



## 2016 Northeastern Division Meeting Abstracts

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### **Pathogenicity of a 16SrIII-L phytoplasma associated with frogskin disease of cassava (*Manihot esculenta* Crantz) in Colombia**

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Frogskin disease (FSD) is the most important constraint to cassava crop as it directly affects the production of roots, causing yield losses as high as 90% for farmers in Cauca, Colombia. The phytoplasma associated with FSD belongs to the 16SrIII-L subgroup and was isolated using an artificial culture medium kept under anaerobic conditions. In this study, our goal was to evaluate the pathogenicity of phytoplasma strains isolated from cassava diseased roots. Cassava clones CM 2952, Col 1, Col 896, Bra 184, SM 909-25, Per 334, Per 326, Per 534, and Per 1999, were evaluated. *In vitro* plants were grown in a greenhouse for 60 days and inoculated with phytoplasma by stem and root injection of liquid phytoplasma cells medium. Inoculated plants were maintained in an insect-proof greenhouse until root symptoms developed. The control plants were inoculated with liquid medium without phytoplasma cells. The inoculated plants were evaluated every two months. Pathogenicity was confirmed in five (CM 2952, Col 1, Col 896, Bra 184, SM 909-25) of ten genotypes exhibiting severe FSD symptoms. The phytoplasma was re-isolated *in vitro* from cassava symptomatic roots and identification confirmed by nested-PCR, restriction fragment length polymorphism and sequence analyses of amplified rDNA products. This study reports for the first time the pathogenicity of phytoplasma isolated on artificial culture medium originated from cassava roots.

### **Structural biology of viruses in the luteoviridae**

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Viruses in the family *Luteoviridae*, collectively referred to as luteovirids, cause economically important diseases on crops worldwide. In nature, luteovirids are transmitted exclusively by aphids in a persistent, circulative manner. As luteovirids move systemically in both aphids and plants as virions, the structure of the viral capsid is critical to their success as pathogens. However, no crystal structure has ever been obtained for a luteovirid virion or structural protein; current models are based on epitope mapping, mutational studies, and cross-linking data. Using Protein Interaction Reporter (PIR) technology, a mass spectrometry-compatible cross-linker, we expanded on structural proteomic studies in *Potato leafroll virus* (PLRV) to include a second luteovirid species, *Turnip yellows virus* (TuYV). We compare and contrast the multimeric structures of PLRV and TuYV capsid proteins, and outline plans to integrate our cross-linking data with cryo-electron microscopy to generate the first atomic resolution structure of a luteovirid virion.

### **When do onion-pathogenic bacteria become associated with growing onions?**

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Bacterial decay of onion bulbs is a serious problem for growers in New York State and elsewhere. Bacteria may enter growing onion bulbs during damaging weather events such as hail, driving rain, during feeding by thrips, or during harvest when green leaf tissue may be cut from the bulb. However, NY growers often report seeing the earliest signs of bacterial disease in their crops in early July, when one or several internal leaves develop lesions. We wished to determine when during the growing season asymptomatic internal onion tissues first become contaminated with bacteria, and which bacteria are commonly found in these plants. In previous years, we isolated and identified strains of *Burkholderia* spp., *Pantoea ananatis*, and *Enterobacter* sp. from onions with symptoms of bacterial rot in NY. During the summer of 2015, we collected growing onion plants from three important onion-growing regions of NY. Internal bacteria were isolated and many were identified using molecular genetic techniques. In general, few plants were found to harbor internal bacteria early in the season compared to later in the season. Surprisingly, pathogenic bacteria were recovered from asymptomatic plants early in the growing season, even though no symptomatic plants were recovered until mid-August. This suggests that some species of onion-pathogenic bacteria can live endophytically for some time without causing disease symptoms.

### **Development of markers for the identification of *Verticillium longisporum* species and lineage in canola plant tissue using real-time PCR**

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Canola is a major crop kind in Canada with multi-billion dollar annual export revenues. Verticillium wilt in canola caused by *Verticillium longisporum* was detected for the first time in 2014 in a canola field in Manitoba Canada. Following this first detection new DNA based diagnostic assays were sought to quickly and reliably identify the pathogen to both species level and lineage instead of using Sanger sequencing. A combined real-time PCR assay to detect and identify the *V. longisporum* species and lineages was developed and the assay's sensitivity on seed material was also evaluated. The threshold of detection of this molecular assay on the IGS (Intergenic Spacer region of ribosomal DNA) has been quantified down to one infected seed in 10,000. Several SNPs (Single Nucleotide Polymorphism) were identified in two gene regions that could be used to determine the lineage of the pathogen and were used for designing the assay. Six novel sets of primers and probes have been designed and tested for specificity and sensitivity on pure culture material and naturally infested environmental samples. Analysis has demonstrated the potential of the assay and allowed for redundancy of two gene regions for the determination of the *V. longisporum* lineages A1/D1, D2 or D3 in pure culture and naturally infested field material. The diagnostic tool was successfully implemented to process samples for the Canadian national survey in 2015.

#### **Detection of internal decay in American elms undergoing injection for control of Dutch elm disease using sonic and electrical resistance tomography**

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Without regular fungicide injection for treatment of Dutch elm disease (DED), caused by *Ophiostoma novo-ulmi*, American elms (*Ulmus americana*) often become infected and die. While the treatments can be effective, the act of injection creates numerous wounds on the root flares that can potentially facilitate invasion by wood-decaying fungal pathogens. The goal of this study is to determine if internal decay occurs more frequently in American elms that are regularly injected for control of DED than those not receiving injections. This was achieved using both sonic (SoT) and electrical resistance (ERT) tomography, currently the most accurate means for non-destructive detection of internal decay. To date, 67 American elms at 10 sites in Massachusetts, Connecticut and Rhode Island have been sampled using SoT and ERT. Based on the SoT scans, 11/67 (16%) trees had measurable decay present in the lower trunk with percent damage ranging from 4–67% of the sampled cross section. For elms with a diameter  $\geq 140$  cm at the sampled cross section, 7/17 (41%) were found to have internal decay. The ERT scans found that over half (36/67; 54%) of all sampled elms exhibited high conductivity in the heartwood relative to the outer sapwood, likely due to wetwood bacteria, which are common in American elm. Further sampling, particularly on American elms that are not injected, will aid in our understanding of how fungicide injection influences tree health with regards to internal decay.

#### **Evaluation of alternative hosts of Grapevine red blotch-associated virus**

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Red blotch is a viral disease of *Vitis* spp. that delays fruit ripening and reduces fruit quality. The causal agent, Grapevine red blotch-associated virus (GRBaV), has a single-stranded, circular DNA genome (3,206 nt) and is the type member of a new genus tentatively named *Grabovirus* in the family *Geminiviridae*. Recently, the three cornered alfalfa treehopper (*Spissistilus festinus* [Homoptera: Membracidae]), a generalist phloem feeder, was reported to vector GRBaV from infected to healthy grapevine. *Vitis* spp. is the only host of GRBaV that has been identified so far but, by analogy with other geminiviruses, the virus host range may be broader. The objective of this research is to evaluate a range of annual plant species as alternative hosts of GRBaV. *Agrobacterium tumefaciens*-mediated delivery of an infectious clone of GRBaV were utilized for plant inoculation. Following inoculation, plants were monitored for GRBaV infection at regular intervals by multiplex PCR and by assessing symptom development. Any alternative hosts were further investigated for their potential to serve as virus inoculum reservoirs for vector-mediated transmission. Determining the host range of GRBaV is an important component to understanding red blotch disease epidemiology and to developing effective disease management strategies in vineyards.

