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A molecular mechanism of age-related resistance

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Age-related resistance (ARR) refers to a gain of disease resistance during shoot or organ maturation. ARR associated with vegetative phase change, a transition from the juvenile to the adult vegetative phase, is a widespread agronomic trait in fruits, vegetables, and row crops, affecting resistance against viruses, bacteria, fungi, oomycete and insects. However, it is still unclear how innate immunity is differentially activated in successive stages of shoot maturation on a plant. MicroRNA156 (miR156) and its targets, the SQUAMOSA-promoter binding protein-like (SPL) transcriptional factors, are conserved regulators of vegetative phase change. We found that *Arabidopsis thaliana* (Arabidopsis) showed ARR against its bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000) during a transition from the juvenile to the adult stage. The timing of ARR activation was associated with a temporal drop of miR156 level. A systematic analysis of the loss- and gain-of-function mutants of 11 *SPL* genes revealed that a subset of *SPL* genes, including *SPL2*, *SPL10*, and *SPL11* activated ARR in the adult stage. The immune function of *SPL10* was independent of its function in regulating morphogenesis. In addition, we found that the *SPL10*-mediated age-dependent activation of the salicylic acid pathway contributes to ARR. Disrupting SA biosynthesis or signaling abolished the age-dependent gain of resistance against *Pto* DC3000. Given the conserved roles of miR156 in regulating developmental timing in plants, miR156 may contribute to age-related resistance in various pathosystems.

Exploration of a novel density-dependent volatile-induced antimicrobial compound produced by *Xanthomonas perforans*

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For most of the 20th century, *Xanthomonas euvesicatoria* (*Xe*) was the only known causal agent of bacterial spot of tomato (BST) in Florida. *X. perforans* (*Xp*) was first isolated in 1991 from Florida tomato fields and quickly replaced *Xe* via production of bacteriocins (BCNs) against *Xe*. However, in a previous study, a *Xp* mutant with nonfunctional BCNs was still able to outcompete *Xe* in the field. We discovered that the BCN mutant produced an antimicrobial compound that is effective against *Xe* in vitro. This compound was volatile-induced, cell density-dependent, and expressed under limited nutritional conditions and at a later growth stage than the previously described BCNs in *Xp*. Production of the volatile-induced antimicrobial compound was not conserved across *Xp* field strains, but other *Xanthomonas* species (*X. gardneri*, *X. vesicatoria*, *X. cynarae*, *X. campestris* pv. *campestris*) also produced a similarly expressed antimicrobial compound against *Xe* and elicited production of the antimicrobial compound by *Xp*. A wide range of other plant pathogenic bacteria, including *Clavibacter michiganensis* subsp. *michiganensis*, *Pantoea stewartii*, and *Pseudomonas cichorii*, also elicited production of this antimicrobial compound by *Xp*. To identify the antimicrobial compound, we performed mass spectrometry of agar where the antimicrobial was present, compared with agar from lower cell density Petri plates where the antimicrobial was not

observed. Further examination of this antimicrobial compound will enable a better understanding of pathogen interactions and fitness factors in the environment.

Determining the distribution of quinone outside inhibitor (QoI) fungicide-resistant *Cercospora sojina* on soybean from Indiana
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Frogeye leaf spot (FLS) is an important foliar disease in soybean (*Glycine max* L. (Merr.)) caused by the fungal pathogen *Cercospora sojina* K. Hara. FLS is predominantly found in the southern United States, but it has become more common in Indiana causing significant yield losses in recent years. Foliar fungicide applications of quinone outside inhibitors (QoIs) have been one of the major tools in the management of this disease, but 21 states have already confirmed the presence of QoI-resistant *C. sojina*, including Indiana. QoI-resistant isolates have been documented in only 4 of the 92 counties in Indiana (Clark, Delaware, Fountain, and Pulaski Counties), but further mapping of these resistant populations will help in directing current management strategies. In the summers of 2019 and 2020, 406 isolates of *C. sojina* were collected from 32 new counties across Indiana and screened for QoI-fungicide resistance using a PCR-RFLP method. An in vitro fungicide sensitivity test was performed on a subset of isolates to evaluate the sensitivity of *C. sojina* isolates to QoI and Demethylation Inhibitors (DMI) fungicides. A discriminatory dose of picoxystrobin (QoI) was established (1 µg/mL) to distinguish between QoI-sensitive and resistant isolates. QoI-resistant isolates were found in 29 new counties. 251 out of the 406 isolates (61.8%) were confirmed as QoI-resistant. Partial nucleotide sequences of the *cyt b* gene from two resistant and two sensitive *C. sojina* isolates were analyzed to corroborate the presence and absence of the G143A mutation, respectively.

Using historical fungicide records to evaluate use patterns

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Poor pesticide application practices such as lack of product rotation and poor timing can be contributing factors to the selection for pesticide resistance in a target organism. The Fungicide Resistance Assessment Mitigation and Extension network has been collecting fungicide records from consenting grape growers in Michigan (MI), Oregon (OR), and Washington (WA) to gain a better understanding of use practices in commercial vineyard settings. Records span from 2015 to 2020, and encompass conventional, organic, and certified sustainable program participants. Across all three regions, the total average annual applications were seven. Within each region, the annual average applications were seven (MI), eight (OR), and six (WA). The mean application interval was 14 days; by state, the mean was 16 (MI), 12 (OR), and 14 (WA). FRAC M02 (sulfur) applications comprised 32% of all applications; 13% were applications of products that were not classified (NC, e.g., mineral oil), 11% were FRAC 3 (demethylation inhibitors), 11% were FRAC 50 (aryl-phenyl ketones). Forty-six percent of all applications were tank mixes. First applications of the season were mostly FRAC M02 (40%) and NC (38%). However, final applications of the season were FRAC 3 (18%), FRAC 13 (15%), and FRAC U06 (13%). These analyses can give us insight into how grower choices can vary by a variety of factors such as operation size, region, and product marketing. This information can be used by extension personnel to identify potentially problematic practices and adjust fungicide stewardship recommendations.

Grapevine protection sensing: Non-destructive fungicide activity monitoring with in situ spectroscopy

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Grapevine powdery mildew (*Erysiphe necator*) is a destructive disease. Management is primarily achieved by fungicide applications at 10- to 14-day intervals, but fungicide overuse has environmental, financial, and resistance consequences. A reliable method to monitor in-field fungicide activity might enable growers to lengthen application intervals based on residual activity. The goal of this study was to determine if in situ spectroscopy can non-destructively quantify synthetic and bio-fungicide activity in grapevine over time. Metrafenone and *Bacillus mycoides* isolate J fungicides were applied in Chardonnay vineyards in Geneva, NY, in 2020. Leaf reflectance in the visible to shortwave infrared (VSWIR) electromagnetic range (350–2500 nm) was measured with a handheld spectroradiometer at 2-, 7-, and 14-days post-application (DPA). Fungicide efficacy was determined as an inverse function of powdery mildew colonization, quantified by a Blackbird imaging robot. At 2 DPA, area under the disease progress curve (AUDPC) was significantly different between control and fungicide treatments, and spectral measurements correlated with cube-root transformed AUDPC ($R^2 = 0.6$ for metrafenone). VSWIR wavelengths significantly discriminated among treatments with a two-class random forest accuracy of 62.2–97.2%. Overall, this study provides preliminary evidence that in situ spectroscopy can be used to determine fungicide activity, which could facilitate strategic fungicide applications and promote sustainable viticulture.

Magnesium-copper hybrid nanomaterials against copper tolerant *Xanthomonas perforans* and tomato bacterial leaf spot

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Bacterial spot of tomato (BST) is one of the most devastating diseases in hot, humid environments. BST is caused by four distinct bacterial pathogens: *Xanthomonas euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. cynarae* pv. *gardneri*. Bacterial resistance to copper-based bactericides and antibiotics makes disease management more challenging. This requires alternative solutions to manage the disease. We studied potential of novel hybrid nano-formulations of copper and magnesium; magnesium double coated (Mg-Db) and magnesium-copper (Mg-Cu) to control copper-tolerant *X. perforans*. In vitro experiments showed that no bacterial colonies were

recovered after 4 h of exposure to 500 ppm of both nano-formulations, while it required 24 h of exposure at 100 and 200 ppm for complete inhibition. In greenhouse experiments, Mg-Db and Mg-Cu at 100 ppm significantly reduced bacterial populations compared to Kocide 3000 (copper hydroxide) and the grower's standard, Kocide 3000 mixed with mancozeb (ethylene-bis-dithiocarbamate) ($P = 0.01$). There was no phytotoxicity observed in plants with the use of any of the nano-formulations. In a field experiment conducted in the fall of 2020, Mg-Db and Mg-Cu at 200 and 500 ppm significantly reduced disease severity compared to untreated controls, while the grower standard did not reduce disease severity ($P = 0.01$). These results suggest that these novel magnesium-based nanomaterials could be used to manage copper-tolerant bacterial pathogens.

Trichothecene NX-3 is required for *Fusarium graminearum* initial infection and disease spread

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Fusarium graminearum causes Fusarium head blight (FHB) on wheat and barley. In North America, three genetically distinct populations of *F. graminearum* have been identified and a different trichothecene chemotype predominates in each of these populations. Although deoxynivalenol (DON) plays a critical role in the ability of *F. graminearum* to spread FHB, it is unclear if 3,7,15-trihydroxy-12,13-epoxytrichothec-9-ene (NX-3) serves a similar function in FHB development. Therefore, we investigated the role of the trichothecene NX-3 in *F. graminearum* infection. Deletion mutants of Tri5 were generated from representative strains of the three different North American populations. No trichothecene production was observed in any of the Δ Tri5 mutants. FHB symptoms were restricted to the inoculated wheat florets when point-inoculated with the Δ Tri5 mutants, confirming the necessity of DON and NX-3 for FHB spread. However, whole head dip inoculations revealed a unique role of NX-3. Significant reductions in number of diseased florets and fungal biomass were observed in 44211 Δ Tri5 mutants compared to its parent strain 44211. Whereas no significant disease difference was observed between PH-1 Δ Tri5 and 46422 Δ Tri5 mutants and their respective parent strains. Additionally, while 44211 displayed less FHB spread, it caused infection comparable to PH1 and 46422 when evaluated by dip inoculation. Our results demonstrate that NX-3 is important for FHB development and provide novel information to control FHB and mycotoxin contamination caused by this newly recognized population.

The role of soil moisture in colonization of *Macrophomina phaseolina* on strawberry plants

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Macrophomina charcoal rot, caused by the soilborne fungus *Macrophomina phaseolina* (Mp), is an economically significant disease threatening California strawberry production, with few management options. In California, plants are typically planted in the fall but do not show symptoms until late spring. Studies in other crops have shown that ideal soil moisture can limit Mp colonization of plants. The objective of this research was to evaluate the role of soil moisture in colonization of Mp on strawberries. A field study was conducted from October 2019 through June 2020 in Irvine, CA. Soil moisture treatments were established at high, optimal, or low soil moisture content (SMC) as determined by soil water potential thresholds monitored using tensiometers. Soil moisture treatments were evaluated in a factorial split-plot design with two cultivars (Monterey, Fronteras) and two inoculation treatments (inoculated, control). Every 4-weeks post-inoculation, roots and crown from randomly sampled plants were cultured on Sorenson's NP-10. Mp was isolated from plants as early as 1-month post-inoculation. For crown isolation, an influence of irrigation was seen where Mp was isolated from significantly fewer Monterey crown pieces under high SMC compared to optimal SMC ($P = 0.0198$). For root isolation, however, a cultivar $\times$ inoculum interaction was observed ($P = 0.0245$) where Mp was isolated from fewer Fronteras roots than Monterey roots. Preliminary results indicate that high SMC may impede Mp colonization, but its role may be cultivar-dependent. The study is currently being repeated.

The *diageotropica* (*dgt*) mutation contributes to tomato resistance to *Ralstonia solanacearum* through a SA-independent mechanism

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Bacterial wilt, caused by the soilborne plant pathogen *Ralstonia solanacearum* (*Rs*), affects more than 200 species of plants and is among the most devastating plant diseases in the world. In tomato, resistance to *Rs* is quantitative, and no resistance genes to the U.S. *Rs* strain K60 have been identified. Transcriptomic analysis of resistant tomato roots showed that at 48-h post-inoculation with *Rs*K60, genes involved in auxin transport and signaling pathways are downregulated. In previous work, we found that a tomato mutant defective in auxin transport and signaling, known as *diageotropica* (*dgt*), has significantly enhanced resistance to *Rs*K60. Compared to wild-type plants, *Rs* colonization of the root xylem is delayed in *dgt*, and mutants did not show any wilting symptoms at 8-days post-inoculation. Auxin acts antagonistically with the plant hormone salicylic acid (SA), and we have found that *dgt* roots have higher levels of SA compared to the susceptible wild-type parent. In addition, SA-response genes are upregulated in non-inoculated *dgt*. However, after inoculation with *Rs*, expression of SA-response genes is not further activated in *dgt*. We crossed *dgt* to *NahG*, a transgenic tomato which constitutively degrades SA and is hypersusceptible to *Rs*. Although SA levels are reduced in *dgtNahG*, the double mutant maintains resistance to *Rs*K60. Our research suggests that resistance in *dgt* is due to defects in auxin transport and signaling, and is largely independent of SA. Understanding how DGT and auxin pathways function during BW is important for Solanaceae crop improvement.

In planta expression and functional characterization of non-coding RNAs involved in detection and response to plant hosts in *Dickeya dadantii*

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Dickeya sp. are necrotrophic bacterial pathogens that cause blackleg symptoms on potato and soft rot disease on many other plants. Similar to other plant pathogenic bacteria, it uses chemotaxis to sense plant defense compounds, find an opening, and enter the plant. Interestingly, *Dickeya* possesses an unusually high number of methyl-accepting chemoreceptors (MCPs) compared to other Enterobacterales, some of which have been shown crucial for disease. Long untranslated regions exist in the 5' extreme of most MCPs in *Dickeya*. However, their role is unknown. RNASeq data from our lab shows they are transcriptionally active in planta. We hypothesize they harbor regulatory sequences such as small non-coding RNAs (ncRNAs) that control expression of downstream genes. Bioinformatic analyses supported this by revealing promoter and terminator motifs in key regulatory positions. As well, transcriptional start sites (TSSs) determined via CappableSeq, matched the start of the RNASeq-identified transcription peaks. The levels of transcription of these regions were validated using qRT-PCR assays in vitro and in planta (in susceptible and tolerant potato lines). RB-TnSeq, mutant generation and testing, transcriptional reporter fusions and two-component sensors, are currently being used to characterize the biological function of these regions and MCPs, as well as determine the signals they are responding to. The project provides important insights into regulatory mechanisms used by *Dickeya* when interacting with plants and thus might provide targets for control of these pathogens.

Mutation of an amino acid ABC transporter reduces chemotaxis in the soft rot bacterium *Pectobacterium carotovorum* subsp. *carotovorum* WPP14

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Some ATP-binding cassette (ABC) transporters participate in bacterial chemotaxis by sensing specific environmental and intracellular molecules with a substrate binding protein and interacting with a methyl-accepting chemotaxis proteins (MCP). The role that ABC importers play in pathogenesis or chemotaxis has never been examined systematically in plant pathogens. In soft rot Pectobacteriaceae, the enrichment of ABC transporters and MCP and their co-localization in the genomes suggests these systems likely participate in common physiological processes. Phylogenetic analyses based on multiple sequence alignments of ATP-binding proteins and structure analyses of the substrate-binding proteins indicated that there are 27 ABC importers that are likely to interact with MCP and their enrichment compared to animal pathogens suggests that these proteins play an important role in phytopathogenesis. Here, we used a transposon-based system to construct a mutant library in *Pectobacterium carotovorum* subsp. *carotovorum* WPP14. From this library, we isolated an amino acid ABC importer mutant that had a reduced chemotaxis response to stem sap extracted from the yellow-skinned cultivar 'Lamoka' compared to the wild-type strain. However, the swimming and swarming motility were similar between the mutant and the wild-type strain. Currently, we are in the process of identifying the insertion site of Tn5SSgusA in this mutant and evaluating if the reduced chemotaxis response can be recovered by a gene complementation assay.

Elucidating the molecular basis of *Phytophthora*–plant interactions: From model plants to crops

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Oomycetes are the most destructive pathogens to agriculture threatening food security, biodiversity, and forestry. This study focuses on two devastating oomycete pathogens, *Phytophthora infestans*, the causal agent of potato and tomato late blight and *P. cinnamomi*, an invasive oomycete capable of infecting over 5,000 plant species including avocados. Despite the importance of *P. cinnamomi* to the avocado industry, the molecular basis of pathogenicity, host adaptation, and plant immunity are largely unknown due to lack of genomic resources and efficient transformation systems. Here, we identified several plant genes and pathogen effectors contributing to plant immunity and pathogenicity, respectively by utilizing reverse genetics, biochemical, omics, and functional genomic approaches. Several signaling proteins including transcription factors, NBS-LRRs, pathogenesis-related proteins, and genes involved in hormone signaling and production of antimicrobial compounds were associated with resistance to these pathogens. Finally, we also identified several *P. cinnamomi* effectors differentially regulated at different stages of pathogen infection. Interestingly, these effectors reside in gene-sparse regions in the *P. cinnamomi* genome when compared with housekeeping genes which reside in gene-dense regions. Ultimately, the identification and characterization of key pathogen and host genes associated with susceptibility and resistance will aid in the development and deployment of *Phytophthora* resistant plants.

Potential adaptive genetic variation in *Macrophomina phaseolina* as revealed by environmental association on the whole-genome data

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Macrophomina phaseolina is a worldwide distributed pathogen of many economically important crops. As causal agent of charcoal rot, it can significantly reduce yield and seed quality in soybean and dry bean mainly in tropical and subtropical regions, but more recently in temperate zones as well. Little is known on the relationship of genomic variation with geography and climate, nor which climatic variables may be driving differentiation in *M. phaseolina*. Genomes of 95 *M. phaseolina* isolates from 13 states across the U.S., Puerto Rico, and Colombia, isolated from soybean and dry bean were sequenced to determine whether populations are structured by geography

or environment. Inference on population structure revealed clustering of Puerto Rican and Colombian isolates from dry bean, while isolates from the U.S. regardless of dry bean or soybean origin were assigned to a separate cluster. To investigate the relative contributions of geography and environmental differences to genetic differentiation between populations and loci associated with environmental variation, partial redundancy analysis (RDA) and genome scans for selection were used. Environmental factors explained more genomic variation than geography, with temperature seasonality and precipitation explaining the most SNP variation among isolates, suggesting that local adaptation to heterogeneous environments may be an important source of genetic variation in *M. phaseolina*. The loci significantly associated with multivariate environment were investigated for their potential roles in mediating adaptation in *M. phaseolina*.

Prevalence of mutations conferring resistance to QoI and SDHI fungicides in the early blight pathogens on tomato (*Alternaria* spp.)

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Early blight of tomato (*Alternaria* spp.) is a common disease in the southeastern United States. Both quinone outside inhibiting (QoI) and succinate dehydrogenase inhibiting (SDHI) fungicides are used to manage this fungal disease, but failure of some of these fungicides has been reported. In 2017–2018, 47 isolates of *Alternaria* spp. were collected from 26 symptomatic tomato fields in 9 counties of NC, TN, and GA. Isolates were screened for mutations conferring resistance to QoI (F129L, G143A, and G137A) and SDHI (H278Y/R, H134R, H133R, and D123E) fungicides using Sanger sequencing. Seven fungicide programs were evaluated for management of early blight (using a QoI-resistant isolate) and compared to a non-treated control. Ninety-six percent of isolates were identified as *Alternaria linariae* and 4% were *A. alternata*. Seventy-six percent of isolates possessed the mutation conferring resistance to QoI fungicides with a frequency of 65% for F129L and 35% for G143A. Forty-nine percent of isolates had a mutation conferring resistance to SDHI fungicides with a frequency of 91% for H278Y and 9% for D123E. At least one isolate collected from 24 of 26 fields possessed a mutation conferring resistance to either or both QoI or SDHI fungicides, while isolates from two fields using organic practices had neither mutation. All seven fungicide programs managed disease better than no treatment. In this geographical region, *A. linariae* is the predominate causal agent of early blight on tomato and resistance to QoI and SDHI fungicides is widespread in this fungal population.

Effector binding element associations with resistance QTL against *Xanthomonas oryzae* pathovars in *Oryza sativa*

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Durable resistance to *Xanthomonas oryzae* pathovars is highly sought after in rice due to the crop yield losses caused by the pathogens. Using a multi-parent advanced generation intercross rice population and genome-wide association studies and interval mapping, quantitative trait loci were previously identified that are significantly associated with resistance to multiple strains of *Xoo* and *Xoc*. What is unknown is the molecular basis for the induction of genes under QTL during infection. We hypothesize that interactions of *Xoo* and *Xoc* transcription activator-like effector proteins with rice gene promoters under the QTL lead to the resistant/susceptible phenotypes exhibited across varieties. To test this, we designed a pipeline that predicts candidate genes involved in quantitative resistance by considering three criteria: the presence of an effector binding element in the gene's promoter, a significant correlation between EBE presence and disease phenotype and overlap of significant genes with resistance QTL. With this analysis, we used the eight MAGIC founders to generate profiles for candidate genes involved in resistance against *Xoo* and *Xoc* strains. Candidate genes include ones encoding an NB-LRR, transposon-related, and CCR4-NOT proteins. Frequently, fine mapping is used to identify genes involved in resistance. While this method is effective, it is also time-consuming. A pipeline that associates EBEs with the induction of candidate genes and disease resistance will streamline the identification of genes involved in quantitative resistance to TALE-producing *Xanthomonas*.

Comparing computer vision disease scouting to human scouting for grapevine downy mildew detection and fungicide efficacy quantification

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Downy mildew (DM, *Plasmopara viticola*) is the leading barrier to production in the eastern U.S. winegrape industry. DM can appear at nearly all stages of vine growth on clusters, canes, and leaves, and can result in total crop loss without chemical protection. We previously developed a deep learning-based image analysis pipeline capable of quantifying DM severity in vineyards with high accuracy (mIOU = 0.8). The goal of this study was to compare our computer vision model's performance to that of human experts for rating DM fungicide efficacy. A custom ATV color imaging system was used to collect georeferenced stereoisimages in the Cornell Pathology Vineyards (Geneva, NY) DM fungicide efficacy study yielding 3,581 image pairs. Human experts manually rated plants for DM during image collection. Image stereo pairs were segregated into panels and duplicates removed based on geolocation. The developed pipeline was used to calculate DM infection severity as the ratio of total infection area to total canopy area. Experimental results showed that imaging-derived severity rates highly correlated ($r = 0.93$) with expert field assessments. ANOVA results found that imaging-derived assessment had adequate statistical power for treatment separation equivalent to human scouting. Our novel approach to disease scouting lays the foundation for automated, in-field DM quantification on robotic platforms for precision disease management.

Bacteria associated with Russian wheat aphid honeydew initiate plant defense responses

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In the phytobiome, plant health outcomes are largely based on the dynamic interactions of multiple organisms. Our previous work demonstrated that variation in Russian wheat aphid (RWA, *Diuraphis noxia*)-induced chlorosis on wheat is determined, in part, by aphid-associated bacteria. Aphids with high titers of bacteria induced gene expression and accumulation of salicylic acid (SA), a hormone that inhibits insect resistance, in susceptible wheat. High, sustained expression of SA biosynthetic genes was followed by downregulation of jasmonic acid (JA) biosynthetic genes; JA is associated with insect resistance. We hypothesize that aphid-associated bacteria contribute to aphid virulence by modulating the plants' insect defense mechanisms. Although saliva from aphids contains culturable bacteria, scanning electron microscopy (SEM) studies did not detect bacteria in high numbers in the aphid salivary glands or foreguts. However, the same genera of bacteria were detected in aphid honeydew as in saliva; thus, we are focusing on the role of honeydew in induction of plant defense. To simplify analysis, we tested barley, an alternate host for RWA with considerable genetic resources and found that aphids with higher bacterial titers were more virulent to barley than aphids with low bacterial titers. In this presentation, we will report on the impact of honeydew on plant defense response pathways in barley.

A small species-specific effector from *Colletotrichum graminicola* that targets the host nucleus and aids in virulence at the early stages of infection

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The fungus *Colletotrichum graminicola* is the causal agent of maize anthracnose. Fungal effector proteins are important for disabling plant defense components and promote successful infection. We have identified *C. graminicola* effector protein 3 (CgEP3). Based on evolutionary analysis, we determined that CgEP3 is specific to *C. graminicola*. Also, the genomic region including *CgEP3* did not have synteny with other closely related species of *Colletotrichum*. We propose that genomic rearrangements led to the evolution of a new gene, *CgEP3*. Using heterologous transient expression in tobacco plants, we discovered that CgEP3 is localized to the nucleus and cytoplasm of host cells. Transcriptional profiling and live cell imaging show that *CgEP3* is specifically expressed in conidia and appressoria suggesting that it is functionally important at this time-point. *CgEP3* null mutants had significantly reduced lesion size, appressorial penetration and biomass accumulation on maize leaves and stems but, internal appressoria pressure and development are normal. Complementation of the *CgEP3* null mutant restored the wild-type phenotype. A transcriptomic analysis designed to understand the plant responses to CgEP3 during the colonization of maize leaves revealed that several plant defense genes are suppressed by CgEP3. We propose that CgEP3 is a pre-penetration effector that can help the fungus to disable plant defenses during the early stages of infection.

Effects of 2,4-diacetylphloroglucinol on disease suppression of *Aphanomyces* root rot (*Aphanomyces euteiches*) of field pea

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Aphanomyces euteiches is an oomycete which causes *Aphanomyces* root rot (ARR) on field pea. Currently, no effective approaches are available to control ARR. Soil beneficial bacteria that are antagonistic to *A. euteiches* have been identified and show promising control effects against ARR. However, antimicrobial compounds that are produced by the antagonistic bacteria and inhibit *A. euteiches* remain unknown. Here, we demonstrated 2,4-diacetylphloroglucinol (DAPG) is the major antibiotic employed by *Pseudomonas protegens* Pf-5 to inhibit *A. euteiches* mycelial growth and suppress ARR on field pea. Mutation of the DAPG biosynthesis gene *phlA*, but not the biosynthesis genes for other five antibiotics including HCN, oframide A, rhizoxins, pyrrolnitrin, and pyoluteorin, abolished the inhibition of Pf-5 against *A. euteiches* on PDA plates. Purified DAPG and its intermediates phloroglucinol (PG) and monoacetylphloroglucinol (MAPG) were also tested in the inhibition assays. DAPG inhibited *A. euteiches* mycelial growth at an amount of 25 nmol. Neither PG nor MAPG inhibited *A. euteiches* at an amount of 50 nmol. DAPG-treated *A. euteiches* hyphae showed extensive branching. Compared to a DAPG-negative mutant, the DAPG-producing strain of Pf-5 showed a significant higher control efficacy against ARR on field pea under greenhouse conditions. We are currently investigating the regulation of DPAG biosynthesis by Pf-5 during its interactions with *A. euteiches* and/or the plant host field pea.

Secretome profiling of *Fusarium oxysporum* f. sp. *albedenis*

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Date palm (*Phoenix dactylifera* L), of palm family (Arecaceae), is considered the most important tree that sustains life in oasis ecosystems. Besides its edible fruits, it provides numerous natural capital assets, such as providing shade and local cooling that creates a microclimate suitable for growing other crops, therefore, extensively contributing to the settlement of populations in oases. Fungal diseases of palm trees pose a threat to the health and value of oasis ecosystems. One such disease is Bayoud caused by the soilborne ascomycete fungus *Fusarium oxysporum* f. sp. *albedenis* (Foa). The Bayoud disease is characterized by discoloration from green to whitish of the middle crown leaf, followed by crown dieback and ultimately the death of infected trees. Here, we aim at investigating the molecular basis of Bayoud disease. We use Foa genome to predict its secretome. Of 1,464 predicted secreted proteins, 1,077 sequences contain less than 500 amino acids. While a third of predicted secreted proteins had no Pfam domain, a large set are predicted

carbohydrate-active enzymes (CAZymes) and cysteine-rich. Foa secretome comprises several necrosis inducing toxins and putative effectors that are predicted to modulate the host immunity. Some of these effector proteins are likely to be key contributing factors that can influence the outcome of the Bayoud disease outbreak.

Effectors of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*

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The obligate biotrophic oomycete pathogen *Pseudoperonospora cubensis* causes downy mildew in cucurbits. Presently, two clades of *P. cubensis*, Clade 1 and Clade 2, have been defined based on phylogenetic analysis with traditional molecular markers. *P. cubensis* isolates belonging to Clade 2 are considered to be a recent introduction to the United States and presumed to be responsible for the downy mildew epidemic in 2004. With the aim of identifying clade-specific effector-encoding genes in *P. cubensis*, the genomes of one representative isolate from both Clade 1 and Clade 2 were sequenced and secretomes predicted. Using the R package effectR and a web-based Galaxy toolshed specifically designed for oomycete effectors, we identified 234 and 252 effectors in Clade 1 and Clade 2 isolates, respectively. The expression pattern of genes in Clade 1 and Clade 2 was profiled by an RNA-seq time-course experiment with samples collected at various timepoints of infection. Several effectors showed upregulation in Clade 2 as compared to Clade 1 but not vice-versa. An orthology analysis was performed and single-copy orthologs were identified in Clade 1 and Clade 2. Single-copy orthologs that showed differential expression patterns were selected for an RT-qPCR analysis with different timepoints of infection. Some of the selected effectors showed differential RT-qPCR expression pattern between the two clades. Functional analysis is being conducted in these effectors with the aim of using them for effector-assisted breeding to identify new sources of resistance to downy mildew in cucurbits.

Understanding the diversity of *Passalora fulva* isolates from high tunnel tomato production

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High tunnels extend the growing season of high-value crops, such as tomatoes. Unfortunately, the environment in a high tunnel is conducive to the spread of the tomato leaf mold pathogen, *Passalora fulva* (syn. *Cladosporium fulvum*). The disease leads to defoliation and, ultimately, yield losses. Despite existing molecular research about *P. fulva*, little is known about current pathogen populations in high tunnel tomato production. From 2016 to 2019, samples were collected from high tunnels in the Northeast and Minnesota, and the fungus was isolated and characterized. We first confirmed Koch's postulates to demonstrate that the *P. fulva* isolates collected were the causal fungus. Given the qualitative nature of resistance, race assays were conducted by inoculating differential sets of tomatoes with each isolate. Of the 50 isolates tested, 44 isolated were race 2, and 6 isolates were race 0. For each isolate, we sequenced four previously characterized effector genes and examined the polymorphisms. *Avr2* had the greatest number of polymorphisms within the coding region, compared to no observed polymorphisms within the *Avr4* coding region. For greater insights on diversity, genotyping-by-sequencing was performed. Regional differentiation between isolates collected from Minnesota and the Northeast were identified. Information from this study will enable better management recommendations to growers, as high tunnel production of tomatoes continues to expand.

Identification and molecular characterization of a novel foveavirus associated with camellia ringspot disease

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Ornamental camellias (*Camellia* spp.) are a group of globally important landscaping plants. Various diseases with viral symptoms have been reported in camellias for many years. In this study, three groups of viral contigs were identified by high throughput sequencing (HTS) from a camellia plant with ringspot symptom. One large contig had the highest nucleotide (nt) sequence identity of 96% to the recently described camellia ringspot associated virus 1 (CRSaV-1), and two contigs were 85–86% identical to CRSaV-2 at nt sequence level. The fourth viral contig of 8,432 nt shared the highest nt sequence identity of 59% with Asian Prunus virus (APV-2), suggesting it was a new species. The complete genome of this new virus was determined to be 8,829 nt long, excluding 3' poly(A) tail. Its genome organization resembles those of known foveaviruses but contains an additional open reading frame at the 3' terminal region. Phylogenetic analysis also places this virus with members of the genus *Foveavirus* in the family *Betaflexiviridae* in the same subgroup. The virus, which is provisionally named as camellia ringspot associated virus 4, share 50–56% nucleotide sequence identities with other foveaviruses and should represent a new species of the genus. The virus was detected in additional two camellia trees from a public garden and a commercial sale. The study provides the information necessary to classify and detect the virus for further study of the diseases associated with camellias.

Beyond the peptidoglycan: Investigating the ability of Gram-positive Actinobacteria to induce plant immunity

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Gram-positive actinobacteria include pathogens of plants with diverse mechanisms, localizations, and interactions with their plant hosts. Despite the impact of these pathogens, little is understood regarding their interactions with the plant immune system. Species within the *Clavibacter* genus, an economically important group of actinobacterial pathogens, are known to colonize the plant xylem vessels to cause canker and wilt disease symptoms. For *C. michiganensis*, a devastating disease of processing tomatoes, there are no commercially available resistant cultivars. However, previous transcriptomic and proteomic studies show some plant immune components are induced

during infection such as the expression of PR1 and the production of ethylene. Since Gram-positive actinobacterial pathogens lack the type three secretion system, we hypothesize the potential for these bacteria to induce immunity may be through the perception microbe-associated molecular patterns (MAMPs). Computational analyses of the well-characterized MAMPs *elf18*, *csp22*, and *flg22* in plant-associated actinobacteria show notable differences in copy number and similarity to the consensus sequence in a MAMP-dependent manner. The *C. michiganensis* MAMPs *elf18* and *csp22* were tested against tomato genotypes and are immunogenic by their ability to induce ROS. The ability of tomato genotypes possessing receptors for *elf18* and *csp22* to restrict infection by *C. michiganensis* as well as the interaction between this pathogen and plant immunity will be reported.

Genome analyses of the avocado root rot pathogen *Phytophthora cinnamomi*

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Phytophthora cinnamomi (*Pc*) is a widely distributed and devastating soilborne oomycete pathogen infecting over 5,000 plant species threatening biodiversity, forestry, and agriculture. Wide genotypic and phenotypic variability regarding growth rate, virulence, and fungicide sensitivity have been observed among *Pc* isolates collected from avocado in California (CA). Pathogenomics will be used to uncover how genomic events lead to pathogen development, environmental adaptation, and increased virulence. The publicly available *Pc* reference genomes are fragmented and may not reflect the true genome of *Pc*; therefore, we generated high-quality reference genomes for two genetically and phenotypically distinct CA *Pc* isolates (strains Pc2109 and Pc2113), using long- and short-read sequencing technologies. Flow cytometry and k-mer analysis revealed that the true *Pc* haploid genome size is approximately 140 Mb. Ploidy analysis using two independent bioinformatic approaches revealed that these two CA field isolates are triploid. Consistent with other *Phytophthora* spp., the genome architecture of these *Pc* isolates fit the two-speed genome model. Notably, genes associated with pathogenicity reside in gene-sparse regions when compared with housekeeping genes which reside in gene-dense regions. These improved genomic resources reflect the most accurate representation of the *Pc* genome and will be used in future population and comparative genomics studies, as well as to study *Pc*-plant interactions to aid in the development of new control methods and *Pc* resistant crops.

A potential New Source of Rust (*Puccinia arachidis*) Resistance from Wild Peanut Relatives *Arachis stenosperma* and *Arachis batizocoi*

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Due to its low genetic diversity, cultivated peanut (*Arachis hypogaea*) is susceptible to many pathogens and pests and relatively few sources of resistance have been found in the cultivated gene pool. *Puccinia arachidis* (peanut rust) is a pathogen that plagues many peanut farmers around the world. This project focuses on a peanut population that has been produced by backcrossing *A. hypogaea* with an allotetraploid derived from the wild peanut relatives *A. batizocoi* and *A. stenosperma*, called GA-BatSten1. Segregant lines have been found to have resistance to various pathogens including rust. The goal of this work is to genetically identify wild segments that confer the resistance to rust to enable the production of new resistant lines using marker assisted selection. Rust is rarely found in U.S. fields, so detached leaf assays were used to assess the fitness of the plants. Individual leaves were inoculated and assessed for resistance by monitoring the number of lesions per leaf area. An index per area was also found using number of lesions, severity of the lesions, and sporulation status. These leaves were then genotyped using the Thermofisher Arachis Array with 36K SNP markers. So far, two highly resistant families from this population have been identified. Using this data will allow us to identify the source of rust resistance introgressed from the wild resistant alleles which will allow us to breed a new cultivar of rust resistant peanut that will be beneficial for peanut farmers around the world.

Cell-density regulated attachment proteins contribute to early disease development in *Ralstonia solanacearum*

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Adhesins (adhesive proteins) help bacteria stick to and colonize diverse surfaces and are important virulence factors. The *Ralstonia solanacearum* (*Rs*) genome encodes over 30 adhesin and adhesin-related genes. Although *Rs* has many putative adhesins and attachment is critical to host colonization, little is known about the role of adhesins in bacterial wilt disease. An in planta transcriptomic study revealed that 4 putative adhesins were upregulated in a quorum sensing mutant ($\Delta phcA$) unable to respond to high cell densities, while 10 others were downregulated. Based on this differential gene expression, we hypothesized that adhesins negatively regulated by PhcA contribute to early stages of disease when the bacteria are likely at low cell density. We used single and poly-mutants to assess the role of three adhesins upregulated in $\Delta phcA$, RadA, B, and C, to virulence and fitness in planta. Root attachment assays and confocal microscopy revealed that all three adhesins function in adhesion to tomato seedlings. However, as hypothesized, the adhesins are not essential for later stages of disease, including colonization of the root endosphere and stems and, subsequently, wilt symptom development in adult tomatoes. Interestingly, together RadB and C are indispensable for competitive fitness in planta. In vitro biofilm assays also indicate that RadA, B, and C may not function in cohesion, interbacterial attachment. Using a suite of plant assays, we have identified three adhesins that contribute to the first critical plant-bacterial interaction in bacterial wilt disease.

Identification of phloem translome alterations during *Candidatus Liberibacter asiaticus* infection in tolerant and susceptible citrus cultivars

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Huanglongbing (HLB) is a devastating disease of citrus worldwide. In the United States, HLB has been associated with the phloem-restricted bacterium *Candidatus Liberibacter asiaticus* (CLAs). CLAs can be spread by phloem feeding Asian citrus psyllids or through grafting. Molecular interactions between CLAs and the plant are required for accumulation and spread of CLAs in phloem tissues. However, many of these interactions are not well characterized. In this study, we have adapted translating ribosome affinity purification (TRAP) methodologies to analyze phloem specific responses to CLAs in citrus trees. This method uses tissue specific promoters to express FLAG-tagged ribosomal proteins for immuno-capture of cell-specific mRNAs. HLB-susceptible sweet orange, HLB-resistant Poncirus, and HLB-tolerant Carrizo (sweet orange × Poncirus) were transformed to express the FLAG-tagged ribosome protein L18 (RPL18) under the control of two phloem-specific promoters (pSULTR2;2 and p396ss), as well as the constitutive CaMV 35S promoter. Tagged ribosome complexes were purified from citrus leaves using anti-FLAG conjugated magnetic beads. High quality translome RNA was recovered from citrus expressing FLAG-RPL18 but not from non-transformed control plants. TRAP citrus lines have been infected with CLAs using no-choice Asian citrus psyllid feeding, and efforts are underway to identify phloem genes that are altered during infection. Identified genes will represent unique phloem specific targets for disrupting the CLAs life cycle.

The occurrence of diverse virus species in Texas cucurbit fields as determined by metagenomic analysis

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Texas is among the top producers of cucurbits in the U.S., but there is little knowledge on cucurbit-infecting viruses in the state. Consequently, field surveys were conducted during fall 2020 in nine cucurbit fields (4.1 to 174 ha) in the two major growing regions, the Lower Rio Grande Valley and Central Texas. Disease incidence ranged from 5 to 100% across fields and 118 plants were sampled for diagnosis. Total RNA extracts from sample subsets that represented the spectrum of observed symptoms were distributed into two composite samples, which were subjected to Illumina high-throughput sequencing (HTS). Analyses of the combined ~37 million single-end raw reads (75 nt each) from both samples revealed 107 contigs (206–10,013 nt) specific to 14 viruses. Among them, the near complete genomes of watermelon crinkle leaf-associated virus 1 (WCLaV-1), WCLaV-2 (putative novel *Phenuiviridae*), cucurbit chlorotic yellows virus (CCYV; genus *Crinivirus*; family *Closteroviridae*), and squash vein yellowing virus (SqVYV; genus *Ipomovirus*; family *Potyviridae*) were reassembled from the HTS contigs and showed high homologies to their respective exemplar isolates. The results were validated via RT-PCR, cloning, and Sanger sequencing of gene-specific virus segments obtained from the field-collected samples, revealing varied incidences of the viruses across fields. This study is the first report of WCLaV-1 and WCLaV-2 in the U.S., and that of CCYV and SqVYV in Texas, indicating the occurrences of diverse and potentially yield-limiting viruses in Texas cucurbit fields.

Association of a novel combination of an umbravirus, a satRNA, and cowpea polerovirus 1 with a bushy top disease of common bean (*Phaseolus vulgaris* L.)

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During surveys for common bean infecting viruses in Zambia in May 2018, bushy top and mosaic symptoms were observed on some plants in one field. Symptomatic leaf tissue samples were collected from five plants, preserved dry on silica gel, and used for total nucleic acid isolations, according to the CTAB method. Metagenomic exploration of the virome of one representative sample resulted in the recovery of genome-length sequences of Ethiopian tobacco bushy top virus (ETBTv; 4,238 nucleotides [nt]), ETBTv satellite RNA (satRNA-E; 521 nt), peanut mottle virus (PeMoV; 9,696 nt), and cowpea polerovirus1 (CPPV1; 5,902 nt) in the sample. The results were validated via RT-PCR and Sanger sequencing of gene-specific fragments for each agent in the five samples. In pairwise comparison, the four genomic RNA sequences shared 88 to 100% nt identities with their respective exemplar isolates. To fulfill Koch's postulate, inoculum prepared from symptomatic leaves of a source common bean plant was rubbed onto five plants each of carborundum-dusted common bean cv. Sutter Pink and *Nicotiana occidentalis*. Systemic leaves of both plant species produced bushy top symptoms 10- to 14-days post-inoculation and they tested positive for ETBTv, satRNA-E, and PeMoV by RT-PCR and Sanger sequencing. But the sap-inoculated plants of both species were negative for CPPV1. The results showed that the observed common bean bushy top disease was likely induced by mixed infection of ETBTv and satRNA-E, with CPPV1 serving as the likely luteovirid helper virus of the umbravirus agent under natural field conditions.

Baseline sensitivity and efficacy of pydiflumetofen to *Venturia effusa*

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Commercial pecan production in Georgia relies heavily on multiple fungicides to control the pecan scab pathogen, *Venturia effusa*. Many of these fungicides are experiencing some level of resistance development. Pydiflumetofen, a novel SDHI fungicide, was just labeled for use on pecan in 2020 and is widely used. In order to monitor resistance development, baseline sensitivity must be

established. Fifty isolates collected in 1996 from two different orchards with no SDHI exposure and stored in sterile water were plated on fungicide amended media containing 0, 0.0002, 0.002, 0.006, 0.02, 0.06, 0.2, 0.6, 2, and 6 ppm pydiflumetofen. Relative growth and EC₅₀ values were calculated. The average EC₅₀ value was 0.0015 ppm, with a range from 0.000001 ppm to 0.0102 ppm. The discriminatory concentration for future sensitivity monitoring will be 0.2 ppm, which suppressed 98.8% of the baseline isolates. Field efficacy of pydiflumetofen at 76 g/ha (P), difenoconazole at 122 g/ha (D) and P + D (as Miravis Top) was evaluated in 11 different pecan orchards around southern Georgia in 2019 and 2020. The P, D and P + D resulted in 97, 75, and 98% control, respectively. Quadris Top, the current industry standard, gave 79% control. Pydiflumetofen is extremely effective against *V. effusa*, but should be monitored for resistance development going forward.

Quorum sensing signal from plant pathogenic bacteria hijack host sterol biosynthesis and plasma membrane integrity to suppress plant defense responses

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Phytopathogenic bacteria rely on quorum sensing signals to coordinate population behaviors, such as motility or expression of virulence factors, which are essential for successful colonization of their hosts. However, little is known about the direct effects of these signaling molecules on plant cell biology, as well as the molecular mechanisms behind these interactions. Here, we report a novel mechanism by which the quorum signaling molecule DSF (cis-11-methyl-dodecanoic acid) produced by *Xanthomonas campestris* pv. *campestris* could interfere with the integrity of plant host plasma membrane by altering plant lipid profile, especially phytosterols that are highly enriched in the plasma membrane. As a result, this perturbation of membrane lipid composition interferes with membrane compartmentalization into nanodomains, as well as the clustering behavior and endocytosis of the flagellin receptor FLS2 and thereby, mitigates plant defense responses. These DSF-induced effects on receptor dynamics and host immune responses could be entirely reversed by pharmacological sterol removal. Our data demonstrate a novel mechanism that plant pathogens may deploy to alter the host membrane properties to their favor, and how membrane remodels intracellular pathways during biotic interactions through sterol homeostasis.

Bacterial warfare: Diversity, function, and role of Rhs polymorphic toxins within the *Ralstonia solanacearum* species complex

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Extracellular polymorphic rearrangement hotspot (Rhs)-toxin effectors are ubiquitous in prokaryotes and are under strong positive selection in bacterial genomes. These antagonistic proteins are understood to be vital mediators of intraspecies competition and contributors to bacterial fitness. Rhs-toxin systems are composed of a *rhs* toxin gene with a conserved N-terminus and a hypervariable C-terminus tip that carries the killing domain (i.e., nucleases, peptidases). Directly downstream of the toxin gene encodes an immunity protein that protects the cell from self-killing. Members of the *Ralstonia solanacearum* species complex (RSSC) are the causal agent of bacterial wilt, an economically devastating vascular disease that affects over 200 species of crops worldwide. Many strains in the RSSC are predicted to have multiple *rhs* toxin genes in their genomes, allowing for diverse inhibition profiles. In this study, we used a hidden Markov model to identify 502 *rhs* genes in RSSC with 44 distinct toxin domains and classified them into 4 subfamilies. We selected a warm-temperate strain K60, containing the highest number of predicted *rhs* toxin genes, to study Rhs toxin diversity, mechanisms of action, and their specific contribution to intraspecies competition. By understanding the diversity and function of these antagonistic proteins, we can elucidate their potential role in shaping host-associated microbiomes, pathogenic and competitive bacterial mechanisms, and novel management strategies against phytopathogenic bacteria.

Migration and recombination as main drivers of *Xanthomonas euvesicatoria* population shifts in Florida

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Xanthomonas euvesicatoria (*Xe*) is the causal agent of bacterial leaf spot (BLS) disease of tomato and pepper. During the 1980s and 1990s, *Xe* experienced multiple population shifts that led to the breakdown of resistance in commercial pepper cultivars and the displacement of *Xe* by *X. perforans* on tomato. To determine the processes that led to the emergence of new races of *Xe* in Florida, we analyzed the effector content and distribution in *Xe* strains, in the context of the species evolutionary history. We used a historical collection of 105 *Xe* genome sequences that spans 53 years from Florida and worldwide. Our evolutionary analysis revealed 7 distinct lineages, each with different temporal and geographic distribution that suggests the emergence of new races of *Xe* in Florida was caused by the introduction of lineages 5 and 7. Evidence of recombination within lineages 3, 4, and 5 are supported by the horizontal gene transfer of rare effectors between those *Xe* lineages. In contrast, the distribution of some effector allelic variants follows a pattern of vertical transmission, maintaining those alleles within a specific lineage and showing a strong phylogenetic signal. Our results demonstrate that *Xe* populations have been structured through processes of migration and recombination, and generate information relevant for the development of management strategies.

Insight into the root-associated microbiome related to brown root rot disease of *Ficus* trees

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Brown root rot (BRR) caused by the white rot fungus *Phellinus noxius* is a destructive tree disease in tropical and subtropical regions. Microbiomes in soil and roots are imperative for health maintenance and stress resistance of plants. However, little is known about how BRR affects the microbiomes in nature and how *P. noxius* interacts with other root-associated microorganisms to impact the health of host trees. In this study, next generation sequencing (NGS) and microbial isolation were conducted to investigate the microbiota associated with the roots of healthy and BRR-diseased ficus, one of the most widely distributed evergreen tree in Asia. The results indicated that the diversity and richness of fungal but not bacterial communities were largely decreased in *P. noxius*-infected roots. Among the fungi detected, *Cosmospora* was positively correlated with *P. noxius*, whereas *Aspergillus* and *Penicillium* were negatively correlated with *P. noxius*. Among the bacteria detected, different groups of *Bacillus* and *Streptomyces* were enriched in healthy and diseased samples. The potential interaction between *Cosmospora* and *P. noxius* was evaluated by dual culture and cultivation on the lignin medium. The growth of *Cosmospora* but not *P. noxius* was limited by high concentration of lignin. This finding indicated that the degradation of lignin by *P. noxius* may help create a suitable environment for other microorganisms to establish. Our results provide a comprehensive overview of the belowground microbiota of trees and the succession of the microbial community under the BRR disease pressure.

Maize yellow mosaic virus interactions with maize chlorotic mottle virus and sugarcane mosaic virus in mixed infections

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A maize-infecting polerovirus, variously named maize yellow dwarf virus RMV2 (MYDV RMV2), MYDV-like, and maize yellow mosaic virus (MaYMV), is frequently found in mixed infections in plants also infected with maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV), known to synergistically cause maize lethal necrosis (MLN). MaYMV was discovered in deep sequencing studies precipitated by recent maize lethal necrosis (MLN) emergence and is prevalent at global locations with MLN, but its role in or contribution to disease was not known. We examined how MaYMV impacted disease development in mixed infections with MCMV, SCMV, and both MCMV and SCMV compared to single infections and mock inoculated plants. Results demonstrated that MaYMV symptoms included stunting as well as leaf reddening in single and mixed infections. However, MaYMV did not recapitulate MLN synergistic disease in double infections in which either MCMV or SCMV was missing (MaYMV + MCMV or MaYMV + SCMV), but did significantly enhance stunting in mixed infections, and suppressed titers of both MCMV and SCMV in double infections. Interestingly, MaYMV strongly suppressed the SCMV-induced titer increase of MCMV in triple infections, but MLN symptoms still occurred with the reduced MCMV titer. These data indicate the potential disease impact of this newly discovered ubiquitous maize virus, alone and in the context of MLN.

Surveys for the resistance-breaking orthotospoviruses in tomato fields in Florida

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Members of the genus *Orthotospovirus* such as tomato spotted wilt virus (TSWV) and tomato chlorotic spot virus (TCSV) are thrips-transmitted and highly destructive viruses of tomato. A critical component of integrated pest management is the use of orthotospovirus-resistant tomato cultivars with the *Sw-5* gene. *Sw-5* is a single dominant gene responsible for resistance to TSWV and is also effective against TCSV and groundnut ringspot virus. Specific amino acid (aa) mutations in the nonstructural viral movement protein (NSm) of TSWV result in *Sw-5* resistance-breaking. In 2019, high incidences of tospovirus-like symptoms were observed in tomato fields established with *Sw-5* tomato cultivars in south Florida. To assess presence of potential resistance-breaking orthotospoviruses, we conducted field surveys in 2019–2021. Samples with orthotospovirus-like symptoms from both resistant and susceptible tomato plants were collected from several counties. The presence of *Sw-5* gene and orthotospovirus infection in all samples were verified by PCR and RT-PCR assays, respectively. Sequences of the NSm from 106 Florida orthotospovirus isolates were analyzed for novel and/or known resistance-breaking aa substitutions (C118Y/T120N). Based on sequence analysis, known aa substitutions were not present in any Florida isolates. However, a novel aa substitution (D17E) in the NSm was observed predominantly in TCSV isolates from resistant tomatoes. Future investigations are needed to determine the association of this mutation with emergence of a potential resistance-breaking virus isolate.

Bulk soil communities of *Fusarium* spp. are resilient to changes induced by crop and tillage practices

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The fungal genus *Fusarium* contains plant pathogenic, beneficial, and commensal strains. Past work suggests that non-pathogenic *Fusarium* strains can contribute to disease-suppression in soil by competition or induced plant resistance. Identifying factors that influence the abundance of non-pathogenic strains could lead to strategies that enhance plant-beneficial interactions and deter pathogen invasion. We developed an EF-1 α amplicon-sequencing method with sub-species resolution for *Fusarium* community analysis. This method reconstructed *Fusarium* EF-1 α sequences with 100% accuracy and performed close to expectations on synthetic communities. Using this method, we observed that non-pathogenic strains of *F. oxysporum* did not colonize different crops at uniform rates; some strains had asymptomatic host preferences. Whereas healthy plant roots were colonized somewhat evenly by different strains, the strawberry pathogen *F. oxysporum* f. sp. *fragariae* (*Fof*) typically accounted for >90% of sequences in susceptible strawberry roots. Bulk soil communities of *Fusarium* were remarkably stable regardless of crop history and tillage practices. Although the density of *Fof* in bulk soil (measured in CFUs/g) was higher after strawberry cultivation than fallow, the relative abundance of *Fof* (measured by EF-1 α amplicon sequencing) was not significantly different. In another cropping system, 18 years of conservation tillage and cover

cropping did not alter bulk soil *Fusarium* communities. We will discuss the implications of these results for the establishment of pathogenic strains in new fields.

Genetic and histochemical characterization of resistance to frogeye leaf spot in soybean

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Cercospora sojina is a fungal pathogen that causes frogeye leaf spot (FLS) in soybean. The proliferation of this disease in Louisiana has led to yield losses of up to 20%. Thorough genetic and phenotypic characterization of resistance is integral to the breeding process, and utilization of varieties resistant to local isolates is crucial to reduce these losses. However, limited knowledge exists on new resistance genes effective against local *C. sojina* isolates or how host cellular responses differ between varieties. To identify novel sources of resistance, we conducted greenhouse trials with lines from the soybean nested association mapping population. Plants were inoculated separately with conidia from four local *C. sojina* isolates at the V2 stage, placed in a dew chamber for 48 h, and evaluated 14-days post-inoculation. Our preliminary results indicate that lines LG04-4717, LG90-2550, and LG94-1128 exhibited significantly lower disease symptoms from each of the isolates used as measured by categorical and severity scales. To further understand mechanisms of resistance to FLS, we are currently examining reactive oxygen species (ROS) production and callose deposition from lines that harbor different resistance genes. Furthermore, an image-based analysis pipeline is being optimized and applied to score disease on multiple parameters, as well as precisely measure ROS and callose. The identification of new resistance genes and precise characterization of early cellular responses occurring in resistant varieties will aid in the development of host resistance to manage FLS.

Influence of potato host susceptibility on biology and virulence of oomycete pathogen *Phytophthora infestans*

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Phytophthora infestans mediates its interaction with a host plant to maintain biotrophy prior to a transition to necrotrophy. This includes circumventing the trigger of pathogen induced cell death. The fully susceptible potato cultivar ‘Katahdin’ does not have *R* genes that recognize *P. infestans* RXLR effectors to induce cell death. The modified potato plant, SP951, carries a single copy of the *RB* gene which can recognize a family of RXLR effectors. We investigated the impact that continued exposure to each plant type had on *P. infestans* virulence over time. We inoculated and re-isolated (passaged) *P. infestans* through each plant type 5 times. We found that after 5 passages, on both plant types, *P. infestans* experienced significant reduction in virulence ($P = 2.2 \times 10^{-16}$). When virulence was assessed on ‘Katahdin’ plants, isolates passaged through SP951 were significantly more virulent than isolates passaged through ‘Katahdin’ ($P < 0.05$). The broad loss in virulence was likely the result of the sub-culturing required between passages, a phenomenon previously investigated. The interaction of *P. infestans* with a partially resistant host significantly slowed this loss of virulence. The nature of these host-pathogen interactions, specifically the impact of molecular changes that a partially resistant host induces in *P. infestans* is yet to be determined; however, we have shown that a partially resistant host induced significantly greater virulence. Our work provides evidence to support the hypothesis that plant defense signals can induce changes in pathogen virulence.

Resistance to lettuce drop (*Sclerotinia* spp.): Cell wall composition analysis to determine the biological basis

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Sclerotinia spp. are one of the most generalist classes of fungal pathogens that release oxalic acid and cell wall degrading enzymes and initiate tissue maceration. The pathogens infect >700 plants worldwide, including lettuce, and capable of transitioning from a biotrophic to a necrotrophic lifestyle. Our understanding of host resistance to *Sclerotinia* spp. is limited. We recently identified soft basal stem as a plausible susceptibility factor to *Sclerotinia* spp. in lettuce. Greenhouse experiments on six lettuce genotypes with varied growth habits and resistance levels provided evidence of host cell wall chemical components on resistance to lettuce drop. Significant variations were observed among lettuce accessions in cellulose, hemicellulose, and lignin composition. Simple carbohydrates are elevated in both the basal stem and root tissues of lettuce genotypes susceptible to *Sclerotinia* spp. The finding implicated cell wall polymers recalcitrant to degradation by lignocellulolytic enzymes as candidates underlying strength-mediated resistance to degradation by *Sclerotinia* spp.

Microneedle extraction and smartphone-based isothermal amplification: A rapid solution for in-field detection of plant pathogens

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Molecular diagnostic assays are widely used to detect and track the spread of plant diseases in agriculture. However, current molecular assays involve sample shipment and in-house analysis, leading to relatively long result turnaround times of up to several days. Here, a portable molecular diagnostic platform consisting of a polymeric microneedle (MN) patch, a 3D-printed loop-mediated isothermal amplification (LAMP) cassette, and a smartphone-based fluorescence reader device has been developed to integrate nucleic acid extraction, amplification, and amplicon detection in a single device. The MN-extracted nucleic acid is purification-free and requires less than one minute to extract without using any equipment. This method was successfully applied to isolate host DNA from various plant species such as potato, tomato, and pepper, as well as pathogen nucleic acid biomarkers of *Phytophthora infestans* and tomato spotted

wilt virus (TSWV) from asymptomatic tomato leaves (e.g., day 1 and day 3 of post-inoculation for *P. infestans* and TSWV, respectively). Finally, the MN extraction was combined with a smartphone-based LAMP assay to detect pathogens directly in the field. A polydimethylsiloxane (PDMS) chip attached to a flexible polyimide heater was utilized to run parallel LAMP assays, and a 3D-printed smartphone microscopy reader was used to capture the fluorescence images of the cassette. This integrated MN-smartphone platform detected both *P. infestans* and TSWV simultaneously from co-infected leaves down to 1-pg sensitivity within 30 min without using benchtop equipment.

Genome-wide association study of citrus microbiome

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Although plant genotypes are known to be a determinant of microbiome composition, we lack an understanding of the mechanisms underlying how genotypes affect the microbiome, which hinders the utilization of genotypes in microbiome engineering. Microbiome engineering has been suggested to be a promising and more environmentally friendly solution for protecting crops against pests and promoting plant growth by reducing the usage of pesticides and chemical fertilizers. Citrus is a globally important, perennial fruit crop that normally contains scion grafted on the rootstock. To investigate the relationship between citrus genotypes and the microbiome, we sequenced the metagenome of leaf, root, and rhizosphere soil samples and the genome of corresponding citrus genotypes (56 scion genotypes and 9 rootstock genotypes) from one location using deep shotgun sequencing. Analyses of the relationship between citrus genotypes and the microbiome suggest that the plant genetic networks of the scion and rootstock are the main determinant of the leaf and root microbiomes, respectively, but they are also coordinated with each other. A genome-wide association study (GWAS) analysis identified genes associated with microbiome compositions and genus-level abundances. This study provides key information that aids the utilization of genotypes for microbiome engineering as well as the editing of specific genes to improve host microbiota.

Detection of the downy mildew pathogen *Pseudoperonospora cubensis* in spore trap samples from cucurbit fields using advanced molecular techniques

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Pseudoperonospora cubensis, the causal agent of cucurbit downy mildew (CDM), is an economically devastating disease to cucurbits in Georgia. The biotrophic oomycete pathogen, *P. cubensis*, is characterized by, and its spread mapped by, dispersal spores that are able to travel via air movement in the warm, humid summers of the southeast. Methods for early detection of CDM are therefore essential for the cucurbit industry, where an integrated management approach relies heavily on timely fungicide applications. The timing of fungicide applications can be greatly improved with early and sensitive molecular detection methods. In this study, we developed a cost-effective and simple spore trap collection method coupled with advanced molecular techniques such as qPCR and LAMP for early detection of *P. cubensis*. Airborne sporangia were collected from two locations in Georgia and amplified with *P. cubensis* specific molecular markers. The results demonstrated that this detection system was highly effective for species-specific *P. cubensis* detection. In addition, we compared the efficiency and the functionality of three different molecular detection techniques (PCR, real-time qPCR and LAMP), which showed real-time qPCR and LAMP were highly sensitive assays, compared to the conventional PCR test. Overall findings from these studies will guide growers in the use of effective fungicides at the appropriate time, which will greatly improve management strategies for cucurbit downy mildew in Georgia.

Fishing for NLRs: Utilization of a bait and capture technique to rapidly advance identification of soybean *Rps* resistance genes to *Phytophthora sojae*

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Seedling, root, and stem rot diseases caused by the soilborne oomycete pathogen *Phytophthora sojae* have historically been managed by deploying dominant resistance genes (*Rps* genes) in soybean (*Glycine max*). Selection pressure has led to the development of *P. sojae* pathotypes that overcome currently deployed *Rps* genes; however, fine mapping and cloning of novel *Rps* genes for breeding efforts can take 5–10 years. To rapidly identify and map novel *Rps* genes, Resistance gene enrichment Sequencing (RenSeq) of nucleotide binding, leucine rich repeat (NLR) encoding genes was conducted on a panel of soybean genotypes with known, potentially novel, or no *Rps* genes. Baits were designed based on 755 NLR genes from *G. max* 'Williams 82' reference genome and used to capture NLR encoding DNA from the soybean panel. Long 1–20 kb reads were obtained using PacBio sequencing and subjected to de-novo assembly, annotation of sequences that contain NLR motifs, and removal of sequences present in genotypes with no *Rps* genes. Putative *Rps* genes were identified that mapped to known resistance loci on chromosomes 3, 13, and 18, corresponding to 8 genes annotated in the 'Williams 82' reference genome. The allele sequences amplified from resistant genotypes were distinct from the susceptible genotypes based on Sanger sequencing. These sequences will be used for gene silencing studies and to develop allele-specific markers for the introgression of novel *Rps* genes into modern germplasm. This work provides a model for the rapid identification of resistance genes in soybean against other pathogens.

A dual sequence and culture-based survey of maize rhizosphere protists reveals dominant, plant-enriched, and culturable community members

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Heterotrophic protists comprise a major component of the phytobiome. They enhance nutrient cycling, promote beneficial microbes, and affect disease outcomes. However, it is still unknown if plant roots recruit a distinct protist microbiome as they do with bacteria. In addition, there are few plant-associated protists in culture, making it difficult to study these protists in experimental settings. We examined the protists associated with the rhizospheres of maize grown in two locations with differing soil types and bulk microbial communities. High throughput amplicon sequencing demonstrated that the rhizosphere contained a reduced diversity of protists found in surrounding bulk soil, enriching for the supergroups Rhizaria and Amoebozoa. Thirteen protist species were enriched in the rhizosphere in one or both locations, of which eight were present in all rhizosphere samples. Most enriched protists belonged to the uncultured Glissomonad genus Group-Te and two genera of amoebae. The core enriched protists were correlated with the abundance of several rhizosphere bacterial species, suggesting potential impact on the bacterial plant microbiome. Simultaneous isolation efforts yielded 103 protist isolates from 6 supergroups, including members of both core and rare genera from the sequencing, providing information about what core and rare protists are most readily culturable. This study demonstrated that maize roots recruit a similar microbiome despite being grown in different types of soil. We also generated a protist library that can be used for future experiments.

