control of fungal plant diseases in agriculture.

Fungal diversity by plant section in the Mississippi soybean production system

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Soybean is considered one of the most important commodities produced in Mississippi and achieved record yields of 41 and 42 bushels per acre in 2011 and 2012, respectively. However, late-season pod and seed diseases are environmentally dependent in the Mid-south and can result in significant yield loss. This study evaluated the fungal diversity associated with seed located at three points within the soybean canopy at physiological maturity (R8). The top, middle, and bottom section of 100 random plants were sampled from each of six commercial fields within the Mississippi Delta (Delta; irrigated) and the Black Belt Prairie (Hills; non-irrigated). Seed were surface disinfested, plated onto acidified potato dextrose agar, and incubated at 22°C for four weeks. Microscopic identification was based on morpho-taxonomy. *Fusarium oxysporum* and *Phomopsis longicolla* were the most predominant fungi identified regardless of region. *F. oxysporum* and *P. longicolla* were isolated from the mid-section and top 8% and 11%, respectively in the Hills. Conversely in the Delta, *F. oxysporum* frequency was numerically greater (14%) in the lower canopy whereas *P. longicolla* was isolated from the mid-section of soybean plants with 16% frequency. *F. moniliforme* and *Alternaria* sp. were consistently isolated from soybean seed collected from the Hills but at frequencies below 6%. Irrigation may play a role in fungal colonization of soybean seed located in the mid- to lower canopy.

The genome of the fern pathogen *Mixia osmundae* reveals hints about its cryptic biology

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Mixia osmundae (Nishida) C.L. Kramer (Pucciniomycotina, Mixiomycetes) is a rarely encountered basidiomycete that infects ferns belonging to the genus *Osmunda* L. (Pteridophyta, Polypodiopsida). The fungus causes small chlorotic lesions on fern leaves that become covered in a white powdery layer. This layer is formed by spores that are exogenously produced on the surface of a large sac-like sporogenous cell, a characteristic structure unique to *M. osmundae*. As *M. osmundae* is not commonly found, many aspects of the biology of this fungus are unknown, including whether the spores are produced via mitosis or meiosis. We conducted whole genome analyses of *M. osmundae* to answer these and other questions. We have determined that the spores produced on fern leaves are haploid and likely produced asexually. We also detected that although *M. osmundae* possesses the genetic pathways for breaking down various plant cell wall components, it is not able to digest and produce glucose or xylose from them. These and other genomic data hint that the pathogen is a biotroph, although not an obligate biotroph since it also has a budding state that can be maintained in culture. To date, *M. osmundae* has been collected only from Asia and the eastern United States; however, our searches through sequence databases indicate that the genus might also be present in Europe and that this monotypic class may have other undiscovered members.

***Melampsora* rusts on weeping willows in the United States**

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Rust fungi in the genus *Melampsora* Castagne (Pucciniales, Pucciniomycotina) infect willow species (*Salix* L.) around the world. The uredinial states—by far the most prevalent stage of infection in this group—of different *Melampsora* species can be very similar in spore morphology making species diagnosis difficult. As a consequence, the name *M. epitea* Thüm., a species originally described from Europe, is most often applied in diagnosis of willow rusts in the Americas. A recent study of *Melampsora* species from North America determined that there are at least 14 different *Melampsora* phenotypes present on the continent and that none of these were referable to European species including *M. epitea*. Although that study included rusts of many different willow species, none were included from weeping willow (*S. babylonica* L.). We examined new collections of *Melampsora* specimens from *S. babylonica* from southern Louisiana. Morphological comparisons revealed

that urediniospore and uredinia characteristics overlapped with the descriptions of several other *Melampsora* species from North America, Europe and Asia. However, comparisons of sequence data from the EF1- α and ribosomal large subunit revealed that the collections from *S. babylonica* in Louisiana represent yet another cryptic North American species. Herein we describe a new species to accommodate the Louisiana material and provide a review of the species of *Melampsora* that occur on weeping willows.

The use of growth promoting bacteria isolated from wild strawberry for the management of strawberry black root rot

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Black Root Rot (BRR) is a disease complex of strawberry conferred by one or more organisms including *Pythium*, *Fusarium*, *Rhizoctonia* spp. and several species of nematodes. The elimination of methyl bromide use has stimulated the search and evaluation of ecologically-based strategies to manage BRR. Plant growth promoting rhizobacteria (PGPR) are bacteria that colonize plant roots and can help to control plant diseases or enhance plant growth. The objective of this work was to evaluate eight bacteria isolated from wild strawberry (*Fragaria chiloensis*). Experiments were done under controlled conditions using *F. ananassa* var. Chandler and inoculating the soil at 0.5% (v/v) with *Fusarium solani* and *Rhizoctonia solani*. Bacteria applications made (every 2 weeks over two months) decreased BRR severity ($p \geq 0.05$) whereas a single application at planting did not impact BRR severity compared to no PGPR controls. Disease severity from 4 of the isolates tested, *Bacillus pumilus* (37%), *Bacillus mycoides* (42.5%), *Bacillus thuringiensis* (22.3%) and *Pseudomonas putida* (16.6%) was similar or lower than the one found when using the biological fungicide Companion™ (39.9%). Further evaluations need to be made to determine the role of this bacteria and their use in an integrated disease management system for strawberry BRR.

Use of PCR technology for the speciation of fungi recovered from dried fruits and tree nuts

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Fungi (moulds and yeasts) are present everywhere in the environment and often find their way into foods. A high number of them are able to grow on foods and several species can produce mycotoxins. Mainly conventional methods have been used to identify foodborne moulds. Some identification problems, however, have arisen because sometimes closely-related species have identical morphologies; in such cases the use of molecular techniques is necessary for more accurate results. The aim of this study was to speciate potentially-toxigenic moulds from dried fruits and tree nuts using a PCR technique. The DNA from 82 fungal specimens recovered from dried fruits and tree nuts was isolated using the Norgen Biotek Fungi/Yeast Genomic DNA Isolation Kit. The isolated DNA was PCR amplified using the primers encoding the *b*-tubulin gene, Bt2a and Bt2b. The PCR conditions used were initial denaturation at 95°C for 10 min, 38 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min and a final extension at 72°C for 7 min. Amplification results were evaluated by gel electrophoresis of the PCR products. The amplified DNA was sequenced and sequence comparisons and species identifications were done using the local alignment search tool (BLAST). The 82 strains identified belonged to 18 species from the *Aspergillus*, *Penicillium*, *Eurotium* and *Fusarium* genera. Percent identifications achieved with this method were above 95 for most isolates tested.

Fungal contaminants recovered from selected tree nuts and dried fruits

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Dried fruits and tree nuts often contain potentially-toxigenic fungi. In this study, 64 tree nut samples (almonds, pecans, pine nuts and walnuts) and 50 dried fruit samples (apricots, cranberries, papaya, pineapple and raisins) obtained from U.S. retail were analyzed for fungal contamination using the BAM method. Isolated strains were identified using conventional plating and molecular methods. Results of the tree nut analysis showed that the highest mould and yeast (MY) counts (2.2×10^5 cfu/g) were found in walnuts and the lowest in pecans. The most common mould in nuts was *Aspergillus niger*; relatively low numbers of *A. flavus* were found across the board, while

Penicillium spp. were very common in pine nuts and walnuts. Low levels (1.0×10^2 cfu/g) of yeasts were recovered from only two pine nut samples. Dried fruits showed minimal fungal contamination ranging from <100 to 7.3×10^3 . The highest fungal levels were present in raisins. No moulds or yeasts were recovered from any of the papaya and most of the cranberry, pineapple and apricot samples. The most common mould in dried fruits was *A. niger* followed by *Penicillium* spp. One apricot sample also contained low levels (1.0×10^2 cfu/g) of yeasts. This research demonstrated that potential mycotoxin-producing moulds (e.g. aspergilli and penicillia) were present in walnuts, pine nuts, almonds and raisins at relatively high levels.

Identification and temporal distribution of potential insect vectors of *Erwinia tracheiphila*, the causal agent of bacterial wilt of cucurbits

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Bacterial wilt of cucurbits (BWC) is caused by *Erwinia tracheiphila* (*Et*). This bacterium is spread from plant to plant by the striped (SCB) and the spotted (SCR) cucumber beetles. *Et* develops and circulates in the xylem, making its control with bactericides impossible. Consequently, to reduce disease incidence, it is crucial to control the vector-insect populations. The aim of this study was to develop a sampling strategy to determine the potential vector – insects harboring *Et* in cucurbit fields, as well as their temporal distributions. Cucurbit pest insects were collected weekly throughout the growing season and frozen until use. Total DNA was extracted from each sample using CTAB and *Et* presence was detected by PCR, using ET-1 and ET-2 primers. The predominant insects collected in l'Acadie field were SCB (116), SCR (5), western corn rootworm beetle (88) and northern corn rootworm beetle (NCR, 15). *Et* was detected in all species, with a total incidence of 8%. *Et*-positive beetles were most common from end of July to mid-August. Surprisingly, *Et* was detected in 5 of the 15 NCR specimens, which to our knowledge, was not reported to be a BWC vector. Our results suggest that the NCR should be considered as a potential vector of BWC. Further research is needed to determine the actual role of the NCR in BWC epidemiology and to elaborate more efficient BWC control with minimal insecticide applications.

Effect of winter rye and wheat used as cover crops to reduce *Pseudomonas syringae* disease incidence and severity in squash

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Cover crops is gaining in popularity because it promotes soil conservation, increases microbial diversity and activity, reduces weed infestation and was reported to reduce the incidence of some plant diseases. In this study, the impact of cover crops on *Pseudomonas syringae* (*Ps*) population sizes and symptom development were investigated. In 2012, the experimental plot was established at the Agriculture and Agri-Food Canada experimental farm (l'Acadie, Qc). Cover crops, rye (*Secale cereale* L.) and wheat (*Triticum* L.), were seeded in a randomized complete block design the preceding fall. The control treatment was conventional tillage. In June, cover crops were terminated using a roller crimper and herbicides. Spaghetti squash (*Cucurbita pepo* L.) were seeded one week later. Population sizes of *Ps* on squash leaves 24 and 45 days after seeding, disease severity, growth and yield at harvest were determined for the different treatments. Bacterial population size of *Ps* was significantly lower for both sampling times ($P < 0.0001$, $F = 30.54$ and $P < 0.004$, $F = 4.67$) for rye and wheat treatments compared to the conventional tillage. This has also resulted in a significant decrease of symptom severity caused by *Ps* on fruit for rye and wheat treatments ($P < 0.004$, $F = 14.03$) compared to the control. According to these results, rye and wheat used as cover crops have the potential to modulate population size of pathogenic bacteria on squash leaves which could help in disease management.

Extracellular DNases contribute to virulence of *Ralstonia solanacearum*

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Root cap border cells have been recently recognized as an important physical defense barrier against soilborne phytopathogens. Proteins, polysaccharides and DNA produced by border cells form the root cap slime, an extracellular matrix that protect plant roots from pathogen invasion, like the well-characterized neutrophil extracellular traps (NET) in mammalian systems. This finding suggests that pathogens able to degrade this matrix with nucleases would have increased virulence on plants. The genome of the root-

infecting bacterial wilt pathogen *Ralstonia solanacearum* encodes two predicted extracellular nucleases, NucA and NucB. Analysis of single and double *nuc* mutants confirmed that both genes encode DNA degradation and revealed that a *nucAB* double mutant has reduced ability to infect both pea and tomato seedling roots. This suggests that these bacterial nucleases degrade the DNA component of the plant root cap extracellular trap to facilitate root infection. Thus, root border cell count may be an important trait to select for protection against soilborne pathogens. Preliminary results also suggest that *R. solanacearum*'s extracellular nucleases may be involved in systemic translocation of the bacterium *in planta* in later stages of infection, possibly by participating in the formation of wild-type biofilms inside the plant host.

WITHDRAWN

High genetic diversity in North American populations of *Phaeoconiella chlamydospora*, causal agent of Petri disease and esca of grapevine

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Phaeoconiella chlamydospora is one of the causal agents of Petri disease and Esca, causing chronic infection of the grape wood that reduces yield and vineyard longevity. Sexual fruiting bodies of the fungus have not been found in the field. Conidia are thought to cause primary and secondary vineyard infections, and the pathogen population is presumably clonal. Using 18 microsatellite markers, we genotyped 96 isolates from three regions of North America: the northeastern U.S., California, and British Columbia. All 96 isolates had a unique haplotype, suggesting that sexual reproduction generates new allelic combinations in individuals of this species. Clustering analyses revealed three genetic groups of isolates. Two genetic groups spanned the California and British Columbia collections, whereas the third group was comprised mainly of northeastern U.S. isolates. High genetic subdivision ($F_{st} = 0.28$; $P < 0.001$) suggests that genetic and/or ecological factors may maintain genetic differentiation among these three groups. The presence of a few immigrant haplotypes in each region may represent pathogen introductions through human transport of infected plant material. Significant differences in levels of genetic diversity were found among the groups, with the highest gene diversity ($H = 0.83$) and allelic richness ($A = 9.4$) in the northeastern U.S. A high diversity of *Vitis* species in this region, and possibly differences in pathogen life history, may explain these findings.

Interactions between tospovirus proteins in mixed infections using bimolecular fluorescence complementation (BiFC)

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Molecular interactions between cognate proteins of economically important tospoviruses may be of crucial significance for the understanding of pathogenesis, evolution, and development of efficient and durable control strategies for these viruses, which frequently exist as mixed infections in the same host plant. Earlier studies in our lab have suggested that during a mixed infection, *Tomato spotted wilt virus* (TSWV) turns a restrictive host into a permissive one for another distinct tospovirus, *Iris yellow spot virus* (IYSV). The mechanism(s) behind this observation are not clear. Previously we reported that IYSV nucleocapsid (N) and movement (NSm) proteins multimerize and interact with each other in infected *Nicotiana benthamiana* plants. Here we describe the findings of interaction studies of IYSV and TSWV proteins in the same host plant infected with both viruses. Viral genes of both viruses were separately cloned into binary pSITE-BiFC vectors using the Gateway system. Expression clones were agroinfiltrated into a nuclear marker line of *N. benthamiana* expressing cyan fluorescent protein fused to Histone 2b. Confocal microscopy was used to visualize the fluorescence of the expressed fusion proteins. Preliminary results suggest that N and NSm proteins of IYSV interact with their counterparts coded by TSWV in a doubly infected plant. Findings of this research could facilitate a better understanding of the virus-virus interactions within host plants.

AvrRxol1 is a virulence factor that suppresses growth of eukaryotic and prokaryotic cells

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The effector AvrRxol1, also called XopAJ, is present in *Xanthomonas*, *Acidovorax*, and *Burkholderia* bacterial plant pathogens. AvrRxol1 triggers resistance on plants expressing the Rxol1 gene and suppresses growth in yeast. Both phenotypes require an ATP binding-site motif. We analyzed the effects of expressing the *avrRxol1* gene from *X. oryzae* pv. *oryzicola* in yeast (*S. cerevisiae*), in *E. coli*, and in an *avrRxol1*-deficient *X. oryzae* strain. Inducible expression of *avrRxol1* suppressed growth of *E. coli* as well as *S. cerevisiae*. Yeast and bacterial growth suppression phenotypes were abolished by the introduction of site-directed mutants at the predicted ATP binding motif and a second predicted catalytic site. Co-expression of *avrRxol1* with the open reading frame immediately downstream, a putative atypical chaperone, abolished or strongly diminished the yeast and bacterial growth suppression phenotypes. *avrRxol1* expression significantly increased *X. oryzae* cell proliferation on rice cv. Kitaake at two days post inoculation, although symptoms were not noticeably affected. The effect of the ATP binding site on the virulence phenotype of AvrRxol1 will be reported. The data suggest that bacterial effector AvrRxol1 is a universal growth-suppressive toxin, with activity against prokaryotic and eukaryotic cells, and that cells are protected from AvrRxol1 activity by co-expression of a chaperone.

Synthetic detection circuits targeting *Xylella* diffusible signal factor in bacteria and plants

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Xylella fastidiosa, a devastating quarantine pathogen of citrus and grape, secretes a small fatty acid diffusible signal factor (XfDSF). Recently, technology was developed to generate transgenic detector plants that develop a measurable response in the presence of particular compounds. Three components were incorporated: a receptor that relays a signal in the presence of a chemical input, a response-relay protein that carries the signal to the nucleus, and a reporter gene activated in response to the signal. We tested a synthetic receptor that triggers the reporter activation in the presence of *X. fastidiosa* DSF. In a bacteria-compatible version of the synthetic circuit, the synthetic receptor detected low nanomolar concentrations of 2-Z-tetradecanoic acid, a chemical component of XfDSF. Reporter induction was stronger resulting from 2-Z-tetradecanoic acid treatments than from similar small molecules varying in double bond number and position and in methylation sites. Variants of the receptor circuit were introduced into a system for testing the XfDSF response in protoplast assays, and the results will be reported in this presentation. These efforts represent the first steps toward the application of plant synthetic biology to detection of pathogens.

Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Lecanoromycetes, Ascomycota)

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In lichen-forming fungi, traditional taxonomical concepts are frequently in conflict with molecular data, and identifying appropriate characters to describe phylogenetic clades remains challenging in many groups. The selection of suitable molecular markers for the reconstruction of robust phylogenetic hypotheses is therefore fundamental. We investigated the phylogenetic relationships of *Usnea* species based on ribosomal DNA ITS and nuLSU, as well as two protein-coding genes *RPB1* and *MCM7*. ITS comprised several highly variable regions, containing substantial genetic signal but susceptible to causing ambiguities in the alignment. We compared several methods of alignment of ITS and found that a simultaneous optimization of alignment and phylogeny (using BALI-phy) improved significantly the topology and the resolution of the phylogenetic tree. However the resolution was better when using protein-coding genes, especially *RPB1*. The phylogeny based on the concatenated dataset revealed that the genus *Usnea* is subdivided into at least four highly-supported clades. The characterization of these clades is however hindered by the high homoplasy of characters within the phylogeny. On the other hand, most of the species were reconstructed as monophyletic, indicating that combinations of phenotypic characters are suitable discriminators for delimitating species, but are inadequate to describe generic subdivisions.