#### **Genome plasticity or “error catastrophe”? Illumina deep sequencing shedding light onto population structure of PVY<sup>N</sup> strain isolates**

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Genetic variation among strains and isolates of *Potato virus Y* (PVY) have been intensively studied; however, little is known about the genetic diversity within PVY populations. Using Illumina next generation sequencing, the population structure of 15 isolates of seven different PVY strains was analyzed. Eight were PVY<sup>N</sup> isolates and six of those came from the same geographic region in two different years. A consensus sequence, without indels or insertions, was successfully extracted from the sequenced reads of each isolate after being mapped to the strain reference genome. RNA-seq reads mapped unevenly throughout the genome of each isolate. The capsid protein (CP), VPg, and 6K1 cistrons had the highest concentration of mapped reads; the P3 cistron the lowest. The isolates within the PVY<sup>N</sup> had a higher population genetic diversity than any other strain evaluated and the population genetic diversity of the PVY<sup>N</sup> isolates, differed between collection years and sites. The highest numbers of single nucleotide variants in the PVY<sup>N</sup> isolate populations were concentrated in the CP and 6K1 cistrons, whereas the lowest numbers of variants were found in the 6K2 cistron. The evidences suggest that N strain has a plastic genome with regions of high and low tolerance to variations.

#### **Leveraging 'omics technologies to develop new initiatives for controlling vector-borne plant pathogens**

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A majority of plant pathogens and a large number of important animal pathogens are transmitted by insect vectors. Plant pathogens are divided between those that are carried on the cuticle linings of mouthparts and foreguts and those that circulate in their vectors. Insects in the order Hemiptera are among the most prolific vectors of plant pathogens. Our lab focuses on plant pathogens that are exclusively transmitted by hemipteran pests in a circulative manner. Phloem-retention facilitates circulative transmission by these insects, which spend prolonged periods of time feeding in the phloem tissue. This presentation will highlight our efforts to develop and apply advanced proteomics technologies to enable us to explore the dynamic pathogen-vector interface. Several examples of proteomics data will be discussed to illustrate the power of these technologies to further our basic understanding of the molecular pathways involved in circulative transmission in plants and aphids and the excellent agreement of our data with previously published studies on the biology of circulative transmission. Finally, examples from our data will also be presented to show how proteomics technologies can enable us to develop novel strategies that disrupt pathogen movement within and between hosts.

#### **Nanoparticles of micronutrients suppress Fusarium wilt of watermelon**

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Nanoparticles (NP) has great potential in agriculture. Micronutrients, such as Cu, Mn, and Zn, activate enzymes that catalyze phenolic defense products against root infecting fungi. Manipulating the micronutrient content in roots is difficult because micronutrients have poor basipetal mobility when foliarly applied and low availability in neutral soils. We investigated whether foliar sprays of NP of micronutrients to young watermelon plants in the greenhouse could affect their growth, yield, and response to *Fusarium wilt* caused by *Fusarium oxysporum* f. sp. *niveum*. Preliminary trials in a greenhouse found

that NP of CuO, MnO, and ZnO were superior to NP of CeO, FeO, NiO, SiO, and TiO when sprayed on young plants and grown disease infested soils. NP of CuO were superior to MnO or ZnO in the greenhouse in increasing fresh and dry weight of the plants and in suppressing Fusarium wilt. Root levels of Cu, Mn or Zn did not differ from controls roots in any element suggesting minimal basipetal movement. Field studies have been more variable, but NP of CuO and ZnO tend promote yield more than untreated plots. Digests of edible flesh found no difference in any of metals. Preliminary transcriptional analyses found increased expression levels of polyphenol oxidase gene, but only in plants exposed to both *F. oxysporum* f. sp. *niveum* and NP of CuO. Potential mechanisms will be discussed.

#### **Winegrape cultivar trials in Connecticut: 2012 - 2015**

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In the past 15 years, the number of wineries in Connecticut has doubled (32 extant) while the number of vineyards (44) and the total acreage planted to winegrapes has tripled (450 A). This burgeoning industry, a form of agro-tourism, brings economic benefits to the rural communities in which most are located. Although Connecticut is a small state, minimum temperature in winter varies from 0° F near the coast to -15°F in the Litchfield hills. The major limitation for the kind of grape that can be economically grown in this area is the survival of grape vines through the winter. Another problem is the length of the growing season, which may be too short to allow late season cultivars to fully ripen. Vineyards are expensive to establish (~\$5500 per acre) and maintain (~\$2700 per acre annually) and do not produce salable product for, at least, three years. Thus, it is of paramount importance to choose the “right” cultivar when planting a new vineyard. It is also very important to choose the proper training and pruning method according to the habit of growth of each cultivar. In 2008, 24 cultivars were planted. Our objectives were: 1) To match the training and pruning method to the habit of growth for each winegrape cultivar. 2) To evaluate the fruit yield, juice quality, susceptibility to disease, and vegetative vigor for each winegrape cultivar.

#### **First report of *Alternaria infectoria* causing leaf spot of wheat in the U.S.A.**

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Distinctive leaf spots were observed on soft winter wheat at variety plots in June, 2015 and 2016 in Monroe County, NY. Irregularly round to elongated lesions with tan centers and purple-brown borders were found on leaves, heads and stems. Disease severity was low, and incidence was consistent across varieties and replicated plots. A number of *Alternaria* spp. were isolated from sterilized symptomatic tissue and identified by morphology and DNA sequence analysis. Several isolates were determined to be members of the *Alternaria infectoria* species group. Koch's postulates were completed using winter wheat seedlings grown in a greenhouse. Four varieties observed with lesions in the field were inoculated and all plants developed lesions at leaf tips. One variety developed lesions typical of those found in the field. *Alternaria infectoria* was reisolated, and species identity reconfirmed with DNA sequencing. This is the first report of *A. infectoria* causing a disease of wheat in the United States. The symptoms caused by these New York isolates differ significantly from those associated with other *Alternaria* diseases, including black point caused by *A. infectoria* and leaf blights caused by *A. triticimaculans* and *triticea*. The presence of disease during two successive growing seasons in only this region suggests a unique set of environmental conditions favoring the disease or a local inoculum source.

#### **Rolling and dew removal effects on dollar spot disease of creeping bentgrass**

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Dollar spot (caused by *Sclerotinia homoeocarpa*) is a frequently occurring disease on golf course turfs. The effect of lightweight rolling on dollar spot incidence of creeping bentgrass (*Agrostis stolonifera* L. 'Independence') was evaluated in a field study mowed at 12.7-mm 3d wk<sup>-1</sup> in North Brunswick, NJ. A 2 × 2 factorial arranged in a randomized complete block design with four replications evaluated time [morning (AM) or afternoon (PM)] and frequency (3 or 6 d wk<sup>-1</sup>) of lightweight rolling during the fall of 2015 and spring of 2016. Two additional treatments included morning removal of dew using an absorbent fabric and an untreated control. Rolling turf in the morning (dew present) had the greatest impact on disease and reduced the area under the disease progress curve (AUDPC) by 52 and 33% compared to PM rolling during the fall and spring, respectively. During the fall, rolling frequency interacted with time of rolling, where rolling 6 d wk<sup>-1</sup> increased the effectiveness of AM rolling at reducing disease incidence compared to AM rolling 3d wk<sup>-1</sup>; however, rolling frequency did not influence the effect of PM rolling on disease. Rolling frequency did not affect disease during the spring, regardless of the time of rolling. Use of an absorbent fabric to remove dew reduced AUDPC by 74 and 53% during the fall and spring, respectively, compared to the control. This was similar to the effect of AM rolling 6 d wk<sup>-1</sup> in the fall. This research will be continued in 2016.