Identifying methylotrophs with biocontrol activity against soilborne pathogens using a comparative genomics platform

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Soilborne pathogens cause economic risk to global agriculture every year. *Fusarium* and *Pythium* species are the main causal agents of these diseases in row crops like soybean. While chemical fungicides are effective at controlling these diseases, biofungicides are practical alternatives due to their persistence on the host plant, decreased risk of pathogen resistance development, and reduced environmental impact. Pink-pigmented facultative methylotrophs (PPFMs or M-trophs) are a unique type of biofungicide due to their robust colonization of plants and their compatibility with existing agricultural technologies. These ubiquitous plant symbionts have been identified as inducers of systemic resistance in many systems; however, not all M-trophs have these effects. NewLeaf's comparative genomics platform—the Prescriptive Biologics Knowledgebase—contains over 6,000 genomes across dozens of species of *Methylobacterium* and *Methylorubrum*. This platform allows for hypothesis-based screening for biocontrol activity, as well as genotype-phenotype associations that inform hypotheses regarding modes of action. Isolate nominations were evaluated in field and greenhouse trials, which led to the identification of several isolates that caused significant protection against *Fusarium* and *Pythium*. These data indicate that genome mining and genotype-phenotype associations can efficiently identify biofungicide candidates. Current efforts are focused on determining the translatability of this activity to high value crop systems such as hydroponic fruits and vegetables.

Puccinia coronata* var. *coronata*, a recently observed fungus in North America, reduces fruit production and seedling fitness of *Frangula alnus

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Puccinia Series *coronata* spp. cause diseases of oats, barley, and turfgrasses around the world. Many native, naturalized, and weedy grass and buckthorn species in North America are also infected by crown rust fungi with potentially significant ecological effects. *Puccinia coronata* var. *coronata* (*Pcc*) has recently been reported in North America where it primarily alternates between two highly invasive species, *Frangula alnus* (glossy buckthorn) and *Phalaris arundinacea* (reed canarygrass). We hypothesize that rust infection affects *F. alnus* fitness by defoliation, increased seedling mortality, and reduction in seed production. In 2020, we observed flowering and fruitset on branches of 39 *F. alnus* plants in natural areas around St. Paul, MN. In June, when *F. alnus* is infected by *Pcc* aecia, approximately 3.7% of flowers produce fruit; however, in August, when rust is absent, on 9 plants still flowering, 35% of flowers produced fruit. In a greenhouse experiment, *F. alnus* seedlings were inoculated with *Pcc* telia on *P. arundinacea*. After two months, following pycnial and aecial infection, rust-treated plants had 18.3% less dry biomass and 40.5% leaf area loss when compared to control plants, and suffered 6.3% mortality. Results from the two experiments suggest *Pcc* reduces the fitness of invasive *F. alnus*, and that *Pcc* may have potential as an augmentative biological control agent of *F. alnus*.

Transcriptomic analysis of *Fusarium oxysporum* f. sp. *vasinfectum* after gossypol treatment uncovers multiple genes involved in virulence on cotton

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Fusarium oxysporum f. sp. *vasinfectum* (*Fov*) is a soilborne filamentous fungus that causes vascular wilt in cotton. In response to pathogens such as *Fov*, cotton plants produce the phytoalexin gossypol as a defense mechanism. This research aims to explore virulence factors adopted by *Fov* during infection and colonization of cotton. To identify these virulence factors, candidate genes that could be responsible for gossypol tolerance were identified through RNA sequencing. Four RNA samples were extracted from germplings of a virulent race 4 *Fov* genotype, grown in minimal medium before and after treatment with 80 µg/mL of gossypol at 1, 2, and 4 h. RNA sequencing data showed upregulation of multiple classes of gene families, and of particular interest a beta-lactamase and a tannase in presence of gossypol. The expression of these genes was validated through RT-qPCR analysis. *Fov* mutants of both the beta-lactamase and the tannase were created using CRISPR/Cas9 system and their role in virulence was assessed. The stem lesion and pot assays in cotton indicated that the genes encoding lactamase and tannase are involved in virulence in cotton. Among these genes we found that the beta-lactamase is highly upregulated during infection in cotton. While beta-lactamases are widely studied in bacterial systems for their role in antibiotic resistance, our findings on a beta-lactamase in *Fov* highlights their importance in fungal virulence and their potential role in antimicrobial compound resistance.

Dual RNAseq reveals transcriptome changes during *Fusarium virguliforme*–*Trichoderma afroharzianum* interactions

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Trichoderma spp. are well-studied biocontrol agents. Nevertheless, some aspects of their interaction with the pathogen-host, including recognition mechanisms and factors governing the intraspecies variability in *Trichoderma* antagonism, are still unclear. This study elucidated the mechanisms underlying phenotypic differences among *T. afroharzianum* isolates Th19A and Th4 during confrontation with *F. virguliforme*, by investigating differences in their transcriptomes. In a dual plate assay, Th19A overgrows *F. virguliforme*, whereas Th4 forms an inhibition zone. RNA isolated during different stages of the interaction was sequenced and aligned to the reference genomes. DESeq2 and GOEnrichment were used to analyze differentially expressed genes (DEG) and GO enrichment. Differences were noted in the *F. virguliforme* transcriptome during interaction with Th19A or Th4 across different stages of the interaction. GO categories enriched for *F. virguliforme* genes differed, indicating possible transcriptional plasticity upon interaction with Th19A versus Th4. Transcriptome changes were also observed in *T. afroharzianum*, with differences in GO enriched categories between isolates. Several DEG encoding secreted proteins, including CAZymes and CBM1-domain-containing proteins, were upregulated in Th19A and Th4 upon interaction with the pathogen, even before physical contact, demonstrating possible volatile-mediated recognition of both isolates *T. afroharzianum* by *F. virguliforme*. This study contributes to a better understanding of the interaction between *T. afroharzianum* and *F. virguliforme*.

Development of a high-throughput sequencing protocol for the detection of cileviruses associated with the Citrus leprosis disease complex

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The citrus leprosis disease (CiLD) complex associated with *Brevipalpus* mite-transmitted cilevirus infection is an emerging threat to Brazil, Colombia, Mexico, and the U.S. First CiLD was reported from US in 1860s but the infection was associated with a Dichorhavirus. Of the three describes cileviruses, only the hibiscus strain of Citrus leprosis virus C2 (CiLV-C2H) has been reported from U.S. The recent discovery of CiLV-C2H in *Citrus sinensis* in Colombia with CiLD symptoms illustrates the potential threat to U.S. citrus production as the pathogen, susceptible hosts, mite vectors, and suitable environments exist. To protect the multibillion dollars citrus industry, a high-throughput sequencing (HTS)-ribo-zero protocol was developed and optimized for the identification of cileviruses infecting *Citrus* spp., *Swinglea glutinosa*, and *Hibiscus rosa-sinensis*. HTS protocol was applied to the plants showing symptoms consistent with cilevirus infection belongs to the families; Orchidaceae, Araceae, Passifloraceae and Zingiberaceae. Total RNA was extracted, and Ribo-Zero libraries were made. Out of 68 samples, 53 were sequenced using either MiSeq or NextSeq platforms. In addition to sequences corresponding to several citrus, orchid and hibiscus viruses, HTS analyses also identified a new reassortment strain of CiLV-C2, consist of RNA1 of CiLV-C2H and RNA2 segment of CiLV-C2 in a *C. sinensis* tree in Colombia. Furthermore, current protocol was successfully utilized to identify cileviruses in *Passiflora* sp., *Dieffenbachia* sp., *Etilingera elatior*, and Dichorhviruses in citrus and orchids.

Distinct transcriptional regulators contribute to growth of *Dickeya dadantii* and *D. dianthicola* in potato tubers

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Members of the soft rot Pectobacteriaceae (*Dickeya* and *Pectobacterium* spp.) are highly damaging to a wide range of crops. These closely-related pathogens share many important virulence traits, such as the production of plant cell wall degrading enzymes. However, as there has been little host-specificity observed for the soft rot pathogens, it is unclear the extent to which virulence traits may vary among strains. To functionally annotate virulence determinants and host colonization traits, we constructed and mapped large (>300,000 mutants) barcoded transposon libraries in the strains *D. dadantii* 3937, *D. dianthicola* ME23, and *D. dianthicola* 67-19. Changes in relative proportion of each mutant within a mixture enable fitness quantification of nearly all non-essential genes in that strain. By inoculating these transposon libraries into potato tubers, we identified genes that contribute to host colonization. Combined with a matrix of homologous gene families among these strains, generated using the PyParanoid computational pipeline, we can directly compare fitness traits in each strain. For example, we identified five putative DNA-binding transcriptional regulators whose presence is significantly detrimental to in planta fitness in a subset of strains. For further characterization of these putative regulators, we aim to identify members of each regulon as well as their distribution within the *Dickeya* and *Pectobacterium* pangenome. Species mixtures are often observed in soft rot, and so understanding of specific virulence traits is crucial for developing disease control methods.

Bioactivity of mellein and its production by *Macrophomina phaseolina*

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Macrophomina phaseolina (*Mp*), a fungus that infects >500 species of commercially important plants, is suspected to infect host roots by a toxin-mediated process involving entry of hyphae through necrotic areas produced on the root surface. *Mp* is known to produce

numerous secondary metabolites including potent phytotoxins, (–)-botryodiplodin and phaseolinone. Recently Khambhati et al. analyzed *Mp* cultures isolated from soybean and identified 12 unreported secondary metabolites including mellein, a bioactive compound produced by many fungi, insects, and marine organisms. The aim of this study was to analyze *Mp* cultures for mellein production, evaluate the phytotoxicity of *Mp* cultures, and determine the phytotoxic effects on soybean seedlings. Among cell-free filtrates of 89 *Mp* cultures analyzed by LC-MS/MS, 28.1% produced mellein (1.66–74.9 µg/L). The same *Mp* culture filtrates were tested for phytotoxicity using soybean seedlings in hydroponic culture with 1:1 and 3:1 growth medium/filtrate ratios. Phytotoxicity symptoms were observed in all cultures (at 1:1 ratio: chlorosis, 61%; necrosis, 82%; wilting, 9%; death, 26%; at 3:1 ratio: chlorosis, 73%; necrosis, 78%; wilting, 7%; death, 16%). Mellein serially diluted from 1.25 to 100 µg/mL in hydroponic culture medium caused only symptoms of wilting and stunted growth in soybean seedlings with increasing severity from 40–100 µg/mL. This suggests that mellein alone does not account for the potent phytotoxic effects of *Mp* culture filtrates, and further investigation is required to determine its role in *Mp* pathogenesis.

Using infrared imaging as an early detection method for bacterial leaf scorch, *Xylella fastidiosa* subsp. *multiplex*, in southern highbush blueberry

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Xylella fastidiosa subsp. *multiplex* (*Xfm*), a xylem-clogging bacterial plant pathogen, causes bacterial leaf scorch (BLS) of blueberry by interfering with host water transport. Reduced water movement likely interferes with evapotranspiration cooling, which could present a diagnostic heat signature in *Xfm*-infected plants prior to the appearance of visible BLS symptoms. We evaluated whether inoculated blueberries generate thermal symptoms prior to the expression of visible symptoms using both infrared (IR) and visible spectrum images captured with a Fotric 220 thermal imaging camera. Tissue-cultured plants of blueberry cultivars ‘Rebel’ and ‘Emerald’ were syringe-inoculated on 1 stem per plant with a suspension of either 1×10^8 cells/mL, 2×10^8 cells/mL, or buffer only (control); other stems were left uninoculated. Five replicates per treatment of ‘Rebel’ and two replicates per treatment of ‘Emerald’ were assessed. Following *Xfm* inoculation, images were captured every 2 days over a 3-month period. When exposed to an IR lamp and direct sunlight, *Xfm*-inoculated plants displayed surface temperatures 2–8°C greater on pre-symptomatic and early symptomatic leaves as compared to leaves on uninoculated stems. All *Xfm*-inoculated plants displayed heat signatures, and thermal symptoms were evident up to 1 week prior to the development of visible symptoms. In addition, disease intensity and progression appeared to be associated with inoculum level and cultivar. Results suggest that IR imaging may be a reliable and useful method for early diagnosis of BLS and similar vascular diseases.

Fungicide sensitivity of *Sclerotinia sclerotiorum* from USA soybean and dry bean compared to different regions and climates

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Increasing fungicide use against *Sclerotinia sclerotiorum* may lead to resistance or reduced sensitivity to the most commonly used fungicides; few studies have investigated this in the USA. We sought to determine if there was a difference in fungicide sensitivity between: a) isolates from dry bean and soybean; b) isolates from fields with different levels of fungicide treatments; and c) if reduced sensitivity was associated with applications of a single active ingredient. We further hypothesized that tropical (Brazil) and sub-tropical (Mexico) isolates were less sensitive compared to temperate ones (USA) due to different management practices and climates. Fungicide sensitivity (EC_{50D}) of 524 *S. sclerotiorum* isolates from the USA ($N = 455$), Brazil ($N = 36$), and Mexico ($N = 33$) was determined using discriminatory concentrations (DC) previously identified of tetraconazole (2.0 ppm; FRAC group 3), boscalid (0.2 ppm; group 7), picoxystrobin (0.01 ppm; group 11), and thiophanate-methyl (group 1), which had a qualitative DC of 10 ppm. A selection of isolates least sensitive to boscalid and picoxystrobin were used for gene sequencing. Among isolates from the USA, Brazil, and Mexico, only two isolates from Mexico had a mutation (A11V in the *SdhB* gene) known to confer qualitative resistance to SDHI fungicides, but were not found to be strongly resistant. This study is the first to establish baseline sensitivity of *S. sclerotiorum* to tetraconazole and picoxystrobin throughout the world and the first to establish a baseline sensitivity to boscalid in the USA.

Genomic regions associated with virulence in *Setosphaeria turcica* identified by linkage mapping in a biparental population

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The ascomycete *Setosphaeria turcica* is a devastating pathogen of maize and sorghum and the causal agent of northern corn leaf blight and sorghum leaf blight. Strains of *S. turcica* are host specific and infect either sorghum or maize, which has been attributed to a single locus for virulence on each host. To identify the genetic basis of host-specificity in *S. turcica*, we performed phenotyping, genotyping, and linkage mapping of a biparental population generated by crossing maize and sorghum specific strains. *S. turcica* progeny ($n = 144$) segregated 1:1:1:1 for virulence to maize, sorghum, both maize and sorghum, and avirulence to both crops ($P = 0.09$). Whole genome sequencing of the parents and progeny using skim sequencing produced over 780 million reads, and 32,635 variants were scored. A genetic map with 17 linkage groups (LGs) spanning 3,069 cM was constructed with 869 high-quality markers. QTL mapping identified a single locus for virulence to sorghum (on LG1) and another single locus for maize (on LG4). In vitro traits, including radial growth and conidiation did not correlate with in planta virulence complexity ($P > 0.05$), suggesting that virulence on both hosts does not apply a

fitness cost to *S. turcica*. Radial growth and conidiation were negatively correlated ($r = -0.37$; $P < 0.01$), indicating differences in hyphal growth versus dispersal ability for this fungal species. Identification of candidate virulence genes have implications towards targeted breeding of *S. turcica* resistant maize and sorghum cultivars, along with a better understanding of this pathosystem.

New encounters with old problems: Orchid fleck dichorhavirus infecting three new ornamental hosts in Florida

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Orchid fleck virus (OFV) the type member of the genus *Dichorhavirus* (Mononegavirales: Rhabdoviridae) and their *Brevipalpus* mite vectors are distributed worldwide. OFV naturally infects over seventy species of plant, including Orchidaceae, Asparagaceae, and *Citrus* sp. Citrus-infecting strains of OFV (OFV-Orc1 and OFV-Orc2), which are typically associated with orchids, have been causing recent outbreaks of citrus leprosis (CL) in South Africa and Hawaii, as well as Mexico (with the strain OFV-Cit). During the summer of 2020, we found OFV infecting three ornamental species of Nolinoid plants (Asparagaceae: Nolinoidae) in the landscape in Leon and Alachua Counties, FL: *Liriope* spp., *Ophiopogon* spp., and *Aspidistra elatior* Blume. Sanger sequencing confirmed two strains of OFV from our samples: OFV-Orc1 and OFV-Orc2 and RT-PCR. We identified three mite species from OFV-infected plants: *Brevipalpus californicus* s.l., *B. obovatus* and *B. confusus*. *B. californicus* s.l. is likely the vector of OFV in these plants. There has not been another outbreak of CL in Florida citrus since 1968, but Hartung et al. (2015) recently identified the historical CL virus as a distantly related dichorhavirus, citrus leprosis virus-N0. This suggests that a widely distributed dichorhavirus like OFV may threaten various native and introduced plant species and possibly citrus in the southeastern U.S.

Changes in subcellular localization of host proteins induced by plant viruses

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Viruses are dependent on host factors at all parts of the infection cycle, such as translation, genome replication, encapsidation, and cell-to-cell and systemic movement. RNA viruses replicate their genome in compartments associated with the endoplasmic reticulum, chloroplasts, and mitochondria or peroxisome membranes. In contrast, DNA viruses replicate in the nucleus. Viral infection causes changes in plant gene expression and in the subcellular localization of some host proteins. These changes may support or inhibit virus accumulation and spread. Here, we review host proteins that change their subcellular localization in the presence of a plant virus. The most frequent change is the movement of host cytoplasmic proteins into the sites of virus replication through interactions with viral proteins, and the protein contributes to essential viral processes. In contrast, only a small number of studies document changes in the subcellular localization of proteins with antiviral activity. Understanding the changes in the subcellular localization of host proteins during plant virus infection provides novel insights into the mechanisms of plant-virus interactions and may help the identification of targets for designing genetic resistance to plant viruses.

Cas12a-based diagnostics for sensitive and specific detection of citrus greening and phytoplasma pathogens

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Highly sensitive and accurate diagnosis of plant pathogens is critical for disease management. In particular, pathogens that exist in low titers within the plant, such as *Candidatus Liberibacter asiaticus* (CLas, the citrus greening or huanglongbing pathogen) and purple potato top (PPT) phytoplasma, are very difficult to be detected prior to the appearance of disease symptoms. To help improve detection sensitivity beyond traditional qPCR, we adapted the Cas12a-based DETECTR (DNA endonuclease-targeted CRISPR trans reporter) assay for specific detection of CLas and PPT DNAs down to the attomolar level (10^{-18}). This assay combines the isothermal recombinase polymerase amplification (RPA) along with the Cas12a collateral cleavage of fluorescent reporter oligonucleotides, allowing for highly sensitive and rapid detection of pathogen nucleic acids. The fluorescent reporter assay result can be read through a microplate reader for high throughput sampling. Furthermore, the method is compatible with lateral flow technology to allow instrument-free and field-deployable diagnostics of plant pathogens. With the adoption of the DETECTR assay for sensitive detection of pathogen nucleic acids, we hope to improve the early detection technology and facilitate plant disease management.

Using a synthetic biology approach to identify the chemical drivers that recruit *Ralstonia* phytopathogens to the host roots

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Many bacteria, including wilt pathogens in the *Ralstonia* species complex, require chemotaxis to locate and colonize host plant roots. Bacterial chemotaxis is mediated by membrane-bound receptors called methyl-accepting chemotaxis proteins (MCPs). Chemical signals are transduced through a two-component system triggering directed swimming up or down a chemical gradient. Soilborne phytopathogens contain dozens of chemoreceptors per genome each with unique chemical sensitivities. *Ralstonia* strains are more

attracted to host root exudate extracts and we hypothesize that chemoreceptor specificity helps drive *Ralstonia*'s selection of plant hosts. Despite an abundance of genomic data, we lack robust bioinformatic tools to predict the chemical specificities of structurally diverse chemoreceptors. We developed a chimeric two-component system that allows us to rapidly probe the ligand-binding of bacterial chemoreceptors with a fluorescent reporter strain. Using the ligand binding domains of MCPs and the output domain of NarQ, a nitrate-sensing receptor from *E. coli*, we constructed chimeras of the MCPs in *R. solanacearum* IBSBF1503 and *R. syzygii* PSI07. We are testing these synthetic constructs to determine which MCPs sense which root exudate chemicals. From this data, we will generate targets for genetic studies that will inform our understanding of how chemoreceptors drive host discovery from the soil reservoir. In the long-term, this research will translate into strategies for pathogen control and deepen our understanding of the role of *Ralstonia* chemotaxis in dictating host-range.

Screening cotton cultivars for resistance to *Fusarium oxysporum* f. sp. *vasinfectum* races 1 and 2 and *Meloidogyne incognita*

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More than 40% of the 1.4 million hectares of cotton grown in the Southern High Plains region of Texas are infested with *Meloidogyne incognita* (Mi). *Fusarium oxysporum* f. sp. *vasinfectum* races 1 and 2 (FOV) interact with Mi to cause Fusarium wilt. However, plant resistance to Mi may involve different genes than plant resistance to FOV. The objective of this study was to screen cotton cultivars for resistance to Fusarium wilt. Cultivars were screened in a greenhouse trial and were inoculated with Mi and four local FOV isolates (including race 1 and 2) using a root-dip technique. Visual symptoms (VS) were rated on a scale of 0–5 over 2 weeks and calculated as area under the disease progress curve. Stem necrosis (SN) and root necrosis% (RN) were also evaluated. FM 1730GLTP (PR = partial resistance to Mi), FM 1911GLT (PR) and ST 4946GLB2 (PR) had the lowest values for VS (av 1.9), SN (av 26%), and RN (av 22%). Varieties with intermediate responses were FM 1621GL (PR), NG 4936 B3XF (S = susceptible to Mi), PHY 400 W3FE (PR) and ST 5600B2XF (HR = highly resistant to Mi), with an average VS rating of 3.6, 50% SN and 55% RN. NG 4098 B3XF (S) had low VS (2.6) but intermediate SN (43%) and RN (42%). Varieties with one or more measured variable with the highest rating included DP 1646 B2XF (S), DP 1747NR B2XF (HR), DP 1840 B3XF (S), PHY 350 W3FE (PR) and PHY 480 W3FE (HR) with average values of 5.1 (VS), 60% (SN) and 57% (RN). There were no significant differences ($P = 0.05$) between races 1 and 2. These results show that Mi resistance does not always correlate to FOV race 1 and 2 resistance.

Social distancing reduces boxwood blight incidence and severity

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Boxwood blight has caused substantial losses to the nursery industry since it was first reported in the United States in 2011. The fungal pathogen, *Calonectria pseudonaviculata*, produces conidia that are splash dispersed. Therefore, growing boxwood plants in nurseries with tight spacing may accelerate disease spread when coupled with frequent overhead irrigation. To test this, we assessed the development and spread of boxwood blight from inoculated plants to healthy plants of the susceptible boxwood cultivar 'Green Velvet' grown in 3.8 L pots. Plants were spaced 0 or 15 cm apart and overhead irrigated once, twice, or three times a day. Healthy plants for each treatment were arranged in a 7 × 7 grid with an inoculated plant placed in the center of the grid. Center plants were inoculated in mid-July and symptoms developed 1 week later. At the 15 cm spacing, initial symptoms on inoculated plants were mild regardless of irrigation frequency ($\leq 5\%$ of canopy) and the disease was never observed to increase in severity or spread to healthy plants for the duration of the study (8 months). However at the 0 cm spacing, initial symptoms were more severe ($\geq 35\%$ of canopy) and the disease spread to adjacent healthy plants over the following 8 months, with generally greater disease severity and faster spread observed at higher irrigation frequencies. Thus, increasing plant spacing and reducing irrigation frequency could potentially mitigate further spread of boxwood blight if the disease is present in a nursery.

Seasonal differences in prevalence of two criniviruses in the low desert melon production regions of California and Arizona

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The whitefly-transmitted cucurbit yellow stunting disorder virus (CYSDV, *Crinivirus*, *Closteroviridae*) is well established in low desert regions of California and Arizona, whereas cucurbit chlorotic yellows virus (CCYV), another crinivirus, that produces identical foliar symptoms, emerged in this region in 2014. To understand seasonal prevalence of these viruses in the low desert melon production region, melon leaf samples were collected from commercial fields and research plots during spring and fall production seasons of 2019 and 2020, and RNA extracts were evaluated with a recently developed multiplex reverse transcription polymerase chain reaction (RT-PCR). Additionally, 20 melon leaf samples collected during the fall season, were assayed by multiplex quantitative RT-PCR to quantify differential virus accumulation. In the spring of 2019 and 2020, CCYV was the predominant virus (100% and 81% infection respectively), but CYSDV coinfecting plants with CCYV in 20% and 40% of samples during 2019 and 2020, respectively. In contrast, CYSDV was the predominant virus during the fall seasons (91% and 90%, respectively), with lower incidence of coinfection with CCYV (30% and 14% for 2019 and 2020 respectively), and CYSDV titer was much higher than CCYV titer in fall melon samples. All whiteflies collected from sampled fields were found to be *Bemisia tabaci* MEAM1. Ongoing studies are evaluating reasons for the seasonal changes in virus prevalence as well as monitoring virus incidence following the recent introduction of these viruses into the California's San Joaquin Valley.

Sweetpotato virome communities in Sub-Saharan Africa: A network analysis framework for complex virome datasets

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Virome network analysis is a useful approach for understanding the structure of virome communities in individual hosts and across regions. We developed a virome network analysis for complex datasets, integrating concepts about viral metagenomics, island biogeography, and bipartite networks. We implemented this virome network analysis framework to analyze a collection of 1287 sweetpotato accessions collected in Sub-Saharan Africa. Our analysis framework assembled small RNA sequences with VirusDetect, followed by sequence clustering by assigning the genus based on a cluster of identities (%) based on species demarcation criteria, generating virome species clusters (VSCs). These VSCs were normalized with read counts. Additionally, we used a cropland connectivity framework, based on a gravity model to describe the risk of pathogen movement, to identify seven regions using unsupervised machine learning methods. These seven sweetpotato regions were used for individual network analyses. The sweetpotato virome has a core of viruses: SPFMV, SPSCV, SPLCV, SPSMV-1, SPVG, SPV2, SPVC, SPPV, and badnavirus-like sequences. We compared the seven sweetpotato virome communities, indicating an independent species dynamic for low compared to high crop densities, and finding a unique virome composition with virome similarity only partly driven by geographic distance. Understanding the virome structure informs management strategies for sweetpotato in the agroecological landscape of Africa.

A MORC1 interacting protein is a bZIP transcription factor that positively regulates plant immunity against *Phytophthora infestans* in Solanaceae

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Phytophthora infestans (*Pi*), the causal agent of potato and tomato late blight, is a global threat to food security, causing multibillion-dollar losses due to reduction in crop production and expenditures in disease control. Microorchidia (MORC) proteins belong to the GHKL ATPase superfamily and play critical roles during plant immunity against different pathogens. This study focuses on understanding how MORC1 regulates plant immunity to *Pi* by the identification of MORC1-interactors (MIPs) using biochemical, genomics, and proteomics approaches. Through a yeast-two hybrid screening, a basic leucine zipper (bZIP) transcription factor (MIP10) was found to physically interact with MORC1 and their C-terminal regions are required for the interaction. MIP10 lacks nuclear localization signal, but its abundance in the nucleus is enhanced after flagellin 22 treatment. Reverse genetics analysis of *MIP10* in *Nicotiana benthamiana* (*Nb*) and *Solanum lycopersicum* (*Sl*) was performed to determine its role during plant immunity against *Pi*. In *N. benthamiana*, silencing *NbMIP10* enhanced pathogen susceptibility and compromised cell death induced by pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). On the contrary, transient overexpression of *SlMIP10* enhanced *Pi* resistance. Finally, silencing *SlMIP10* in *S. lycopersicum* compromised ETI induced by *Pto-AvrPto* recognition. Together, these results argue that this bZIP transcription factor is a positive regulator of plant immunity and can be used to engineer *P. infestans* resistant crops.

Seasonal trends in host reservoirs for *Impatiens necrotic spot virus* (INSV): An emerging pathogen affecting lettuce production in California

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The emergence of thrips-transmitted, *Impatiens necrotic spot virus* (INSV) has rapidly become a major concern for lettuce production in the Salinas Valley on the Central Coast of California, resulting in millions of dollars in losses during the 2019 and 2020 seasons. While conventional chemical control tactics have shown to be insufficient in managing the thrips vector, *Frankliniella occidentalis*, genetic-based resistance to the virus remains unavailable in commercial lettuce cultivars. Both the vector and virus have an extensive host range that includes cultivated and uncultivated plants, creating additional management challenges in lettuce production. Here, we sought to identify the host range for INSV to understand the importance of alternative hosts as virus reservoirs in the Salinas Valley. Plant collections of non-lettuce species were made in four regions that were situated in varying environments and were identified as areas that experienced either a low, medium, or high incidence of INSV in lettuce crops during the 2019 season. In total, over 3,000 plants representing over 70 species were tested for INSV and numerous hosts were identified as reservoirs that can support the virus during the non-lettuce season. Furthermore, the INSV infection rate of several key hosts varied based on their spatial and temporal positions, which raises additional interesting epidemiological questions regarding the emergence and spread of the virus in the region.

Transcriptomic analysis of changes in *Citrus × microcarpa* gene expression post *Xanthomonas citri* subsp. *citri* infection

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Citrus canker (CC) is an economically important bacterial disease caused by *Xanthomonas citri* subsp. *citri* (*Xcc*). There is variability in resistance to CC among *Citrus* species. Previous transcriptomic studies have shown that *Xcc* downregulates biosynthesis of secondary metabolites at early stages of infection in susceptible citrus. However, in partially resistant calamondin (*Citrus × microcarpa*), increased secondary metabolite production has been reported post *Xcc* inoculation. We analyzed the calamondin transcriptome and discovered 6,645 and 11,799 differentially expressed (DE) genes between *Xcc*-inoculated and control samples at 2- and 5-days post inoculation

(dpi), respectively. Among the DE genes, the phenylpropanoid biosynthesis (PPB) pathway, a major secondary metabolites synthesis pathway, was upregulated at early stages (2 dpi) of *Xcc* infection. Furthermore, in contrast to previous studies of susceptible *Citrus* species, pathways related to a resistance response (such as plant-pathogen interaction and MAPK signaling pathway) were upregulated whereas the pathways related to a susceptible response (such as auxin and gibberellin biosynthesis and cell wall degradation) were not significantly upregulated in calamondin. The plant hormone biosynthesis pattern in calamondin was also different from the susceptible citrus. Thus, PPB may represent the principal biochemical pathway underlying CC resistance.

Soybean nodule non-rhizobial bacteria for plant protection and growth promotion

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Legume nodules host a number of other microbial occupants besides the rhizobia. To identify beneficial bacteria for plant disease control and growth promotion, soybean nodules were studied as the source of beneficial bacteria as our metagenomics analysis of a single soybean nodule identified a large number of diverse bacterial species. A total of 500 colonies from 10 nodules were tested individually against the tomato wilt causing bacterial pathogen *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) on solid media plates for inhibition of pathogen growth. From the initial screening, 54 colonies were selected based on significant growth inhibition of the pathogen. These colonies were further tested in vitro on another bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst) and two fungal pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum* on solid plates. Bacterial metabolites were extracted from 15 selected colonies out of the 54 colonies with ethanol and tested against the Cmm and Pst pathogens on solid plates. These 15 colonies were identified by using MALDI-TOF mass spectrometry and 16S rRNA gene sequencing; *Pseudomonas* spp. was found to be the dominant bacterial group among soybean nodule-associated bacteria. In planta testing using 15 selected nodule-associated colonies as a protectant, a number of them imparted significant protection against the Cmm pathogen and/or plant growth promotion on tomato seedlings. The results showed that nodule-associated bacteria have great potential for plant health management as well as growth promotion.

Mycobiome activity in Lophodermella needle cast pathosystem on *Pinus contorta* revealed through metatranscriptomics

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The increasing emergence of needle diseases prompts the need to better understand needle pathogens and mechanisms of disease development. This study aims to examine the gene expression of the mycobiome of asymptomatic and symptomatic needles of *Pinus contorta* infected with pathogenic *Lophodermella concolor* and *L. montivaga*. We hypothesized that the mycobiome expression will differ significantly between symptomatic and asymptomatic needles, and needles symptomatic of *L. concolor* and *L. montivaga*. These differences are driven by pathogenicity-related mechanisms distinctly employed by the pathogens which also provide them with a competitive advantage over other fungal endophytes. This study identified higher expression of fungal transcripts in symptomatic needles that may be involved in pathogenicity. Despite a small proportion of shared transcripts between needles symptomatic each of *L. concolor* and *L. montivaga*, their functional annotations were highly similar which may suggest that mechanisms involved by the mycobiome at the necrotrophic phase of the disease may be similar between these two species. Despite the presence of *Lophodermella* pathogens, the differentially expressed pathogenicity-related transcripts in asymptomatic needles were marginal, thus reinforcing our previous findings of *Lophodermella* species as likely latent pathogens. Results of this study provide insights on biological mechanisms in emerging forest diseases caused by latent pathogens, which will be useful in forest disease management.

Identification of quinone outside inhibitor fungicide-resistant *Parastagonospora nodorum* isolates from Kentucky wheat fields

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Winter wheat (*Triticum aestivum*) in Kentucky is commonly grown in a two year rotation under no till management. Persistent inoculum left on the soil surface promotes Stagonospora leaf and glume blotch of wheat caused by *Parastagonospora nodorum*. This disease can be difficult to manage due to abundant inoculum and high adaptability conferred by a short life cycle that incorporates both sexual and asexual reproduction. Resistance to *P. nodorum* in wheat is currently partial, increasing the importance of effective fungicide applications. Quinone outside inhibitor (QoI) fungicides are commonly used to manage foliar diseases in wheat. Fungal pathogens, especially those that are highly adaptable, often develop resistance to this class of fungicide. Isolates of *P. nodorum* were collected from Kentucky wheat fields in 2018, 2019, and 2020. The cytochrome b gene was sequenced, revealing a mutation conferring a change from glycine to alanine at codon 143 (G143A) in some isolates. Spore germination assays on azoxystrobin-amended media confirmed that isolates with the G143A mutation lacked sensitivity to QoI fungicides. Results of greenhouse trials showed that an application of a QoI fungicide did not control Stagonospora leaf blotch caused by isolates with the G143A mutation. In total, 46% of *P. nodorum* isolates recovered had the G143A mutation.

Multi-omics analysis reveals bHLHs act as master transcription factor regulators of reproductive development in *Pospiviroid* infection in tomato

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Viroids are angiosperm pathogens constituted of non-coding RNA that cause serious diseases in crops of agronomic interest. The transition from vegetative growth to reproductive development requires the coordination of gene networks, where transcription factors act as essential components for organ morphogenesis. Reproductive development symptoms have been associated with alterations in hormonal pathways that affect expression levels in bHLH-type transcription factors (TFs-bHLH), including regulation by small interfering RNAs derived from the viroid genome as pathogenic effectors (vd-siRNAs). A network of gene associations of the 161 bHLH TFs in the tomato genome and their targets is proposed to infer a mechanism of interaction during viroid infection. Our study aims to determine interactions between TF-bHLH master regulators and vd-siRNAs/targets as effectors of the disease that could be key in the expression of genes involved in biological processes linked to viroid symptoms in a susceptible host. The multi-omics approach contemplated integrating transcriptomic data, which involve co-expression and regulation networks, as well as signaling pathways, with comparative genomics analysis to study evolutionary events in susceptible and tolerant hosts. Our study suggests that vd-siRNAs are modulators of target genes in hormone pathways and represents the first approach to study the role of bHLH-miPs in the genetic regulation of reproductive developmental processes during viroid infection.

E-probe diagnostic nucleic acid analysis for quarantine viruses on Poaceae

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Infections of viruses in Poaceae crops can occur as single or mixed infections, in any case leading to a significant decline of yields. At least 31 viruses have been identified infecting Poaceae and several of quarantine interest. Current methods for detection and diagnosis are laborious and time-consuming since they target one virus at a time. The goal is to introduce high-throughput sequencing (HTS) technology into routine quarantine diagnostics using the E-probe Diagnostic Nucleic Acid Analysis (EDNA), which can detect all regulated viruses infecting the Poaceae family simultaneously. The Mi-Fi® server was developed to build databases containing unique genomic sequences (e-probes) that target specific virus sequences with full genome coverage. Synthetic genome sequences simulating output from HTS data were created for 31 viruses, including seven hosts (barley, corn, miscanthus, sugarcane, sorghum, switchgrass, and rice). Further BLAST alignments and tests using Mi-Fi® were performed for e-probe curation. HTS (Illumina) outputs from 13 sugarcane infected samples were tested with Mi-Fi® using the previously developed e-probes databases created for the detection of Poaceae quarantine viruses. The e-probes detected the 31 quarantine viruses in silico. Validation of results in vitro using the 13 sugarcane selected HTS outputs indicates Mi-Fi® is able to detect nine sugarcane quarantine viruses simultaneously. EDNA is a flexible bioinformatic diagnostic approach that can assist during quarantine pathogen detection.

Spatiotemporal dynamics of Stemphylium leaf blight epidemics in New York onion fields

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Stemphylium leaf blight (SLB) caused by *Stemphylium vesicarium* has become the dominant foliar disease affecting onions in New York. The epidemiology of the disease is poorly understood. Information about the spatial and temporal dynamics can be used to create hypotheses about inoculum survival and dispersal. To understand the spatiotemporal dynamics of SLB epidemics, three onion fields were evaluated in each of 2017 and 2018. In each field, five leaves were arbitrarily selected at 31 locations along three linear transects. Five (2017) and four (2018) disease assessments were performed in each field. The average SLB incidence was 41.2%, and severity (lesion length with *S. vesicarium* sporulation) was 7.2 cm. The logistic model best fitted the disease progress data for 72.2% of the datasets, typical of a polycyclic epidemic. The spatial distribution of the disease was adequately described by the binomial distribution for 66.7% of the datasets, and the random pattern was more frequently detected in 2017 than 2018. The dispersion index ranged from 0.62 to 2.32 with a median of 1.13 and detected aggregation in only 25% of the datasets. A random pattern was also predominantly observed by the geostatistical-based approaches. In addition, there was no spatial association between successive sampling periods. The relative contributions of primary and secondary inoculum sources for SLB epidemics are hypothesized and discussed.

Scalable grapevine viral disease detection with NASA imaging spectroscopy

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Viral disease, including that caused by grapevine (*Vitis spp.*) leafroll virus complex 3 (GLRV), causes >\$3 billion in damages and losses to the US wine industry annually. GLRV has a long latent period in which vines are infectious but do not display visible symptoms, making it an ideal model pathosystem to evaluate both symptomatic and asymptomatic remote sensing based disease detection. Vegetative imaging spectroscopy quantifies how light interacts with plants and is a promising tool for crop disease detection. Here, we use airborne spectroscopic data collected in September 2020 by NASA's Airborne Visible/Infrared Imaging Spectrometer Next Generation (AVIRIS-NG) to detect GLRV in California vineyards. In collaboration with industry and academic colleagues, 280 acres of Aglianico, Cabernet Sauvignon, and Petite Sirah grapes were scouted for disease symptoms at peak time of year and a subset collected for confirmation testing. Multiple endmember spectral mixture analysis (MESMA) and ColorSpace2 were used to prepare data for

analysis. Using random forest classification and partial least squares regression, we observed clear spectral differences that allowed for differentiation between healthy and GLRV infected vines at 1-m resolution with 80% accuracy. We hypothesize these spectral differences are linked to inherent disease physiology and will scale as has been seen with other physiology-linked processes. This work aims to develop a generalizable, rapid, scalable, and non-destructive viral detection method for use with NASA's forthcoming Surface Biology and Geology satellite.

High-polarity components of plant extracts stimulate *Colletotrichum acutatum* sensu lato to germinate

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Colletotrichum acutatum sensu lato causes citrus postbloom fruit drop and strawberry anthracnose. Although these diseases mainly affect citrus flowers and strawberry fruit, the pathogens survive on vegetative tissues on both hosts. Previous work showed that plant extracts and sugar solutions stimulate the quiescent pathogens to germinate on vegetative plant tissues. Conidia produced on vegetative tissues serve as the primary inoculum for disease. However, there is no information on the precise factor in the extract that stimulates germination, thus our objective was to identify the component responsible for the stimulus. We prepared water-based floral extracts with strawberry flowers, citrus flowers, and citrus leaves. Solutions containing all the sugars present in the floral extracts were prepared. We conducted bioassays to assess the effect of floral extracts, sugar solutions, and water on conidial germination of strawberry and citrus *C. acutatum* isolates. Germination was much higher with the floral and leaf extracts than with the sugar solutions. High-polarity and medium-polarity fractions of the leaf extract were separated and tested in the bioassays. The high polarity extracts stimulated germination the most. Untargeted metabolomics identified 13 major compounds in the high polarity fraction. Experiments are underway to test the individual components of the high-polarity portion of the leaf extracts.

Dissection of the SDS2-mediated cell death and defense pathway to *Magnaporthe oryzae* in rice

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Programmed cell death (PCD) plays an important role in plant immunity against pathogens. We found that SPL11 cell-death suppressor 2 (SDS2) is a receptor-like protein kinase and positively regulates PCD by phosphorylating the U-box E3 ligase SPL11. The protein also interacts with and phosphorylates OsRLCK118 and OsRLCK176 to regulate the NADPH oxidase OsRbohB for ROS generation. However, the co-receptor of SDS2 and the downstream pathways of the SDS2-mediated resistance are still unknown. In this study, we performed RNA-seq to identify genes that were upregulated by the overexpression of *SDS2*. Among the upregulated genes, 23 genes were chosen for further confirmation with qRT-PCR. The analysis shows that five out of 23 selected genes were upregulated in the *SDS2* overexpression line compared to that in the wild type (WT) Kitaaki. Among the five genes, we confirmed that OsBDG1 interacts with the SDS2 kinase-dead mutant but not with the WT in different interaction assays. The subcellular localization assay shows that OsBDG1 is localized both on the cell membrane and in the nucleus. The luciferase assay indicates that co-expression of *SDS2* and *OsBDG1* in rice protoplasts leads to decreased luciferase activity compared to the single expression of each gene, suggesting that the SDS2-OsBDG1 interaction may cause cell death. These results show that OsBDG1 might be a co-receptor of SDS2 that is involved in PCD and defense in rice.

Identification and characterization of long intergenic non-coding RNAs (lincRNAs) modulated during '*Candidatus Liberibacter* spp.' infection of potato

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Long non-coding RNAs are non-translational products of transcription which play a crucial role in gene regulation. Of them, a sub-class that resides in the intergenic regions, lincRNAs, remains largely uncharacterized in plants, especially in response to biotic stresses. Here, we assembled 4,397 reference lincRNAs in healthy potato and plants challenged with '*Candidatus Liberibacter* spp.' (CLso) using high throughput RNA-sequencing and bioinformatics approaches. Of the 4,397, less than 1% (9 lincRNAs) had a bonafide orthologs among other plant species (*Arabidopsis* and rice) based on reciprocal-BLAST analysis. Approximately 30% (1,308 lincRNAs) were differentially expressed by CLso during various stages of disease progression (7-, 14- and 21-days post infection) when compared to mock and insect-vector alone challenged plants. We further constructed co-expression networks of lincRNAs and protein-coding (PC) transcripts to understand the potential coordinated regulation of PC transcripts by lincRNAs. This analysis identified highly correlated lincRNAs and PC transcript modules encoding GST, expansin, and SAUR proteins, all of which could influence potato defense responses to CLso infection. Together, our study provides novel insights into the identity and expression dynamics of lincRNAs that underpin CLso-potato-insect vector interactions.

Studying the impact of bacterial communities on calcium availability in the peanut pegging zone

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Interactions between bacterial communities and plants have been shown to influence nutrition availability, further altering plants' growth and diseases susceptibility. A critical nutrient for proper peanut development is sufficient calcium within the peanut pegging zone during flowering. Without sufficient calcium, seed quality is reduced and disease susceptibility is increased. However, little research has shown the direct relationship between specific bacteria and their ability to improve seed quality. We have isolated a group

of bacteria known as calcite dissolving bacteria (CDB) from field soil, which are able to dissolve calcite (mineral form of calcium). CDB represent bacteria from multiple genera. We found that CDB could increase soluble calcium level in field soil and had positive effect on seed germination. As a geocarpic plant, peanut pods grow in the same soil environment as roots do, which sets an obstacle to distinguish pod-specific response to calcium deficiency from secondary responses derived from root physiology. We developed an “In-Tube-Growth” system, which allows us to study individual peanut pod development grown in a separated environment from roots. This system can be used to study pod-specific response to biotic and abiotic stresses. We aim to increase the soluble calcium level within the pegging zone through application of CDB and monitor individual pod responses to calcium level through the “In-Tube Growth” system.

Mining Fusarium head blight resistance in gene bank accessions of bread wheat

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Fusarium head blight (FHB) is devastating to wheat production and economic return due to loss of yield, quality, and compromised end use. Genetic resistance to FHB is a key integrated pest management strategy. Additional sources of resistance are continually needed to improve FHB resistance. There are five types of resistance; type II is resistance to spread of the pathogen within the spike. This project aims to characterize newly identified sources of FHB resistance in bread wheat (*Triticum aestivum* L.) among Plant Gene Resources of Canada accessions. Ten thousand accessions were evaluated for in-field resistance over the course of 6 years, of which 4,000 were re-evaluated over 3 years. The 200 lines with the greatest FHB resistance in the field disease nursery were selected for type II resistance evaluation in the greenhouse by point inoculation with *Fusarium graminearum* and phenotyping 21 days post inoculation. Genotypic data was obtained via a 90K Infinium SNP array. Greenhouse phenotypic data was combined with genotypic data to generate a genome wide association study (GWAS) for FHB type II resistance quantitative trait loci (QTL). The results from this GWAS will be compared to GWAS from previous field nursery evaluation, where a large number of non-*Fhb1* QTL for FHB resistance were identified on chromosomes 1A, 4A, 6A, 2B, 5B, 7B, 1D, 2D, and 3D. The knowledge, germplasm characterization, and markers generated from this project will aid in breeding for resistance to FHB and benefit the wheat industry.

Viruses of southern grapes in Mississippi

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Muscadines (*Vitis rotundifolia*) and southern-bred interspecific hybrid bunch grapes are the prevalent grapevines grown in the southern U.S. This study, designed to identify viruses and viroids infecting grapes in Mississippi, was carried out in 2018 and 2019. For this purpose, nucleic acids were extracted from individual muscadine (majority of samples) and southern hybrid vines, reverse transcribed, and custom sequenced on Illumina platform applying 2 × 150 nt pair-end methodology. Several grapevine-infecting viruses, belonging to genera *Closterovirus*, *Ampelovirus*, *Vitivirus* and *Marafivirus* considered economically important for the European (*Vitis vinifera*) grapes, were found to be relatively frequent in tested samples. Our results represent an original report on a few of these viruses for the state. In addition, our study furthers the general knowledge of grape-infecting viruses by discovery of some previously unreported viruses for this host.

Screening of commercial soybean cultivars for resistance to aerial blight caused by *Rhizoctonia solani* AG1-IA

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Rhizoctonia solani AG1-IA, the causal agent of aerial blight (AB) on soybean is a major production constraint in the mid-Southern US. In this region, rice/soybean rotation systems are common, and since the same pathogen infects rice (causing sheath blight), management of disease in one crop affects management in the other. Resistance to strobilurin fungicides was first documented in this pathogen in 2011 (from rice), and poor fungicide efficacy has since become common in both rice and soybean. Thus, long-term management of AB depends on the identification and use of resistant soybean cultivars. Currently, however, there is little information on soybean cultivar resistance to AB and field screening can be challenging due to the patchy distribution of this soilborne pathogen. The current project aims to develop a greenhouse cultivar screening protocol to screen the reaction of soybean cultivars grown in Louisiana for resistance to AB. For this, symptomatic soybean samples were collected from four parishes in Louisiana in 2020 and collection will be repeated in 2021. Identification of Isolates with AG1-IA specific and universal ITS primers have thus far confirmed that 87.5% were *R. solani* AG1-IA. Identification of the remaining isolates is currently underway. Virulence of the isolates is currently being tested using a detached-leaf assay and results suggest substantial variation in virulence among isolates (percent disease severity 8% to 57.46%). Whole-plant inoculations will be carried out using the most virulent isolates in order to screen commercial soybean varieties.

Outreach in the Big Sky Country—Promoting sustainable pest management practices in Montana

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Diverse sectors of the Montana economy depend on research-based integrated pest management (IPM) information. Agriculture is the largest segment of Montana's economy and organic crop acreage is steadily increasing, creating a need for IPM information specific to organic crop production. The state's growing urban population increases the demand for ornamental nursery products and urban IPM information. The Montana State University Extension IPM Program is supported by an interdisciplinary team of 11 specialists and

addresses the critical need for readily available, up to date IPM information. Our program is designed to reduce health and environmental risk from pest management and focuses on IPM implementation in agronomic crops and urban communities, supports diagnostic facilities, and delivers IPM education to pesticide applicators. We utilize various communication channels including workshops, grower meetings, traditional media, online resources, and social media to reach our audience. Our audience is diverse, including farmers, home and landowners, agricultural professionals, pesticide applicators, state and federal organizations, and private businesses. The Schutter Diagnostic Laboratory is an integral part of the IPM program serving Montanans with pest identification and management recommendations. Its economic impact on the state through diagnostic services amounted to \$14.9 million from 2018 through 2020. The impacts of our diagnostic lab along with the contributions of the other IPM specialists make our program vital to the implementation of IPM practices in Montana.

The distribution of *Fusarium* species and their chemotypes across Western Canadian producer wheat fields

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Significant wheat yield is lost annually due to *Fusarium* head blight (FHB), a fungal disease that affects small grain cereal crops worldwide. This project aims to investigate the leading players of FHB in Western Canadian producer fields. Analysis of deoxynivalenol in spring and winter wheat samples from 65 participating fields across Western Canada revealed that spring wheat samples contained 0.01 ppm to 1.7 ppm, 0.01 ppm to 8.5 ppm and 0.01 ppm to 1.7 ppm of DON in 2019 samples from Manitoba, Saskatchewan, and Alberta, respectively. The samples from 2020 had a lower DON in grain and chaff compared to 2019. DON levels for winter wheat were low (<0.2 ppm) for all three provinces in both years. Samples of chaff collected from the same spring wheat heads showed a higher DON content and higher *Fusarium* species diversity than in grain. Most samples collected were infected by *F. graminearum* (Fg), with the highest Fg percentage recovered from the spring wheat from Manitoba. Most of the Fg isolates contained the 3ADON chemotype. The 15ADON chemotype percentage was significantly lower in Alberta. The Fg abundance within spring wheat stalks is higher in Manitoba and Saskatchewan than in Alberta. However, a two-way ANOVA test showed that the differences were not significant for either Fg abundance between provinces ($P = 0.310$) or Fg abundance from three different sections of the stalks ($P = 0.148$). This study confirmed that Fg and 3ADON chemotype is becoming dominant in FHB in Western Canada.

Development of molecular tools for detecting genetically distinct biotypes of *Austropuccinia psidii*

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Myrtle rust is a disease caused by a biotrophic fungal pathogen, *Austropuccinia psidii*, thought to have originated in Central/South America. Recently, *A. psidii* has appeared in all continents, except Antarctica. A recent study revealed three major genetic groups of *A. psidii*: (1) a eucalypt/rose apple biotype found in Brazil, (2) a guava biotype found in Brazil, and (3) a pandemic biotype that occurs on multiple hosts with a wide global distribution. Subsequently, a distinct biotype from South Africa has been identified. Bioclimatic modeling was used to predict areas with suitable climate for *A. psidii* biotypes 1, 2, and 3 that are at risk from invasion. The bioclimatic modeling found that the eucalypt/rose biotype poses a threat to Hawai'i, Australia, and New Zealand, while the pandemic biotype threatens regions in Brazil/Uruguay. Our research objectives are to: identify genetic markers and develop loop-mediated isothermal amplification (LAMP) assays to easily distinguish among the four *A. psidii* biotypes; use LAMP assays to further document the distribution and host associations of each *A. psidii* biotype, and provide stakeholder groups with LAMP assays and associated training. Preliminary LAMP assays detect and distinguish the four *A. psidii* biotypes. Further testing is ongoing with more isolates from different regions and diverse hosts. The developed tools are critical to evaluate invasive threats by *A. psidii* biotypes, develop regulatory protocols for preventing spread into new areas, and improve management strategies for minimizing the damage caused by *A. psidii*.

A new *Diaphorina citri*-associated reo-like virus in Asian citrus psyllid populations in Florida citrus groves

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Huanglongbing (HLB) or citrus greening, is a devastating citrus disease currently jeopardizing Florida's citrus industry. *Candidatus Liberibacter asiaticus* (CLas), one of the putative bacterial pathogens causing HLB, is vectored and dispersed by the Asian citrus psyllid (ACP; *Diaphorina citri*). HLB and the ACP are endemic in Florida, further stressing the need for long-term control of this pathosystem. Microorganisms in the ACP, specifically viruses, may offer the potential to biologically control this damaging pest or to interfere with the transmission of CLas to citrus trees. This study builds upon ongoing research utilizing PCR and high-throughput sequencing (HTS) to analyze the virome of ACP populations collected from citrus groves throughout Florida. We collected thousands of ACPs from over 20 commercial citrus-producing counties throughout Florida. Using HTS and PCR, we detected known and novel viruses associated with the ACP. Here, we further characterize a novel reo-like (Family *Reoviridae*) double-stranded RNA virus tentatively named *Diaphorina citri* Cimodo-like virus (DcCLV). Nine segments belonging to this putative reo-like virus were identified using HTS and

subsequently verified back in positive samples using RT-PCR. RT-PCR surveys of the predicted RNA dependent RNA polymerase (RdRp) also detected this novel virus throughout Florida ACP populations not analyzed with HTS. Phylogenetic analysis of the DcCLV RdRp gene places this virus in an unassigned genus of the subfamily *Spinareovirinae*.

Soil texture and nutrients influence the diversity and abundance of beneficial bacterial genera in agricultural fields

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Soil nutrients and texture are important determinants of crop productivity and can have profound impacts on soil bacterial community structure and dynamics. A landscape scale study was conducted by collecting bulk soil samples from 14 farms across Pennsylvania to assess the influence of soil nutrients and texture on beneficial bacterial genera. Nutrients (P, K, Mg, Ca, S, Zn, Cu), pH, CEC, organic matter (OM), and texture (sand/silt/clay) of samples were assessed. DNA extracted from the same soil samples were PCR amplified targeting bacterial 16S rRNA genes (515F/806R primers) and sequenced (MiSeq). For each soil attribute, samples were categorized into three groups: high, medium, and low. The effect of each attribute on bacterial ($n = 23$) genera based β -diversity was assessed using PERMANOVA and beta dispersion tests. Soil pH, Mg, S, Zn, Cu were found to be the most important factors ($\alpha = 0.05$), explaining 6, 4, 5, 5, 4, and 4% of the total variation, respectively. Linear correlation analysis revealed significant ($\alpha = 0.05$) positive correlations between certain soil attributes and some genera (ex: OM and *Arthrobacter*, *Flavobacterium*, *Gaiella*, *Mycobacterium*). Some attributes were negatively correlated with certain genera (Ca and *Bryobacter*, *Ellin6067*, *Gemmatimonas*, *Haliangium*, *Sphingomonas*). *Bacillus*, *Bradyrhizobium*, *Kribbella*, *Lysobacter*, *Pseudomonas*, and *Streptomyces* read counts were not correlated with any of the measured soil attributes. Overall, results showed how bacteria-based soil health can be improved by manipulation of soil nutrients.

Potential of antidesiccants as a physical barrier to prevent infection of boxwood plants by *Calonectria pseudonaviculata* under controlled environments

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Boxwood blight caused by *Calonectria pseudonaviculata* (*Cps*) is an emerging disease affecting everyone on the horticultural supply chain from growers to consumers. Antidesiccants are film-forming polymers that are used at production and landscapes for mitigating environmental stresses including transplant shock, drought, drying winds, heat and sunburn, frost and freeze, as well as salt injury. The goal of this study was to evaluate the potential of six commonly used antidesiccant products as a physical barrier to prevent infection of boxwood by *Cps*. These products were Anti-Stress 2000, AquaLock, Moisture-Loc, TransFilm, Vapor Gard, and Wilt-Pruf. They were evaluated at recommended rates using *Buxus sempervirens* 'Justin Brouwers'. Container-grown boxwood plants pretreated with an antidesiccant were inoculated with *Cps* at 2×10^4 conidia/ml then incubated in close storage bins for 24 h to facilitate infection. Percent leaves blighted were assessed 14 days after inoculation. This study was repeated three times. TransFilm, Vapor Gard, and Wilt-Pruf consistently reduced blight incidence by 93 to 94% when compared to nontreated controls. Statistically, these three products performed as well as the fungicide standard—Concert II with chlorothalonil and propiconazole as its active ingredients. The other three products also reduced the infection but at lesser extents. Practical implications of these results are also discussed.

Genetic mapping of new necrotrophic effector genes in *Pyrenophora tritici-repentis*

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The ascomycete *Pyrenophora tritici-repentis* (*Ptr*) is the causal agent of tan spot of wheat, a common and economically important disease worldwide. Three necrotrophic effectors (NE), including *Ptr ToxA*, *Ptr ToxB*, and *Ptr ToxC*, have been identified from this fungal pathogen that act as important virulence factors. However, many studies have suggested that the fungal pathogen produces additional NEs. To identify new NEs from the race 2 isolate 86-124, we developed a fungal population from a cross between this isolate and the race 5 isolate DW5. The population was subjected to genotyping with SNP and SSR markers as well as the *ToxA*, *ToxB*, and mating type gene makers. For phenotyping, each progeny was inoculated onto the *Ptr ToxA*-insensitive wheat line CDC-Osler which was susceptible to 86-124, but resistant to DW5. The constructed genetic map consisted of eleven linkage groups corresponding to the eleven *P. tritici-repentis* chromosomes (Ch.) of the reference isolate Pt-1C-BFP. The multiple copy *ToxB* was mapped to two independent loci with one containing five copies and the other containing a single copy. QTL analysis revealed two genomic regions associated with virulence of 86-124 toward CDC-Osler, designated as *VirOsler*. The first region was identified on Ch. 2 as a major QTL (*VirOsler1*) accounting for 80% disease variations, and the second one was mapped as a minor QTL (*VirOsler2*). The identification of new effector genes is a significant step toward understanding fungal virulence in the *Ptr*-wheat host pathogen interaction.

Highly efficient generation of canker resistant sweet orange enabled by optimized CRISPR/Cas9 system

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Sweet orange (*Citrus sinensis*) is the most economically important species for citrus industry. However, it is susceptible to citrus bacterial canker (CBC) and Huanglongbing (HLB). The diseases already caused about 70% reduction of citrus production in Florida, one of the major citrus production regions in the world. Due to the hybrid nature of sweet orange, most genes exist as two heterozygous alleles. Generation of biallelic mutants remains difficult for sweet orange due to low transformation rate, existence of heterozygous alleles for target genes and low biallelic editing efficacy. Therefore, it is highly desirable to significantly increase editing efficacy to generate biallelic mutants. In this study we optimized CRISPR/Cas9 system to generate biallelic mutants to a rate as high as 89% by using albino (*PDS* null mutation) as readout. By using this improved system, we obtained more than 78% biallelic mutants for two different alleles of TAL effector binding element (EBE) of canker susceptibility gene *CsLOB1* in sweet orange. We have generated 6 different biallelic mutant lines in the EBE region of *CsLOB1*. The generated biallelic hybrid sweet orange mutant plants are resistant to

citrus canker. Importantly, we achieved high editing efficiency of biallelic mutation for hybrid sweet orange to the rate comparable to that in model plant rice with homozygous genome. This improvement in citrus genome editing makes functional genomics study on hybrid sweet orange more feasible. Particularly it will help speed up citrus breeding for varieties resistant to CBC and HLB.

Plant health in a pandemic: Challenges and impacts of the COVID-19 crisis in developing countries

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The COVID-19 pandemic has challenged the health, safety, and economic wellbeing of communities around the world. Government-mandated lockdowns, travel restrictions, and limitations on in-person gatherings have impacted agriculture in various ways. The agricultural sector in developing countries have been particularly hard hit by the pandemic. Presented here is an analysis of COVID-19 risk assessments conducted by ACIDI/VOCA implemented USDA and USAID projects, across seven developing countries. These assessments illuminate the impacts of COVID-19 across various aspects of these agricultural systems, including production and operations, finances, sales, jobs, and food and nutrition security. Specific groups, including women and small/micro-sized business, have been more severely affected by the COVID-19 pandemic. We examine how these findings, such as decreased access to extension services or inputs like pesticides or fertilizer, are directly connected to current and potential repercussions for plant health and the resilience of these producers. These impacts also provide insight into necessary measures for promoting future resiliency in these agricultural systems.

Identification of powdery mildew resistant marker in watermelon by metabolomics and genomics approach

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Watermelon (*Citrullus lanatus*) is an important cucurbitaceous crop species with high economic and nutritive value. It is widely cultivated in USA with an approximate global production of >100 million tons. However, its production in U.S. is severely affected by Powdery mildew (PM) disease caused by *Podosphaera xanthii*. Due to wide host-range, and presence of highly variable pathogen population structure throughout the year, the pathogen poses serious threat to watermelon production in USA and globally. This enable growers to depend heavily on fungicides to control the spread of the disease. However, the overuse of fungicides has an impact to environment and development of fungicide insensitivity in *P. xanthii* isolates. Given the global problem associated with current management strategies, here we identified a metabolized-based marker (phytomelatonin) through resistant root stock grafting approach which can impart both local and systemic resistance response against *P. xanthii*. Comparative whole-genome resequencing analysis of PM-resistant USVL531-PMR, PM-susceptible USVL677-PMS and 4 PM resistant RIL and F2 lines (USVL531-PMR × USVL677-PMS) has led us to identify a region of PM resistance introgressed break point ClaPMR2. This region was further validated on a F2 population with KASP (R vs S) markers, which can be utilized in resistance breeding in commercial cultivated watermelon lines. The sustainable approach adopted in the project has potential to improve watermelon production and increase protection from *P. xanthii* by serving as an alternative to overuse of fungicides.

Assessing hyperspectral reflectance of soybean sudden death syndrome (SDS) infected plants prior to foliar symptoms

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Soybean (*Glycine max* (L.) Merrill) is the world's most important seed legume; it serves as a source of protein and oil. Soybean production is affected by more than 200 plant pathogens of which sudden death syndrome (SDS) is one of the top five soybean yield-limiting diseases. SDS is caused by a soilborne fungus, *Fusarium virguliforme* (Fv), which results in two phases (root necrosis followed by foliar interveinal chlorosis and necrosis). Foliar symptoms usually occur late in the growing season and as such a destructive method is typically used to identify root rot phase. There is the need for a nondestructive technique to identify SDS infected soybeans prior to visual foliar symptoms. Our aim is to use spectral reflectance to assess any spectral signature that occurs as a result of physiological responses in soybean plants infected with SDS prior to foliar symptoms. A SDS susceptible variety was planted in the greenhouse with three soil treatments (water control, 5,000 Fv spores/ml, and 10,000 Fv spores/ml) in a randomized complete block design with four replicates. Reflectance data was collected at 21, 28, 35, and 42 days after planting using a high-throughput phenotyping platform with red green blue (RGB) and hyperspectral cameras. Real-time polymerase chain reaction (qPCR) was used to quantify Fv in the roots. Successful completion of this work will help us to understand SDS percentage reflectance in the appropriate wavebands under different inoculum densities; this could potentially serve as a beneficial technique in early identification of SDS prior to foliar symptoms.

Reduction of the G143A mutation in *Erysiphe necator* overwintering chasmothecia

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Erysiphe necator, a major grape pathogen, has recently been shown to have widespread resistance to quinone outside inhibitor (QoI) fungicides. Qualitative resistance results from the G143A substitution in the mitochondrial *cytb* gene that can be monitored via a competitive TaqMan qPCR assay. A decrease in the mutation frequency in asexual conidia from the fall to spring has been observed, suggesting a potential fitness cost from the mutation in overwintering sexual chasmothecia. To examine this hypothesis, chasmothecia were collected from vineyards in the Willamette Valley of OR prior to leaf drop (late Oct) and prior to budbreak (Mar). Leaves or bark were placed in bags, agitated with ice water, and filtered through mosquito netting to capture chasmothecia. The detected G143A frequency reduced from 81 to 46% in 2019–2020 ($n = 107$). There was an initial G143A frequency of 92% ($n = 131$) for fall 2020, with spring 2021 sampling still in progress. Additionally, in fall 2019 chasmothecia populations from 4 vineyards were inoculated onto artificial grape trunks, maintained outside, and ascospore release monitored using impaction samplers from February to May 2020.