Resolving species boundaries in the lichen-forming *Peltigera canina* complex (Lecanoromycetes, Ascomycota)

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Morphology alone can confound an accurate understanding of biodiversity, especially when morphological traits are influenced by the environment, or when cryptic speciation is rampant. Our study focuses on the lichen-forming ascomycete *Peltigera*, a monophyletic genus of more than 90 species that plays a major ecological role in nitrogen cycling. We chose the *P. canina* complex, a group of ca. 35 species whose phenotypic variation is poorly understood, and in which the potential for finding new or cryptic species is high. We tested if morphologically circumscribed species are supported by molecular data, using ITS rDNA and highly variable non-coding sequences developed using genomic data mostly from other lichen-forming fungi. Simultaneously, we sequenced the *rbclX* region of the photobiont partner – the cyanobacterial genus *Nostoc* – to investigate the level of reciprocal mycobiont-photobiont specificity and its putative role in shaping fungal speciation. We confirmed that hypervariable regions of ITS1 and ITS2 can act

as diagnostic characters to differentiate species in all currently described species within the *P. canina* complex. In addition, several new species were discovered or confirmed. A strong biogeographical signal was detected across the phylogeny, as well as within selected species. Notably, European, North American and Asiatic populations of *P. degenii* formed distinct monophyletic lineages.

Effect of planting date on peanut stem rot epidemics and efficacy of early season prothioconazole applications

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The effects of four planting dates (April 25, May 8, May 21 and June 4) on peanut (*Arachis hypogaea*) stem rot (*Sclerotium rolfsii*) epidemics and efficacy of early-season prothioconazole sprays were evaluated in 2012. Plots were either not treated or treated with prothioconazole (0.16 kg a.i./ha) at 21 or 35 days after planting (DAP) applied in a 30 cm band (all received chlorothalonil for leaf spot control). Final stem rot incidence in untreated plots was 32, 18, 13, and 9%, for planting dates 1-4, respectively. Prothioconazole plots had a similar pattern, but disease incidence was reduced by an average of 42 and 50% for the 21 and 35 DAP applications, respectively. Pod yields for untreated plots were 6388, 6519, 5431 and 2589 kg/ha for planting dates 1-4, respectively. Prothioconazole plot yields averaged 558 and 531 kg/ha higher for the 21 and 35 DAP applications, respectively, but most differences were not significant ($P=0.05$). The first symptoms appeared at 100, 100, 70 and 40 DAP for planting dates 1-4, respectively.

Friend or foe: Defense response and growth regulation of *Zea mays* in response to *Phialocephala fortinii*

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Endophytic fungi are common plant inhabitants and form complex relationships with the plant host. The fungus can stimulate optimal growing conditions and tolerance to biotic and abiotic stressors. The plant-fungal symbioses can be very fragile and stressed environments can trigger pathogenic activities. These relationships have been widely studied however little is known about how the plant responds to dark septate fungi at the molecular level. *Phialocephala fortinii* is a common dark septate endophyte found around the world. The purpose of this study was to identify genes that are involved in the establishment of the interaction of *Phialocephala fortinii* and corn plants. Corn seeds inoculated with a strain of the fungus showed a significant increase in germination and growth and the plant did not show signs of physical damage caused by the fungus. RNA was purified from stem and root tissues to conduct microarray analysis and qRT-PCR. In both tissues, genes involved in the expression of plant defenses, growth, photosynthesis and stress tolerance were differentially stimulated by the fungus. Though the plants showed no visible damage, plant defense genes were up-regulated suggesting biotic stress from the presence of the fungus. Our data suggests that the plant is regulating the interaction with this particular fungus and additional studies need to be conducted to determine the specific role of defense genes in the presence of endophytes.

Pathogenicity and aggressiveness of *Alternaria solani*, *A. alternata*, and *A. triticina* on potato

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Alternaria isolates were collected from potato foliage showing symptoms of early blight and brown spot in the Columbia Basin, WA, and Bonners Ferry and Rupert, ID in 2009, 2010, and 2011. Morphological characterization and ITS sequencing identified *Alternaria alternata* (*Aa*), *A. solani* (*As*) and a novel species identified as *A. triticina* (*At*). This is the first documented occurrence of *At* associated with potato. *At* has been typically associated with grasses such as durum wheat and barley, which may be grown in rotation with potato in the Columbia Basin. Aggressiveness of *At*, *As* and *Aa* was quantified on unwounded and wounded leaflets of cv Russet Norkotah. Disease incidence, infection frequency (IF), and lesion expansion (LE) of *As* were significantly greater than *Aa* or *At* on both wounded and unwounded leaflets. Wounding of tissue significantly increased infection frequency (IF) and lesion expansion (LE) of *Aa* and *At* relative to unwounded tissue. *Aa* was aggressive on wounded tissue and *At* was a weak pathogen regardless of wounding suggesting that *At* is a weak pathogen or saprophyte on potato. Management

of *Alternaria* diseases on potato should continue to be targeted towards *As* and *Aa* as *At* contributes little to overall disease severity in potato fields.

Molecular phylogenetic studies in the phytopathogenic genus *Diaporthe*

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Species of *Diaporthe* cause diseases of a wide range of plants including many that are economically important worldwide. Molecular data are required to accurately identify species of *Diaporthe* in nearly every situation. The nuclear ribosomal internal transcribed spacer (ITS) region performs adequately as a barcode for identifying previously well-defined species of *Diaporthe*; however, multiple markers are required to accurately resolve phylogenetic species. Species of *Diaporthe* associated with *Citrus* diseases in United States and elsewhere were resolved based on a multigene phylogeny in conjunction with morphological, host and geographic data. Sequences from gene regions of ITS, translation elongation factor 1-alpha, beta-tubulin, calmodulin, actin and FG 1093 were used in the phylogenetic analysis. Some species, such as *Diaporthe citri*, *D. vaccinii*, *D. neoviticola*, *D. perijuncta* are shown to be highly host specific while others, such as *D. foeniculina*, *D. rudis* and *D. eres* have an extensive host range. In addition the long standing phylogenetic and taxonomic problems associated with defining the generic type species *Diaporthe eres* were resolved based on molecular data and observation of the type specimens. Multiple molecular markers were used to compare intraspecific ITS variability with the other genes including partial sequences of translation elongation factor 1-alpha, beta-tubulin, calmodulin, actin, RPB2 and FG 1093.

Ectomycorrhizal fungi associated with *Dicymbe altsonii* trees and *Coccoloba lianas* at Mabura Ecological Reserve, Guyana

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Recent meta-analyses of diversity patterns in ectomycorrhizal (ECM) fungi indicated that plant host lineage, habitat isolation, and edaphic factors are contributing drivers of local ECM fungal species richness. Within-stand ECM fungal host preference has been indicated in several temperate studies, but is generally weaker in tropical studies. In some studies of both temperate and tropical ECM communities, phylogenetic clustering of ECM fungi in relation to soil type has been observed. In Guyana, ECM fungal host preference is low for different sympatric species of ECM Fabaceae and Dipterocarpaceae in rainforests dominated by ECM trees. Lianas of ECM *Coccoloba* spp. (Polygonaceae) occur in Guyana in low densities in otherwise non-ECM forests, and also in close spatial proximity to discrete stands dominated by large ECM canopy trees in edaphically heterogeneous local landscapes. In this study, we asked whether ECM fungal communities are influenced by host plant identity, isolation from ECM fungal inoculum, or soil type. We characterized ECM fungal communities at the Mabura Ecological Reserve, Guyana by sequencing ITS rDNA from individual ECM root tips. We sampled discrete stands of the ECM canopy tree *Dicymbe altsonii* (Caesalpiniaceae) and lianas of two *Coccoloba* species across two soil types. Here we present preliminary results on the relative influence of soil, isolation, and host phylogenetic affinities for tropical ECM fungal communities.

Evidence for iron competition as a mechanism for biocontrol of *Erwinia amylovora* by *Aureobasidium pullulans*

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Aureobasidium pullulans strains CF10 and CF40, the biocontrol agents in the product Blossom Protect, suppress infection of pear and apple flowers by *Erwinia amylovora* with the mechanism of biocontrol attributed to nutrient and site competition on floral surfaces. Iron has been shown previously to be a limiting resource on surfaces of pome fruit flowers, and thus we hypothesized that competition for iron via siderophore sequestration is also a potential mechanism of biocontrol. In siderophore production medium, both CF10 and CF40 produced a hydroxamate siderophore, the synthesis of which was suppressed by amendment of 0.5 mM FeCl₃. Moreover, on solid nutrient agar plus 10% sucrose, zones of inhibition were apparent when 6 and 7 day old cultures of CF40 were overlaid with *E. amylovora*; these zones were not

observed if the medium was amended with 0.5 mM FeCl₃. In the field, CF10 and CF40 were excellent colonists of both the stigmatic and hypanthial surfaces of pear and apple flowers. The effect of iron supplementation on biocontrol of fire blight and additional treatments to enhance siderophore activity by *A. pullulans* will be evaluated in 2013 orchards trials.

Gut-inhabiting yeasts from the gut of Australian passalid beetles

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Passalidae (Polyphaga, Coleoptera) is a family of beetles with approximately 960 species that are distributed worldwide. Preliminary studies that characterized ascomycete and basidiomycete yeasts in the gut of these wood-eating beetles from the USA, Guatemala, and Thailand, demonstrated associations between certain yeast taxa and passalids. We extended the study to include yeasts and beetles from tropical forests near Cairns and Brisbane, Queensland, Australia. We isolated more than 1000 yeast strains from about 150 beetles belonging to 10 species. LSU and ITS rRNA markers were used to identify a subset of 250 yeast strains, which revealed that the gut of Australian passalids contained undescribed ascomycetes in the *Debaryomyces*, *Dipodascus*, *Kazakhstania*, *Ogataea*, *Scheffersomyces*, *Sugiyamaella*, *Spathaspora*, *Torulaspora*, and *Zygowillipsia* clades, as well as basidiomycetes in the genera *Cryptococcus* and *Trichosporon*. A close relative of *Candida subhashii* (*Spathaspora* clade) and the xylose-fermenting yeast *Scheffersomyces stipitis* were the most common species isolated in Queensland. These results agree with those of previous studies that showed a common association of xylose-fermenting yeasts in the gut of lignicolous insects. Species and higher taxa of yeasts, however, vary between Queensland passalids and those previously collected in distant regions.

A culture-based and culture-free assessment of the geographic and temporal variation of boreal endophytic and endolichenic fungal communities

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Although species-rich in all terrestrial communities, endophytic and endolichenic fungi reach their greatest phylogenetic diversity in boreal forests -- earth's largest and most threatened forest biome. Previous studies have used single sampling events and culture-based methods to infer their spatial-, geographic-, and host affiliations. Here we pair culture-based and culture-free methods to examine endophytic and endolichenic communities in sets of 20 plant and lichen species sampled in multiple years at one boreal site, and in multiple temperate, temperate-montane, and boreal forest communities across the western hemisphere. In addition to revealing previously unexplored temporal variation in symbiotroph associations, detecting geographic structure among congeneric but allopatric hosts, and highlighting the complementarity of culture-based and culture-free methods, our application of 454 pyrosequencing shows how different tissue-storage and sequence analysis methods yield markedly different estimates of diversity. Further, we show that mycobiont sequences can provide controls for detecting pyrosequencing errors that would go undetected by the otherwise stringent quality control measures that are current in the field. Together these efforts reveal the relative contributions and biases of culture-based and culture-free methods in endophyte studies, and provide a first perspective on the symbionts of iconic hosts that are the foundation of boreal forest communities.

Effect of crop rotation on *Pythium* spp. population composition in Arkansas soybean fields

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Pythium are an ecologically diverse group of microorganisms found in virtually all soils. The more than 100 species in this genus have a wide range of environmental and host preferences, but little is known about the effect of crop rotation on *Pythium* communities. To understand the effect of crop rotation on species diversity, soil was collected from plots following long-term rotation treatments including rice, corn, soybean and wheat. Soil from each plot was placed in cups, wetted to saturation, planted with the soybean cultivar Hutcheson, and incubated at 25°C. After three days, seeds were collected and washed in running water and placed on 2% water agar. Hyphal tips were transferred to a *Pythium* P5ARP selective medium. Molecular identification was performed by sequencing the ITS region and Blast analysis to a curated reference database. A total of 320 isolates were identified representing 12 species. Overall, the most frequently recovered species were *P. spinosum*, *P. irregulare*, *P. pereocandrum* and *P. sylvaticum*. In continuous rice production *P. spinosum* was the most prevalent species isolated. In the soybean-wheat-rice and rice-wheat-soybean-wheat rotations, *P. spinosum* and *P. irregulare* were the most frequently recovered, while *P. irregulare* and *P. sylvaticum* were the most frequently recovered species in the soybean-rice and rice-soybean rotations. *Pythium* species composition reflected the frequency at which a susceptible host was planted in the rotation.

***Alternaria* species in the Bogotá plateau, Colombia**

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In Colombia genus *Alternaria* is reported attacking important crops like citrus, ornamentals, and the family Solanaceae. However, frequently the diagnostic does not include determining the species. This study was done to increase the knowledge on diversity of the genus occurring in Colombia. Samples with characteristic lesions, from different sites in the Bogotá plateau, were collected and taken into the laboratory. Once in there, samples were surface sterilized, and lesions were sectioned and cultured on Potato Dextrose Agar. The Taxonomical determination of the isolates was done following Simmons (2007). Fifty isolates were recovered, and 10 species were identified. All the recovered species belonged to the small spores section. Species most frequently occurring was *A. alternata*, isolated from 17 plant hosts, mainly Solanaceae and ornamentals. Occurrence of *A. solani*, archetypal species in the Solanaceae, was not recorded despite the high frequency of this botanical family among the hosts. We performed a principal components analysis with morphometric characteristics of conidia, resulting conidial width the character that brings greater separation between species. However, using only morphological parameters difficult identifying species, consequently, we propose the combination of identification strategies that, in addition, to the morphology include molecular tools and profiling of the secondary metabolite production.

Resistance of gladiolus cultivars to *Uromyces transversalis* in field trials in Mexico: Preliminary results

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Uromyces transversalis, which causes gladiolus rust, is endemic to commercial gladiolus-producing areas of Mexico, but it is not established in the USA. It can be introduced on cut-flowers shipped from Mexico, which results in a quarantine of the shipment. Therefore, resistance of cultivars of gladiolus (*Gladiolus ×hortelanus*) to *U. transversalis* is being studied in commercial fields in two locations in Mexico: Cuautla, Morelos and Santa Isabel Cholula, Puebla. Initial trials were conducted in fall 2011 and early 2013. The percentage of foliage with disease symptoms and signs from natural infections was assessed weekly for 7 weeks after mature leaves were present. Disease pressure was low (<13%) in Cuautla (2011 and 2013) and higher in Santa Isabel Cholula in 2011 (up to 51%). Lowest foliage disease was observed on cultivars Romulo Cantante, Primavera, Lupe, and Red Beauty with significantly higher levels of disease observed on Sanserri, Ibadan, Cartago, and Roja Borrega. Flower color did not correlate with disease severity. Incorporating disease resistant cultivars into a long-term management strategy for gladiolus rust will help reduce accidental introductions of this pathogen into the USA on cut-flowers.

Variation in fungal soil community structure across substrate age and elevation gradients in native forests on Hawaii Island, USA

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Hawaiian microfungi are very poorly documented and very poorly understood; we know very little about their population structure and how they respond to environmental gradients. Macro and micro fungi alike are very important for ecosystem function, playing a variety of roles. The lack of previous studies performed in Hawaii lead us to ask our own questions about soil fungal diversity; does substrate age (lava flow age) influence soil fungal diversity? Do annual temperature and precipitation contribute to changes in soil fungal community diversity? Not only does Hawaii contain various substrate ages in the form of lava flows, native *Metrosideros polymorpha* ('ohi'a) forests span from sea level to over 2500 meters in elevation. Because of these inimitable gradients, the Big Island is an ideal place to examine the roles of precipitation, temperature, substrate age and elevation in determining fungal soil community structure. This is a novel study that investigates the functioning of these fungal organisms and how they respond to variation in substrate age, rainfall and temperature. Soil samples were collected on four dissimilar aged lava flows and at five elevations in native Hawaiian forests. Total genomic DNA was extracted from approximately one hundred soil samples followed with the amplification of the ITS region. This region was sequenced using next generation sequencing technology. The number of Operational Taxonomic Units (OTUs) were reported and analyzed to determine soil fungal diversity.

Phenological status influence the antifungal phytoconstituents of a wild Brassicaceae

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Among plants, Brassicaceae have been reported having significant antifungal activity. In this work the antifungal effect of extracts from aerial part (ethanol, *n*-hexane, chloroform and water-soluble) of *Raphanus raphanistrum*, bearing white flowers (WF) and purple flowers (PF), was evaluated against the pathogens *Alternaria* sp., *Botrytis* sp. and *Colletotrichum* sp. Flower color in *R. raphanistrum* is a clue of the pollination status (purple flowers have not been pollinated; white flowers have already been pollinated). Evaluation was completed by using PDA amended with PF and WF extracts at different final concentration in order to generate a dose-response curve, by measuring the radial growth of the colonies and amount of conidia after 8 days of incubation. Extracts effect was found to be dose dependent for both phenological status, and extracts were further established as having fungistatic effect. Extract of WF and PF plants showed inhibition against the three fungi for doses above 1.0 and 0.1 µg/µL, respectively. The conidia counting for PF extract revealed no production, while conidia production for WF extract showed a negative dose-response relationship. Best inhibition was obtained by *n*-hexane-soluble extract on both phenological statuses. Results indicate that the aerial part of *R. raphanistrum* might be considered a good source of antifungal phytoconstituents, however its effectivity depends on the phenological status of the donor plant.

Geostatistics analysis of the relationship between plant nutrition and coffee rust

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Precision agriculture is an important tool in the management of many cultures. Especially in relation to soil fertility. The goal of this study was to characterize the spatial distribution pattern of coffee rust (*Hemileia vastatrix*) in non-irrigated crops. The area have 15 ha of Acaia 474/19 cultivar, at 6 years of age and spacing of 3.6 x 0.70m (3968 plants.ha⁻¹). In this area was sampled 51 point (50 x 50m). The evaluation of the incidence of the disease was performed in 8 leaves per plant, 5 plants per sampling point, in the middle third of the plant. Four evaluations were done at intervals of 60 days (August 2012 to February 2013). The area under the disease progress curves of coffee rust incidence (AUDPCI) was calculated. According to the results, there was moderate spatial dependence with the AUDPCI, for nutrients potassium and boron. For variable calcium, pH and zinc, there was strong spatial dependence. For all variables, the best model was the exponential.