#### **Using Fe<sub>3</sub>O<sub>4</sub> super paramagnetic nanoparticles for isolation of genomic DNA from soil for detection of root knot nematode**

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Quantifying levels of plant-parasitic nematodes prior to planting using a DNA-based soil test could provide a more efficient and accurate estimation of potential crop damage or yield loss, leading to informed management decisions, including pesticide application. Current techniques for extracting DNA from soil include commercial kits and phenol-chloroform based methods. However, these are often difficult, time consuming, produce hazardous waste, and may only be suitable for analyzing small volumes of soil. Variability in estimation of pathogen population densities is greatly reduced when larger volumes of soil are able to be analyzed. A method for the isolation of DNA from soil using Fe<sub>3</sub>O<sub>4</sub> superparamagnetic nanoparticles and magnetic bioseparation was investigated. DNA was isolated from 1, 25, 50, and 100 g of soil containing *Meoloidogyne hapla* (root-knot nematode) using a nanoparticle suspension. Quality and quantity of the resultant DNA was assessed using spectroscopy (NanoDrop) and flurometry (Qubit®). The ability to amplify the internal transcribed spacer (ITS) region of *M. hapla* was also tested by qualitative PCR. Genomic DNA was extracted from all soil volumes and the ITS region was successfully amplified. Further amendments to the extraction protocol are required to optimize the procedure and ensure efficient isolation of total DNA from soil.

#### **Towards a better understanding of the etiology and management of sour rot in grapes**

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Sour rot is a disease affecting grapes in viticultural regions worldwide, yet etiology and management strategies are not well understood. Symptoms are characterized by fruit rot accompanied by the smell of acetic acid and presence of *Drosophila* (fruit fly) species. We have successfully reproduced visual and olfactory disease symptoms and the accompanying characteristic acetic acid, along with its ethanol precursor, within affected berries, in the lab. Healthy fruit were wounded, inoculated with *Saccharomyces cerevisiae* and *Acetobacter aceti*, and exposed to *D. melanogaster* adults. Inoculation without exposure to flies significantly promoted ethanol production but not acetic acid generation, whereas concomitant exposure to flies resulted in both. In field trials conducted on interspecific hybrid cv. 'Vignoles' in 2013–15 in the Finger Lakes region of New York, both insecticide and antimicrobial treatments significantly reduced sour rot development. In 2015, sour rot severity was reduced by 73–81% on vines treated prophylactically post-veraison with weekly sprays containing a combination of the insecticide zeta-cypermethrin (Mustang MAX) plus the antimicrobial potassium

metabisulfite or hydrogen dioxide (Oximate 2.0) in comparison to the untreated vines which averaged 20.5% sour rot severity; severity was reduced by 49% on vines receiving only insecticide sprays.

#### **Investigation of tumorigenic and non-tumorigenic *Agrobacterium vitis* strains on grape graft take**

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*Agrobacterium vitis* causes gall disease on grape that is initiated at wounds. It also causes necrosis which inhibits wound-healing and thus negatively affects graft-take. We studied the impacts of strains CG49 (tumorigenic) and F2/5 (non-tumorigenic) on graft take and plant growth. Dormant grape cuttings were soaked in CG49 or F2/5 cell suspensions ( $1 \times 10^6$  CFU/mL) or H<sub>2</sub>O for ~ 2h. A hand graft tool was used to make a V-cut and the upper and lower cutting sections were self-grafted. All grafts were kept in moist perlite for 30 days. A subset was then transplanted into soil for another 30 days, and plant traits were assessed at both time points. At 30-day post-inoculation (d.p.i.), CG49 treated grafts developed more callus (crown gall) at graft unions and the basal ends than the F2/5 or H<sub>2</sub>O ( $p < 0.01$  for both) treatments. Graft strength was measured with an Instron testing machine in a 3-point bending configuration. CG49 treated grafts had an average (avg) strength of 34.6 newtons (N), significantly higher than those of the other treatments (22N for F2/5,  $p < 0.01$ ; 27.45N for H<sub>2</sub>O,  $p=0.05$ ). At 60 d.p.i., CG49 treated grafts developed less shoot biomass (avg=5.5g) and were weaker in strength (avg=52.1N) compared to F2/5 (avg=9.5g, 58.2N;  $p < 0.01$ ) or H<sub>2</sub>O (avg=11.5g, 70N;  $p < 0.01$ ) treatments. *A. vitis* population increases by ~1000-fold after 5 d.i. and maintained a high level. Our data shows that *A. vitis* negatively affects graft strength and subsequent plant growth.

#### **Responses of potato varieties and effects of chemical compounds to *Pectobacterium* spp. and *Dickeya* spp. that cause blackleg of potato**

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Blackleg and bacterial soft rot of potato, caused by *Pectobacterium* spp. and *Dickeya* spp. has caused a drastic problem in the Eastern states of the US since 2015. Nine commercial varieties (cv. Atlantic, Green Mountain, Kathadin, Red Norland, Russet Burbank, Sebago, Shepody, Snowden, Yukon Gold) and 25 lines from the Maine breeding program were evaluated for pathogen susceptibility. Bacterial suspensions of *Dickeya dianthicola* strain ME30, *D. dadantii* 3937, *Pectobacterium carotovorum* subsp. *carotovorum* WPP14, and *P. wasabiae* WPP163 were used to challenge potato tubers. Tubers were perforated with 1 cm-deep holes using a sterile pipette tip. Each hole was filled with 20  $\mu$ l of bacterial inoculum, and taped to avoid loss of moisture. Water was used as a control. The inoculated tubers were incubated in the dark at 28°C for 5 days and then assessed for lesion sizes. ‘Sebago’ was the most tolerant variety to *P. carotovorum*; AF5312-1 was the most tolerant to *P. wasabiae*; ‘Yukon Gold’, AF 4172-2, and ‘Shepody’ were the most tolerant to *Dickeya* spp. In a second experiment, a filter paper was added with 10  $\mu$ l of the following products: 9 essential oils, isothiocyanate, or copper sulfate, and then placed onto tryptic soy agar, which was previously spread with bacterial suspension. After incubation for 2 days at 28°C, the inhibition zone was measured. Oregano essential oil and isothiocyanate had an effect in inhibiting both *Dickeya* spp. and *Pectobacterium* spp.