From an initial chasmothecia G143A frequency of 83%, detection of the G143A mutation in the resulting ascospores decreased from 60% to 17% by bud break (April 11). These data indicate that either the G143A mutation decreases the survival of chasmothecia or increases their maturation rate, and therefore pre-budbreak ascospore release. Both mechanisms would result in a lower frequency of the G143A population in the spring epidemic.

Field scale genotypic diversity of *Sclerotinia sclerotiorum* in soybeans in Pennsylvania and Minas Gerais, Brazil

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White mold (WM), caused by *Sclerotinia sclerotiorum* (*Ss*), is an economically important disease affecting soybean production. Currently, the literature is unclear as to whether the genetic structure of the pathogen is predominately defined by selfing or sexual outcrossing. The goal of this research is to determine the genotypic diversity of Pennsylvania (PA) *Ss* populations at the field scale. In 2020, six soybean fields with a history of white mold in PA were gridded and soil sampled to quantify the number of sclerotia. Harvested sclerotia were germinated on potato dextrose agar, and hyphal tip isolation was performed. From five fields, 216 isolates were obtained, and DNA was extracted. Isolates will be genotyped using nine microsatellite markers identified from the literature. Genotypes will be determined by fragment analysis using capillary electrophoresis. Results will be compared at a field scale to determine the population structure. The population structure identified in PA will be compared with that obtained from Minas Gerais, Brazil, using the same genetic markers. Comparisons will identify any differences in population structure and genetic variation at a field scale between PA and Brazilian populations. Expected results will improve our understanding of how quickly the pathogen overcomes resistance, adapts to fungicide use, and increase our understanding of the potential local spatial movement of inoculum.

Toward pyramiding resistance to early and late leaf spot in peanut with alleles from the wild species *Arachis cardenasii*

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Early (ELS) and late (LLS) leaf spots caused by *Passalora arachidicola* and *Nothopassalora personata* respectively, produce severe losses on peanut yield. Both are managed by fungicide programs but the use of resistant cultivars is still the most economical way to mitigate them. This study aims to evaluate resistance to ELS and LLS in peanut hybrids with wild-derived chromosome segments from *Arachis cardenasii*. Hybrids were obtained by crossing Bailey, with resistance to various diseases including ELS and IAC 321, with resistance to LLS and rust. Hybrids were backcrossed and BC₁F₁s with selected combinations of segments were evaluated for ELS and LLS resistance using a detached leaf bioassay. Six disease measures were examined: incubation period (IP), lesion number per leaf area (LN/LA), number of sporulating lesions per leaf area (SL/LA), percent diseased leaf area (DLA), area under the disease progress curve of lesion number per leaf area (AUDPC_{LN}) and area under the disease progress curve of number of sporulating lesions per leaf area (AUDPC_{SL}). ELS bioassay results showed that hybrids and controls varied on all disease measures except IP. Results of LLS bioassays revealed differences among hybrids and controls on LN/LA, DLA, AUDPC_{LN} and AUDPC_{SL}. The results suggest that hybrids with segments from Bailey and IAC 321 derived from *A. cardenasii* exhibited higher level of resistance to both ELS and LLS than the parents. Development of advanced lines with multiple resistances that combine wild-derived segments will provide long-term, high level of resistance to these diseases.

Segregation of the resistance in wild potato hybrids against soft rot caused by *Dickeya dianthicola*

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Soft rot and blackleg of potato (*Solanum tuberosum*) are caused by soft rot Pectobacteriaceae, including *Pectobacterium* and *Dickeya*. Since 2014, there have been outbreaks of soft rot and blackleg caused by *Dickeya dianthicola* in the potato growing regions of the eastern US and Canada. Therefore, host resistant materials for potato breeding are needed. In our previous study, we found that high resistance was exhibited in inoculated tubers of the wild potato species *S. microdontum*. We crossed one extremely resistant *S. microdontum* clone "SCAN1110" = PI 458355 (female), with best linear unbiased prediction (BLUP) lesion size 2.8 mm, with a self-compatible and *Dickeya* susceptible clone "Yoshi6" = PI 654351, with lesion size 4.4 mm. We selected three F₁ hybrid potato plants for their self-compatibility and tuberizing ability. When the tubers of 37 S₁ plants were inoculated with *D. dianthicola*, the BLUP of lesion sizes in tubers fell into a broad spectrum ranging from 2.8 to 7.5 mm. Thus, we appear to have created an unbiased diploid F₂ population that encompasses the extremes of *Dickeya dianthicola* resistance. It should be useful for study of the genetics and physiology of the trait.

Microbial and insect consumers impose increasing cumulative costs on perennial plant productivity over time

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The effects of biodiversity on ecosystem productivity may be underestimated as they ignore losses in productivity due to consumers, including foliar, rhizosphere fungi, and arthropods. Moreover, the costs of consumers to perennial plants may be amplified across seasons, as stored carbon reserves can play important roles in subsequent year productivity. Here we characterize the cumulative effects of consumers on productivity in perennial grassland communities varying in plant diversity. We used pesticides to remove arthropods, soil fungi, foliar fungi, and all consumers in combination from plots maintained at 1, 4, and 16 plant species, and estimated 9 years of ecosystem productivity using biweekly plot reflectance (NDVI) of aboveground vegetation and with annual root cores. The positive

effects of removing foliar fungi and arthropods on aboveground productivity increased across nearly a decade. This increase was fastest in monoculture plots, suggesting that diverse communities were more resilient to consumer impacts. Finally, while consumer removal generally increased root biomass, we found no cumulative increases in benefits across growing seasons. This work showcases the successive, cumulative impacts of consumers on aboveground primary productivity and their disproportionate effects in low-diversity plant communities. Moreover, these results suggest that foliar consumers impose greater costs to belowground investment in monoculture than in polyculture, highlighting potentially critical connections between plant diversity, consumers, and belowground carbon investments.

Investigating chemical control options for halo blight of hops

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Halo blight of hops, caused by the *Diaporthe humulicola*, is an emerging fungal disease, causing yield loss in the eastern United States. This disease can cause between 20–50% yield loss when untreated and currently no chemical treatment options are available for growers. That said, currently registered fungicides that are available for powdery mildew could be used to prevent yield loss and reduce symptoms based on research in related pathosystems. Active ingredients *Bacillus mycoides*, cyflufenamid, flutriafol, metrafenone, pyraclostrobin + boscalid, quinoxifen, tebuconazole + fluopyram, trifloxystrobins were evaluated for their ability to control halo blight in both in vivo and in vitro conditions. Field trials of these fungicides were conducted in a low trellised hopyard in East Lansing, Michigan, in 2020 and 2021 using a randomized block design, and incidence and severity data was collected throughout the growing season. In 2021 a poison agar assay using six different concentrations of flutriafol or cyflufenamid was used to assess conidia germination and radial fungal growth, in which radial growth was measured at days 3 and 10 and conidial germination was assessed at hours 5 and 24. Field experiments show that the ingredient flutriafol can reduce the incidence and severity of disease symptoms in leaf tissue by 15 and 30% respectively when compared to our untreated control treatment. With data collected from these experiments hop growers will be able to utilize specific powdery mildew products to help combat this emerging disease.

Regenerative potato cropping effects on diseases, insects, and rhizosphere microbiome in organic potatoes

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Soil disturbance before planting, hilling and harvesting of potatoes as well as lack of soil cover early and late in the season often lead to depletion of organic matter and soil nutrients. Regenerative potato cropping is based on the shallow incorporation of a well-developed winter cover crop (4–10 cm) before planting without ploughing, and application of a nutrient rich dead mulch after the first hilling operation instead of further hilling. Experiments were conducted from 2014 to 2020 under natural inoculum pressure. When *Phytophthora infestans* and/or *Alternaria solani* occurred, disease was consistently reduced by at least one third by regenerative cropping. In addition, potato beetle (*Leptionotarsa decemlineata*) egg clutches and larval abundance were reduced by 30 to 60% while larval development and damage to the potatoes was dramatically reduced in mulched crops. Preliminary results show that macro- and micronutrient levels differ in mulched potatoes while water stress is reduced. Tillage was the main driver shaping the rhizosphere fungal communities. Indicator taxa associated with reduced tillage among others included fungal species with biocontrol potential against insect pests (e.g., *Actinomyces elegans*) as well as fungi that produce mushrooms known to efficiently decompose fresh organic material. Potato yields in minimum tillage after leguminous cover crops combined with nutritious mulch were equal to ploughed and regularly hilled potatoes with no difference in tuber health. Succeeding crops greatly profit from improved soil fertility and soil water conservation.

Influence of spore traits on the microbiome composition of plant organs

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Microbiomes from maize, soybean, and wheat were characterized in a long-term three-crop rotation research site to begin unraveling the origins of the fungal and bacterial communities of leaves, stems, and roots. Communities of plant organs were characterized across the growing season using amplicon sequencing. Communities differed across hosts, and among plant growth stages and organs. Roots consistently showed the highest number of bacterial OTUs compared to aboveground organs, whereas the alpha diversity of fungi was similar between above- and belowground organs. The fungal taxa specific to roots, stems, or leaves were examined to determine if the specificity reflected their life histories based on previous studies. The analysis suggests that fungal spore traits are drivers of organ specificity in the fungal community. Understanding how community structure of specific crop organs is formed, will provide a critical resource for manipulations of microbial communities. The ability to predict how organ specific communities are influenced by spore traits will enhance our ability to introduce them sustainably.

Understanding the infection mechanism of *Cercospora beticola* of sugar beet using advanced staining technique and confocal laser scanning microscopy

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Cercospora leaf spot, caused by *Cercospora beticola* Sacc. is a major disease in sugar beet. *C. beticola* produces toxins and enzymes to alter the host membrane permeability, triggering the hypersensitive response (HR). A 12-h interval time-course experiment up to 120 h

was conducted to determine the progression of *C. beticola* growth on sugar beet varieties. Leaf samples were stained with DAB solution to detect the production of HR-related H₂O₂. Samples were also stained with WGA-Alexa-Fluor-488 dye for in planta fungal growth visualization on confocal laser scanning microscope. In DAB staining, susceptible variety exhibited an early HR starting from 48 h post-inoculation (hpi), whereas in resistant variety HR was not detected until 60 hpi. At later time-points, the amount and spread of HR in susceptible variety was significantly higher than resistant variety indicating the nutritional success of pathogen. In fluorescently stained samples, pathogen penetration followed by conidia germination was either direct or by producing appressorium on stomata starting at 48 hpi in susceptible variety while at 60 hpi in resistant variety, later followed by secondary hyphae produced appressorium onto stomatal guard cells. Thus, pathogen entry and HR were correlated indicating importance of early establishment in pathogens' infection success. We hypothesized that a spatio-temporally controlled host cell-death machinery is in constant struggle with evolving *C. beticola* pathogen effectors. RNAseq analysis is underway to decipher the molecular mechanism of this pathosystem.

Comparing the efficiency of spore traps to capture airborne inoculum of *Cercospora* spp. on soybean

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Cercospora leaf blight (CLB) of soybean is a devastating foliar disease in Louisiana caused by *Cercospora* cf. *flagellaris* and *Cercospora* cf. *siganotica*. Although seeds were considered the primary inoculum, recent studies suggest that CLB epidemics are more likely initiated by airborne conidia. With the goal to use spore traps to target fungicide applications, we compared the relative efficiency of three spore traps to capture air-borne fungi in soybean fields. An active Burkard spore trap and two passive spore traps—wind vane and windsock mesh-based (latter from Phenotype Screening, TN)—were used. Spore traps were set-up around a soybean field in Winnsboro, LA, from May–November 2019, with weekly sampling (total of 216 samples collected). DNA was extracted, pooled every two weeks, the ITS2 region was amplified with fITS9-ITS4 primers, and the amplicon library was sequenced on an Illumina MiSeq. Operational taxonomic units (OTU) that likely correspond to *C. cf. siganotica* and one other *Cercospora* were observed in high frequency in the first 2 weeks of August and September and OTUs likely *C. cf. flagellaris*, in October. Because the ITS2 haplotypes are identical to multiple species of *Cercospora*, the identity of these OTUs and spore peaks are being verified by quantitative PCR using species-specific primers/probes. The active spore trap and the wind vane traps performed better than the windsock trap in capturing greater numbers and diversity of fungi. Experiments targeting fungicide applications during spore peaks to manage CLB are currently underway.

Comparing prediction models for sclerotinia stem rot (*Sclerotinia sclerotiorum*) in soybean (*Glycine max*) in Quebec

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In Quebec, the disease Sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) causes yield losses in soybean (*Glycine max* (L.) Merr). Producers often rely on prophylactic fungicides to manage the risk of disease incidence. However, whether pesticides are needed depends on the risk of disease development, which is largely influenced by environmental conditions prevailing prior to and during the soybean flowering period. Inappropriate fungicide use is costly economically and environmentally. To evaluate the risk of SSR disease development, researchers have created multiple SSR prediction models. Models either predict end-of-season disease incidence directly or indirectly by forecasting the risk of inoculum presence during the soybean flowering period. Apothecia and disease severity observations from 2019 and 2020 were used to evaluate the ability of previously published bioclimatic models to predict apothecia production and/or disease incidence in Quebec commercial and research fields through receiver operating characteristic (ROC) curve analysis. Upon validation, models were customized using the data collected in Quebec to improve their predictive ability in the local context. The most accurate apothecia formation model used maximum temperature, while the best disease incidence model used maximum temperature, wind speed, and relative humidity, with AUCs of 0.84 and 0.71, respectively. These results suggest that published models provide a reliable basis for the development of an adapted SSR forecasting system for Quebec soybean growers.

Characterization and prevalence of Potato virus Y strains in Michigan

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Potato virus Y (PVY) is a major concern throughout the US and is one of the primary diseases monitored and tested for in the seed certification process. Detection of PVY in early generation potato seed lots will help prevent infected material from entering the production chain and will prevent unnecessary yield and profit loss. PVY is a complex of multiple strains that cause a variety of foliar and tuber symptoms. PVY^{NTN}, a recombinant strain, is the main cause of potato tuber ringspot disease (PTNRD), which causes yield loss and reduces tuber quality for susceptible potato cultivars. Understanding the local PVY strain population is critical for detection and management. In this study, we investigated PVY strains in Michigan. In 2019, samples were collected from a seed lot with high levels of visual foliar symptoms of PVY in the field (4.9%). In 2020, samples were collected from seed lots with three levels of visual foliar symptoms of PVY in the field, high (0.9%), medium (0.23%) and low incidence (0.01%). We conducted reverse-transcriptase high-fidelity polymerase chain reaction (RT-PCR) tuber testing. In 2019, high levels of PVY (12.9–100%) were observed in a visually high-incidence lot. The majority of tested samples were positive for PVY^{N:Wi}. In 2020, more than 90% of tested samples were positive for the PVY strain N:Wi. However, N:O, NTN, and O strains were also present. Overall, our results suggest that PVY^{N:Wi} is the prevalent strain in Michigan. However, testing is ongoing to better understand impacts on seed production and the Michigan certification program.

Alaskan fungi attributed to cause bud blight disease in spruce share several similarities

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Bud blight disease of spruce (*Picea* spp.) in Alaska is attributed to three different fungal organisms *Gemmamyces piceae*, *Dichomera gemmicola*, and *Camarosporium* sp. Except for *G. piceae*, the exact role of these organisms in bud blight disease remains unclear. Interestingly, these fungi have been found to share several similarities. First, they share the same ecological niche and sometimes are found in the same symptomatic buds. Secondly, local alignments of the ITS, RPB2, BTUB, and TEF1 genes showed that partial gene regions were identical and only a small number of nucleotide changes were observed between species. Third, phylogenetic analyses using several methods (maximum-likelihood and Bayesian methods) placed these species in family Melanommataceae with strong support values. To date, five whole genomes of two of these species have been sequenced, *G. piceae* (HiSeq platform, 9, 18 Gb raw data) and *Camarosporium* sp. (MiSeq platform, 8, 8, 8 Gb raw data). De novo assemblies were performed using Spades and the number of contigs produced were 2,895, 7,571, 1,051, 1,028 and 3,364, respectively. Total length for each assembly was 40, 49, 50, 41, and 41 Mb, while GC% content was 47.1, 48.9, 51.6, 48.7, and 50.9, respectively. Whole genome information will be useful to identify shared genetic regions and aid identification of genes that could be involved in pathogenesis, such as effectors, carbohydrate active enzymes, peptidases, and sugar transporters. Diagnostic assays to differentiate *G. piceae* from *Camarosporium* sp. in nature will also be developed based on genomic differences.

Susceptibility of rosaceous and citrus fruits to infection by the notorious food spoiling and mycotoxin-producing fungus *Paecilomyces niveus*

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Paecilomyces rot is a recently described postharvest disease of apples. The causal agent, *Paecilomyces niveus*, is a long-standing food spoiling agent of fruit products. Its thermotolerant ascospores can withstand some pasteurization processes used to sterilize food and germinate in finished product. *Paecilomyces niveus* produces the FDA-regulated mycotoxin patulin, an important contaminant of apple, orange, and other various fruit juices that can cause gastrointestinal and neurological damage. *P. niveus* contamination was assumed to arise solely through environmental sources, but now it is known that apples infected with *P. niveus* can also serve as sources of spoilage inoculum. In this study our objective was to assess the host range of *Paecilomyces* rot in cultivated fruits. We show that multiple popular apple cultivars in addition to other rosaceous fruits: peaches, pears, and cherries are susceptible to *Paecilomyces* rot. We also demonstrate the ability of *P. niveus* to infect, cause symptoms, and reproduce in oranges and clementines—distinguishing it from another infamous patulin producer and postharvest pathogen, *Penicillium expansum*. Lastly, we found that two closely related food spoiling species *P. fulvus* and *P. variotii* are unable to act as wound-infecting pathogens of apples. Our results challenge the assumption that *P. niveus* contamination can be solely environmental and raises concern about the role infected fruits play in *P. niveus* contamination of foods.

Discrimination of two closely related spinach leaf spot pathogens, caused by *Stemphylium vesicarium* and *S. beticola* based on species specific primers

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Leaf spot diseases of spinach, caused by *Stemphylium vesicarium* and *S. beticola*, are important leaf spot pathogens of spinach. Although isolates were initially characterized as *S. botryosum*, more recent studies indicate that the isolates can be identified as *S. vesicarium* or *S. beticola* based on a multilocus gene sequencing. Accurate detection and identification is necessary for epidemiological studies and effective disease management. Based on the sequence alignment of the glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and calmodulin (*cmdA*) genes, four sets of species-specific primers were designed and evaluated for their species-specificity to *S. vesicarium* (Sb-1-St001, Sb-2-St002, A-St416, TX-1, and TX-3) and *S. beticola* (Sb-4-St037, Sb-5-St030, Sb-6-St257, TX-8, and CA-1). Results showed that the primer pairs from each species based on either *gapdh* or *cmdA* genes were highly sensitive and specific for detection and differentiation of each species based on DNA isolations from cultured isolates. Efforts are underway to evaluate the robustness of the species specific primers with a broader set of isolates of each species and from field samples. The PCR based diagnostic analysis can be completed within 24 h, compared with 1–2 weeks for doing isolations and molecular identification. Differentiation the two species is important in that the biology, epidemiology, and fungicide resistance can vary between the two species and therefore impact disease management decisions.

Lessons from two years of citrus disease surveys in Georgia, USA

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Exponential growth of Georgia's citrus industry during recent years has seen commercial acreage in the state increase from essentially no acres in 2012 to an estimated 2,700 acres in 2021. This expansion is largely due to market opportunities available for cold-hardy citrus varieties following the extended decline in Florida citrus production due to huanglongbing (HLB) and other diseases. As citrus acreage increases in Georgia, concerns about citrus diseases increase as well. To better understand the prevalence and distribution of citrus diseases in Georgia, disease surveys of commercial citrus orchards, nurseries, and residential trees were conducted in 2019 and 2020. To test for *Candidatus* Liberibacter asiaticus (CLAs), causal agent of HLB, a total of 249 samples were collected from trees in 9 commercial and 28 residential sites in 15 Georgia counties; 11 of 249 trees (4.4%) tested positive for CLAs, with 8 sites in 7 counties having at least one positive tree. All 11 CLAs-positive trees came from residential sites, and CLAs-positive trees were confirmed for the first time in Bryan, Glynn, Lowndes, Pierce, and Ware counties. Sampled trees were also screened for citrus canker (caused by

Xanthomonas citri subsp. *citri*), but to date no citrus canker has been identified in Georgia. In addition, testing efforts also identified *Citrus tristeza virus* and Hop stunt viroid in Georgia citrus for the first time. To protect Georgia's young citrus industry and make growers aware of disease issues, continued monitoring efforts are warranted for these and other citrus pathogens.

Triazole resistance in human pathogen *Aspergillus fumigatus* on commercial plant products

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Aspergillus fumigatus, a fungus associated with decaying plant material, is the major cause of aspergillosis. The primary treatment is triazole antifungals. An increasing number of patients have presented with pan-azole-resistant strains of *A. fumigatus* prior to treatment with azoles. The TR₃₄/L98H and TR₄₆/Y121F/T289A alleles in the *cyp51A* gene are the most common mutations conferring pan-azole resistance. Several studies have concluded that these mutations arose in agricultural settings due to long-term use of triazole fungicides to combat plant-pathogenic fungi. Several studies have identified azole-resistant *A. fumigatus* on commercial plant products, but none have looked at plant products sold or produced in the United States. The purpose of this study was to identify if azole-resistant *A. fumigatus* is present on commercial products in the United States. Ten categories of retail plant products were screened for azole-resistant *A. fumigatus*. Of the approximately 500 isolates that have been collected, 6 from peanut and compost have been identified as pan-azole-resistant through minimum inhibitory concentration (MIC) testing. The mechanism of resistance of azole-resistant isolates will be investigated by *cyp51A* sequencing and population genetic analyses based on STRAf genotyping will be conducted to determine the relatedness of strains collected in this study to clinical and environmental strains worldwide. We provide additional evidence for azole-resistance on commercial products and attempts to elucidate the origin and hotspots of azole-resistance in the environment.

Silverleaf disease in nectarines and its molecular and immunochromatographic diagnosis

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Chile is one of the main stone fruit exporting countries. Nectarine (*Prunus persica* var. *nectarine*) is grown in Chile, mainly in the central area, between the Valparaíso and O'Higgins Regions. *Prunus* genus is host of various wood diseases such as Silverleaf which is a growing problem in Chile, reducing the yield and quality of fruit and decreasing orchards longevity. The presence of *C. purpureum* in the wood of commercial orchard trees showing foliar silver symptoms was confirmed by isolating wood sections in acidified potato dextrose agar and by PCR using species-specific primers. The intensity of foliar symptoms of infected plants was recorded, based on a severity scale. Leaves were tested through ELISA to detect and quantify the endoPG that *C. purpureum* produces. Leaves were macerated with an extraction buffer, incubated in primary and secondary antibodies, and the optical density (OD) was measured at 450 nm. Both the monitoring of symptoms and the collection of leaves were carried out every 30 days, until the leaves fell. It was possible to reproduce and quantify endoPG from nectarines leaves at different phenological stages, and showing different intensity of foliar symptoms, allowing *C. purpureum* detection at different stages of infection. It was determined that there is a relationship between the intensity of foliar symptoms and the concentration of endoPG in the leaves.

Harvester ants show no preference for diseased or asymptomatic sorghum seeds

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Red harvester ants (*Pogonomyrmex barbatus*), the primary food source for the endangered Texas horned lizard, are native to south Texas, where they perform many ecological functions such as soil aeration and nutrient cycling. Harvester ants scavenge seeds, including weed and agricultural seeds, from areas surrounding their nests and store the seeds underground in granaries for future consumption. Many of these seeds are affected by fungal pathogens that can hasten the decomposition process and shorten the storage life of seeds but may also serve to pre-digest difficult to degrade seed coats. Sorghum (*Sorghum bicolor*) seeds affected by head mold were collected from a local field, and sorted into symptomatic and asymptomatic categories. We conducted a choice assay on harvester ants from a suburban setting without prior exposure to sorghum seeds to determine whether there was preference for symptomatic seeds. Seed trays were placed along the harvesting trail of 20 unique colonies, and monitored at 1, 2, 4, and 24 h after placement for the number of remaining seeds. Survival analysis suggests that there were no significant differences between harvesting preferences for the symptomatic and asymptomatic seeds. This suggests that harvester ants may not be able to detect mold-affected sorghum seeds and may incidentally collect them. Furthermore, the scavenging of contaminated seeds into underground granaries suggests that the ants may unwittingly be creating buried pathogen inoculum sources for future epidemics, particularly in agricultural fields under routine tillage.

Field evaluation of rice resistance to seedling blight caused by *Rhizoctonia solani* AG-11 and AG-4

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Seedling blight is one of the most important diseases in rice in the southern United States. Seedling blight has been recently reported to be caused primarily by two *Rhizoctonia solani* anastomosis groups (AG-11 and AG-4). Use of resistant cultivars can be the most effective means to minimize economic loss caused by this disease. However, no information is available on rice resistance against both important pathogens. Field evaluation of 45 rice genotypes, consisting of 13 inbred and 13 hybrid cultivars, and 19 elite inbred breeding lines, for resistance to *R. solani* AG-11 and AG-4 was conducted in the field at Beaumont and Eagle Lake, TX. Significant differences in percent stand loss caused by either *R. solani* pathogen were observed among these rice genotypes. However, there were no cultivars

and breeding lines showing a high level of resistance to either pathogen. Stand loss caused by *R. solani* AG-11 ranged from 47 to 96%, while stand loss caused by *R. solani* AG-4 ranged from 80 to 97%. Only two cultivars RT7521 FP (hybrid) and CLL15 (inbred) showed partial resistance against *R. solani* AG-11, with stand loss being less than 50%. There was a positive correlation ($r = 0.5$, $P = 0.0004$) in stand loss between *R. solani* AG-11 and AG-4 although *R. solani* AG-4 tended to cause a higher level of stand loss in most rice genotypes than *R. solani* AG-11. These results can provide the foundation to developing effective seedling blight management strategies.

Effect of solitary and combined use of cover crops on soilborne disease suppressiveness in woody ornamental nursery production systems

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Cover crops are known to be effective in reducing soilborne diseases in woody ornamentals. However, it remains unknown how solitary and combined uses of cover crops influence soilborne disease, and whether these effects differ with seed rate. We carried out this study to explore the impacts of cover crops, in solitary and combined use, on soilborne disease suppressiveness in woody ornamental nursery production systems. Soils were sampled from the established red maple (*Acer rubrum* L.) plantation grown with and without cover crops (crimson clover [*Trifolium incarnatum* L.] or triticale [$\times$ *Triticosecale* W.] or [their mixture]) following the cover crop senescence. Greenhouse bioassays were carried out using red maple cuttings on inoculated (with *Rhizoctonia solani*, *Phytophthora vexans*, or *Phytophthora nicotianae*) and non-inoculated field soils. Plant height, total plant weight, and fresh root weight were measured and plant roots were assessed for root rot disease severity using a scale of 0 to 100% roots affected. There were no significant differences in plant height among the treatments. Total plant and fresh root weights were significantly or numerically greater for red maple plants grown in cover cropped soil. Although all the cover crop treatments demonstrated effective control of root rot diseases, a high rate of crimson clover and a high rate of the mixture were the most effective. These findings may be helpful to nursery growers in making soilborne disease management decisions.

Spatiotemporal pathogen tracking and histological responses of swamp bay, *Persea palustris*, during colonization by *Raffaelea lauricola*

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Laurel wilt disease, caused by the fungus *Raffaelea lauricola*, has devastated native members of the Lauraceae family throughout the southeastern United States and threatens avocado production in southern Florida. In swamp bay trees (*Persea palustris*) it causes wilt symptoms and mortality within weeks following inoculation. Disease symptoms are caused by a loss of hydraulic conductivity closely associated with the formation of tyloses in xylem vessels. We investigated the colonization of swamp bay trees by inoculating them with a green fluorescent protein (GFP)–expressing strain of *R. lauricola* and compared spatial location morphology and titers of the pathogen with the histological responses of the host. Data were collected at 3, 7, 10, 14, and 21 days after inoculation from three trees per time point (15 trees total) and three mock-inoculated trees. Trees were sampled every 10 cm above the inoculation point along the main stem and branches and 10 cm below the inoculation point. Transverse sections from each sample were observed by epifluorescence microscopy and both propagule numbers and the morphology of the pathogen were recorded. Sections of the same samples were also fixed, embedded, sectioned and stained to observe the histological responses of the host. The results provide a spatial-temporal understanding of *R. lauricola* dynamics during colonization relative to xylem occlusion and demonstrate why laurel wilt disease progresses so rapidly in susceptible hosts.

Economic impacts of downy mildew and reduced fungicide efficacy on slicing cucumber

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Fungicides remain the most important tool to manage downy mildew on cucumber. The objective of this study was to estimate yield losses that might occur if a fungicide to which *Pseudoperonospora cubensis* has demonstrated reduced efficacy was used. Downy mildew-susceptible ‘Speedway’ slicing cucumber was grown in Charleston, SC, in spring and fall 2020, sprayed seven times with one of four fungicides applied in alternation with chlorothalonil, and harvested 12 times. Chlorothalonil applied biweekly and water were controls. *P. cubensis* clade 2 isolates ($N = 3$) were present on foliage in both seasons. Final disease severity reached 80% in spring and 82% in fall in the water control and was $\geq 45\%$ in all four fungicide treatments. Severity was lowest in treatments with Presidio (fluopicolide) and Previcur Flex (propamocarb), intermediate with Orondis Opti (oxathiapiprolin + chlorothalonil), and highest with Curzate (cymoxanil) and both controls ($P < 0.05$). Nevertheless, mean marketable weight across the four fungicide treatments in both seasons was greater than marketable weight across the two control treatments, 1,087 and 655 cartons/ha, respectively ($P = 0.0001$). Profits also were greater for the four fungicide treatments than the controls, a mean of \$11,193/ha versus \$3,264/ha, respectively. Although cymoxanil did not reduce disease severity, yield did not differ significantly from yields in the other three more effective fungicide treatments. Fungicides with reduced efficacy may still protect hybrid slicing cucumber from yield losses due to downy mildew.

Csp1, a cold-shock protein homolog in grapevine pathogen *Xylella fastidiosa* is involved in biofilm formation, stress response, and virulence

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Bacterial cold shock proteins are conserved nucleic acid binding chaperones. Csp1 is a temperature-independent cold shock protein homolog in *Xylella fastidiosa*, a bacterial pathogen of grapevine and other economically important crops. Csp1 contributes to stress tolerance and virulence in *X. fastidiosa*. However, little is known about the specific function(s) of Csp1. To investigate the role(s) of Csp1, phenotypic differences between wild type *X. fastidiosa* strain Stag's Leap and a *csp1* deletion mutant ($\Delta csp1$) were compared. A significant decrease in cellular aggregation and surface attachment was observed in $\Delta csp1$. The $\Delta csp1$ strain was also less viable than wild type after long-term growth, in vitro. The role of Csp1 on gene expression was also investigated. Transcriptomes of wild type and $\Delta csp1$ grown under standard conditions were sequenced with Nanopore's MinION. RNA-Seq analysis showed changes in expression of several motility and biofilm-related genes. One gene of interest, *pilAI*, encodes a type IV pili subunit protein and was up regulated in $\Delta csp1$. Type IV pili contribute to virulence by facilitating bacterial movement inside host vascular tissue. Deleting *pilAI* from the Stag's Leap genome increased biofilm formation in vitro and reduced virulence in grapevines. *X. fastidiosa* virulence depends on attachment and movement within xylem vessels. Studies in other bacteria have also linked biofilm formation with stress responses. These results suggest Csp1 may play roles in virulence and stress tolerance by influencing expression of genes involved in biofilm formation.

Ceratocystis wilt and canker pathogens: A new diagnostic assay to detect them all

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All the 42 known species in the genus *Ceratocystis* are plant pathogens, mostly causing wilt and canker diseases on woody hosts. While many of them are aggressive and significant pathogens, molecular detection methods exist for only three species, making diagnostics of the remaining time-consuming and laborious. This is a significant risk considering the impact that some of these pathogens have had on newly adopted hosts, e.g., kiwifruit in Brazil and 'ōhi'a trees in Hawaii. To enable fast and sensitive detection of any *Ceratocystis* species in plant material, a two-probe based TaqMan real-time PCR assay was developed for the amplification of *Ceratocystis* DNA down to the concentration of 0.2 pg. The assay was tested using infected fig stem material: *C. ficicola* was successfully detected from symptomatic and asymptomatic tissues, even when bulking one part of infected tissue with nine parts of healthy stem tissue. This demonstrates that the assay can be a powerful tool for screening plant material presenting early or latent infections, which is crucial to ensure that pathogens are not introduced into new areas with imported plant material. To determine the species of *Ceratocystis* that was detected with the real-time PCR assay, a complementary end-point PCR was developed for DNA sequencing. Using these assays enables to reveal future *Ceratocystis* disease risks and allows timely detection of cryptic species that might become significant pathogens in the currently changing climatic conditions or when introduced into new habitats.

Phytotoxicity and toxin production from *Macrophomina phaseolina* isolated from strawberry in Florida

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Macrophomina phaseolina (*Mp*) causes charcoal rot disease in >500 cultivated and wild plants. Strawberry, one of the most important crops in Florida, can be severely damaged by *Mp* due to wilting of foliage, crown decay, drying and defoliation of older leaves, and plant death, resulting in major economic losses. We evaluated phytotoxicity and toxin production by *Mp* cultures isolated from various strawberry fields in Florida from 2001 to 2012. Forty *Mp* cultures were single-spored, and >85% found positive for (–)-botryodiplodin production using an in-culture colorimetric assay. *Mp* cultures grown on Czapek-Dox liquid medium for ~10 days at 28°C were filtered through 0.45µm membrane to produce cell-free filtrates. Ninety-eight percent of *Mp* culture filtrates induced phytotoxicity symptoms in soybean seedlings in hydroponic culture with 5% wilting, 23% chlorosis, 35% necrosis, and 78% death. LC-MS/MS analysis of *Mp* culture filtrates identified one or more mycotoxins in each, including 3% 3-nitropropionic acid, 85% botryodiplodin, 3% brevianamide F, 65% cordycepin, 5% cyclo(L-Pro-Tyr), 5% cyclo(L-Pro-L-Val), 53% gliocladiic acid, 90% kojic acid, 3% mellein, 83% moniliformin, and 85% orsellinic acid. Phaseolinone was also identified. These toxins may play a role in the virulence of *Mp*. Further research is needed to identify any additional toxins in *Mp* cultures and to determine their role in the infection mechanism in order to develop better approaches to managing charcoal rot in strawberry and other crops.

Development of molecular methods for genotyping isolates of *Polymyxa betae*

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Despite the fact that *Polymyxa betae* is a vector of different soilborne viruses that cause important diseases in sugar beet, it is still poorly studied. High throughput Illumina sequencing and comparative genomics were used to develop molecular methods for genotyping isolates of *P. betae*. A single cystosorus culture collection was established for a range of isolates recovered from soil samples collected from sugar beet growing areas across the U.S.A. DNA from heavily infected roots from seven geographically diverse isolates were submitted for Illumina sequencing. Comparative genomics, using a Belgium isolate as reference sequence, was used to identify polymorphic regions among the eight genomes. Eighteen genotyping markers were tested on ten *P. betae* isolates, most of the primers tested performed well and the amplicons obtained were sequenced. Multiple sequence alignments identified polymorphisms in the sequences obtained with a set of twelve primer pairs. A total of 132 DNA sequences were concatenated, including the reference sequence, for each locus. The neighbor-joining method was used for phylogenetic reconstruction as implemented in Geneious 2020.2.3 and network analysis was performed using the neighborNet method from the SplitsTree5 software. Both analyses showed different clades independent of the geographic origin, revealing genetic variability among the *P. betae* isolates. Currently, we are testing field samples from sugar beet production areas to validate the marker set for population studies in commercial production fields.

Seed transmission of a cacao virus from the Americas and the implication on crop cultivation and movement of germplasm
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Cacao mild mosaic virus (CaMMV), previously known as cacao *Trinidad virus* (CTV) strain A, was recently reported in Puerto Rico and Brazil, and may be widespread in the Americas. The virus causes mosaic and mottling on leaves and pods of *Theobroma cacao*, and is associated with dieback symptoms in the field. Improved cacao clones developed by breeding programs are primarily propagated through grafting, which can lead to an accumulation of viruses in germplasm. The importance of screening cacao budwood for viruses is well understood, but seedlings used as rootstock are not routinely screened for viruses. Most known cacao viruses belong to the *Badnavirus* genus, in which vertical transmission is rare. However, a grow-out test with seeds from three cacao clones (one pod per clone), provided evidence of high rates of seed transmission of two strains of CaMMV. Nearly 50% of seedlings grown from symptomatic and healthy-looking pods harvested from infected trees, tested positive for CaMMV 6 weeks after planting using a recently developed nested PCR test. Results were confirmed with Sanger sequencing. Approximately one third of the plants showed symptoms such as vein banding and mosaic. The potential for international spread of CaMMV through seeds, and the impact of seed transmission on farmers and breeders will be discussed.

Testing garden soil infectivity at landscape sites once infested by *Calonectria pseudonaviculata* in eastern United States with detector boxwood plants

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Calonectria pseudonaviculata (*Cps*) has ruined many boxwood gardens since 2011. This pathogen is known to produce microsclerotia which were shown to be able to survive the soil environment in the U.K. for at least 5 years. The objective of this study was to determine whether garden soil at once-infested sites continued to be infective after blighted boxwood had been removed and the sites replanted with another plant or left unplanted for a period of time. The testing was done in five gardens with three in New York and one each in South Carolina and Virginia. Boxwood in two of these gardens were diagnosed as having boxwood blight 4 years ago and the others 1, 3, or 7 years ago. All but the Virginia garden were then replanted with *Ilex* spp. Container-grown *B. sempervirens* 'Justin Brouwers' was used as a detector plant at the Virginia garden where affected boxwood had been cut to low stumps in early 2016 without regrowth. Two batches of detector plants were placed near stumps in early summer and another two in mid fall of 2019, with each being exposed to once-infested soil for 2 weeks, then taken to the lab to be tested for infection by *Cps*. Similar testing was done in New York and South Carolina gardens using *B. × 'Green Mountain'* and *B. sempervirens* 'Suffruticosa', respectively. Boxwood blight was not observed on any detector plants on-site or after they were taken to a lab and exposed to disease-conducive conditions. These results indicate that *Cps* may not survive in the soil environment in eastern U.S. as long as it was shown to do in the U.K.

Boxwood Blight Insight Group (BBIG): An international consortium to protect a major evergreen shrub crop and iconic landscape plant

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Boxwood blight caused by *Calonectria pseudonaviculata* was first reported from the U.K. and New Zealand in the 1990s. In the U.S., this disease was first confirmed in eight states (CT, MA, MD, NC, NY, OR, RI, and VA) in late 2011. Boxwood blight has since spread to 22 additional states, decimating boxwood crops and gardens. To fight this emerging disease, 14 scientists from 12 labs have teamed up with stakeholders and international colleagues to form the Boxwood Blight Insight Group (BBIG). The BBIG mission is to improve blight mitigation through innovation, economic analysis and education. Specifically, the BBIG is developing a better understanding of boxwood blight in the Pacific Northwest and identifying the critical control points to help growers produce blight-free plant stock. The BBIG also will develop several major innovations: robust diagnostic kits, more resistant cultivars, boxwood self-defense enhancers, antidesiccants as physical barriers, and biological controls. Additionally, the BBIG will integrate recently developed technologies, and provide economic analyses to promote their adoption and synergy. Furthermore, it will investigate boxwood genetics and microbiome, pathogen colonization and epidemiology, and analyze the cost/benefit of individual innovations and their integration in the form of best management practices at production and post-production sites. The BBIG emphasizes research translation into products and practical recommendations and delivers them to end users in an expedited fashion for sustainable boxwood production and gardening.

Development of a loop-mediated isothermal amplification assay for rapid and specific detection of *Xanthomonas perforans*

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Bacterial spot of pepper and tomato caused by *Xanthomonas* species is one of the most widespread and economically devastating diseases of pepper and tomato in the southeastern US. The four species that cause bacterial spot on pepper and tomato: *X. euvesicatoria*, *X. vesicatoria*, *X. gardneri*, and *X. perforans*, can be found on all aboveground plant parts. Species-specific identification can be vital in understanding the spread of the specific pathogens throughout various geographical areas. Incorrect diagnosis can lead to disease

control failure, which increases the use of chemicals and potential resistance development. A quick and accurate diagnosis tool for identifying bacterial spot in early stage is necessary for effective disease management. This study describes the development of a loop-mediated isothermal amplification (LAMP) assay for sensitive and rapid detection of one of the bacterial pathogens *Xanthomonas perforans* that causes bacterial spot of pepper and tomato. LAMP primers were developed using the sequence “*Xanthomonas perforans* 91-118 chromosome” and tested using *X. perforans* DNA and other *Xanthomonas* spp. The detection limit of this LAMP assay was 0.1 pg/μl of genomic DNA per reaction with no cross reactivity with other *Xanthomonas* species. This simple, rapid LAMP method is suitable to identify *X. perforans* in laboratory and field conditions. Moving forward, this method could be adapted for use directly in low-resource field settings

Identification and characterization of fungal pathogens associated with loblolly pine needle defoliation (LPND) in the southeastern USA

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Loblolly pine is a leading productive timber species in the southeastern USA. Over the past 3 years, an emerging threat is expressed by successive needle defoliation (LPND) followed by stunted growth and tree mortality in loblolly pine plantations. Besides, the symptoms of the disease were perplexed somewhat with pine needle rust and recurrently attributed to invasive *Phytophthora* species due to the similarity of disease nature and devastation. Therefore, the study investigated the potential causal agents of the disease and characterized the fungal pathogens associated with LPND in the southeastern USA. Moreover, 70 trees were randomly tagged at seven long-term monitoring plots in Chatom, Alabama to monitor and record the annual disease incidence and severity. Based on colony morphology and ITS-rDNA sequence data, a total of 28 species of fungi representing 17 families were recovered from diseased loblolly pine needles. Native brown-spot pathogen, *Lecanosticta acicola*, was the species most frequently recovered from unhealthy loblolly pine needles in combination with some other common needle cast and rust pathogen (s). Tagged trees were consistently found chlorotic and defoliated from 2019 to 2020. The current emergence of the brown-spot pathogen causing loblolly pine mortality necessitates the investigation of the role of changing climatic conditions which might be associated with increased pathogen pressure to loblolly pines in the southeastern USA.

Physiological root response of huanglongbing infection

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Huanglongbing (HLB), caused by *Candidatus Liberibacter asiaticus* (CLAs), has reduced citrus production in Florida over 70%. After infection, CLAs quickly colonizes the root system before visible canopy symptoms develop. Understanding early and late effects of CLAs on the roots is fundamental to HLB management. Using split root rhizotrons and one-side graft inoculation below the trunk split in late summer was used to study root infection and disease development. HLB caused differential root responses to infection depending on tree age at infection. Root lifespan was affected by the infection and the period of root growth (seasonal root flushes). Plants inoculated at 1.5 years old had more root growth in the non-inoculated half of HLB-affected trees before CLAs detection in the roots (late summer root flush). The cumulative root mortality of late summer roots was higher on the inoculated side, with 50% root death 6 weeks after emergence (wae) compared to 10 wae for non-inoculated side of HLB trees and mock-inoculated (Healthy) trees. The fall root flush took 22 wae to reach 50% mortality in both sides of HLB trees while Healthy trees took 28 wae. In contrast, 2.5-year-old plants had inhibited root growth in both sides of HLB-infected plants as early root response. HLB and Healthy trees had similar root lifespans. Roots emerged during fall flushes reached 50% mortality at 23 wae. Independent of tree age at infection, after CLAs was present and established in the root system, the infection caused stimulation of root growth of the spring root flush in all HLB-affected plants.

Clade and mating type distribution and population structure of *P. cubensis* on *Cucumis melo* in the eastern United States

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Pseudoperonospora cubensis, causal organism of cucurbit downy mildew, is the largest threat to melon (*Cucumis melo*) production in the eastern United States. Currently, there is little genotypic information on the populations of *P. cubensis* affecting melon in the U. S. In 2019 and 2020, 106 *P. cubensis* isolates were collected from symptomatic melon cultivars ‘Halona’ and ‘Hale’s Best Jumbo’ in CDMIPM-PIPE sentinel plots in nine states. Isolates were genotyped using 11 microsatellite markers, while clade and mating type (MT) were determined using diagnostic PCR protocols. To date, all isolates displayed an inverse relationship between clade and MT, with 18 and 88 isolates in clade1/MT2 and clade 2/MT1, respectively. Only leaves collected from ‘Hale’s Best Jumbo’ plots contained isolates representing both clades and MT within a single plot. Using microsatellite allele data, a pairwise population matrix of Nei’s unbiased genetic distance and gene diversity were calculated using GenAlex for each sentinel plot from each state for each year. In general, genetic differentiation between sentinel plots was low across the country, except in South Carolina and Louisiana; these locations were the only two where clade 1/MT2 isolates were found. South Carolina had the greatest genetic diversity, as it was the only location where both clade/MT combinations were recovered. This genotypic information will help contextualize future downy mildew breeding efforts and other control strategies in melon.

Evaluation of ultraviolet light for suppression of strawberry powdery mildew and post-harvest botrytis fruit rot

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Strawberry powdery mildew (PM), caused by *Podosphaera aphanis*, and botrytis fruit rot (BFR), caused by *Botrytis cinerea*, are diseases that cause significant loss in strawberry production and post-harvest. Our objective was to determine the efficacy of germicidal ultraviolet light (UVC) on severity of foliar PM and post-harvest incidence of BFR. UVC was applied twice weekly during darkness at a dose of 200 J·m⁻² for 14 weeks on an organic field of ‘Florida 127’. The UVC lamp array was carried by an autonomous robot (THORVALD, Saga Robotics). Treatments were arranged in a RCBD consisting of six replicated plots. PM severity was estimated on five immature trifoliated leaves per plant in six plants per plot. For BFR, 24 fruit were harvested from each plot and the percentage of symptomatic fruit with BFR was determined after 3 to 7 days of incubation. The area under the disease progress curve (AUDPC) for PM severity was reduced by 80% compared to non-treated controls (NTC). Seasonal UVC treatment also reduced the incidence of BFR post-harvest by 88, 42, and 18% after 3, 5, and 7 days of incubation, respectively, compared to the NTC. UVC applications alone provided substantial suppression of foliar powdery mildew. Suppression of BFR by UVC declined as incubation times increased, but BFR reduction was still significant for up to 7 days of incubation and merit further study as either stand-alone treatments or supplements to fungicidal treatment.

Forecasting white mold development on dry bean using machine learning algorithms

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Modeling disease development as a function of environmental factors improves our understanding of how epidemics occurs and it could help growers to make informed-spraying decisions. In this study, multiple classification, and regression machine learning (ML) algorithms were evaluated for their accuracy in predicting the development of white mold, caused by *Sclerotinia sclerotiorum*, on dry bean. Leaf wetness duration and dry period were used as predictors. Ascospore-laden dry bean flowers were placed on leaves of seedling plants at V-1 stage. Plants were incubated at 18–20°C in alternating 8–16 h wet/12–24 h dry periods for 196 h. Development of foliar lesions was recorded daily after inoculation and disease incidence was used as the response variable. Five ML models including artificial neural network (ANN), random forest (RF), decision tree (DT), linear regression (LNR), and support-vector machines (SVM) were used in regression analyses while LNR was replaced with logistic regression (LGR) in classification analyses. Training and testing of the algorithms were conducted using 20-fold cross validation method. In regression analyses, ANN was the best-fit model to the data, with R^2 of 95% and RMSE of 5.82, followed by RF and DT with R^2 of 90% and 88% and RMSE of 8.46 and 9.54, respectively. In classification analyses, ANN had the highest accuracy of 92%, followed by SVM and LGR models with 90% and 88%, respectively. Results of this study represent the potential of ML methods for the development of models that evaluate risk of plant disease development.

Dual transcriptome analysis of the susceptible interaction between basil and *Peronospora belbahrii*

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Downy mildew is a disease of sweet basil production worldwide. Investigating the genetic response of how the pathogen successfully infects basil could help identify targets for control products or discover basil genes that may promote susceptibility to downy mildew. The expression levels of all the genes in basil leaves were analyzed using RNA sequencing 3 and 6 days after inoculation with spores from an Illinois strain of *Peronospora belbahrii*. A number of basil genes that were identified with the gene ontology term “defense response to oomycetes” were upregulated in the inoculated basil leaves, while many genes contributing to photosynthesis in the infected leaves were downregulated after 3 days. Many other genes were differentially expressed in the basil leaves after 6 days as the pathogen began to produce spores in the leaves. Seven of the eight basil genes identified as orthologues of *Arabidopsis thaliana* DOWNY MILDEW RESISTANT 6 were upregulated with infection. After 3 and 6 days the pathogen was expressing high transcript levels of genes that code for carbohydrate degrading enzymes, which likely are involved in the release of sugars from the plant cell walls. Many of the same genes encoding carbohydrate degrading enzymes were well expressed by the pathogen infecting basil leaves in similar experiments performed at the University of Hawaii. These results contribute to a better understanding of the infection process and spread of downy mildew and would contribute to the development of more effective measures for reducing the severity of the disease.

Identification and functional characterization of candidate effector proteins from the maize tar spot pathogen *Phyllachora maydis*

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Effector proteins secreted by plant pathogens play a vital role in plant pathogenesis by suppressing host immune responses and promoting virulence. Though the effector repertoires of bacterial and oomycete pathogens have been well-characterized, our knowledge of fungal effector proteins is still quite limited even though fungal pathogens are a serious threat to global food and nutritional security. One such fungal pathogen is *Phyllachora maydis*, which causes tar spot in maize. Tar spot is considered one of the most economically important diseases of maize and, though it was only recently discovered in the United States, it has spread to most maize production areas in the United States and is responsible for substantial yield losses. Here, we identified several *P. maydis* virulence proteins that likely have a vital role in plant pathogenesis and, thus, may serve as new sources of genetic-based resistances. Using EffectorP, we identified 59 candidate effectors from the *P. maydis* secretome, 15 of which are predicted with high confidence. We next employed TMHMM to identify potential transmembrane motifs and LOCALIZER to identify putative nuclear localization signals, chloroplast and mitochondrial transit peptides. Of the 15 candidate effectors predicted with high-confidence, 11 lack an identifiable transmembrane

motif, 3 have putative nuclear localization signals, and 1 has an identifiable chloroplast transit peptide. Future work will assess whether we can harness effector-triggered immunity to introduce new resistance traits against *P. maydis* in maize.

Understanding the epidemiology of gray mold caused by *Botrytis cinerea* in pome fruit systems of the Pacific Northwest

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Recent surveys in the Pacific Northwest (PNW) showed gray mold, caused by *Botrytis cinerea*, to be the second most important postharvest decay of pome fruit after blue mold. Although *B. cinerea* thrives better under humid conditions, it's intriguing why it causes important losses in the region where conditions during the growing season are semi-arid. From 2018 to 2021 we collected samples from different Fuji apple and d'Anjou pear orchards located in central Washington and Oregon. Two to eight orchards per year were sampled and collected at bloom, fruitlet, green fruit, commercial maturity stages preharvest and after 6 months of storage in regular atmosphere at 1°C. *Botrytis* incidence was determined by plating flowers and different organs of fruit (calyx, peduncle, flesh) on a *Botrytis* selective medium (BSM), whereas fungal biomass was determined using qPCR. Our preliminary results show that *Botrytis* is present in all orchards and organs tested. Furthermore, we detected *Botrytis* in the different parts of the fruit at different phenological stages. Although variability between orchards was observed, the overall *Botrytis* incidence for the apple orchards was 10, 11, 14, 18, and 21% at bloom, fruitlet stage, green fruit, commercial ripe fruit, and after 4 months of storage, respectively. Respective overall *Botrytis* incidences for pear samples across the four orchards were 5, 9, 11, 19, and 10%. Our preliminary data indicate increase of infection rates during the growing season which would require an adjustment of preharvest management sprays to curb gray mold development in storage.

Impact of acetic acid vapors on the growth and survival of the emerging soilborne pathogenic fungus *Neopestalotiopsis clavispora*

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Since the phase out of methyl bromide in the 1990s, strawberry growers have searched for an efficient and environmentally friendly alternative to control soilborne pathogens. An approach, called anaerobic soil disinfestation (ASD), seems a promising alternative to chemical soil fumigation. ASD consists in adding a labile carbon source to the soil before covering it with a tarp and flooding it for up to 6 weeks. Organic acids produced by the soil anaerobic bacterial populations are among the processes believed to be involved in the mechanisms by which ASD represses soilborne plant pathogens. This study evaluated the effect of acetic acid vapors on the growth and survival of *Neopestalotiopsis clavispora*, an emerging soilborne pathogenic fungus known to cause root and crown rot in strawberry. To achieve this, an agar disc covered with actively growing mycelium was deposited on potato dextrose agar (PDA) and placed in an airtight glass jar containing a filter paper saturated with different concentrations (0–25%) of acetic acid. Once their exposition time completed (2–168 h), the agar discs showing no growth were placed on a new PDA and left to incubate in an atmosphere free of acetic acid vapors for a week to determine if the treatment had a fungistatic or a fungicidal effect. Results showed that the exposition of *N. clavispora* to acetic acid vapors causes a significant reduction of growth at 5%. For 10% acetic acid, exposition up to 96 h was fungistatic while 168 h was fungitoxic. Exposition of 6 h at 15% and 4 h at 20% or 25% were fungitoxic.

Characterization of *Pseudomonas* spp. and *Xanthomonas* sp. isolated from cranberry upright stem galls

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Introduction: Upright stem galls are a disease manifestation associated with indole-3-acetic acid (IAA) producing microbes in cultivated cranberry bogs. Although important for normal development of plant organs and tissues, exogenous IAA produced by bacteria in excess can result in hyperplasia within the stems of cranberry plants, which can result in loss of fruit set and plant death from stem girdling. Stem gall isolates from cultivated cranberry bogs of Southeast Massachusetts include members of the genera *Bacillus*, *Pseudomonas*, and *Xanthomonas*, and include uncharacterized species. At least one of the *Pseudomonas* isolates produces large amounts of IAA in vitro. Genotypic and phenotypic analyses were used to characterize two new species of *Pseudomonas* and one new species of *Xanthomonas*. Results: Digital DNA-DNA hybridization results suggested that two *Pseudomonas* isolates and one *Xanthomonas* isolate from the gall tissue share insufficient homology with each of their closest relatives to be considered the same species. This was also supported by several differences in the metabolic capabilities of each isolate compared to each of their closest relatives. In addition to verifying that each isolate was potentially a novel species, the metabolic profiles produced allowed for characterization of each isolate. Conclusion: One of the *Pseudomonas* isolates from cranberry upright gall tissue represents a new species within the genus. The other *Pseudomonas* isolate and the *Xanthomonas* isolate likely represent recent speciation events.

Targeted management: Delineating *Pythium* population dynamics in creeping bentgrass putting greens

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Pythium root rot (PRR) is a detrimental disease of creeping bentgrass (CRB) putting greens. Although the disease is typically managed with late spring/early summer fungicides, the timing of preventative applications is poorly understood, as are the *Pythium* species responsible for the initial inoculum. The objective of this study is to delineate the population shifts of *Pythium* species in a CRB putting green over time. From Jun 2020 until Feb 2021, ten 25.5-cm² soil cores were randomly collected from each of six adjacent 2.4-m² plots. Soil moisture and soil temperature data were recorded monthly. After washing cores, root tissue (2 cm) from each group were plated onto P₅ARP media and pure cultures were obtained for sequencing. Candidates (*n* = 38) were identified as *Pythium* species by comparing the ITS region with verified *Pythium* spp. using NCBI BLAST®. *P. torulosum* (47.3%) was consistently isolated throughout

the study. The second most frequently isolated species were *P. catenulatum* (21.1%) and *P. volutum* (21.1%), with 75% of *P. catenulatum* isolates collected in Sep 2020 and 100% of *P. volutum* isolates collected in Feb 2021. *P. vanterpoolii* (7.8%) was isolated in July, August, and December 2020. *P. spinosum* (2.6%) was the least abundant species isolated, collected in Oct 2020. These data indicate that *P.* populations shift throughout the year. Upon the completion of this study, linking species pathogenicity and population fluctuations with soil temperature and moisture will assist turfgrass managers with optimal PRR management through targeted fungicide programs.

A comparative genomics investigation into cool-virulence in *Ralstonia solanacearum* to improve its phytosanitary regulation

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Ralstonia solanacearum (*Rs*) is a soilborne pathogen of hundreds of plant species including many economically important crops. A group of *Rs* strains, known as Race 3 biovar 2 (R3b2), cause potato brown rot at cooler temperatures than other *Rs* strains and threaten the US potato industry. R3b2 strains are not established in the US, but have been accidentally imported. R3b2 are hard to distinguish from other *Rs* strains, and R3b2 strains are not a formally described taxon. Thus, the entire *Rs* species is included by USDA APHIS in the list of highly regulated select agents. To improve the regulation of cool-virulent *Rs* strains, we applied comparative genomics to develop a genome-based classification and identification framework capable of precisely distinguishing the cool-virulent R3b2 strains from non-cool virulent *Rs* strains. Towards this goal, the genomes of 144 *Rs* strains closely related to well-characterized R3b2 strains were sequenced and a core genome phylogenetic tree was constructed that also included strains representative of the genetic diversity of *Rs*. Phylogenetic relationships among very closely related strains were improved using single nucleotide polymorphism-based methods. The extent of horizontal gene transfer and the existence of ecologically relevant populations within *Rs* was determined as well. Virulence assays at different temperatures are being performed to correlate genotype with phenotype. We expect this study to yield new insight into the genetic basis of cool virulence and to improve regulation of cool-virulent *Rs* in the USA.

Viral quantification and viability determination in mechanical inoculation of *Wheat streak mosaic virus*

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Wheat streak mosaic virus (WSMV) is a common wheat virus causing significant economic losses all over the wheat-growing regions of the world. Mechanical inoculation is a common practice to evaluate plant resistance in breeding lines. Successful inoculation relies on the infectivity and viability of the virus particles. A time-course study was designed to quantify viral load and viability of WSMV in phosphate buffer inoculum and infected wheat leaves. Accurate quantification of viral copy number was calculated by reverse transcription quantitative PCR (RT-qPCR) using a standard curve generated from a serial dilution of plasmid DNA carrying cloned WSMV fragment. RT-qPCR revealed that WSMV was stable in the buffer for at least 96 h after preparation with an average viral genome copy number $1.86 \times 10^6 \pm 4.85 \times 10^5$. WSMV genome copy numbers in the infected leaves ranged between 2.66×10^4 and 4.69×10^6 at 21 to 28 days post-inoculation. Viral copy numbers in wheat leaves inoculated at different times were statistically similar to leaves inoculated at 0 h after inoculum preparation. There was a weak linear relationship between viral copy number and phenotypic rating score. This work provides an accurate quantification of virus concentration in the inoculum and in the infected plants, which are essential information to compare viral load and phenotypic rating score for unbiased viral disease assessment.

Impact of selected chemical contaminants found in recycled wastewater on soilborne plant pathogens

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California agriculture is endangered by water shortages and soilborne plant pathogens such as *Verticillium dahliae* and *Fusarium oxysporum*. Recycled wastewater (RWW) has been used to address water supply issues. However, pharmaceuticals, known as chemicals of emerging concern (CECs), are not completely removed from RWW and they can accumulate in soils and plant tissues that are irrigated with RWW. CECs can potentially interact with soilborne pathogens and exacerbate plant diseases. Four CECs commonly found in RWW -Acetaminophen, trimethoprim, sulfamethoxazole, and gemfibrozil -were evaluated to determine their impacts on growth and spore germination of *V. dahliae* or *F. oxysporum* in vitro. A greenhouse assay was used to determine how these CECs affected the severity of Verticillium wilt in *Solanum melongena*, an important California crop. All assays in this study used single CEC concentrations at levels found in RWW. The greenhouse assay indicated that the CECs impacted disease severity during certain stages of plant growth. Growth rate assays indicated that the pathogens tested were sensitive to the compounds, but the effects were strain specific. Acetaminophen, sulfamethoxazole, and gemfibrozil decreased spore germination in *V. dahliae*, while trimethoprim decreased spore germination in both *V. dahliae* and *F. oxysporum*. Our findings are important to growers and suggest that CECs in RWW can impact the growth of plant pathogens and may play a role in plant disease severity.

Chemical warfare: The mycotoxins aflatoxin and fumonisin govern *Aspergillus flavus*-*Fusarium verticillioides* interactions

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Contamination of maize by mycotoxins affects food safety worldwide. Mycotoxin consumption can lead to a variety of life-threatening health problems for animals and humans. Consequently, there is a large economic cost associated with mycotoxin contamination, including quality-price downgrades and even complete crop loss from required destruction. Two mycotoxins commonly contaminating maize, aflatoxin and fumonisin, are produced by the plant pathogens *Aspergillus flavus* (*Af*) and *Fusarium verticillioides* (*Fv*) respectively. Multiple studies/surveys have found these fungi co-colonizing maize in the field they have not examined their direct interaction in detail. In this study, we investigated in vitro interactions between *Af* and *Fv* by employing wild-type and mycotoxin non-producing mutant strains. We show using these strains that fumonisin inhibits the growth of *Af*, while, in contrast, aflatoxin did not impact the growth of *Fv*. However, when confronted, the ability of each fungus to produce its primary mycotoxin correlates to reduced mycotoxin production by the other species. The responses were also positionally variable with *Af* demonstrating a reduction in aflatoxin proximal to a fumonisin producing *Fv* strain, while fumonisin production was suppressed colony-wide in response to an aflatoxin producing *Af*. This work gives insights into the ecological role of mycotoxins as well as their potential to be used in fungal-fungal communication. The interactions observed here suggest that, in co-colonized ears, each species likely reduces the level of mycotoxin contamination by the other.

Biofumigant green manure soil amendments influence soil bacterial communities and lettuce yield

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Biofumigant green manure soil amendments can improve plant health by suppressing soilborne plant pathogens. They also serve as a carbon and energy source that supports microbial activity in soil which can feed back on plant yield through multiple mechanisms. The goal of this study was to test for a relationship between biofumigant green manure soil amendment type, soil bacterial communities, and lettuce yield. Broccoli, marigold, and sudangrass were harvested at maturity and applied as green manures to potted field soil. Lettuce was transplanted into amended field soil and a non-amended fallow treatment and maintained under growth-chamber conditions. Soil was sampled following green manure amendment and bacterial communities were characterized from extracted soil DNA using nanopore sequencing. Lettuce yield was measured at the termination of the experiment. Soil bacterial community composition was significantly different between fallow and green manure amended soils. In addition, the relative abundance of individual bacterial taxa was significantly different among green manure types. Lettuce yield was highest in soil amended with marigold green manure and lowest in sudangrass amended soil. The results of this study show green manure amendment type influences soil bacterial community composition and lettuce yield, providing a platform for future research focused on understanding how the soil microbiome mediates the effect of green manure amendments on vegetable health.

Mapping rice genes involved in blast resistance, chalkiness, and yield related components

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Plant genes for disease resistance, quality and productivity are often co-located and the same molecules and resources of plants are often simultaneously allocated for disease resistance, quality, and productivity. ‘Minghui 63’ is a late maturing long grain indica cultivar resistant to rice blast disease, has good yield potential, and is one of the most widely used fertility restorers in hybrid rice production. ‘M-202’ is an early maturing, semi dwarf medium grain temperate japonica cultivar susceptible to rice blast disease and is one the parents for many rice cultivars with premium quality. Genomic regions in Minghui 63 and M-202 involved in reactions to the US blast races, chalkiness, and yield components are unclear. In this study, we evaluated Minghui 63 and M-202 with 12 US blast races, percent chalk, number of days to heading, tiller angle, plant height, panicle length, number of primary branch/panicle, panicle weight, grain length, and thousand grain weight. A recombinant inbred line population consisting of 275 individuals was developed from the cross of Minghui 63 with M-202. This Minghui 63/M-202 population was genotyped with 156 polymorphic simple sequence repeat (SSR) markers. A linkage map with 1,022.84 cM was constructed using Inclusive Composite Interval Mapping (ICIM) software, and the entire population was evaluated for the abovementioned traits and underlying genomic regions in both ‘Minghui 63’ and M-202. The possibility of trade-offs among blast resistance, quality, and productivity and its implication for rice breeding will be presented.