The southern states of the United States as the part of a possible Caribbean center of fungal biodiversity

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The southern states of the United States—including such examples as Arkansas, Florida, Louisiana, Mississippi, Missouri and Texas—harbor peculiar

species of pyrenomycetous fungi that do not occur in other parts of the world. Thus, *Camillea signata* has been reported only from the states listed above. The same preference for the southeastern United States is observed in *Biscogniauxia schweinitzii*, and a number of species—*Biscogniauxia arima*, *Hypoxydon lividipigmentum*, *H. thouarsianum* var. *macrosporum* and *Jumillera viridis*—reported for adjacent areas in Mexico also occur in Texas and some of the other southern states. Several of these species have distributions that extend to northern parts of South America. As such, *Hypoxydon venezuelense*, described from Venezuela, also has been found in Texas. *Hypoxydon rickii* is known from French Guiana, Mexico and St. John (U.S. Virgin Islands), as well as from Louisiana and Texas. The islands of the Caribbean seem to share some other species of pyrenomycetous fungi with continental areas around that body of water. Such a peculiar species as *Vivantia guadalupensis*, described from Guadeloupe, has been found in Texas. The restriction of many species of fungi to countries around the Caribbean suggests the existence of a special center of fungal biodiversity. The concepts currently in use for many species believed to be distributed widely in tropics might be reconsidered if one takes into consideration such a biogeographical pattern.

Development of transgenic grapefruit cultivars with a calcium signal modifier (CSM-1) gene using a nptII- and GUS-free selection method

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The bacterium '*Ca. Liberibacter asiaticus*' (Las) and its vector, the Asian Citrus Psyllid (ACP), have crippled the citrus industries of many regions and it now threatens the Texas citrus industry as well. The fact that there is no known cure or effective treatment for the control of HLB and there are other destructive and economically damaging diseases such as citrus canker and *Phytophthora*, the development of disease resistant plants is very important. The production of genetically modified grapefruit and sweet orange cultivars overexpressing a calcium signal modifier gene (CSM-1) from citrus, which boosts the plants' calcium signaling ability, was achieved through an *Agrobacterium* mediated genetic transformation. The CSM-1 gene shows promise of conferring the necessary broad-spectrum disease resistance to fend against the many diseases threatening the citrus industry. Preliminary results using the CSM-1 gene shows resistance to *P. nicotianae*, *Alternaria alternata*, and *X. axonopodis* pv. *citri*. In this research, we are developing CSM-1 transgenic plants with additional benefit by eliminating the *nptII* gene for antibiotic resistance, and the *uidA* screenable marker gene, which may be more appealing and acceptable to consumers. Selection of putative transgenic plants is being performed by direct inoculation with *P. nicotianae*. Quantitative PCR and southern blot analyses are planned in order to determine gene activity as well as gene copy number in the plants.

Competitiveness of field QoI-resistant isolates of *Alternaria alternata*, the causal agent of Alternaria brown spot (ABS) of tangerine

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QoI fungicides have been used extensively for ABS control in Florida; but resistance has become widespread among citrus-producing counties, making ABS control difficult. In order to evaluate if QoI-resistance in *A. alternata* is associated with fitness costs and resistance stability, five each of QoI-resistant and -sensitive isolates were selected. The fitness parameters evaluated were mycelial growth, spore production and germination. Additionally, incubation period, lesion number and disease severity were evaluated on detached leaves of four tangerine cultivars. In the greenhouse, Murcott plants were inoculated with and without Abound (azoxystrobin) and the number of lesions counted. High variation among isolates was found for all fitness components; but when compared between phenotypes (resistant and sensitive), no significant differences ($P > 0.05$) were found. Cultivars Minneola and Dancy had the shortest incubation time and the highest lesion number, independent of phenotype group inoculated. After 10 transfers on unamended PDA, the azoxystrobin and pyraclostrobin resistance levels were the same as the initial level in all resistant isolates. Fungicide protected Murcott plants inoculated with sensitive isolates showed significantly ($P < 0.0001$) lower lesion number than plants inoculated with resistant isolates. This study suggests that resistant isolates will be stable over time and they did not significantly differ in fitness compared to sensitive isolates.

Xylanase production by *Lasiodiplodia theobromae* using two inducers

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Lasiodiplodia theobromae, one of the fungus responsible of Botryosphaeria dieback in grapevines, uses diverse strategies to colonize their host, including the secretion of a wide diversity of extracellular enzymes. Interest in xylanases has increased due to their catalytic capacity and versatility as well their possible biotechnological uses. The aim of this study was to evaluate the xylanolytic capacity of different *Lasiodiplodia theobromae* strains under the influence of two inducers. Three isolates (L28, MXCS01 and UCD256Ma) were grown onto Vogel's minimal media supplemented with either 0.1% beechwood xylan or 2% grapevine wood as inducers. Cultures were incubated in the dark at 28 °C for 21 days and each third day, crude extracts were obtained by filtration, lyophilized, and the xylanase activity evaluated using plate assays. Degradation halos were measured. All obtained extracts exhibit xylanolytic activity, but the halos were considerably higher in extracts obtained from fungi grown with grapevine wood as inducer. The activity of extracts obtained using xylan as inducer increased with time incubation. Isolate L28 showed higher activity when induced in grapevine wood compared with other isolates. Current work is done to discriminate the enzymatic capacity among fungal strains in order to carry on further characterization analysis.

Diversity and community structure of marine ascomycetes from twelve coastal beaches of the western Gulf of Mexico

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Marine fungi are an important component on trophic interactions in sandy beaches. However hardly anything is known about their diversity and community structure. The intermareal zone of twelve sandy beaches spread along the coast of the Mexican portion of the Gulf of Mexico was sampled for evaluating the diversity of marine fungi. In each of the beaches, 50 samples of washed-up detritus were collected, and were incubated in the laboratory up to a period of 12 months. A total of twenty-two taxa were registered. The highest richness was observed in the south of Veracruz State in Coatzacoalcos beach, and the lowest richness in the beaches of Miramar and Tuxpan. Diversity results showed, that the highest levels of diversity were registered in Progreso and Nautla beaches, whereas the lowest diversity corresponds to Tuxpan and Miramar beaches. These results agree with the amount of organic detritus and the development of tourism respectively. Dominant ascomycetes species were *Corollospora maritima*, *Lindra thalassiae*, and *Arenariomyces triseptatus*, whereas, *Arenariomyces majusculus*, *Lineolata rhizophorae*, and *Naïa inornata* are recorded for the first time from Mexican beaches.

Detection of *Xanthomonas oryzae* pathovars from rice seeds: An assay potentially viable for use in seed trade and germplasm exchange

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Xanthomonas oryzae pathovars *oryzae* and *oryzicola*, causal agents of bacterial blight and bacterial leaf streak, respectively, are regulated pathogens. Phytosanitary regulations in most countries require that seed to be imported must be certified free from these pathogens. However, a uniform detection protocol is still needed. Existing protocols for their detection is usually culture-based, of which the main constraint is that these pathogens are relatively slow-growers in agar media, easily overgrown by other bacteria present in the seeds. In order to apply an existing multiplex primer set for these pathogens (developed by Lang and co-workers) to seed health testing, a simple protocol was designed in order to have PCR-ready extracts from seedlots without the need to perform a full DNA extraction. The qualitative assay provides an option to detect the pathogen by isolation and identification by PCR and an option to detect the pathogen directly from seeds. An enrichment step has also been incorporated for seedlots with an expected low bacterial load. The assay was validated during a training-workshop on harmonizing detection strategies for *Xanthomonas oryzae* pathovars *oryzae* and *oryzicola* contamination on rice seeds. A harmonized protocol for the detection of these pathovars would be useful not only for research but also for regulatory purposes, as this would ensure understanding between and among end-users and clients.

A quantitative PCR assay for detection of *Fusarium oxysporum* f. sp. *phaseoli* in bean seeds

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The fungus *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*) is an important pathogen of bean that can be disseminated over large distances by seeds. A rapid, sensitive and accurate seed health testing method is needed to prevent pathogen spread and ensure that seed quality and phytosanitary requirements are met. In order to improve routine testing for this pathogen in seed samples, a bulk DNA extraction and quantitative PCR (qPCR) protocol was developed to screen seeds for the presence of *Fop*. Primer design was based on the sequence of the *Fusarium* transcription factor 1 gene that produces a 63-bp product. The SYBR Green assay was optimized for both specificity and sensitivity. Using this method the target genomic DNA of highly virulent strains was detectable at 20 pg/μL. Contaminated seed lots were prepared by mixing known amounts of *Fop*-infested seeds with non-infested seed; four replications of 100, 200 or 400 seeds each were ground into a fine powder prior to DNA extraction. The qPCR method developed herein resulted in the detection of 0.25% *Fop* infestation in bean seeds (1 contaminated seed and 399 *Fop*-free seeds) and the primer set was demonstrated to be specific for *Fop*, showing a high sensitivity for the target pathogen. These results were confirmed with naturally infested seeds. As this assay reliably and rapidly detects *Fop* in seeds, it can help to prevent the long-distance spread of this agricultural pathogen via contaminated seed lots.

***Ramularia eucalypti* species complex untangled**

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Ramularia eucalypti is a plant pathogen known to infect *Eucalyptus* trees. It was reported to cause severe leaf spotting symptoms in mature *Eucalyptus* trees in Italy and has also been found in Australia, where *Eucalyptus* are native. However, strains identified as *R. eucalypti* based on morphology and on sequence data of the ITS region of the nrDNA operon have also been subsequently isolated from human tissue (skin, lung and bone marrow), from environmental samples (rubber from a refrigerator, apple in storage) and other plant hosts (*Phragmites*, *Pinus*). Further analyses by multi-locus sequence typing based on seven genes, namely LSU, ITS, ACT, EF1- α , HIS3, GPDH and Rpb2, have shown this taxon to represent a complex consisting of at least nine species. All strains were grown in a serial incubator with temperatures ranging from 6 °C to 40°C for morphological characterization. One strain, isolated from human tissue, is able to grow at 40°C and thus may pose a significant threat as a human pathogen.

***CYP51A1* upstream anomalies and overexpression in myclobutanil and difenoconazole resistant *Venturia inaequalis* isolates**

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Upstream insertions of genetic elements and subsequent overexpression of the *CYP51A1* gene were previously found to promote resistance to myclobutanil in populations of *Venturia inaequalis* from Michigan. Adoption of DMI fungicides with different levels of intrinsic activity in apple scab management programs necessitates a reexamination of *CYP51A1* expression in isolates with varying DMI sensitivity phenotypes. 36 *V. inaequalis* isolates with ranges in sensitivity to myclobutanil and difenoconazole (0.1 μg/ml) were collected from a NY state research and baseline orchard and analyzed for *CYP51A1* upstream anomalies and expression. Amplification upstream of *CYP51A1* identified 2 novel insertions of 352bp and 183bp, and 2 previously documented insertions of 499bp and 224bp. Within the insertions, 4 different promoters were identified, but were not associated with resistance to either fungicide. Additionally, several isolates with resistance to difenoconazole, but not myclobutanil, had insertions of 5kb or 8kb in length located 149bp upstream of the *CYP51A1* start codon. *CYP51A1* expression was evaluated for all isolates using qRT-PCR. While relative *CYP51A1* expression of difenoconazole-sensitive isolates was significantly lower than difenoconazole-resistant isolates, there was no relationship between myclobutanil sensitivity and *CYP51A1* expression. The results suggest a lack of DMI cross resistance in *V. inaequalis* and involvement of different mechanisms of resistance.

Characterization of ectotrophic root-infecting fungi isolated from ultradwarf bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) root materials

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The growth and quality of bermudagrass putting greens decline during the summer and early fall months in the Deep South. Root systems appear blackened, rotted, or stunted and are frequently colonized with dark, runner hyphae that are characteristic of ectotrophic root-infecting (ERI) fungi. The focus of this study was to characterize ERI fungi isolated from ultradwarf bermudagrass roots. Colonized roots were cut into 1-cm sections, surface disinfested with a 0.6% NaOCl solution, rinsed three consecutive times with sterile-distilled water, plated on modified potato dextrose agar (PDA), and incubated for 7 days at room temperature under 24 hour fluorescent light. Hyphal tips were transferred to PDA. Genomic DNA was extracted from sterile, pure cultures of isolated fungi. The internal transcribed spacer (ITS) regions of rDNA were amplified by PCR using ITS1 and ITS4 primers and compared to known ITS sequences retrieved from the GenBank via BLAST. Evolutionary analyses of rDNA-ITS regions suggest that MSU isolates belong to six distinct, well-supported clades. Temperature controlled colony growth studies and pathogenicity tests were conducted on representative samples from each of the six clades.

Diversity of saprotrophic oomycetes from a mangrove swamp of Ilha do Cardoso, Cananéia, São Paulo state, Brazil

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Mangrove swamps are found in tropical and subtropical coastal areas throughout the world and are well-distributed along the Brazilian coast. *Halophytophthora* spp. are considered to be the most important decomposers of fallen mangrove leaves in these ecosystems worldwide. Zoospores of *Halophytophthora* appear to be well adapted to the fluctuating conditions of salinity, pH and temperature caused by tides. The knowledge of the diversity of straminipiles in Brazilian mangrove swamps is very limited, and there are no previous records of *Halophytophthora* species for Brazil. Our aims were to (i) assess the diversity of oomycetes in a mangrove swamp of “Ilha do Cardoso”, and (ii) identify the species on the basis of morphological and molecular characterization (18S, ITS and 28S regions of the rDNA and COX-I and COX-II of mtDNA). At three sampling dates we collected samples of fallen mangrove leaves and water at different salinities along the Perequê river, the sea and in a shallow lagoon. Structural parameters of oomycete assemblages were characterized. We obtained 81 isolates, 58 belonged to *Halophytophthora* and 23 to other oomycetes. We recorded six species of *Halophytophthora*: *H. batemanensis*, *H. kandeliae*, *H. operculata*, *H. porrigovesica*, *H. spinosa* and *H. vesicula*. The most frequent and abundant species were *H. vesicula* and *H. spinosa*. We thank FAPESP and CAPES (Science Without Borders Program) for financial support.

Diagnostic outreach trainings in the Caribbean, and Central and South Americas

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The shift to globalization is increasing the introduction of new and exotic plants and pathogens to the U.S. Ports of entry into the United States are only able to check a small percentage of arriving shipments for plant pests. To help slow the ingress of new pathogens and alleviate the inspection burden on port inspectors, laboratories in countries of origin are being outfitted with appropriate equipment and diagnosticians are being trained for diagnosis of diseases of interest. These trainings, which take place both in-country and in the U.S. at the UF-IFAS Plant Diagnostic Center, have included diagnosticians and inspectors from the Caribbean, Central America, Eastern Europe, and South America. Several hands-on capacity-building trainings held in-country were hindered by inadequately-equipped laboratories or inadequate staffing levels, which made some training difficult to standardize or even

complete. The new facility at the University of Florida Plant Diagnostic Center is designed to accommodate hands-on trainings for up to 24 people on a wide range of techniques. The facility will help to standardize training and provide a more structured laboratory learning environment. In-person and distance-education trainings are being planned for the future on such topics as diagnosis of viruses, bacteria, fungi, and other organisms, use of molecular diagnostic tools, warm-season turfgrass disease diagnosis, and general plant disease diagnosis.

Testing leaf-shredding as a component of an integrated management strategy for apple scab in South African orchards

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The South African apple industry currently relies solely on chemical fungicides to control apple scab (*Venturia inaequalis*). South African orchards are very different to those overseas and sanitation strategies used commonly overseas are not necessarily successful in South Africa. The objective of this trial is to determine if leaf-shredding could be used with current chemical control to reduce scab incidence and severity, and reliance on fungicides, in spring, on a commercial scale. In winter 2011 and 2012, leaf-shredding was tested against a non-sprayed negative control, a positive control sprayed with a commercial spray programme and a combined treatment of leaf-shredding with a commercial spray programme. Two treatment repetitions were applied in a randomized block design in each of two orchards. Scab incidence and severity on fruit and leaves were assessed weekly from green-tip until fruit-set in the following spring. In the leaf-shredding plots, incidence and severity were significantly lower ($p < 0.05$) on fruit in both years, but not on shoots in 2011, than in the negative control and were significantly higher than in the combined treatment and positive control plots in both years on fruit and shoots. Incidence and severity in the combined treatment were lower than in the positive control and, although not significantly different ($p < 0.05$), the increased quality and exportability of fruit may benefit producers economically. The trial will be repeated in 2013.

Pseudothecial and ascal densities of *Venturia inaequalis* in a warm and a cold winter region in the Western Cape, South Africa

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Pseudothecial density (PD) and ascal density (AD) of *Venturia inaequalis* were studied under field conditions in 2012 in the Western Cape Province, South Africa. Scabbed apple leaves were collected in Koue Bokkeveld (KB), a cold winter region, and in Elgin (EL), a warm winter region. Leaves were overwintered either in their region of origin (KB-KB or EL-EL) or in the other region (KB-EL or EL-KB) in a randomized experimental design to investigate temperature influence on number of pseudothecia per fertile lesion (PD) and average number of asci per pseudothecium (AD). KB-KB leaves had a PD (25.1) that was significantly higher ($p < 0.05$) than in the other treatments (KB-EL=16.1, EL-KB=12.9 and EL-EL=11.7). Average temperature during June and July 2012 was 8°C in KB and 10.3°C in EL, when pseudothecial production occurred. According to previous studies, pseudothecial number increased as temperature decreased from 20 to 4°C during the 28 days after leaf-fall. Differences in average AD were significant only between treatments KB-EL (124.4) and EL-KB (161.6). AD in KB-KB (159.1) and EL-EL (155.6) did not differ significantly from KB-EL or EL-KB. Average temperatures in August and September 2012 were 6.7°C and 10.1°C in KB, and 9.8°C and 12°C in EL, respectively. According to previous studies, ascal production is optimal at 6°C. The data suggest that factors other than winter temperatures may have influenced PD and AD in treatments. The trial will be repeated in 2013.