#### **Predictability of dollar spot disease development on bentgrasses using weather-based models**

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Dollar spot epidemics (caused by *Sclerotinia homoeocarpa* F.T. Bennett) differ among bentgrass (*Agrostis* spp.) species and cultivars. The objectives of this research were to evaluate disease incidence on bentgrasses that vary in tolerance to dollar spot under fairway conditions and to assess the reliability of two existing weather-based models for predicting dollar spot epidemics on these grasses. Six bentgrass cultivars [‘Independence’, ‘Penncross’, ‘Shark’, ‘007’, and ‘Declaration’ creeping bentgrass (*A. stolonifera*), and ‘Capri’ colonial bentgrass (*A. capillaris*)] were seeded in a randomized complete block design with five blocks in North Brunswick, NJ during September 2014. Disease severity was assessed every 2- to 5-d and compared to a growing degree day (GDD) model for predicting the onset of disease symptoms and a logistic regression model for predicting season-long disease activity. The GDD model accurately predicted the onset of disease symptoms in highly susceptible cultivars during 2015 but not 2016. The logistic regression model forecasted a high risk of dollar spot one week before symptoms first appeared in highly susceptible cultivars during both years. Disease forecasting by the logistic regression model was fairly accurate for highly susceptible cultivars throughout 2015, but over-predicted disease activity during 2016. Accurate disease forecasting on tolerant cultivars was more problematic with either model in both years of this study.

#### **Resistance screen of potato variety to pink rot caused by *Phytophthora erythroseptica***

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Pink rot (*Phytophthora erythroseptica*) of potato (*Solanum tuberosum*) has been a factor causing yield loss of potato. To examine tolerant varieties, field trials were conducted in Aroostook Research Farm, Presque Isle, ME from 2014 to 2016. Six commercial varieties (cv. Atlantic, Dark Red Norland, Pike, Snowden, Red Gold, and Russet Norkotah) and 20 lines of potato were studied. Randomized complete block design was applied in the trial with three blocks. Plots size was one 3-ft-wide row with 10 feet in length and one foot of plant space. Inoculum was prepared by incubating three isolates of *P. erythroseptica* in mushroom spawn bags containing 6 L of vermiculite and 3 L V8 broth for four weeks at 22°C. The inoculum was evenly hand distributed at 200 ml/foot in furrow, followed by placing seed pieces. Fertilizer, insecticides and herbicides were applied as standard practice to the area. Bravo WeatherStik (1260 ga/ha) was applied to the foliage during the season to prevent the spread of late blight. The emergence and vigor of potato were evaluated four weeks after planting. The harvested tubers were examined for disease and yield. According to the results of 2014 and 2015, ‘Atlantic’ and ‘Snowden’ were the most tolerant varieties to pink rot. AF4172-2, AF4157-6, and AF4648-2 were also tolerant to pink rot.

#### **Natural development of head smut (*Tilletia maclaganii*) in seven cultivars of switchgrass over five years**

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Surveys of ‘Cave-in-Rock’ switchgrass in Iowa suggested that plants infected by the head smut fungus, *Tilletia maclaganii*, were drastically reduced in biomass yield. However, no study to date has examined the multi-year progress of head smut in cultivated switchgrass or the capacity of *T. maclaganii* to incite disease in multiple, commercially important switchgrass cultivars. We, therefore, studied the natural development of head smut in replicated plots of seven upland switchgrass cultivars in Big Flats, New York over five years. Cultivars were planted in 2010 using a complete randomized design and positioned downwind of a field of ‘Shawnee’ with greater than 90% incidence of head smut. Smut became evident only in the second year after planting (2012) and mean incidence increased in 2013 (25%), 2014 (41%), and 2015 (74%). All cultivars were susceptible, but, the highest mean incidences in 2015 were observed in ‘Cave-in-Rock’ (94%), ‘Shawnee’ (91%), ‘Blackwell’ (83%), and ‘Summer’ (81%). Somewhat lower incidences were observed in ‘SW1655’ (67%), ‘Shelter’ (65%), and ‘SW1657’ (45%). Progressive intensification of head smut – from zero to high incidence – was demonstrated to occur in as little as five years and on all cultivars examined.

#### **Evaluating the disease resistance profile of brown midrib silage corn**

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Observing severe damage by northern leaf blight (NLB) on brown midrib (bm) silage corn in upstate NY led us to hypothesize that the bm allele(s) enhance disease susceptibility. The reduced lignin content in bm lines is desirable in silage corn because it is more digestible for ruminants; could it also be more digestible by pathogens? These genes belong to phenylpropanoid pathway, which plays roles in both structural support, and also plant defense. Several bm genes co-localized with QTLs for NLB. To date, six bm mutants have been identified in maize, with four of them described at the gene level. Challenging *bm1*, *bm2*, *bm3* and *bm4* mutant lines with NLB, Stewart's Wilt (SW) and gray leaf spot (GLS) indicated that the mutants are more susceptible to the foliar fungal and bacterial diseases. The *bm3* mutant line was consistently one of the most susceptible of the bm mutants. Genic diversity in *bm1*, *bm2*, *bm3* and *bm4* and associations with disease resistance will be discussed in relation to natural genetic variation for resistance to NLB in maize. Understanding of the susceptibility associated with the bm genes is important for advancing both disease management and our understanding of resistance in maize to multiple diseases.

#### **Comparative proteomics to identify critical proteins for transmission of *Candidatus Liberibacter asiaticus* by the Asian citrus psyllid**

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Citrus greening is associated with *Candidatus Liberibacter asiaticus*, which is spread by the Asian citrus psyllid, *Diaphorina citri*. Prior to transmission to a new tree, *C. L. asiaticus* must cross the psyllid gut. To reveal interactions between the psyllid gut and *C. L. asiaticus*, we used quantitative mass spectrometry to compare the gut proteomes of psyllids reared on healthy and *C. L. asiaticus*-infected trees. We also performed fluorescent in situ hybridization and confocal microscopy using probes specific to *C. L. asiaticus* and *Wolbachia*, a bacterial symbiont of the psyllid. The results show that *C. L. asiaticus* and *Wolbachia* co-habitate within the same cells, but do not co-localize. *C. L. asiaticus* negatively regulates the expression of many *Wolbachia* signaling proteins in the gut and up-regulates actin cytoskeletal proteins. *C. L. asiaticus* has a negative impact on gut cell morphology, inducing the rupture of midgut cell nuclei and down regulating mitochondrial metabolism in a localized manner. *C. L. asiaticus* is also putatively observed in the psyllid midgut cell nuclei, a localization that may explain the observed nuclear lysis in *C. L. asiaticus*-exposed insects. These data show that *Wolbachia* plays an important yet unknown role in the acquisition of *C. L. asiaticus* and that *C. L. asiaticus* invokes an unusual immune response upon acquisition that involves nuclear lysis, mitochondrial impairment and cytoskeletal remodeling.