Fungi impacting maple health in sugar bushes in the Mid-Atlantic U.S.

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Maple syrup production is a culturally and economically important activity in the Mid-Atlantic U.S. and faces unique challenges different than those of producers in northern forests. Up-to-date regional surveys with a focus on phytopathogenic fungi will provide much-needed recommendations to help identify fungi contributing to decline and mortality to inform management strategies and empower various stakeholders. In 2020, we initiated fungal surveys across sugar bushes with variable intensity of management and different species compositions in PA, VA, and WV. Symptomatic maple trees were evaluated and sampled from managed sugar bushes and unmanaged forests. This included maples with symptoms associated with well-known diseases such as Eutypella canker and damage from pests such as the sugar maple borer. Bark plugs were collected and once plated, unique morphotypes were quantified. Representative stains were retained for morphological and molecular study. Thus far, a total of 40 trees from two stands have been processed and yielded at least 53 fungal morphotypes. Of these, 20 have been identified using ITS barcoding. Putative plant pathogenic fungi include *Climacodon septentrionale*, *Fusarium solani*, *Phoma* spp., and *Stegonsporium acerinum*. Surprisingly, efforts to culture from Eutypella cankers yielded mostly *Daldinea*, *Nemania*, and *Xylaria* (Xylariaceae), yet their role remains unclear. By delimiting the occurrence of known and novel pathogens in Mid-Atlantic sugar bushes, we hope to help grow syrup production in the region.

A practical model for huanglongbing research

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Huanglongbing (HLB) is widely regarded as the most devastating of all citrus diseases and threatens sustainability of citrus worldwide. HLB develops in citrus trees infected with the phloem-limited alpha proteobacter *Candidatus Liberibacter asiaticus* (CLAs) that is vectored by the Asian citrus psyllid (ACP). HLB research is especially problematic due not only to the complexities of the pathosystem, but also to citrus as a crop. A model system to facilitate HLB research has been identified as a priority by both the citrus research community and the citrus industry. Various “models” have been utilized to study HLB, including non-citrus surrogate hosts, surrogate pathogens, and graft—rather than ACP—inoculation of citrus. In this report, a practical HLB model that includes true citrus (*C. medica*, citron), CLAs and transmission of CLAs to citrus by ACP that is amenable to physiological studies and for screening agricultural chemicals to mitigate HLB is described. Citron was chosen as the host not only because it is in the genus citrus, but also because it roots readily from stem cuttings (clonal, highly uniform), a large number of plants can be produced with a very small footprint, and citrons, although are highly susceptible to CLAs infection and develop characteristic HLB symptoms but do not decline as rapidly as other citrus types. Protocols for production of citrons and CLAs-infected ACP and inoculation of citrus via ACP transmission are described along with responses of citrons to CLAs infection.

Development of a consistent and effective inoculation method for Alternaria leaf spot in sugar beet in the field

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Alternaria leaf spot (ALS), caused by *Alternaria* spp., is a foliar disease in many crops including sugar beet. ALS has been an increasing issue in Michigan since 2015, which makes the development of ALS management strategies important for the region. Having a consistent inoculation method for ALS is an important tool to assess management strategies. For example, identification of resistant varieties under natural infection can be convenient but may be subject to inconsistency, and it is not practicable when natural infection is low. Therefore, this project aims to develop a consistent artificial inoculation method in the field. Two sugar beet varieties (susceptible and moderately resistant) were grown in the field in 2020. *Alternaria alternata* strain P23 was grown on ½ strength V8 agar plates in the dark. Inoculum (1×10^3 spores/ml) was prepared by suspending conidia in malt extract broth (MEB) at 0.2% or 0.5%. Controls were MEB or water. Inoculation was with a backpack sprayer at a rate of 20 ml per plant. Disease rating was started one week after the inoculation and continued for 5 weeks using a 0–10 rating scale where 0 = no spots and 10 = complete defoliation. We observed a significant difference in disease score between the two varieties. The moderately resistant variety gave a significantly lower disease rating than the susceptible variety by 2 weeks after inoculation and throughout the rating period thereafter. The inoculum in 0.2% MEB gave significantly higher disease than the 0.2% MEB control. Testing continues to verify the consistency of the inoculation method.

Baseline sensitivity of *Phytophthora erythroseptica* isolates to oxathiapiprolin

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Pink rot of potato, caused by the oomycete pathogen *Phytophthora erythroseptica*, affects tuber yield, quality, and storability each year. Metalaxyl/mefenoxam seed treatments have been used for decades to manage the disease. However, given the presence of mefenoxam insensitive isolates and lack of cultivars with complete resistance, alternative management options are needed. Oxathiapiprolin is shown to be effective against other *Phytophthora* spp. and downy mildew pathogens. To assess whether it could be used to manage pink rot, 56 isolates of *P. erythroseptica* collected from seven states in 2012 or earlier were used to establish the baseline sensitivity. All but two had been previously tested for sensitivity to mefenoxam and ranged from sensitive to highly insensitive. All isolates were pathogenic on tubers following recovery from long-term storage. A mycelial growth assay was conducted using technical grade product from 1×10^{-6} to 1×10^{-1} µg active ingredient (a.i.)/ml. When the colony diameter on the unamended control plate reached approximately 70 mm, mean diameters were used to calculate EC₅₀ values. All isolates were sensitive to oxathiapiprolin, with EC₅₀ values ranging from 6.21×10^{-5} to 4.7×10^{-4} µg a.i./ml and a mean of 2.66×10^{-4} µg a.i./ml. These results are comparable to reports for other *Phytophthora* spp. and indicate that oxathiapiprolin is a good candidate to use in field studies on pink rot management. These results also establish an important baseline that can be used to monitor pathogen populations for the emergence of oxathiapiprolin insensitivity.

Growth phase regulation of copper tolerance and phenotype comparison between copper tolerant and copper sensitive strains of *Erwinia amylovora*

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We found *E. amylovora* isolates which are highly sensitive to copper (Cu). The isolates show differences in virulence, growth rate, resistance to cadmium and paraquat and induction of exopolysaccharides in the presence of Cu when compared to type strains Ea273 and CFBP1430. These results suggest that Cu tolerance in *E. amylovora* might be controlled by global regulators in charge of other aspects of the bacterium's biology. In vitro Cu adaptation and survival assays demonstrated that entry into stationary phase but not pre-exposure of cells to sublethal Cu concentrations confers increased resistance to Cu. This does not happen with other toxic compounds like H₂O₂, which pre-exposure to sublethal concentrations confers protection against higher doses of the same compound. Stationary phase cells of the Cu sensitive strains showed similar Cu-shock responses as the type strains Ea273 and CFBP 1430 when pre-exposed low Cu concentrations (0.1 mM), but were more sensitive to Cu-shock when pre-exposed to higher Cu concentrations. However, exponentially growing cells of strains Ea273 and CFBP 1430 developed counterintuitive, lower survival rates to Cu-shock than the Cu

sensitive strains, with pre-exposure to 0.5 mM Cu inducing a higher sensitivity to Cu-shock than that observed in the Cu-sensitive strains. An RNA-Seq analysis is ongoing to determine the basis for these phenotypes in *E. amylovora*.

Large-scale transposon mutagenesis reveals type III secretion effector HopR1 is a major virulence factor in *Pseudomonas syringae* pv. *actinidiae*

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Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is a serious threat to kiwifruit production worldwide. Four biovars (Psa biovar 1; Psa1, Psa biovar 3; Psa3, Psa biovar 5; Psa5, and Psa biovar 6; Psa6) were reported in Japan and high virulence Psa3 strains spread rapidly to kiwifruit production areas worldwide. Therefore, there is an urgent need to develop critical management strategies for bacterial canker based on dissecting the dynamic interactions between Psa and kiwifruit. To elucidate the molecular mechanism of Psa3 infection, we developed a rapid and reliable flood-inoculation method and a high-throughput inoculation method using kiwifruit seedlings. Using this inoculation method, we screened 3000 Psa3 transposon insertion mutants and identified virulence related genes. We revealed that the type III secretion effector HopR1 is a major Psa3 virulence factor. We also demonstrated that *hopR1* mutants of other biovars showed reduced virulence. These results suggest that HopR1 universally contributes to virulence in all Psa biovars.

A population genomics approach to characterize diversity and structure of *Cercospora janseana* on rice in the southern United States

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Cercospora janseana is a fungal pathogen that causes narrow brown leaf spot (NBLS) on rice. Common commercial cultivars are susceptible to NBLS and yield losses can range from 10 to 40%. Increased NBLS outbreaks in recent years have highlighted a need to develop improved disease management strategies. However, managing NBLS with genetic resistance is hindered by a lack of information on the genetic diversity and population structure of the pathogen. To address these issues, we conducted a population genomics study to investigate the diversity and structure of *C. janseana* in the southern United States. *C. janseana* was isolated from foliar lesions of rice collected from Louisiana and Texas in 2019 and 2020. High-molecular weight DNA was extracted from isolate RL44 and sequenced using an Oxford Nanopore MinION. Sequencing reads were de novo assembled into 15 nuclear contigs with an N50 of 3.59 Mb. RNAseq enabled annotation of 9,757 high-confidence gene models, including the prediction of 720 secreted proteins and 105 effectors. Whole-genome sequencing of 46 isolates resulted in the identification of 385,912 SNPs and 21,915 InDels. Principal components and STRUCTURE analyses revealed two distinct clusters of individuals unrelated to geographical origin. A pairwise homoplasmy index test and equal presence of mating type genes indicated that sexual reproduction is likely occurring in the field. Taken together, *C. janseana* harbors high levels of genetic variation and the potential for recombination, which poses challenges for genetic resistance management efforts.

Molecular characterization of a *Pseudomonas* species associated with disease in Hibiscus

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During the summer of 2019, leaf spots and crinkling symptoms were found on hibiscus (*Hibiscus rosa-sinensis*) brought into New York from a nursery in Florida. Initial identification using MLST with several housekeeping genes suggested this isolate was a member of the genus *Pseudomonas* but the species was unclear. This result was consistent with *H. rosa-sinensis* being a host to members of the genus *Pseudomonas*. To definitively determine the species, whole genome sequencing using Nanopore and Illumina sequencing technologies was performed. The genomic sequence of the pathogen shares highest homology to two pathovars of *P. amygdali*. The genus *Pseudomonas* continually undergoes extensive taxonomic reclassifications. *P. amygdali* (reported synonyms *P. ficuserectae*, *P. meliae*, and *P. savastanoi*) has been recently proposed to be a separate species, and includes *P. amygdali* pv. *hibisci* which is sometimes referred to as *P. syringae* pv. *hibisci*, as well. Our data is consistent with this new classification. Additionally, the genome content and structure were compared against widely known *P. syringae* pathovars: tomato DC3000 and B728a. A group of pathogenicity islands containing known effectors from other Pseudomonads were identified. Pathogenicity assays showed that the isolate causes disease in tomato and caused a hypersensitivity reaction in tobacco. The genomic information is being used to better understand the pathogenicity of this pathogen and closely related Pseudomonads that infect floriculture crops.

Deciphering the novel role of AtMIN7 in cuticle formation and defense against the bacterial pathogen infection

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Cuticle is the outermost layer of plant aerial tissues that interacts with the environment and protects plants against various biotic and abiotic stresses. ADP ribosylation factor guanine nucleotide exchange factor proteins (ARF-GEFs) are the key components of vesicle trafficking system. Our study discovers that AtMIN7, an *Arabidopsis* ARF-GEF, is critical for *Arabidopsis* cuticle formation and related defense against the bacterial pathogen *Pseudomonas syringae* pathovar tomato (*Pto*). Our transmission electron microscopy and scanning electron microscopy results indicate that the *atmin7* mutant leaves have the thinner cuticular layer, defective stomata structure, and impaired cuticle ledge of stomata compared to the leaves of wild type plants. GC-MS analysis further revealed that the amount of cutin monomers was significantly reduced in *atmin7* mutant plants. Furthermore, the exogenous application of either of three plant

hormones, including salicylic acid, jasmonic acid, and abscisic acid, could enhance the cuticle formation in *atmin7* mutant leaves and the related defense against the bacterial *Pto* infection. Thus, the transport of cutin-related components by AtMIN7 may contribute to its impact on plant cuticle formation and related defense function.

North Dakota survey of *Fusarium* species associated with root rot of lentil (*Lens culinaris* L.)

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Root rot of lentil, caused by a pathogen complex which includes several *Fusarium* species, is the most important disease of this crop in the U.S. Northern Great Plains. Surveys of North Dakota lentil fields were conducted in 2016 and 2018 to identify *Fusarium* species associated with root rot. Nearly 400 *Fusarium* isolates were collected from 114 lentil fields. *F. oxysporum* was isolated from 71% of fields, the highest frequency among the 10 species identified. Based on correlation with disease severity data, it is likely that *F. oxysporum* is an important contributor to root rot of lentil. These results are the first to evaluate the *Fusarium* root rot complex of lentil in the U.S. Northern Great Plains and represent the first step in managing this devastating disease. Further research is needed to determine virulence of the *Fusarium* species identified here, as well as the effect of agronomic practices, such as crop rotation, and seasonal climatic variation on the *Fusarium* species complex causing root rot of lentil.

Exogenous application of jasmonic acid reduces Tomato spotted wilt orthotospovirus infection in tomato under control conditions and drought stress

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Disease pressure is likely to increase in the future due to the rise in temperatures and extreme weather events. This can have detrimental impacts on the sustainability of disease management, especially of viral infections. Management of viral diseases is highly dependent on environmental conditions, as viruses are reliant on their plant host's physiological machinery. However, there is limited knowledge about how viral disease dynamics in specific crops like tomato (*Solanum lycopersicum*) would be affected by plants exposed to simultaneously occurring abiotic and biotic stresses. In this study, we evaluated the effect of drought stress on Tomato spotted wilt (TSW) disease incidence in tomato and the effect of exogenously applied phytohormones. Our research indicated that prolonged drought stress exacerbates disease progression as both incidence and disease severity of TSW disease were higher under drought stress. Application of jasmonic acid (JA) reduced disease incidence and severity under both well-watered and drought stress conditions, while application of salicylic acid (SA) or abscisic acid (ABA) did not have significant impact on disease under drought. Thus, we found that inhibitory effect on TSW is exerted by induction of JA-mediated, rather than SA-mediated, pathway, which is well known for biotrophic pathogen-related plant responses.

***Ralstonia* type III effector arsenal targets diverse regions inside solanaceous host cells**

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Phytopathogens such as *Ralstonia solanacearum* utilize the type III secretion system (T3SS) to inject virulence proteins, called T3Es, into the host plant cell. Once inside the host, T3Es localize to different cellular compartments and interact with host proteins and structures. T3Es can suppress plant immune pathways, hijack host metabolic pathways, influence hormone regulation, and/or alter plant development. As a first step toward understanding the function of T3Es in *Ralstonia* species, we examined the subcellular localization of a collection of 19 T3Es from *R. pseudosolanacearum* and *R. solanacearum*. *Ralstonia* species have a vast effectome compared to other bacterial phytopathogens, with the number of T3Es ranging from 45 to 76 across *Ralstonia* strains. For our localization work, we used two systems: tomato (*Solanum lycopersicum*) hairy roots, and *Nicotiana* leaves. T3E localization was not impacted between the two *Solanum* species and tissue types. Nearly half of the T3Es localized to the cell periphery and nucleus, and approximately 30% were present at the cell periphery or the nucleus. A quarter of our T3Es localized to organelles such as the tonoplast or peroxisomes. We found that in silico protein prediction programs were not reliable indicators of T3E subcellular localization, emphasizing the importance of in planta localization. We conclude that *Ralstonia* T3Es have a diverse subcellular localization and that subcellular localization experiments are crucial in establishing a foundation for future studies.

Management of *Phytophthora cinnamomi* using fungicides and host plant defense inducers under drought conditions

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Phytophthora, combined with stress conditions, are detrimental to ornamental crops. However, very few studies combining ornamentals and drought have been conducted. In this study, different fungicides and host plant defense inducers were tested for their ability to manage *P. cinnamomi* under drought conditions. Dogwood plants were grown in a pine bark medium in 10.2-cm containers with four controls. Treatments were drench applied as curative and preventative. The pathogen was inoculated 4 weeks before introducing drought. Seedlings were irrigated using overhead irrigation for a month after inoculation and later regulated by drip irrigation. Moisture content was maintained at 15–18% of moisture holding capacity of the substrate for 15 days. Data recording was done on different physiological and morphological parameters in various stages. In both preventative and curative applications, all treatments significantly suppressed disease compared to the inoculated stressed control, except Orchestra in curative application. In the curative

application, net photosynthesis, stomatal conductance and mid-day leaf moisture potential (PMS) were significantly higher in Subdue MAXX, Signature Xtra and Empress treated plants, whereas effective quantum yield of Photosystem II (PhiPS2) and net chlorophyll content were not significantly different. In the preventative application, Interface, Signature Xtra and Subdue MAXX-treated plants showed significantly higher net photosynthesis, stomatal conductance and net chlorophyll content. PhiPS2 and PMS were also higher in Subdue MAXX-treated plants.

Development of a seedling assay for high-throughput virulence screening of *Ralstonia solanacearum* species complex strains

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Members of the *Ralstonia solanacearum* species complex (*Rssc*) are the causal agents of bacterial wilt of many plant species, and impact economically important crops such as tomato, tobacco and potato. We developed a high-throughput virulence screen for the *Rssc* that is implemented in 96-well plates using *Nicotiana glutinosa* seedlings grown in soft water agar to save space, effort, and resources. In our assay, *N. glutinosa* seeds were sown quickly and easily on top of water agar in individual wells of a 96-well plate by pipetting out the desired number of seeds in an aqueous suspension. Wells were inoculated on the same day by first touching a bacterial colony with an autoclaved toothpick and then stabbing the toothpick into the center of the water agar well. This inoculation method resulted in sufficient delivery of inoculum, consistent virulence results, and confirmed bacterial growth and systemic spread in inoculated seedlings, allowing reduction of inoculum preparation efforts and facilitating high-throughput screening. Our assay is suitable for forward genetic screening of a large number of strains, isolates, or mutants for disease symptoms under both cool (20°C) and warm (28°C) temperature conditions before detailed studies can be narrowed down to a manageable number of desired candidates. Our virulence screen method provides a valuable tool for future work in understanding genetics of virulence of *Rssc*, especially cool virulence of the highly regulated race 3 biovar 2 group of *R. solanacearum*.

Genome-based marker identification for molecular detection of the avocado fungal pathogen *Raffaelea lauricola*

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Genome characterization of fungal pathogens is crucial for the development of novel disease management strategies, from early detection to manipulation of host defense mechanisms. Highly sensitive molecular detection methods include the use of disease-related genes encoding for proteins secreted by fungal pathogens early in the infection process. In Florida, the avocado industry has been severely affected by laurel wilt (LW), a lethal disease caused by the fungal pathogen *Raffaelea lauricola*. This disease has also caused extensive damage to native *Lauraceae*-dominated forests in the Southeastern United States. Here, we aim to identify sequence conserved (core) of genes in the *R. lauricola* genome as potential molecular markers for early pathogen detection. We used publicly available genomes of *R. lauricola* (strains RL4, RL570 and C2646) and of the non-pathogenic species *R. aguacate* (strains PL1004 and PCDF011). Using the Orthofinder software we identified 1,246 out of 6,433 orthogroups specific to *R. lauricola*. These specific regions will be evaluated for marker development and will be used to design DNA and/or protein-based diagnostic tools. The development of specific markers that can detect this lethal pathogen at early stages during the infection and from asymptomatic hosts will aid to the implementation of efficient control methods to prevent the spread of the pathogen within commercial avocado orchards and the establishment of the pathogen in new areas.

Potential roles of unfolded protein responses in the salicylic acid-dependent resistance induced by phytoplasma infection in plants

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Phytoplasmas are minute, cell wall-less obligate intracellular bacteria that infect a wide range of plant species, including agronomically important crops and ornamental plants. As with other plant pathogenic agents, phytoplasma infection induces salicylic acid (SA)-dependent defense responses in host plants. In this study, we employed potato purple top (PPT) phytoplasma and its alternative host, tomato as pathogen-plant model to investigate the potential roles of unfolded protein responses (UPRs) in SA-dependent resistance induced by phytoplasma infection. The results of real time quantitative PCR assay showed that the UPR marker genes, SIBip (a tomato Bip ortholog), bZip60 and bZip17 were significantly upregulated in PPT phytoplasma-infected tomato plants compared with mock graft-inoculated controls. In addition, WRKY75, a transcriptional activator of SA-dependent resistance, was also upregulated. Two lines of evidence indicated that SA-dependent PPT phytoplasma resistance was triggered by UPRs. First, in PPT phytoplasma-infected plants treated with UPR inducers DTT and TM, SA-dependent resistance increased along with enhanced UPRs, and PPT phytoplasma titer decreased. Second, in PPT phytoplasma-infected SIBiP gene knockdown tomato line (via virus-induced gene silencing approach), SA-dependent resistance was suppressed, and PPT phytoplasma titer was higher than that of control plants. The results from this study suggest that UPRs play a key role in SA-dependent resistance in plants induced by phytoplasmas.

A novel rod-shaped virus isolated from maize in Ecuador

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Maize leaf samples collected from the highlands of Ecuador were subjected to transmission electron microscopy (TEM) analysis using partially purified extracts to look for virus particles. Rod-shaped virus-like particles of 13 by 120–160 nm in size were observed. High-throughput sequencing (HTS) applied to total RNA extracts yielded a single virus-like contig of 11.9 kb. The complete genomic sequence was obtained and consisted of a mono-partite genome of 11,988 nt with four open reading frames (ORFs) encoding putative proteins of 42 kDa, 25 kDa, 73 kDa, and 264 kDa, respectively. The largest protein showed homology, with up to 30% amino acid identity, to the viral polymerase of several putative members of the *Mononegavirales*. The genome of the new virus has highly conserved intergenic sequences and complementary ends. The virus was detected in several Andean white maize cultivars and its seed-transmission was confirmed by grow-out experiments under greenhouse conditions. Although the original leaf samples displayed light-green concentric rings, infected plants from seed-transmission experiments did not show the symptoms, suggesting that the virus-like symptoms observed in the original sample were likely due to additional co-infecting viruses, which were not detected by HTS. Based on sequence identity and distinctive virion morphology, the new virus, for which we propose the name maize suscal virus, probably represents a new group of viruses in the Mononegavirales order.

Biocontrol activity of *Trichoderma* strains recovered from Florida against *Fusarium oxysporum* f. sp. *lycopersici* race 3 (*FOL3*)

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Trichoderma spp. are common inhabitants of sandy soils, including many utilized as biological control agents (BCAs). However, limited information is available regarding the efficacy of *Trichoderma* spp. against *FOL3* and their persistence in a Myakka fine sand common to vegetable production areas of Florida. A growth room trial evaluated nine native *Trichoderma* spp. recovered from local soils and one commercial BCA against *FOL3* on tomato over time. Each *Trichoderma* strain was inoculated in pasteurized field soil at 10^6 or 10^7 CFU/plant and challenged with two levels of *FOL3* (10^5 or 10^6 CFU/plant) 10 days after sowing tomato seeds (cv. HM1823). Soil was sampled (~ 4 cm³) from each cell plug every 2 weeks over 60 days. Recovery of *Trichoderma* and *FOL3* from soil was quantified (CFU/g) on modified Potato Dextrose Agar (PDAm) and malachite green agar (MGA), respectively. There were no significant differences in *Trichoderma* recovery among different strains over time, but significant differences based on application rate. Local *Trichoderma* sp. (JC161), *T. hamatum*, *T. lixii*, and *T. asperelloides* strains reduced Fusarium wilt incidence compared to the non-treated *FOL3* control ($P < 0.05$); while the commercial BCA product did not significantly differ from the non-treated *FOL3* control. The two applied rates of *Trichoderma* had no significant effect on plant height compared to the control while the applied *FOL3* rates had a significant effect on height. The results demonstrate the biocontrol potential of local *Trichoderma* for the management of *FOL3* on tomato in Florida.

Metagenomic sequencing for rapid identification of *Xylella fastidiosa* from leaf samples

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Xylella fastidiosa (*Xf*) is a globally distributed plant pathogenic bacterium. To date, 595 plant species have been reported as *Xf* hosts (EFSA 2020). Because the primary control strategy for *Xf* diseases is the eradication of infected plants, timely and accurate detection is necessary to prevent crop losses and further pathogen dispersal. Current standard *Xf* diagnostics primarily relies on a quantitative PCR (qPCR) assays. Nevertheless, these methods do not consider new or emerging variants due to pathogen genetic recombination and are limited by their sensitivity. In this study, we developed and tested a metagenomics pipeline using in-house short-read sequencing as a complementary approach for affordable and accurate *Xf* detection. We were able to use metagenomics to identify *Xf* to strain level in single and mixed infected plant samples, at concentrations as low as one picogram of bacterial DNA per gram of tissue. We also tested naturally infected samples from a variety of plant species originating from France, Italy and the United States. We identified *Xf* subspecies in samples that were previously considered inconclusive ($Cq > 35$) with real-time PCR (EPPO 2019). Overall, we showed the flexibility of the pipeline by using seven different plant hosts and DNA extraction methods. Our pipeline provides taxonomic and functional information for *Xf* diagnostics without extensive knowledge of the disease. We hope this pipeline can be used for early detection of *Xf* and other plant pathogens and incorporated as a tool to inform disease management strategies.

First record of stripe rust, *Puccinia striiformis*, on a North American native grass host, *Vahlodea atropurpurea*

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Puccinia striiformis is a destructive rust pathogen that occurs on wheat and other grasses throughout the world. Spreading of this pathogen can cause extreme loss in crop yields leading to global economic losses. In North America, the disease is the most damaging to wheat, barley, and rye in the western states, most commonly in eastern Washington, northeastern Oregon, and northern Idaho. Eleven different plant genera are affected by *P. striiformis* in North America. In 2015, *P. striiformis* was found on *Vahlodea atropurpurea* in Douglas Co., Washington. Dark colored, blister-like sori covered by the epidermis developed on infected stems and leaves, and gradually ruptured exposing the blackish brown, powdery mass of teliospores. Teliospores were subglobose or ovoid, $11\text{--}15 \times 12\text{--}17.5$ μm diam.,

reddish brown with smooth ~1-µm thick walls. Molecular analysis was used to confirm the identification. Rust28SF/LR5 and Rust2inv/LR6 primers were used to amplify the 5' end of the nuclear large ribosomal subunit (28S) gene region. The 28S sequence from *V. atropurpurea* showed 99.9% similarity with sequences of *P. striiformis* previously deposited in GenBank. A representative specimen was deposited in the Friesner Herbarium (Butler University). *V. atropurpurea* is a perennial grass found in the region from Alaska south to California and throughout Canada and New Hampshire. To our knowledge, there were not rust species found on *V. atropurpurea* thus far. In the future, we would like to examine more species in genus *Vahlodea* to determine if there may be any other rust pathogens present.

You wouldn't download a plant: Lessons learned (and future applications) from bringing plant diseases from field to screen in a fully online course

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Many lessons were learned in adapting the lab component of an upper-level introductory plant pathology course, which previously relied heavily on laboratory time and field experiences, to a fully online experience during the Covid-19 pandemic. Innovations included a larger and more independent team of teaching assistants, smaller sections, and simultaneous instruction of multiple sections. Novel course design included live interactive demonstrations, active learning in groups, and a self-guided disease foray. Technology adaptations included the use of consumer-grade video and lighting equipment. Benefits included more student-student interaction; direct demonstration of macro- and microscopic specimens by projecting from microscope cameras; immersive recordings from the field using portable audio and video recording equipment; engagement of students with citizen science; and improved accessibility. Rather than leave these efforts and insights behind, they can help inform and improve the future delivery of class content even in a traditional semester. Included are example clips from the semester, a demonstration of benchtop and field videography setups for the sharing and demonstration of plant pathology specimens at microscopic and macroscopic scales, and a discussion on how these adaptations can continue to be boons in the return to the classroom.

Evaluating the effect of field temperature on the growth of fungi causing fruit rot in deciduous holly

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Fruit rot of deciduous holly (*Ilex* spp.) is an emerging disease caused by a complex of seven fungal pathogens. While inoculations on wounded, mature *Ilex* fruits with all seven pathogens led to symptoms development, *Diaporthe ilicicola* also consistently caused disease when inoculated on flowers at bloom. Despite the timing of infection (spring), symptoms development does not occur until late-summer/early-fall when field temperatures drop. Controlled temperature experiments were conducted to monitor the hyphal growth and spore germination rates of *D. ilicicola*, *A. alternata*, *E. nigrum*, and *C. fioriniae* between 5 and 35°C to determine whether temperature alone is responsible for the latency in symptoms development. Results show that all isolates germinated and grew optimally between 25 and 30°C. The faster growing fungi in the complex, *A. alternata* and *E. nigrum*, were able to grow at both temperature extremes, 5°C and 35°C, while *D. ilicicola* and *C. fioriniae* were almost completely inhibited at 5°C. Interestingly, at 35°C, *D. ilicicola* spores not only germinated but also formed appressoria within 24 h. These results do not support the hypothesis that the late-season temperature drop directly induces changes in fungal growth consistent with the onset of symptoms development; however, they do suggest that infection of fruit by *D. ilicicola* likely occurs shortly following flower bloom when field temperatures increase during the summer months. These results advance our understanding of this pathosystem and will be used to help develop an integrated management plan for reducing crop loss.

Endophytic microbiota in apple and their relation with transcriptional reprogramming in response to *Chondrostereum purpureum*

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Silverleaf disease caused by *Chondrostereum purpureum* is a major economic disease for several fruit crops in Chile. Apple is one of the most susceptible species and silverleaf occurs throughout all the production area, having a negative impact on fruit yield and quality, and longevity of apple orchards and nurseries. In order to develop strategies for Silverleaf disease management by biological control agents, it is necessary to evaluate the intrinsic capacity of individual endophytic microorganisms to trigger plant natural defense responses. Thus, the objective of this work was to compare the transcriptional profile of apple plants cv. Brookfield, inoculated with isolates of *Bacillus*, *Paenibacillus* and *Streptomyces*, and later challenged with *C. purpureum*. The antagonistic ability against virulent *C. purpureum* strains was tested by the use of the three mentioned genera isolated from reverted and healthy apple plants. RNA was isolated from sawdust collected from the trunk of inoculated plants and candidate genes, which participate in different defense mechanisms, were analyzed by quantitative RT-PCR (qPCR). Results showed that meanwhile all evaluated bacteria can suppress *C. purpureum* development, major effects were observed in plants inoculated with *Bacillus* spp., according with the expression of defense-related genes like NPR1, which was highly upregulated in plants inoculated with *Bacillus* spp.

Real-time and conventional PCR assays for identifying a newly named species of root-lesion nematode (*Pratylenchus dakotaensis*)

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Root-lesion nematodes, *Pratylenchus* spp. are one of the economically important nematode pests on soybean. New PCR-based assays were developed for detecting and identifying a new species of root-lesion nematode (*P. dakotaensis*) recently discovered in a soybean

field in Richland County, North Dakota (ND). Species-specific primers, targeting the internal transcribed spacer of ribosomal DNA (ITS-rDNA), were designed to be used in both conventional and quantitative real-time PCR assays. The specificity of the primers was evaluated in silico analysis and laboratory PCR experiments. Results showed that only *P. dakotaensis* DNA was amplified in the conventional and real-time PCR assays but none of the DNA from other control species tested had amplification. Detection sensitivity analysis revealed that an equivalent to 1/8 and 1/32 of the DNA of a single nematode was detected by using the conventional PCR and real-time PCR, respectively. According to the standard curve generated, the amplification efficiency (E) of the primers in real-time PCR was 94% with an R^2 value of 0.95 between quantification cycle (Cq) number and log number of *P. dakotaensis*. Twenty soil samples collected from soybean fields in seven counties of ND were tested to validate the assays. The PCR assays successfully amplified the DNA of *P. dakotaensis* and discriminated it from other *Pratylenchus* spp. commonly present in ND soybean fields. Rapid and accurate detection of *P. dakotaensis* is essential for effective management of this new root-lesion nematode species in infested fields.

Anaerobic soil disinfestation amendment lignin to nitrogen ratio affects mortality of *Fusarium oxysporum* inoculum

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Cover crops, such as winter wheat, are potential carbon sources for anaerobic/biological soil disinfestation (ASD). Cover crop costs are often lower than other amendment sources and also improve soil quality and provide other agroecosystem services. However, lignin and nitrogen (N) content of residue varies according to wheat cover crop growth stage and may have a significant impact on amendment decomposition, and subsequent population dynamics of soilborne plant pathogens and indigenous soil microorganisms. We evaluated wheat cover crop residue as an ASD amendment with varying lignin to N ratio (L:N) on survival of indigenous soil fungi and inoculum of the strawberry root rot pathogen, *Fusarium oxysporum* (Fo), following soil treatment with ASD. Treatments included 1) vegetative stage wheat residue, 1:1 L:N; 2) reproductive stage wheat residue, 2:1 L:N; 3) mature wheat residue, 7:1 L:N; 4) a wheat bran control; and 5) a non-amended anaerobic control. The ASD experiment was conducted in pots with sandy soil in a growth chamber at 25 to 35°C, and repeated. All treated pots showed high anaerobic condition, except for the mature wheat stage amendment and the anaerobic control. Wheat residue with low L:N ratio (1:1 and 2:1) substantially reduced recovered Fo inoculum populations compared to other treatments, with no negative impact on indigenous *Trichoderma* spp. or *Aspergillus* spp. Our results suggest that low L:N ratio of wheat cover crop residue increases suppression of pathogenic Fo during ASD.

***Fusarium oxysporum* f. sp. *vasinfectum* race 4 soil quantification in Pima and Upland cotton cultivars**

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Fusarium oxysporum f. sp. *vasinfectum* race 4 (FOV4) is a soilborne pathogen that causes devastating losses in cotton (*Gossypium* spp.). Reported in El Paso in 2017, cotton stakeholders in Texas seek to understand the inoculum dynamics, dispersal, and control. The objective of this research is to quantify FOV4 in soil in response to growing resistant and susceptible Pima (*G. barbadense* L.) and Upland (*G. hirsutum* L.) cultivars. This trial consisted of a resistant and susceptible Pima, and tolerant and a susceptible Upland planted in sixteen replications in El Paso, TX, in 2020. Plant survival, root vascular necrosis, and yield were collected in the field. DNA was extracted from soil samples at planting (0 DAP), midseason (55 DAP), and harvest (143 DAP). FOV4 was quantified using real-time PCR. Significant differences among cultivars were observed for all field measures and midseason FOV4 quantity. Susceptible cultivars showed lower survival, higher root necrosis, lower yield, and elevated FOV4 quantity. Upland cultivars showed fewer symptoms than Pima cultivars; however, susceptible Upland and susceptible Pima did not have significant differences in midseason FOV4 quantities. FOV4 quantity at planting was negatively correlated with plant survival, and positively correlated with root necrosis showing that inoculum presence at planting can impact disease progression during the season. This research shows that the planting of resistant cultivars prevented an increase of FOV4 in the soil during the growing season, thus suggesting potential for disease management.

Advances in study of Cotton leafroll dwarf virus in Mississippi

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Cotton leafroll dwarf virus (CLRDV, genus *Polerovirus*, family *Solemoviridae*), a virus known to affect cotton production in South America and Asia, was recently identified in several states of the United States, including Mississippi. The initial discovery of the virus in commercial cotton production fields in Mississippi in 2018/2019 prompted a more comprehensive study on CLRDV in the state in 2020 focusing primarily on: 1) evaluation of distribution of the virus in Mississippi by field scouting and lab testing; 2) amelioration of molecular diagnostics; 3) studying genetic structure of the virus population in MS; 4) CLRDV genome sequencing of multiple isolates in the region; and 5) evaluation of cotton germplasm for possible sources of resistance. Furthermore, we also provided support to some neighboring states by providing the initial testing of their CLRDV-suspected samples. The subsequent presentation reports the progress on CLRDV-focused research and specific results obtained during the 2020 season.

Molecular mapping of QTL for a transformation-related trait and FHB resistance in a mapping population derived from the Bobwhite × PI 277012 cross

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Bobwhite is one of the few wheat cultivars most widely used for wheat transformation, but the genetic mechanism underlying transformability is not well understood. To identify quantitative trait loci (QTL) associated with plant regeneration capability from immature embryo-derived calli, a mapping population consisting of 186 recombinant inbred lines (RILs) derived from the cross between Bobwhite and a Fusarium head blight (FHB) resistant wheat line (PI 277012) were developed. Immature embryos were used as explants for tissue culture, and plant regeneration rates from the calli were recorded for the parents and RILs. The average regeneration rate was 0% and 42.5% for PI 277012 and Bobwhite, respectively. The RILs had an average regeneration rate ranging from 0 to 33%. The mapping population was genotyped using the genotyping by sequencing approach and a genetic linkage map was constructed with 3,779 SNP markers distributed on all 21 chromosomes, spanning 2,291 cM. QTL analysis detected two QTLs on chromosome 1A and 6D controlling plant regeneration capability in Bobwhite, which accounted for 10.5% and 9.8% of the phenotypic variation, respectively. To confirm the FHB resistance QTL (*Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*) previously identified in PI 277012, the Bobwhite × PI 277012 RIL population was phenotyped for FHB resistance in greenhouse and field experiments. However, only one QTL (*Qfhb.rwg-5A.2*) for FHB resistance was identified in this population, which explained 33.0% and 36.2% of the phenotypic variance under the field and greenhouse environments, respectively.

A polyketide synthetase gene cluster is responsible for antibacterial activity of *Burkholderia contaminans* MS14

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Burkholderia contaminans MS14, isolated from a soil sample in Mississippi, is known for producing the novel antifungal compound occidiofungin. In addition to this, MS14 possesses a broad range of antibacterial activities against common plant pathogens. Random mutagenesis and gene complementation indicate that four genes are required for antibacterial activity observed in MS14. With the aim of finding the biosynthetic gene cluster for the unknown antibacterial compound, we used RNA-Seq to analyze the transcriptome of MS14 wild type and mutants lacking antibacterial activity. The lower expressed genes in all mutants were studied and a polyketide synthases (PKS) gene cluster was predicted to be directly involved in MS14 antibacterial activities. The *nptII*-resistance cassette and CRISPR-Cas9 systems were used to mutate the PKS gene cluster. Plate bioassays showed that either insertion or frame-shifting one of the PKS genes resulted in a loss of antibacterial activity. Considering the antibacterial-defective mutants keep the same antifungal activities as the wild type strain, the results suggest that this PKS gene cluster is highly likely to be involved or directly responsible for the production of MS14 antibacterial activity.

The transcriptomic response of papaya plants (*Carica papaya*) to the *Babaco mosaic virus* (BabMV)

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The *Babaco mosaic virus* (BabMV) damage the babaco plants (*Vasconcellea heilbornii*) and compromise the plants' productivity. Recently, the pathogenicity in papaya hosts has been demonstrated but the plant's response mechanism is poorly understood. This research aimed to characterize the response of papaya plants to BabMV infection using transcriptomics. Young leaves of 3–4 month old papaya plants were infected with BabMV and the transcriptome was evaluated at 2, 10, 15, and 30 days post infection (dpi). Illumina reads were processed using Galaxy and high-quality reads were mapped to both papaya and BabMV genomes using HiSat. Statistical analysis was performed using the Wald test from the Deseq2 package in R. Bonferroni-corrected *P*-values were used to select the differentially expressed genes and HCL as well as expressions graphs (QTC) were constructed using MeV. Results showed that >90% of the reads at 2 and 10 dpi mapped to the papaya genome but 69% of the reads from BabMV-treated plants at 15 and 30 dpi aligned to the virus genome, suggesting a high viral load in the sampled tissues. HCL yielded two clades with samples from 2 and 30 dpi as well as 10 and 15 dpi. QTC showed that most genes were upregulated at 30 dpi in infected plants. About 15,449 genes of the papaya genome were annotated using Omicsbox and GSEA identified gene ontologies at 10 and 30 dpi related to the response to heat, temperature stimulus, abiotic stimulus, oxidative stress, inorganic substance, and protein folding. This is the first report showing the transcriptomic response of papaya plants to BabMV.

Analysis of leaf-associated bacterial and fungal microbiomes of corn inbred lines related with tar spot disease

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Tar spot is a foliar disease of corn, caused by the obligate biotrophic pathogen *Phyllachora maydis*, that has recently emerged as an economic concern for corn production in the United States. We investigated leaf microbiomes in relation to corn cultivars with differential tar spot symptoms under *P. maydis* infestation in natural field conditions. Leaf samples from 16 corn cultivars were assessed for tar spot development and both bacterial and fungal microbiomes were characterized by paired-end sequencing on the Illumina MiSeq platform. Comparative metagenomics analysis of the leaf microbiomes revealed clear differences in overall microbial communities between resistant and susceptible corn cultivars. Bacterial communities in both resistant and susceptible lines were dominated by a single operational taxonomic unit (OTU) identified as *Methylobacterium* spp. The least-abundant OTU in resistant lines was in the genus *Pseudomonas*, whereas species of *Microbacterium* and *Deinococcus* were the least-abundant OTUs in susceptible lines. For fungal communities, genera *Fusarium*, *Genoleuria*, *Zymoseptoria*, and *Colletotrichum* were least abundant, whereas *Papiliotrema* and *Alternaria* were the most abundant OTUs in the resistant lines. By contrast, fungal communities in susceptible lines were dominated by two OTUs in the genera *Puccinia* and *Sphaerellopsis*. Further analyses of the differences in microbiome

composition between susceptible and resistant cultivars could lead to the development of novel and more effective biological control agents against tar spot.

Development of a LAMP assay based on nanotechnology tools to detect *Neofusicoccum* spp. and *Diplodia* spp. associated with grapevine trunk diseases

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Grapevine trunk diseases represent one of the most important problems for viticulture worldwide, therefore it is necessary to use a sensitive method for early diagnostic. LAMP is highly sensitive technique and is widely used in molecular diagnosis, having a principal disadvantage, the crossing contamination. In the present investigation, we design a new strategy to detect the two main phytopathogenic fungi responsible for grapevine trunk diseases, using conjugated probes with quantum dots biomimetically synthesized, helping in decrease the occurrence of false negatives for cross-contamination. The conjugated quantum dot-ssDNA it will be done through ligands interchange, joining directly on the surface of the quantum dot, obtaining a nanoprobe with higher optical properties and the recognize properties of the DNA, detecting specifically conserved regions of the amplicon by fluorescence quenching via Förster resonance energy transfer. The analyzed samples were from pure cultures of the fungus *Neofusicoccum parvum*, *Diplodia seriata*, and wood DNA. Preliminary results indicate that the LAMP system was able to detect *N. parvum* and *D. seriata* from pure cultures and from wood DNA. As negatives controls, we use DNA samples of the pathogens, *Eutypa lata*, *Phaeomoniella chlamydospora*, and *D. seriata/N. parvum* according to the analysis. This investigation results useful for the future molecular detection on field samples and contribute to the improvement of the diagnosis system that allow preventing earlier and timely the dissemination of these pathogens.

Characterization of ice nucleation activity (INA) in *Fusarium avenaceum*

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Fusarium avenaceum is a plant pathogen with a broad host range, causing Fusarium head blight (FHB) on maize and small-grain cereals. *F. avenaceum* is also known for ice nucleation activity (INA), which can produce ice nucleating particles (INPs) that catalyze ice formation at a higher temperature (-5°C) than the one at which pure water freezes (-38°C). Biological INPs are among the most efficient INPs and may impact atmospheric processes. Besides fungi, they include bacteria, pollen, and lichens. INA in fungi was first discovered in *F. avenaceum* in 1992, but little is known about its INA. This study aimed at characterizing INA in *F. avenaceum* and investigating its genetic basis. Multiple *F. avenaceum* strains were screened for INA using a droplet freezing assay. Differential filtration experiments were conducted to estimate the size of INPs. Whole genome sequencing (WGS) on the Illumina platform was performed of all strains. Comparative genomic studies have been initiated to identify the differences in gene content between ice nucleation-active (Ice^{+}) and inactive (Ice^{-}) strains. As a result, 11 *F. avenaceum* strains were found to be Ice^{+} and 3 strains to be Ice^{-} . *F. avenaceum* INPs appear to consist of aggregates. Ice^{+} and Ice^{-} strains were found to share high similarity in gene content. Therefore, we expect to find a relatively small number of putative INA genes that can be experimentally tested for contributing to INA. Knowledge of *Fusarium* INA gene sequences will be instrumental in obtaining further insight into the role of fungal INA in atmospheric processes.

Carlavirus: Is it a risk for beans in Australia?

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A carlavirus, closely related to *Cowpea mild mottle virus* and spread by the whitefly *Bemisia tabaci* (MEAM1), was reported affecting fresh market beans (*Phaseolus vulgaris*) in a major Australian growing district in autumn 2016. The virus caused losses of up to AUD\$400,000 during that season but rarely causes economic impacts in other districts. Regular surveys of crops in the district were done during autumn and spring/summer from early November 2016 to June 2019. No production occurs in late summer and winter. Each survey consisted of inspecting ~15,000 plants for symptoms and 150 plants for adult whitefly. Symptomatic plant samples were indexed for carlavirus by RT-PCR. Results showed that virus incidence was seasonal, with bean crops only affected during autumn and disease incidences only high in some years. The virus was not detected in spring or early summer crops, despite presence of the vector at similar levels to autumn populations. Whitefly levels varied between and through the seasons and showed some relationship with rainfall. Finer details of results showing the variation in virus incidence within and between seasons and years will be presented. In addition how virus incidence relates to whitefly population levels will be discussed. These results supports our conclusion that this carlavirus is a very low risk for Australian beans unless conditions are favorable for vector populations to become high.

Cropland connectivity: Best practices for incorporation in geographic risk analyses

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A cropland connectivity risk index (CCRI) can be an important new component of geographic disease risk assessment to support planning of surveillance and mitigation strategies. Cropland connectivity measures how readily crop-specific pathogens can move through crop landscapes, based on their geographic proximity and host density. Here we discuss some key features for effective use of CCRI algorithms. CCRI uses as input the mapped density of a target crop or plant species at a global or regional scale, as well as parameters describing the probability of pathogen movement as a function of distance. Because good estimates of these parameters may not be available, especially for new and under-studied diseases, analyses can evaluate a range of parameter values in an uncertainty quantification. We discuss other considerations for a cropland connectivity analysis, such as potential thresholds for the minimum cropland density to be included in analysis, and the spatial resolution of the density maps. We also provide advice on interpreting the resulting maps of a CCRI in an uncertainty quantification. Locations with consistently high mean CCRI might be important for pathogens with many types of dispersal, while locations with high variance in CCRI might be important only for some pathogen species. A CCRI can be integrated with other data layers, such as climate-based risk and crop trade, as interpretation and considerations for combining these types of risk measures.

Use of *Trichoderma koningiopsis* to reduce viability of *Calonectria* spp. in boxwood leaf litter incubated at 0, 10, or 20°C

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Boxwood is threatened by *Calonectria pseudonaviculata* (Cps) in the US and by both Cps and *C. henricotiae* (Che) in Europe. The pathogen persists in fallen litter, so an attempt was made to reduce survival with application of a biocontrol agent. Leaves infested with Cps or Che were placed in plastic boxes containing sterile sand and moisture adjusted to carrying capacity using either sterile water or a spore suspension of *Trichoderma koningiopsis*. Boxes were incubated at 0, 10, and 20°C in three trials lasting 6 months. Twenty leaves were removed from each box monthly and plated on GYET agar media. The slope of the first order linear regression line was calculated for each expt-by-trt-by-isolate-by-temp-by-box set of values in the 0–6 month observation period. The slope variable, SPcal was analyzed as a three-factor mixed model using Proc Mixed for Trt, Isolate, and Temp with Expt as the random block. *Trichoderma* significantly affected pathogen viability ($P < 0.0001$), there was no difference in the survival of Cps & Che ($P = 0.16$) and incubation temperature was not significant ($P = 0.24$). This suggests that altering the topsoil or mulch environment under boxwood plants could reduce survival of the pathogen.

Identifying specificity mechanisms in *Zymoseptoria tritici* in host and non-host interactions

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Wheat is the second-most important cereal crop, grown by 30 million farmers worldwide. It is affected by *Zymoseptoria tritici*, the cause of Septoria tritici blotch. This disease has an asymptomatic stage for 1–13 days, followed by a rapid transition to necrotrophy. The lifestyle of *Z. tritici* makes this pathogen an attractive model to investigate infection phase-specific gene expression, and host-pathogen specificity mechanisms in compatible, incompatible and non-host interactions. Differential gene expression and KEGG pathway enrichment were determined in *Z. tritici* during a compatible interaction with the susceptible cultivar Taichung 29, two incompatible interactions with the resistant cultivars Veranopolis (*Stb2*) and Israel 493 (*Stb3*), and one non-host interaction with barley. Differential gene expression was calculated at 1, 3, 6, 10, 17, and 23 days after inoculation (DAI). The highest number of differentially expressed genes (DEGs) was observed at 10 DAI in the compatible interaction compared to the incompatible interactions with Veranopolis and Israel 483. This suggests that *Z. tritici* activated genes to initiate and maintain the necrotrophic lifestyle around 10 DAI. The enriched pathways from 10 to 23 DAI were biosynthesis of secondary metabolites and antibiotics, and protein processing in endoplasmic reticulum. More DEGs were obtained at 3 DAI in the compatible and incompatible interactions compared to the non-host. This suggests that 3 DAI is the point at which the fungus recognizes the plant as a host and activates specific genes to trigger subsequent responses.

Evaluation of two main ribosomal RNA depletion kits for plant virus detection using high-throughput sequencing: Ribo-Zero versus FastSelect

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High-throughput sequencing (HTS) is becoming a common approach to detect viruses in a host. Removing highly expressed ribosomal RNA is a crucial step to produce a high-quality RNA-seq library. Illumina TruSeq Stranded Total RNA with Ribo-Zero Plant is a traditional method for removing cytoplasmic, mitochondrial, and chloroplast ribosomal RNA from plant tissues in RNA-seq, but it's a time-consuming process with many steps. Recently, QIAGEN developed a low-cost one-step rapid rRNA removing kit, QIAseq FastSelect, which can be completed in 20 min. To evaluate these two methods, we developed an RNA-seq experiment with eight plant samples, where six had at least one virus present: *Ipomoea batatas* (3), *I. setosa* (1), and *Solanum tuberosum* (4). Our analyses showed that FastSelect had a similar rRNA removal capability as Ribo-Zero, and it's more stable than Ribo-Zero. However, FastSelect significantly reduced sequencing yields, which were less than half of Ribo-Zero. This caused virus mapped reads significantly lower than Ribo-Zero, although both methods detected the same viruses. Thus, we recommend doubling input materials when using FastSelect kits for RNA-seq to improve the sequencing depth of the virus.

Susceptibility of spinach cultivars to *Stemphylium* leaf spot (*Stemphylium vesicarium*) and white rust (*Albugo occidentalis*) in Texas

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Two spinach foliar diseases of economic importance in the Texas Wintergarden region are *Stemphylium* leaf spot, caused by *Stemphylium vesicarium*, and white rust, caused by *Albugo occidentalis*. During the 2020–2021 season, 79 commercial spinach cultivars were each planted in 3 replicate beds in La Pryor, TX, and inoculated 5 and 7 weeks after planting with a mix of three isolates of *S. vesicarium*. Each plot was rated for severity of white rust (natural infection) 8 weeks after planting, and for severity of *Stemphylium* leaf spot a week later (1 to 10 scale, with 1 = no symptoms and 10 = 100% of the foliage symptomatic). The severity of *Stemphylium* leaf spot averaged 2.9 (range of 1.0 to 8.0 per plot). Of the 79 cultivars, 21 had a mean rating of 1.0, 30 had a mean severity ranging from 1.1 to 3.0 (partially resistant), 6 had a mean severity of 3.1 to 5.0 (moderate), and 22 had a mean severity from 5.1 to 7.0 (susceptible). The white rust severity ratings averaged 4.0 (range of 1.0 to 10.0 per plot). Five cultivars had no white rust (mean of 1.0), 24 had partially resistant reactions (1.1–3.0), 29 had moderate mean ratings (3.1–5.0), 14 had partially susceptible ratings (5.1–7.0), and 7 had highly susceptible ratings (7.1–8.7). No cultivar was completely resistant to both diseases, but Salamander, PV 1569, Colusa, Sunangel, Spiros, Baboon, and Fantail had mean ratings ≤ 2.0 for both diseases. The results can be used by growers and breeders to select spinach cultivars with complete or partial resistance to *Stemphylium* leaf spot and/or white rust.

Genome assembly of the sugar beet root rot pathogen *Aphanomyces cochlioides* using Nanopore and Illumina sequencing

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The soil-dwelling oomycete, *Aphanomyces cochlioides*, is responsible for damping-off and root rot of sugar beet, causing yield losses in major sugar beet growing regions. Genetic and genomic sequences for this pathogen are currently lacking, including a complete reference genome. Availability of genome sequences allow us to gain valuable insights into the fundamental biology of this organism. To improve the genomic resources for *A. cochlioides*, we used a combination of long-read and short-read sequencing platforms to generate a genome assembly of isolate 103-1 originally collected from Breckenridge, MN. We used CANU to assemble the genome into 76.36 Mb, which is 92.7% of the estimated genome size of 82 Mb. The resulting assembly consisted of 97 contigs with a contig N50 size of 2.6 Mb. Genome completeness was also assessed using BUSCO with the Alveolata-Stramenopiles dataset, of which the assembly had a 93.2% completeness score. Gene prediction using Funannotate predicted 21,892 gene models and the RepeatModeler pipeline estimated a genome content of 32.1% repetitive elements. The genome was used in an analysis of variation among five *A. cochlioides* isolates from Minnesota, North Dakota, and Texas. This resource adds an important oomycete reference genome for comparative studies and will facilitate future research into interspecific and intraspecific variability, mining for pathogenicity and virulence factors such as RXLR and CRN effectors, and population structure.

Roles of apple laccase gene regulation and lignin deposition in apple root resistance to *Pythium ultimum*

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Development and deployment of resistant or tolerant rootstocks offers a cost-effective, ecologically friendly, and durable approach for apple replant disease management. In a recent study, major microRNA (miRNA) families and their corresponding target genes were identified using a large-scale approach of miRNA profiling and degradome sequencing for three resistant and three susceptible apple rootstock genotypes to *P. ultimum* infection. One of the most noticeable groups of target genes are laccase genes which are targeted by mi397b. The expression patterns for mi397b demonstrated differential regulation between resistant (R) and susceptible (S) genotype groups and in response to pathogen infection; the stronger cleavage intensity was observed in susceptible genotypes group for three apple laccase encoding genes (lac -3, -5 and -7), as indicated by degradome sequencing. In current study, the size and phylogenetic features of apple laccase families was analyzed using online bioinformatic tools and the genotype-specific expression of lac -3, -5 and -7 in response to *P. ultimum* infection were examined using qRT-PCR method. The variation of lignin deposition patterns between genotypes and in response to *P. ultimum* infection were investigated by tissue sectioning and Wisner staining. The data are being analyzed to understand the relationship between mi397b and laccase encoding genes as well as the effect on lignin deposition at the initial phase of defense activation in apple root to this necrotrophic pathogen.

Long-term effects of different potato cropping systems on soil and crop health

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Beneficial soil and crop management practices, such as longer rotations, cover crops and green manures, organic amendments, and reduced tillage, may improve soil and crop health when incorporated into cropping systems. However, long-term trials are needed to assess the full impacts and effects of these systems. In field trials originally established in 2004, three different 3-year potato cropping systems focused on management goals of soil conservation (SC), soil improvement (SI), and disease suppression (DS) were evaluated and compared to a standard 2-year rotation (SQ) and a non-rotation control (PP). After 15 years and results compiled over the last 4 years (2015–2018), the SI system (with history of compost amendments) improved soil properties water-holding capacity, organic matter and nutrient contents, aggregate stability, and CEC, relative to all other systems, as well as higher tuber yields (by 26%) and higher microbial activity (by 44%) relative to the standard SQ system. The DS system, which included a disease-suppressive green manure rotation crop and fall cover crops, also improved yield (by 16%), had higher OM content, and increased microbial activity relative to SQ, as well as reducing soilborne diseases (black scurf and common scab) by 10–30%. The nonrotation PP system resulted in notable degradation of soil properties and yield over time. These results demonstrate that soil health management practices can be

effectively incorporated into viable potato cropping systems to improve soil properties and crop health and may enhance long-term sustainability.

Combating onion bacterial diseases with pathogenomics tools and enhanced management strategies: Research objectives and preliminary results

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‘Stop the Rot’ is a USDA NIFA SCRI project (2019–2023) with 25 scientists from diverse disciplines in the US and South Africa conducting research on the host, bacterial pathogens, and environmental factors influencing bacterial diseases of onion. The primary objectives are to: generate regional characterization of bacterial pathogens through field surveys, pathogenomics, and microbiome studies; develop robust molecular diagnostic tools for timely detection, identification, and management of bacterial diseases; establish a National Onion Bacterial Strain Collection to support research; use stakeholder feedback from trials to evaluate and optimize management strategies and identify risk-based management options; employ economic evaluations to ensure management recommendations are practical, viable, and financially sustainable. Although five bacterial genera are most commonly associated with onion diseases (*Pantoea*, *Burkholderia*, *Enterobacter*, *Xanthomonas*, and *Pseudomonas*), the first year of surveys revealed a broader diversity of bacteria (at least 51 genera) associated with symptomatic onions. However, few of these genera produced symptoms in pathogenicity tests. Initial research on developing species-specific diagnostic tests is targeting *Pantoea agglomerans*, a pathogen of national concern. Irrigation, soil fertility, cultural, and post-harvest practices as well as bactericides are being evaluated. Results to date indicate that managing irrigation timing and mode of application could reduce onion crop losses from bacterial diseases.

Unexpectedly large peaks of *Aspergillus Flavus* propagules observed at cotton fields during corn harvest

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Aspergillus flavus is an airborne pathogen of several important crops, including corn, cotton, and peanuts. It is also a human pathogen, allergen and mycotoxin producer. Aflatoxins produced by *A. flavus* emerged as known foodborne poisonous substances in the 1960s. Propagules move from field to field and crop to crop. In this study we analyze *A. flavus* spore capture data from a 2019 project sponsored by Cotton Inc. Nine cotton sites were monitored. A mechanistic model of *A. flavus* environmental suitability for sporulation was developed using the Battilani model. Measured spore counts were compared to modeled environmental suitability in order to validate data. At eight sites a large peak of *A. flavus* spores was noted at a time when environmental suitability for sporulation was very low. At those sites, the peak of *A. flavus* spore count corresponded with corn harvest dates as published by USDA. The prevailing wind during the time of peak spore count was determined from hourly wind data for each of the nine cotton sites. The USDA CropScape database confirmed that corn fields were upwind of our cotton fields. At the one site where no large peak was seen, that site was downwind from a city. We conclude that corn harvest is a significant event in the origin of *A. flavus* propagule spikes that were seen at cotton fields.

Sporulation timing of the invasive oak wilt fungus, *Bretziella fagacearum* in Michigan

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Oak wilt (OW), caused by the fungal pathogen *Bretziella fagacearum* (Bf), blocks the vascular system of *Quercus rubra* and other red oaks (Lobatae) within 4 weeks of infection. Sap beetles (Nitidulidae), which are attracted to sporulating mycelial mats produced on *Q. rubra* trees killed by OW, can vector Bf if they visit fresh wounds on a healthy oak. Root grafts facilitate further spread of the fungus. Ongoing spread of OW and *Q. rubra* mortality is of increasing concern in urban and forestry communities throughout 24 US states where OW occurs. We sought to identify fungal sporulation periods and peaks, which correspond to high-risk periods of Bf overland infection by nitidulid beetles. We tagged *Q. rubra* trees at 12 OW centers in northern Michigan forests. Trees were monitored for development of OW symptoms and mat production at 2-week intervals from March to November in 2019 and 2020. Mats were collected, assigned to morphological classes, and evaluated for spore viability using a serial dilution plating technique. Mycelial mats were produced from April to November in both years. In 2019, viable spores were produced by mycelial mats throughout the same period, except for July and November. In 2020, viable, sporulating mycelial mats were produced from April to November. Peak periods of sporulation occurred in late spring and early fall in both years. These data will help define relative risk of overland Bf transmission in Michigan and subsequent guidelines for minimizing activities that could cause *Q. rubra* wounds.

Assessing the effectiveness and timing of fungicides on southern rust of corn (*Puccinia polysora*) in Indiana

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Southern rust of corn is caused by the obligate pathogen *Puccinia polysora*. *P. polysora* does not overwinter in Indiana, but each year urediniospores are carried north in weather systems. Southern rust was first documented in the Midwest in 1949 and can be a concern for growers when the disease appears mid-season. Warm and moist conditions are favorable for this tropical pathogen and secondary infections quickly accelerate the severity resulting in significant yield loss. Fungicide efficacy and timing were evaluated for their effectiveness against *P. polysora*. Trials were conducted in 2019 and 2020 in Knox County, Indiana. Eight fungicides were evaluated,

applied at tassel/silk (VT/R1) and at milk (R3) growth stages, and were compared to a nontreated control. Severity was rated by visually assessing the percentage of southern rust pustules per leaf on five plants in each plot at early and late R5 (dent) growth stage. Southern rust was first detected in Indiana on 25 July in 2019 and 2020 growing seasons and reached moderate severity in the trials (12.0% and 16.9% in 2019 and 2020, respectively). In both years, all fungicides applied at both VT/R1 and R3 growth stages significantly decreased southern rust severity over the nontreated control. Even though fungicides reduced southern rust, no significant differences in yield were detected between treatments in both years.

Emergence, identification, and characterization of *Diplocarpon coronariae* causing apple blotch disease in the eastern United States

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Apple blotch disease, which is caused by *Diplocarpon coronariae* and leads to severe premature defoliation and fruit spotting, has become an increasing problem in apple production regions of the Eastern United States. We recovered 54 isolates from symptomatic apple leaves and fruit in New York (NY), Pennsylvania (PA) and Virginia (VA) which all showed uniform, slow growing, and dark grey colonies on peptone potato dextrose agar (PPDA) after 3 weeks of incubation at 25°C. Phylogenetic analysis of rDNA internal transcribed spacer confirmed all isolates as *D. coronariae*. Pathogenicity tests on apple leaves and fruit revealed that all isolates were pathogenic. Leaves of cultivars ‘Fuji’ and ‘Honeycrisp’ were infected with *D. coronariae* and the transcriptome profile of seven genes at 0-, 2-, 5-, 8-, 12-, and 15-days post inoculation (dpi) showed increased expression of chitin-elicitor receptor kinase 1, beta-1,3-glucanase and class III endo-chitinase in ‘Honeycrisp’ (disease severity of 55%) compared to ‘Fuji’ (disease severity of 87%) at 2- and 5-dpi. Fungicides fluxapyroxad, thiophanate methyl and difenoconazole effectively inhibited mycelial growth of isolates in vitro with mean EC₅₀ values of 0.015, 0.018, and 0.023 µg/ml, respectively. The EC₅₀ values of isolates from NY were significantly higher. Our results lay a foundation for future studies on origin of this pathogen in the U.S., introduce potential gene candidates for breeding resistant cultivars, and will facilitate development of best management practices to control this disease.

Open-source hardware and software in the plant sciences can affordably improve reproducibility, throughput, and work-life balance

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The year 2020 was challenging for productivity in many research laboratories due to personal hardships, funding disruptions, laboratory inaccessibility, etc. One silver lining observed from these hardships was the innovation that blossomed from researchers involved in the maker movement. Through the use of free open-source hardware (FOSH) and software (FOSS), researchers can readily improve upon extant designs or create their own by using basic tools and affordable fabrication technologies, such as 3D printing. Many designs are thoroughly documented, published in peer-reviewed journals, and often present data meeting reproducibility benchmarks of commercial laboratory equipment for a fraction of the cost. Published FOSH relevant to plant sciences include peristaltic pumps, liquid handlers, reactive environmental sensors, automated multispectral acquisition systems, and more. While DIY laboratory equipment has become more available, documentation is often not tailored to scientists without a foundation in engineering. Here we present a review of extant FOSH relevant to plant sciences, resources to improve accessibility for the non-engineer, and our thinking on FOSH limitations. In response to our own laboratory accessibility limitations, we began implementing FOSH into our laboratory starting with a re-design of the open-source liquid handler, Ottobot. Accordingly, we present design simplifications, expanded documentation for the non-engineer, reproducibility benchmarking data, total build cost, and total time spent from initial research to build for a non-engineer.