WITHDRAWN

Effects of rootstock on *Xylella fastidiosa* infection and grapevine sap phenolics

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Pierce's disease, caused by the bacterium *Xylella fastidiosa*, poses a threat to grape production in the United States and warm climates elsewhere. There are numerous grapevine rootstocks available that may impart increased vigor or tolerance to soil-borne pests. However, little is known about the potential of rootstocks to provide increased resistance to pathogens that infect scions. In this study, host biochemical responses to *X. fastidiosa* infection were compared between potted Chardonnay vines grown on 101-14 MG, 110R, RS3, and Salt Creek, as well as Cabernet Sauvignon vines grown on 101-MG and 110R. Plants were sampled three- and six-months after *X. fastidiosa* inoculation, with bacterial titers determined by qPCR and phenolics quantified by high-performance liquid chromatography. Pierce's disease symptoms were rated at six months. We observed differences due to rootstocks in bacterial titers and symptom development, as well as associated changes in many phenolic compounds. To examine the effects of rootstocks on the production of constitutive and induced phenolic compounds, root vigor also was calculated by weighing root balls at the end of the experiment. Root vigor was then correlated with phenolic production in both non-infected and *X. fastidiosa*-infected plants. These results improve understanding of the role that different rootstocks play in imparting resistance to xylem-limited bacterial pathogens.

Relationship of potato biochemical responses to '*Candidatus Liberibacter solanacearum*', causal agent of zebra chip, to disease progression

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Zebra chip disease is an emerging threat to potato production in the United States and elsewhere. Knowledge of how potato hosts respond to the causal agent, '*Candidatus Liberibacter solanacearum*' (CLS), will aid with efforts to breed potatoes more tolerant to infections by this bacterium. To this end, multiple aspects of potato physiological responses to CLS were examined. Changes in host biochemistry over the course of infection, responses of different cultivars of potatoes to CLS, and differences in biochemical responses in tubers versus shoots and leaves were assessed. Host biochemical responses to CLS were consistently observed to be positively associated with zebra chip symptom severity. That is, CLS-infection resulted in greater levels of phenolic compounds, reducing sugars, and certain amino acids, which were associated with greater symptom progression over time. Therefore, one target for breeding programs should be to develop potato cultivars that do not respond to infection by major shifts in host biochemistry. These cultivars are expected to be less symptomatic than cultivars currently used in commercial production.

Effects of grapevine sap phenolics on the *in vitro* growth of *Xylella fastidiosa*

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Pierce's disease, caused by the bacterium *Xylella fastidiosa*, poses a serious threat to grape production in the United States. Previous work indicated that grapevines infected with *Xylella fastidiosa* respond by producing greater levels of phenolic compounds in xylem sap and tissues, presumably to limit bacterial growth. This study examined the ability of xylem sap phenolics, such as procyanidins and coumaric acid derivatives, to affect *Xylella fastidiosa*

growth *in vitro*. Concentrations of phenolics utilized were similar to levels detected in both non-infected and infected grapevines. The ability of phenolic compounds to affect aggregation of *Xylella fastidiosa* also was observed. These results increase knowledge about the role of host-produced phenolics on *Xylella fastidiosa* colonization within its grapevine hosts.

Effects of root-knot nematode parasitism on host gene silencing

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Plant-parasitic nematodes cause significant damage to crops worldwide. The root-knot nematode (RKN, *Meloidogyne* spp.), one of the most damaging nematodes due to its broad host range, establishes intimate feeding sites (giant cells) within the roots of a variety of plants. How RKN cause such dramatic physiological changes whilst evading plant defenses is unknown. Recently it has been demonstrated that a variety of plant pathogens interfere with their host's silencing pathways. This work aims to provide a more refined look into how root-knot nematodes alter their host's silencing pathways. Interference of silencing pathways during nematode invasion was indicated in microarray datasets generated from laser-captured giant cells in *A. thaliana* roots. Subsets of genes regulated by small RNAs were upregulated during the infection process. Results examining the effects of compromising these pathways in *A. thaliana* and *N. tabacum*, suggest that these components influence the host's susceptibility to RKN by allowing more adult females to form and increasing fecundity. We have generated multiple transgenic *N. tabacum* lines expressing a silenced reporter gene to detect the disruption of these pathways. During the course of infection, it is evident that the silenced reporter gene is recovered, specifically within giant cells. Better insight into this interaction will be invaluable to our growing understanding of the roles of host gene silencing during parasitic interactions.

Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2012

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Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), was widespread and severe throughout the U.S. in 2012, although not as bad as the epidemics in 2010 and 2011 for the Pacific Northwest and the 2010 throughout the country. Stripe rust of barley, caused by *P. striiformis* f. sp. *hordei* (*Psh*), occurred in California, Oregon, Idaho, and Washington continually at low levels. Stripe rust samples collected from 25 states were tested on 18 wheat and 12 barley differentials for identifying *Pst* and *Psh* races, respectively. Seven previously existing *Psh* races were detected, of which PSH-48 (virulent only on Topper of the barley differentials) and PSH-33 (virulent only on Topper and Abed Binder 12 of the differentials) were predominant with frequencies of 49% and 37%, respectively. A total of 23 *Pst* races were detected including 2 new races. PSTv-37 (48%), PSTv-11 (12%), PSTv-14 (10%), PSTv-52 (9%), and PSTv-48 (4%) were the top five frequent races. Races PSTv-11 and PSTv-14, which were predominant in the western U.S., appeared in the eastern U.S. for the first time in 2012. High virulence frequencies (>80%) were detected for *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, and *YrExp2*; moderate (20-80%) for *Yr1*, *YrTr1*, and *YrTye*; low (<10%) for *Yr10*, *Yr24*, *Yr32*, and *YrSP*; and none for *Yr5* and *Yr15*.

Association mapping for stripe rust resistance genes in spring wheat germplasm lines

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is an important disease of wheat worldwide. Genetic resistance plays an important role in the disease control. We previously developed 70 spring wheat germplasm lines with stripe rust resistance through crossing 66 common and 4 durum wheat lines originally from 28 countries with common wheat variety 'Avocet Susceptible' (AvS). To identify stripe rust resistance genes, these lines were phenotyped with four *Pst* races (PST-43, PST-100, PST-114 and PST-127) in greenhouse seedling tests and in fields tests in 2010-2012; and genotyped with the 9K wheat single nucleotide polymorphism (SNP) chip, together with 18 *Yr* near-isogenic lines (NILs). Principal component analysis (PCA) showed that

20% of the variation was explained by the first three components. Hierarchical clustering analysis of 5,232 polymorphic SNPs separated the lines into two distinct groups, one consisting of all NILs and AvS, and another including the remaining lines. Fifty-nine SNPs on 12 chromosomes were associated ($P < 0.005$) with stripe rust resistance, of which eight on five chromosomes (1A, 3A, 3B, 5A, and 6B) remained significant after correcting for multiple testing using the positive false discovery rate (FDR) criterion ($Q < 0.05$). Two SNPs on chromosome 1AS were spanned a genetic region of 1.3 cM, which has a 33-35% disease severity effect. Because no *Yr* genes have been previously mapped in this region, this gene is likely novel.

Monitoring the infection process of *Xanthomonas fragariae* in strawberry with a GFP-labeled strain

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Xanthomonas fragariae causes angular leaf spot of strawberry, which is an important disease in strawberry growing regions worldwide. To further understand the infection process of this pathogen *in planta*, we constructed a *X. fragariae* strain containing a p519ngfp plasmid that produces green fluorescent protein (GFP). The fluorescent Xf100-GFP strain displayed identical pathogenicity as the wild-type strain Xf100. The leaves of strawberry plants were inoculated with the Xf100-GFP strain. Multiplication and systemic movement of GFP-labeled bacteria inside the plants at different intervals after inoculation were examined via epifluorescent microscopy. The quantity of bacteria in different plant tissues was estimated by isolation and plating on sucrose-peptone agar, as well as with quantitative PCR. This study demonstrated that the GFP-labeled *X. fragariae* strain is an efficient tool for examining this pathogen's infection process in strawberry plants, and the knowledge resulting from the use of this tool should be useful in future studies on disease management of angular leaf spot.

Roles of the Gac-Rsm pathway in the regulation of phenazine biosynthesis in *Pseudomonas chlororaphis* 30-84

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Pseudomonas chlororaphis 30-84 is a phenazine-producing bacterium capable of suppressing take-all disease of wheat. The GacS/GacA two component regulatory system regulates the production of secondary metabolites including phenazines crucial for the biological control activity of 30-84. To better understand the role of the Gac system on phenazine regulation, we conducted a transcriptomic analysis comparing the wild type and a *gacA* mutant. RNA-seq analysis identified 771 genes under GacA control. Consistent with previous findings, the transcript abundance of the phenazine biosynthetic genes was significantly reduced in the *gacA* mutant as was the transcript abundance of several phenazine regulatory genes such as *phzI*, *phzR*, *rpoS* and *pip*. Results also demonstrated that the non-coding RNA *rsmZ* and the RNA-binding protein RsmE were involved in phenazine regulation. Constitutive expression of the quorum sensing transcriptional activator *phzR* together with the addition of *N*-acyl-homoserine lactones did not rescue phenazine production in the *gacA* mutant, indicating the direct post-transcriptional control by Gac of the phenazine biosynthetic genes. Based on these results, we proposed a model to illustrate the hierarchic role of phenazine regulators modulated by Gac in the control of phenazine production in 30-84. Transcriptomic analysis identified additional genes regulated by GacA that may contribute to the biological control capability of strain 30-84.

First detection and molecular identification of *Phytophthora parasitica* from annual vinca in Nevada

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A stem and leaf blight disease, causing wilting and death of annual vinca (*Catharanthus roseus*), was first noticed in Las Vegas landscaping sites in 2012. Many vinca plants were killed during the season without knowing its etiology. To identify the cause, symptomatic plants were taken from an affected annual vinca garden and submitted to the plant pathology laboratory. *Phytophthora* was positively detected from the plants using an immunostrip test. To isolate *Phytophthora* species, fresh stem tissue was placed on PARP

medium and incubated at 22°C in the dark. Isolates of *Phytophthora* were transferred to corn meal agar and V8 juice agar for morphological identification. On corn meal agar, the isolate produces abundant sporangia with dimension of 29.8 to 59.5 µm long x 24.8 to 47.1 µm wide. To further confirm its identity, regions of rDNA including partial 18S ribosomal RNA gene, ITS1, 5.8S ribosomal RNA gene, ITS2 and partial 28S ribosomal RNA gene were amplified, subcloned into pGEM®-T vector, and then sequenced. A consensus DNA sequence of 892-bp PCR fragment (KC768775) was obtained by aligning 4 cloned sequences. BLASTn search and sequence alignments revealed 99% homology to the corresponding regions of *P. parasitica* strain TARI 22073 reported from Taiwan (GU111667.1). The morphological and molecular data suggest that the isolate from annual vinca belongs to *P. parasitica*. To our knowledge, this is the first detection of *P. parasitica* from annual vinca in Nevada.

Molecular diversity of *Citrus tristeza virus* in California

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Citrus tristeza virus (CTV) is a serious citrus pathogen worldwide. Recent genetic studies have identified five CTV standard genotypes namely T30, VT, T36, T3, and B165/T68. Two diverse collections of CTV isolates, intercepted or collected from all major California citrus-growing regions in the past 100 years, are maintained *in planta* by the Citrus Clonal Protection Program (CCPP), University of California, Riverside, and the CTV eradication program of the Citrus Pest Detection Agency (CPDA), Tulare, CA, respectively. The molecular genotypes of more than 300 isolates from the CCPP and CPDA collections were determined. T30 was most abundant genotype found but a few isolates with the T36, VT, T3 and B165/T68 genotypes were also determined. Some isolates could not be categorized into any of the standard genotypes and were referred to as having a non-standard genotype. CTV isolates collected in 2008-2010 from Central California field surveys had primarily the T30 genotype. However, some T30 genotypes were present in mixtures with the non-standard, VT, or T3 genotypes but were limited in distribution and abundance. These data provides evidence that CTV quarantine and eradication programs delimit CTV genetic diversity and abundance. Furthermore, the CCPP and CPDA programs are critical for the detection and eradication of exotic CTV isolates. Moreover, the genetic diversity described here will help improve CTV management strategies such as cross-protection.

Development of loop mediated isothermal amplification reaction and TaqMan real-time PCR for the detection of *Gooseberry vein banding associated virus*

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A loop mediated isothermal amplification reaction (LAMP) and a TaqMan real-time PCR were developed and compared for rapid, efficient and specific detection of *Gooseberry vein banding associated virus*. A set of six primers based on the conserved RNase H region was selected for evaluation of the LAMP assay. The optimized LAMP reaction was performed in a single tube at 63°C for 2 hrs and 85°C for 10 min, with a closed-tube visual detection system in which a fluorescent dye was added. Light green color or intense green fluorescence was observed in samples from 10 GVBaV-infected plants. For the TaqMan real-time PCR, a pair of primers and a probe was designed to target the RNase H region. The assay also detected all 10 GVBaV isolates. The sensitivities of the LAMP and TaqMan real-time PCR were compared with that of conventional PCR, and results showed that the detection limit of 10⁻⁶ for the TaqMan real-time PCR was 10-fold more sensitive than the conventional PCR (10⁻⁵) and 100-fold more sensitive than the LAMP assay (10⁻⁴). Four other badnaviruses were not detected by either assay, indicating their specificity for GVBaV. The TaqMan real-time PCR is more sensitive, faster and less labor intensive than the conventional PCR. Although the sensitivity of LAMP was lower than the other two methods, it is simple, fast and cost-effective. The two assays will be useful in quarantine and certification programs where large numbers of samples need to be tested for the virus.

Characterization of the mating-type locus (MAT) of *Guignardia citricarpa*, the fungal causal agent of citrus black spot

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The economically important *G. citricarpa* causes citrus black spot and was recently introduced in Florida leading to fruit blemishes and yield reduction. *G. citricarpa* produces pseudothecia in decomposing leaf litter under intermittent wet and dry cycles. Understanding key environmental and genetic regulators of pseudothecia formation may lead to effective control measures, but the underlying mating compatibility regulation remains unknown. To characterize the mating type locus (MAT) and begin to understand other aspects of *G. citricarpa* biology, we constructed a draft genome sequence with next-generation sequencing technology, assembled the sequence data with the de Bruijn graph algorithm, and predicted gene models with GeneMarkES. BlastP queries revealed one predicted gene on a 30.7 kb contig with significant similarity (2e-27) to the *mat1-2-1* gene of related Dothidiomycete fungi. The *G. citricarpa mat1-2-1* gene is 3,091 bp and encodes a predicted 790 amino acid HMG-domain protein. The conserved *apn2* gene is linked to the *mat1-2-1* idiomorph, and one hypothetical protein is encoded downstream of MAT1-2-1 marking the contig end. Conserved genes flanking other MAT loci, including *sla2* or *gap*, were not linked to the MAT locus. Sequence matching the *mat1-1-1* idiomorph was not identified from our genomic data, implying that *G. citricarpa* may be heterothallic. The *mat* structure in *G. citricarpa* can facilitate further studies to unravel conditions for sexual reproduction.

WITHDRAWN

Association of single nucleotide polymorphism markers based on secreted protein genes of *Puccinia striiformis* f. sp. *tritici* to avirulence genes

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Puccinia striiformis f. sp. *tritici* (*Pst*) causes stripe rust (yellow rust, *Yr*), one of the most destructive diseases of wheat worldwide. The interaction between resistance and avirulence in the pathosystem fits the gene-for-gene model. Over 50 *Yr* genes for stripe rust resistance have been officially named in wheat; however, no any avirulence genes have been molecularly identified in *Pst*. We identified over 500 secreted protein genes from the cDNA libraries and genomic sequences of *Pst*, some of which may be related to avirulence of the pathogen. Single nucleotide polymorphism (SNP) markers were developed from the secreted protein genes polymorphic among two U.S. *Pst* races (PST-78 and PST-130) and one Chinese race (CYR32). A total of 170 U.S. isolates collected in 2010 and 2011 were phenotyped on 20 *Yr* single-gene lines and genotyped with 46 SNP markers. Hierarchical analysis of the 46 SNPs separated the 170 isolates into two distinct groups. The general liner model analysis showed that 16 of the SNPs were significantly associated ($P < 0.01$) with 11 avirulence loci (*AvirYr5*, *AvirYr6*, *AvirYr7*, *AvirYr8*, *AvirYr9*, *AvirYr15*, *AvirYr17*, *AvirYr43*, *AvirYr44*, *AvirYrSP*, and *AvirYrExp2*). Markers for two of the avirulence genes, *avirYr7* and *avirYr44*, were also identified using a

mixed liner model ($P < 0.01$). More markers are under testing. The markers will be useful in mapping and cloning *Pst* avirulence genes.

Characterization of Sec-translocon-dependent extracytoplasmic proteins of 'Candidatus Liberibacter asiaticus' based on PhoA assay

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Huanglongbing (HLB) disease is a destructive disease of citrus globally, which is caused by 'Candidatus Liberibacter asiaticus' (Las). Las is a phloem-limited fastidious pathogen which is transmitted by the Asian citrus psyllid, *Diaphorina citri*, and appears to be an intracellular pathogen that maintains an intimate association with the psyllid and the plant throughout its life cycle. The understanding of the molecular basis of the interaction of Las with its hosts is limited owing to the difficulty in culturing the bacterium. Interestingly, Las contains a complete Sec apparatus, the major route for bacterial protein secretion from the cytoplasm, which represents a mechanism for the secretion of Las proteins, including virulence factors, into the extra-cytoplasmic milieu. Many extracytoplasmic proteins contribute to bacterial attachment, entry, and pathogenesis. In this study, we intended to characterize the Sec-translocon-dependent proteins of Las. A total of 150 Las proteins were predicted to contain signal peptides targeting them out of the cytoplasm via the Sec translocon using algorithms: LipoP, SignalP 3.0, SignalP 4.1, and Phobius. Of these proteins containing putative signal peptides, 55 proteins have been tested using an *Escherichia coli* based alkaline phosphatase (*phoA*) gene fusion system and 35 (63%) of these candidates exhibited positive signal peptide activity in *E. coli*.