#### **Susceptibility of *Pachysandra* species and cultivars to the boxwood blight pathogen *Calonectria pseudonaviculata***

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*Calonectria pseudonaviculata* (*Cps*) can infect *Pachysandra* species. *Pachysandra axillaris*, *P. procumbens*, and five cultivars of *P. terminalis* were evaluated for susceptibility to *Cps*. Three experiments used 10 replicate potted plants each with or without thiophanate-methyl plus chlorothalonil at label rates. Plants were inoculated 2 days after treatment with  $1.5 \times 10^5$  *Cps* conidia and grown under overhead irrigation between infected boxwoods. Leaf and stem lesions were recorded after 2 wks. *P. terminalis* 'Common', 'Variegated' and *P. procumbens* were the most susceptible. *P. axillaris* 'Windcliff', *P. terminalis* 'Green Carpet' and 'Crinkled' were least susceptible and 'Green Sheen' was intermediate. Fungicides reduced disease by 85% with no plant interaction. *P. axillaris* and the *P. terminalis* cultivars were also evaluated with detached leaf assays in humidity chambers. Four mature leaves of each cultivar were inoculated with 2,000 to 4,000 *Cps* conidia in a single drop on adaxial or abaxial surfaces. Disease development and sporulation were recorded after one or two wks. The experiment was conducted 5 times. Disease was most severe when detached leaves were inoculated on the abaxial surface. Green Sheen and Crinkled were the most susceptible cultivars; *P. axillaris*, Variegated, Common and Green Carpet had less disease and sporulation, especially on the adaxial leaf surface. While *Pachysandra axillaris* appears less susceptible than other species, it can be infected by *Cps*.

#### **Biological control of soilborne diseases in organic potato production as affected by varying environmental conditions**

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Soilborne diseases are persistent problems in potato production and alternative management practices are needed, particularly in organic production, where control options are limited. Selected biocontrol organisms, including two naturally-occurring hypovirulent strains of *Rhizoctonia solani* (Rhs1a1 and Bs69) and a commercially available *Bacillus subtilis* (GB03), were evaluated individually and in combination, for control of soilborne diseases of potato under organic production practices over three field seasons in Maine. Varying rainfall conditions over the three growing seasons significantly affected crop growth, disease development, tuber yield, and biocontrol efficacy. However, multiple biocontrol treatments resulted in modest but significant reductions in black scurf and common scab under a variety of environmental conditions, reducing incidence and severity of each by 15–47%. Most biocontrol treatments reduced black scurf, but only specific treatments reduced common scab and silver scurf (by 10–25%). Combinations including both a hypovirulent strain and GB03 tended to be most effective. Although tuber yield varied greatly by year, Rhs1a1 and GB03 treatments, as well as combinations, increased yield by 11 to 37% over all three seasons. Use of hypovirulent *R. solani*, along with other biocontrol organisms, may provide reductions in soilborne diseases and enhanced yield in organic potato production.

#### **A transcriptomic approach to elucidate the mode of action of the biocontrol agent *Pseudozyma flocculosa* toward powdery mildews**

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In the last decades, the ever-growing awareness towards pesticide impact on health and the environment has led to the development of new approaches in plant disease management. Initially ignored in agricultural systems, beneficial microorganisms have recently gained popularity as potential biological control agents (BCA). Multiple efforts have been invested in trying to describe with precision the underlying mode of action that explains antagonism of a microorganism towards another. In the present study, we used a transcriptome profiling of the tritrophic interaction *Pseudozyma flocculosa*-*Blumeria graminis* f.sp. *hordei*-barley to investigate the mode of action by which the fungal BCA *P. flocculosa* antagonizes plant powdery mildews. Results show that *P. flocculosa* antagonistic properties are unrelated to the ones of other biocontrol agents. None of the hypotheses of competition, parasitism, induced resistance or antibiosis, first pointed as a potential mode of action for *P. flocculosa*, were supported by transcriptomic data. On the other hand, a detailed analysis of highly expressed genes has led us to unravel a completely unexpected strategy for a BCA.

#### **Comparing the genetic diversity of *Sclerotinia sclerotiorum* populations from United States and Brazil**

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Genetic variability of *Sclerotinia sclerotiorum* has been suggested to be higher in the tropics compared to those in temperate zones. In this study, populations of *S. sclerotiorum* from North America (temperate) and central Brazil (tropical) were compared using mycelial compatibility groups (MCGs) and 13 microsatellite (SSR) markers. Populations were sourced from diseased plants within leguminous crops in New York State, U.S.A. (NY; n = 78 isolates), and Minas Gerais, Brazil (MG; n = 109). Twenty MCGs were identified in NY and 14 in MG. The NY population had 22 multilocus genotypes (MLGs) and MG had 24, but after rarefaction 20 MLGs were estimated for MG. Clonal fractions of MLGs were 71.8% (NY) and 78% (MG). In MG,

evenness ( $E_5$ ) calculated from SSR data was significantly higher than in the NY population. The Stoddart and Taylor and the Simpson indexes of genotypic diversity were higher in MG, but no difference between the populations was found using the Shannon-Wiener index. The populations were highly differentiated, with 29% of total variance attributed to differences between them and  $G'_{ST}$  and Jost's  $D$  indexes higher than 0.35. Cluster analysis revealed dissimilarity higher than 80% among most MLGs from both populations. Our results do not support the hypothesis of higher levels of variability associated with *S. sclerotiorum* populations from tropical climate zones.

#### ***Pseudomonas syringae* - tomato interactions: an unfolding New York story**

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Characterization of the complex interplay of bacterial factors and host defense components that govern *P. syringae*-host interactions represents an important foundation for identifying new sources of host resistance. System redundancies in both pathogen virulence and host defense pathways have presented significant challenges, as has the considerable variation in virulence associated features among even closely related *P. syringae* strains. Availability of an increasing number genome sequences for closely related *P. syringae* Group I strains pathogenic on tomato and/or brassica species reveals patterns in Type III effector (T3E) repertoire associated with host range. More recently, isolates have been isolated from upstate New York tomatoes exhibiting significantly higher levels of virulence relative to previously characterized *P. syringae* pv. *tomato* strains DC3000 and T1. One of these, Pto NYS-T1, has been sequenced for diagnostic purposes and overcomes resistance in certain wild tomato species. Other recent isolates show varying levels of virulence on specific tomato lines. Comparison among these strains reveals interesting differences in their repertoire of T3Es and other virulence-associated features. Screening these strains against diverse tomato lines is pointing to candidate sources of host resistance.

#### **Black cherry crown health, mortality, and seed production on the Allegheny National Forest**

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In the past decade foresters have reported changes in black cherry (*Prunus serotina* Ehrh.) crown health and seed production adversely affecting forest management goals. Ninety-two Forest Health Monitoring plots, originally measured during 1998–2001 on the Allegheny National Forest, were re-surveyed in 2014–2015 to assess health trends. The percent standing dead black cherry basal area increased to 15.7% compared with 10.2% and 8.3% in earlier surveys. Almost 70% of this mortality is composed of trees  $\geq 11$  inches DBH. Estimates of live crown ratio indicate that 60% of the black cherry basal area is associated with trees having  $< 25\%$  live crown ratios. Plots defoliated by cherry scallop shell moth (*Hydria prunivorata* Ferg.) in 1994–1996 and by fall web worm (*Hyphantria cunea* Drury) in 2011–2013 average about 18% standing dead black cherry basal area compared with 12% for putatively undefoliated plots. The cherry leaf spot foliar pathogen, *Blumeriella jaapii* (Rehm) Arx, is widespread, poorly quantified, and injures mature black cherry crowns and seedling regeneration. Seed production has been monitored in paired 70 and 110 year-old cherry stands at two locations since 2010. Older stands produce as much or more seed than younger stands and seed production has averaged  $< 212,000$  seeds/acre in all stands except in 2010. These results suggest that complex biotic factors interact to affect cherry health and new research is assessing probable causal agents.