Development of infectious clone of novel blueberry carlavirus

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A full genome of a novel blueberry carlavirus, resembling (BIScV), was cloned into a modified pORE-E4 *Agrobacterium* binary vector between the ENT2CUP2 promoter and the hepatitis delta virus ribozyme—Nos-t terminator. Leaf agroinfiltration of the binary vector construct combined with the p19 virus suppressor, failed to infect *Nicotiana benthamiana* and *N. tabacum* but produced severe systemic symptoms in *N. occidentalis* consisting of mosaic, leaf blister and deformation and overall stunting. Mechanical transmission using sap from agroinfiltrated *N. occidentalis* produced identical symptoms in the same host. Purified virus particles from infected *N. occidentalis* showed flexuous carlavirus-like rod particles. However, unlike the other carlavirus infecting blueberry, Blueberry scorch virus (BIScV), the novel carlavirus, caused symptomless infection in *Chenopodium quinoa* and reacted very poorly to BIScV antibodies in ELISA. The availability of an infectious clone for this novel blueberry carlavirus provides tools for future studies on the biology of the virus.

More than just chemistry: Characterization of fungicide physical mode of action in the hop powdery mildew pathosystem

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Understanding the physical mode of action of fungicides is beneficial to build more efficacious and efficient disease management programs. To this end, post-infection activity and translocation of commonly used fungicides to control hop powdery mildew, caused by *Podosphaera macularis*, were quantified in greenhouse, laboratory, and field studies. Representative fungicides were applied at different time points pre- or post-inoculation with *P. macularis* and then rated 10 days later to quantify levels of disease. In separate laboratory studies, fungicides were applied to the abaxial side of leaves, inoculated with the *P. macularis* 24 h later, and subsequent zones of inhibition were measured to quantify translocative properties. In greenhouse and field studies, all fungicides effectively suppressed disease development when applied 24 h prior to inoculation. Efficacy varied by fungicide when applied 6–72 h after inoculation, with a general tendency for efficacy to decrease with time since inoculation for most fungicides except those containing fluopyram, paraffinic oil, and polyoxin D in greenhouse studies. Field studies with a subset of the fungicides produced similar trends of decreasing efficacy with increasing time from inoculation to application. Translaminar movement varied substantially between fungicides, with fluopyram producing the largest zone of inhibition while trifloxystrobin had none. This research will help inform the development of effective spray programs for hop powdery mildew.

Construction of a knowledge base to diagnose postharvest diseases of apple

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Apple fruit (*Malus domestica*) can be stored under controlled atmosphere conditions for a period of up to 12 months. In the course of the postharvest process, diseases caused by different fungal pathogens can lead to quality deterioration and losses of fruit. Reliable disease diagnosis is therefore crucial to contain the damages or to define preharvest plant protection measures. In order to bring the diagnosis process closer to practitioners, the goal of the present study was to construct a knowledge base that could be implemented into a diagnostic decision support system. Seven different postharvest diseases of apple commonly occurring in South Tyrol (northern Italy) were considered, namely Alternaria rot, Alternaria spot, blue mold rot, bitter rot, bull's eye rot, grey mold rot, and Mucor rot. For each disease, character traits of the external lesions and internal rots were accurately described based on information provided in specialist literature and the observation of images of apples, on which the abovementioned diseases were determined by microbiological and/or molecular genetic assays. The knowledge base was modeled in a Bayesian network resulting in a theoretical prediction accuracy of 90%. While the representation of medical knowledge for computer-based diagnosis is well advanced, it is less commonly applied to plant diseases. Therefore, the present study provides a valuable contribution to this field. Furthermore, the opportunities and challenges of knowledge-based diagnostic systems for postharvest diseases of apple are addressed.

Characterization of rhizospheric microbiomes associated with sheath blight resistant and susceptible rice lines

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Sheath blight (ShB) caused by the fungal pathogen *Rhizoctonia solani* is one of the most economically important rice diseases worldwide. Especially, the disease is considered a major threat to the rice production in the southern United States due to the conducive environmental condition and the lack of resistant cultivars. Given the fact that plant health is greatly influenced by plant microbiota, in-depth study of the microbiomes associated with ShB would give an insight into the development of innovative strategies for ShB management using microbial agents. In this study, 23 rice lines were evaluated for their ShB resistance under greenhouse and field conditions. Among those lines, three resistant and three susceptible lines were selected to characterize their root-associated microbiomes. Using high-throughput sequencing of the 16S and 18S rDNA regions, we obtained the profiles of rhizospheric microbial community in each rice line. Results showed that numerous taxa were significantly enriched in the rice rhizosphere compared to the adjacent bulk soil samples, but no significant clustering was found between the resistant and susceptible rice lines. Nevertheless, several taxa at the genus level showed differential abundance between the rhizospheres of resistant and susceptible lines, which include *Bacillus*, *Sphingomonas*, and *Nitrospira*. Our study provides valuable information about the effect of ShB disease to the rhizospheric microbiomes of resistant and susceptible rice lines, and the rice-microbiome interactions related to ShB resistance.

Characterization of *Pythium* spp. causing a putative new disease of annual bluegrass golf course putting greens

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First noticed in 2005, a unique disease of annual bluegrass (*Poa annua* L.; ABG) has been observed on golf course putting greens in the northeastern US. Occurring during warm, humid conditions, disease symptoms on ABG produce yellow to orange, circular patches. Coenocytic mycelium and embedded oospores were visualized in leaf tissue and *Pythium* isolates recovered after pathogen isolation from nine golf courses. The objectives of this research were to characterize these pathogens by 1) completing Koch's postulates to confirm causal agent of disease on ABG; and 2) investigating molecular and morphological characteristics to identify causal pathogens. Collected *Pythium* isolates were able to cause disease on seedling ABG in controlled environment pathogenicity assays. After isolate inoculation, ABG putting green field plots produced similarly observed disease symptoms and inoculated pathogens were reisolated out of symptomatic plant tissue. Sequencing of internal transcribed spacer region of rDNA, cytochrome c oxidase subunit I and II, and β -tubulin loci revealed the 13 isolates were nested within the *Pythium aristosporum*/*P. arrhenomanes* species complex. Current results from morphological characterization are ambiguous and not sufficient to assign isolates definitively to one of the two *Pythium* species. This previously unreported disease of annual bluegrass is caused by the species complex of *P. aristosporum*/*P. arrhenomanes* and is termed Pythium patch. Further morphological and molecular studies are needed to disentangle *P. aristosporum*/*P. arrhenomanes* taxonomy.

Evaluating soybean breeding lines for resistance to *Phomopsis* seed decay: Results from 2020 seed plating assays

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Soybean *Phomopsis* seed decay (PSD) causes poor seed quality in most soybean production areas worldwide. Although PSD could be caused by different *Phomopsis* and *Diaporthe* species, *P. longicolla* (syn. *D. longicolla*) is the primary cause of PSD. Breeding for PSD-resistance is the most cost-effective long-term strategy to control this disease. To develop high yielding cultivars and breeding lines with PSD-resistance, an extended effort of over a decade has been made to achieve this goal, evaluating multiple breeding lines annually. In 2020, a total of 168 soybean seed samples from 36 breeding lines and 26 checks were assayed for the percent seed infection by *P. longicolla*. Those breeding lines were derived from multiple pedigrees and utilized at least six sources of PSD-resistance. They were delay-harvested from field trials inoculated with *P. longicolla* in 2019. Results showed that there were significant differences ($P \leq 0.05$) in seed infection by *P. longicolla* among soybean lines, with some lines having as little as 4% infection (DA1239-09), while others had levels as high as 83% infection (cultivar LD00-7620). Twelve breeding lines had levels of infection less than 10%, which were all significantly less than the infection levels of susceptible checks. We expect this research will lead to the release of improved germplasm lines with PSD resistance, high seed germinability, and lower seed damage. The improved germplasm lines will be utilized in breeding programs to develop high-yielding cultivars that have reduced elevator dockage caused by damaged seed.

Development of loop-mediated isothermal amplification assay for rapid detection of *Pectobacterium parmentieri* in infected potato and soil samples

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Blackleg and soft rot caused by bacterial species of *Pectobacterium* and *Dickeya*, are among the most significant plant diseases leading to huge economic impact on crop production. Among all the *Pectobacterium* species, *P. parmentieri* (formerly *P. wasabiae*) is one of the most aggressive species causing soft rot disease in potatoes. In this study, a real time loop-mediated isothermal amplification (LAMP) assay was developed for specific and sensitive detection of *P. parmentieri*. LAMP primers targeting the unique *petF1* gene region of *P. parmentieri* were designed using comparative genomic approach and validated in silico using NCBI GenBank database. Specificity of the developed assay was tested against 16 strains of *P. parmentieri* and 95 strains including all other species within the genus *Pectobacterium* in inclusivity and exclusivity panels, respectively. The detection limit of 10 CFU/ml and 100 fg was obtained using serially diluted bacterial cell lysate and purified gDNA, respectively. No adverse effect was observed when host gDNA was added to the reactions containing 10-fold serially diluted gDNA and bacterial cell lysate. The developed assay unambiguously detected *P. parmentieri* with naturally and artificially infected plant and soil samples, and directly from boiled bacterial colonies. No conflicting results were obtained by multiple operators confirming applicability of the assay in routine diagnostics, seed certification programs, biosecurity, and epidemiological studies.

Monitoring of *Botrytis cinerea* resistance to pydiflumetofen in Florida strawberry fields

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Botrytis fruit rot (BFR), caused by *Botrytis cinerea*, is a major fruit rot disease in Florida strawberry fields. The intensive use of fungicides to control BFR has resulted in resistance to several fungicide groups over the years, consequently requiring prolonged monitoring of resistant populations for appropriate management recommendations. Pydiflumetofen (Miravis®) is the newest single-site fungicide within FRAC group 7, and the development of resistance in the fields is unknown. In this study, 261 and 181 *B. cinerea* isolates were collected from commercial fields during 2019–2020 and 2020–2021 strawberry seasons, respectively. Resistance frequencies to pydiflumetofen were evaluated using a conidial germination assay at discriminatory doses of 1, 3, and 5 µg/ml. Isolates were considered resistant based on the percent conidial germination (>50%) and the germ tube length (germ tube twice the length of the conidium). In the 2019–2020 season, resistance frequency to pydiflumetofen was 0.4% (only one isolate). The evaluation of resistance frequency of isolates collected in 2020–2021 is currently in progress. Resistance frequency to cyprodinil, one of the key active ingredients in Switch®, has been high in Florida strawberry fields. Given only a low-level resistant population of *B. cinerea* to pydiflumetofen was detected, Miravis Prime®, a mixture of pydiflumetofen and fludioxonil, might be a better product for managing BFR in Florida, though precautionary measures should be taken to prevent further selection of resistant isolates.

Copper and streptomycin resistance in *Pseudomonas syringae* pv. *papulans* isolated from infected fruit and asymptomatic buds of apple cultivar NY-1

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Pseudomonas syringae pv. *papulans* (*Psp*) is considered a major issue for apple cultivar Mutsu during wet springs, although it can infect other cultivars when close to ‘Mutsu’ trees. Between 2018 and 2020 we isolated *Psp* from fruit and buds of cultivar ‘NY-1’, i.e., SnapDragon, in orchards in New York State without ‘Mutsu’ trees nearby. The *Psp* isolates grew on LB plus 3.5 mM CuSO₄ and 25 µg/mL streptomycin (Sm). A hybrid Nanopore and Illumina assembly of one of the new strain’s genome sequence predicted the presence of Sm and Cu resistance genes in a separate 191-Mbp plasmid. A conjugation assay demonstrated the transferability of Cu and Sm resistance to the sensitive *Psp* strains. Preliminary assays showed that strains acquiring the new plasmid additionally developed

increased resistance to cadmium and H₂O₂, which correlates with the presence of other genes in the conjugative plasmid linked to the resistance against heavy metals and oxidative stress.

Adaptation to the partial resistance of the most prevalent hop genotype in the landscape by *Podosphaera macularis*

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Deployment of host resistance places a strong selection pressure on pathogen populations for virulence. Partial resistance conferred by multiple quantitative trait loci is hypothesized to be more durable than qualitative resistance based on a single, dominant gene. The hop powdery mildew fungus, *Podosphaera macularis*, displays phenotypic plasticity and has developed virulence on cultivars with disease resistance based on both major R-gene and partial resistance. As of 2020, Citra was the most widely planted hop cultivar in Washington, Oregon, and Idaho with acreage of Citra harvested in all three states nearly doubling from 2018 to 2020. Research was initiated to characterize the pathogenic diversity of isolates of *P. macularis* on Citra and determine whether isolates with local adaption to this cultivar have emerged coincident with shifts in planting of Citra. The intended outcome of this research is to inform hop growers of the relative disease risk associated with this cultivar and ultimately to assist with powdery mildew management programs. This poster will summarize research progress towards elucidating the disease severity caused by *P. macularis* isolates originating from Citra and determining if the cultivar is at an increased risk for disease in the Pacific Northwest.

Detection of *Phytophthora capsici* from irrigation ponds in South Georgia

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Phytophthora capsici, the causal agent of Phytophthora blight, is a prominent and economically damaging oomycete pathogen in South Georgia. *P. capsici* causes crown, root, leaf, stem, and fruit infections on a wide range of vegetable crops. Oomycete pathogens such as *P. capsici* are dispersed in water as their zoospores are flagellated and can move through runoff. Irrigation ponds are often reservoirs for different pathogens and reusing the captured runoff is increasing in popularity to decrease irrigation costs. This combination allows for unintended outbreaks of diseases by pumping the contaminated runoff onto susceptible crops. Detection and identification of these pathogens is a crucial step in disease management and rapid detection can ensure timely application of disease control measures. In this study, 42 irrigation ponds in nine counties from South Georgia were surveyed for the presence of *P. capsici* using a novel filtration method in conjunction with a LAMP assay specific for *P. capsici*. Ten ponds in five counties were found to have *P. capsici* as detected from the assay, suggesting that testing of irrigation ponds for *P. capsici* and other pathogens should be conducted to assist in preventing disease outbreaks.

Revealing the NLRome of cultivated hexaploid sweetpotato

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Sweetpotato (*Ipomoea batatas*) is an important staple crop worldwide that can become infected by several pathogens in the field and postharvest. Challenges to develop sweetpotato resistant lines include the presence of few breeding programs, limited knowledge of the genetic basis of resistance, and hexaploid nature of the crop. Plants evolved a complex and multilayered immune system to defend against pathogens. Plant intracellular immune receptors known as nucleotide-binding domain and leucine-rich repeat (NLR) proteins represent a key component of the plant immune system by recognizing pathogen proteins and triggering an immune response. To catalog NLR diversity in historical (Apache) and contemporary (Covington) sweetpotato accessions, we conducted resistance gene enrichment sequencing (RenSeq), a genome reduction approach to capture and sequence full NLR genes. A custom designed NLR bait-library allowed us to enrich NLR genes with 96% target capture rate. Our analysis detected 2,821 contigs associated with NLR motifs for the most commonly grown cultivar in the United States (Covington), while Apache, a 1950s sweetpotato cultivar, exhibited 3,556 contigs associated with NLR motifs. To reveal the NLR diversity in sweetpotato, we employed a curated database of cloned and functionally characterized NLRs to assign sequenced sweetpotato NLRs to functional phylogenetic clades. Our study provides a catalog of NLR genes that will serve as a resource for future breeding initiatives and improve our understanding of evolutionary dynamics of NLRs in sweetpotato.

Evaluating efficacy of intelligent sprayer technology and warning systems for apple diseases

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Apples are the most valuable fruit crop in the United States however, managing the insect pests and diseases that attack apples is costly and imposes environmental and public health risks. Air-assisted (airblast) sprayers are widely used to apply pesticides in apple orchards, but their inaccuracy wastes pesticide and contributes to off-target damage from spray drift. The Intelligent Sprayer (IS) uses Light Detection and Ranging (LiDAR) technology to direct sprays more accurately. In a 2020 field trial in Iowa, using IS technology reduced spray volume by more than 30% with equivalent pest control compared to a standard airblast sprayer that was set to deliver 100 gal/acre. In addition, a disease-warning system that is based on the number of accumulated hours of relative humidity over 90% from the first cover spray was used to manage the fungal disease complex sooty blotch and flyspeck. Four fungicide spray applications were saved using the warning system compared to a calendar-based spray timing during a relatively dry growing season. The ability of IS

technology to combine with the warning system to minimize pesticide load will continue to be evaluated during the 2021 and 2022 growing seasons.

Intra- and interspecific variation in outcomes of competition between *Aspergillus* section *Flavi* species co-infecting maize and groundnuts

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Aspergillus section *Flavi* species co-occur and contaminate crops including maize and groundnuts with aflatoxins. Previous studies demonstrated outcomes of competition between *A. flavus* genotypes vary by crop host, but host influences on competition between different *Aspergillus* species have not been examined. The current study assessed intra- and interspecific variation in outcomes of competition between *A. flavus* (AF), *A. parasiticus* (AP), *A. aflatoxiformans* (AA), and an unnamed taxon known as lethal Aflatoxicosis fungus (LAF) following co-infection on maize and groundnuts. Three isolates from each of the four species were co-inoculated with a representative isolate of a competing species on kernels of maize and groundnuts and incubated (30°C, 7 days). Proportions of species-specific SNPs in DNA from kernel-infecting mycelia and conidia were quantified to assess outcomes of competition during colonization and sporulation, respectively. The extent to which isolate, competing species, and host influenced competition varied. AF isolates were consistently competitive on maize during colonization, while LAF isolates were competitive on groundnuts during sporulation. Responses of AP and AA isolates to co-infecting species and host were variable. Results suggest both host and fungal genotype-specific factors influence competition among species within *Aspergillus* section *Flavi*, thus altering *Aspergillus* community structure and subsequent aflatoxin contamination.

Evaluation of seed treatment fungicides for management of root rot and foliar symptoms of sudden death syndrome and grain yield response of soybean

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Seed treatments for sudden death syndrome (SDS) caused by *Fusarium virguliforme* management are available in the U.S., however side by side comparisons of these seed treatments are lacking. Sixteen field experiments were established in Illinois, Indiana, Iowa, Michigan, Wisconsin, and Ontario Canada in 2019 and 2020 to evaluate seed treatment combinations. Treatments included a nontreated check (NTC), fungicide and insecticide base seed treatments (base), fluopyram, base + fluopyram, base + saponins extract from *Chenopodium quinoa*, base + fluopyram + heat-killed *Burkholderia* spp., base + pydiflumetofen, base + thiabendazole + heat-killed *Burkholderia* spp., and base + thiabendazole + *C. quinoa* extract + heat-killed *Burkholderia* spp. Treatments were tested on SDS-resistant and -susceptible cultivars at each location. Overall, NTC and base showed the highest level of root rot, highest foliar SDS index (FDX), and lowest yield. Fluopyram and pydiflumetofen were the two most effective treatments for managing SDS. Resistant cultivars reduced FDX in both years but root rot was greater on resistant cultivar compared to susceptible cultivar in 2020. Yield response to cultivar was also inconsistent between the two years. In 2020, the susceptible cultivar provided significantly more yield than resistant cultivar. These results reinforce the need to include root rot evaluations in the breeding process and highlights the importance of an integrated SDS management plan since not a single management tactic alone provides adequate control of the disease.

U.S. wheat powdery mildew pathogen population adapting to multiple fungicides

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Blumeria graminis f. sp. *tritici* (Bgt) causes powdery mildew of wheat throughout the eastern U.S. and in many other countries. U.S. epidemics are most frequent and severe along the Atlantic seaboard. Two main classes of fungicides, demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs), are used against this disease. A total of 381 Bgt isolates from five central and eastern U.S. regions were assayed for sensitivity to two DMIs, tebuconazole and prothioconazole, and two QoIs, pyraclostrobin and picoxystrobin. DMI sensitivity was significantly reduced in the eastern regions (Great Lakes, Mid-Atlantic, and Southeast) as compared to centrally located Missouri, Arkansas, and three Plains states. The Y136F mutation in the Cyp51 gene was associated with reduced DMI sensitivity and was more common in eastern states, where Bgt epidemics occur more often. Among these mutant isolates, there was also a weak but significant association in sensitivity levels to the two fungicide classes, especially for pyraclostrobin and either DMI (adj. $R^2 \geq 0.24$, $P < 1 \times 10^{-8}$), suggesting a common mechanism such as overexpression of efflux transporters. QoI sensitivity did not follow a clear regional pattern, and the resistance-conferring mutation G143A was not detected. However, the collection exhibited significant quantitative differences in QoI sensitivity. It appears that subsets of the U.S. Bgt population are adapting to fungicide selection pressure with quantitative sensitivity reductions based on multiple mechanisms, including Cyp51 mutation and possible multi-drug resistance.

Development of a screening method for soybean cultivar resistance to target spot caused by *Corynespora cassiicola*

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Recent outbreaks of target spot, caused by *Corynespora cassiicola*, have caused widespread yield losses in the midsouth. Differences in cultivar reaction to target spot were observed in the field, but greenhouse screening has been hindered by lack of conidial production and insufficient symptom development on inoculated seedlings. To develop an effective screening method, initial experiments used a soybean isolate and the field-rated soybean cultivars CZ4748 (susceptible) and Hutcheson (moderately resistant). Unable to produce conidia, the isolate was grown in V8 liquid media for 3 days, the mycelium was washed, ground in 200 mL of water, and sprayed on 10-14-day old plants. Within 4 days, small red-brown lesions with or without chlorosis appeared on the leaves, stems and petioles, but did not progress. A detached leaf assay was also conducted using mycelial suspension on unifoliate leaves, with and without wounding. Lesions began at 3 days and progressed, forming typical target spot lesions, especially with wounded leaves. Symptoms were more severe in CZ4748 than with Hutcheson. Newly collected soybean isolates produced spores and inoculations with spores resulted in symptoms consistent with those produced by mycelium, but with lower variability and slower progression. Detached leaves of 20 soybean cultivars were inoculated with spores resulting in symptom development in 2 cultivars that were significantly greater and 6 significantly less than in Hutcheson. Using detached, wounded leaves could be an effective method for target spot screening of soybean cultivars.

Field assessment of *Phomopsis longicolla*-inoculated soybean entries in Mississippi with and without a fungicide

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Phomopsis longicolla (PL), the causal agent of Phomopsis seed decay of soybean, results in economic losses by reducing harvested grain quality in the mid-southern U.S. Extended periods of hot, wet weather preceding physiological maturity (R8) oftentimes results in PL infection. Our objective was to evaluate the agronomics and grain damage between 21 soybean entries consisting of breeding lines ($n = 17$) and PL-susceptible checks ($n = 4$) in a controlled, outdoor environment. Field trials were established between 2018 and 2020 under greenhouse cold frames ($n = 2$) covered in plastic to eliminate rainfall. Plots consisted of a single row of each entry, replicated three times separated by a fallow alley. A suspension consisting of PL alpha conidia (1×10^5) was used to inoculate plots at the R5.5 growth stage. Shelter-1 remained fungicide non-treated while Shelter-2 received a 293 ml/ha fungicide application consisting of prothioconazole + trifloxystrobin at beginning maturity (R7). Shelters were overhead irrigated for approximately 200 h between R5.5 and R8. Plots were hand-harvested 2 weeks post-R8 to determine plot weight and grain damage. Damage was reduced by as much as 75%, albeit not significant, by some entries in the non-fungicide plots. On average, DB06X06-093 reduced damage by 27% in non-fungicide treated plots when compared to PL susceptible-checks. Significant differences in plot weight and damage were observed between entries in the fungicide-treated plots. On average, entries reduced visual damage by up to 64% when compared to the PL-susceptible checks.

Effectiveness of seed-applied fungicides for managing soybean seedling diseases in Pennsylvania

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Fungicide seed treatments have become an integral component of current day soybean production. To date, evidence on the response of soybeans to fungicide seed treatments is inconsistent. The objective of this study was to investigate the effectiveness of ApronMaxx (mefenoxam + fludioxonil) fungicide seed treatment on soybean seedling diseases, vigor, and yield. During 2018 and 2019, on-farm, multi-location strip trials were conducted in 11 different environments in Pennsylvania. The experimental design was a randomized complete block at each location. Pre-plant soil samples were used to quantify the density of four soilborne pathogens: *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., and *Phytophthora* spp. Growth and disease incidence were measured using initial plant stand at three weeks after planting, and normalized difference vegetation index, plant height, tap root length, and root weight to shoot weight ratio at the R1 and V4 growth stages during 2018 and 2019, respectively. Plot yield was recorded at maturity. Statistical analysis indicated non-significant treatment effects for all measured parameters. Additionally, no relationships between density of soilborne pathogens and the grain yield was observed. Further tests are needed to examine pathogenicity of recovered isolates and fungicide sensitivity, but our current results help PA farmers to improve management decisions on the necessity of fungicide seed treatments.

Predicting potential distribution of the Armillaria root disease pathogen, *Armillaria solidipes*, within the Intermountain Region of the United States

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The impacts of root disease are expected to escalate under predicted rapid changes in climate and presumed increases in extreme climate events over the next century. Observational evidence suggests damage from Armillaria root disease (caused by the fungal pathogen, *Armillaria solidipes*) is rapidly increasing on climatically maladapted trees within the Intermountain Region of the United States. *A. solidipes* is well documented as a primary pathogen that causes reduced tree growth and increased tree mortality in this region and many neighboring regions in North America. Information on the geographic extent and scale of Armillaria root disease across the landscape will help forest managers to implement management practices to mitigate damage from this disease. We used the MaxEnt algorithm of species distribution modeling to predict where *A. solidipes* is likely to occur during both contemporary and future time periods using georeferenced occurrence locations for DNA sequence-confirmed *A. solidipes*. These locations were coupled with WorldClim v2.1 climatic grids based on CMIP6 downscaled future climate projections under select global circulation models (GCMs) and various Shared Socioeconomic Pathways (SSPs) to run the MaxEnt predictions. With this information, we can determine geographic areas

where 1) suitable climate space (potential distribution) for *A. solidipes* occurs within the region; 2) climatically maladapted trees are likely to occur; and 3) these trends are likely to continue across the landscape in the future.

Clustering analyses of secreted proteins of the sugarcane orange rust pathogen *Puccinia kuehnii*

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Effector proteins secreted by fungi can alter plant functions, which then promotes parasitic growth. Sequence identification and functional annotation of secreted effectors are essential steps to understand the biology and molecular basis of host-pathogen interactions. Like other biotrophic fungi, *Puccinia* species possess large repertoires of secreted proteins, the vast majority of which have unknown functions or no sequence annotations. Using sequence clustering, we investigated the functional diversity of secreted effector protein families of *P. kuehnii*, the causal agent of sugarcane orange rust. We performed a large-scale family analysis with the predicted secretomes of isolates 1040 and 2143 of *P. kuehnii* and the secretomes of five other Pucciniales species (*P. coronata*, *P. graminis*, *P. sorghi*, *P. striiformis*, and *P. tritricina*) using TribeMCL software. A total of 2,236 protein clusters were identified of which 436 were specific to *P. kuehnii*. Our long-term goal is to provide insights into the evolution of secreted protein families from rust fungal genomes.

High-quality genome assemblies of *Phytophthora ramorum* clonal lineages NA1 and EU1 from PacBio sequencing

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Phytophthora ramorum is the causal agent of sudden oak death. Currently, two clonal lineages of the oomycete pathogen, EU1 and NA1, cause epidemics in West Coast forests. The two lineages are thought to differ in aggressiveness on oaks and tanoak as well as in epidemiological behavior. Novel, high-quality genome assemblies provide a necessary resource for understanding the genetic differences among these clonal lineages and accurately tracking the evolution of these lineages in the landscape. Currently, genome assemblies for both NA1 and EU1 are fragmented into hundreds or thousands of scaffolds. Here we report on two high-quality assemblies of EU1 and NA1 individuals sequenced with PacBio long-read technology. To compensate for the high error rate of this technology, we sequenced at high depth, achieving 181× and 344× coverage for the EU1 and NA1 assemblies, respectively. Both assemblies have a total size of 57.5 Mb and are assembled with high contiguity into fewer than 30 scaffolds, resulting in N50 lengths of approximately 3.5 Mb. The EU1 assembly captures 99% of BUSCO genes and NA1 100%, and the genomes are predicted to encode 15,352 and 15,265 protein-coding genes, respectively. These novel, high-quality genomes balance high completeness with a low number of duplications in genes expected to be conserved in single copy. Together, these genomes provide a valuable resource in understanding the genetics, evolution, and adaptation of the NA1 and EU1 clonal lineages in West Coast forests.

Molecular characterization of two new viruses possibly associated with mealybug wilt of pineapple in Hawaii

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Pineapple (*Ananas comosus*) is a pantropical crop whose production is severely affected by a viral disease, mealybug wilt of pineapple (MWP). MWP is associated with the presence of pineapple mealybug wilt-associated virus 2 (PMWaV-2) in Hawaii, Cuba, and Brazil, but not in Australia and China. Recently, new viruses potentially involved with MWP in Hawaii were characterized from MWP-symptomatic pineapple plants and a pineapple germplasm accession by high-throughput sequencing (HTS) analyses. The genomic sequences of a new sadwavirus and a new ampelovirus were determined. The positive single-stranded RNA genome of the new sadwavirus encodes two RNA molecules of 6,128 and 4,161 nucleotides in length. The assembled viral sequence for the novel ampelovirus genome has a total size of 17,854 bp. In preliminary surveys on Oahu-Hawaii, the presence of the two new viruses was confirmed by RT-PCR analyses in MWP-symptomatic pineapple plants. The names pineapple secovirus A (PSV-A) and pineapple mealybug wilt-associated virus 6 (PMWaV-6) are proposed for the new member in the family *Secoviridae* and for the new distinct *Ampelovirus* member, respectively. Results of a preliminary field study suggested that PSV-A and PMWaV-6 may potentially be involved in MWP. These new viruses may be associated with MWP in Australia and China and other countries, where PMWaV-2 has not been closely associated with the disease. Further studies are needed to evaluate the role of the new viruses in MWP.

Rainfastness of fungicides for *Fusarium* head blight and deoxynivalenol reduction in soft red winter wheat

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Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is a disease of wheat and other small grain crops that leads to grain contamination with mycotoxins such as deoxynivalenol (DON). Fungicides are most warranted for FHB control when wet, rainy weather occurs during and shortly after anthesis, as these conditions favor FHB development and DON contamination. However, rainfall during this period may negatively affect fungicide performance against FHB and DON. In this study, we investigated the effects of simulated rainfall of different durations (0, 15, 30, 60, and 120 min) and start times after fungicide application (0, 15, 30, and 60 min) on the efficacy (in terms of percent control relative to the nontreated check, C) of the fungicides Prosaro, Caramba, and Miravis Ace

when applied to wheat spikes at anthesis, with or without the nonionic surfactant Induce. On average, fungicide treatments with Induce reduced FHB index (IND) by 63 to 86% and DON by 53 to 64%. When applied with the surfactant, the performance of the three fungicides was fairly consistent across rainfall treatments. For DON, C ranging from 48 to 68% across rainfall durations and 47 to 66% across rainfall start times. However, efficacy was substantially lower ($C < 40\%$ for both IND and DON) when the fungicides were applied without the surfactant and plots were subjected to 120 min of simulated rainfall, beginning immediately after the treatment was applied. These results suggested that Prosaro, Caramba, and Miravis Ace are very rainfast once applied with Induce.

A multifaceted investigation into the causes of emerging needle diseases in loblolly pine (*Pinus taeda*)

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Many fungi are known to cause foliar diseases in conifers. By causing the death or premature abscission of needles, these diseases impair photosynthesis, resulting in lost growth and sometimes mortality. Observers such as state and industrial pine managers are reporting an increase in the frequency and severity of needle disease outbreaks on native pines throughout the southeastern United States. Traditionally, foliar diseases are not considered a serious threat to loblolly pine (*Pinus taeda*), the principal timber species in the region, and the scale and severity of the outbreaks are both novel and concerning to pine growers. Our research uses an array of emerging technologies and traditional tools to identify the fungal pathogens potentially responsible for the outbreaks in two southern states. Using LAMP, we screened foliage samples for the presence of *Lecanosticta acicola*, the endemic causal agent of brown spot needle blight on longleaf pine (*P. palustris*), confirming its presence among diseased loblolly in Arkansas sites, but not in Florida. Similarly, analysis by ITS2 metabarcoding revealed distinct shifts in the fungal community composition between healthy and diseased needles and increases in the relative abundance of *Lecanosticta*, *Rhizosphaera*, and other known pathogens. Our team is working to isolate the pathogens identified in our molecular analyses and demonstrate their pathogenicity. We also found that recent outbreaks observed throughout the commercial range of loblolly pine differ in the fungi identified and are likely caused by multiple fungal pathogens.

A rapid greenhouse screening method for cereal cyst nematode (CCN) resistance in wheat

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The cereal cyst nematodes (CCN, *Heterodera avenae* and *H. filipjevi*) cause substantial yield loss in wheat. In the Pacific Northwest (PNW) of the U.S., *H. avenae* was first detected in 1974 and *H. filipjevi* in 2008. CCN related annual yield loss has been estimated to be \$78 billion globally and at least \$3.4 million in the PNW. Methods for control of CCN, including fallow and rotation, nematicides, and environment-friendly biological control, are all infeasible while breeding for resistant cultivars is the most efficient and cost-effective approach. However, the breeding progress has been slow due to lack of a fast and convenient screening procedure. In this study, a dependable method for CCN resistance screening was developed, enabling the screening to be conducted under greenhouse conditions, within 6 weeks in limited space, using naturally infested soil collected from the field. With this method, we 1) screened over 1,000 wheat breeding lines and identified more than 200 with useful resistance; 2) discovered that the PNW *H. filipjevi* reacts differently to the CCN differential lines with the existing Ha23 and Ha33 pathotypes, indicating that the PNW *H. filipjevi* is likely to be a new pathotype; and 3) determined that none of the previously identified *Cre* genes confer full resistance to the PNW *H. filipjevi* pathotype, supporting a new pathotype status. These results indicate that CCN resistance should be screened using the PNW *H. filipjevi* pathotype and there are useful levels of genetic resistance in adapted PNW wheat breeding lines.

PCR detection of cacao swollen shoot viruses indicates the prevalence of Cacao swollen shoot Togo B virus in cacao fields in Côte d'Ivoire and Ghana

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Cacao swollen shoot disease (CSSD) in West Africa is caused by a complex of badnaviruses (genus *Badnavirus*, family *Caulimoviridae*). Disease symptoms in cacao trees include foliar discoloration, swellings on branches and roots, pod deformation, reduced pod number and quality, and death in 1–5 years. Four primer pairs, herein referred to as CSSD1 through CSSD4, were designed and used for polymerase chain reaction (PCR) amplification of the CSSD-badnavirus movement protein (MP) domain of cacao swollen shoot CD virus (CSSCDV), cacao swollen shoot Togo B virus (CSSTBV), cacao swollen shoot CE virus (CSSCEV), and cacao swollen shoot Ghana M virus (CSSGMV). Leaf samples were collected from symptomatic ($n = 430$) and asymptomatic ($n = 97$) cocoa trees in producing-areas of Côte d'Ivoire and Ghana during 2017–2020. The CSSD1 primers detected CSSD-badnaviruses in 194 of 310 and 84 of 120 samples from Côte d'Ivoire and Ghana, respectively. Sequence analyses of the MP amplicons indicated that CSSD1 primers detected CSSTBV, CSSCDV, and CSSCEV, three of seven CSSD-associated recognized badnaviruses. Amplicons obtained with the CSSD2, CSSD3, and CSSD4 MP primers yielded sequences that shared their highest nucleotide (nt) identity with CSSTBV, CSSCEV, and CSSGMV, respectively. Of the 450 CSSD-badnavirus MP sequences obtained, 393 were most closely related to CSSTBV, at 81.0–98.0% shared nt identity, and grouped in a single clade on the phylogenetic tree. These results indicate that CSSTBV was the predominant CSSD-badnavirus detected in cacao leaf samples collected in Côte d'Ivoire and Ghana.

Control of root rot of field pea (*Pisum sativum*) caused by *Fusarium avenaceum* from *Fusarium* head blight diseased grain

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Dry pea has become an important component of dryland farming systems in the northern Great Plains. As dry pea production has increased, so has *Fusarium* root rot caused primarily by numerous *Fusarium* spp., including *F. avenaceum* (FAV). At the same time, *Fusarium* head blight of cereals (FHB) has become a growing issue in this region due to the increase in no-till cropping systems that include corn production. The practice of turning the fan speed of a combine to remove FHB diseased kernels (FDK) is a method to lower mycotoxins but it is unknown if this practice may pose a threat to other rotational crops. *Fusarium* spp. causing FHB symptoms collected in 2016 found 45% FAV within the complex. To determine if this is a threat to pea production when applied to a field, a study was performed in 2019 and 2020 at two dryland no-till research locations in Montana to determine the amount of FDK infested with FAV that can cause pea root rot and to show the efficacy of various seed treatments under these conditions. Results indicated that increasing FDK applied to the field increased the severity and incidence of root rot but had no effect on emergence or yield. Seed treatments significantly lowered root rot severity and incidence in three of the four location years and increased yield at one location in 2019. This indicates that blowing diseased grain into a field should not be performed when rotating with pea but some of the negative effects from this can be mitigated, at least in the short-term, by the application of effective seed treatments.

Occurrence of carbendazim resistance in soybean anthracnose pathogen in northern Thailand

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Anthrachnose disease, caused by *Colletotrichum truncatum*, is becoming a serious problem in soybean cultivation in Thailand. Fungicide, especially carbendazim, is commonly applied in soybean disease control, however, the reduction of fungicide sensitivity has been frequently complained by farmers. During 2017–2020, total of 137 isolates of *C. truncatum* were obtained from various fields in northern Thailand and screened the carbendazim resistance using mycelial growth inhibition assay. The EC₅₀ values to carbendazim of almost all isolates were greater than 1,000 µg/ml, and they were considered as highly resistance, while sensitive isolates displayed their EC₅₀ values of less than 1 µg/ml. Moreover, the characterization study of *TUB2* gene of both sensitive and resistant isolates was investigated. A substitution of encoded amino acid in resistant isolates was majorly detected at codon 198 from glutamic acid to alanine (E198A) or minorly detected at codon 200 from phenylalanine to tyrosine (F200Y). This information is informative not only for soybean growers and related organizations to monitor the fungicide resistance situation in the fields but also researchers to fulfill understanding of molecular mechanisms responding to carbendazim resistance in this pathogen.

Host-pathogen-vector dynamics point to complex life cycle of *Exobasidium maculosum*, which causes *Exobasidium* leaf and fruit spot disease of blueberry

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Exobasidium maculosum is a blueberry pathogen that has increased in prevalence in the southeastern United States. The US is the top blueberry producer in the world and Georgia is consistently in the top five producing states. *Exobasidium* fruit and leaf spot can cause significant yield losses, but the complete life cycle of the pathogen is still unknown. Our goal was to investigate whether arthropods play a role in disease transmission and if ploidy variation in the pathogen might be a factor which influences the high level of genetic diversity that is observed. Insect exclusion studies that involved placing covered and uncovered trap plants under diseased bushes and noting differential disease incidence and severity were conducted in Alma and Toccoa, Georgia. Eighty percent of the covered plants had 0–2 leaf spots while uncovered plants had numerous leaf spots. This finding suggests that an arthropod is involved in disease transmission. Ploidy variation was assessed on 15 isolates, equally from three distinct populations, of *E. maculosum* using flow cytometry. Our results show that there is ploidy variation among strains of the pathogen and that aneuploidy exists within its populations. These findings are suggestive of host-pathogen-vector associations that may be more complex than previously thought and that ploidy variation is likely a factor influencing the high genetic diversity observed in the pathogen. Both findings are important in epidemiology of the disease and highlights new factors that should be considered in its management.

Phytoviroomics and datamining added insights on the recent intercontinental detection of an iflavirus and kitavirus in tomato

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Phytoviroomics studies, or analysis of all viral sequences from plant samples, were intensified by a recent increase in accessibility of high-throughput sequencing. This opened possibilities for novel discoveries, and wider understanding of the role of plant viruses across ecological scales. Here, we report the detection of tomato matilda virus (TMaV, *Iflaviridae*) and tomato fruit blotch virus (ToFBV, *Kitaviridae*) from an extensive phytoviroome survey of selected tomato-growing areas in Slovenia (2019–2020). TMaV was discovered in Australia (2015) and recently detected in Italy (2018), while ToFBV was discovered from Australian and Italian tomato samples collected in 2018–2019, and recently in Spain (2020). In our study, TMaV was detected in 66 symptomatic and asymptomatic tomato and 4 weed samples, while ToFBV was detected in 2 symptomatic tomatoes, also infected with TMaV. Re-analysis of sequence read archive (SRA) datasets in NCBI from a survey of tomatoes and *Solanum nigrum* (2017–2018) revealed the presence of the two viruses

in France. Full sequence comparison showed low diversity of TMaV and ToFBV from the countries where they were detected. Bioinformatic scanning of sequences of common organisms revealed presence of *Tetranychus* spp. (spider mite) in the SRAs and in our datasets. Biological assays are needed to study the possible impact of TMaV and ToFBV on tomato health, as well as the possible link with *Tetranychus* spp. Overall, our phytoviroomic survey coupled with datamining of SRAs, provided new insights on the distribution of TMaV and ToFBV in Europe and Australia.

Identification, role in defense responses, and a comprehensive web repository of transcription factors in alfalfa (*Medicago sativa* L.)

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Transcription factors (TFs) are proteins that govern organismal development and response to the environment by regulating gene expression. The information on the specific TFs repertoires within individual plant species is critical for the understanding of their biological roles and evolutionary history across the plant kingdom. For years, only a scattered knowledge on a few individual TFs was available for alfalfa, one of the most widely planted forage legumes in the world. In this study, taking advantage of the most recent developments in the field of alfalfa genomics, we have re-analyzed and significantly expanded the alfalfa TFs repertoire, which is now comprised of 8,465 TFs, distributed among 53 families. TF superfamilies in alfalfa, such as WKRY, bHLH, NAC and others that are implicated in a variety of biological processes including defense responses, were found to be substantially larger than those of a close alfalfa relative, *M. truncatula*. We have also updated the AlfalfaTFDB, (<https://plantpathology.ba.ars.usda.gov/alfalfatfdb.html>), a simple open-access database offering an essential tool for studying the regulation of gene expression in alfalfa, to include new TFs, identified in our lab since 2014. This newly gained information on alfalfa TFs presents unique opportunities to decipher molecular mechanisms involved in defense responses to pathogens, the crop adaptation to the environment, evolution, plant productivity and biomass increase.

A landscape analysis of seed system stakeholders in Sub-Saharan Africa

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Availability of high quality seed is a key factor for crop productivity, especially for roots, tubers and bananas (RTB) which are vegetatively propagated. Pathogens and pests can accumulate in planting material, reducing yield and market value. CGIAR Toolbox for working with RTB seed systems was created in response to seed degeneration and other seed system bottlenecks. It houses 11 tools to diagnose, evaluate and improve seed systems worldwide, designed for stakeholders including donors, development and humanitarian organizations working to increase food and seed security outcomes. To better target the toolbox, we evaluated the landscape of seed system stakeholders in terms of geographical footprint and networks of collaboration, and identified key areas of intervention for over 60 stakeholders in Sub-Saharan Africa. Based on publicly available information, stakeholders are most active in Eastern Africa, followed by several countries in Southern Africa, and least active in Central Africa. Stakeholder networks are generally complex, forming clusters of engagement throughout the landscape. In terms of the seed value chain, most development organizations prioritize policy and capacity building of the seed sector, as well as seed quality and health. Humanitarian organizations focus more on delivery and access, although some are increasingly shifting from seed aid to longer-term seed security goals. This study is designed to better target stakeholders and help scale seed system improvements where access to high quality seed is in high demand but in chronically short supply.

Exploration of the interactions between *Xanthomonas* spp. and phyllosphere residents

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Plant-pathogen interactions are influenced by several biotic and abiotic factors, among which microbiome component has gained a particular attention in the recent years due to advances in sequencing technologies. The goal of this study is to dissect interactions of the pathogen with the host microbiome and assess their influence on overall host susceptibility. The model pathosystem utilized is a foliar pathogen, *Xanthomonas*, of the host tomato. In this study, the phyllosphere of tomato plants was surveyed through culture-dependent competition assays of isolated bacterial residents from the phyllosphere against *X. perforans*. A library of 128 phyllosphere residents, composed of approximately 14 genera, was constructed from tomato fields and greenhouses and identified through 16S sequencing. Prior research from our lab indicates contribution of type VI secretion system (T6SS) towards overall disease dynamics by *X. perforans* on tomato. T6SS has been explored for its role in mediating prokaryotic interactions and interbacterial competition in other pathosystems. Here we test the hypothesis that T6SS of *X. perforans* is important for creating its niche in the phyllosphere and its ability overcome competition with resident microflora. In vitro competition assays were conducted to explore interaction of WT *X. perforans* and T6SS mutant. A total of 17 phyllosphere isolates were observed to be influenced by functional T6SS. This study sheds light on how pathogens adapt to/modify the phyllosphere microbiome during host colonization.

Recovery of potato blackleg pathogens and impact of temperature on interactions between *Pectobacterium parmentieri* and *Dickeya dianthicola*

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The aggressiveness of soft rot and blackleg (SRB) causing bacteria in potatoes is elevated by co-infection of *Dickeya* and *Pectobacterium* species, especially *D. dianthicola* and *P. parmentieri*, but conducive environmental conditions for these two genera differ and the effect of the environment on their interaction is not well understood. We sought to identify SRB present in positive field samples and understand the effects of temperature and relative humidity on co-infection of *D. dianthicola* and *P. parmentieri*. We isolated pure cultures of SRB bacteria from a subset of previously collected and stored positive field samples collected in WI. Morphological and sequence-based identification indicated that *Pectobacterium* spp. are more easily recovered from previously frozen samples than *D. dianthicola*. Tuber maceration and cut stem assays were conducted to test the aggressiveness of five *D. dianthicola* and three *P. parmentieri* strains that had been previously characterized under various growth temperatures. Initial tuber maceration assays showed that tissue loss increases as the temperature increases, regardless of strain or strain combination. Further, co-infection had a more significant effect on *D. dianthicola* than *P. parmentieri*, except for one strain of *D. dianthicola* that showed reduced aggressiveness when co-infected with *P. parmentieri*. These results identify the SRB isolates found in SRB positive samples from Wisconsin and aid in further investigation on the impact of environmental factors on the interaction between *D. dianthicola* and *P. parmentieri*.

The diversity and virulence of oomycetes associated with cotton seedlings in Alabama and options for biocontrol

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Many oomycete species infect seedlings of crops, including cotton (*Gossypium hirsutum*). This study aims to 1) survey the diversity of oomycetes associated with cotton seedlings in Alabama, 2) evaluate correlations with soil edaphic factors, 3) test virulence toward cotton seeds, and 4) explore oomycete biocontrol using spore-forming *Bacillus* species. In 2021, 30 symptomatic cotton seedlings were collected from 13 fields each (390 total), representing four major Alabama soils. Oomycetes were isolated from roots of two- to four-week-old cotton by plating onto a semi-selective agar medium. Internal transcribed spacer region sequencing identified the resulting hyphal tipped isolates. A seed virulence assay was conducted to verify the pathogenic species. 57 *Bacillus* strains across 16 species were screened in vitro for biocontrol against *Pythium ultimum*. Preliminary results indicate that 303 oomycete isolates were obtained, with Northern Alabama having the highest incidence, richest diversity, and soils with the greatest cation exchange capacity. *Phytophthora nicotianae* was present throughout the state but most abundant in Northern Alabama and virulent toward cotton seeds. Lastly, 20 *Bacillus* strains reduced *Pythium ultimum* disease severity by at least 75%. Future work will include sequencing the cotton spermosphere microbiome with and without a biocontrol agent and continued assessment of oomycete diversity in Alabama. This knowledge will facilitate management strategies for cotton seedling diseases caused by oomycetes in Alabama and the US.

Grapevine red blotch virus is absent in registered nurseries in Washington State

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Grapevine leafroll-associated virus 3 (GLRaV-3) and Grapevine red blotch virus (GRBV) are considered the most damaging to vineyards in Washington State. Since registered nurseries are the main source for grapevine planting stock, a project was initiated to test grapevines for GLRaV-3 and GRBV to ensure virus-tested vines are maintained in nurseries. A composite sampling method in combination with simple and affordable sample preparation protocol using inexpensive reagents were used for processing leaf samples during the crop season and cane samples during the winter. Sample extracts were tested in RT-qPCR and PCR assays using SYBR Green-based dye chemistry for the presence, respectively, of GLRaV-3 and GRBV in leaf and cane samples. When a composite sample tested positive for GLRaV-3 and/or GRBV, leaf or cane samples from individual vines corresponding to each virus-positive composite sample were collected and tested to identify virus-positive vine(s). Appropriate controls for each virus were included for reliable interpretation of test results. During the past six years (2015–2020), a total of 14,048 composite and 3,313 single vine samples from nearly 80 wine grape (*Vitis vinifera*) cultivars were tested for GLRaV-3 and GRBV. The results indicated that 6.7% (951/14,048) of composite and 28.6% (949/3,313) of single vine samples from registered nurseries tested positive for GLRaV-3. In contrast, none of the samples tested positive for GRBV. Overall, the results indicate the absence of GRBV and low incidence of GLRaV-3 in grapevines maintained in registered nurseries.

Minor but important: Susceptibility genes targeted by minor effect virulence effector Tal7b

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Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is the most globally devastating bacterial disease of rice. The optimal management method for BB is to deploy disease-resistant cultivars. The challenges, however, are Xoo's ability to overcome deployed resistance. Transcription activator-like (TAL) effectors are not only crucial to Xoo's virulence but are essential to its fitness. A

minor effect virulence effector, Tal7b, significantly contributes to disease severity. Unique QTL has been identified to Tal7b by screening a Multi-Parent Advanced Generation Inter-Cross (MAGIC) population. We hypothesize that Tal7b targets are contained within these QTL and that polymorphisms in the predicted TAL effector binding elements (EBEs) of putative target genes can be exploited to achieve durable disease resistance. To test this hypothesis, we inoculated seven MAGIC lines with Xoo PXO99A carrying the *tal7b* gene or the empty vector and conducted RNA-seq analysis. We identified 210 genes differentially expressed between the two treatments. Of these, nine that were putative susceptibility genes, some with antibacterial potential, were contained within one of the previously identified 13-BB resistance QTL. Altogether, our data suggest that the TAL7b targets disease susceptibility genes contributing to Xoo fitness in rice. The new EBE and susceptibility genes identified may harbor polymorphisms that disable the susceptibility gene's activation and contribute to resistance. Future research will focus on elucidating the function of novel susceptibility gene candidates.

Identification of the causal agents of tip rot in carrot

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Carrot is the main field vegetable in Norway and stored in 0–1°C for up to 8 months. In long-term storage, a large amount of carrots is lost and wasted due to postharvest disorders. Tip rot, which is a light brown to dark necrosis starting from the tip of the tap root and a dry rot sometimes wet rot progressing upward, is a major problem in stored carrots and contribute to the high level of carrot rejection during sorting and packaging. The causal agents of tip rot are not known. The objectives were to identify the causal agent of tip rot, determine their pathogenicity and latent period. Carrot with tip rot symptoms were collected at harvest from four fields, and from 8 to 10 storages 5–7 months after harvest over 2 years, and candidate pathogens were identified by both morphological and molecular methods. The pathogenicity of the candidate pathogens was tested on four carrot cultivars. In addition, the latent period was determined by incubating carrots inoculated with the candidate pathogens at 0°C, 3°C, and 6°C. The predominant pathogens were *Mycocentrospora acerina*, *Cylindrocaryon* spp., and *Fusarium* spp. Tip rot severity significantly varied among cultivars and storage temperatures. The latent period significantly varied by storage temperature and pathogen. The latent period for *M. acerina* varied from 6 days at 6°C to 12 days at 0°C. Overall results indicate that tip rot of stored carrots is caused by several fungi, and their interaction with bacteria and nematodes. The identification of the causal agent(s) of tip rot is crucial to implement appropriate control measures.

Optimizing media and temperature conditions for culturing of the dry berry fungal pathogen of *Rubus* spp.

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Dry berry is a caneberry disease first described in the late 1950s that is re-emerging as a disease affecting raspberry production in the Pacific Northwest of the United States and British Columbia, Canada. While first classified as *Rhizoctonia rubi* based upon growth morphology, using molecular genetic tools we found that the fungus groups tightly within *Monilinia* spp. Little research has been conducted on dry berry disease since it was first described in 1959, leaving numerous aspects of the pathogen's foundational growth ecology and epidemiology unknown. Our initial focus has been on identifying optimal growth media, both in solidified media and broth culture, as well as characterizing pathogen growth across temperatures spanning 10 to 30°C. These studies indicated that the dry berry pathogen grows most profusely, in terms of growth rate and colony mass, on fruit juice-based growth media including a blackberry juice medium (BA) and a tart cherry juice medium. When cultured on both 1× potato dextrose agar (PDA) and BA, fungal colonies expand most rapidly between the temperatures of 20 and 25°C. The presence of a daily 12-h photoperiod did not appear to affect growth rate or morphology. Future studies will aim to characterize the conditions necessary to induce pathogen sporulation and to establish preliminary baseline fungicide sensitivity levels for the most widely deployed chemistries within raspberry production, including those of FRAC group 3.

How abiotic stress plays ally to pathogenic attack in trees

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Prolonged drought and elevated temperatures associated with climate change (CC) affect tree growth and productivity and may predispose them to greater disease susceptibility. However, the physiological mechanisms by which trees become prone to pathogenic attack due to CC are poorly characterized. We designed an experiment with 4-year-old Austrian pine trees to test the effects of combined drought and heat stress (CC treatment) on susceptibility to infections by *Diplodia* spp. of contrasting aggressiveness. We found that trees were more susceptible to both pathogens under CC, irrespective of baseline aggressiveness. Induced susceptibility was associated with reduced levels of phenolic compounds 72 h after inoculation. Host gene expression analysis indicated a decrease in photosynthetic activity, plant growth hormones and pathogenesis-related (PR) proteins, while pathways associated with abscisic and jasmonic acids, sucrose and starch metabolism, and programmed cell death were all upregulated. Meanwhile, pathogen gene expression analysis showed possible enhanced ability to (1) respond to iron starvation and toxic environments; (2) utilize diverse carbon and nitrogen sources; and (3) neutralize the host's pathogen-detection machinery. Our results significantly advance our understanding of the physiological basis of increased pathogen aggressiveness towards trees subjected to climate change conditions.

Effect of deep application of non-fumigant nematicides on root-knot nematode in a tomato plasticulture system

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Meloidogyne incognita is detrimental to vegetable crops due to the damage it causes. To manage this nematode, placement of a single or double drip tape at a depth of 1–3 in. below the plastic mulch to aid in chemigation is a common practice in vegetable plasticulture systems. However, nematicide application at this depth might offer little or no control of *M. incognita* populations existing deep in the soil that may move upward and infect host crops later in the season. A split-plot RCBD field trial was conducted in the spring of 2019 and 2020 to evaluate the best combination of drip tapes (surface and sub-surface drip tapes [SSDT] vs. only surface drip tape [SDT]) and nematicides (fluensulfone, fluazaindolizine, and fluopyram) on *M. incognita* in tomato. Surface and sub-surface drip tapes were placed at a depth of 2 and 12 in. below the soil surface, respectively. In 2019, the three nematicides reduced ($P < 0.05$) both root gall and *M. incognita* populations compared with 2020 where no effect was observed. The placement depth of drip tape (SSDT vs. SDT) had no effect on root gall and the nematode population density in both years. Tomato yield was not impacted by the drip tape placement and nematicides. This study shows that the drip tape utilized for nematicide application plays little role in the control of *M. incognita* in a single crop system. However, this technique might be effective in double or multiple cropping operations and requires further investigation.

Solutions for *Phytophthora*-infested landscape beds in the southeastern United States: Identifying resistant annuals and herbaceous perennials

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Many annual and herbaceous perennial ornamental bedding plants grown in the southeastern United States are susceptible to *Phytophthora* root and crown rot. This disease is caused by several species of *Phytophthora*, an oomycete that thrives in moist soil and can persist for many years once introduced to a landscape. Because practical management options for this disease are limited, knowledge of annual and herbaceous perennial plant cultivars that exhibit resistance or tolerance to this disease is desirable. In 2019 and 2020, one to three cultivars of 17 annuals and 21 herbaceous perennials were evaluated for their susceptibility to *Phytophthora* root or crown rot in three landscape beds distributed between western and central North Carolina. Landscape beds were artificially infested with *Phytophthora nicotianae*, *P. tropicalis*, *P. drechsleri*, and *P. cryptogea*. Plants were visually rated every 2 weeks for disease incidence and severity, and symptomatic plants were evaluated to identify the cause of disease. *P. nicotianae* was the only species confirmed to be active in all landscape beds in both years of the study. Disease incidence and severity varied by location and by year, and variation in the susceptibility of cultivars belonging to the same species was observed. Thirteen cultivars of annuals and eleven cultivars of herbaceous perennials displayed resistance to *Phytophthora* root and crown rot caused by *P. nicotianae* and will be recommended to growers and homeowners in the southeastern United States.

Optimizing cover cropping and tillage for suppression of soilborne pathogens in a bare-ground vegetable agrosystem

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Variation in agrosystems can lead to inconsistent disease suppression by cover cropping. Host status, biomass production and tillage practices influence disease suppression. Therefore, we conducted a crop rotation study to compare the impact of season and tillage practices on cover crop productivity and suppression of plant-parasitic nematodes and fungal soilborne pathogens in the southeastern United States. Treatments were laid out in 2019 and 2020 in a 2×4 factorial split-plot design in randomized complete blocks. Whole-plot treatments consisted of deep (45 cm) and shallow (15 cm) tillage. The first factor of the factorial subplots had two rotation treatments of either cover cropping or fallowing, while the second factor consisted of four season-treatments (spring and fall = sunn hemp [*Crotalaria juncea*]; winter = rye [*Secale cereale*] or vegetables for all seasons). Tomato cv. BHN 602, squash cv. Spineless Beauty, and cabbage cv. Bravo were grown in spring, fall and winter, respectively. Sunn hemp in the spring and fall suppressed ($P < 0.01$) root-knot nematode, *Meloidogyne incognita*, abundance while winter rye had no effect. Only sunn hemp grown in the fall with a higher biomass, suppressed *M. incognita* beyond fallowing. Deep tillage had no effect on *M. incognita*, but suppressed incidence of damping-off of squash caused by *Rhizoctonia solani* and white mold of cabbage caused by *Sclerotinia sclerotiorum* in the fall and winter, respectively. Therefore, optimization of season and tillage practices depends on the targeted pathogen.

Differential epigenetic modifications among *Xylella fastidiosa* strains correlate with type I restriction-modification system variation

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Xylella fastidiosa is a bacterial plant pathogen with genetically distinct subspecies which collectively cause disease in diverse host plants. Genomes of *X. fastidiosa* strains are enriched in genes encoding restriction-modification systems, which methylate native DNA and cut unmethylated DNA at specific sequence motifs. Restriction-modification systems can provide defense against phages and limit horizontal gene transfer between closely related strains, but can also influence expression of virulence-related genes in some animal-pathogenic bacteria. Type I restriction-modification systems function as a protein complex, with sequence specificity determined by the two target recognition domains of a specificity subunit, HsdS. Analysis of 117 *X. fastidiosa* strains reveals most strains contain three conserved type I restriction-modification systems, while strains of subspecies *multiplex* and *pauca* contain a fourth system. Up to 22 unique *hsdS* alleles were identified per system; in total, at least 30 aggregate *hsdS* allele profiles were identified across *X. fastidiosa* strains, suggesting differential genomic methylation patterns exist across strains. Genomic methylation profiles of 12 *X. fastidiosa* strains were determined and contain two to four methylated motifs characteristic of type I restriction-modification recognition sites. Variation among these motif sequences was identified both within and between *X. fastidiosa* subspecies and is consistent with variation among *hsdS* alleles.

Fungicide resistance is prevalent in postharvest fungal pathogens and impacted by temporal-spatial and host-pathogen associations across the globe

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Fungicides are the primary tools to control a wide range of postharvest fungal pathogens. Fungicide resistance is a widespread problem that has reduced fungicide efficacy. Resistance to FRAC 1 chemistries are associated with mutations in amino acid position 198 in the β -tubulin gene. Hence, we conducted a meta-analysis of β -tubulin sequences to infer temporal, spatial, host and genus level patterns of fungicide resistance in postharvest pathogens. In total, data was acquired from 2,647 specimens from 12 genera of fungal phytopathogens residing in 53 countries, on over 200 hosts collected between 1926 and 2020. The specimens containing a position 198 mutation were globally distributed and highly conserved in a variety of pathosystems. Analyses showed that there is an association between the mutation with the year an isolate was collected, the pathogen genus, the pathogen host and the collection region. The resistance phenotype has been in a decline since its peak between 2005 and 2009. FRAC 1 fungicide usage data followed a similar pattern in that application has been in a decline since its peak between 1997 and 2003. The data shows that, with the reduction of selection pressure, FRAC 1 fungicide resistance in fungal populations will decline within 5–10 years. Based on this line of evidence, we contend that a β -tubulin position 198 mutation has uncharacterized fitness cost(s) on fungi in nature. The dataset can inform end users on the regions and hosts that are most prone to contain resistant pathogens and assist decisions concerning fungicide resistant management strategies.

Encrypted genome of endohyphal bacterium: Unique genetic islands and prophages of *Enterobacter* sp. associated with fungal pathogen *Rhizoctonia solani*

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Endobacteria living in fungal hyphae have been identified in diverse classes of fungal hosts, ranging from plant pathogens to beneficial mycorrhizae. They have profound impact on the biology of associated fungi and plant hosts, as well as the ecology of inhabited environment. The genome sizes and features of these endobacteria vary drastically depending on their host fungi and living niches, while the key genes corresponding to the bacterial–fungal interaction still need further investigation. Previously in our lab, an *Enterobacter* sp. strain, named En-Cren, was isolated from the hyphae of the soil pathogen fungus *Rhizoctonia solani* AG2-2IIIB, the causal agent of turfgrass brown patch disease. This endobacterium does not have a reduced genome size, with the plausible reason that it can be released from damaged hyphae as a free-living bacterium and potentially live as a saprophyte. The annotated genome of En-Cren revealed several unique genetic islands and prophage regions. Annotated genes in these regions encode type VI secretion system related genes, transcription regulators, transporter proteins, shock proteins, and toxins. Considering its special living environment, these proteins may be related adaptation for new metabolism and signaling pathways. En-Cren can also migrate along the fungal mycelia. Genes affecting the hyphal movement include flagellum synthesis genes and stress response regulator genes. Further study of these genetic features may allow us to better understand the nature of the interaction between this endobacteria and the pathogenic fungal host.

Shift in *Pyrenopeziza tritici-repentis* sensitivity to picoxystrobin in South Dakota

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Picoxystrobin, a quinone outside inhibitor (QoI) fungicide, is extensively used in the management of fungal leaf spot diseases. QoI fungicides targets the quinone outside (Qo) binding site in the cytochrome *b* gene that inhibits fungal mitochondrial respiration. The QoI-insensitive fungal isolates are known to have three mutations, such as G143A, F129L, G137R. Tan spot caused by the fungus *P. tritici-repentis* is a significant disease in wheat in South Dakota. Many QoIs including pyraclostrobin, azoxystrobin and picoxystrobin have been used for tan spot management for several years in SD which warrants the monitoring of *Ptr* sensitivity to QoI fungicides. In this study, 162 *P. tritici-repentis* isolates collected from wheat fields in South Dakota were evaluated for their sensitivity to Picoxystrobin. Of 162 isolates, 26 isolates (16%) exhibited insensitivity based on G143A mutation and EC₅₀ values using spore germination assay. Our results indicate reduced sensitivity to insensitivity to picoxystrobin in some isolates suggesting regular monitoring of the *P. tritici-repentis* population to QoI fungicides is essential to track the evolution of insensitive population. The fungicide picoxystrobin seems an effective fungicide when used with another fungicide with different chemistry to manage tan spot of wheat. Our results will provide stakeholders with information to consider chemical applications integrating fungicides belonging to different modes of action to manage wheat tan spot.