Temporal dynamics of soybean root colonization by *Fusarium virguliforme*

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Soybean sudden death syndrome, caused by *Fusarium virguliforme*, is one of the most devastating diseases in the Midwest. Selection of resistant soybean varieties is the most effective disease control strategy. Separate genetic loci control foliar and root rot resistance to sudden death syndrome in soybean. The ability to rapidly and accurately phenotype root resistance to *F. virguliforme* colonization would be extremely valuable for soybean breeding and epidemiological studies. Roots were collected from soybean plants from the V3 growth stage every two weeks until after harvest. Root samples were ground and DNA was extracted for *F. virguliforme* quantification using a real-time qPCR assay. Field experiments were conducted at two locations, utilizing two susceptible and two partially resistant soybean cultivars. *Fusarium virguliforme* was detected at the initial V3 sampling time point in both locations; however, foliar symptoms were not observed until plants reached R1 growth stage. A general trend of increasing *F. virguliforme* DNA concentration was observed in roots overtime, with a decrease in concentration noted at the R5 growth stage. Cultivars demonstrated distinct DNA accumulation rates of *F. virguliforme*. Partially resistant cultivars demonstrated minimal or no foliar disease symptoms throughout the trial; however, the *F. virguliforme* DNA concentrations among cultivar root systems were only significantly different ($p < 0.05$) at V3 and post-harvest sampling times.

WITHDRAWN

Sensitive detection of *Spiroplasma citri* by targeting prophage sequences

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Spiroplasma citri is a phloem limited and nutritionally fastidious wall-less bacterium causing citrus stubborn disease (CSD) in California. An important step in CSD management is early detection of *S. citri*. Because isolation of *S. citri* is technical demanding and time consuming, current detection protocols are exclusively PCR-based. PCR primers were developed from sequences of house-keeping genes, which have low copy numbers in the bacterial genome. Recent advance in genome sequencing of *S. citri* revealed that the bacterium harbors multiple copies of prophage genes. The multi-copy DNAs provides higher template concentration, leading to more sensitive PCR detection. In this study, two primer sets (Php-orf1 and Php-orf3) were developed from prophage sequences. SYBR Green-based real-time PCR was performed to evaluate detection sensitivity with 18 *S. citri* cultures isolated from different hosts including citrus, peach, horseradish and carrot. Compared with the primer sets based on spiralin and P58 gene, primer set Php-orf1 reduced Ct values by 3.02 ± 0.62 and 1.76 ± 0.51 , respectively. Similarly, primer set Php-orf3 reduced Ct value by 4.91 ± 0.49 and 3.65 ± 0.62 , respectively. With >250 field samples collected in two citrus orchards in California from 2007 to 2011, an over three log increase of detection sensitivity was also observed. In conclusion, results from this study showed PCR targeting prophage sequences was a highly sensitive technique for detection of *S. citri*.

WITHDRAWN

The impact of soil composition on *Meloidogyne incognita* and identifying resistant soybean cultivars in Illinois

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Meloidogyne incognita is a widely distributed pathogen of soybean in the United States. The nematode has spread into many counties in Illinois since its detection in the state 18 years ago. A microplot study was conducted to determine the impact of various sand:silt:clay ratios on nematode reproduction and disease severity. The ratios were chosen that best represent fields found in southern Illinois. Soil was collected from Jackson, Macon and White counties of Illinois and steam-pasteurized. Two nematode infestation levels were used in a factorial treatment arrangement with six replications. At harvest, root weight, top dry weight and soybean yield were determined for each treatment. Roots were rated for gall severity and the population density of the nematode in the soil and roots was quantified. Soil type had a significant impact on nematode reproduction and gall severity. A second project identified resistant varieties

for soybean producers in Illinois. Over 450 commercial varieties in the maturity groups 2.5-4.9 were evaluated for resistance to *M. incognita*. Seed of each variety was planted in cone-tainers with 150 cc of steam-pasteurized soil. After emergence, the soil was infested with 3,000 eggs of *M. incognita*. Fifty days after the soil was infested the roots were rated for gall severity. A very small percentage (less than 10%) was resistant to the nematode.

Effects of minor elements on Cercospora leaf blight of soybean and production of cercosporin

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Cercospora kikuchii is the causal agent of both Cercospora leaf blight (CLB) and purple seed stain in soybean. CLB, which appears late in the season and is exacerbated by high temperatures, is a serious disease of soybean in Louisiana. Yield losses of 10 to 15 percent are common, and complete crop failure may occur under severe conditions. The disease is currently managed by early planting and fungicide applications; however, fungicide protocols are still being evaluated to determine the most efficacious materials and times of application. In addition, evidence suggests that the pathogen is developing resistance to many of the currently used fungicides. Furthermore, disease resistant varieties often succumb to the disease after a few years. Previous experiments have shown that some minor elements, including iron, aluminum, and zinc, may suppress disease symptoms. This study investigated the effects these and other minor elements on in vitro cercosporin production. Furthermore, the possible siderophore-like activity of cercosporin and other fungal metabolites was examined using mass spectrometry and other analytical techniques.

A multi-state screen to identify seedling fungal pathogens of soybean

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Seedling diseases are both common and very destructive on soybean. They are caused by a variety of plant pathogens including fungi, bacteria, and oomycetes. Through a multi-state effort, fungal pathogens that cause seedling diseases of soybean are being identified. Over 1,200 fungal isolates were processed for identification. We are using two different approaches for genus and species determination. The first approach relies on morphological features using microscopy and the second through the sequencing of specific genetic loci. In the sequencing approach, up to three different barcodes are targeted. The internal transcribed spacer (ITS) is being sequenced due to the high copy number of rRNA genes, allowing it to be easily amplified. To help identify *Fusarium* spp., the elongation factor EF-1 α is also sequenced. Finally, the intergenic spacer (IGS) region is sequenced in some of the isolates to confirm the identification. The presented data is from fungal isolates originating from eight soybean producing states and collected from a total of 48 soybean fields.

Reuse of spent mushroom compost for Agaricus bisporus production

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Agaricus bisporus, the white button mushroom, is economically important in the United States. National *Agaricus* production is valued at over 1 billion dollars (USDA 2011-2012). As costs rise, raw material cost and movement of spent mushroom compost (SMC) off the farm continue to be an economic burden. In this study, yields of two standard compost formulas with and without 20% SMC (DWT) are compared to a 20% SMC formula containing lignocellulolytic-rich materials. Previous research has indicated that SMC has lower hemicellulose and cellulose values than fresh compost as a result of the breakdown of the compost by *A. bisporus* over the course of the crop. To further establish the relationship between hemicellulose and yield, the compost was supplemented at spawning with corn-based substrates containing varying levels of hemicellulose: 24.6, 36.0, and 48.0 percent hemicellulose by dry weight. Yield, biological efficiency, and bulk density analysis indicate that SMC may be complemented with lignocellulolytic materials with the potential to match or increase yields compared to a standard formula.

Isolation of monoclonal scFv phage that bind to polygalacturonases produced by two bacterial pathogens of grapevines

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Polygalacturonase (PG) enzymes have shown to be virulence factors of a number of plant pathogens. Phytopathogens utilize PG to degrade pectic

polymers in their host plants to provide nutrients and allow movement within the infected plant. *Xylella fastidiosa* (Xf) is a xylem-limited, gram-negative bacterium that causes Pierce's disease of grapevines. Xf possesses a single polygalacturonase gene and it was shown that if the PG gene was disrupted the resulting PG-mutant was non-pathogenic in grapevines. Similarly a PG mutant of *Agrobacterium vitis* (Av) was shown to be less pathogenic on grapevines. Thus, identifying peptides or proteins that could inhibit the activity of polygalacturonase may provide a viable means for protecting plants. In order to isolate putative inhibitors, a single chain fragment variable antibody (scFv) phage library was utilized in panning experiments using PG enzymes of both Xf and Av, as well as a commercially available PG from *Aspergillus aculeatus* (Aa). Panning experiments identified unique scFvs that bind to each of the PGs. Additionally scFvs that bound to Aa PG also bound to Xf PG. We are currently testing these scFvs for their ability to inhibit Av and Aa PGs because to date we have been unable to produce enough enzymatically active Xf PG to perform inhibition assays.

Genetic diversity of viruses causing mosaic in Louisiana sugarcane

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Mosaic caused by *Sugarcane mosaic virus* (SCMV) contributed to the near collapse of Louisiana's sugarcane industry in the early 20th Century. By the 1950s, the cultivation of resistant cultivars eliminated mosaic as a major disease problem; however, new strains arose among previously resistant cultivars. These new strains were placed in a new taxon, *Sorghum mosaic virus* (SrMV), on the basis of molecular studies conducted in the 1990s. Between 1978 and 1995, over 90% of the virus isolates from plants with mosaic symptoms in Louisiana were identified as SrMV strain H. A reverse transcriptase-polymerase chain reaction (RT-PCR)-based restriction fragment length polymorphism (RFLP) analysis was used to determine that 67% of isolates collected between 2000 and 2003 were identified as SrMV strain I, representing a shift from strain H as the predominant strain causing mosaic. Among isolates collected from 2005 to the present, SrMV strain I remains the predominant virus and strain recovered from sugarcane with mosaic symptoms. However, for the first time since the 1950s, isolates of SCMV were also identified. In approximately 8% of the samples from plants with mosaic symptoms, the causal agent has not been identified. Due to the economical threat posed by these viruses, an awareness of their genetic diversity is needed by pathologists and breeders when screening new germplasm in search of new sources of resistance to mosaic.

Aflatoxin management in corn with Afla-Guard

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Aflatoxin contamination is a perennial threat to corn production in the southern United States. *Aspergillus flavus* is the predominant species associated with aflatoxin production; however, not all strains produce the toxin. Two non-aflatoxigenic strains of *A. flavus* were evaluated at five field sites in 2011 and 2012. A commercially available formulation of non-toxicogenic *A. flavus* (Afla-Guard, NRRL 21882), was evaluated at three application timings (V10, VT and R2 in 2011 and V5, V10 and V6 in 2012) and at two rates (11 and 22 kg/ha) alongside untreated control plots and other plots treated with *A. flavus* strain K49 (NRRL 30797), a Mississippi-native biocontrol strain. June and July were exceptionally hot and dry in 2011, while 2012 had more normal June and July temperatures and above-average precipitation. Of the 10 plots examined over two years, five had ≤ 5 ppb aflatoxin, so it was not possible to attribute any aflatoxin reduction to the biological control strain application. The remaining five sites, in contrast, were heavily contaminated with aflatoxin, with concentrations from some individual plots over 1,000 ppb aflatoxin. Higher application rates (22 kg/ha) and early application (V5 and V10) generally resulted in the greatest reduction in aflatoxin concentration and strain K49 applied at V10 resulted in the lowest contamination concentrations, but the differences were not statistically significant.

Emergence and impact of two tospoviruses in Florida

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A unique strain of *Groundnut ringspot virus* (GRSV), which has undergone genome reassortment with, and contains the medium RNA segment of, *Tomato chlorotic spot virus* (TCSV) was identified in tomato in south Florida in late 2009. A typical (non-reassorted) strain of TCSV was reported from tomato in this same area in 2012. The known geographic range of both GRSV and TCSV has expanded since their initial discovery in Florida. The potential for these newly established tospoviruses to spread to other important crop and weed hosts was examined by mechanical inoculation of plants in multiple families in the greenhouse. Concurrent field surveys have been used to complement the experimental host range studies to identify natural hosts for GRSV and TCSV. Combined results of greenhouse and field studies demonstrate that solanaceous plant species are most commonly infected, and several new host species have been identified. Both GRSV and TCSV, and the related *Tomato spotted wilt virus*, are now widespread in peninsular Florida. The potential impacts of these viruses are being evaluated.

Isolation and characterization of rhizobacteria Jdm1 antagonist of root-knot nematodes

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A rhizobacteria strain, Jdm1, was isolated from rhizosphere of traditional Chinese medicine *Trichosanthes kirilowii* in Jiangsu province, China and was identified as *Bacillus subtilis*. Exposure free-cell filtrate of the strain to the root-knot nematode *Meloidogyne incognita* under in vitro condition significantly reduced egg hatch and caused substantial mortality of the juveniles. In the green house trials, it showed that 56 days after treated with strain Jdm1, the number of galls in the tomato (*Lycopersicon esculentum*) rhizosphere reduced significantly and the disease severity corresponding to the treatment of antagonist Jdm1 against *M. incognita* was 36.43%, significantly lower than that of control 75.00%. Consistently, the biocontrol efficacy of Jdm1 reached 69.05%, 51.13% and 48.15% in the field in Shangqiu Henan 30, 60 and 90 days after transplanting respectively. The strain Jdm1 inoculation in field condition had no significantly effect on the bacterial community in the tomato rhizosphere by PCR-DGGE analysis. The bacteria strain which enhances plant growth and inhibits nematodes activity offers a new promising prospect on the research and has a potential to be a safe and effective microbial pesticide.

Exploring the characteristics of *Pythium* communities: Can knowledge about pathogen communities improve disease control?

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Pythium species are an important component of the soilborne pathogen complex causing damping-off of tree seedlings in forest nurseries. However, little is known about the phenotypic or genotypic diversity of forest nursery *Pythium* communities because disease control relies almost exclusively on chemical fumigants with broad spectrum efficacy. As fumigant use declines worldwide, knowledge about these communities will be necessary to develop new and more selective disease control measures. To this end, we are characterizing *Pythium* communities at three forest nurseries. To date, 19 *Pythium* species have been identified and population analyses indicate different degrees of genetic diversity for three commonly isolated species, *P. irregulare*, *P. sylvaticum*, and *P. ultimum*. In greenhouse trials with inoculated Douglas-fir seedlings, eight species each were categorized as either highly virulent or weakly virulent. In paired-culture assays, variation was observed among *Pythium* species and isolates in suppression by *Streptomyces lydicus*, a biocontrol agent. The variability found within these *Pythium* communities exemplifies the difficulty encountered in trying to find a single disease control measure to replace fumigation. An integrated pest management approach involving coordinated measures, each of which target a different component of a given pathogen complex, may be necessary to manage diseases caused by multiple pathogen species.

WITHDRAWN

Development of '*Candidatus Liberibacter solanacearum*' haplotyping assay

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Two haplotypes of '*Candidatus Liberibacter solanacearum*' (Lso), A (LsoA) and B (LsoB), have been reported to be associated with zebra chip (ZC) disease of potato. Currently, a PCR assay using SSR markers is routinely employed to determine Lso haplotype. However, this PCR assay cannot quantify Lso haplotypes, and is not capable of calculating the ratios of haplotypes in co-infected potato plants and potato-psyllid samples. Based on the published Lso genome sequence (LsoB) and the draft LsoA genome sequence, forty sets of Lso typing primers were designed. Evaluation of the new primers was conducted on potato and psyllid samples infected by LsoA and LsoB. Two sets of LsoA specific primers and two sets of LsoB specific primers were found to consistently type Lso infections in potato and psyllid samples. These primers were selected and subjected to more extensive testing and compared to the existing SSR markers. Haplotype specific primers can be used in conventional PCR format as well as in a SYBR green real-time PCR format. Real-time PCR can quantify the number of genomes of each specific haplotype in Lso-infected potato and psyllid samples, and can also calculate the ratios of each haplotype in co-infected potato and psyllid samples. With the new primers, epidemiological studies and ecological studies evaluating Lso haplotypes associated with ZC can be conducted.

New products for management of lesion and ring nematode on walnuts

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Two trials were conducted to determine the effectiveness of DiTera (a toxin produced by *Myrothecium verrucaria*) and Nema-Q (an extract of *Quillaja*, the soapbark tree) for management of lesion (*Pratylenchus vulnus*) and ring (*Mesocriconema xenoplax*) nematode on walnuts. In an orchard in Sutter County, CA, spring and fall treatments of DiTera at 56.1 kg/ha, and Nema-Q at 23.4 l/ha, were applied in a 50-percent banded spray followed by sprinkler irrigation, each year for four years to Chandler on seedling Paradox rootstock, and to own-rooted Chandler trees. Five individual tree replicates per treatment were compared to untreated controls in a randomized complete block design. On Paradox rootstock, both DiTera and Nema-Q increased walnut yields and produced more vigorous trees ($p=0.05$), but did not reduce nematode numbers. The second trial, conducted in San Joaquin County, CA, consisted of 17 treatments with 6 individual tree replicates per treatment in a randomized complete block design with rates of DiTera at 56.1, 28, and 14 kg/ha; rates of Nema-Q at 56, 37.4, 23.4, and 12 l/ha; combinations of the two products; and an untreated control. Products were applied in a 50-percent banded spray followed by drip irrigation to Chandler variety trees on Paradox rootstock. The combination treatments increased trunk circumference, and reduced populations of lesion and ring nematode ($p=0.05$). Individual treatments with each product reduced numbers of ring nematode ($p=0.05$).

***Verticillium dahliae* in soil, roots and stems of green manure crops**

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Green manure crops are planted in rotation with crops susceptible to *V. dahliae* (Vd) to reduce wilt expression. However, asymptomatic infections of green manure crops may result in formation of microsclerotia that serve as inoculum for subsequent crops. The objective of this study was to quantify Vd pathotypes of potato and mint from soil, roots, and stems of potato, mints and

selected green manure crops grown under greenhouse conditions. *Brassica juncea* 'ISCI 199', *B. juncea* 'Pacific Gold', *Sinapis alba* 'Martigena', *Sorghum bicolor sudanense* 'Piper', *Zea mays* 'Marvel', *Triticum aestivum* 'Alpowa', *Solanum tuberosum* 'Norkotah', *Mentha x piperita* 'Black Mitchum', and *M. spicata* were planted in soil infested with *Vd* pathotypes of potato and mint, grown to maturity, and harvested. *Vd* was quantified from soil, roots and stems on semi-selective media. Significantly greater colony-forming units (CFUs) of the mint pathotype were detected in soils from all crops except *Z. mays*, where the potato pathotype was greater and *M. spicata* where no difference was observed. Greater CFUs/g of soil were detected from asymptomatic hosts, *B. juncea* 'Pacific Gold' and *M. spicata*, soil relative to soil where symptomatic hosts were grown. Fewer CFU/60 cm of root tissue of both pathotypes were detected from green manure roots than *S. tuberosum*. Differential microsclerotia formation of *Vd* potato and mint pathotypes were observed in soil, roots and stems of asymptomatic and symptomatic hosts.