#### **Accurately accounting for decay and carbon loss in trees: a novel nondestructive approach using sonic and electrical-resistance tomography (SoT-ERT)**

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Because of their capacity for uptake and storage of atmospheric carbon (C), forests are critical to global warming mitigation efforts. Forest carbon-cycle studies and land-use management strategies require accurate estimates of C density, yet current allometric methods do not account for the widespread but largely ignored process of internal tree decay. SoT-ERT constitute a nondestructive means by which decay and cavity volumes are inferred from cross-sectional images depicting variation in density and moisture. We present a method that uses SoT-ERT data to indirectly estimate C content. For validation, C content was indirectly estimated using SoT-ERT data and C-density (w/v) metrics associated with these data for 105 stem disks corresponding to SoT-ERT cross sections from 12 *Acer saccharum*, 11 *Betula alleghaniensis*, and 16 *Fagus grandifolia*, and validated against direct estimates based on these stem disks' mass; this validation was compared to a similar validation using the indirect method but assuming no decay. "Limits of agreement" and prediction-interval statistical analyses showed that the tomography-based method accounting for decay and cavities had much stronger agreement with the direct mass-based method than did the method that assumed no decay. These results confirm tomography's potential to accurately quantify the extent of internal decay in large-scale forest studies, thereby improving the accuracy of forest carbon accounting.

#### **Evaluation of hop cultivation feasibility in Connecticut based on yield, growing characteristics, and susceptibility to diseases and pests**

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Interest in hop (*Humulus lupulus*) cultivation in the Northeast of the U.S. is on the rise because of the increasing popularity of brewing and demand for regional products. Production had moved to the Pacific Northwest in the last century. This study examined the feasibility of hop cultivation regarding yield, growing characteristics, and susceptibility to diseases and pests in CT. Five varieties - AlphaAroma, Cascade, Newport, Summit, and Perle - were evaluated in low and high trellis systems at two locations over three years. The most common diseases and pests were downy mildew (DM), spider mites, aphids, and potato leafhoppers. DM, caused by *Pseudoperonospora humuli*, is the most severe disease in New England, as the pathogen can infect all plant parts and cause up to 100% yield losses. Symptoms include stunted shoots, black sporulation, angular leaf lesions, and brown cone discoloration. DM was observed. AlphaAroma, regarded as moderately resistant, was heavily infected, suggesting differences in pathogen virulence. Downy mildew was controlled by an IPM program including phytosanitary measures and fungicide application. Cascade and Summit were well suited for CT production and the high trellis system resulted in better yields except for the semi-dwarf variety Summit. Perle, a German breeding line, had the smallest growth and lowest yield. This study demonstrated the feasibility of hop production in CT by using proper varieties, cultural practice, and IPM.

#### **Evaluation of tomato cultivars for late blight resistance and yield in Oman**

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Late blight of tomato, caused by *Phytophthora infestans*, first appeared in Oman in 2013 and continued to cause significant yield loss in 2014 and 2015. Tomato is an important vegetable crop with approximately 1400 hectares grown annually in Oman. In 2016, several field and laboratory trials were conducted to evaluate cultivar resistance in material originating from the mid-east region, Europe and the U.S.A. Concurrent to the disease evaluations yield and agronomic quality of tomato cultivars was also conducted. For both agronomic and late blight resistance evaluations, 36 determinate cultivars

and 12 indeterminate tomato cultivars were seeded in December 2015 in the greenhouse and transplanted to the field in late January and early February of 2016. Trials consisted of four replications planted in randomized complete block designs. Detached leaflet analysis clearly showed high levels (R-gene) of resistance in U.S.A. cultivars Iron Lady and Mountain Merit. Little to no resistance was observed in material originating from Europe, Turkey and Oman. Matt's Wild Cherry with reported levels of high resistance from trials in the U.S.A. was very susceptible. Mountain Merit and Iron Lady had yields of 75 and 61 tons per hectare, respectively. These yields were comparable with commonly grown tomato cultivars in Oman, Ginan and Alissa, which yielded 57 and 65 tons per hectare, respectively.

#### **Optimizing the timing and rate of nitrogen application to reduce onion center rot losses in Pennsylvania**

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Center rot disease of onion, caused by *Pantoea ananatis* and *P. agglomerans*, can cause losses up to 50% in Pennsylvania fields. This research objective was to evaluate the relationship between timing and rate of nitrogen (N) application with disease incidence and marketable yield. In 2015 and 2016, three randomized complete block split-split plot field trials were conducted in Centre and Lancaster Co., to evaluate the weekly fertigation of urea ammonium nitrate at four rates (0, 56, 117 and 179 kg N/ha) either half-season before bulbing or full-season (whole plot). A subset of plants in each plot was inoculated with a mix of *P. ananatis* and *P. agglomerans* isolates as the sub-sub plot (inoculation proximity). Foliar N content, marketable yield, disease incidence and severity were evaluated. In 2016, inoculation proximity was highly significant in determining center rot incidence ( $P \leq 0.0001$  for both trials) and there was a significant interaction between rate and timing of N application ( $P = 0.0417$ ;  $P = 0.0376$ ). In 2015, only N rate significantly affected disease incidence ( $P \leq 0.0001$ ). In all three trials, marketable yield was not significantly different between the three N rates, excluding the control, and the reduced rate of 117 kg N/ha had the highest numerical marketable yield. The results of this research will be used to identify the optimum rate and timing of N application to reduce center rot losses and thus provide growers with higher precision disease management.

#### **Variation in the *Fusarium verticillioides*-maize pathosystem and implications for sorting mycotoxin-contaminated maize grain**

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*Fusarium verticillioides* is an ascomycete fungus ubiquitously associated with maize that can cause Fusarium ear rot and produce fumonisin, a mycotoxin linked to human and animal health risks (e.g. neural tube defects, growth retardation, esophageal cancer). The high cost and resources required for fumonisin quantification limit the extent of efforts to monitor fumonisin in the food system and to breed resistant maize varieties. Other indicators of *F. verticillioides* infection, such as kernel symptom severity, grain bulk density and levels of the fungal pigment bikaverin, can potentially serve as proxies for fumonisin contamination. However, the associations among these indicators and the effect of maize genotype on them can vary greatly under different modes of pathogenesis. Here we present data from a field experiment comparing two inoculation methods intended to represent colonization pathways via rain/wind-borne dispersal and insect damage. Ear rot, density, bikaverin and fumonisin were significantly correlated and trait heritabilities were relatively high, especially under silk channel inoculation. Discriminant analysis using ear rot, bulk density and bikaverin as covariates differentiated samples with fumonisin above and below the legal limit with up to 74% specificity and 67% sensitivity. Our results show promise for developing cheaper, more accessible methods for detecting contamination, breeding resistance, and sorting fumonisin-contaminated maize grain.