A GPI-anchored protein gene, *CpGpi-Api1*, from the chestnut blight fungus *Cryphonectria parasitica* is a viral-specific antioxidant virulence factor

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The chestnut blight fungus, *Cryphonectria parasitica*, and its interaction with hypovirus, *Cryphonectria hypovirus* 1 (CHV1), is a model to study the fungus-virus infection. The gene (*CpGpi-Api1*) encoding a glycosylphosphatidylinositol (GPI)-anchored protein (GPI-AP), which has been differentially expressed by sectorization and CHV1 infection, is characterized in this study. Sequence analysis of deduced amino acid of *CpGpi-Api1* revealed the high similarity and phylogenetic clustering to the known fungal GPI-APs with canonical N-terminal leader peptide and C-terminal GPI-anchoring signal. The *CpGpi-Api1*-null mutant showed no phenotypic changes from the wild type in growth rate, sporulation, and pigmentation. However, the *CpGpi-Api1*-null mutant showed hypersensitivity to ROS and

synthetic peptides of the *CpGpi-Ap1* exhibited the potent antioxidant activity. Interestingly, virulence of the *CpGpi-Ap1*-null mutant was significantly reduced. In addition, when the CHV1 was transferred to the *CpGpi-Ap1*-null mutant, severely retarded colonial growth was observed. These results indicate that the *CpGpi-Ap1* gene plays an important role in fungal virulence by acting against the host plant defense such as ROS. These also suggest that CHV1 infection specifically downregulates the *CpGpi-Ap1* gene to result in fungal hypovirulence, while the presence of the *CpGpi-Ap1* gene is required for the physiological tolerance of the host fungus against hypoviral infection. This is first report on the biological function of a fungal GPI-AP as a virulence factor.

Vector ecology of Grapevine red blotch virus in southeastern vineyard

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Grapevine red blotch virus (GRBV), which is the causative agent of red blotch disease, is a member of the family *Geminiviridae*. Horizontal transfer of GRBV has been confirmed in vineyards, with the three-cornered alfalfa hopper (*Spissistilus festinus*) as the major vector. *S. festinus* is unable to complete its lifecycle on grapevine. In western U.S. vineyards, it is hypothesized that as cover crops in vineyards are removed closer to harvest, *S. festinus* will move into the canopy when vegetation is limited. In the Southeast, *S. festinus* is a common insect of the area but is not a pest of grapevine. In 2019, an estate vineyard in northern Georgia was surveyed for GRBV. Findings suggest potential spread between an infected vineyard block and a free-living grapevine across the street adjacent to the vineyard property. Results suggest that the infected block and wild grapevine were genetically similar to each other, but different from other GRBV isolates found at another site on the property. Preliminary insect surveys have found *S. festinus* in ground cover between vineyards rows. Our current efforts are to determine if spread of GRBV is occurring in the vineyard and if *S. festinus* is the vector responsible in this ecosystem.

Transcriptomic analysis of NAD⁺ induced resistance in squash against powdery mildew caused by *Podosphaera xanthii*

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Beta-nicotinamide adenine dinucleotide (NAD⁺) is a ubiquitous electron carrier involved in many metabolic pathways of the plants. Application of NAD⁺ can induce systemic acquired resistance (SAR)-like defense responses in *Arabidopsis*. In this study, we investigated the plant responses in squash powdery mildew caused by *Podosphaera xanthii* after treatment with NAD⁺ using Illumina RNA-Seq technology. Disease resistance related differentially expressed genes (DEGs) were further analyzed by qRT-PCR. Squash samples were collected at 0, 4, 24, and 48 h after NAD⁺ treatment and pathogen treatment. Total RNA was extracted from each sample. Data analysis revealed that more than 5,000 genes were significantly regulated among the 25,000 mapped genes. These results indicate that exogenous NAD⁺ induces profound transcriptomic changes in squash. Furthermore, 67 lectin receptor kinase (*LecRK*) genes, which are homologous to the *Arabidopsis* extracellular NAD⁺ (eNAD⁺) receptor gene *LecRK-I.8*, were found in squash. Meanwhile, the homology relationship among these *LecRK* genes and *Arabidopsis LecRK* genes were presented. These results suggest that squash may also use *LecRKs* to sense eNAD⁺. The Gene Ontology function enrichment and Kyoto Encyclopedia of Genes and Genomes enrichment analysis indicates a few pathways that related with resistance and signaling. This study could serve as the basis for further in-depth research on the protein function and identification of the eNAD⁺ receptors in squash.

Assessing the sensitivity of *Pythium* spp. causing Pythium wilt of lettuce to commercial fungicides

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Pythium wilt of lettuce, caused by the oomycete *Pythium uncinulatum*, has emerged as a major soilborne disease of lettuce in the Salinas Valley of California, with some growers reporting up to 100% crop loss in 2020. In most cases, chemical control is being sought as the sole strategy to control Pythium wilt, and metalaxyl (mefenoxam) is used as the primary fungicide due to the limited number of commercial products registered for use on leafy greens. Although metalaxyl-insensitive species of *Pythium* have been reported in many crops across the US, this information is lacking for *Pythium* spp. associated with lettuce. *Pythium* spp. isolates ($n = 120$) were obtained from infected lettuce roots in 2020, and are currently being tested for metalaxyl-sensitivity at various concentrations (0, 1, 10, and 100 µg/mL) in in vitro assays by measuring mycelial radial growth overtime. Results obtained so far demonstrated that metalaxyl significantly reduced the growth of the majority of isolates (88.6%), although a small number (11.4%) were insensitive to the highest concentrations, and the observed sensitivity by individual isolates was significantly reduced overtime. Future work will include testing the efficacy of seed and soil drench applications of metalaxyl to protect lettuce plants following inoculation with either sensitive or insensitive *Pythium* isolates. Overall, this research provides novel information and contributes to improve management of Pythium wilt.

Comparative susceptibility of ten mungbean cultivars to Septoria brown spot and frogeye leaf spot in the greenhouse

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Consumer preference for plant-based protein is driving an increase in mungbean (*Vigna radiata*) production. Nearly all of the mungbean consumed in the U.S. are imported. Mungbean's ability to tolerate drought and high temperature gives U.S. farmers a greater option in their crop rotations. Mungbean is a short season crop of 60 to 90 days and fits well with our agronomic infrastructure of equipment, chemicals, inputs, and storage. However, diseases can be a major constraint for production. As U.S. farmers adapt to new markets and sustainable food production, plant breeding programs must screen for disease. To aid in the development of varieties for the Midwest, two common soybean pathogens, *Cercospora sojina* (frogeye leaf spot) and *Septoria glycines* (brown spot) were compared on

10 mungbean cultivars. Greenhouse experiments were conducted twice. Five potted plants of each cultivar were randomly placed in three flats containing 18 pots each. Suspensions (10^6 conidia/ml) from 3-week-old cultures were sprayed on the first trifoliate stage (V1) until drip, (~1 ml per plant). A clear dome was secured over each flat for one week after inoculation and misted twice daily. Presence of leafspots was observed 3 weeks after inoculation, examined for presence of conidia, and re-isolations were made. All 10 varieties showed symptoms of both diseases; a wide range of disease susceptibility was observed across cultivars. This study is the beginning of the development of pre-emptive management efforts that address anticipated problems in mungbean production in the Midwest.

Sensitive detection of nematode-transmitted viruses in vector nematodes and small fruit crops in the Pacific Northwest (USA)

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The Pacific Northwest (PNW) maintains a \$840 million small fruit industry susceptible to nematode-transmitted nepoviruses. Washington has documented nepoviruses on small fruit crops, including *Tobacco ringspot virus* (TRSV) on blueberry and grape and *Tomato ringspot virus* (ToRSV) on blueberry and raspberry. ToRSV has been detected on wine grapes in Oregon. The known dagger nematode vectors of these viruses, *Xiphinema americanum* sensu stricto, *X. californicum*, and *X. rivesi*, have been reported widely in the PNW, increasing the risk of virus transmission among and between plant hosts. Conventional methods of virus detection (ELISA and PCR) are limited in sensitivity and unable to detect low virus titers found in nematodes. The objective of this research is to develop quantitative (qPCR) and digital droplet PCR (ddPCR) assays to detect naturally occurring titers of plant virus in individual nematodes. In fall 2020, plant and soil samples were collected from five and eight nepovirus-positive field sites, respectively. Thirteen nepovirus-positive plant samples were obtained from greenhouse collections. Additional material will be collected in spring 2021. RNA and dagger nematodes were extracted from plant and soil samples, respectively, for use in assay validation and optimization. Specificity and sensitivity of ToRSV and TRSV qPCR and ddPCR assays will be compared and discussed. Virus detection in nematodes will allow for virus transmission and virus-vector relationships to be studied, ultimately informing risk and management practices for PNW small fruit growers.

Detection of *Phytophthora vexans* in different sources using loop-mediated isothermal amplification

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Phytophthora vexans is an important water borne and soil inhabiting oomycete pathogen causing root and crown rot of plants including certain woody ornamentals, fruits, and forest trees. Early and accurate detection of *Phytophthora* in the nursery production system is critical, as this pathogen is quickly transported to neighboring healthy plants through the irrigation water system. Detection of this pathogen in the irrigation water and infected plant material using conventional bait-based methods or serological assays is difficult and tedious. It is most likely due to the presence of other oomycetes in the stagnant water or plant tissue. Hence, a specific, sensitive, and rapid molecular diagnostic method is required to overcome the limitations of traditional identification. In this study, a novel loop-mediated isothermal amplification (LAMP) assay was developed for the amplification and identification of *P. vexans*. Several sets of LAMP primers were designed, but LSU2 was found to be specific to *P. vexans* as it did not amplify other closely related species. Also, the developed assay was sensitive enough to amplify pathogen DNA up to 300 fg/μl. The reaction was optimized at 71°C for 60 min in a 25-μl reaction mixture. The reaction results were observed by real-time LAMP, gel electrophoresis, warm-start colorimetric dye, and SYBR™ Green I DNA staining for visual observation of amplified products. In summary, LAMP assay for detection of *P. vexans* was developed which could aid in a rapid detection of inoculum from potential sources like irrigation water and infected plants.

Identification of two novel pathovars of *Pantoea stewartii* subsp. *indologenes* affecting *Allium* sp. and millets

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Pantoea stewartii subsp. *indologenes* (*Psi*) is a causative agent of leafspot of foxtail millet and pearl millet; however, novel strains have been identified that can cause center-rot like symptoms in onion. We performed genotypic and phenotypic characterization of 17 *Psi* strains isolated from various sources including onion. Based on the host range evaluation, we propose two pathovars *Psi* pv. *cepacicola* pv. nov. and *Psi* pv. *setariae* pv. nov. *Psi* pv. *cepacicola* causes symptoms on *Allium* species (leek, onion, chive, and Japanese bunching onion) along with the members of *Poaceae* (foxtail millet, pearl millet, and oat). However, *Psi* pv. *setariae* can only infect foxtail millet, pearl millet and oat. We also propose that the type strain of *Psi* (LMG 2632T) should be designated as a pathotype strain of *Psi* pv. *setariae* and the strain 'PNA 14-12' as the pathotype strain of *Psi* pv. *cepacicola*. The two pathovars are genotypically closely related and cannot be differentiated by digital DNA-DNA hybridization and average nucleotide identity. Although the newly proposed pathovars are not clearly distinguishable by their fatty acid and methyl esterase profiles, and substrate utilization patterns, a fatty acid (unknown 10.9525) and few metabolites (3-methyl glucose, Na butyrate, and fusidic acid) can be potentially used to distinguish them. We also report the distribution of previously known pathogenicity (HiVir, *hrcC*) and virulence (*alt*) factors of *Pantoea* sp. in the new pathovars. The impact of these new pathovars in the center rot pathosystem of onion is yet to be determined.

Blackleg-resistant *Solanum chacoense* lines inhibit bacterial quorum sensing and plant cell wall degrading enzymes

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Some lines of the wild diploid potato *Solanum chacoense* are resistant or tolerant to blackleg and soft rot caused by *Dickeya*. To identify candidate metabolites that might contribute to resistance, we screened 154 accessions of *S. chacoense* with a virulence assay to identify resistant and susceptible *S. chacoense* lines. We then used bio-chemical assays and non-targeted liquid chromatography mass spectrometry (LC-MS) to further characterize two resistant and two susceptible lines. Stem extracts from these selected accessions were tested for their effects on exoenzyme activity, cell morphology, AHL production, and bacterial motility of *Dickeya dianthicola* (ME23). *D. dianthicola* grown in stem extract from resistant lines had reduced protease (Prt), cellulase (Cel), pectate lyase (Pel) activity and reduced AHL synthesis compared to cells grown in the stem extracts from susceptible lines. However, stem extracts caused no difference in bacterial motility. A metabolic profile was created for resistant and susceptible lines, with and without *D. dianthicola* infection, using reverse phase LCMS. Analysis of metabolomic data showed a difference in the metabolites and their abundance between different *S. chacoense* lines. We also found a shift in metabolic abundance in *S. chacoense* lines when infected with *D. dianthicola* at a different timepoint. Detailed analysis and annotation identified phenolic acids, alkaloids, terpenes, and fatty acids as potential contributors to resistance, potentially through interference with bacterial quorum sensing.

Structure-activity and molecular modeling studies of strobilurin-based dual fungicides for the control of rice blast pathogen *Pyricularia oryzae*

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Fungicide resistance in plant pathogens is becoming a serious problem for sustainable crop disease management and requires urgent and innovative solutions. Here, we summarize our research on the design and development of innovative dual strobilurin-based fungicides, and the improvement of lead compounds based on structural investigations and molecular modeling. The first-generation dual molecules containing strobilurin and succinate dehydrogenase pharmacophores showed in some cases good activity against wild-type *Pyricularia oryzae*, but lacked activity against strobilurin-resistant strains. Therefore, structure-activity relationship studies were performed with the aim to improve the activity of novel compounds also against strobilurin-resistant strains. The design of the novel molecules was supported also by molecular modeling. The first three-dimensional model of *P. oryzae* cytochrome *bc1* complex containing azoxystrobin as a ligand was developed. The model was validated with a set of commercially available strobilurins, and it well explains the resistance mechanism of the mutation G143A to strobilurins caused by the sterical hindrance of alanine residue. Moreover, the model explains the activity of metyltetraprole against strobilurin-resistant strains. This research contributes to identification of the key recognition determinants of strobilurin-like compounds in the cytochrome *bc1* active site. A compound with >80% in vitro inhibitory activity against both wild-type and mutant *P. oryzae* was identified and will be further optimized and tested in vivo.

Assessing pathogen fitness cost in fungicide-resistant *Penicillium expansum* that impact postharvest decay management of pome fruit

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Penicillium expansum, the main causal agent of blue mold (BM) of pome fruit, is responsible for the majority of postharvest decay of apple and pear in the Pacific Northwest (PNW). Besides sanitation, BM is controlled with fungicides applied postharvest. Fungicide resistant-*P. expansum* isolates are difficult to control due to the development of fitness penalties. Therefore, we evaluated fitness costs of fourteen *P. expansum* isolates with different fungicide resistance phenotypes to fludioxonil, pyrimethanil, and thiabendazole, the three most widely used postharvest fungicides. Isolates were tested in vitro and in vivo to assess risks incurred by the packer via spore germination, mycelial growth, sensitivity to reactive oxygen species, osmotic stress, and resistance stability. In vitro fitness cost evaluations included growth, sporulation, and virulence with or without selection pressure. The impact of single, dual, and triple fungicide resistance is discussed to provide valuable information on the importance of fungicide rotation to enhance BM management postharvest.

Fungicide application timing provides effective and efficient *Stemphylium* leaf blight control in onion

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Michigan growers produce approximately 1,010 ha of pungent storage onions valued at \$10 million. *Stemphylium* leaf blight (SLB) caused by *Stemphylium vesicarium* has surpassed purple blotch to become the state's most common foliar disease of onion. In 2020, replicated field experiments were established to evaluate the following: (i) initiation of fungicide applications according to five growth stages; (ii) application intervals timed according to the TOM-CAST disease forecaster or the calendar; and (iii) alternation of site-specific fungicides. Fungicide applications initiated at the 3–4 and 5–7 leaf stages had less severe disease symptoms and higher yield compared to applications initiated at the 8–12 leaf stage or later. Fungicide applications initiated at the 5–7 leaf stage required two fewer fungicide applications and similar yields compared to the treatment with fungicides initiated at the 3–4 leaf stage. TOM-CAST treatments resulted in significantly reduced disease control and yield than the 7-day calendar application schedule. Fungicides applied every 10-days resulted in similar disease control and yield to that of the 7-day application schedule but required three fewer fungicide applications. Pydiflumetofen + fludioxonil ($n = 3$ applications) alternated with chlorothalonil ($n = 7$ applications) improved disease control and increased yield when pydiflumetofen + fludioxonil was used earlier than later in the rotation. The early-season fungicide applications applied every 10 days provided improved SLB control and yields while reducing fungicide inputs.

Convolutional neural network approaches for smartphone-based rapid detection of tomato diseases

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Tomatoes are a critical high-value crop for the livelihoods of many farmers around the world. However, a diverse range of biotic and abiotic issues impede the production and marketability of the crop, resulting in a devastating loss to the farmers. With laboratory-based early detection mechanisms being inaccessible in remote regions, there is the need for alternative rapid-detection mechanisms to aid in the timely implementation of disease control measures. Prior research conducted at the macroscopic level has resulted in high validation metrics, but low detection capabilities in the field. This study assessed Convolutional Neural Network approaches based on their ability to distinguish 17 biotic and abiotic issues of tomatoes at the microscopic (30×) level using a smartphone attachable lens. Disease symptoms ($n = 100$), either on the upper or underside of the leaves or on the surface of fruits, were diagnostically validated in the lab and imaged. Classifiers unique to the part of the plant the image is taken of, or a combined classifier consisting of all classes, were trained and tested. Architectures created from scratch tuned to that separation of the dataset were compared to transfer learning and pretrained models. The combined architecture composed from scratch and hyperparameter tuned proved to be the optimal classifier, with an F1 Score of 98.76% and a KFold Accuracy Score of 93.45%. This new AI approach is expected to aid extension pathologists, farmers, and supply chain management personnel to make reliable assessments to initiate management plans for disease control.

Identification of a novel basil downy mildew resistance gene candidate using de novo comparative transcriptomics

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Basil downy mildew (BDM) is a challenging agricultural disease caused by the oomycete pathogen *Peronospora belbahrii*. Newly available BDM-resistant cultivars have successfully mitigated crop losses but have incomplete resistance. Identification of specific genes associated with qualitative and quantitative resistance will hasten marker assisted breeding of basil cultivars with durable resistance. This study reports the discovery of a candidate resistance gene employing comparative transcriptomics of a BDM-resistant ('Mrihani') versus a BDM-susceptible ('Newton') cultivar. Expression analyses revealed 376 genes uniquely regulated during the resistance response. Candidate gene MRI-R1 was significantly upregulated in 'Mrihani' following pathogen inoculation, and is homologous to the canonical resistance gene NLR family. Polymerase chain reaction (PCR) confirmed its unique presence in 'Mrihani' and absence in 'Newton'. Sequencing of MRI-R1 clones identified several alleles, indicating allelic polymorphism of MRI-R1, which is possible due to the tetraploid nature of the basil genome. Two of the identified alleles were observed in two resistant offspring from the genetic cross using 'Mrihani' and 'Newton' as breeding parents. MRI-R1 likely represents an allelic series contributing to quantitative resistance against basil downy mildew, and further analyses will determine the utility of this gene as a breeding marker. This study demonstrates the feasibility of identifying specific candidate resistance genes in a challenging pathosystem without extensive genomic resources.

Effect of number of microsatellite markers on estimates of population genetic diversity and structure in the pecan scab pathogen, *Venturia effusa*

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Scab (caused by *Venturia effusa*) is the major biotic cause of pecan yield loss in the southeastern US. The pathogen population has been characterized as genetically diverse with limited differentiation, with mating types in equilibrium indicating regular recombination (the sexual stage has been identified in vitro). We compared the ability of 30, 15, and 7 previously developed microsatellites to characterize the population genetic diversity and structure of *V. effusa* in 11 pecan orchards ($n = 784$ isolates). Markers were from 7 of 20 chromosomes. Genotype accumulation curves were prepared. There were slight reductions in the number of multilocus genotypes (MLGs) identified using 7 (691 MLGs) or 15 (721 MLGs) compared to 30 (739 MLGs) markers, but the impact was small and the effect on measures of genotypic and gene diversity was negligible ($H_{exp} = 0.793, 0.803, \text{ and } 0.793$, respectively). Based on clone corrected data, linkage disequilibrium was observed in 10 populations using 30 or 15 markers, but in only 5 populations when using 7 markers. Population structure as determined by analysis of molecular variance, minimum spanning networks and discriminant analysis by principal components resulted in similar conclusions (differences were slight). Knowledge of population genetics of *V. effusa* are urgently needed to understand pathogen population interactions and gene flow, knowledge which will help underpin future breeding efforts to develop durable resistance in this long-lived orchard tree. Using fewer markers will conserve resources while maintaining integrity of results.

Aflatoxin production and gene expression analysis of *Aspergillus flavus* strains isolated from peanut seeds in Georgia

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Aspergillus flavus, a mycotoxin producing fungus, infects peanut seed resulting in reduced quality, viability, and germination, as well as losses in edible peanuts due to aflatoxin contamination. In 2020, peanut seed from Georgia had low germination and a high frequency of *A. flavus* contamination. *Aspergillus* isolates ($n = 102$) were collected from seven seed lots around southern Georgia. The isolates were identified as *A. flavus* by morphological characteristics and confirmed with a PCR using species-specific primers. The identity and expression of aflatoxin-producing strains were determined by targeting four genes (*nor1*, *ord1*, *omtA*, and *aflR*) involved in the aflatoxin biosynthetic pathway. In all examined strains, 80 out of 102 (78%) were successfully amplified using all four genes, indicating aflatoxigenic activity, and ELISA analysis confirmed that the isolates produced high levels of aflatoxins (B1). A real-time PCR was

used to analyze the level of the gene expression in all collected isolates. The results showed a significant variation in expression of these regulatory genes among aflatoxigenic and non-aflatoxigenic *A. flavus* isolates. Overall, this research indicates that 4 out of the 25 biosynthesis-related genes may play a role in the regulation of aflatoxin production in these *A. flavus* isolates. More research is needed on the functional importance of specific regulatory genes in *A. flavus* and the potential risk of aflatoxin production in stored peanut seed throughout Georgia.

Development of weather-based models to predict the contamination of maize grain with deoxynivalenol

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Gibberella ear rot (GER; *Fusarium graminearum*) is one of the most economically important ear diseases of maize in Ohio. This is largely because it leads to grain contamination with mycotoxins such as deoxynivalenol (DON) and other trichothecenes that reduce the utilization and market value of the crop. From 2015 to 2018, GER epidemics and associated weather conditions and grain contamination with DON were monitored on 15 to 16 hybrids at 10 locations across the state of Ohio. A total of 37 weather-based variables, derived from temperature, relative humidity, surface wetness, and rainfall, were summarized over six 15-day windows relative to the R1 (silking) growth stage to generate 222 potential predictors of DON contamination. These variables were screened through correlation analyses followed by LASSO and all-subset variable selection, then logistic regression with 10-fold cross-validation were used to develop models to predict the risk of DON contamination ≥ 1 ppm. A set of 11 one- and two-predictor models were selected based on performance and simplicity. The accuracy of these models ranged from 0.78 to 0.83, with sensitivity (proportion of cases with DON ≥ 1 ppm correctly predicted) and specificity (proportion of cases with DON < 1 ppm correctly predicted) ranging from 0.68 to 0.77 and 0.80 to 0.89, respectively. In general, warm temperatures and high moisture between 7 to 21 days after R1 were most predictive of the risk of DON contamination. These results are important first steps for the development of tools to help guide DON mitigation and grain marketing decisions.

Effect of moisture on wheat grain contaminating with zearalenone, an estrogenic metabolite produced by the fungus *Fusarium graminearum*

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Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is associated with grain contamination with mycotoxins such as zearalenone (ZEA), a potent estrogenic secondary metabolite. The objectives of this study were to quantify the effects of 1) temperature (20, 25, and 30°C) and relative humidity (RH, 70, 80, 90, and 100%) on ZEA contamination of grain in wheat spikes with different levels of FHB index (IND, mean percent diseased spikelets per spike) under controlled conditions and 2) simulated last-season rainfall (0, 5, and 10 days of pre-harvest rainfall) and harvest timing on ZEA contamination of grain in the field. ZEA contamination was highest at 100% RH across all temperatures and mean levels of IND, and much lower at RH $\leq 90\%$, even at mean IND $> 92\%$. At 100% RH for 28 days, ZEA contamination was higher at 20 and 25°C than at 30°C, suggesting that cool, wet conditions are required for ZEA production. Five or 10 consecutive days of simulated rainfall immediately before harvest increased ZEA contamination. Grain harvested early and not exposed to simulated rainfall had lower mean ZEA compared to grain harvested late and subjected to pre-harvest rainfall, suggesting that early harvest could be a useful strategy to minimize grain contamination with ZEA. This study was the first to associate ZEA contamination of wheat grain with temperature, RH, and rainfall, and show that early grain harvest could minimize contamination. These findings could be used to better understand and predict the risk of ZEA contamination and minimize its impact on grain quality.

Quantifying host range in the *Ralstonia solanacearum* IIB-4 clade

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Bacterial wilt diseases caused by *Ralstonia solanacearum* are important global crop production constraints. DNA sequence analysis indicates the plant pathogenic species complex comprises four divergent branches (phylotypes) native to Africa, Asia, the Americas, and the South Pacific. The species complex has a remarkably broad host range, infecting over 450 plant species, but the breadth of host range is not uniform among the thousands of known strains. The phylogroup IIB, sequevar 4 (IIB-4) clade exhibits a particularly broad host range and has caused multiple economically important wilt outbreaks in the Americas. Using stem inoculations of 18 non-clonal strains on diverse plant hosts, we are quantifying virulence and a snapshot of the host range in the IIB-4 clade. The 18 strains were isolated from 5 crop species in Florida, the Caribbean, and Latin America, encompassing a broad swath of the phylogroup II ancestral range. Fifteen strains were highly virulent on tomato, completely wilting plants within 5 days. Three strains were moderately virulent, wilting a majority of leaflets within 14 days. Virulence on melons was more variable. By comparable metrics, 1 strain was highly virulent on melon, 11 were moderately virulent, 5 showed low virulence, and 1 was avirulent. Assays on two additional hosts (banana and impatiens) are ongoing. Preliminary results for impatiens suggest similar variability to melon. Despite a high degree of genetic similarity, we find that *R. solanacearum* strains in the IIB-4 clade exhibit considerable variation in their virulence on diverse hosts.

Will fungal pathogens of *Acrocomia aculeata* endanger its bright biofuel future?

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Acrocomia aculeata (macaw palm, macaúba in Brazil) is a native palm regarded as one of the most promising sources of biofuel. Although there is relatively little published information on this species, commercial plantations are being established in Brazil. Plant pathogens compose a menace to such plantations. Here, an account of the pioneering survey, started in 2009, fungi associated with the palm in Brazil is presented. The aim of the present work was to preliminarily detect and study the mycobiota of *A. aculeata* and investigate the possible threat represented by pathogenic species. A combination of molecular and morphological studies provided identification of these fungi. Although most of them are considered pathogenic to other crops the pathogenic status of each species has yet to be verified. *Camarotella* sp. belongs to a genus known to include biotrophic pathogens and was found to be capable of causing severe damage to leaves in natural stands of the palm, being associated with black leaf scab. *Pleopassalora* sp. is suspected to cause leaf spots and *Neopestalotiopsis* sp. along with *Hysterostomella* sp. were found causing similar necrotic lesions on leaves. *Ophiostoma* sp. was regularly found producing fruiting bodies on leaves as well as *Thielaviopsis* sp. on fruits. *Phytophthora palmivora* causes root and meristem rot of young and mature plants. The flowering stage of macaw can present *Fusarium proliferatum* causing floral abortion. Further verification and complete assessment of the mycobiota are in need, but all occurrences can represent new host-pathogen records.

Citrus fruit (Meyer lemon) phenology and susceptibility to *Phyllosticta citricarpa* and continued mating type screening of the Floridian population

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Phyllosticta citricarpa is the causal agent of citrus black spot, a disease that first emerged in Florida from sweet orange fruit in a grove near Immokalee, FL in 2010. *P. citricarpa* has since become established in Florida, with the potential to cause heavy yield loss and reduce the marketability of fresh fruit. Initial studies revealed that only the MAT1-2 idiomorph was present in Florida. With only asexual spores present, our understanding of disease spread changes and this may have regulatory implications since the sexual spores become airborne and spread further and likely faster. Therefore, monitoring for the presence of both mating type genes and determining the phenology of fruit susceptibility in Florida is vital to understand the disease progression. To date, we have screened 26 isolates surveyed between 2016 to 2020 in Florida, in addition to 113 isolates previously screened. The MAT1-1 idiomorph remains absent in Florida. This means our Floridian population of *P. citricarpa* reproduces asexually. The critical fruit stage of susceptibility to asexual spore infection in Florida is still unclear. To address this, 97 fruit were used for a phenology study; 25 served as controls and 72 were inoculated with *P. citricarpa*. Fruit size ranged in diameter from 1.9 to 6.99 cm and fruit color at time of inoculation was classified as green or yellow. First symptoms were observed after 5 months on green fruit with a diameter between 4 and 5.5 cm and symptoms were severe on ripening fruit. Our preliminary result indicates that fruit are susceptible at all developmental stages.

Evaluation of *Solanum sisymbriifolium* as a rootstock to control *Meloidogyne incognita* in tomato

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The use of resistant rootstocks is one of the most effective methods to manage root-knot nematodes (*Meloidogyne* spp.) in tomato production. Our goal was to determine the effects of grafting a tomato cultivar on the rootstock *Solanum sisymbriifolium* to control *M. incognita* and improve crop yield. We studied the efficacy of four nematicides, including Nimitz, Velum Prime, Vydate, and Majestene, on the grafted, self-grafted, and non-grafted (susceptible 'Roadster' and resistant 'Red Bounty' cultivars) tomato plants grown on plastic beds. Nematicides were applied through the drip irrigation system utilizing one drip tape per bed. The interaction between nematicide and grafting was significant ($P < 0.0001$) only for root gall severity. The rootstock and Red Bounty reduced ($P < 0.05$) gall severity more than self-grafted and non-grafted Roadster; however, no difference in gall severity was observed between the rootstock and Red Bounty, regardless of which nematicide was used. A similar trend in the nematode population density in the soil was observed at harvest when both the rootstock and Red Bounty treatments had significantly lower nematode counts than the self-grafted and non-grafted plants. There was no difference in tomato fruit weights of small, medium, large, and extra-large sizes among the rootstock, self-grafted, and non-grafted plants regardless of which nematicide was used. These results demonstrate that grafting onto highly resistant rootstocks is a practical management approach for producing susceptible tomato cultivars in soils infested with root-knot nematodes.

Surveillance and mitigation strategies for bacterial wilt and other diseases in a potato seed network in Kenya

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Protecting seed systems from invasive pathogens is a key step in protecting crop health. We used network analysis to evaluate disease risk in a potato seed distribution network in Meru, Kenya. We integrated cropland connectivity networks, international trade networks, and local seed trade networks, to identify locations that are likely to be particularly important for the spread of the bacterial wilt pathogen, *Ralstonia solanacearum*, and other key pathogens. Information about households, seed sources, quantities sold, pest incidence, pest management, knowledge about seed quality and degeneration, and farmers' sources of information related to potato production were analyzed. The survey revealed that the main seed sources were neighbors, own seed, seed companies, friends, exchange, and markets. Certified seed accounted for 43% of total seed exchange. Influential actors were identified based on their

number of interactions and by their importance for surveillance as evaluated using the impact network analysis framework in the INA R package. We also compared four scenarios for potential disease management in this seed system. Users of uncertified seed have high disease risk, and this is especially important if their positions in the network give them the potential to be super-spreaders of disease. Understanding the key actors in a seed system can help target efficient disease management for improving regional outcomes.

Populations of *Gemmamyces piceae* causing bud blight disease of spruce in Alaska are different from European populations

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Gemmamyces piceae has been found in Alaska since 2016 causing bud blight disease of spruce (*Picea* spp.) although its status as a native or invasive pathogen has not been fully elucidated. Isolates from European and Alaskan populations of *G. piceae* were analyzed using phylogenetics of single and concatenated genes of the ITS, RPB2 and TEF1 but could not be differentiated since no important differences in nucleotides were observed. In order to understand the population structure of *G. piceae* present in Alaska and the relationship with *G. piceae* from Europe a population genetics approach was used using Short Sequence Repeats (SSR). Primers for 24 SSR loci were designed from one de novo assembly from a European isolate and used to genotype several isolates of *G. piceae* collected in Alaska in 2020. Results showed only 10 primers had consistent amplification in all isolates evaluated and fragment analysis showed important differences between these populations. Among seven European isolates genotyped, polymorphisms were observed in five loci, yielding seven haplotypes. Among 18 Alaskan isolates, ignoring null alleles, only one locus was polymorphic, which produced a total of two haplotypes. Due to the constricted variation observed in the Alaskan population, it remains unclear whether reduced polymorphism is due to a bottleneck event or is simply ascertainment bias. Several tests are required to conclude these findings, which will be combined with an analysis of mating types present in Alaska and used to create a diagnostic assay to differentiate European from Alaskan populations.

Pathogenicity of soilborne *Fusarium* spp. from Pennsylvania

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Fusarium root rot in soybean is a common disease caused by key soilborne pathogens of the genus *Fusarium*. Pathogenicity assays on new *Fusarium* isolates should improve our understanding on their impact on soybean seedling disease incidence. The objective of this study was to investigate pathogenicity of 20 *Fusarium* isolates representing phylogenetically distinct clades based on a previous molecular characterization. One to three isolates from 12 phylogenetic clades including *F. solani* species complex (FSSC 3+4, FSSC 5, FSSC 9, FSSC 11, and FSSC 15), *F. oxysporum*, *F. incarnatum-equiseti*, *F. nisikadoi*, and *F. sambucinum* species complexes were used in this study. About five mycelium cores from each single-spore culture were incubated in potato dextrose broth at 150 r.min⁻¹ and 25°C on an orbital shaker for 7 days. Pathogenicity of isolates will be tested following a rolled-towel assay using soybean variety SC9277R arranged in randomized complete block design with three replicates. Each replicate will comprise 20 soybean seeds inoculated with a suspension of 2.5×10^5 conidia/ml or sterile water as the control. After 7 days, seeds will be assessed, and disease severity index will be calculated. Results will provide insight into selecting phylogenetic clades for further investigations on the aggressiveness, and fungicide sensitivity of *Fusarium* spp. We also expect that the outcome from these studies will improve recommendations for managing pathogenic *Fusarium* spp. in PA field crops.

Metatranscriptomic identification of novel amalgaviruses (*Amalgaviridae*) in Orchidaceae family

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Orchidaceae is one of the largest family of flowering plants in the world, with more than 800 genera and approximately 25,000 described species, comprising ~10% of all angiosperms. Orchids are widely distributed in tropical and subtropical areas. Although there have been recent efforts to study orchid-associated microbiome, viruses have been largely ignored because they do not generally cause disease. Recent approaches and tools, such as high-throughput sequencing (HTS), allowed the discovery of novel viruses, including apparent non-symptomatic ones. In the current study, we performed a metatranscriptomics analysis of five orchid transcriptomes publicly available in the Transcriptome Shotgun Assembly (TSA) database. We identified the near full-length genomes of six amalgavirus. The identified viral contigs, within 3,384–3,438 nucleotides (nt), exhibited a typical genomic organization of amalgavirus, showing two overlapping ORFs. They were detected in the transcriptome of *Blettia striata*, *Epipogium roseum*, *Gymnadenia conopsea*, *G. rhellicani*, and *Ophrys fusca* subsp. *iricolor*. The amalgavirus found in *G. conopsea* and *G. rhellicani* shared high identity, indicating they belong to the same species. Thus, those five new virus species, provisionally named *B. amalgavirus* 1, *E. amalgavirus* 1, *G. amalgavirus* 1, *G. amalgavirus* 2 and *O. amalgavirus* 1 could constitute novel members in the *Amalgavirus* genus.

Whole-genome mapping of eyespot resistance to *Oculimacula yallundae* and *Oculimacula acuformis* in winter wheat cultivar Madsen

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Oculimacula yallundae (OY) and *O. acuformis* (OA) are the soilborne pathogens of eyespot, an economically important disease of winter wheat. Madsen has been one of the most popular cultivars in the U.S. Pacific Northwest (PNW) for more than 30 years, mainly due to its high-level of eyespot resistance. Despite its popularity, the genetic control of resistance to OY and OA has not been reported. To map all eyespot resistance genes in Madsen, 180 recombinant inbred lines (RIL) developed from a cross between Madsen and Liangxing 99 were inoculated with β -glucuronidase (GUS)-transformed OY and OA isolates separately at the seedling stage in

replicated growth chamber assays. The RIL were genotyped with the 90K wheat SNP chip and 3219 SNP and 8 SSR markers covering the whole genome were used for QTL analysis. Resistance of Madsen was more effective against OY than OA based on the phenotypic tests. A major QTL for resistance to both OY and OA was present on chromosome 7DL covering a 14.8-cM interval with 23 markers that was more effective to OY than OA. This locus is expected to be *Pchl* previously identified for eyespot resistance. QTL were also detected on chromosomes 1A, 2AS, 5A, and 6A for both pathogen species. QTL on chromosomes 3B and 5B were only effective against OY. Another minor QTL detected on chromosome 2A was effective for OA only. This study provides a complete list of eyespot resistance loci and their associated markers in the Madsen genome. Markers tightly linked to these loci will facilitate selection of eyespot-resistant plants from PNW-adapted wheat cultivars.

Survey of Sugarcane yellow leaf virus in second clonal stage of canal point breeding and cultivar development program

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Sugarcane yellow leaf virus (SCYLV) is a widespread global pathogen that causes sugarcane yield losses in both symptomatic and asymptomatic varieties. The objective of this study was to survey SCYLV incidence in the second clonal stage of Canal Point's breeding and cultivar development program (CP program). The CP program selection process begins with greenhouse seedling (i.e., germinated from true seed versus clonal propagation; January–February, year 1) transplantation to the field in May–June. Seedlings that are selected, based on visual ratings for field vigor and disease response, progress to the first clonal stage (Stage 1; January–February, year 2) before moving into the second clonal stage (Stage 2; ~1,500 clones) in late November. SCYLV-infected commercial cultivars (41 plots) were randomly planted throughout the Stage 2 field. The first dewlap leaf of five plants for each genotype were randomly collected from all Stage 2 plots (February, year 3). The cross-sections of leaf-midrib samples were imprinted on 0.45-μm nitrocellulose membranes, for detection of SCYLV by tissue-blot immunoassay using polyclonal antibodies. After 3 years of field exposure from seedling transplantation to leaf collection in Stage 2, 11% of the tested genotypes were positive for SCYLV. The results suggest that incidence of SCYLV in second clonal stage is not significant enough to select for SCYLV-resistant genotypes with confidence.

Characterization of viruses associated with yellow leaf spot disease of *Spiraea* spp.

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Spiraea (*Spiraea* spp., Fam. Rosaceae) are deciduous shrubs widely cultivated as landscape ornamentals in northern zones because of their winter hardiness. Two aphid-transmitted viruses *Spiraea yellow leafspot virus* (SYLSV) (genus: *Badnavirus*) and *Spiraea leafspot spherical virus* (SLSSV) (Totivirus-like) have been found in co-infections in *Spiraea* plants showing yellow leafspot disease, suggesting that both viruses are required to cause the disease. Between 2018 and 2020 an increase in the occurrence of yellow leafspot disease was observed in both commercial nurseries and landscape plants in the Midwestern region of the United States. The re-emergence of both SYLSV and SLSSV was suspected. Samples from several cultivars and species of *Spiraea* were tested for the presence of viruses by a combination of transmission electron microscopy (TEM), high-throughput sequencing (HTS), polymerase chain-reaction (PCR), and reverse-transcription (RT)-PCR. Neither TEM nor molecular analyses detected the SLSSV (Totivirus-like) in symptomatic plants. However, the complete sequence of two isolates of SYLSV, from Maryland and Minnesota, respectively, were assembled by HTS. Both isolates share 95% nucleotide identity. SYLSV was found consistently associated with the disease symptoms, suggesting that SYLSV can induce symptoms without the presence of the co-infecting spherical virus SLSSV. This finding is important for the regulation of disease-causing viruses in plant certification schemes.

Sampling nursery-grown ornamental plants for *Phytophthora* species: The challenge of obtaining pathogen-free plants

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Phytophthora root and crown rot (PRCR) has become a major disease of lavender (*Lavandula* spp.) in the United States. While the impact of PRCR is most detrimental to lavender growers, the pathogen is mainly spread through infected or infested nursery stock. Therefore, it is critical to test plants before transplanting to avoid contamination of field sites. Detection of *Phytophthora* species often relies on destructive sampling of and direct isolation from relatively few plants. Our objective is to develop non-destructive sampling methods for reliable detection of *Phytophthora* spp. from nursery-grown plants. Lavender growers have been encouraged to submit samples after receiving trays of nursery-grown plants. Predetermined plants from each tray (19 to 44%) were strategically sampled, based on the number and orientation of cells in a tray, by clipping the bottom 5 mm of each plant. Clippings were combined into a composite sample and tested for *Phytophthora* spp. with a standard baiting bioassay. To date, we have processed 59 samples from the two most common species of *Lavandula* (*L. angustifolia* and *L. × intermedia*) and 18 cultivars. Plants originally came from six nurseries in three states. Eight samples (14%) tested positive for *Phytophthora* spp., including *P. cactorum*, *P. cryptogea*, and *P. nicotianae*; positive plants came from four of the six nurseries. However, we detected sporangium-inhibiting residues in the baiting water of 12 samples (20%), and these residues may have masked the presence of *Phytophthora* spp. on sampled plants.

Spore trapping and quantification of lettuce and spinach downy mildews in the Salinas and Coachella Valleys of California

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Downy mildews (DM) caused by *Peronospora effusa* and *Bremia lactucae* are major disease constraints for spinach and lettuce industries in California, respectively. DMs cause severe damage to leaves rendering these fresh products unmarketable. Management can be achieved with the application of fungicides, but routine applications result in large expenditures and can lead to fungicide resistance in the pathogen. There is also an increasing demand to limit chemical inputs due to the potentially negative environmental impacts. In this work we prepared a multiplex quantitative PCR TaqMan detection assay for both the spinach and lettuce DM pathogens for dual pathogen detection in a single reaction. We used this assay to determine levels of these pathogens in two different regions, the Salinas (spring to fall production) and Coachella (fall to spring production) Valleys, for two 8- to 10-week time periods. One to two Rotorod spore traps were placed at two locations in each valley, and rods were collected three times per week. Interestingly, the results revealed year-round detection of spores in the Salinas Valley, but during months of warmer weather and periods of reduced crop production both airborne DM pathogens were not detected in the Coachella Valley. This information can be used to associate spore load with increased or decreased disease risk in the different regions examined, and will enable a more comprehensive assessment of pathogen inoculum density during the season and the relationship of environmental parameters on disease severity.

Culturing of *Candidatus Liberibacter asiaticus* is practically impossible and likely requires a synthetic biology approach

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Candidatus L. asiaticus (Las) is an insect-transmitted phloem-limited bacterial pathogen associated with citrus huanglongbing. *Liberibacter crescens* (Lcr) is the only axenically cultured *Liberibacter* (1.5-Mb genome) and serves as a surrogate for many uncultured *Ca. Liberibacter* spp., including Las. All attempts to achieve axenic growth and complete Koch's postulates with Las (1.2-Mb genome) have failed to date, at best resulting in mixed cocultures, all with very low Las "titers." Genome comparisons revealed Las is deficient in key biosynthetic metabolic and structural pathways found in Lcr. Called loci unique to Lcr (e-values <3e-30, <40% identity cut-off by both InterProScan and Prokka) were identified using a custom Perl script and reciprocal blasts were conducted using ORHOMCL. A total of 406 unique genes were found present in Lcr and absent (or truncated) in all sequenced Las strains. Twenty-five genes have been eliminated by ongoing site-directed mutagenesis experiments. Based on published Tn5 transposon mutagenesis data, 317 of the 406 genes were eliminated as nonessential for viability, leaving 89 Lcr genes as potentially essential for growth. Nine of these have published experimental support indicating they are needed for growth of Lcr. Some (*gloA*, *lpxL*, *acpXL* etc.) are highly unlikely to be rescued by media supplementation alone. Conjugation of large plasmids from *E. coli* to Lcr was successful. Upon experimental validation, the remaining genes will be cloned in a combinatorial vector system and transferred via conjugation into transient Las cultures.

Infectivity of *Cacao swollen shoot Ghana M virus* (CSSGMV) cloned 1.2-mer in cacao plants by gene gun- and agro-inoculation

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At least seven badnaviral species have been associated with cacao swollen shoot disease (CSSD), a re-emerging disease of cacao (*Theobroma cacao* L.). Symptoms include foliar chlorosis, mottling, red-vein banding, pod deformation, stem and root swelling, and death within 5 years. A 1.2-mer infectious clone of *Cacao swollen shoot Ghana M virus* (CSSGMV)-Nig5 (MH029281), genus *Badnavirus* (Caulimoviridae), was constructed to demonstrate Koch's postulates and determine the latent period. Cacao 'Amelonado' seedlings were experimentally inoculated with the cloned CSSGMV biolistically ($n = 5$) or by agroinoculation ($n = 15$). Presence of CSSGMV in the developing leaves was determined by PCR amplification of a 1068 bp product. Three of the five biolistically inoculated plants developed symptoms of leaf deformation and red vein-banding 60 days post-inoculation (dpi). The three symptomatic plants were positive by PCR amplification, while mock-controls were negative. All 15 agroinoculated cacao seedlings developed foliar mosaic and yellow-vein banding symptoms 30 dpi, and 3 developed swollen shoot symptoms 120 dpi. All 15 plants yielded the expected size PCR product, and a sequenced subset shared >99.8% nucleotide identity with CSSGMV-Nig5 (MH029281). Total DNA and RNA isolated from plants inoculated by both methods were subjected to Illumina sequencing, revealing complete CSSGMV genomes with >99.5% nt identity to CSSGMV-Nig5. These results provide evidence to fulfill the Koch's postulates of CSSGMV as an etiological agent inducing CSSD symptoms like those found in cacao fields of Nigeria.

Rose rosette emaravirus: Understanding the vector is a key to combat the disease

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Rose rosette, a devastating disease and a threat to virtually all cultivated roses, is caused by *Rose rosette emaravirus*, a multi-segmented ssRNA virus endemic to North America. The virus is vectored by microscopic eriophyoid mites, dispersing passively by wind currents. The epidemic in the US has grown to the point where roses are not planted in disease hot spots, leaving the rose industry in jeopardy. From breeders and nurseries to home growers, all parts of the supply chain look to an evidence-based approach to mitigate the disease. Despite considerable progress in virus epidemiology, little is known about the vector, and there are no compelling products to control it. Our systems-based approach to combat rose rosette aims to: (i) determine species boundaries between vector and non-vector eriophyoid mites; (ii) identify the predatory mite fauna associated with the vector; and (iii) evaluate two entomopathogenic fungi; a member of the Hypocreales and Exobasidiales as potential biopesticides to control eriophyoid mites on rose. Cryptic diversity within the confirmed vector, *Phyllocoptes fructiphilus*, was revealed by mitochondrial, nuclear, and morphometric data. In-depth vector characteristics are pivotal in future studies involving virus-vector interactions and disease management. Effective biocontrol solutions should be implemented in the IPM strategy to enhance the biosecurity of rose.

Gene expression profiling of *Aspergillus flavus* after *Vibrio gazogenes* treatment

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Aspergillus flavus produces the carcinogenic aflatoxins in agricultural crops such as maize, peanut, cottonseed, and tree nut. Aflatoxin contamination is a great concern for the food and feed industry worldwide. In past studies, we observed inhibition of overall growth and aflatoxin biosynthesis in *A. flavus* upon exposure to the marine bacterium, *Vibrio gazogenes* (Vg). However, the molecular mechanism responsible for this phenomenon is still unclear. In this study, *A. flavus* was co-cultivated with live and dead Vg in the nutrient medium, while fungal culture without bacterial treatment was used for the control samples. Total RNA was extracted from the Vg-treated mycelia harvested at 24 h, 30 h, and 40 h post-inoculation (hpi). RNA samples were used to prepare cDNA libraries and these libraries were sequenced using Illumina HiSeq platform. Differential gene expression was determined by comparing the expression values in live and dead bacteria-treated fungal culture versus control samples at 24, 30, and 40 hpi. A total of 223, 806, and 275 genes were significantly upregulated and 337, 828, and 154 genes were downregulated in live bacteria treated fungal culture, while 115, 95, and 24 genes were upregulated and 60, 121, and 25 genes were downregulated in fungal cultures treated with dead bacteria, respectively. At 40 hpi, numerous aflatoxin biosynthesis related genes were significantly downregulated upon treatment with live or dead bacteria. Our study provides preliminary information on the molecular basis of the antagonistic effect of Vg to *A. flavus* aflatoxin biosynthesis.

Updates on field assessment of mutations conferring differential resistance to SDHI fungicides in dollar spot caused by *Claviceptis jacksonii*

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Dollar spot, caused by *Claviceptis jacksonii* is one of the important turfgrass diseases on golf courses. While cultural practices are available for reducing dollar spot infection, repeated fungicide applications are often necessary for highly managed turfgrasses. Since boscalid was launched in the early 2000s, several new succinate dehydrogenase inhibitors (SDHI) are currently available for controlling dollar spot. However, resistance to this SDHI class has recently been reported. Following SDHI field failure on 6 golf courses in USA and Japan and 1 university research plot, the UMass Turf Pathology Laboratory has identified a total of seven unique SDH mutations conferring differential in vitro sensitivity to SDHIs in *C. jacksonii*. In 2018, 2019, and 2020 turf research plots at the Joseph Troll Turf Research Facility in South Deerfield, Massachusetts, were inoculated with sensitive, non-mutated isolates of *C. jacksonii*, as well as resistant isolates harboring each unique mutation. Field efficacy trials were conducted on inoculated plots to assess differential sensitivity to 5 SDHI active ingredients (boscalid, fluxapyroxad, isofetamid, fluopyram, pydiflumetofen) and non-SDHI fungicides across mutations. It was observed that there is partial cross-resistance to SDHIs and fluopyram displays negative cross-resistance on particular mutation. Results indicate unique mutations are associated with distinct SDHI sensitivity profiles. This information will be useful in developing sustainable management strategies for controlling resistant populations of *C. jacksonii*.

Sensitivity of *Cercospora sojina* to demethylation inhibitor and methyl benzimidazole carbamate fungicides

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Frogeye leaf spot (FLS), caused by *Cercospora sojina*, can cause significant yield losses to soybean. Foliar fungicide application is an important method used to control FLS; however, *C. sojina* strains resistant to quinone outside inhibitor (QoI) fungicides have become widespread throughout U.S. soybean production regions. Because of QoI-resistance, demethylation inhibitor (DMI) and methyl benzimidazole carbamate (MBC) fungicides have become more important in managing FLS. Sensitivity of the DMI fungicides flutriafol and tetraconazole and the MBC fungicide thiophanate methyl were established for *C. sojina* isolates collected from 2013 and 2017 to 2020. A total of 222 *C. sojina* single-spore isolates were tested to assess effective fungicide concentration at which 50% of mycelium growth was inhibited relative to a non-fungicide control (EC50) for each isolate-fungicide combination. EC50 value means for isolates collected from 2013 and 2017 to 2020 were compared, and significant difference ($P < 0.01$) was observed for sensitivities to flutriafol, tetraconazole and thiophanate methyl. These findings indicate a minor shift towards reduced sensitivity to flutriafol and thiophanate methyl. For tetraconazole, no shift towards reduced sensitivity was observed. Based on these results, continued monitoring of *C. sojina* population sensitivity to DMI and MBC fungicides should occur.

Cross resistance of SDHIs in *Botrytis cinerea* and response of SDHB mutations

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Succinate dehydrogenase inhibitors (SDHIs) are cornerstone synthetic fungicides that are used to manage gray mold caused by *Botrytis cinerea* in several hosts including grapes. In this study, we investigated the cross resistance between new SDHIs including pyraziflumid, isofetamid, benzovindiflupyr, fluxapyroxad, and pydiflumetofen with commonly used SDHIs, boscalid and fluopyram. Different SDHB mutations were investigated in *B. cinerea* collection of grapes and their frequency and EC50 value were determined. Among 207 *B. cinerea* isolates, five different mutations were detected including H272R/Y, P225F/H, and N230I at frequencies of 72, 4, 11, 0.4, and 4.9% respectively. Five isolates of each genotype were used to screen the cross resistance of the SDHIs. Isolates with the 225-F allele were associated with strong cross resistance with all SDHIs except benzovindiflupyr and pydiflumetofen; isolates with the 230-I allele had moderate resistance while isolates containing the 225-H allele were sensitive to isofetamid and fluxapyroxad; isolates containing the 272-R allele were associated with stronger resistance to boscalid than isolates containing the 272-Y allele and isolates containing both 272-R and Y alleles had moderate resistance to pyraziflumid while isolates containing the 272-Y allele had higher EC50 than isolates containing the 272-R allele. These results provide essential information for industry and growers about the potential efficiency of new SDHIs for management of gray mold.

Diversity in species composition and fungicide resistance profiles in *Colletotrichum* isolates from apples

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In 2018, bitter rot outbreaks occurred in three commercial apple orchards in Illinois. Three isolates obtained from symptomatic fruit in these orchards and were identified as *Colletotrichum siamense* (*C. gloeosporioides* species complex) based on morphology and calmodulin, G3PDH, and β -tubulin gene sequences. Mycelial growth tests in vitro and on detached fruit revealed that two isolates were dual-resistant to azoxystrobin and thiophanate-methyl ($EC_{50} > 100 \mu\text{g/ml}$). The other isolate was resistant to only azoxystrobin. Sequencing revealed that resistance to thiophanate-methyl and azoxystrobin was based on the E198A mutation in β -tubulin gene, and the G143A mutation in cytochrome b. Interestingly, one isolate highly resistant to azoxystrobin ($EC_{50} > 100 \mu\text{g/ml}$) had no amino acid variation in CYTB. This study shows that quinone outside inhibitor fungicide resistance in *Colletotrichum siamense* has emerged on apple and is being selected by current spray strategies. Diversity in fungicide resistance was present in our sample of just three isolates, with isolates that were dual resistant, single resistant, and resistant with no point mutation. Resistance monitoring may alert growers to potential threats, but based on the results of this study, we recommend non-molecular methods for identifying resistance, since detecting the G143A mutation may not reveal all resistant isolates.

Xanthomonas strains isolated from Araceae reveal diverse phylogenetic relationships and origins

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The family Araceae (aroids) comprises economically important ornamental genera including *Anthurium*, *Dieffenbachia*, and *Philodendron*. *Xanthomonas phaseoli* pv. *dieffenbachiae* (*Xpd*) causes bacterial leaf blight on aroids and since 2009 has been listed as an EPPO A2 quarantine organism. In this study, multi-locus sequence typing (MLST) with housekeeping genes *atpD*, *dnaA*, *dnaK*, *gltA*, and *gyrB* was used to analyze 59 representative aroid strains selected from many hosts, temporal and geographical origins. Sequences of related *Xanthomonas* species/pathovars and *Stenotrophomonas*, a member of the Xanthomonadaceae, were retrieved from the NCBI GenBank database. Phylogenetic analysis of 161 concatenated sequences revealed that representative strains clustered in clades with six *Xanthomonas* species/pathovars and two *Stenotrophomonas* species. The 39 strains that cause anthurium blight clustered close to *Xpd*, whereas two strains from *Syngonium* and *Xanthosoma* showed sequence homology with a *X. citri* pv. *aracearum* strain from *Dieffenbachia*. Strains from *Caladium*, *Dieffenbachia*, and *Philodendron* grouped into three host-specific subclades within the polyphyletic *X. euvesicatoria* clade. Eleven strains clustered with *X. arboricola*, *X. sacchari*, and *S. maltophilia*/*S. rhizophila* clades. Based on Biolog GEN III microplate analysis, the latter strains grouped in *X. sacchari* and *Stenotrophomonas* clades and clustered separately from other aroid strains. Overall, this study revealed high heterogeneity among strains from diverse hosts and origins and suggested the existence of new taxonomic species.

Fungal wheat endophytes protect against *F. graminearum* and reduce mycotoxins

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Fusarium graminearum is a devastating fungal disease of wheat that infects wheat heads and reduces grain quality, yield, and releases mycotoxins that prevent grain consumption from humans and animals. Since no complete genetic resistance exists within wheat germplasm, fungicides remain a primary management tool for *F. graminearum*. However, reliance on fungicides can lead to fungicide resistance and alternative management strategies are desirable. Fungal endophytes live inside plants asymptotically and can provide protection to the plant from a variety of biotic or abiotic stresses. In a previous study, 1,634 fungal wheat endophytes were isolated. Three of these isolates were highly protective against root rotting caused by *F. graminearum*. In this study, we identified these isolates using multiple loci and a phylogenetic approach, assessed competition for space in vitro, and examined protection of wheat heads. Two *Fusarium* endophytes were identified as *F. oxysporum* and *F. commune*. The third endophyte was identified as an *Alternaria* sp. that was closely related to *A. destruens*. The mechanism of competition for space in vitro for all three endophytes was competitive exclusion by antagonism at a distance with metabolites followed by preemption of non-occupied space to restrict *F. graminearum* spread. When these endophytes were applied to wheat heads two endophytes significantly increased seed weight and reduced mycotoxin contamination. A field trial is currently being conducted to determine if these endophytes would be candidates for biological control.

Applied learning and active engagement through virtual platforms

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Active learning modules and problem based exercises are key components of an upper level introductory plant pathology course taught at Cornell University. Students directly engage with teaching assistants, professors, and local growers to learn about plant pathology and understand its direct applications. Transitioning the curriculum and accompanying activities online was a unique undertaking this past year. We took the course's learning outcomes and designed digital projects and lesson plans. While some activities, such as interactive disease scenarios, adapted well to the online format, larger challenges included transitioning labs and tests. Standard exams were replaced by live, biweekly group quizzes focused on problem solving. One of the labs is a problem based learning project (PBL) which includes farm visits, crop observations, interactions with growers, creating a disease fact sheet, and a final presentation detailing farm history and management recommendations. We created interactive scouting maps with tagged photos of disease, pest incidence, and crop symptoms. Students then "walked" the farm by creating a scouting plan and came up with a list of questions for the grower. TA's posing as growers then answered their questions. Student groups used the symptoms they "found," their knowledge of the farm's

growing practices, and the answers to their questions to create a comprehensive final presentation with their findings and recommendations for the grower. These activities may serve as a means for remote students to participate in active learning in a virtual setting.

Infection biology of the Eucalyptus leaf blight pathogen, *Teratosphaeria destructans*

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Teratosphaeria destructans is one of the most important leaf pathogens of *Eucalyptus* planted in tropical and sub-tropical areas. Consequently, its dispersal and management are of growing concern, but limited by a lack of knowledge regarding its infection biology. In this study, we compared the conditions for germination of the aggressive *T. destructans*, with *T. epicoccoides*, a less aggressive *Eucalyptus* foliar pathogen, distributed in temperate and sub-tropical areas of the world. The infection process and disease development for both pathogens was studied on a *E. grandis* × *E. urophylla* hybrid clone utilizing light and transmission electron microscopy. The optimal temperature for the germination of *T. destructans* ranged from 25 to 30°C and 15 to 20°C for *T. epicoccoides*. Conidia of both pathogens germinated independent of exposure to light and was dependent on high levels of relative humidity, close to 100%. Both pathogens also infected the host via stomata 48 h after inoculation and the hyphae grew intercellularly. No specialized structures such as haustoria were observed. Symptoms were visible 3 weeks post-inoculation and pycnidia containing conidia developed below the substomatal cavities from 4 weeks post-inoculation. Results of the study provide a foundation for controlled inoculations to screen *Eucalyptus* genotypes for resistance and to understand mechanisms involved in the *Eucalyptus*-*T. destructans* interaction.

Development and characterization of polymorphic microsatellite markers for *Neopestalotiopsis* from strawberry and alternative hosts

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Pestalotia leaf spot and fruit rot of strawberry is an emerging fungal disease having come into the spotlight in recent years in the strawberry industry in the U.S. due to its substantial economic impact. This disease severely affects strawberry leaves and fruit and, with progress, can also affect crowns and roots. Currently, the paucity of knowledge on disease epidemiology is hampering the development of effective control strategies. Our previous research revealed that a new *Neopestalotiopsis* species is responsible for the recent disease outbreak; however, the limited phylogenetic resolution based on multilocus sequencing analysis has impeded the species designation and identification of the geographical origin of the pathogen. To address these questions, we developed microsatellite (simple sequence repeat, SSR) markers through draft genome sequencing of three species, including the new *Neopestalotiopsis* species, *N. rosae*, and the ex-type species *Pestalotia longisetula*. SSR markers were identified using MISA and compared manually to ensure polymorphisms among the three species. Markers with distinct banding profiles for six *Neopestalotiopsis* species were further confirmed by sequencing. The evaluation of *Neopestalotiopsis* isolates spanning a wide geotemporal range is underway to provide new insight into the taxonomic status of the *Neopestalotiopsis* genus and the potential origin and movement of the pathogen populations across the strawberry-producing areas in the U.S.

Populations of *Pseudomonas syringae* pv. *phaseolicola* use different kinds of persistence to survive biological and chemical control treatments

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Persistence is a temporary state of dormancy that a tiny proportion of the bacterial population uses to survive antibiotic treatments and other stress. Pathogen persisters are thought to contribute to new disease cycles and the emergence of resistance. Membrane-disrupting biological controls, such as the protein complex tailocin, also leave a small proportion of persister-like survivors. In this study we asked whether *Pseudomonas syringae* pv. *phaseolicola* (*Pph*) persists to tailocin are different from the cells surviving the antibiotic streptomycin. Both types of persisters comprised 0.05% of the initial population. Microscopic profiling with fluorescent dyes showed that streptomycin treatment only killed 25% of the population and induced diverse physiological states in the remaining fraction, while tailocin rapidly killed all but the persister cells. Cell sorting showed that the streptomycin persisters occupied a state of low redox activity, while tailocin *Pph* persisters were metabolically active. The persisters were able to infect plants, but nonculturable cells with metabolic activity were not. A combination treatment of tailocin and streptomycin was highly effective in eradicating the entire population. This work shows that pathogens maintain different physiological subpopulations that can tolerate different types of disease controls, illustrating a mechanism by which integrating chemical and biological controls could prevent disease recurrence and limit emergence of resistance.

Effect of alternative application methods of fungicides to control charcoal rot (*Macrophomina phaseolina*) of strawberry in greenhouse conditions

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Charcoal rot, caused by *Macrophomina phaseolina*, is an important disease in Florida strawberry production that leads to wilting and collapse of plants. Pre-transplant soil fumigation is the main management tool used by growers; however, other strategies are required

during the season. The efficacy of alternative application methods of fungicides to control charcoal rot was determined in a greenhouse trial. Bare-root, cut-top 'Strawberry Festival' plants were inoculated two weeks after transplanting by placing toothpicks colonized with two *M. phaseolina* isolates and containing microsclerotia. Treatments were organized in a factorial design with six fungicides (thiophanate-methyl, flutriafol, fluopyram, isofetamid, difenoconazole, and pyraziflumid) and five application methods (dipping plants in a fungicide suspension for 5 min before transplanting, application in the planting hole, drenching 3 and 15 days after inoculation (DAI), drenching with first observed symptoms (FOS), and dipping plants and drenching with FOS). Non-treated inoculated and non-inoculated controls were included. Disease incidence was evaluated weekly for 16 weeks. Overall, drenching 3 and 15 DAI with thiophanate-methyl, pyraziflumid, fluopyram, or isofetamid were the most effective in reducing disease incidence ($P = 0.0002$). Drenching soon after transplanting might be used as a tool in the integrated management program for control of charcoal rot.