Use of a fungal "cocktail" to inhibit growth of *Phytophthora cinnamomi*

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Phytophthora cinnamomi is problematic throughout the world and has a very wide host range. The diseases that it causes are often a threat to biodiversity within a specific region. Once in the soil, it can persist for many years without a host and is extremely difficult to mitigate. To date, a regimen of fungicide and fumigation is the only method that has shown some promise to limit the spread of this pathogen from small pockets. However, this is costly and often impractical depending upon the environment. The purpose of this research was to examine the potential of specific biological control agents individually or in combination to remediate soil infested with *P. cinnamomi*. Colonies of *Penicillium herquei*, *Penicillium daleae*, or *Metarhizium anisopliae* inhibited *P. cinnamomi* growth in dual culture bioassays. Soil populations of *P. cinnamomi* were reduced significantly when treated with a *Trichoderma* sp. alone or in combination with the antagonists above. However, roots of lupine seedlings planted in soil with *P. cinnamomi* and the antagonists became infected. Further studies will be conducted to determine if efficacy can be increased with higher initial populations of the antagonists, and whether in the absence of a host, *P. cinnamomi* populations can be eliminated completely over a longer period of time.

Integrating foundational topics in an undergraduate biology curriculum

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The design of the new undergraduate Biology curriculum at Misericordia University was inspired by the BIO Core curriculum at the University of Wisconsin-Madison. In their first three semesters, students in our Biology program take courses covering foundational topics in biology, including evolution, ecology, genetics, organismal, cell and molecular biology. Biological Interactions, the final course in the sequence, aims to integrate the biological principles covered in the previous courses. Positioned at the end of the sophomore year, it also serves as a transition point for students to move into the upper level elective courses and prepares students for the research emphasis of the junior and senior years. "Biological Interactions" is an inherently broad topic and allows faculty flexibility in defining how they will approach the teaching of this course. In its current form, the fundamental principles of several biological interactions, such as host pathogen relationships, predator-prey relationships, competition, and mutualism are discussed at different levels of biological organization, including the cell and molecular, organismal, and ecological levels. Additionally, these interactions are examined in an evolutionary context. The examples selected to highlight the interactions are intentionally broad and not limited to a biological domain or kingdom.

Ectomycorrhizal communities on pine and oak seedlings converge in the absence of canopy tree influence

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The succession of mycorrhizal fungi often mirrors plant succession, and ectomycorrhizal (EM) community composition may affect the outcome of competition among trees during late succession. Previously, a reciprocal transplant of white oak (*Quercus alba*) and loblolly pine (*Pinus taeda*) seedlings was conducted in paired plots of oak-dominated and pine-dominated forest. This study suggested that the fungal associations of adult trees

determine which EM species are dominant in the soil and thus available for seedlings to associate with. Here, we investigate the degree to which adult trees influence the availability of EM fungi for seedlings by removing that influence. Soil was collected from oak- and pine-dominated stands and dried to kill off mature mycelium, leaving only the spore bank as a source of inoculum. Pine and oak seedlings were then planted in this soil in laboratory conditions. EM root tips were collected for molecular identification of fungal species based on ITS barcoding. Soil samples from field and laboratory conditions were also analyzed for fungal and bacterial diversity using 454 sequencing. We found a reduced influence of canopy type and a more pronounced influence of seedling identity when compared to the EM communities on seedlings planted in the field, suggesting that adult trees do alter the availability of fungi by promoting the growth of their preferred EM associates. The fungal and bacterial communities in the soil will also be discussed.

Genome characterization of Tomato necrotic dwarf virus, a *Torradovirus* from southern California

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Tomato necrotic dwarf virus (ToNDV) is a whitefly-transmitted virus that caused significant losses for tomato production in southern California during the 1980s, but was never fully characterized. The virus produces icosahedral virions approximately 30 nm in diameter, and can be transmitted by three whitefly species; *Bemisia tabaci*, *Trialeurodes abutilonea*, and *T. vaporariorum*, as well as mechanically and by grafting. Symptoms and transmission characteristics of ToNDV resemble those of other members of the emerging genus, *Torradovirus*, family *Secoviridae*. An isolate of ToNDV originally collected from Imperial County, CA and maintained in tomato was sequenced to determine its relationship to other viruses. The ToNDV genome is composed of two RNA molecules of 7.2 and 4.9 kb. RNA1 contains a large ORF encoding a 2150 aa polyprotein that has homology to other viruses within the genus, *Torradovirus*. RNA2 encodes two ORFs of 189 and 1190 aa, respectively, with the latter expressed as a polyprotein. The closest relative of ToNDV is *Tomato marchitez virus* at 80% and 91% identity for the RNA1 and RNA2 polyproteins, respectively. Sequence identity for other members of the genus range from 62-81% for the RNA1, and 69-83% for the RNA2 polyproteins. Results of sequence analysis and comparison of genomic and biological features confirm ToNDV should be recognized as a distinct member of the genus *Torradovirus*.

Genome analysis and biological characterization of Moroccan pepper virus (MPV), and reclassification of Lettuce necrotic stunt virus as MPV

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Moroccan pepper virus (MPV) and *Lettuce necrotic stunt virus* (LNSV) have been steadily increasing in prevalence in central Asia and western North America, respectively over the past decade, and are responsible for diseases of lettuce, tomato, pepper and some floral crops. Sequence analysis of a California LNSV isolate and three isolates of MPV demonstrated 97% genomic identity between LNSV and MPV, and 97 to 100% identity in the coat protein, which is usually quite divergent among distinct members of the genus. Aside from one another, the closest relative of both viruses appears to be *Tomato bushy stunt virus*, sharing 82% genomic identity and 65% identity in the coat protein. A full-length clone of a California LNSV isolate was developed and virus derived from infectious transcripts was used to evaluate host plant reactions under controlled conditions. Symptoms of LNSV matched those described previously for MPV on most of a select series of host plants although some differences were observed. Collectively these molecular and biological results demonstrate that LNSV should be classified as MPV within the family *Tombusviridae*, genus *Tombusvirus*, and confirm the presence of MPV in North America.

A postharvest fruit rot of apple caused by *Lambertella* sp. in Washington State

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During surveys for postharvest diseases of apples in 2003-05, a fruit rot disease was observed on stored apples collected from packinghouses. The disease appeared to originate from infections of wounds on the fruit, and lesions were brown and decayed tissues were spongy. *Lambertella* sp. was

consistently isolated from the decayed fruit. Isolates differed from *Lambertella corni-maris* by only 0-2 base pairs in the sequences within the combined LSU, ITS, and SSU regions. The fungus grew at -0.5 to 25°C and formed apothecia on artificial media after ca. 8-24 weeks. On PDA under 12/12 light/darkness, apothecia were 1-2.75 mm in diameter with stipes of 1-4 mm x 0.5 mm. Asci were 76-125 x 3.5-5.5 µm, inoperculate, eight-spored, clavate, and narrowed at the base. Ascospores were aseptate, 7-10 x 2.5-4.5 µm, uniseriate to biseriate, and initially hyaline then changing to orange-brown within the ascus. Colony characteristics included: growth with little/no aerial mycelium, dark-yellow to gray-black in color, orange crystals secreted after 10-14 days, gray-black pseudosclerotia, and yellow pigmentation in the agar. Morphological characteristics of the fungus overlap with those of *L. corni-maris*. 'Fuji' apple fruit that were wounded, inoculated with representative isolates, and incubated at 0°C yielded the same symptoms, and the fungus was re-isolated from the diseased fruit. This is the first report of a fruit rot in apple caused by *L. corni-maris* in Washington State.

Mating type distribution and the absence of cleistothecia of *Podosphaera macularis* in the Pacific Northwest

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The hop powdery mildew pathogen is known to produce cleistothecia in the eastern North America and Europe, but ascocarps have not been reported from the Pacific Northwestern region of the U.S. (PNW). Sexual reproduction is regulated by the mating-type locus MAT1 in the Erysiphales and other Ascomycetes. For sexual reproduction to occur both mating type idiomorphs, MAT1-1 and MAT1-2 need to be present. The absence of the ascigerous stage of the fungus in the PNW may be due to only one mating type being present. To characterize the frequency of the mating type idiomorphs in populations of *P. macularis*, PCR assays were developed with primers designed from conserved domains specific to each mating type idiomorph. A survey in 2012 of *P. macularis* isolates from the PNW found only the MAT1-1 idiomorph among the 183 isolates collected; MAT1-2 was not detected. In contrast, among 43 isolates from the eastern United States and Europe, mating type idiomorphs were detected at a frequency consistent with a 1:1 ratio (Chi square $P=0.879$). Although our findings do not exclude other environmental, host, or epidemiological impediments to ascocarp formation, their current absence can be explained by the absence of the MAT1-2 mating type idiomorph. Present difficulties in managing this disease in the PNW could be exacerbated unless quarantine measures can prevent introduction of the MAT1-2 mating type.

Genomic comparison of closely related boreal endophytes

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Fungal endophytes live in plant and lichen tissue without inducing obvious disease symptoms. Endophytes constitute a large and hyperdiverse group found in every host sampled and across diverse biomes from the tropics to the boreal zone. Fundamental questions such as function and host range remain unanswered for nearly all of these species. To understand better the genetic variation underlying endophyte biology, we are developing a model system of closely related endophytes in the genus *Daldinia*. This *Daldinia* clade shares 95% sequence similarity by ITS, yet includes isolates from disparate geographic locales ranging from Florida to Alaska and from phylogenetically diverse hosts ranging from lichens to gymnosperms. Multi-gene phylogenetic analyses place this clade in a single monophyletic group with *D. loculata* and its potential anamorph, *Annelosporium nemorosum*, indicating that the clade is at least closely related to *D. loculata*. We present analyses from whole genome sequencing of four strains from this model clade and one strain from a neighboring outgroup, all from Alaska. The ~38Mb genomes from the model clade show remarkable similarity at the genomic level despite isolation from different types of hosts. One or more bacteria were associated with all strains. These bacteria, mostly in the Alpha- and Gammaproteobacteria and

one each in the Betaproteobacteria and Bacteroidetes, are by 16S sequence highly similar but not identical to other endohyphal bacteria.

Factors affecting black dot development in storage

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Colletotrichum coccodes, the causal agent of black dot, is an economically important pathogen of potato causing stem blights and tuber blemishes, which the latter is primarily an issue in storage. Tuber infection is typically characterized by extremely small black spots and dark sooty lesions, which can lead to rejection of the crop. In order to investigate the role of crop duration on disease development, two susceptible potato cultivars (Rosara and Agata) were grown in 2011 and 2012 and harvested at four different 10-day intervals. Tubers were graded and stored for 3-4 months at 12°C and rated for disease development visually and using qPCR. Both visual ratings from 2011 and qPCR results from 2012, indicated that tubers with longer crop durations had greater disease severity. To determine how black dot develops over time in storage, symptomatic tubers were collected concurrently and rated at 0, 3, and 5 months. qPCR quantification showed an increase in black dot symptoms over time with an average 0.02, 0.71, 2.95 ng/gram of tissue at 0, 3 and 5 months, respectively. Finally, to determine when tubers are the most susceptible to *C. coccodes* infection, tubers were inoculated at different times before and after skin set and visually rated for disease one week post-inoculation. Results demonstrated that tubers were the most susceptible before skin set. Results from these studies increase our understanding of black dot and may be useful for future disease management.

Impact of Verticillium wilt on cotton fiber quality

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Cotton (*Gossypium hirsutum* L.) is planted on approximately 1.5M ha on the High Plains of Texas. Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb. is widespread throughout this region. Under severe conditions, the disease is known to reduce fiber quality, specifically length, strength and uniformity; however, the impact of wilt on micronaire is poorly understood. The relationship between wilt incidence and fiber quality was determined using data from field studies conducted in 2010 and 2012 evaluating a partially resistant cultivar 'FM 9180B2F' (FM) and a susceptible cultivar 'DP 0912B2RF' (DP) using linear regression. Wilt incidence ranged from 0.9 to 67.7% and 0 to 56.3% for DP and 0 to 57.8% and 0 to 43.8% for FM in 2010 and 2012, respectively. Micronaire was negatively correlated ($P<0.0001$) with wilt incidence for DP and FM in 2010 and 2012, respectively. Positive correlations between wilt and fiber length were found for DP ($P<0.001$) and FM ($P<0.05$). Strength was not affected by wilt incidence in 2010, but exhibited a positive relationship ($P<0.009$) with the disease in 2012. Improvements in fiber length and strength did not offset the discounts received from reduced micronaire. When combined across years, the disease negatively impacted ($P<0.05$) value of the fiber of both DP and FM. Such reductions, in conjunction with yield losses associated with Verticillium wilt greatly affect profitability in fields infested with *V. dahliae*.

Effects of rainfall on leaf endophyte communities associated with five grass species

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Grass-endophyte symbioses may play an important role in many ecosystems due to the ability of some fungal endophytes to enhance stress tolerance in their plant host. Despite the ecological significance of these symbioses, the forces controlling endophyte distributions remain unknown. We tested whether leaf endophyte communities were differentially associated with five plant hosts exposed to a range of five precipitation levels. Specifically, endophytic fungi were characterized by culturing and sequencing fungi from leaves of five native, C4 bunchgrass species: *Andropogon gerardii*, *Bouteloua curtipendula*, *Panicum virgatum*, *Schizachyrium scoparium*, and *Sorghum nutans*. Precipitation treatments ranged from severe drought (325 mm yr⁻¹) to extreme wet (1330 mm yr⁻¹) based on historical records. Host specificity was low, with only the endophyte community colonizing *Bouteloua curtipendula* significantly different from all other grass species. Endophytes also varied very little across precipitation treatments. Given evidence from other regional studies that endophytes sort across habitats based on historical levels of

precipitation, we suspect dispersal limitation may partly explain the lack of response to altered rainfall at this site.

Assessments of potential management and environmental factors affecting regional occurrence of potato zebra chip disease

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Potato zebra chip (ZC), putatively caused by the bacterial pathogen '*Candidatus Liberibacter solanacearum*', vectored by the potato psyllid (*Bactericera cockerelli*), is a newly emerging disease, which has caused widespread damage in much of the U.S. potato producing regions. Potato products from affected tubers show unacceptable dark coloration upon frying rendering them unmarketable. To characterize potential factors which influence regional variation in ZC occurrence, management and environmental variables were collected from six locations from south Texas to Nebraska over a three-year period (2010-2012). ZC occurrence was assessed in 26 fields in systematically selected plots (20 m × 30 m) in field edges and centers of the fields. The number of symptomatic plants/plot were classified into 2 categories (ZC ≤ 3 or ZC > 3) and subjected to discriminant function analysis to determine the association of the variables with the two ZC intensity categories. Latitudinal location, planting date, and maximum temperature were found to be the most important factors in distinguishing between the two categories. When individually subjected to logistic regression analysis, the three variables accounted for 90, 86, and 70% of the area under the curve, respectively. There was a significant but low negative correlation between ZC intensity and latitudinal location ($r = -0.499$; $P = 0.0094$), indicating that the occurrence of ZC was greater in the southern- than in the northern regions.

Phylogenetic lineages within *Alternaria* and allied genera

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Alternaria is an omnipresent fungal genus that includes saprobic, endophytic and pathogenic species, associated with a wide variety of substrates. Recent DNA-based studies reveal the *Alternaria* complex to consist of multiple polyphyletic genera and that morphology-based species-groups do not always correlate with *Alternaria* species-clades. The *Alternaria* complex currently comprises nine genera and seven species-groups. The aim of this study was to delineate phylogenetic lineages within *Alternaria* and allied genera based on sequence data of parts of the SSU, LSU, ITS, GAPDH, RPB2 and TEF1- α gene regions. Our data reveal a basal monophyletic *Pleospora/Stemphylium*-clade sister to *Embellisia annulata*, and a well-supported terminal *Alternaria* clade. This *Alternaria* clade comprises 24 internal clades and six monotypic lineages, the assemblage of which we recognize as *Alternaria*. *Embellisia annulata* is synonymized with *Dendryphiella salina*, and together with *Dendryphiella arenariae*, are placed in the new genus *Cicatricea*. The sexual genera *Clathrospora* and *Comoclathris*, which were previously connected to *Alternaria*, cluster within the *Pleosporaceae*, outside *Alternaria s.str.* whereas *Alternariaster*, a genus formerly seen as part of *Alternaria*, clusters within the *Leptosphaeriaceae*.

WITHDRAWN

WITHDRAWN

Effects of agricultural management practices on soil microbial communities and disease development in vegetable production

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Soil microbial communities and their relationship with development of southern blight (*Sclerotium rolfsii*) on tomato under no-tillage, tillage and organic farming practices in southern Georgia were studied. Length heterogeneity polymerase chain reaction (LH-PCR) combined with cloning and sequencing was used to assess microbial communities. Relative abundance of amplified PCR products was analyzed using PRIMER-E software. Bacterial communities in soils with no-tillage and organic cultivation were relatively similar, but significantly different from those with tillage cultivation. Differences in bacterial communities were correlated to soil organic matter ($R^2 = 0.558$), cation exchange capacity ($R^2 = 0.583$) and percentage of Mg ($R^2 = 0.590$). The SIMPER analysis indicated that a 346 bp fragment contributed 8.51% and 8.77% of similarity in no-tillage and organic farming respectively, but only 6.18% in the tillage farming. Cloning and sequencing indicated that the 346 bp fragment was closely related to actinomycetes. Another unidentified 315 bp fragment contributed 20.1% and 29.4% of similarity in no-tillage and organic farming respectively, but only 1.59 % in the tillage farming. Disease severity on tomato plants grown in organic and no-tillage soils in a greenhouse bioassay was significantly lower than in conventional tillage soils. Bacterial communities in the soils may have played a role in reducing southern blight on tomato.

WITHDRAWN

Characterization of culturable bacterial endophytes of switchgrass (*Panicum virgatum* L.) and their capacity to effect plant growth

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Switchgrass (*Panicum virgatum* L.) is a perennial warm season grass that is native to the plains of North America and is widely grown as a forage, bioenergy or groundcover crop. Despite its importance, a bottleneck in switchgrass production is poor seedling vigor, which as a perennial crop represents an important time for management. Herein, data identify a suite of culturable bacterial microflora extracted from switchgrass, and show their capability to influence host plant growth and development. A total of 307 bacterial isolates were cultured and isolated from surface sterilized switchgrass biomass and sequence identified into 76 strains (subspecies classification), 36 species and 5 phyla. Approximately 58% of bacterial strains, when reintroduced into surface-sterilized switchgrass seeds, were documented to increase lamina length (cm from base to tip after 60 days growth) relative to uninoculated controls. Ecologically, Phylum Firmicutes was the most abundant bacterial classification and encompassed 75% of all isolates. Although the culturable bacterial community studies herein represent an unknown and assumedly minor proportion of the total microbiome, by focusing on culturable bacteria, we delineate functional feedback between the presence of isolated bacteria and switchgrass seedling growth.