#### **Genes influencing quantitative resistance to northern leaf blight of maize**

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Northern Leaf Blight (NLB), caused by *Setosphaeria turcica*, is causing alarming losses to maize producers in the U.S.A. and elsewhere. We have sought to understand the genetic architecture of resistance and to identify the genes underlying the resistance loci identified. Certain genes have been implicated in resistance through fine mapping, association analysis and expression studies. For some of these genes, mutant lines were selected from the *UniformMu* population and other sources. For a minority of the genes tested, inoculation studies conducted in the greenhouse and field showed that the mutants had altered disease phenotypes. For most genes, the mutants were more susceptible, but for others the mutants were more resistant. Genes with confirmed mutant effects include the tasselseed1 and tasselseed2 genes; brown midrib genes; the pangloss gene; a remorin gene; an F-box domain protein, a guanylyl cyclase, and a calcium-dependent metal ion binding domain. These findings suggest diverse mechanisms for quantitative disease resistance and susceptibility. Some mutant lines showed altered disease phenotypes not only for NLB, but also for other diseases. There is growing evidence that multiple genes underlie some quantitative trait loci.

#### **Molecular determinants of Grapevine fanleaf virus in *Nicotiana benthamiana***

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Grapevine fanleaf virus (GFLV) is the primary causal agent of fanleaf degeneration, one of the most devastating viral diseases of grapevine. GFLV, from the genus *Nepovirus*, family *Secoviridae*, has a bipartite (+) sense RNA genome and both RNAs are necessary for systemic plant infection. RNA1 encodes proteins for polyprotein maturation and genome replication. RNA2 encodes proteins for replication of RNA2, cell-to-cell movement of the virus and genome encapsidation. Grapevines infected with GFLV show a variety of symptoms, including abnormally lobed (fan-shaped) leaves, mosaics, vein yellowing, and internode shortening. Infected vines also produce fewer and poorer quality berries and have a reduced productive lifespan. GFLV has a limited host range and little is known of the virus-host interactions which lead to symptoms. Previous work using chimeras of symptomatic (strain GHu) and asymptomatic (strain F13) isolates of GFLV in the plant model species *Nicotiana benthamiana* have shown that vein-clearing symptoms in this host are dependent on a 408-nucleotide sequence at the 3' end of the RNA1-encoded RNA-dependent RNA polymerase gene of GFLV-GHu. Recent work has shown that the 5' proximal nucleotides of the GFLV-GHu symptom determinant can be replaced with the cognate sequence from GFLV-F13 without affecting symptom development in *N. benthamiana*. Increasing the knowledge of GFLV's basic biology will provide new insights into how this virus causes symptoms in its hosts.

#### **Citrusgreening.org – A systems biology resource for vector biologists**

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Huanglongbing (HLB) is a tritrophic disease complex involving citrus host trees, the Asian citrus psyllid (ACP) insect and a phloem restricted, bacterial pathogen *Candidatus Liberibacter asiaticus* (CLAs). It is considered to be the most devastating of all citrus diseases, and there is currently no adequate control strategy. We have designed a web portal with information for consumers and growers as well as genomics resources for citrus, ACP and CLAs. This is an open source software project with the goal of developing a system for storing, integrating and analyzing all -omics data related to HLB. The JBrowse browser provides the context for expression data and annotated features on the genome. Pathway/Genome Database model biochemical pathways within each organism and will be used to explore the entire disease complex. This will allow systems-based analysis and the ability to target genes involved in multiple pathways using transcriptomics, proteomics and metabolomics data. Micro-CT analysis of the ACP will be combined with

transcriptomics data from different tissues, life stages and sexes to create a 3D atlas that will reveal the internal anatomy of ACP overlaid with the expression profile of different tissues across major life stages. The portal will allow manual curation so that the community can continuously improve the knowledge base as more experimental research is published. The portal can be accessed at <https://citrusgreening.org/>.

#### **Functional analysis of the uracil transporter (UraA) of *Erwinia amylovora***

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*Erwinia amylovora* is the causal agent for fire blight - a devastating bacterial disease of apples. Previous research showed that *E. amylovora* can obtain uracil from the host during disease development, however little is known about uracil transport into *E. amylovora*. In this study we functionally characterize the uracil transporter UraA from *E. amylovora*. The *EaUraA* open reading frame was cloned into the inducible plasmid pQE80L and introduced into the *E. coli* Keio strain JW2482-1 that lacks its endogenous *UraA* gene. Growth studies reveal that *EaUraA* allows transport of toxic nucleobase analogue 5-fluorouracil, but not 5-fluorocytosine, 8-azaguanine or 8-azaadenine, into cells. Radiolabeled uptake studies show that *EaUraA* transports [<sup>3</sup>H]-uracil with a high affinity ( $K_m = 0.56 \mu M$ ). Nucleobases adenine, guanine, cytosine, hypoxanthine, xanthine and the analogue 6-thioguanine act as weak competitive inhibitors of [<sup>3</sup>H]-uracil uptake. We have recently cloned the *E. amylovora* ORFs for each of the putative adenine; guanine/hypoxanthine; xanthine and cytosine transporters into pQE80L and are pursuing functional characterization. Future research will investigate the effects of *E. amylovora* nucleobase transporter mutants on fire blight disease development.

#### **Development of real-time isothermal amplification assays for the detection of *Phytophthora infestans* in leaf samples**

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Real-time Loop-Mediated Isothermal Amplification (LAMP) and Recombinase Polymerase Amplification (RPA) assays were developed targeting the ITS2 region of the ribosomal DNA of *Phytophthora infestans*, the causal agent of potato late blight. A rapid plant crude extract (PCE) preparation method from infected potato leaves was developed for on-site testing. The assays specificity was tested using several species of *Phytophthora* and other potato fungal pathogens. Both LAMP and RPA assays showed specificity to *P. infestans* and the closely related species *P. andina*, *P. mirabilis*, *P. phaseoli* and *P. ipomoeae*, but not reported as potato pathogen species. No cross-reaction occurred with the potato pathogens tested including *P. erythroseptica* and *P. nicotiana*. The sensitivity was determined using *P. infestans* pure genomic DNA incorporated into healthy PCE samples. Both LAMP and RPA assays detected DNA at 50 fg/ul and showed to be insensitive to PCE inhibition. The isothermal assays were validated with artificially inoculated potato plants using a Smart-DART platform for the PCE preparation and the real-time isothermal reactions. The LAMP and RPA assays effectively detected *P. infestans* DNA in symptomless leaf tissue as soon as 24h after inoculation. A rapid and accurate on-site detection of *P. infestans* in plant material will contribute to improved late blight diagnosis, early detection of first infections and facilitate prompt management decisions.

#### **Perspectives on the transition from bacterial phytopathogen genomics studies to applications enhancing disease control: from promise to practice**

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The advent of genomics has advanced science into a new era providing a plethora of “toys” for researchers in many related and disparate fields. Genomics has also spawned many new fields including proteomics, metabolomics, etc. furthering our ability to gain a more comprehensive view of individual organisms and of interacting organisms. Genomic information of both bacterial pathogens and their hosts has provided the critical starting point in understanding the molecular bases of how pathogens disrupt host cells to cause disease. In addition, knowledge of the complete genome sequence of the pathogen provides a potentially broad slate of targets for the development of novel virulence inhibitors that are desperately needed for disease management. Regarding plant bacterial pathogens and disease management, the potential for utilizing genomics resources in the development of durable resistance is enhanced because of developing technologies that enable targeted modification of the host. Here, we summarize the role of genomics studies in furthering efforts to manage bacterial plant diseases and highlight novel genomics-enabled strategies heading down this path.