Uncovering cryptic species among *Neonectria* fungi causing perennial target cankers in the central Appalachian Mountains

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Fungi in the Nectriaceae include important plant pathogens that cause annual and perennial canker diseases. In the central Appalachian Mountains, a recent survey uncovered *Neonectria magnoliae* causing perennial cankers on Fraser magnolia (*Magnolia fraseri*) and tulip-poplar (*Liriodendron tulipifera*) but in different forests at different elevations. Phylogenetic data indicate *N. magnoliae* may include at least two cryptic species, but more data are needed to confirm these findings. To help further resolve species boundaries, additional isolates of *N. magnoliae* were collected from both hosts at three locations in West Virginia. Morphological studies revealed strains from one clade (from Fraser magnolia) produced both macro- and microconidia, while isolates from a second clade (from tulip-poplar) only produced microconidia. Phylogenetic analysis of elongation factor 1- α (EF1- α) DNA sequences confirmed our previous findings: isolates from each host fell within separate lineages and together formed a monophyletic clade. Recently, an additional strain of *N. magnoliae* was recovered from alder and was identical to strains from magnolia. Cross-pathogenicity studies are underway to determine pathogenicity of *N. magnoliae* strains from both lineages on tulip-poplar. These findings highlight the importance of combining phylogenetic approaches with morphological studies and host range testing to better delimit species boundaries and understand the threats these species pose.

Studying the subcellular localization of *Citrus tristeza virus* (CTV) proteins

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Citrus tristeza virus (CTV) is the causal agent responsible for a number of citrus diseases which has killed millions of trees, almost destroying whole industries. Many attempts have been made to characterize the role of CTV encoded proteins in different viral processes. However, there are many unanswered questions about the functions of these proteins. Here, we tried to provide information on the subcellular localization of 13 kDa (p13), 18 kDa (p18), 23 kDa (p23), 61 kDa (p61), and HSp70h (p65) proteins of CTV inside the host cells. The localization of these proteins was studied using agroinfiltration of p13, p18, p23, p61, and p65 fused to green fluorescent protein (GFP) into the leaf epidermal cells of *Nicotiana benthamiana* followed by confocal laser scanning microscopy. Intracellular distribution of the proteins and their colocalization with the endoplasmic reticulum (ER), Golgi complex (GC) and plasmodesmata were investigated. Our finding indicated that all these proteins are associated with ER. Co-localization of putative CTV proteins with ER show that they are capable to traffic intracellularly via the ER system. Furthermore, p23, p61, and p65 exhibited some degrees of ER remodeling and modification that led us to suggest that these proteins might have a possible role with the formation of viral replication complexes.

Investigating azoxystrobin resistance in the potato silver scurf pathogen *Helminthosporium solani*

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Silver scurf is a tuber blemish disease causing negative impacts of increasing concern in potato production. There is little disease resistance in commercial cultivars and resistance is poorly understood. Growers have utilized variably effective cultural controls and fungicides for disease management. After *H. solani* developed resistance to the fungicide thiabendazole, QoI fungicides (such as azoxystrobin) were adopted. There has been an increase in silver scurf severely affecting quality and storability. Genetic mutations associated with QoI resistance have been identified in other ascomycetes in the cytochrome *b* gene. Therefore, *H. solani* might currently be poorly controlled due to QoI resistance after over two decades of use. We established a novel protocol to evaluate *H. solani* isolates for azoxystrobin sensitivity. In both assays tested (liquid culture and solid media), salicylhydroxamic acid (SHAM) significantly reduced *H. solani* growth ($P = 1.249e-05$). In addition, 40 days of incubation were essential to discern the greatest differences between azoxystrobin doses and individual isolates. While liquid culture assays can offer multiple advantages, solid media assays resulted in less variability within treatments and replications. We also sequenced the *H. solani* genome and developed five primer pairs that can be used to study the region of cytochrome *b* critical for determination of mutations associated with azoxystrobin resistance in ascomycetes. Our work provides substantial advancement in the understanding of QoI resistance in *H. solani* for improved management.

TIR domains: Not just for R-genes! A phyto bacterial TIR domain effector manipulates NAD⁺ to promote virulence

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One of the primary types of plant NLRs encoded by R-genes are TIR-NB-LRRs that cleave NAD⁺ and activate plant immunity. The pathogen *Pseudomonas syringae* secretes a type III effector HopAM1 that suppresses plant immunity and contains a TIR domain homologous to TIR-NB-LRRs. In vitro and in vivo assays were conducted to determine if HopAM1's TIR domain cleaves NAD⁺ and is essential for HopAM1's suppression of plant immunity and contribution to virulence. HPLC and LC-MS were used to analyze metabolites produced from NAD⁺ by HopAM1 in vitro and in both yeast and plants. *Agrobacterium*-mediated transient expression and in planta inoculation assays were performed to determine HopAM1's enzymatic activity and virulence contribution in plants. HopAM1's TIR domain is catalytically active and cleaves NAD⁺ to produce nicotinamide and a novel cADPR variant (v2-cADPR). Expression of HopAM1 triggers cell death in yeast and plants in a TIR domain-dependent manner in association with depletion of endogenous NAD⁺. Furthermore, HopAM1's TIR domain is required to suppress both PTI and ETI and promote *P. syringae* virulence. HopAM1 modulates endogenous NAD⁺ to produce a novel cADPR variant to promote pathogenesis. This work suggests that HopAM1's TIR domain possesses different catalytic specificity than that of TIR domain-containing NLRs encoded by R-genes and that pathogens exploit this activity to sabotage NAD⁺ metabolism for immune suppression and virulence.

Cereal and non-cereal crop sequences affect Fusarium head blight across the Canadian prairies

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Fusarium head blight (FHB) is a frequent disease of cereal producers across western Canada. Approximately 60% of the area seeded to annual crops in the Canadian prairies consists of wheat and canola; this low crop diversity within rotations increases cereal disease risk. Growers have included non-cereal crops in their rotations; however, the impact of the various crops is not well understood. This study focused on the effect of a 3-year crop sequence that included cereal and non-cereal crops to determine their effects on the severity of FHB and the *Fusarium* species involved. From 2018 to 2020, five locations were seeded with a core set of five crops including wheat, barley, canola, pea, and maize, and a sixth crop appropriate to each location. The experiment consisted of a 6 × 6 matrix arranged in a split block design. Yield, crop quality and FHB severity were recorded. *Fusarium* spp. were isolated and identified from cereal kernels. The crop stubble immediately preceding the cereal crop played a key role in FHB severity, the *Fusarium* spp. detected, and the amount of Fusarium damaged kernels in the harvest sample. Differences in *Fusarium* spp. kernel infection in wheat and barley were noted among previous crop sequences; the frequency of *F. poae* was similar in sequences with cereals and pulses. Including pulses and other non-cereal crops in a 3-year crop sequence benefitted yields, but did not have a major impact on cereal diseases. The population of *Fusarium* spp. differed depending on experimental location and the previous crop.

Pathogen and tree fruit health-map (PATH-Map): A smart phone application and dashboard for tree fruit pathogen, pest, and disorder tracking

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Fruit quality is routinely impacted by diseases, insect pests, and physiological disorders both pre and postharvest. USDA-ARS Partnerships for Data Innovations (PDI) in conjunction with the Fruit, Pathogen, Pest, Disorder Team (FPPD), have developed PATH-Map, Pathogen And Tree fruit Health-MAP, that can be used to record symptoms and observations in the field and during storage. The user-friendly mobile phone app is accessed through Survey123 and the corresponding dashboard has outputs to record issues based upon visual symptoms. The graphical interface can display occurrences at state and county levels over time with color and data filters. The database is open access and is being utilized by scientists, support specialists, and extension agents to record and document abiotic or biotic fruit problems. There is an option to upload photos of unknown symptoms/problems which can be visualized by experts for tracking issues impacting fruit quality. Visual confirmation is achieved as users flag their uploads requesting help, both through a comments section on existing posts, or by obtaining more specific diagnostic tests (i.e., molecular tools). PATH-Map is a new, innovative, comprehensive tool to monitor, analyze, and map diseases, pests, and/or physiological disorders that negatively impact fruit quality. Future applications include using stored entries for epidemiological modeling, tracking, and generating predictive models to manage pathogens, pests and physiological disorders that negatively impact fruit quality.

Evaluation of fungicides for management of head diseases in malting barley

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Fusarium head blight (FHB) caused by *Fusarium* spp. and Septoria nodorum blotch (SNB) caused by *Parastagonospora nodorum* are fungal diseases of small grain crops that can reduce grain yield and quality. In Delaware, wet spring conditions can favor both SNB and FHB in malting barley. In October 2019, malting barley 'Violetta' was planted into a field with corn stubble to evaluate fungicide efficacy and optimal timing of a newly released product, Miravis Ace for management of FHB and SNB. Treatments included Prosaro and Caramba applied at Feekes 10.5.1 and Miravis Ace applied at Feekes 10.3, Feekes 10.5.1, and 4 days after anthesis compared to a non-treated control. Twenty barley heads in each plot were evaluated for FHB incidence (% symptomatic heads) and severity (% symptomatic glumes per head) as well as SNB incidence and severity 5 weeks after heading. All treatments reduced FHB incidence and severity compared to the control. Incidence of FHB averaged 53% in non-treated plots, while treated plots ranged from 12 to 23% ($P =$

0.0006). All treatments reduced SNB incidence compared to the control ($P < 0.0001$), and all except Caramba reduced SNB severity ($P = 0.0004$). All treatments significantly improved yield compared to the non-treated control (86.7 bu/A, $P = 0.0012$), with the highest yield observed in Miravis Ace applied at anthesis (105.6 bu/A).

Linking microbial taxa with function in artificially structured apple rhizosphere microbiomes

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In the search for new ways to promote positive plant-microbe associations or suppress negative ones, the application of metagenomics to the study system is a promising approach. Previous work has demonstrated that certain mustard seed meal formulations, when applied to soil, alter the apple rhizosphere microbiome in a positive manner and effectively suppress soilborne pathogens. In this study, microbial metabolic attributes and environmental resources associated with seed meal-induced suppression of apple replant disease were identified in the rhizosphere of different apple rootstock genotypes using metabolic network approaches for the analysis of metagenomic data. Network approaches also led to the identification of environmental resources/metabolic activities which were linked with particular microbial taxa. We highlight computational predictions that were subsequently tested experimentally, including preliminary evidence for the targeted recruitment of disease-suppressing microbial groups using catecholamine compounds. Beyond the period of initial orchard establishment, the ability to predict how particular source metabolites may be used to “artificially structure” the indigenous soil microbiome is highly relevant to managing a whole host of soilborne diseases.

How can a plant virus help in managing the SARS-CoV-2 pandemic

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Since the declaration of the pandemic of SARS-CoV-2, in March 2020, the scientific community has joined forces to rapidly develop methods for virus detection and epidemiological monitoring. We have contributed to such effort by developing and performing continual analysis of municipal wastewater across Slovenia for the presence and quantity of the virus. As one of the EU reference laboratories for plant viruses with background in environmental studies, we found an unlikely ally in the form of a plant virus, the pepper mild mottle virus (PMMoV), which infects plants but it is also highly prevalent in the environment due to its stability. PMMoV is able to survive the human digestive tract and reach the sewage system, where its RNA can be detected by qPCR. Our previous studies showed that PMMoV is ubiquitously present in wastewater in Slovenia in high quantities. This is the basis for implementation of PMMoV as an internal control and correction factor in detection of SARS-CoV-2 in wastewater. We measure PMMoV for two purposes: 1) to assess the efficiency of our concentration method by comparing the PMMoV concentrations obtained from the non-concentrated and concentrated fraction of the sample; 2) as a fecal indicator to normalize the measured SARS CoV 2 concentrations by correcting for the influence of external factors, such as rain or industrial discharges. This two-way correction of the SARS-CoV-2 measurements in wastewater is more stable against lower level of environment-related fluctuations and shows a better correlation with the official SARS-CoV-2 positive case reports.

Interspecific interactions between *Pythium* species coexisting in float-bed tobacco transplant production greenhouses

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Pythium root rot is a common disease in hydroponic tobacco transplant production greenhouses and previous research suggested multiple *Pythium* species may be involved. However, there is little information on the role of each *Pythium* species in tobacco greenhouses and how they interact with each other. Our previous survey revealed that *Pythium* communities in tobacco greenhouses are highly diverse in regard to species composition and virulence. The most aggressive species identified in the survey, *Pythium myriotylum*, coexisted with multiple other *Pythium* species within multiple surveyed tobacco greenhouses. In this study, significant interactions between *P. myriotylum* and some naturally coexisting species were observed. *P. catenulatum*, *P. pectinolyticum*, *P. adhaerens*, and *P. porphyrae* formed clear zones against *P. myriotylum* on 10% V8 agar dual cultures. These zones may be associated with reduced *P. myriotylum* mycelial growth and spore production. *P. catenulatum* significantly ($P < 0.01$) increased while *P. dissotocum* isolate 3 (subgroup) significantly ($P < 0.01$) reduced the growth rate of *P. myriotylum* on 10% V8 medium. Co-inoculation experiment results suggested *P. porphyrae* significantly ($P < 0.01$) reduced the virulence of *P. myriotylum* on tobacco seeds and seedlings, but *P. adhaerens* significantly ($P < 0.01$) increased *P. myriotylum* virulence. These results suggest that there are significant interspecific interactions within *Pythium* communities occurring in tobacco greenhouses and *P. porphyrae* may have the potential to control *P. myriotylum* in tobacco greenhouses.

Identification and host-range evaluation of *Alternaria* species complex affecting broccoli in Georgia

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Alternaria leaf blight and head rot (ABHR) caused by *Alternaria* species complex, has been an emerging issue for brassica crops in eastern United States particularly in Georgia. Brassica crops including broccoli, cabbage, kale, and collard can be severely affected by this pathogen under favorable conditions. A statewide survey was conducted in fall 2020 in commercial broccoli fields in Georgia to

understand the dominant *Alternaria* species complex prevalent in this area. Also, the pathogenicity and aggressiveness of the *Alternaria* sp. was evaluated using a detached-leaf assay under laboratory conditions. A total of 92 isolates were collected from six commercial broccoli fields from three predominant broccoli growing counties (Colquitt County, Tift County, and Grady County) in Georgia. The isolates were identified as *Alternaria* sp. based on morphological characteristics and further single spore isolates were identified to the species-level using Illumina sequencing. One hundred percent of the *Alternaria* sp. collected from broccoli were identified as pathogenic on broccoli and cabbage in a detached leaf assay; however, they differed in their aggressiveness in these tested hosts. Further characterization of isolates for fungicide resistance and host-range evaluation is underway. Overall, *Alternaria* sp. affecting broccoli crops is prevalent in Georgia that pose an emerging threat for sustainable production.

Seed treatment effects of soybean-associated beneficial bacteria on the microbiomes in soybean plants

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We formulated consortia of soybean-associated beneficial bacteria for seed treatment, which showed growth-promoting effects on soybean plants. In this study, we characterized the microbial communities of soybean plants in the endosphere and rhizosphere compartments influenced by two sets of soybean-associated bacterial consortia (SBC), SBC1, and SBC2. These SBCs are composed of multiple beneficial bacteria predominantly belonging to genera of *Bacillus*, *Pseudomonas*, *Streptomyces*, *Enterobacter*, *Achromobacter*, and *Ensifer*. According to the 16S rDNA sequence data, the bacterial genera in the SBCs used for seed treatment showed highest relative abundance in both compartments of soybean roots, suggesting that the bacteria of the SBCs colonized the roots during plant growth. The microbial community patterns were not significantly different among different seed treatments in the level of species richness and diversity (alpha-diversity), although SBC1 showed the highest of alpha-diversity among the treatments in both compartments, followed by SBC2 and the untreated control. However, the microbiome structure (beta diversity) in the rhizosphere compartment was significantly different among the treatments (49.3% of variation, $P = 0.018$). The differential heat tree analysis showed that in comparison with the untreated, SBC2 and SBC1 had more differentially abundant taxa in the endosphere and rhizosphere compartments, respectively. The result of this study contributes to the development of innovative seed treatment using beneficial microorganisms for more sustainable soybean culture.

Aphanomyces root rot of alfalfa disease survey in eastern South Dakota establishes widespread pathogen distribution

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Alfalfa is the third most valuable crop grown in the United States. *Aphanomyces euteiches* is an oomycete pathogen that causes Aphanomyces root rot of alfalfa, which inhibits alfalfa growth in both seedling and adult stages. Common disease management practices are crop rotation and planting of resistant alfalfa varieties. *A. euteiches* can survive for long periods in soil, and its high genetic diversity allows it to remain virulent against many alfalfa varieties. Without current studies establishing the presence and distribution of *A. euteiches* in South Dakota, there was a need for soil surveillance and isolation of *A. euteiches* to guide growers in the selection of proper alfalfa varieties to increase production. In the summer of 2020, 42 fields in 14 different counties across eastern South Dakota were surveyed to collect composite soil samples. In growth chamber assays, alfalfa check cultivars of differing resistance were grown in the collected soil for *Aphanomyces* isolation and race-typing. Seedlings were rated for disease severity using a standardized rating system. *A. euteiches* detection was further validated through DNA extraction from infected root tissue and PCR with *Aphanomyces*-specific primers. Also, samples of infected root tissue were placed on selective media, and *A. euteiches* was isolated. Race-typing, PCR, and root isolation assays all demonstrated the presence of *A. euteiches* in South Dakota. This study establishes the widespread presence of race 1 and race 2 of *A. euteiches* in South Dakota and will aid growers in alfalfa variety selection to maximize yields.

Effector screening of secreted in xylem (SIX) genes from *Fusarium oxysporum* f. sp. *lycopersici* reveals several race 3 isolates containing AVR1

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Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a soilborne disease present in many global production regions. Three FOL races are recognized, FOL3 is the predominant race in Florida, as most commercial varieties are resistant to FOL1 and FOL2. Specific secreted in xylem (SIX) genes that encode protein effectors corresponded to several immunity (I) genes which confer effective host resistance in tomato are associated with race designations. SIX4 (AVR1) is present in FOL1 isolates but absent in FOL2 as well as FOL3 and known to suppress I2 and I3 based host resistance. However, five recovered isolates from multiple production regions in Florida were identified as FOL3 through pathogenicity assays with tomato Fusarium wilt differentials, despite containing SIX4 as confirmed through sanger sequencing. Whole genome sequencing further confirmed the presence of SIX1-7 genes in two isolates in addition to SIX4. The sequences recovered from both approaches were 100% match to known FOL1 isolate IPO1530/B1 (Fol004). Further characterization is in progress. This is the first report of FOL3 isolates with this unique effector profile and emphasizes the need for continued monitoring and characterization of these evolving soilborne pathogens for resistance breeding, as well as for the development of reliable detection methods.

Wound healing and its role in the prevention of Rhizopus soft rot on sweetpotato (*Ipomoea batatas*) roots

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Rhizopus soft rot (RSR) caused by the fungus *Rhizopus stolonifer* is a common postharvest disease of sweetpotato. Infection occurs through wounds on roots to cause a soft watery rot within a few days. To reduce the risk of RSR in storage, roots are cured immediately after harvest to heal wounds. However, once they are removed from storage, they undergo packing operations that create new wounds and subsequent infection by the fungus. Preventative applications of synthetic fungicides have been relied on, but increasing concerns of fungicide residues on roots, presents a need to develop other methods of managing RSR. Promotion of wound healing is a desirable approach which could be included in integrated management programs because healed wounds have been shown to be an effective barrier to infection by the fungus. The objective of this study was therefore to understand the development of RSR in relation to wound healing under curing (29°C, 85% RH) and storage (15°C, 85% RH) conditions. Wound healing measured by lignification in wounded roots of three sweetpotato cultivars was assessed by staining root sections with phloroglucinol. Concomitantly, roots were inoculated with a spore suspension of *R. stolonifer* isolate 92-Rs-2 on each day and RSR incidence recorded up to 14 days. Preliminary results showed increased lignification and a decline in RSR incidence with increased incubation time. Future studies will seek to identify genes associated with enhanced wound healing in different cultivars and environments for possible incorporation into sweetpotato breeding programs.

Sequence-anchored genetic mapping and pyramiding of adult plant resistance loci against oat crown rust (*Puccinia coronata* f. sp. *avenae*)

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Crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is a widespread disease of oat that causes 10–40% yield losses. Mutations and genetic recombination in its alternate host, buckthorn (*Rhamnus cathartica*), make the rust fungus genetically variable. Managing the disease can be achieved sustainably using resistant varieties; thus, sources of adult plant resistance (APR) are needed. In this study, we mapped nine biparental populations generated from crosses between APR donors and a susceptible variety, Otana, namely: OtanaA/CI4706-2, OtanaA/CI9416-2, Otana/PI189733, OtanaD/PI260616-1, OtanaA/CI8000-4, OtanaA/CI7035-1, OtanaA/CI1712-5, OtanaA/PI266887-1, and Otana/PI263412-1. Populations were phenotyped in buckthorn nursery in St. Paul, MN, where a sexual population of crown rust is present. Individual lines were genotyped using Illumina NovaSeq and reads were aligned to the OT3098 oat reference genome prior to SNP calling. Redundant SNPs were removed for linkage map construction and composite interval mapping. Genetic mapping results revealed a major QTL in Mrg21 in Otana/PI189733, with sequence-based markers anchored in oat chromosome 4D (Chr4D). Another QTL in Chr4D was detected in OtanaA/CI9416-2, while two QTL were detected in Chr7C and Chr5D in OtanaA/CI1712-5. All QTL identified so far contain clusters of disease resistance genes based on BLAST results. qPCR markers were developed for each QTL and used to pyramid APR loci. Preliminary data from the advanced pyramided lines showed a decreasing coefficient of infection when 2–4 QTL are combined.

Diversity of *Verticillium dahliae* isolates affecting chile pepper production in New Mexico

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Chile pepper is an important crop in New Mexico both for its cultural heritage and its cash receipts. *Verticillium dahliae* caused wilt is one of the largest constraints on chile production in southern New Mexico. Identification of natural resistance sources, like the Ve locus used in commercial tomato lines, would be a significant help to New Mexico chile growers. Understanding the diversity and race structure of *V. dahliae* affecting chile production in New Mexico is a necessary first step toward identifying and deploying genetic resistance in chile peppers. Here we report race characterization and diversity studies which show that New Mexico is affected by a diverse population of *V. dahliae* strains, and many of which overcome the Ve mediated resistance in tomato. The results of this work suggest that Ve like resistance, either discovered within the chile gene pool or introduced from tomato via genetic engineering, is unlikely to be useful for generating chile cultivars resistant to Verticillium wilt.

Sooty blotch in the mango variety Ataulfo and options for management

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Sooty blotch is a fungal disease that can cause substantial economic losses in mango, as well as in other fruit such as apple and peach. Blotches often reduce the value of mango fruit, from premium fresh-market grade to processing use. The disease is often associated with the secretion of sugary substances that promote the growth of dematiaceous fungi. *Gloeodes pomigena* appears to be one of the primary causal agents of this disease. Due to difficulties in isolation and characterization of causal agents, several fungi have been identified as causal agents of this disease. There is a great likelihood that the pathogens have sometimes been misidentified, which can make it harder to interpret the various studies of sooty blotch. In this study, we review current information in English, French, and Spanish related to sooty blotch, with an emphasis on management options for mango. Based on this initial review of literature, an expert elicitation instrument was conceived to fill in data gaps, which helped us find more obscure literature and draw on unpublished knowledge. The disease is a particular concern for the mango variety Ataulfo, also known as Honey, and we provide recommendations for next steps for research and management for this variety and for the mango industry in general.

Sensitivity of *Cercospora sojina* to tetraconazole

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Cercospora sojina, causal agent of frogeye leaf spot (FLS), is a plant pathogen that effects soybean fields in the United States. Disease is most severe in warm and humid weather conditions, where it can cause yield losses of up to 60%. QoI fungicides were intensively used to manage FLS; however, a single point mutation known as G143A resulted in QoI resistance and solo-QoI fungicides failure to manage FLS. Hence, DMI fungicides have been used for disease management. The goal of this project was to a) monitor *C. sojina* resistance to QoI; and b) investigate *C. sojina* sensitivity to tetraconazole, an active ingredient in DMI fungicides. 33 isolates were collected from symptomatic leaves across TN, IL, GA, and LA during the 2006-2020 soybean seasons, and were screened for the presence of the G143A mutation. The effective concentration to inhibit 50% mycelia growth (EC50) of tetraconazole was calculated through a mycelial growth assay using V8 media amended with 0, 0.01, 0.1, 1, and 10 ppm of tetraconazole, using three replications. EC50 values were calculated using the Probit analysis in JMP software. As a result, 80% of the isolates tested had an EC50 in the range of 0.1 to 0.5 ppm. There were no isolates with an EC50 higher than 1.0 ppm. Tetraconazole was effective in inhibiting FLS growth and, therefore, can continue to be used in FLS management. Further results on the G143A mutation will be presented in the meeting.

Economic return and efficacy of foliar fungicides used for management of tar spot in Indiana corn

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Tar spot caused by *Phyllachora maydis*, a foliar disease of corn was first detected in Indiana and Illinois in 2015 and now is confirmed in 10 states and Ontario, CA. Initially, only 7 counties in Indiana confirmed tar spot, but now is confirmed in 78 counties. Currently, yield loss estimates 241 M/Bu of corn valued at \$1.3 T. Fungicides are available, but are they an economically viable management option? We assessed the cost effectiveness of fungicides on tar spot in Indiana over 2 years at two locations: Wanatah and West Lafayette. Fungicides were applied at VT/R1 and compared to a nontreated control. Disease was assessed during the season and final rate taken at R6. Tar spot severity was rated by visually assessing stroma% and chlorotic/necrotic tissue per leaf on five plants in each plot at the ear leaf, ear leaf+2 and -2 leaves. Stay green% and yield were calculated. Economics of each fungicide was estimated by formula: Net Return (Rn) = Yield Response (kg ha⁻¹) * Corn Price (\$ kg⁻¹) – Fungicide Program Cost (\$ ha⁻¹). Tar spot severity was moderate-high at Wanatah and low-none at West Lafayette. All fungicides significantly reduced tar spot severity and increased stay green%. Significant yield difference occurred with yield increase >11.8% averaged across fungicides. Avg. Rn were ground (\$160.7 and \$-32.7 ha⁻¹) and aerial (\$116.7 and \$-46.1 ha⁻¹) at Wanatah and West Lafayette respectively. Results indicates that it is not economically viable to apply a fungicide when disease is low and a significantly positive Rn on investment is achievable when a fungicide can increase yield by 13.2% to nontreated control.

Investigation into the loss of fungicide efficacy for Alternaria leaf blight and head rot of broccoli and cruciferous crops in the eastern USA

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Cruciferous vegetables are widely consumed in the USA and represent a national economic value over \$1 billion per year. *Alternaria* leaf blight and head rot is one of the most common and destructive diseases in broccoli and other crucifers crops and is caused by *Alternaria brassicicola*, representing a pre- and post-harvest loss of hundreds of millions of dollars per year. While fungicide applications are integral to reduce yield loss, resistance is suspected to have led to a loss of QoI fungicide efficacy in multiple locations. However, a competing hypothesis for this loss of efficacy may be due to the emergence of a novel species or sub-species that is less sensitive to QoI fungicides. As such, the goal of this preliminary investigation was to determine whether there is resistance to QoI fungicides and determine the genetic relationships among populations from different geographic regions. A selection of 15 isolates from broccoli and other cruciferous hosts from NY, VA, and GA were used in our study. Fungicide sensitivity will be determined using a conidial germination assay on a series of seven azoxystrobin (QoI) concentrations to estimate the EC₅₀. The least sensitive isolates will be used for cytochrome *b* gene sequencing and aligned to the *A. solani* reference genome to determine whether mutations conferring fungicide resistance are present (F129L, G137R, and G143A/S). Results will be used to guide subsequent studies to investigate the population genetics and fungicide resistance profiles across the eastern USA and develop diagnostic tools to detect fungicide resistance.

Elucidating mosaic-symptom causing viruses in Wisconsin iris production

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Iris severe mosaic virus (ISMV), a potyvirus known to cause mosaic symptoms in iris and crocus, is a pathogen thought to severely limit iris production and salability and is of growing concern in Wisconsin. A survey aimed at determining the presence of ISMV in Wisconsin was conducted. Plant material was collected from various sources including a commercial iris garden, where it was possible to systematically test with respect to source grower, year of variety introduction, and year of acquisition by the grower. Using ELISA and end-point PCR with potyvirus-specific primers, we discovered that ISMV is not always associated with characteristic mosaic symptoms. Further, we were able to evaluate the effect of source, year of introduction, and time grown in Wisconsin on virus presence. PCR amplicons were cloned and sequenced, allowing us to identify potyviruses in addition to ISMV associated with symptomatic plants. To further determine the virome of these ISMV infected plants, high-throughput sequencing on extracted RNA from a representative subsample of plants will be performed. Results from such sequencing will determine the role other viruses play in shaping the severity of mosaic symptom development in ISMV-infected plants. Our results indicate that other viruses should be monitored in iris, especially ones that may be more able than ISMV to change hosts.

Vacuum-enhanced heat treatment eradicates *Ceratocystis* pathogens in logs from ohia trees (*Metrosideros polymorpha*) affected by rapid ohia death

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Ohia is the most widespread and highest-volume native hardwood tree in Hawaii. Off-island shipments of ohia logs is regulated by a state quarantine due to epidemics of rapid ohia death (ROD) caused by two *Ceratocystis* species. Only pathogen-free logs or those subjected to approved heat treatments may be shipped from ROD-affected islands. Two vacuum-enhanced heat treatments of *Ceratocystis*-colonized logs were evaluated for eradicating the ROD pathogens while minimizing quality degrade. Replicate trials were conducted with a portable vacuum steam system (VS) to treat 24- to 44-cm diam × 2-m long debarked logs from naturally infected trees. Additionally, 16- to 26-cm diam × 1.7-m long de-barked logs from artificially inoculated ohias were heat-treated using a vacuum drying kiln (VK). Carrot bait assays were used to determine presence of viable *Ceratocystis* in sapwood of two disks (7 cm thick) from each log before and after treatment. Either *C. lukuohia* or *C. huliohia* were commonly isolated from outer and inner sapwood of all logs prior to treatment. VS treatment of either 60°C or 56°C at 70% log radius depth were held for 60 and 30 min, respectively, for chamber loads of three logs and resulted in >99.5% pathogen eradication in 7 to 19 h. Heating of five logs per load in a VK to 56°C at similar depths and held for 30 min, followed by a drying cycle (45°C) resulted in complete elimination of the pathogens within 48 h. Log quality was not reduced by any of the treatments. These results are under review by the Hawaii Department of Agriculture for use as approved log treatments.

Stem rust and crown rust of tall fescue in Georgia and the development of an F1 population to identify stem rust resistance genes

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Stem rust, caused by *Puccinia graminis*, and crown rust, caused by *P. coronata*, are common rust diseases on cool-season grasses for which long-distance spore dispersal has been recorded in the northern US. During the summers of 2019 and 2020, severe infections of stem rust and crown rust were observed on > 60% of tall fescue (*Festuca arundinacea*) germplasm plants in a breeding nursery located at the University of Georgia (UGA), Griffin GA. Urediniospores of both rusts were collected from infected plants in the field, morphologically characterized and used to extract genomic DNA, followed by amplification of the internal transcribed spacer (ITS) region of the ribosomal DNA. BLASTn analyses revealed that the sequence of stem rust and crown rust pathogens had >99% similarity with *P. graminis* and *P. coronata* var. *avenae* f. sp. *avenae*, respectively. Pathogenicity tests were performed, and stem and crown rust pathogens were re-isolated from artificially inoculated tall fescue plants, whereas control plants had no symptoms. In addition, an F1 mapping population was generated by crossing susceptible and resistant tall fescue lines identified in the breeding nursery at UGA. Using two microsatellite makers polymorphic for the parents, 156 F1 hybrid progenies were confirmed. Quantitative trait locus analysis is currently underway to depict the genetic basis of stem rust resistance in tall fescue. This information will be valuable for developing rust management strategies and breeding for resistance to this pathogen.

Distribution and genetic variability of natural infection by Mexican papita viroid on tomato fields in central Mexico

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In 2013, tomato plants (*Solanum lycopersicum*) grown in fields located in the Jocotitlan locality of Mexico State exhibited remarkable stunting, leaf chlorosis, and reduced fruit number, among other symptoms frequently related to viroid diseases. Diseased samples were collected and bioassays through mechanical inoculation with leaf extracts of samples demonstrated the causal agent's transmissibility to tomato plants under greenhouse conditions. Inoculated plants expressed symptoms that were similar to those on the source plants. RT-PCR assays of RNA samples were positive with expected size product (~170 bp) consistently detected using Bostan et al., 2004 pospiviroid-specific primers in all symptomatic samples or from the mechanically inoculated tomato plants. Analysis of sequences obtained from direct sequencing of amplicons revealed one dominant sequence with 85% identity to Mexican papita viroid (MPVd) isolate VF2 complete sequence reported in Canada infecting greenhouse tomato (GenBank Accession No. FJ824844.1). In follow-up studies, MPVd infections were identified in tomato fields in the Atlacomulco locality of Mexico State during 2017–2018 and in Ixmiquilpan in the state of Hidalgo in 2020. A total collection of 11 isolates were partially sequenced and analyzed with MAFFT software for phylogenetic relationships. The close relationship between the Mexican and the Canadian isolates suggests that MPVd in these two countries may share a common origin.

Pan-genome of novel *Pantoea stewartii* subsp. *indologenes* identifies genes involved in onion pathogenicity and lateral gene transfer

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Pantoea stewartii subsp. *indologenes* (*Psi*) is a causative agent of leafspot of foxtail millet and pearl millet; however, novel strains were recently identified that are pathogenic on onion. Our recent host range evaluation study identified two pathovars *P. stewartii* subsp. *indologenes* pv. *cepacicola* pv. nov. and *P. stewartii* subsp. *indologenes* pv. *setariae* pv. nov. In the current study we developed a pan-genome using the whole genome sequencing of newly identified seventeen *Psi* strains. The full spectrum of the pan-genome contained

7,030 genes. Among these, 3,546 (present in genomes of all 17 strains) were the core genes that were a subset of 3,682 soft-core genes (present in ≥ 16 strains). The accessory genome included 1,308 shell genes and 2,040 cloud genes (present in ≤ 2 strains). The pan-genome showed a clear linear progression with $>6,000$ genes, suggesting the pan-genome of *Psi* is open. Further, we conducted a horizontal gene transfer (HGT) study including three other *Pantoea* species namely, *P. ananatis*, *P. agglomerans* and *P. allii*. A total of 20 HGT events among the three *Pantoea* species were identified with most gene transfers observed between *P. ananatis* and *Psi* pv. *setariae* pv. nov. Pan-GWAS analysis predicted a total of 154 genes including seven cluster of genes associated with the pathogenicity phenotype on onion. One of the clusters contain 11 genes with known functions and are found to be chromosomally located.

PhONA: An R package for integration of system phenotypes in microbiome networks

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Understanding factors influencing microbial interactions, and designing methods to identify key taxa, are complex challenges for achieving microbiome-based agriculture. Here we developed an R package, PhONA (Phenotype-OTU Network Analyses), to integrate system phenotypes in a microbiome network, and provided a framework to select a testable and manageable number of OTUs/ASVs to support microbiome-based applications. The package was evaluated using a fungal OTU dataset generated for a grafted tomato system, aimed at understanding how grafting and the choice of rootstock influenced root-associated fungal communities. Three tomato rootstocks (BHN589, RST-04-106, and Maxifort) were grafted to a BHN589 scion and the associated endosphere and rhizosphere fungal communities were profiled by metabarcoding using the fungal Internal Transcribed Spacer 2. Diversity analyses provided evidence for a rootstock effect (explaining $\sim 2\%$ of the total variation, $P < 0.01$) on the fungal community. Differentially abundant OTUs specific to each rootstock were identified for both endosphere and rhizosphere. PhONA first identifies OTUs predictive of phenotype using lasso regression, then uses the predicted OTUs from lasso regression to build a reduced GLM. PhONA then combines the results of GLM to indicate positive or negative associations of the predicted OTUs with the host phenotype, and with other OTUs in a network model. Future studies of taxa associated with effective rootstocks identified using PhONA could support the design of synthetic communities for microbiome-based agriculture.

Analysis of population structure and differentiation, linkage disequilibrium decay, and demographic history of *Fusarium graminearum* from the U.S.

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Fusarium graminearum (*Fg*) is an ascomycete fungus that causes Fusarium head blight (FHB) of wheat and barley. In addition to yield loss, the fungus also can contaminate grain with trichothecene mycotoxins. Strains are divided into chemotypes based on the trichothecene mycotoxin produced (e.g., 3ADON, 15ADON, NX2, and NIV). Large-scale population genomics studies with *Fg* are limited. We analyzed 454 isolates of *Fg* from the upper Midwest, New York, Louisiana, and South America based on $>90,000$ SNP markers obtained from genotyping by sequencing. Three populations, as well as several admixed strains, were apparent in our U.S. sample based on structure analysis; isolates group into populations based on chemotype and geographic origin. Demographic inference suggests that the three populations separated from a common ancestor at roughly the same time. These models are consistent with changes in size after the split and moderate gene flow between populations. Differentiation between pairs of populations was fairly high (F_{ST} 0.385–0.551), reflecting a long time since the split. Recombination has been frequent, as linkage disequilibrium decays to half of the highest value within 15 kb and reaches background levels within 300 kb for all populations. Understanding structure and the evolutionary potential of populations of *Fg* is critical to effective FHB management. Despite high divergence, these populations are not genetically isolated, and they have a high evolutionary potential given a greater rate of recent intermixing (apparent from admixed strains) and frequent recombination.

Emerging *Penicillium* species in commercial apple packinghouses in the Pacific Northwest

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Blue mold (BM), caused by several *Penicillium* species, is the most destructive postharvest disease of apple and pear worldwide. Although *P. expansum* has been recognized as the main causal species, BM caused by several other uncharacterized species may impose an emerging threat to the apple industry in the Pacific Northwest (PNW). A total of 966 isolates were collected between 2016 and 2018 from BM-symptomatic apples in various packinghouses in the PNW and tentatively grouped into a larger group including *Penicillium* spp. based on initial phenotyping. A total of 577 isolates were identified as candidates for further evaluations on CYA, MEA, PDA, and YES media for 10 days in 23°C. Based on the growth characteristics of *Penicillium* on aforementioned media, a subset of 166 single-spore representative isolates was obtained and subjected to morphological and molecular characterizations. The morphological characterization and species distribution of BM-causing species are presented. The information on species distribution in the given pool of *Penicillium* isolates will determine the potentially emerging BM species in the PNW. Furthermore, the data on fungicide sensitivity and in vivo pathogenicity will determine the scope of future disease management strategies and acquire new knowledge to mitigate the development of fungicide resistance in this persistent postharvest pathogen in pomes.

Response of spinach varieties to anthracnose (*Colletotrichum dematium*) in the Texas Winter Garden

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Anthrachnose, caused by *Colletotrichum dematium*, is a challenge to US spinach production, particularly in the Texas Winter Garden. Producers have voiced interest in optimizing management practices, including evaluating disease resistance of existing varieties. The objective of this work was to determine the response of spinach varieties to inoculation with *C. dematium*. Eighty-four varieties of interest to Texas producers were selected, planted in a randomized complete block design with 3 reps, and managed under standard field production practices with sprinkler irrigation. Plots were inoculated with *C. dematium* infested oats at a rate of 10 g/m² dry wt at 28 dap. Plots were rated for disease symptoms at 21, 35, and 42 dpi using a scale of 0–10 (0 = 0% symptoms, 1 = 1–10%, 2 = 10–20%, etc.) reflecting the amount of diseased plants in the plot. Of the 84 varieties tested, 3 had a rating of 3 or less; 51–354, Canapus, and E1. Two additional varieties, E3 and E4 had average ratings of 5.3 and 6, respectively. Overall, the majority of commercial spinach varieties in this study were highly susceptible to *C. dematium* under current production practices with respect to planting density and irrigation regimens. Experimentation with reduced planting density, alternate irrigation, and combining multiple management methods preventatively or early in an epidemic are currently the most promising strategies until improved varieties are available commercially.

The perfect storm: Hurricane damage, lower stem and root feeding beetles and their associated blue stain fungi within the longleaf pine savanna

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Wind disturbance events can allow insects and their associated mycobiome to rapidly exploit host material and increase their populations. We sought to better understand these interactions, and evaluate longleaf pine management techniques (including prescribed fire and salvage logging) for their effects on relative abundance of root infesting insects and fungi. In October 2018, the Jones Center at Ichauway, a 12,000-ha longleaf pine-dominated, frequent fire ecosystem, was heavily damaged by hurricane Michael. We used three management treatments in wind disturbed areas: 1) Prescribed burning with salvage logging; 2) prescribed burning without salvage logging; and 3) no prescribed burning, no salvage logging. We replicated these treatments five times, creating 15 plots across the study site. We sought to answer three questions: Are there differences in beetle abundance across treatments? At this regard, we are analyzing beetle abundance over a 2-year period by using Lindgren funnel traps baited with beetle attractants. What blue stain fungi are commonly associated with target beetles? To answer this, we have cultured fungi from live target beetle species to isolate and identify blue stain species. What blue stain fungi are commonly found within the roots of healthy and symptomatic longleaf pine roots? For this purpose, we collected samples from roots of two healthy and two symptomatic trees from each plot and plated them to isolate and identify blue stain species.

Species composition and genetic diversity of soilborne *Fusarium* species from 17 counties in Pennsylvania

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Fusarium root rot and wilt are important soilborne diseases observed in many parts of Pennsylvania (PA). However, knowledge on diversity among *Fusarium* species in PA causing such diseases is scarce. Therefore, this study was focused on determining species composition and genetic diversity of *Fusarium* isolates across PA. Soil samples collected from 17 farmer fields during summer 2018 and 2019 were used to acquire 313 putative *Fusarium* isolates using Nash and Snyder medium. For species confirmation, single-spore cultures of the isolates were morphologically characterized on PDA and subjected to PCR targeting TEF-1 α and RPB2 genes. PCR amplicons were sequenced, and homology was explored using *Fusarium* MLST and NCBI databases. Multiple sequence alignment was performed using ClustalW and a phylogenetic tree was constructed by MEGA 7 software using maximum likelihood method. Analysis revealed that the majority (67.1%) of isolates belonged to the *F. solani* species complex (FSSC 3+4, FSSC 5, FSSC 9, FSSC 11, and FSSC 15). The rest were categorized into *F. oxysporum* (FOSC) (27.8%), *F. incarnatum-equiseti* (2.9%), *F. nisikadoi* (1.9%), and *F. sambucinum* (0.3%) species complexes. Molecular phylogeny results resolved isolates from each complex into separate monophyletic clades while FSSC and FOSC isolates were further divided into nine and five sub-clades, respectively. Outcome of this study provides a sound base for strategizing future experiments towards establishing best management methods to control *Fusarium* diseases in PA crop fields.

Multilocus detection of *Ganoderma zonatum* from environmental samples

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Ganoderma zonatum, a white rot fungus, is responsible for butt rot disease on palms. Typical symptoms include wilting of fronds that starts from the lower part of the canopy and moves to the top. As wilting symptoms are also associated with other diseases and disorders, the appearance of conks on the trunk is necessary to confirm the presence of this fungus. Early detection of *G. zonatum* in symptomatic palms is a crucial step towards building and implementing better disease management strategies. The objective of this study was to develop a DNA-based molecular diagnostic assay that could be used as a diagnostic feature to confirm the presence of *G. zonatum* in palm trunks before conks were observed. In order to design a species-specific test, primers were tailored to end on SNPs that could differentiate *G. zonatum* from eleven other *Ganoderma* species. Multiple regions in four genes, ITS, *tefla*, *rpb1*, and *rpb2*, were targeted and tested before narrowing down to a set of two primer pairs to detect *G. zonatum* with high specificity and sensitivity. This assay could successfully determine the incidence of *G. zonatum* in different environmental samples, including, saw dust collected from naturally infected palm trunks as well as soil samples containing *G. zonatum* basidiospores. Development of this PCR-based assay reduces the time needed to diagnose *G. zonatum* in palm samples by several months. Additionally, it is a potential tool to detect and track the presence of the fungus in different palm tissues and environments.

In-depth virome study linking tomato, associated weed plants, and irrigation water sources in Slovenia reveals many new and known plant viruses

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Tomato is one of the most important vegetable crops, and is associated with the highest number of plant viruses known for any plant species. To better understand the impact of viruses on tomato health and understand their epidemiology in an ecosystem context, a holistic overview of the complete virome of tomato, and of other associated parts of the specific agroecosystem (other plants, environmental samples) is needed. In the frame of the ongoing INEXTVIR project (www.inextvir.eu), we have used high-throughput sequencing to define the virome of tomatoes, surrounding weed species, and linked irrigation water samples in Slovenia. Close to 500 samples of asymptomatic and symptomatic tomatoes, surrounding weed plants and irrigation water sources were collected on 14 localities, from 2019 to 2020. Sequencing of systematically prepared plant pools revealed the presence of 41 known, and at least 68 putative new viral species in tomatoes and weeds, with high fraction of new virus discoveries (~80%) in weeds. Diverse plant viral species were also detected in irrigation waters, in which the virome structure was dependent on the type of the sampled water (e.g., surface, river, underground). Most of the viruses were detected in one type of the sample (tomatoes/weeds/water); however, few of them were also detected across different sample types, implying their persistence in the ecosystem. The comprehensive virome datasets obtained in this study represent a baseline data, which will help us detecting and better understanding possible future emergences of viral diseases in tomato.

Evaluating the effects of at-plant fungicides on potato soilborne diseases and soil microbial communities

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Soilborne diseases such as Rhizoctonia and silver scurf represent a significant limitation to potato productivity and tuber quality in the U.S. Due to limited host resistance, these diseases are typically managed with fungicides. Concerns with adverse effects of pesticides on beneficial soil microorganisms, which mediate many key soil functions including disease suppression, have led to consideration of biological fungicide alternatives. The performance of biological fungicides against soilborne disease, however, is often inconsistent in performance. In 2020, field trials were conducted to evaluate the effects of at-plant chemical fungicide and biological control treatments on the severity of Rhizoctonia and silver scurf diseases of potato, tuber yield, and soil microbial communities. There was no disease incidence in the Rhizoctonia trial. However, the conventional chemical fungicide Cruiser Maxx Vibrance and the biocontrols AmyProtec42 (28 ml per 4 plot), Howler, and Serenade resulted in significantly higher yield than the non-treated control. There was low silver scurf disease pressure, and Maxim MZ resulted in significantly lower disease severity than the non-treated control. The chemical fungicides, Maxim MZ + Quadris, Quadris, Cruiser Maxx, and the biological control treatment, Regalia, resulted in significantly higher yield compared to the non-treated control. 16S and ITS rRNA gene amplicon sequencing to characterize the bacterial and fungal communities under different treatments is underway.

Cladosporium* species are potential antagonists of emerging fungal pathogens *Bipolaris yamadae* and *B. gigantea

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Leaf spot, blight, crown rot and melting out are symptoms of diseases caused by *Bipolaris* pathogens on major crops and native, invasive, and weedy grasses. In recent survey efforts, we isolated *B. gigantea* and *B. yamadae* from native and invasive understory grasses in multiple U.S. states by single-spore transfer from sporulating leaves. Our isolation efforts frequently were thwarted by colonies of a contaminant identified as *Cladosporium* fungi. Species of *Cladosporium* are common saprophytes, but some also have been reported to be antagonistic to fungal pathogens that cause chrysanthemum white rust and apple scab diseases. Our objectives were to 1) identify these *Cladosporium* isolates, 2) determine if they were antagonistic to *Bipolaris* in co-plating assays, and 3) co-inoculate host grasses with *Cladosporium* and *Bipolaris* to test for an effect on disease severity. Multi-locus sequence analysis identified isolates as members of the *C. cladosporioides* species complex. Some *Cladosporium* isolates produced a zone of inhibition that extended beyond the colony and through which *Bipolaris* would not grow. The invasive grass *Microstegium vimineum* inoculated with *B. gigantea* and *B. yamadae* developed 10% and 20% less disease severity, respectively, when co-inoculated with an antagonistic *Cladosporium* isolate. These experiments suggest *C. cladosporioides* has potential antagonistic effects on *Bipolaris* pathogens.

A systematic survey of grapevine trunk disease pathogens in Oregon vineyards

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Grapevine trunk diseases (GTDs) are caused by a variety of fungal pathogens which have been grouped into the following complexes: Petri disease, Blackfoot, Esca, Botryosphaeria dieback, Eutypa dieback, and Phomopsis dieback. Pathogens commonly enter pruning wounds, colonizing and blocking xylem vessels leading to stunted growth and eventual death of the vine. GTDs have been recorded around the world including in continents such as Asia, Europe, North America, and Australia. This study has sought to provide systematic data on which types of GTDs are most prevalent in Oregon by surveying and collecting samples from vineyards in southern and northern Oregon. Symptoms data was collected from vines identified following a systematic random sampling procedure in 30 vineyards. Depending on the trunk diameter, one to three inch holes were drilled into the trunk to collect wood tissue samples. Fungal

isolation followed both morphology and DNA based identification. Based on symptoms data, some vines appeared to have dead arm, stunted shoot growth, and/or yellowing or reddening leaves while other vines appeared healthy. Pathogens causing Botryosphaeria and Eutypa dieback, Esca, and Blackfoot have been morphologically identified and DNA sequencing of the ITS and elongation factor regions is under progress to identify the pathogenic species. This study provides a comprehensive list of not only the different GTD complexes found in Oregon, but also the most common fungi found in Oregon vineyards. This knowledge can be used to more effectively manage vineyards in order to control GTDs.

Tad pilus-mediated twitching motility is essential for ComEC-dependent DNA uptake and survival of *Candidatus Liberibacter* spp.

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L. crescens (Lcr) is the only axenically cultured Liberibacter to date and serves as a surrogate for functional genomic studies of the uncultured pathogenic *Candidatus L. asiaticus* (Las) and *Ca. L. solanacearum* (Lso). All Liberibacters encode a complete gene repertoire for Tad (tight adherence) pili, primarily associated with surface adherence. Presence and function of a Tad pilus was demonstrated for twitching motility in Lcr. Repetitive extension and retraction of Tad pilus resulting in twitching also implicated its role in uptake of extracellular DNA since no other competence-associated pili are present in Lcr, Las, or Lso. Disruption of the Tad pilus genes *cpaF*, *cpaA*, *cpaB*, and *tadC* resulted in severely reduced bacterial growth. Given that all Liberibacters are deficient in nucleotide biosynthetic genes, loss of twitching motility and viability for the *cpaF* mutant indicated a critical requirement for Tad pilus-mediated uptake of extracellular DNA for food. Mutation in the inner membrane competence protein gene *comEC* had no effect on either motility or growth but completely abolished natural transformation in Lcr. The Lcr *comEC* mutation was complemented by cloned *comEC* from Lcr but not from Las strain psy62. Lcr CpaF was essential for uptake of fluorescent dsDNA through the outer membrane and Lcr ComEC was essential for uptake of fluorescent ssDNA through the inner membrane in Lcr. Tad pili facilitate adherence, motility, and uptake of extracellular DNA for food in all Liberibacters. However, their role in natural competence is not conserved within pathogenic Liberibacters.

Comparative genomics and phylogenetic analyses suggest a taxonomic re-organization and inclusion of a new species in the genus *Clavibacter*

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Clavibacter is a Gram-positive bacterial phytopathogen of important crops including tomato, wheat, corn, and alfalfa. The genus currently has six recognized species and three subspecies. Here we examined the phylogeny and taxonomic status of three *C. michiganensis* subspecies (*californiensis*, *chilensis*, and *phaseoli*) by sequencing genomes of their type strains, plus *C. tessellarius*, and a seed-associated *Clavibacter* strain, A6099. Complete genomes were sequenced using PacBio RS II. The in silico DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) revealed numbers lower than the 70% and 96% threshold values, respectively, for species delineation among all three *Clavibacter* subspecies, thus supporting the elevation of these subspecies to species level. Additionally, orthologous analysis using OrthoMCL, phylogeny, ANI and dDDH data showed a genome homology of 99% between *C. m.* subsp. *chilensis* and *C. m.* subsp. *phaseoli*, indicating that these two members should be merged into a single species. Lastly, comparative genomics allowed differentiation of a separate clade composed of three tomato seed-associated strains, and above procedures indicated that this clade should be considered a new species, for which the name *C. lycopersicum* sp. nov. is proposed with strain A6099 as the Type strain. Based on our polyphasic approach, taxonomic classification in the genus *Clavibacter* should be emended to provide an accurate distinction among the lineages especially during surveillance and epidemiological studies.

Testing and utilization of grapevine-isolated *Trichoderma* spp. for improved control of fungal grapevine trunk diseases and Pierce's disease

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Grapevine trunk diseases, including canker diseases caused by fungi and Pierce's disease caused by the bacterium *Xylella fastidiosa*, can be difficult to control once established within a vineyard. Current management involves the use of pesticides to reduce inoculations. However, sustainable management will require alternative, longer-lasting control options. Therefore, grapevine isolates of *Trichoderma* spp. were obtained in California vineyards to assess ability to reduce incidence and severity of different grapevine trunk diseases. Thus far, a total of six new Californian *Trichoderma* spp. isolates from grapevines have exhibited strong ability to limit growth of the fungal pathogens *Diplodia seriata*, *Eutypa lata*, and *Neofusicoccum parvum* in culture plate assays. Two of these isolates also have been tested for the ability to limit growth in planta. Grapevines treated with these isolates had significantly reduced *D. seriata* and *X. fastidiosa* infection progression. Currently, additional isolates are being obtained and both further in vitro and in planta testing is proceeding. Identification of new *Trichoderma* spp. isolates to control fungal pathogens and *X. fastidiosa* should provide environmentally-friendly options for grapevine trunk disease management.

Characterization of cystosorus-associated microbes as antagonists of the soilborne protist *Spongospora subterranea* f. sp. *subterranea*

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Spongospora subterranea f. sp. *subterranea* (Sss) is a soilborne protist responsible for causing powdery scab on tubers, reducing yield, and vectoring potato mop-top virus (PMTV) on potato plants. There is currently no effective chemical or biological control against Sss. We hypothesize that some microbes naturally associated with Sss are particularly effective as natural antagonists. Thus, we aim to identify these antagonists by exploring microbes co-existing with Sss and investigating their capacities to prevent Sss root colonization and plant growth reduction. Sss-associated bacteria and fungi were isolated from antimicrobial-treated and/or vortexed cystosori suspensions, then identified by 16s and ITS PCR, respectively. Out of 59 isolates screened, we identified 22 bacterial and 15 fungal isolates with antagonistic potential, such as *Bacillus* spp. and *Trichoderma* spp. Significantly higher rates of isolates with established antagonist-potential were identified among the fungi collected from antimicrobial-treated cystosori compared to fungi collected from non-treated cystosori. This suggests potential antagonists may live in close association with Sss, since antimicrobial treatment cleansed the suspension and cystosori surface, selecting for microbes most closely associated with Sss. Greenhouse assays are in progress to investigate the antagonistic activities of these microbes. This project is an important first step in identification of novel biological control opportunities for Sss, which may lead to critical breakthroughs in management of this challenging disease.

Exogenous application of coat protein-specific dsRNA inhibits Cognate cucumber mosaic virus (CMV) of bhut jolokia (ghost pepper)

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Cucumber mosaic virus (CMV) is the major virus infecting bhut jolokia (interspecific hybrid between *Capsicum chinense* and *C. frutescens*) that causes considerable yield losses. We hypothesized that the exogenously applied double-stranded RNA (dsRNA) in plants can mimic the viral RNA intermediate that facilitates in planta virus replication and trigger post-transcriptional gene silencing via the RNAi pathway. The specific objectives of this study were to develop a dsRNA construct for CMV resistance in bhut jolokia and to conduct a proof-of-concept study on CMV silencing in bhut jolokia using exogenously applied dsRNA targeting the CMV coat protein (CP). CMV infected leaf samples were collected from bhut jolokia fields and CMV infection was confirmed by RT-PCR. In vivo production of dsRNA was successful in *E. coli* with the use of T7 RNA polymerase. To investigate resistance induction against CMV, crude extracts of dsRNA derived from CMV-CP gene (dsRNA_CMV-CP) expressed in *E. coli* were exogenously applied with CMV onto healthy bhut jolokia plants and compared with plants inoculated with CMV alone. Symptoms are scored by disease severity scale and treatments were screened by RT-PCR to check the presence of CMV virus. Exogenously applied dsRNA on leaves inhibited CMV symptoms on bhut jolokia plants relative to plants inoculated with CMV alone. Thus, exogenous application of dsRNA molecules derived from CMV CP gene can be a novel tool to suppress CMV and improve yield in these peppers.

Characterization of scabin, a mono ADP-ribosyltransferase toxin produced by the common scab pathogen *Streptomyces scabiei*

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Common scab is a widespread bacterial disease of potato and other crops. The disease creates superficial, raised, or pitted corky lesions on the surface of potato tubers that reduce their market value and cause major economic losses. To date, no fully resistant potato cultivars exist, and current management strategies are inadequate or inconsistent. Studies of the virulence factors of the main causative agent, *Streptomyces scabiei*, are therefore of high importance. Recently, a new putative virulence factor called scabin was discovered in the genome of *S. scabiei*. Scabin is a mono-ADP ribosyltransferase toxin that has been shown to possess in vitro activity toward DNA, RNA, and small guanosine-containing molecules, a rare activity among these toxins. However, the in vivo target of scabin remains unknown. The objective of this study was to investigate the function of scabin and determine its role in the pathogenesis of *S. scabiei*. To achieve this, a scabin knockout mutant of *S. scabiei* was created. The mutant was then tested in leaf infiltration and potato tuber slice bioassays to evaluate the virulence phenotype of the mutant compared to the wild-type strain. The conditions under which the scabin gene is expressed was also investigated using RT-PCR analysis. Subsequently, it was discovered that a neighboring gene is co-transcribed with the scabin gene, suggesting that they may be functionally linked. The distribution of proteins homologous to scabin in other *Streptomyces* species, and the relative position of those genes to other genes of interest, was also mapped out.

A good inoculation method to be used in the disease assay of *Rhizoctonia solani* in sugar beet

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Sugar beet (*Beta vulgaris* L.) is one of the major sugar sources for the world. *Rhizoctonia solani* causes damping-off, and crown and root rot that can result in significant economic losses. *R. solani* AG 2-2 IIIB is the most damaging anastomosis group in the world beet production. In this study, we evaluated different types of inocula-fungal mycelium on barley seeds, mycelial plug and sclerotia on their efficacy to induce disease in sugar beet. First, the fungal growth rate and sclerotia production were compared on six types of media, including clarified V8 (CV8), potato dextrose agar (PDA), metalaxyl benomyl vancomycin (MBV), yeast malt agar (YMA), corn meal agar (CMA), and oatmeal agar (OMA). The fungus grew faster and produced more sclerotia in CV8 media than any other media. The different types of inoculum were then used to inoculate 4-leaf stage sugar beet plant and disease severity recorded. Results from the study suggest mycelia on barely seeds would be the best for *R. solani* disease evaluations due to its ease of production, cost, and ability to consistently produce disease.

Nematode virome: Complexity and phylogenetic implications

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In recent years, several new viruses infecting free-living and plant-parasitic nematodes have been described. The Orsay virus was the first virus found to infect a free-living nematode, *Caenorhabditis elegans*. Afterwards, several viruses have been identified in sedentary plant-parasitic nematodes, such as the soybean cyst nematode, *Heterodera glycines*; two potato cyst nematode species, *Globodera pallida* and *G. rostochiensis*; the clover cyst nematode, *H. trifolii*; sugar beet cyst nematode, *H. schachtii* (from the family Heteroderidae); and the migratory root lesion nematode, *Pratylenchus penetrans* (from the family Pratylenchidae). Thus far, comprehensive research focusing exclusively on the metagenomic analysis of the nematode virome has not been carried out. We have attempted to fill this gap in our knowledge by investigating viral communities associated with soil-inhabiting nematodes, which included both free-living and plant-parasitic lifestyles. This study has demonstrated a remarkable diversity of RNA viruses infecting nematodes in the natural environment and has revealed a number of novel, undescribed viral species.

In vitro testing of *Pseudomonas* for antagonism against *Pythium aphanidermatum* and assessment of plant growth promotion on hydroponically grown spinach

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Pseudomonas has been extensively studied for their plant growth promoting potential and disease suppressing potential. These qualities present *Pseudomonas* as a potentially useful tool in the management of *Pythium* infections on hydroponically grown crops while simultaneously increasing plant yield. Utilizing a collection of *Pseudomonas* strains we aimed to: 1) use dual plating, top agar, and volatile indirect exposure in vitro assays to identify strains with antagonistic activity against *Pythium aphanidermatum* isolate PA5-18 (previously collected from hydroponically grown spinach); and 2), assess the plant growth promoting potential of these *Pythium* suppressing strains on hydroponically grown spinach under greenhouse conditions. In dual plating assays, *Pseudomonas* strains Darke and 1B1 inhibited (>50%) the mycelial growth of PA5-18. Ten *Pseudomonas* strains were found to be inhibitory (>50%) in shared-air volatile exposure assays and two strains were inhibitory (>70%) using the top agar method. For the assessment of plant growth promotion, *Pseudomonas* strains Darke and 14B11 increased fresh and dry root weight by 20% while strain 1B1 decreased fresh root and dry root weight as well as aboveground biomass measurements (by 30%). Future experiments will revolve around determining antagonistic activity of the best performing strains in planta against *P. aphanidermatum* and further evaluation of plant growth promoting potential on spinach.

Increase in soybean cyst nematode prevalence in Kansas from 2010 to 2020

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The soybean cyst nematode (SCN), *Heterodera glycines*, is a major threat to soybean production worldwide. First detected in Kansas in 1985, SCN continues to be an emerging issue for the state. Two surveys of Kansas soybean production fields were conducted a decade apart (2010 and 2020) to assess changing SCN prevalence and abundance. A total of 635 and 302 soil samples were collected during the 2010 and 2020 surveys, respectively, with sampling ongoing in 2021. Cysts were extracted from 100-cm³ subsamples and mechanically ruptured for egg enumeration. Additionally, HG Type was determined for positive samples collected during the 2010 survey. The nematode was detected in 11% of samples (15% based on bioassays), including 37 of 76 counties and 7 of 8 crop reporting districts (CRDs) sampled during the 2010 survey. Maximum egg counts were observed for the South Central, Southeast, and Southwest CRDs with 10,880, 10,720, and 10,720 eggs/100cm³ soil, respectively. Prevalence of the nematode increased to 35% and the average egg density doubled during the 2020 survey, with the largest increases in prevalence observed for the Central, South Central, East Central, and Northeast CRDs. Nearly 60% of populations from the 2010 survey were classified as HG Type 2. Although prevalence is still relatively low, counties with positive samples account for about 85% of total soybean production in the state. As SCN continues to progress from an emerging to a prevailing threat to Kansas soybean production, greater emphasis on monitoring will be a critical component of future management strategies.