Comparison of *Fusarium virguliforme* and *F. tucumaniae* isolates on selected soybean genotypes from Argentina and the United States

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Sudden death syndrome (SDS) causes yield losses from slight to nearly 100% depending on the onset and severity during the growing season. *Fusarium virguliforme* is the causal agent of SDS in North America, and *F. virguliforme* along with *F. tucumaniae* and other species of *Fusarium* are reported as the causal agent of SDS in South America. In both Argentina and the U.S., isolates of *F. virguliforme* are known to vary in their aggressiveness, which may be measured by foliar disease severity and shoot and root tissue reduction. The objective of this study was to test isolates of *F. virguliforme* and *F. tucumaniae* for aggressiveness on a set of soybean genotypes from Argentina and the U.S. that have been reported to have some level of SDS resistance. Nine soybean genotypes were selected based on different levels of susceptibility and different geographic origins, to be inoculated with two *F. virguliforme* isolates, M5 and Mont-1, and two *F. tucumaniae* isolates, Puj and 8-1. There was no interaction between genotypes and isolates when assessing the area under disease progress curve (AUDPC) values for whole plants and stem-cuttings immersed in toxic culture filtrate ($P > 0.05$). Variations among both isolates and genotypes occurred for the AUDPC and root and shoot weight loss using the sorghum inoculum method in Cone-Tainers and the AUDPC using the stem-cutting assay ($P < 0.05$). The AUDPC from both methods were significantly positive correlated with each other ($P < 0.05$).

Sources and availability of *Sphaeropsis pyriputrescens* inoculum in apple orchards

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Sphaeropsis pyriputrescens (SP) is the cause of Sphaeropsis rot, a recently reported postharvest fruit rot disease of apple. Infections of apple fruit by the fungus occur in the orchard, and symptoms develop during storage or in the market. SP also is the cause of a twig dieback and canker disease of apple and crabapple trees. To determine sources of SP inoculum in the orchard, twigs

with dieback and canker symptoms, dead fruit spurs and bark, and fruit mummies on the trees were collected and examined for the presence of SP pycnidia. To monitor inoculum availability, 3 twigs with dieback from each of 10 crabapple trees and 3 dead fruit spurs or twigs from each of 10 trees in a Fuji orchard as well as 5 pieces of dead fruit spurs and 10 pieces of dead bark tissues from each of 10 trees in a Red Delicious orchard were sampled periodically during the fruit-growing season. Samples were examined for the presence of SP pycnidia and viability of pycnidia was assessed. SP pycnidia were observed on diseased twigs, dead fruit spurs and bark, and mummified fruit on both apple and crabapple trees, suggesting that these tissues were the sources of SP inoculum in the field for fruit infection. During the three growing seasons, viable SP pycnidia were observed in 50-100% of the Fuji trees, >90% crabapple trees, and 10-50% of the Red Delicious trees, indicating that viable inoculum for potential fruit infection was available at any sampling time during the fruit-growing season.

Proteomic analysis reveals that the changes induced by RBSDV infection in rice are associated with an elevated accumulation of hydrogen peroxide

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Rice black streaked dwarf virus (RBSDV) is the agent of rice black streaked dwarf disease and causes severe crop yield losses. To understand the pathological mechanism of RBSDV, a gel based proteomic analysis was performed to compare proteomes of rice with or without a viral infection. 72 proteins were differentially expressed, and 69 of these proteins were successfully identified by MALDI-TOF/TOF-MS. These proteins were linked to 13 cellular processes. The changes in the expression of some proteins, as determined by proteomics, were validated by quantitative real-time RT-PCR. Furthermore, measuring the photosynthesis and H_2O_2 levels showed that the RBSDV infection impaired photosynthesis and resulted in the accumulation of H_2O_2 . Moreover, a comparison of the rice proteome profiles during RBSDV infection and under H_2O_2 stress showed that 19 different proteins were co-regulated by both H_2O_2 stress and RBSDV infection, indicating that these two stresses had a convergent regulation network. These proteins were involved in several processes, including photosynthesis, metabolism, redox homeostasis, transcription and translation. Together, our results reveal the global picture of the rice proteome during RBSDV infection, uncover the potential links between H_2O_2 and RBSDV pathogenesis and contribute to a better understanding of the plant-RBSDV interaction.

Thousand cankers disease in California English walnut: Incidence, distribution, and characterization of *Geosmithia morbida*

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Thousand cankers disease (TCD) is an emerging fungal disease responsible for serious decline and death of eastern black walnut (*Juglans nigra*) in Colorado. In California, TCD occurs on native *Juglans californica* and *Juglans hindsii*, and in orchard trees of English walnut (*Juglans regia*). The causal agent, *Geosmithia morbida*, is vectored by the walnut twig beetle (WTB; *Pityophthorus juglandis*). Twelve commercial orchards in six counties in California were surveyed for TCD incidence. Disease incidence ranged from 17% to 65% of the trees in these orchards. One orchard that is bordered by a riparian area with mature *J. hindsii* trees showing WTB damage was surveyed and sampled extensively. The orchard was surveyed in quadrats each containing 5-6 rows. Disease incidence based on symptoms in the 2,217 trees that were examined was 37%. *G. morbida* was isolated from 18% of 61 cankers associated with insect galleries, and was isolated from 36% of WTB collected within the orchard and in the riparian area. Joint-count analysis showed disease aggregation in sections of the field, while disease distribution was random in other parts of the field. Sequence analysis of *EF-1a* of 22 *G. morbida* isolates from different counties revealed little diversity among these isolates at this level of resolution.

WITHDRAWN

Real-time PCR detection and differentiation of four *Colletotrichum* species causing soybean anthracnose

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Colletotrichum species were isolated from soybean (*Glycine max*) with anthracnose symptoms during 2009-2011 in AL, AR, IL, and MS states. Thirty representative strains from 200 isolates were selected and identified by morphological characteristics and sequence analyses. Among them, four *Colletotrichum* species were obtained, including *C. chlorophyti*, *C. destructivum* (sexual stage *Glomerella glycines*), *C. truncatum*, and a new *Colletotrichum* species (*C. maxi*). To increase the efficiency and accuracy of diagnosis on soybean anthracnose, real-time multiplex quantitative PCR (RT-qPCR) assays were designed to discriminate these four *Colletotrichum* species. Fungal mycelial DNAs were extracted from pure cultures, and a partial region of cytochrome c oxidase subunit 1 (*cox1*) gene was amplified for RT-qPCR design. Two sets of EvaGreen RT-qPCR assays were established and the differentiation was based on different Tm values in the melting curve analysis. The first set (Set1) was able to separate *C. chlorophyti* and *C. destructivum*, and the second set (Set2) was for distinguishing *C. truncatum* and the new *Colletotrichum* species (*C. maxi*). The negative signals and/or different Tm values of controls, *C. acutatum* from chili and *C. orbiculare* from watermelon, confirmed the specificity of the assays. This two-set assay was applied to identify more than 100 isolates in our collection and proved to be a rapid and accurate method to detect major *Colletotrichum* species infecting soybean.

The function of the *acr* genes in phenazine regulation and transport in the biological control strain *Pseudomonas chlororaphis* 30-84

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Pseudomonas chlororaphis 30-84 is a phenazine-producing biological control strain that has the capacity to suppress take-all disease of wheat. Phenazines are important for the strain's ability to inhibit pathogens and persist in the rhizosphere. Previous work demonstrated that phenazine production is controlled by quorum-sensing (QS) and multiple two-component signal transduction systems, including RpeA/RpeB. RpeA (a sensor protein) and RpeB (the cognate response regulator) regulate phenazine biosynthesis by controlling the *phzR/phzI* QS genes and other regulatory genes. To better understand the function of RpeA/RpeB in phenazine regulation, transcriptomic analyses were conducted comparing the wild type strain to an *rpeA* mutant. RNA-seq analysis demonstrated that two genes, *acrA* and *acrB*, located adjacent to *rpeA* and *rpeB* were highly up-regulated in the *rpeA* mutant and this was confirmed by quantitative real-time PCR. *acrA* and *acrB* are annotated as acriflavin resistance genes, which are thought to function as multi-drug efflux pumps (e.g. transporters of secondary metabolites, including those with structural similarity to phenazines). Data presented here include characterization of phenazine transport by *acrA* deficient and *acrA/acrB* overexpressing derivatives. We hypothesize that AcrA and AcrB function in phenazine transport and that RpeA/RpeB regulates the production and the transport of phenazines in a coordinated manner.

Three novel *Phytophthora* species from irrigation water in Mississippi

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The genus *Phytophthora* includes a number of destructive plant pathogens. Here we report three new taxa recovered from the irrigation systems at an ornamental crop nursery in Mississippi. Isolates of these taxa were recovered by baiting with rhododendron leaves in 2012. Isolates from each of these taxa produced an identical single-strand conformation polymorphism (SSCP) fingerprint that is distinct from those of all known *Phytophthora* species. These three new taxa belong to the ITS clades 2, 6 and 8, respectively. They are provisionally named as *Phytophthora stricta*, *P. mississippiae*, and *P. macilentosa* by morphology and phylogenetic analysis. All isolates from these species examined in this study are A1 mating type, producing golden-walled oogonia after being paired with an A2 tester of *P. cinnamomi*. Morphologically, *P. stricta* is featured by semi-papillate sporangia and constriction in the sporangiochore. *P. mississippiae* produces semi-papillate, non-caducous sporangia and catenulate intercalary hyphal swellings. *P. macilentosa* is named after its skinny sporangia. *P. macilentosa* is also characterized by its massive growth at 35°C. The pathogenicity of these three species is being tested on rhododendron, pieris, hydrangea, daphne plants. Recovery and description of these novel species will help assess the *Phytophthora* disease risk locally and protect the plant biosecurity globally.

Molecular characterization of a novel soybean-infecting nepovirus from North Dakota

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Recently, three novel and phylogenetically diverse viruses have been discovered infecting soybean, *Glycine max* L., in the United States. In July and August of 2010, 200 soybean fields from 25 counties in eastern North Dakota were surveyed for virus infection. Fields were sampled with a grid pattern across the area with at least 8 km between fields. Twenty leaves were collected at random along transects of approximately 170 m through each field. Total RNA was extracted from all field samples, pooled, depleted of ribosomal RNA, reverse transcribed, and sequenced using an Illumina HiSeq2000. Sequence reads were assembled and compared to all available viral amino acid and nucleotide sequences. The analysis detected *Alfalfa mosaic virus*, *Soybean dwarf virus*, and partial sequences for RNA1 and RNA2 of a novel nepovirus that was most closely related to *Blueberry latent spherical virus* and other nepoviruses in Subgroup C. The assembled sequences for RNAs 1 and 2 were 6,660 nt and 5,735 nt in length, respectively. Both RNAs contained single large open reading frames followed by 3' noncoding regions of more than 1,300 nt. The presence of the new virus in the collected leaf samples was confirmed by RT-PCR using virus-specific primers. As the leaf samples were collected at random, it was not possible to associate infection with disease symptoms. The distribution of the new virus and its effects on soybean yields remain to be determined.

Functional analysis of conserved genes from rust fungi *Puccinia graminis* pv. *tritici*

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Rust fungi cause devastating diseases of wheat and are major constraints to wheat production globally. Three species of *Puccinia* rusts attack wheat, *P. graminis* pv. *tritici* causes stem rust, *P. tritici* causes leaf rust and *P. striiformis* causes stripe rust. The optimal strategy for rust diseases control is to provide durable resistance. In this study, total 1036 genes from *P. graminis* pv. *tritici* were obtained whose transcripts were enriched in haustoria by comparing RNAseq libraries of purified haustoria to libraries made from total RNA of infected leaves. And 583 genes had clear homologs in all three wheat-infecting rust species as determined by standard homology searches (E score cut off 0.00). Seventy-five genes were selected for transient silencing by VIGS expression in wheat plants, nine genes interfered with pathogenicity of stem rust on wheat. The other 66 genes showed no noticeable effects on the rust pathogenicity or reproduction.

Exploring endophyte diversity across the Pooideae

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Cool season grasses from the subfamily Pooideae have extremely wide distributions, inhabiting many different ecological niches. For some, their success can be attributed to fungal symbiotic partners known collectively as the epichloae (*Epichloë* and *Neotyphodium* species). The epichloae can produce a range of bioactive alkaloids (ergot alkaloids, indole-diterpenes, lolines and peramine) and exhibit considerable chemotypic diversity within these biosynthetic pathways that likely equates to fitness benefits to the host. A high throughput pipeline was established to rapidly evaluate endophyte incidence, species identification and potential bioactivity across grass collections representing multiple host tribes. Total DNA isolated from seed or tillers are genotyped with markers designed to key alkaloid biosynthesis genes and mating type genes to determine endophyte diversity among and between grass populations. Phylogenetic analyses of housekeeping and mating-type genes inferred hybrid versus nonhybrid origins and hybrid ancestral progenitors. Sequence analyses of alkaloid genes encoding key pathway steps provide copy number and progenitor origins. Multiple endophyte species were found to associate independently with some grass hosts. The diverse evolutionary histories identified within the collections provide insight into the broader ecological implications of endophyte-plant symbioses.

Epidemiology and population diversity of fungicide resistant and sensitive strains of *Cercospora sojina*, the causal agent of frog-eye leaf spot

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Frog-eye leaf spot (FLS), caused by the fungus *Cercospora sojina*, is a common foliar disease of soybean in regions with warm (25-30°C) and humid (>90% relative humidity) conditions. Since 2010 isolates of *C. sojina* in the United States have been confirmed to be resistant to fungicides that are quinone outside inhibitors (QoI). Two soybean fields in west Tennessee were used to assess the epidemiology and population dynamics between QoI fungicide resistant and sensitive strains of *C. sojina* under different selection pressures. Both fields were in continuous soybean for the past 2 years and previously confirmed to have QoI fungicide resistant and sensitive strains. Four fungicides with different modes of action were applied at soybean growth stage R3 (beginning pod). FLS samples were collected on a weekly basis from disease onset to soybean maturation and continuous weather data was recorded at both locations (air temperature, relative humidity, and precipitation). The proportions of QoI fungicide resistant isolates to sensitive isolates were determined using a spore germination assay with a discriminatory dose of the QoI fungicide azoxystrobin at 0.1 ppm. Subsamples of the same isolates were also screened using single nucleotide polymorphism markers to assess population diversity. The influence of environmental conditions and fungicide selection pressures on the epidemiology of the disease and pathogen population will be discussed.

A new species of *Alternaria* from Korea

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A new species of *Alternaria*, *A. dendropanacies* is described from leaf blight lesions of *Dendropanax moribifera* belonging to the Araliaceae. Its identity as a new species is based on molecular sequence data, morphology and pathogenicity. Conidial morphology of the species is very similar to that of *Alternaria panax*, and we considered it as *A. panax* before. However, the species could be differentiated from *A. panax* by producing more larger conidia and secreting yellow pigment on PDA media. In addition, it could be differentiated by the sequence analyses of combined data of ITS, Alt a 1 and *gpd* genes. Pathogenicity tests also revealed that the isolates of the species induced severe symptoms on *Dendropanax moribifera* but not on *Panax ginseng*.

Characterizing the promoter of the phenazine biosynthesis operon in the biological control strain *Pseudomonas chlororaphis* 30-84

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Phenazines, bacterial secondary metabolites produced by *Pseudomonas chlororaphis* 30-84, are essential for pathogen inhibition and rhizosphere competence. Phenazine biosynthesis is regulated by the PhzR/PhzI quorum sensing system. PhzI is an *N*-Acyl homoserine lactone (AHL) synthase that produces the AHL signal hexanoyl-homoserine lactone. PhzR, in the presence of sufficient AHL, binds to the phenazine promoter and activates expression of the phenazine biosynthesis operon (*phzXYFABCD*). Bioinformatic analysis identified several features within the promoter region upstream from the predicted transcription start site (TSS) including a *lux* box (located at ca. -35 nt) and a -10 sequence as well as several repeat motifs potentially capable of providing significant secondary structure. To better understand the possible role of these features in *phz* regulation, subclones of the promoter region with deletions or site-specific mutations within the motifs were cloned upstream of a promoterless *lacZ* gene and the reporter plasmids were introduced into 30-84. Results indicated that a 127 bp region including the *lux* box, the -10 sequence, and the TSS were required for transcription. Deletion of a 150 bp region downstream of the TSS including the start of translation resulted in >5 fold higher *lacZ* expression compared to the wild type promoter. We discuss the potential roles of these repeat motifs in the formation of stemloop structures and in modulating phenazine promoter activity.

Development and application of scFv for '*Ca. Liberibacter asiaticus*', the pathogen associated with huanglongbing

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We have developed and applied immunization and affinity screening methods to develop a primary library of recombinant single chain variable fragment (scFv) antibodies against '*Ca. Liberibacter asiaticus*' and its insect vector. The primary library has more than 10⁷ unique antibodies and the genes that encode them. We have screened this library for antibodies that bind to specifically chosen CLAs proteins used as bait for affinity-based selection of scFvs. We have selected scFv against several proteins of the surface of CLAs that bind to the major outer membrane protein, including OmpA; the polysaccharide capsule expressing protein KpsF; protein components of the type IV pilus (CapB and CapF); and flagellar proteins (FlhA and FlgI). We have also selected scFv that bind to proteins involved in virulence, including TolC. The scFvs have been used in ELISA and dot blot assays against purified protein antigens and CLAs infected plant and psyllid extracts. We have also recombined many of these scFvs into a plasmid expression vector designed for production of scFvs. The scFv have been used in tissue print assays of infected and healthy plant parts. We have demonstrated a technology to produce antibodies at will and against any protein target encoded by '*Ca. Liberibacter asiaticus*' and the psyllid vector. Future applications will include advanced diagnostic methods for huanglongbing and the development of immune labeling of CLAs in infected plants and psyllids.