#### **An *Epichloë festucae* endophyte antifungal protein with activity against the dollar spot pathogen**

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*Festuca rubra* subsp. *rubra* (strong creeping red fescue) is an important cool-season low maintenance turfgrass species, often infected with the fungal endophyte *Epichloë festucae*. Endophyte infected strong creeping red fescue exhibits fungal disease resistance to *Sclerotinia homoeocarpa* (the causal agent of dollar spot disease) and *Laetisaria fuciformis* (the causal agent of red thread disease). Endophyte-mediated disease resistance is not a general feature of other grass/*Epichloë* endophyte symbioses, and the mechanism of the disease resistance is currently unknown. In a previous SOLiD-SAGE transcriptome comparison between endophyte infected and endophyte free strong creeping red fescue, a highly expressed fungal gene that may be involved was discovered. The encoded protein is similar to a *Penicillium* antifungal protein and an *Aspergillus* antifungal protein. Because of the similarity of the endophyte abundant transcript to other antifungal protein genes, the endophyte protein could be a potential antifungal protein that confers disease resistance to the plant host. We have extracted the protein from the apoplast of endophyte infected strong creeping red fescue and also expressed the protein in yeast. We confirmed the antifungal activity of the partially purified protein through four different assays. We are also attempting to knockout the endophyte antifungal protein gene to assess its effect on dollar spot disease resistance in strong creeping red fescue.

#### **Structure of *Cercospora beticola* populations in organic and conventional table beet production in New York and implications for disease management**

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New York is the second largest producer of processing and fresh market table beet in the United States. *Cercospora beticola* is one of the most damaging pathogens of *Beta vulgaris* (sugar beet, table beet and swiss chard) worldwide, and a major limiting factor to table beet production in New York. The objective of this study was to understand the genetic diversity and structure of the *C. beticola* population in local table beet fields representing organic and conventional production, and investigate the presence of sexual reproduction in New York. A *de novo* genome assembly of *C. beticola* was constructed and used for identification of microsatellite loci. Eight novel microsatellite markers in addition to four previously published loci were used to genotype more than 360 isolates. The results showed significant structuring of *C. beticola* population in New York, and differences in the allelic and genotypic composition between production systems. The contribution of the results to better understanding the epidemiology of *Cercospora* leaf spot in New York and implications for disease management are discussed.



### **Establishing a baseline dataset of factors associated with mycotoxin risk in Indian village food systems**

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Mycotoxin contamination in food systems has serious implications for agricultural output and human health. Here, we report on the results of a preliminary survey of mycotoxin risk factors in 1 village in Telangana and a subsequent survey across 9 villages spanning cultural and agroecological contexts. In the preliminary survey, aflatoxin was detected in 6.5% of household food samples, of which 35.7% exceeded the Indian legal limit (30 ppb) for consumption. Fumonisin was detected in 20% of all samples analyzed, but at low levels (etc.) or grown on a villager's own farm had higher aflatoxin levels than food items acquired via market transactions. Surveys in 9 villages yielded 811 samples of staple food items and a semi-qualitative database of household- and vendor-level risk factors. Aflatoxin B<sub>1</sub> was detected ( $\geq 1$  ppb) in 21.6% of food samples. Overall, 10.5% of samples were contaminated with aflatoxin B<sub>1</sub> at levels greater than the Indian legal limit. Groundnut, maize, sorghum, and rice were the most contaminated commodities in both surveys. Observed food consumption behaviors suggest that these commodities may be important contributors to dietary aflatoxin intake across all sites. Ongoing analyses will enable deeper evaluation of the major behavioral, geospatial, and biological contributors to mycotoxin accumulation within and across study sites.

### **Dual RNA-seq of maize and *Setosphaeria turcica***

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Northern leaf blight (NLB) is a foliar disease of maize, caused by the hemibiotrophic fungus *Setosphaeria turcica*. Outbreaks of this disease can cause high yield losses and have been especially severe in recent years. We used RNA-seq to characterize the transcriptomes of both host and pathogen throughout pathogenesis. To explore the transcriptional differences between compatible (susceptible) and incompatible (resistant) interactions, we analyzed interactions between maize lines carrying or lacking the maize resistance (R) gene *Ht2* and *S. turcica* isolates carrying or lacking the complementary AVR gene *AVRHt2*. In the incompatible reaction between *Ht2*-carrying maize and *AVRHt2*-carrying *S. turcica*, both host and pathogen are seemingly arrested in the biotrophic phase, while in susceptible reactions, the biotrophy-necrotrophy transition is characterized by sudden and extreme shifts in both the host and pathogen transcriptomes. In *S. turcica*, gene-sparse regions are enriched for putative virulence-related genes, including predicted effector proteins and genes that are highly expressed in *planta* but not in culture. Ongoing research seeks to use transcriptome data to identify the *Ht2* and *AVRHt2* genes in concordance with mapping data.

### **CRISPR/Cas9 genome editing and its broad applications in plant pathology and crop breeding**

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The bacterial cluster regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 nuclease (Cas9) system has recently emerged as an efficient and versatile genome editing tool for various animal, plant and microbial organisms. In this study, we have performed precise genome editing and targeted mutagenesis in both rice (*Oryza sativa*) and rice blast fungus (*Magnaporthe oryzae*) with the CRISPR-Cas9 system. The engineered guide RNAs (gRNAs) were shown to direct the Cas9 nuclease for precise cleavages at the desired genomic sites and introduce specific mutations in rice and rice blast fungus at high efficiencies via non-homologous end joining repair or homology-dependent repair, respectively. In addition, a polycistronic tRNA-gRNA editing strategy was developed to facilitate multiplex genome engineering for simultaneous mutation of multiple genes, deletion of chromosomal fragments, and other more sophisticated applications in plants, fungi and other organisms. With improved bioinformatics and molecular tools, the CRISPR/Cas9 genome editing technology is being demonstrated to have broad applications in studying plant-pathogen interactions and engineering crop disease resistance.

### **Identification of host targets of the novel Gr29D09 effector family from the potato cyst nematode *Globodera rostochiensis***

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Along the course of parasitism, sedentary plant-parasitic nematodes secrete effector proteins into their hosts. These effectors exert different functions to promote successful infection, ranging from modifying the host's cell wall to suppressing plant defense system. 29D09 is a conserved effector family found in several cyst nematode species. Our research has shown that Gr29D09 from the potato cyst nematode *Globodera rostochiensis* can suppress plant immunity thereby facilitating nematode parasitism. However, the molecular mechanisms of defense suppression by Gr29D09 in host cells are largely unknown. Gr29D09 is expressed in the dorsal gland cell and highly up-regulated in nematode parasitic stages. Additionally, transgenic potato lines with *Gr29D09* overexpression became more susceptible to *G. rostochiensis* and an unrelated pathogen *Streptomyces scabies*. We further found that Gr29D09 can suppress both pattern-triggered immunity and effector-triggered immunity demonstrated by agrobacterium-mediated transient expression assays in *Nicotiana benthamiana*. To further reveal the role of Gr29D09 in plant immunity suppression, we used ectopic expression coupled with mass spectrometry analysis and identified a list of Gr29D09-targeting candidates in host cells, including hexokinase1 and heat-shock protein 90. Our on-going *in planta* interaction assays will provide insights into how Gr29D09 effectors may modulate plant immune systems to facilitate nematode parasitism.