Putting endophytic and epiphytic fungi into a meaningful phylogenetic context: A study of the vine *Smilax rotundifolia*

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Until we begin to map species level relationships accurately between plants and their fungal symbionts and pool this information across multiple studies, there will be a lack of progress in understanding the structure of ecological networks and host specificity among these organisms. The present study stands as a model for this goal. I tested a hypothesis within the context of one plant and its fungal community; at the same time I generated taxonomically specific data that can be used towards future large scale mapping of plant-fungus networks. First, I tested the hypothesis that surface fungal communities of the woody vine *Smilax rotundifolia* vary according to environment more than its endophytic fungal communities. This hypothesis was found statistically unsupported. Both epiphytic and endophytic fungal communities were highly consistent across sites, and thus both appear to be highly selected for by the plant. The second objective was to place the dominant taxa into phylogenetic context at the species level using recently published multi-locus phylogenies. Multiple isolates from three widely separated sites were used to

verify putative species categories. The genera investigated include *Pestalotiopsis*, *Colletotrichum*, *Phyllosticta*, and *Phomopsis*. While ITS sequences alone seemed effective in discriminating between species isolated in this study, multiple genes were needed to give a meaningful species identity in the context of the entire known phylogenies.

Soil bacteria for broad-spectrum mycotoxigenic fungi control in maize

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Fusarium verticillioides and *Aspergillus flavus* are two important mycotoxigenic fungi in maize. Sustainable methods may reduce mycotoxin contamination. The present work aims to select bacterial strains for the biological control of *A. flavus* and *F. verticillioides* associated to maize kernels. Two *Bacillus* spp. isolated by the bait technique and six actinomycetes pre-selected to reduce mycotoxin and control *A. flavus* in peanuts. They were cultivated in Petri dishes with YPD medium at 28°C for 7 days. For each one, 45 disinfected hominy grains were transferred to a plate containing the bacterial growth and shaken vigorously to have maximum grain coating. They were transferred to new Petri dishes in dark for 7 days at 28°C, and then inoculated with 1.5mL of a 10⁵ conidia/ml suspension of each mycotoxigenic fungi and the effect of each treatment was evaluated by a blotter test and grains scored according to a 0-5 scale based on the mycelium colonization extent on the grains. For consistency of results, tests were repeated 3 times for each pathogenic fungi and using two different hominy lots. Two bacterial strains (one *Bacillus* sp and one actinomycete) reduced consistently the development of both fungi up to 37% for *A. flavus* and 66% for *F. verticillioides* respectively. Under high pathogen inoculum pressure, these bacteria can reduce maize processed food contamination by mycotoxigenic fungi.

Characterization and molecular diagnosis and quantification of quinone outside inhibitor fungicide-resistant isolates of *Cercospora soja*

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Phytopathology 103(Suppl. 2):S2.166

Frogeye leaf spot of soybean, caused by the fungus *Cercospora soja*, is a common foliar disease of soybean that often is managed with foliar fungicides. In 2010, isolates of *C. soja* resistant to quinone outside inhibitor (QoI) fungicides were detected in 3 different states. Currently, QoI-resistant isolates have been detected in a total of 8 different states. Characterization of these QoI-resistant isolates revealed that they contain the G143A mutation in the *Cytb* gene, a guanine to cytosine transversion at the second position in codon 143 that caused an amino acid substitution of alanine for glycine and conditions resistance to QoI fungicides. Using SSR molecular markers, QoI-resistant *C. soja* isolates from different states were found to have genetically diverse backgrounds, which indicate that the selection for these QoI-resistant isolates occurred independently at each location. Specific PCR primer sets were developed to efficiently discriminate QoI-resistant and sensitive isolates. In addition, efficient and economical quantification assays using quantitative-PCR (q-PCR) have been developed to quantify the presence of QoI-resistant and sensitive *C. soja* isolates. These q-PCR assays will be used to identify QoI-resistant isolates from new areas and to characterize and compare the fitness of QoI-resistant and sensitive isolates.

Resistance to infection by *Potato virus Y* among selected varieties for improved seed potato production

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Potato virus Y (PVY), an aphid-transmitted Potyvirus, has evolved to cause asymptomatic infection on both plants and tubers in recent years. The new necrotic strains pose serious challenges to seed potato certification programs. Identification of potato varieties conferring mature plant resistance to the new PVY strain would improve our current IPM strategies to limit the transmission and spread of the virus in the seed potato. Replicated experiments were established in May, 2012 to investigate the response of four commonly used potato varieties to PVY infection at different stages of potato development. Replicates sets of potato varieties were mechanically inoculated with ordinary (PVY⁰) and recombinant (PVY^{N:O}) strains at pre- and post-flowering stages, and compared with untreated controls. Two weeks after each inoculation interval, one leaf from each inoculated plant was collected above the inoculation site and tested via ELISA to confirm systemic infection. At the

end of season, tubers were harvested and graded for overt symptoms of tuber necrosis and placed into storage for a post-harvest summer grow-out to be completed in 2013. Significant reductions (F=5.1947, df=(2,42), P-value=0.0096) in disease incidence were observed in the potato varieties 'Dark Red Noland', 'Silverton' and 'Yukon Gold' when infected with PVY^{N:O} during post-flowering stage. No differences in incidence were observed among varieties or inoculation times when comparing the ordinary of PVY.

WITHDRAWN

Development of a loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Colletotrichum acutatum* on strawberry

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Anthraxnose fruit rot (AFR), caused by *Colletotrichum acutatum*, is among the most destructive strawberry diseases worldwide. *C. acutatum* can enter and spread in commercial fields via asymptomatic infected nursery plants. In order to detect the pathogen on symptomless plants rapidly, conveniently, and cost-effectively, a loop-mediated isothermal amplification method (LAMP) is being developed. A LAMP assay amplifies target DNA at 65°C within one hour, and test results can be evaluated visually. Two sets of LAMP primers, G1 and tub2, amplified the internal transcribed spacer (ITS) and β -tubulin 2 genes, respectively. The G1 primer detected as little as 20 pg of genomic *C. acutatum* DNA from pure culture; however, it also amplified some isolates of non-target *C. fragariae* and *C. gloeosporioides*. The tub2 primer amplified only *C. acutatum*; however, it was 10 times less sensitive than G1. In order to improve the sensitivity of both primer sets, modification of primers, DNA extraction for higher DNA quality and better visual detection methods are being evaluated. Validation of the primer sets for detection of *C. acutatum* on greenhouse and field-grown strawberry plants is pending.

WITHDRAWN

Diverse plant genes are targeted by TAL effectors for disease susceptibility

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Bacterial citrus canker is incited by distinct yet related strains of *Xanthomonas*. *X. citri* subsp. *citri* strain Xcc306 harbors four type III transcription activator-like (TAL) effector genes, only one of which, *pthA4*, is required for pustule formation in Xcc306. Transcriptional profiles of citrus leaf tissue infected with the mutant strain Xcc306 Δ *pthA4* compared to profiles from infections by the same strain harboring either *pthA4* or *pthAw*, a variant gene from strain XccAw, revealed two up-regulated candidate susceptibility (S) genes that were potentially targeted by *pthA4* or *pthAw*. *CsLOB1* is a member of the LOB transcription factor family, while *CsN3-1* encodes a member of the SWEET family (MtN3) of sugar transporters. *CsN3-1* was shown to transport glucose preferentially in contrast to the transport of sucrose by the major S gene products in rice during bacterial blight disease. Artificial designer TAL effectors (dTALes), targeting the *CsLOB1* promoter region but not the *CsN3-1* promoter, restored pustule formation to Xcc306 Δ *pthA4*. Two additional TAL effectors, PthB and PthC, also target *CsLOB1* expression, yet have no effect on *CsN3-1* expression. The results indicate that distinct TAL effectors target a single S gene for pustule formation in citrus. Other disease complexes involving *Xanthomonas* also involve TAL effector genes, and a comparison of the S genes from different diseases will be presented.

WITHDRAWN

Fusarium verticillioides FvStr2, a putative fungal striatin-interacting protein, participates in maize stalk rot virulence

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Fusarium verticillioides is an important maize pathogen that causes ear rots and stalk rots worldwide. While maize stalk rot is considered as one of the more serious diseases of maize, the molecular mechanism associated with this disease is not well understood. Previously, we identified a striatin ortholog, FvStr1 (formerly Fsr1), which plays an important role in *Fusarium* stalk rot virulence. In animals, striatin interacts with other proteins to form the STRIPAK (striatin-interacting phosphatases and kinase) complex and regulates a variety of developmental process and cellular mechanisms. Here, we report the identification of *FvSTR2* gene in *F. verticillioides*, which encodes a STRIPAK component, and characterization of its role in fungal virulence. We have generated a gene-deletion mutant of *FvSTR2*, and tested its infectious growth and virulence in maize stalks. *In silico* protein-protein interaction (PPI) network study revealed that FvStr1 forms complex with peroxins as well as other STRIPAK components. Two key peroxins, Pex14 and Hex1, play a role in *F. verticillioides* virulence, and are hypothesized to form complex with other peroxins. Yeast two hybrid and split luciferase assays are being used to verify these putative interactions between FvStr2 and FvStr1/peroxins/STRIPAK components *in vivo*. Results suggest that FvStr2, together with STRIPAK and peroxins, carry out an important regulatory role in maize stalk rot virulence.

Identification of Wscs and Mid2 as putative upstream sensors of the cell wall integrity signaling in *Magnaporthe oryzae*

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Rice blast caused by *Magnaporthe oryzae* is one of the most severe fungal diseases of rice throughout the world and *M. oryzae* has been used as a model system to study fungal -plant interactions. Wsc1, -2, and -3, Mid2, and Mtl1 sense environmental stimuli and transmit cell wall status through in the cell wall integrity (CWI) signaling pathway consisting of small G-protein Rho1, protein kinase C (Pkc1) and a MAP kinase cascade in *Saccharomyces cerevisiae*. Wscs and Mid2 are the sensors of temperature, heat shock and pheromone related to Mpk1, Ras /cyclic AMP pathway respectively. In this study, we searched *M. oryzae* genome for Wscs and Mid2 homolog according to the conserved domain, and got 3 homologs for wscs family and one for mid2 and mtl1. MAPK cascade is conserved in fungal pathogens and also essential for virulence and viability in *M. oryzae*. MoWscs and MoMid2 should be responsible for responding the cell wall stress, as well as fungal pathogenicity as the putative sensors for MAPK pathway. For now single deletion mutants of Wscs and Mid2 did not attenuated fungal virulence on both rice and barley because the Wscs and Mid2 mutants have redundant function. Therefore the double, triple and quadruple mutants have been constructed to determine the relationship of the four sensors and the phenotype of the double, triple and quadruple mutants will be characterized and tested as the putative upstream sensors of the CWI pathway.

Detection of *Ralstonia solanacearum* using portable surface plasmon resonance technology

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Surface plasmon resonance (SPR)-based technology provides rapid, sensitive and specific detection methods for plant quarantine pathogens. We have used a portable SPR device to detect one of the important quarantine plant pathogens, *Ralstonia solanacearum* (Race 3 biovar 2). Using sensor chips coupled with monoclonal antibody against *Ralstonia solanacearum*, we have

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demonstrated that as low as 15 mg/ml *Ralstonia solanacearum* (Race 3 biovar 2) could generate sufficiently detectable signal with the portable SPR device. The detection of *Ralstonia solanacearum* (Race 3 biovar 2) was rapid and in real-time. We have also shown that sensors coupled with *R. solanacearum*-specific antibody can be regenerated and re-used. Additionally, we have demonstrated that sensor chips coupled with biotinylated oligo DNA specific for *Ralstonia solanacearum* (Race 3 biovar 2) can be used for the detection of this quarantine pathogen with the portable SPR device.

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Efficacy of *Bacillus* biocontrol agents for management of sheath blight and narrow brown leaf spot in organic rice

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Organic rice production has significantly increased in the U.S. over the last decade. Growers lack effective tools to manage sheath blight, caused by *Rhizoctonia solani*, and narrow brown leaf spot (NBLS), caused by *Cercospora janseana*, two major diseases affecting organic rice production. An experiment was conducted in a field under organic management in Texas in 2010 and 2011 to evaluate the efficacy of seven *Bacillus* biocontrol agents for management of sheath blight and NBLS. Plots were inoculated with *R. solani* at panicle differentiation. NBLS developed from natural inoculum. When applied at the boot stage, Serenade Max (*B. subtilis* strain QST713, 14.6% a. i.) and Serenade ASO (*B. subtilis* strain QST713, 1.34% a.i.) were effective in reducing sheath blight severity in 2011. Serenade Max also reduced NBLS severity in both years. Strain MBI600 (*B. subtilis*) reduced severity of both sheath blight and NBLS. The other four *Bacillus* biocontrol agents, including Ballad Plus (*B. pumilus*), did not effectively reduce both diseases and increase yield. Only Serenade Max consistently increased yield by more than 15% over 2 years. Serenade Max can be used as an effective tool to reduce the damage caused by sheath blight and NBLS in organic rice. Serenade Max is a commercially available biopesticide certified for organic production of rice and other crops.

Efficacy of seed treatment fungicides for control of seedling diseases of rice

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Seedling diseases are a major problem leading to losses or replanting of rice due to thin and nonuniform stands in Texas and other rice-producing states. Seedling diseases are caused by numerous fungi including *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium* spp. Seven field trials were conducted in naturally infested fields at two locations in Texas over 3 years (2010 to 2012) to evaluate the efficacy of 14 registered and experimental fungicides for control of seedling diseases and yield improvement of rice. Seed treatments with CruiserMaxx Rice, containing Maxim 4FS (fludioxonil), Dynasty (azoxystrobin), Apron XL (mefenoxam) and Cruiser 5FS (thiamethoxam), at the full recommended rate and the half rate performed similarly, increasing stands by 18 to 60% and increasing grain yield by 3 to 22% depending on the test location and year. Seeds treated with Allegiance LS (metalaxyl) or Trilex 2000 (trifloxystrobin and metalaxyl) resulted in a 7-to-25% increase in stand but no significant increase in yield.

All other seed treatments with 11 experimental fungicides had similar effects as did the Allegiance LS and Trilex 2000 seed treatments. Fungicide seed treatment can be an effective tool to control seedling diseases of rice under Texas environments.

Multistate evaluation of PGPR strain MBI600 and its combined use with azoxystrobin for control of sheath blight in rice

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Rice growers heavily rely on fungicides for control of sheath blight (*Rhizoctonia solani*), the most important rice disease in the southern U.S. A field experiment was conducted in *R. solani*-inoculated plots in TX, AR and MS to evaluate the efficacy of plant growth promoting rhizobacteria (PGPR) strain MBI600 alone and in combination with a reduced rate of azoxystrobin for the control of sheath blight in 2010 (TX only), 2011 (TX only) and 2012. Strain MBI600, belonging to *Bacillus subtilis*, is the active ingredient in the biopesticide Integral®. The seeds of Cocodrie (very susceptible) and Presidio (susceptible) were treated with strain MBI600 prior to seeding. At the boot stage, plots were sprayed with strain MBI600 at 10^9 CFU/ml alone or in combination with azoxystrobin at 0.08 (half rate) or 0.16 kg a.i./ha (full rate). In TX, strain MBI600 alone reduced sheath blight severity in all 3 years and increased yield in 1 of 3 years. Combined use of strain MBI600 with azoxystrobin at the half rate further reduced disease and increased yield to the levels similar to azoxystrobin at the full rate in each year. In AR, strain MBI600 alone did not affect disease severity and yield but its combination with azoxystrobin at the half rate reduced disease and increased yield. Its efficacy was comparable to that of azoxystrobin at the full rate. In MS, strain MBI600 applied alone or in combination with azoxystrobin at the half rate was effective in increasing yield on Cocodrie.

Dissecting the mechanism of avirulence factor AvrGfI of *Xanthomonas citri* in inducing hypersensitive response (HR) on citrus

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Citrus canker is one of the most important diseases on citrus. Genetically distinct xanthomonads including *Xanthomonas citri* subsp. *citri* (Xcc) (strains A, A^w, and A*) and *X. fuscans* pv. *aurantifolii* (Xau) (strains B and C) cause citrus canker. XccA has a broad host range, whereas XccA^w causes canker on Mexican lime but an HR on grapefruit. In a previous study *avrGfI* was shown to be responsible for the HR. To shed light on the biochemical mechanism underlying the AvrGfI-induced HR, we screened the citrus cDNA library using the yeast two-hybrid system. A 180 bp cDNA fragment was identified; the corresponding amino acid sequence was blasted against the citrus genome through Phytozome (www.phytozome.net/search.php). The identified cDNA fragment was shown to encode CsRING1, an E3 ubiquitin ligase. E3 ubiquitin ligase has been known to be involved in eliciting HR on pepper (*Capsicum annuum*) by avirulent *X. campestris* pv. *vesicatoria* infection. Here, we will report our progress in testing our hypothesis that AvrGfI activates CsRING1 to elicit HR in grapefruit. In addition, AvrGfI also plays a significant role in virulence of XccA^w on Mexican lime based on a competitive index assay.

pilG in the biocontrol agent *Lysobacter enzymogenes* strain C3 positively regulates surface motility and negatively regulates antibiotic production

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Lysobacter enzymogenes strain C3 is a gliding bacterium which produces the antifungal secondary metabolite HSAF as an important mechanism in biological control activity against fungal pathogens. To search for regulatory mechanisms in strain C3 that control HSAF production, putative two-component signal transduction (TCST) genes were identified from the genomic sequence database for strain OH11 of *L. enzymogenes*. Mutants of strain C3 were made in which specific TCST genes were disrupted or deleted, and they were evaluated for production of HSAF as measured by HPLC. Strain Lys1909, which contains a deletion mutation in *pilG* encoding for a TCST response regulator, was found to produce higher amounts of HSAF than

wildtype C3, indicating the gene is involved in negative regulation of HSAF production. *pilG* in *Pseudomonas aeruginosa* positively regulates production of type IV pilus (T4P) which has been implicated in surface motility and attachment. Lys1909 exhibited reduced surface spread compared to wildtype C3, evidence that the *pilG* homolog in strain C3 also functions in positive regulation of T4P production. These results suggest that key ecological functions in *L. enzymogenes* strain C3 can be regulated in opposite directions by the same regulatory system.

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