Organic disease management potential of essential oils in managing root rot and damping-off pathogens (*Pythium* spp.) of pulse crops

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Pythium spp. are important soilborne pathogens of pulse crops, with crop losses estimated up to 50%. Management relies on synthetic fungicides, and resistance has developed in some areas of the USA. There is a considerable interest in developing alternative management options for root rots of pulse crops, particularly in the organic industry. Amongst 38 essential oils (EOs), palmarosa, oregano, clove, cinnamon, and thyme oils showed potential to inhibit growth of 10 important pulse crop pathogens. For this study, EOs were tested across five *Pythium* isolates representing *P. ultimum*, *P. sylvaticum*, and *P. irregulare*. In vitro efficacy was evaluated using food poison method, where isolates were grown on PDA amended with EOs (1:250 dilution). Negative control with plain PDA and two positive controls with PDA amended with metalaxyl, and ethaboxam fungicides (100 µg/ml a.i.) were used. A 100% mycelial growth inhibition was noted for all the isolates in presence of EOs. *P. sylvaticum* (LPY17) isolate was resistant to ethaboxam (0% growth inhibition) and showed reduced sensitivity to metalaxyl (24% growth inhibition). EO-seed treatments (0.4 ml/kg seeds) were tested for potential phytotoxic effects on seed germination and root nodulation on chickpea, pea and lentil. The results indicated no significant phytotoxic effect of EOs on root nodulation or seed germination ($P > 0.05$). Additionally, EO seed treatments, along with fungicide seed

treatments, are currently being used to determine in vivo efficacy in managing Pythium rot and damping-off on chickpea, pea, and lentil in greenhouse.

Impact of phosphorus and potassium fertilizers on endophytic *Fusarium* spp. in soybean

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Application of phosphorus and potassium fertilizers is shown to increase resistance in soybean (*Glycine max*) to plant pathogens. However, no research has looked at the effect of fertilizers on endophytes, in particular species of *Fusarium* that are known to dominate the root endophyte community present and cause Fusarium root rot in soybean. Thus, this study was undertaken to determine if phosphorous and potassium fertilizers can affect endophytic *Fusarium*. In 2021, a field trial was established in Brookings, SD, in a randomized complete block design consisting of five treatments (phosphorus [46.6 and 67.0 kg/ha P₂O₅], potassium [33.6 and 67.3 kg/ha K₂O], and a no-fertilizer control). At second trifoliate growth stage, six plants were randomly sampled from each plot, following which the roots were cut, surface-sterilized and incubated on potato dextrose agar at 22 ± 2°C. Seven days post-incubation, seventy-seven isolates of *Fusarium* were recovered. The fungal identity was confirmed by morphology and sequencing of translation elongation factor-1 alpha genes. At six trifoliate growth stage, stand counts were determined and Normalized Difference Vegetation Index (NDVI) values were obtained using an active sensor (GreenSeeker, Trimble, CA). Analysis of variance results showed that treatments have no significant effect on stand counts, isolation frequency of *Fusarium* and NDVI values ($\alpha = 0.05$). Future studies are needed to understand how application of fertilizers can affect severity of Fusarium root rot in soybean, when the endophytic community is dominated by species of *Fusarium*.

Emergence of a new *Melampsoridium* rust epidemic on hornbeam and hophornbeam in the southeastern United States

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Hornbeam and hophornbeam are naturally occurring as understory components in the forests of eastern North America. Fragmented populations of the latter are also present in Central America. Yellow pustules developing on the lower surface of the leaves of both species were first observed in fall 2018 in Gainesville, Florida. An initial survey in this region revealed incidence values of 100% on 20 randomly selected individuals of both host species, with severity measurements ranging from 7 to 15% in the middle of summer. DNA was extracted and amplified from urediniospores collected from both hosts. ITS and nuLSU sequences were searched against the NCBI GenBank database and respectively revealed a 97% homology with *Melampsoridium carpini* isolated from European hornbeam and 97% homology with *M. hiratsukanum* sampled from white alder in California. By the end of year 2020, individuals from both host species with similar sori were reported in Tennessee, Texas, the Carolinas and Georgia in natural areas, landscape settings as well as nurseries. Interestingly, infection on *Ostrya virginiana* var. *guatemalensis* from El Salvador was reported to us as well and seems to be conspecific with the rust observed across the southeastern US. Based on DNA, morphology, and ecology observations, and by comparing with herbarium specimens of the different species within *Melampsoridium* genus, this significant rust epidemic appears to be due to a novel fungal taxon and represents the first report of a *Melampsoridium* infection on *Carpinus* spp. and *O. virginiana* in North and Central America.

Informative *Fusarium oxysporum* protein-coding sequence markers for population and community analyses, identification, and improved diagnostics

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The *Fusarium oxysporum* species complex (FOSC) comprises many economically important soilborne pathogens that cause vascular wilt disease in a broad range of plant species. Putatively non-pathogenic isolates are also prevalent in natural and agricultural ecosystems and can be readily isolated from soil and roots of asymptomatic plants as endophytes or saprophytes. Despite this diversity, the presence of non-orthologous copies of ribosomal RNA genes, combined with low levels of sequence divergence among FOSC isolates, has hampered sequence-based classification within FOSC. We aimed to develop a multi-locus genotyping approach for an improved identification of FOSC isolates. Using 41 phylogenetic informative single copy nuclear protein coding genes, we retrieved their sequences from 167 clone-corrected *F. oxysporum* genomes representing 49 formae speciales and 54 isolates non-pathogenic on the host of recovery. In silico analysis of this dataset showed that five loci are capable of differentiating 89% of the isolates while all 41 loci are needed to reach 100%. Multi-locus diagnostic amplicon markers targeting the five loci were developed and validated by performing PCR assays followed by Sanger sequencing of DNAs from diverse FOSC taxa. A curated database cataloging *F. oxysporum* sequence types based on the five loci will be made available to the public so that a more reliable diagnosis of Fusarium wilt can be performed by diverse stakeholders.

Biological control of citrus bacterial canker by native endophytic *Bacillus* spp. isolates

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Citrus canker caused by *Xanthomonas citri* subsp. *citri* (Xcc) is one of the most devastating citrus diseases worldwide. Effective eco-friendly measures are lacking for control of citrus canker. Here we identified and evaluated three native citrus endophytic *Bacillus* spp. isolates for their potential to control canker disease. The isolates ATY16, PT6, and PT26A were determined to inhibit the growth of Xcc, promote citrus plant growth, and induce systemic defense responses, respectively. The three isolates were able to survive on citrus

leaves and established similar population sizes of 10^3 – 10^4 CFU/cm² leaf tissue at four weeks after foliar application under greenhouse and field conditions. A bioformulation developed with the isolates in combination significantly reduced both the number of canker lesions and bacterial populations of Xcc on citrus host leaves, and induced the expression of plant defense-related genes both in the salicylic acid (SA)- and jasmonic acid (JA)-dependent signaling pathways, after foliar spray in greenhouse assays. In the field, the bioformulation sprayed monthly provided effective control of foliar canker on young citrus trees, reducing the disease incidence by 54% (averaged from four replicated field trials), whereas a copper-based bactericide reduced the disease incidence by 71%. This study presents a useful source of biological control for managing citrus canker in greenhouse and the field.

Developing sustainable solutions to boxwood blight (*Calonectria pseudonaviculata*) with biological control agents

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Boxwood blight caused by *Calonectria pseudonaviculata* (Cps) is an emerging disease of global significance. In the U.S., this disease was first confirmed in Connecticut, Maryland, Massachusetts, New York, North Carolina, Oregon, Rhode Island, and Virginia in late 2011, and it has since spread to 22 other states. Current approach to this disease relies largely on fungicide protection, plus less susceptible cultivars. This fungicide-based approach is not sustainable due to human and environmental health risks, high cost, and potential chemical resistance issues. To address these issues, we have identified several new biological control agents (BCAs), while evaluating existing products on the market. This presentation highlights some potent BCAs we have identified to date. These included *Trichoderma koningiopsis* Mb2, *Pseudomonas protegens* (three strains), *P. lactis* SW, and *Burkholderia* sp. SSG. Specifically, this presentation brings to light the accidental nature of these discoveries, features individual BCAs' outstanding performance against Cps in the in vitro, in vivo, and in planta studies, points out additional traits of practical significance as appropriate, and discusses major steps being taken to move them from lab to field to serve the purposes, providing the horticulture industry and consumers with more sustainable solutions to the blight disease.

Late season infections of *Colletotrichum* spp. result in severe ripe rot of wine grapes

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The correlation between phenological stages of grape clusters and the infection of the devastating disease ripe rot (*Colletotrichum* spp.) is unclear. In a commercial Maryland vineyard with historically severe ripe rot, 20 clusters of the ripe rot susceptible cultivars Merlot and Cabernet Sauvignon were covered with wax paper bags at either bloom, bb-size, pea-size, berry touch, veraison, pre-harvest, or were left non-bagged. The late season bagging and harvest dates tended to be later for Cabernet Sauvignon than Merlot. Vaseline covered microscope slides were also placed in the vineyard every 7–11 days throughout the season and spores of *C. acutatum* and *C. gloeosporioides* complexes were quantified with qPCR. At harvest, disease severity was assessed, and *Colletotrichum* spp. were isolated from each cluster. In 2019, non-bagged and pre-harvest bagged clusters had statistically higher severity than clusters bagged at other times of the season for both cultivars. No significant difference was observed in ripe rot severity among the other treatments. A similar trend was observed in 2020. *C. fioriniae* and *C. nymphaeae* (*C. acutatum* complex) and *C. aenigma* and *C. fruticola* (*C. gloeosporioides* complex) were isolated in both seasons, and *C. fioriniae* was the most frequently isolated species. The spore traps detected DNA of both species complexes throughout the 2019 and 2020 seasons. Collectively, ripe rot occurrence in our trials seemed to be attributed to late-season infections, despite the presence of *Colletotrichum* spores throughout the growing season in both years.

Monitoring and identification of weed hosts of *Cucurbit leaf crumple virus* in Georgia

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Cucurbit leaf crumple virus (CuLCrV) is a begomovirus which can infect most cucurbits including cucumber, muskmelon, squash, pumpkin, and watermelon, as well as snap bean. Many weed species are known hosts of other viruses including weeds in the families *Amaranthaceae*, *Asclepiadaceae*, *Chenopodiaceae*, *Convolvulaceae*, *Euphorbiaceae*, *Solanaceae*, *Asteraceae*, *Malvaceae*, *Plantaginaceae*, *Fabaceae*, and *Apiaceae*. Weeds infected by CuLCrV may not show disease symptoms. However, weeds that harbor CuLCrV can serve as reservoirs of the virus even during non-crop grown seasons, which potentially influences epidemiology and spread of CuLCrV. During crop grown seasons, the virus can be transmitted from infected weeds to crop plants by whiteflies. In this project, we conducted a statewide survey to investigate the presence and prevalence of CuLCrV in different weed species within and around CuLCrV-infected vegetable fields in six counties in GA. Samples were tested for the presence of Cucurbit leaf crumple virus using molecular PCR-based assay at the Molecular Diagnostic Laboratory in Tifton. Based on our survey result, only two weed samples, namely wild cucumber (*Cucurbitaceae*) and wild radish (*Brassicaceae*) were detected as positive for the CuLCrV. *Cucurbitaceae* family plants are well-known hosts for begomoviruses; however, *Brassicaceae* family plants are not common for this group of viruses. Further research will be beneficial to conduct detailed pathogenicity study to confirm wild radish acts as a reservoir for this virus.

Defense-related secondary metabolism is species-specific in *Coffea* spp.

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Coffee is one of the most consumed beverages in the world, and the top agricultural export for many developing countries. High yields and grain quality that lead to the final product excellence depend on plant health. However, high pathogen pressure on coffee plants reduces productivity and beverage quality. New sources of resistance are therefore essential to provide breeders the best tools to defend coffee plants against disease and reduce production cost. Metabolomics is an outstanding approach to detecting and quantifying potential defense compounds in plants, as it identifies very small abundance compounds and track subtle changes in tissue chemistry. For that purpose, untargeted and targeted metabolomics were performed on wild *Coffea* spp. and commercial varieties using gas chromatography with mass spectrometry and liquid chromatography with tandem mass spectrometry, respectively. 160 Phenolic compounds were used as standards, because of their importance in plant defense. Leaves were collected from the Agronomic Institute (IAC) germplasm, in Brazil. Metabolic profiling revealed that even though these plants are closely related, their metabolism is intrinsic to each species. Defense related compounds, like 4-caffeoylquinic acid and the monolignin coniferyl alcohol, were found in wild species but not commercial varieties. Also, some yet-to-be-identified compounds were detected in high abundance only in wild plants. These promising results will complement genome data to facilitate the selection of wild *Coffea* sp. for breeding programs and improved coffee resistance.

Investigating a potential synergistic relationship between *Xylaria necrophora* and economically important nematodes of soybean
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Soybean is grown on more acres in Tennessee than any other row crop, with an annual value of \$583 million. Recently a fungal pathogen, *Xylaria necrophora*, has been discovered spreading throughout southern states. *X. necrophora* is the causal agent of taproot decline (TRD) in soybeans, and it has the potential to negatively impact yield. This disease can infect plants during any growth stage up until R6 with symptoms including interveinal leaf chlorosis, brown streaking of the vascular tissue, white mycelial growth in the pith, and a blackened appearance of the taproot. Although recent work has shed light on TRD susceptible varieties, fungicide efficacy, and alternate crop hosts, potential field risk factors and *X. necrophora*'s interactions with other pathogens has yet to be studied. Because nematodes have been known to interact with soilborne fungal pathogens to increase disease incidence and severity, it is crucial to know if this could occur with TRD. Therefore, the goal of this study is to investigate a potential synergistic relationship between plant parasitic nematodes (*Meloidogyne incognita*, *Heterodera glycines*, and *Rotylenchulus reniformis*) and *X. necrophora* using inoculated greenhouse trials.

Population structure and trichothecene chemotype of *Fusarium* species associated with maize kernels in Ohio
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Gibberella ear rot (GER), caused by members of the *Fusarium graminearum* (*Fg*) species complex, and associated mycotoxin contamination of grain have increased in frequency and severity in Ohio over the last decade. One of the many explanations given for this trend is a possible change in the population structure of the pathogen. The most common mycotoxins associated with *Fg*-infected grain are zearalenone and the type B trichothecenes nivalenol, deoxynivalenol (DON), and its acetylated derivatives 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol (15ADON). We previously determined the type B trichothecene content of 320 grain samples collected from 16 hybrids planted at 10 locations across the state during the 2017 and 2018 growing seasons and only detected DON and 15ADON. Here we used molecular tools to formally characterize and study the population structure and trichothecene chemotype composition of *Fusarium* species isolated from more diverse samples of maize grain. A total of 270 isolates of *Fusarium* spp. were isolated from kernels harvested from fields in 13 Ohio counties from 2015 to 2018, of which 90 were morphologically identified as *Fg*. Trichothecene chemotyping of 46 of these isolates using *Tri3* and *Tri12* multiplex PCR assays showed that all isolates were of the 15ADON chemotype. This supports previous reports of 15ADON being the predominant *Fg* chemotype in Ohio, but additional isolates are being characterized to help confirm these results. Findings from this study would be important for determining the baseline population structure of *Fg* causing GER in Ohio.

Adaptation of the genus *Pantoea* (Gammaproteobacteria: Enterobacteriales: Erwiniaceae) to multiple hosts and environments
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Pantoea is a diverse bacterial genus that includes strains with the ability to colonize plants as commensals or pathogens while other strains have been found in association with humans, insects, animals, and the environment. Some strains have the ice nucleation activity gene and cause frost damage on crops and may contribute to the formation of precipitation in clouds. Some encode one or more type three secretion systems and respective effectors injected into plants or insects. Other strains encode enzymes and toxins that either contribute to plant colonization or help suppress growth of competitors. However, for many *Pantoea* species we do not know if they are specifically adapted to different environments and occupy different ecological niches or if they were isolated from different environments but are actually generalists. To answer this question, we are performing comparative, evolutionary genomics analyses of publicly available *Pantoea* genomes using comparative evolutionary genomics tools, such as LINflow, pyani, and PIRATE. Since recombination plays an important role in bacterial evolution, ClonalframeML and Gubbins are being used to detect regions with frequent recombination and PopCOGent is being used to cluster isolates into ecologically meaningful groups. This analysis is expected to lead to an improved classification and characterization of the genus *Pantoea* necessary for precise identification of strains as plant pathogens, human pathogens, or beneficials with important implication for the safe use of *Pantoea* strains as biocontrol agents and for disease control.

Determining baseline fungicide sensitivity in *Cercospora* spp. from Arkansas to DMI and QoI Fungicides

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Cercospora foliar diseases (*Cercospora* leaf blight-CLB and frog-eye leaf spot-FLS) are one of the most yield limiting foliar disease of soybean in Arkansas. Control is mainly based on the use of fungicides and/or resistant cultivars. Increased use of foliar fungicides and limited diversity of fungicide active ingredients can result in the development of fungicide resistant pathogen populations. The goal of this study is to establish a *Cercospora* spp. baseline sensitivity against the demethylation inhibitors (DMI) fungicide tetraconazole (FRAC 3) and Quinone outside Inhibitors (QoI) fungicide azoxystrobin (FRAC 11). Symptomatic leaves with CLB or FLS and seeds with purple seed stain were sampled from fields in six Arkansas counties. Isolates were obtained from single spores or hyphal tips. Isolates were grown for 7 days on potato dextrose agar (PDA), and a plug was taken from the edge of the colony to transfer to fungicide amended plates with either tetraconazole at six concentrations (ranging from 0 to 50 mg/L) or azoxystrobin with SHAM (60 mg/L) at seven concentrations (ranging from 0 to 10 mg/L). Isolates were incubated at 27°C, and colony diameters measured at 7 days. Some isolates show tolerance to both tetraconazole (EC₅₀ ranged from 5 to 18 mg/L) and azoxystrobin (EC₅₀ ranged from 3 to 18 mg/L). Based on the results, *Cercospora* spp. have tolerance to QoI as reported in nearby states (Louisiana and Tennessee) and this establishes a base for monitoring DMI fungicides resistance program in Arkansas, which could help to address challenges in the future.

Parsing the profiles of fungicide resistance, pathogenicity, and genetic variation in *Botrytis*

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Botrytis bunch rot is one of the most devastating grape diseases. The population diversity of *Botrytis cinerea* contributes to the adaptation to environment change and pathogenic strategies. In this study, morphological classification was performed with a *Botrytis* collection isolated from the vineyards of Ningxia province, China and Washington State, USA, based on the colony color, mycelial morphology, spore sporulation and sclerotium. Four representative *Botrytis* strains 101V3Dd, 207cb, HDQ, and R1 were selected for further profiling fungicide resistance and pathogenicity. Results showed that HDQ strain exhibited multiple fungicide resistance to carbendazim, fludioxonil, iprodione, imazalole, pyridylamide, and cyprodinil, while R1 was sensitive to all these fungicides except pyridylamide. High virulent *Botrytis* strains, such as 207cb and HDQ, presented high laccase production *in vivo* and suppressed the defense responses by decreasing the production of phenolic compounds in the berries. Low virulence strain R1 strain increased phenolic compounds in grape berries and lost the laccase-producing ability *in vivo*. R1 strain was re-sequenced and aligned with previously sequenced 05.10 reference genome. Comparative analyses revealed variation in virulence between *Botrytis* isolates was related to cell-wall-degrading enzymes and biosynthesis pathways of toxin and melanin. Our study provides helpful information for understanding the strategies of environmental adaption and pathogenicity in *B. cinerea*.

Synthetic microbial consortia protect wheat against a soilborne fungal pathogen

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The beneficial effects of single bacterial isolates on plants have been extensively studied. However, monocultures usually will not sufficiently improve plant resilience against stressful conditions due to reduced microbial diversity. Synthetic microbial communities (SynComs) could potentially replace the functions of the plant microbiome to promote crop growth. Here, we characterized a collection of bacteria previously isolated from the wheat rhizosphere for antifungal activity against soilborne fungal pathogens. Fourteen isolated rhizobacteria displayed antifungal activity against *Rhizoctonia solani* AG8 (AG8) *in vitro* and *in vivo*, nine of them impacted the root development of *Arabidopsis* by producing auxin (IAA) or indole derivatives, and ten isolates produced volatile organic compounds (VOCs) that inhibited the growth of AG8. Subsequently, ten SynComs with different compositions from 14 isolates were designed. Four of them significantly enhanced wheat performance upon AG8 infection, compared to the corresponding single bacterial isolate. Together, this study demonstrated the potential for improving crop resilience by designing SynComs.

Viburnum downy mildew control: An action plan for growers

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Central Florida nursery growers have reported disease management challenges impacting the production of ornamental *Viburnum* spp. Growers reported symptoms of blighting and rapid defoliation indicative of downy mildew and a failure of common labeled fungicides to provide acceptable disease control. Subsequently, two trials were conducted at a commercial nursery, to evaluate the performance of fungicides available to nursery growers. Trials utilized natural pathogen populations present on diseased plant materials and included thirteen (Trial 1; July-August) and seven (Trial 2; September-October) fungicides and a non-treated control, with each treatment replicated ($n = 6$) and arranged in randomized complete blocks. Disease severity, based on weekly ratings of percent symptomatic foliage, was used to calculate AUDPC. Samples collected throughout both trials isolated *Colletotrichum*, *Corynespora*, *Pestalotiopsis*, and *Phyllosticta*, causing leaf symptoms similar to those commonly associated with downy mildew, but no *Plasmopara* sp. was observed during trials. Not surprisingly, all oomycete-specific fungicides failed to reduce disease severity relative to the non-treated control. However, fungicides containing benzovindiflupyr, difenoconazole, fluxapyroxad, and pyraclostrobin, statistically reduced disease severity. Copper sulfate, mancozeb, and flutriafol also failed to reduce disease severity, while a generic phosphite gave an intermediate level of control. Results stress the importance for nursery growers to make an appropriate disease diagnosis to avoid ineffective fungicide applications.

Field evaluation of novel compounds for management of bacterial spot of tomato

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Bacterial spot, caused by *Xanthomonas perforans*, is an important disease affecting tomato production in Florida. Alternative control measures are needed to overcome copper-resistance in pathogen populations and improve the disease control. In this study, treatments, mainly consisting of small molecules, were evaluated for their effects on reducing bacterial spot on tomato in two trials under field conditions. Eight applications were conducted starting at 2 weeks after transplanting following a weekly schedule in each trial. Tomato plants were inoculated with a copper-tolerant strain (1×10^8 CFU/ml) the day after the third application of treatments. The final disease severity, area under the disease progress curve (AUDPC), and fruit yield were analyzed using SAS program. Except Kocide 3000 in trial II, all treatments tested have significantly ($P < 0.05$) reduced the disease severity and AUDPC compared to the untreated control. Mankocide slightly improved efficacy in disease management compared to Kocide 3000, but lowered the fruit yield. Eugenol and tannic acid in the solution of cohere as a surfactant and caffeic acid also showed beneficial effects on increasing total fruit yield and the amount of extra-large and large sized fruit in both trials. Sodium salicylate and its rotation with actigard showed beneficial effects on fruit yield in trial I only. In this study, several compounds showed promising effects on reducing bacterial spot and improving fruit yield and further studies were needed to optimize their uses as potential tools in this disease management.

A novel agglutination assay to characterize the interaction between seed gall nematodes, *Anguina tritici*, *Afrina sporoboliae*, and *Rathayibacter* spp.

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Seed gall nematodes in the genus *Anguina* and *Afrina* are globally distributed and economically important. These nematodes are quarantine pests in some countries that impact agriculture by inducing seed galls in cereals like wheat and other forage grasses, and by vectoring the Gram-positive bacterial *Rathayibacter* spp. *R. toxicus* is listed as a select agent by USDA-APHIS because it produces tunicaminyluracil antibiotics in seed galls of ryegrass that results in a fatal livestock poisoning in Australia known as annual ryegrass toxicity. The specificity of interaction between *Anguinid* nematodes and the *Rathayibacter* spp. is unknown. The goal of this research was to determine whether the relationship between *A. tritici*, which affects wheat, and *Af. sporoboliae*, which was recently described from *Sporobolus cryptandrus*, and the *Rathayibacter* spp. they transmit is specific. An agglutination assay was developed as a proxy for adhesion of bacteria to juvenile nematodes (J2) that results in agglutination in compatible interactions. Fifteen bacterial species were incubated with J2 of *A. tritici* and *Af. sporoboliae* and agglutination rated for up to 24 h. *A. tritici* J2s strongly agglutinated only with *R. tritici* and *Af. sporoboliae* J2s only with *R. 'sporobolus'*. These data suggest the *Anguinid-Rathayibacter* interaction is highly specific; however, it remains to be determined whether *Anguinid* nematodes present in the U.S. can serve as vectors for *R. toxicus* since its interactions with nematode vectors are more promiscuous than other species.

Genetic changes correlated with host range divergence within a clade of plant pathogenic *Ralstonia*

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Diverse strains of the *Ralstonia* species complex cause bacterial wilt disease on dozens of different plant hosts. Prior work suggests that strains in the phylotype IIB sequevar 4 (IIB-4) clade exhibit two patterns of host range: Moko disease strains that wilt banana and tomato and “not pathogenic to banana” strains that wilt melon and tomato. Our goal is to identify genetic determinants, such as type III effectors, that underlie these patterns of host specificity. We have selected 18 *Ralstonia* IIB-4 strains isolated from six different hosts across the Americas for comparative genomics. We are sequencing genomes at 30x coverage with Illumina sequencing to assemble genomes. To identify the type III effectors in each strain, we will use the Ralsto T3E database. We will present a phylogenetic analysis of the strains overlaid with their effector repertoires and virulence patterns. Furthermore, we will compare between strains the prevalence of other classes of genes to determine if other traits, such as metabolic adaptations, also contribute to divergent host range within the clade. The specific genetic differences among these closely related strains may suggest which evolutionary forces contribute to shifts in host range among plant pathogenic bacteria.

Phenotypic variability among *Erysiphe necator* isolates

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Phenotypic variation among clonal isolates is an important issue in the study of plant pathogens, especially in obligate biotrophic fungi like *Erysiphe necator*. Through the maintenance of over 100 *E. necator* isolates collected from across the United States we have observed marked growth variability among isolates. To investigate these differences, we collected germination, latency, and colony expansion data from eight representative *E. necator* isolates exposed to temperatures from 10 to 34°C. Isolates were collected from Oregon vineyards during the 2017 growing season and equally represented both mating types and quinone outside inhibitor (QoI) fungicide resistance status. Percent germination was measured as the number of germinated conidia out of 50, latency in days from inoculation to the appearance of the first matured conidium, and expansion rate (mm²/day) over 20 days. There were no significant differences observed among isolate latency or colony expansion. Pairwise comparisons indicated that one of the eight isolates had greater total germination ($P < 0.05$). In addition, germination of the QoI resistant isolates was significantly greater than sensitive isolates at 19°C ($P = 0.036$), and mating type 1 had greater overall germination than mating type 2 ($P < 0.001$). These results indicate isolate germination differences that may lead to incorrect conclusions if single isolates are examined in future studies and suggest that multiple

factors such as mating type and fungicide resistance can contribute to this variation and may need to be considered during isolate selection.

Transcriptomic analysis of the effect of *Ca. P. pruni* infection on gene expression during sweet cherry fruit development

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Candidatus Phytoplasma pruni has reached epidemic levels in Washington state and is causing significant loss to cherry growers as infection results in small, misshapen fruit with poor color and taste. To understand how this pathogen disrupts fruit development a transcriptomic study was conducted, collecting samples of developing fruit and somatic tissues during the 2020 growing season from healthy and infected 'Bing' cherries in eastern Washington. Total RNA was extracted, submitted for Illumina sequencing, and differential gene expression analysis was performed. Differences in expression were observed in the fruit and somatic tissues. Specifically, cytochrome P450 78A9, a known regulator of fruit size, was downregulated in infected fruit. Additional genes of interest that were differentially expressed in the fruit are associated with cell wall development and abscisic acid regulation. In the leaves, differentially expressed genes of interest were associated with pathogen response, secondary metabolism, and ethylene biosynthesis. Also of interest was increased expression of (E,E)- α -farnesene synthase, a putative insect chemoattractant. These data will allow for identification of pathways disrupted by the phytoplasma, enabling the identification of candidate host proteins the phytoplasma effectors may interact with to cause the observed symptoms. This work could aid in the development of resistant cultivars, thereby preventing the current crisis from recurring.

Development and deployment of impactation spore traps for an epidemiological study of *Caliciopsis* spp. on *Pinus strobus*

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Understanding the timing of and environmental factors that influence sporulation of fungal plant pathogens is critical to mitigating the diseases they cause. One of the most cost-effective options to monitor fungal sporulation are impactation spore traps. These traps can be built for as little as \$90 USD per unit and are a popular option for studying sporulation of numerous agricultural pathogens. Here we optimize their design for use in forested settings to understand the epidemiology of *Caliciopsis* spp. which cause *Caliciopsis* canker disease (CCD) of *Pinus strobus* (eastern white pine; EWP). CCD has been reported in the eastern USA causing mortality in seedling/sapling age trees. While reports of CCD in the Great Lakes Region have not yet reached the level of severity observed in the eastern USA, it threatens the substantial regenerating white pine resource found in this region. At each of nine *Caliciopsis*-positive field sites, three traps and one weather station were installed and sampled weekly in New England, Michigan, and Wisconsin. These traps will be deployed from Spring 2021 through Fall 2022. The detection and quantification of airborne *Caliciopsis* inoculum will be used to inform timing and strategies of forest management and develop our understanding of *Caliciopsis* biology in North America. Additionally, this study will grow our understanding of how traditional, agricultural pathogen-monitoring methods can be applied to forestry.

Identification of candidate rice genes for bacterial panicle blight and sheath blight resistance through QTL linkage analysis and QTL-seq

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Bacterial panicle blight (BPB) and sheath blight (SB) are major diseases of rice causing significant yield reduction in conducive conditions. Few cultivars have shown reliable resistance to these diseases. In this study, a recombinant inbred line (RIL) population derived from the U.S. cultivars Jupiter (moderately resistant) and Trenasse (susceptible) was investigated to identify genomic regions associated with BPB and SB resistance. Phenotypes in BPB and SB, as well as days to heading (DH), of the RIL population were evaluated in the field. DH was found to be correlated to BPB and SB diseases. BPB was also positively correlated to SB. High throughput genotyping was conducted using Kompetitive allele specific polymerase chain reaction (KASP) assays and whole genome sequence (WGS) analyses. A linkage mapping combined with a bulk segregant analysis with whole-genome sequence data (QTL-seq) detected a major QTL on the upper arm of chromosome 3 for BPB, SB, and DH traits within the 1.0- to 1.9-Mb position. Additional QTLs for BPB and SB resistance were identified from other chromosomes by QTL-seq. Nine candidate genes were identified within these QTLs, however, sequence analysis revealed only four genes contained polymorphism between the parents. These genes, which are involved in jasmonic acid synthesis, salicylic acid signaling and flowering, also show polymorphism between resistant and susceptible RILs. These findings provide an insight into the complex expression of polygenic resistance for BPB and SB, which may also be closely linked to a flowering trait.

The effect of azoxystrobin and *Trichoderma harzianum* on grey mold in strawberry and lettuce

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Grey mold, caused by *Botrytis cinerea*, is a significant disease across a wide host range. Soilborne sclerotia and hyphae may persist or overwinter in soil and on plant debris, serving as primary inocula for the following year's infection. A field study was conducted at WSU NWREC to determine whether applications of commercially available products could reduce grey mold incidence in strawberry and lettuce. In 2020, Quadris (azoxystrobin) or RootShield WP (*Trichoderma harzianum* KRL-AG2) was applied to lettuce and

strawberry plots either by drench application (DA) or preplant incorporation (PPI). Two plantings of lettuce were completed and strawberry fruit were harvested three times within one year. Disease incidence was assessed visually on the harvested lettuce heads and strawberry fruit. No grey mold symptoms were observed on the first harvest of lettuce. For the second lettuce harvest, no significant differences across fungicides and application methods ($P = 0.87$) were observed. Although, the mean disease incidence was lowest for lettuce when fungicides were applied by drench (6.6% for Quadris and 7.6% for RootShield WP) compared to the control (12.2%). Strawberry plants treated with the azoxystrobin DA had the lowest mean disease incidence across treatments only in the first two harvests (3.1% and 2.8%) whereas disease incidence of those treated with the RootShield WP PPI (0.87% and 7.3%) was lower compared to the DA (8.6% and 7.7%) but not always compared to the control (4.6% and 5.8%). No significant differences were observed among strawberry in the third harvest.

New species of *Urocystis* on *Anemone acutiloba* from Indiana, USA

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Urocystis is one of the largest genera of smut fungi (Ustilaginomycetes) with more than 180 obligate-pathogenic species reported from almost every country of the world. *Urocystis* is easily transmitted from plant to plant, with spores being soil-born. The systematics and biodiversity of *Urocystis* are poorly studied in many parts of the world, including the United States. Some species of *Urocystis* are of great economic importance since they are able to affect wheat, barley, onion, and turfgrass, among the other hosts. However, *Urocystis* spp. are not only restricted to crops, but they are also commonly found on wild and ornamental plants as well. Ranunculaceae is one of the largest host families for *Urocystis*. A great number of plants from this family, like *Ranunculus*, *Anemone*, and *Aconitum* are widely cultivated. This project aimed to reveal the phylogeny and diversity of *Urocystis* on *Anemone* spp. and to test the hypothesis that *U. anemones* occurring on 31 host species is polyphyletic. The morphological and molecular analysis revealed a previously undescribed species of *Urocystis* on the North American *A. acutiloba*. From the phylogenetic analysis it can be seen that the new species is closely related to *U. anemones* from the European host *A. nemorosa*. The novel species will be described and illustrated.

Pathological and molecular biology of *Xanthomonas* strains causing bacterial leaf blight of panax (*Polyscias guilfoylei*) in Hawaii

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Panax, *Polyscias guilfoylei*, family Araliaceae, is widely cultivated as an ornamental hedge and windbreak in Hawaii. *Xanthomonas hortorum* pv. *hederiae* (*Xhh*) was reported to cause of leaf spot diseases on araliaceous hosts including *Hedera*, *Polyscias*, and *Schefflera*. In this study, five Hawaiian strains isolated from panax were selected for pathogenicity studies and complete genome analysis. Three strains (PL35, A3651, A3808) strains caused water-soaked lesions within 98 h when spray inoculated onto leaves without wounding and caused similar lesions when inoculated by injection-infiltration. Strain A1891 produced no symptoms when spray inoculated, but caused necrotic lesions following injection-infiltration. Strain A3650 caused no symptoms on panax. A phylogenetic tree based on MLST analysis revealed that all five strains were phylogenetically distinct from the *Xhh* pathotype strain WHRI7744 isolated from *H. helix*; four strains (PL35, A3651, A3808, A1891) formed a monophyletic subclade closely related to *X. euvesicatoria* (*Xe*) whereas the nonpathogenic strain A3650 clustered with *X. arboricola*. We sequenced the whole genome of PL35, the most aggressive strain on panax. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) analyses indicated that strain PL35 was distinct from *Xhh* with both values below the species delineation, but was closely related to pathovars in the *Xe* phylogenetic clade with 98.56–98.84% ANI and 86.7–88.5% dDDH. Here, we propose that four strains in the unique monophyletic group are considered a pathovar of *Xe*.

Regional network analysis to understand the effects of farmer management decisions on the avocado laurel wilt epidemic in Florida

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Avocado production in Florida and beyond is threatened by laurel wilt, caused by the fungus *Raffaelea lauricola* and vectored by ambrosia beetles. Avocado trees die shortly after infection, and good decision-making for disease management is critical for survival of avocado groves. We used the impact network analysis framework for scenario analysis of how regional management strategies may affect laurel wilt epidemics. We evaluated the rate of within-grove spread and used this to estimate the effects of potential management strategies on within- and between-grove spread in Florida. We evaluated the potential effects of growers' varying management strategies on 1,132 avocado groves, social behaviors, and the regional policies about management requirements. We included different levels of "stubbornness", or hesitancy to adopt new treatment options, and evaluated how parameter combinations may influence the epidemic. For example, in comparisons of potential policies using "carrot" (support for the cost of management) and "stick" (penalties for poor management) approaches, carrot approaches provided better regional management outcomes. Even for high levels of stubbornness, groves that persist will tend to practice good management. This agent-based model can be used to simulate regional epidemics more generally, and to model how these social influence and epidemic networks together impact disease spread. We are

developing this tool to support rapid evaluation of epidemic management strategies in new regions, and policy options for invasive pathogens and pests more broadly.

Survival and detection of *Erwinia amylovora* in cankers and effect of environmental and host factors

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There is limited information on how environmental and host factors impact *Erwinia amylovora* populations in cankers. In an assay repeated over two growing seasons, different cultivars of apple (irrigated and non-irrigated trees), pear and Asian pear were shoot-inoculated with the pathogen, and the developed cankers on wood were harvested throughout the year. Live *E. amylovora* populations in cankers were quantified by viability digital PCR. Both the percent of cankers positive for *E. amylovora* detection and the pathogen viable cell numbers declined during fall and winter. A logistic regression analysis revealed that the chance for detecting *E. amylovora* in cankers decreased in samples collected from non-irrigated trees and/or from trees challenged by the severe drought in 2016. Positive *E. amylovora* detection in cankers after winter was linked to reduced damage of host tissues, with chances for detecting the pathogen increasing in more resistant, i.e., overall less blighted, host species and cultivars (apple and pear versus Asian pear).

The *Ralstonia solanacearum* cool virulence trait is not limited to the Race 3 biovar 2 subgroup

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Ralstonia solanacearum is a heterogeneous species of plant pathogens that all colonize host xylem vessels and cause bacterial wilt diseases like potato brown rot, primarily in warm temperate and tropical climates. Several *R. solanacearum* strains are widely present in the US but the phylotype II-sequevar 1 subgroup, known for regulatory purposes as Race 3 biovar 2 (R3b2), is not established in the US. R3b2 is strictly regulated by USDA-APHIS as a Select Agent. The justification for this intense regulation is that R3b2 strains can cause disease at cooler temperatures and could threaten the US potato industry. However, reports suggest some non-R3b2 strains also have this cool virulence trait. We combined comparative analyses of whole genomes with direct phenotypic testing to see if cool virulence correlates exclusively with the R3b2 genotype. We defined cool virulence as the ability to wilt >50% of tomato and potato plants at 22°C, with well-characterized R3b2 strain UW551 as a cool virulent standard in each assay. Unexpectedly, strains from several other phylogenetically distinct subgroups were cool virulent under these conditions. Among these were strains UW729 (phyl. II-seq 4, French West Indies, aka CFBP6783); UW768 (phyl. II-seq 4, Florida, aka P673); and K60^T (phyl. II-seq. 7, N. Carolina). These results suggest that *R. solanacearum* cool virulence is a polyphyletic trait present in multiple non-R3b2 strains, including two from the southeastern US. Thus, current DNA-based tests for R3b2 will not reliably identify strains that cause potato brown rot at cooler temperatures.

***Hemileia vastatrix* aerial inoculum dynamics and its relationship with coffee leaf rust epidemics on susceptible and resistant crops at Huila, Colombia**

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Coffee leaf rust (CLR) causes 30 to 80% losses on susceptible crops and its management is mainly based on chemical control or resistant varieties. CLR epidemics is related to plant's phenology and berries production dynamics, depending on regional weather. To control CLR requires acting opportunely and efficiently at a low incidence. This study monitored continuously (24 h/7 day) in 2018 and 2019, the aerial inoculum of *H. vastatrix* urediniospores in four altitudes (1,280, 1,524, 1,659, and 1,898 m.a.s.l.), and its relationship with microclimate and CLR incidence and severity, in Pitalito (Huila, Colombia). Lanzoni® VPPS2010 spore collectors coupled with weather stations were located inside two CLR susceptible cv. Caturra crops and two resistant cv. Castillo® crops. The highest spores' concentrations occurred between September and December along the main coffee harvesting, just after the CLR epidemics peak. Caturra crop at 1,280 m.a.s.l. had the highest monthly concentrations between 15,000 and 25,000 spores/m³ air/month, followed by Castillo® at 1,898 m between 8,000 and 15,000 spores/m³ air/month. The highest hourly concentrations occurred between the 17:00h and 21:00h below 1,600 m.a.s.l., and from the 7:00 h to 12:00 h above 1,600 m, depending on winds. CLR was higher in Caturra with maximum incidences of 70% in 2018 and 72% in 2019, versus 2.5% and 8.9% in Castillo®, despite high inoculum availability. These results reinforce the importance of planting a composite resistant variety such Castillo® developed by FNC CENICAFÉ, for sustainable and productive systems in Colombia.

Sensitivity of *Colletotrichum acutatum* of strawberry to fungicide alternatives to quinone-outside inhibitors

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Colletotrichum nymphaeae and *C. fioriniae* belong to the *C. acutatum* species complex and cause strawberry anthracnose fruit rot (AFR). Quinone-outside inhibitor (QoI) fungicides are frequently used to control AFR, however, resistance has been recently reported in U.S. strawberry fields. Thus, alternative modes of action, such as the succinate dehydrogenase inhibitors (SDHIs), phenylpyrroles

(PP), and anilinopyrimidines (AP) were investigated to clarify their efficacy and provide tools for disease management. The SDHI pydiflumetofen and the AP cyprodinil are registered for AFR control as pre-packaged mixtures with the PP fludioxonil. Thus, sensitivity of isolates from diverse strawberry fields in the U.S. was evaluated for pydiflumetofen, fludioxonil, and cyprodinil by using the spiral gradient dilution (SGD) method. Mean EC₅₀ values for *C. nymphaeae* (*n* = 88) were 0.04 and 0.12 µg/ml for fludioxonil and cyprodinil, respectively; whereas mean of EC₅₀ value for *C. fioriniae* (*n* = 12) was 0.10 µg/ml for fludioxonil. *C. nymphaeae* and *C. fioriniae* were insensitive to pydiflumetofen and *C. fioriniae* was not inhibited by cyprodinil. Disease incidence, in detached fruit assays and field trials, was reduced only by fludioxonil and cyprodinil confirming their effectiveness in controlling AFR. Considering that *C. nymphaeae* is the dominant species in Florida, the combination fludioxonil + cyprodinil is the best option as an alternative to QoI fungicides for management of AFR in strawberry production fields.

Evaluation of chemical seed treatment against fungal and oomycete pathogens in managing rice diseases

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Rice is the primary staple for more than half of the global population and is the second most important cereal worldwide. In the US, it is primarily grown in the south. Diseases cause significant yield losses in rice. Major diseases include sheath blight caused by *Rhizoctonia solani* AG 1-IA. *Pythium* species are associated with poor seedling development in rice. Currently, most of these are managed by chemical control as there are no completely resistant varieties. The objective of this study is to determine the efficacy of different chemical seed treatments against fungal and oomycete pathogens in controlled environment. Chemically treated seeds of two rice varieties, Diamond and RT7301, are being evaluated against isolates of *R. solani* AG 1-IA, AG 4, AG 7, AG 9, and different *Pythium* spp. (*P. graminicola*, *P. irregulare*, and *P. ultimum*). In this experiment, eight chemical seed treatments are being evaluated. All eight treatments have the active ingredients mefenoxam (oomycetocide) and thiamethoxam as the base treatment with single, double and triple treatments of fungicide in different combinations (azoxystrobin, fludioxonil or sedaxane), which are used to control different soilborne pathogens. Germination rate was taken, and plants were harvested for root trait measurements. Results from preliminary trials show varying effect of the chemical seed treatments against isolates of *R. solani* and *Pythium* species. Results from this study would indicate the efficacy of different chemistries or combination of chemistries in effectively controlling soilborne pathogens.

Evaluating strawberry genetic resistance to Verticillium wilt under California growing conditions

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Soilborne diseases have been a historical constraint to strawberry (*Fragaria × ananassa*) production in California. Verticillium wilt is a major disease found in all strawberry growing districts affecting a variety of crops. Due to stringent restrictions on fumigants, host plant resistance has become increasingly important as a means of managing the disease. During the 2020–2021 season at Cal Poly State University, 51 strawberry cultivars from four different breeding programs are being examined. Plants were established in November 2020 in a randomized complete block design with four replicated blocks and a fumigated control. Prior to planting, lettuce (cv. Black Seeded Simpson) was planted in June 2020 to increase pathogen inoculum and the ground was disked and beds shaped in September 2020. The pre-plant inoculum level for *Verticillium* spp. microsclerotia was 7 g⁻¹ soil. Plant mortality (%) is being recorded weekly in order to identify resistant or susceptible cultivars. Symptomatic plants are diagnosed by plating surface disinfected petiole tissue on selective media. As of April 15, 2021, the current average mortality rate of all cultivars is 3.8%. Cultivars UCD_Royal Royce, UCD_Moxie, Ruby June, 152X18, 078X01, and 16C100P023 all have the current lowest mortality of 0%, whereas cultivars UCD_Victor, Ravina, and Rosalind have the current highest mortality of 19%, 18%, and 12%, respectively. Final percent mortality for all cultivars will be available in August 2021.

Survey for maize viruses in the United States

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Viruses are among pathogens that can impact production of maize (*Zea mays* L.) in the United States and globally. In 2018, maize samples were selected and collected from various states in the U.S. to assess viruses by RNA sequencing. Maize samples were selected from sweet corn due to its greater virus susceptibility than observed for field corn. Samples from sweet corn plants, weedy monocots within or adjacent to maize fields, or both were collected from Ohio, Indiana, Iowa, Illinois, Kentucky, Tennessee, Pennsylvania, Nebraska, New Hampshire, Louisiana, and Washington states, and RNA was Illumina sequenced and analyzed using an in-house bioinformatics pipeline for virus sequence identification. Sequences with top hits to *Maize dwarf mosaic virus* (MDMV), *Foxtail mosaic virus* (FoMV), and *High Plains wheat mosaic virus* (HPWMoV) were each identified in multiple sweet corn samples. Sequences matching *Brome mosaic virus* (BMV) and *Maize chlorotic dwarf virus* (MCDV) were identified in weedy monocots but not in the sampled maize. Putative novel virus sequences were also identified. Distribution and sequence comparison of the identified virus sequences provides information on variability and distribution of maize viruses in the United States, although sampling was not extensive enough to indicate absence of any virus by lack of detection.

Esendo™: A unique broad-spectrum fungicide combining synthetic chemistry and a high-performance biological

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AgBiome is developing new products combining both synthetic chemistries with high-performance biologicals as well as combination products with multiple biologicals. The first product in this line is Esendo™—a unique premix of azoxystrobin and *Pseudomonas chlororaphis*, the active ingredient in Howler® fungicide. The product concept, profile, and performance examples are introduced in this presentation.

Global pangenome analysis of *Pyrenophora tritici-repentis* reveals high plasticity and translocation of the *ToxA* gene between different chromosomes

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Pyrenophora tritici-repentis (Ptr) is a fungal pathogen that causes tan spot, a destructive foliar disease of wheat. Ptr secretes effectors (*ToxA*, *ToxB*, and *ToxC*), which induce necrosis or chlorosis in susceptible genotypes. A pangenome analysis of forty isolates representing different races and global origins was performed. Additionally, the chromosome organization of two isolates, races 3 and 8, was examined after long-read sequencing. Results show that Ptrs genome is highly plastic with a genome size of 34.8 ± 2.1 Mb, with 43% of its predicted genes present in the core while 57% are accessories. Phylogenetics show that isolates cluster based on location and their ability to secrete Ptr effectors. Long-read assemblies confirmed the translocation of *ToxA* between chromosomes and revealed considerable large-scale re-arrangements between races. Transposable element content varied between 7 and 17.5% of the total genome size, and these elements likely play an essential role in driving Ptr genome plasticity.

Assessing diversity, frequency, and potential pathways of introduction of rust fungi intercepted at Arizona ports of entry from 2015–2020

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Rust fungi are complex, highly specialized obligate parasites that can cause significant damage to agricultural and forestry crops, native plants, and natural ecosystems. Information regarding host range, aggressiveness to native hosts, and presence in the United States is scarce for many of these fungi, making them easily overlooked in ecosystem diversity management. The rust fungi intercepted at Arizona ports of entry transit primarily from Mexico and are considered quarantine-significant pests constituting a substantial portion of pathogens requiring phytosanitary action. Over a 6-year period (January 1, 2015, to December 31, 2020), a total of 1,586 interceptions of rust fungi were found at 8 Arizona ports of entry. The 1,586 rust fungi belonged to 7 known rust families, 11 genera, and 35 unique species. The most prevalent quarantine-significant rust fungus was *Puccinia horiana* Henn., the causal agent of Chrysanthemum white rust. A majority of the rusts that were intercepted are not known to occur in the United States, and those that are present often are limited in their distribution. Identifying and cataloging rusts intercepted at Arizona ports of entry is critical in assessing the risk of introduction and establishment of exotic plant pathogens that may pose a threat to the health and biodiversity of ecosystems in the United States.

CsrD is a cyclic-di-GMP binding protein that in *Erwinia amylovora* regulates *amsG* expression through the modulation of RNase E specificity towards CsrB

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Erwinia amylovora is an economically impactful phytopathogen that causes fire blight disease in apple and pear. The exopolysaccharide amylovoran is a pathogenicity determinant in *E. amylovora* and is a major component of the extracellular matrix of biofilms formed within the xylem vasculature of the host plant. The second messenger cyclic-di-GMP has been reported to positively regulate the transcription of *amsG* (the first gene in the amylovoran biosynthetic operon), thus impacting amylovoran production. However, the regulatory mechanism by which this interaction occurs is not fully understood. Here, we report that c-di-GMP binds to CsrD, a process that alters the degradation specificity of RNase E towards the sRNA, CsrB. Changes in the available levels of CsrB can impact the amount of sequestration imposed by this sRNA on the global regulator, CsrA, thus generating a downstream effect on *amsG* transcription, through the RcsBCD phosphorelay. We highlight the critical residues (within the EAL motif) in CsrD that are involved in its interaction with c-di-GMP. Overall, our findings highlight how the c-di-GMP dependent regulatory circuit channels through CsrD, RNase E and CsrB to impact the *ams* operon, in conjunction with the currently understood mechanism known to be dependent on CsrA and RcsBCD.

Routes of expansion and population diversification in a globally distributed plant pathogen

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Globally distributed plant pathogens pose a significant threat for global economies and food security. Plant disease outbreaks are often linked to human-mediated movement of susceptible plants into pathogenic zones or the introduction of infected plant material into naïve locations. Following each introduction event, new pathogenic populations are formed and develop a unique set of genomic traits. *Xylella fastidiosa*, a fastidious bacterial pathogen, has experienced numerous introduction events thorough its evolutionary history. As such, it represents an ideal model to study such population-based variations. Using worldwide whole genome sequence data, we have performed core genome-based phylogeographic analyses to identify introduction routes leading to new *X. fastidiosa* outbreaks. We have also used genomic data to measure levels of genomic diversity, homologous recombination, and gene gain/loss across populations with different potential ages. Our findings suggest that *X. fastidiosa*'s distribution is currently expanding across the globe and that multiple recent introduction events are linked to diverse regions in the American continent. In addition, we have found that genetic diversification (both via SNP changes and variations in gene content) as well as homologous recombination are likely to have an

accumulative effect on *X. fastidiosa* populations. These findings are key in understanding evolutionary trends within *X. fastidiosa* itself, but also shed light into the evolutionary mechanism important to bacterial plant pathogens.

Estimating rates of bacterial evolution over the course of a single growing season

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Emerging and rapidly evolving bacterial pathogens pose a unique challenge for long term disease management. *Xanthomonas perforans* causes bacterial spot of tomato and continual shifts in the pathogen population have destabilized disease management strategies. We hypothesize that local pathogen evolution may contribute to observed genetic diversity and that *X. perforans* strains experience mutation and recombination events over a single growing season. To estimate the rate of genomic changes in *X. perforans* during a disease epidemic, we marked, released, and recaptured rifampicin resistant strains GEV872 and GEV1001, representing two phylogenetic groups of the pathogen found in Florida. The center plants in replicated plots were inoculated with one of the two strains, and strains were recaptured weekly as the pathogen spread through the plots during the 10-week growing season. Whole genome sequences of recaptured strains were compared to the wild-type reference sequence to identify single nucleotide polymorphisms (SNPs). Estimated mutation rates using uncorrelated relaxed molecular clocks were 44.4 (30.7–60.5) and 1.49 (0.344–4.97) substitutions at end of the season for GEV872 and GEV1001 strains, respectively. Approximately, 24 and 52% of SNPs were shared in more than half of the recaptured strains from GEV872 and GEV1001 plots. Our findings suggest directional mutation occurred at specific sites in both strains. These observations will improve our understanding of bacterial plant pathogen evolution in field conditions.

Biological control of *Fusarium oxysporum* and *Pythium myriotylum* causing damping-off, root, and crown rot on cannabis (*Cannabis sativa* L.) plants

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Damping-off, crown and root caused by *Fusarium oxysporum* and *Pythium* spp. are important pathogens that impact growth of cannabis plants and cause mortality. Producers have limited options to manage these diseases as traditional fungicides are not registered. The effectiveness of biological control agents for disease management was evaluated. Rhapsody® ASO (*Bacillus subtilis*), Stargus® (*B. amyloliquefaciens*), Lalstop® (*Gliocladium catenulatum*, Prestop®), RootShield® Plus WP (*Trichoderma harzianum* and *T. virens*) and Asperello® (*T. asperellum*) were applied as a drench to cuttings of the strain 'White Rhino' in rockwool blocks. After 48 h, inoculation with a *F. oxysporum* spore and mycelial suspension was conducted. Disease was rated 7 and 14 days post inoculation. The most effective treatments ($P < 0.05$) were Lalstop, Rootshield, Asperello and Stargus, resulting in a 56.3% to 30% reduction in mean disease severity, respectively, at 14 days post inoculation. For *Pythium* biocontrol, Rootshield Plus WP, Asperello, and Lalstop were applied as a drench to rooted plants of strain 'Island Honey', followed one week later by *P. myriotylum* inoculation. Disease severity was rated 7, 14, and 18 days post inoculation. The most effective treatments were Rootshield and Lalstop. Plants receiving Lalstop had significantly ($P < 0.05$) higher fresh root weights compared to the pathogen only treatment. These results indicate that biological control agents can reduce the impact of *Fusarium* and *Pythium* on cannabis when applied prior to pathogen infection.

Effect of a quinone outside inhibitor (QoI) fungicide on sunflower endophyte fungal community

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Quinone outside inhibitor (QoI) fungicides are known to increase the incidence of endophytic fungi in plant species. The objective of this study was to determine the effect of applying a QoI fungicide on the endophyte community in sunflower (*Helianthus annuus* L.). Field trials were conducted at one location each in Nebraska, North Dakota, and South Dakota in 2019 and 2020. Trials were conducted in a randomized complete block design with four replications. Treatments comprised of a single application of a QoI fungicide (pyraclostrobin) at floral head formation (R1) growth stage and a no-fungicide control. Stems were sampled 2–3 weeks following fungicide application from each plot, cut, surface-sterilized, and incubated on potato dextrose agar. The trials yielded 535 isolates belonging to five fungal genera, which were identified by morphology, quantitative polymerase chain reaction assays and sequence analyses of internal transcribed spacer and/or translation elongation factor 1- α genes. Among the five genera, *Alternaria*, *Diaporthe*, and *Fusarium* were dominant. A multivariate community analysis was performed to compare differences in the incidence of three dominant genera between pyraclostrobin-treated and no-fungicide control. Results indicated that application of pyraclostrobin did not affect the incidence of *Diaporthe* and *Alternaria*; however, there is a significant increase in the incidence of *Fusarium* ($P = 0.026$). In the absence of disease pressure, application of a QoI fungicide may not reduce the incidence of endophytic fungi that can cause diseases in sunflower.

Efficacy of acibenzolar-S-methyl on grape downy mildew and ripe rot, preliminary results

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Acibenzolar-S-methyl (ASM, Actigard, Syngenta) is a plant defense activator often used for protection against bacterial diseases in various crops. It is currently not registered for grapes, and information on its efficacy against fungal diseases is limited. With ASM

application to grapevine leaves (cv. Chardonnay), we did not observe phytotoxicity symptoms at rates of 10, 50, 100, and 200 ppm. The recommended rate for other crops ranges from 12.4 to 119.8 ppm. In a greenhouse experiment with five weekly ASM applications at 0, 50, 100, or 200 ppm, we observed a significant reduction in powdery mildew leaf disease severity ($F = 19.2$, $P < 0.01$) ranged from 63.7 to 97.2% with all ASM treatments. In a field experiment in 2019, ASM at 75 ppm was applied twice in a 14-day interval to cv. Chardonnay. We observed a significant reduction in mean downy mildew leaf severity ($F = 9.9$, $P < 0.01$). ASM resulted in a 42.3% reduction from the negative control, but we did not observe a decrease in powdery mildew. In a field trial in 2020, we applied ASM to cv. Cabernet Sauvignon bi-weekly from bloom to harvest in addition to a standard treatment, which was mancozeb (3 lb/A) alternated with polyoxin-D (Oso, Certis USA, 10 fl oz/A). We observed a significant reduction of the mean percent ripe rot infected-berry per cluster, ($\chi^2 = 48.4$, $P < 0.01$). The ASM plus standard treatment resulted in a 13.6% reduction from the standard treatment.

Examining the role of *tzs* and *idi* genes of *Agrobacterium rhizogenes* on adventitious roots formation

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Manipulation of plant hormones is a strategy that is often used by plant pathogens during the disease process. *Agrobacterium* spp. are known to produce plant hormones that help in its virulence. For instance, the cytokinin, trans-zeatin (tz) can be produced via the *idi-tzs* genes located in a non-T-DNA region of the Ri plasmid. Previous work by others on adventitious root formation has shown that cytokinin can counter auxin-induced adventitious root formation. We hypothesize that tz production by *A. rhizogenes* inhibits the development of competing adventitious roots during transgenic hairy root formation. To test this hypothesis, we transformed *A. rhizogenes* strain K599 (wild-type), the K599 disarmed strain 18r12v, and its Ri-plasmid cured strain, K599c, with a plasmid expressing *idi*, *tzs*, or *idi* and *tzs* genes. Soybean composite plants were inoculated with all strains and adventitious root numbers were measured. As expected, K599 and 18r12v produced few adventitious roots, while K599c produced high levels of adventitious roots. Transformation of K599c with *tzs*, *idi* or *tzs*, and *idi* were able to restore inhibition of adventitious root formation. To further confirm the presence of tz in our strains we used LC-MS/MS to measure tz production and secretion into bacterial growth medium. LC-MS/MS results corroborate the plant assays results. We also demonstrated that tz production is regulated in part by the *vir* operon. Our results demonstrate the importance of tz in the disease process of *A. rhizogenes* during the induction and formation of transgenic hairy roots.

Emerging fungal canker pathogens in southern sweet cherry orchards in Chile

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The most important and growing fruit crops in Chile is sweet cherry, and has turned into the most profitable. One of the main threats for it are canker pathogens. Orchards are traditionally been affected by bacterial canker and *Cytospora*, in a lesser extent. However, in the last years more canker symptoms and fruiting bodies resembling new species like *Calosphaeria* and *Eutypa* are frequent. In order to determine their etiology, a survey was conducted in Ñuble Region, in the south of Chile. Wood from branches with cankers and dieback were collected from 52 orchards. Wood sections 0.5-cm were cut off from the necrosis progress area, surface disinfected and plated on a quarter-strength potato dextrose agar amended with tetracycline. Plates were incubated at 25°C and purified by transferring fungal tips to PDA. Three different colonies were obtained. They were identified microscopically as *Calosphaeria*, *Cytospora*, and *Eutypa*. DNA was extracted and bt2A/bt2B, ITS1/ITS4 and β TUB 1 genes were amplified by PCR. DNA was sequenced and analyzed, determining that species were *Cytospora leucostoma*, *C. pulchella* and *E. lata*. Representative isolates were inoculated on healthy plants cv. Kordia. After 72 days of incubation, plants were cut and the internal necrosis measured. Fungi was reisolated and compared with the inoculated one, fulfilling Koch's postulates. These findings put Chile's highest valued fruit crop at risk and further studies must be conducted to determine the extent of the problem and suitable management measures.

Krisp: A lightweight kmer-based algorithm for designing diagnostic CRISPR assays using genome data

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The SHERLOCK and DETECTR diagnostic protocols have demonstrated single-nucleotide specificity in simple, low-cost, and field-deployable platforms. At their core, these protocols utilize amplification and CRISPR-Cas proteins loaded with guide crRNA's designed to detect a unique target sequence. Here we introduce krisp, a lightweight kmer-based software package for finding CRISPR diagnostic regions from genome data. Krisp takes sequence information for each sample and builds kmer lookup tables to find candidate primer pairs and diagnostic crRNA regions. Candidate regions are output as alignments, highlighting the conserved primer pairs to be used for amplification, and the diagnostic SNP's for designing guide crRNA's. Krisp is compatible with both RNA and DNA sequences and contains additional user options for parallel execution and for omitting nucleotide regions, soft or hard-masked based on repetitive content or other features associated with unsuitable diagnostic regions. As proof-of-concept, we apply the krisp algorithm to the *Phytophthora* genus of plant pathogens. Krisp is able to find candidate diagnostic regions which classify an isolate as belonging to the genus *Phytophthora*, belonging to the species of *P. ramorum*, or belonging to a specific clonal lineage within *P. ramorum*, illustrating the utility of krisp for diagnostics at multiple hierarchical levels. The krisp software package is an open source python package and downloadable from github along with user tutorials.

Genetic analysis of sequences in the 5' non-translated region of Grapevine leafroll-associated virus 1 required for RNA replication

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Grapevine leafroll-associated virus 1 (GLRaV-1, genus *Ampelovirus*, family *Closteroviridae*) has a long 5′ non-translated region (5′-NTR) with size variable between 857 and 922 nucleotides (nt) and high sequence heterogeneity among the genetic variants. In this study, a mini-replicon cDNA clone of GLRaV-1 (isolate WA-CH; accession: KU674796) was built to map sequences in the 5′-NTR required for RNA replication. The mini-replicon cDNA clone in a modified pCAM1380 binary vector contained the 857-nt long 5′-NTR, 8,146-nt replicase gene module (ORF1a/b), 729-nt Green Fluorescent Protein (GFP) gene, and the 456-nt 3′-NTR. To drive GFP expression, a 200-nt putative controller sequence located upstream of the p24 gene of GLRaV-1 (isolate WA-CH) was inserted upstream of the GFP gene. Functionality of the mini-replicon was validated by stable GFP expression in *Nicotiana benthamiana* leaves via agro-co-infiltration assays in the presence of heterologous silencing suppressors. The mini-replicon cDNA clone remained functional when the 5′-NTR was replaced with corresponding sequences from other genetic variants of GLRaV-1. However, the mini-replicon clone was not functional when the 5′-NTR was replaced with corresponding sequences from other GLRaV species. Testing the mini-replicon clone with deletions in different parts of the 5′-NTR indicated that the first 32 nt at the 5′-terminus are indispensable to drive GFP expression in agro-co-infiltrated leaves of *N. benthamiana*. The GLRaV-1 mini-replicon system established in this study will permit further examination of RNA replication in planta.

Molecular characterization of *Potato Virus Y* strains from the Kashmir Valley, India

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Potato virus Y (PVY) ranks fifth among the top ten economically important viruses worldwide. PVY isolates are historically grouped into three main strains which can cause different symptoms on different parts of the host plants. Characterizing the type of PVY strains is therefore essential for breeding PVY resistant cultivars. A survey to determine the incidence of PVY in the potato fields of Kashmir valley was conducted in 2018–2019. Two hundred potato leaf samples collected from four districts of the valley were tested for PVY by DAS-ELISA that yielded 74 PVY-positive samples. Forty (ten from each district) positive samples were reconfirmed by RT-PCR using coat protein (CP)-specific primers. The disease incidence ranged from 24 to 52%, with the highest incidence recorded in Srinagar (52%) followed by Anantnag (40%), Baramullah (34%), and Budgam (24%) districts. The samples that were positive for PVY were further characterized using strain specific primers, with NTN, N, and O being the most prevalent strains. Some samples also contained a mixture of strains. This study revealed the presence of three PVY strains in the Kashmir Valley and mixed infections by other potato viruses. It is, therefore, important to test and deploy only certified potato seed to minimize losses from these viruses.

Pod blight threatens commercial sunn hemp seed production in south Texas

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Sunn hemp (*Crotalaria juncea*) is an important cover crop that is widely used in south Texas due to its resilience to drought stress and its ability to fix atmospheric nitrogen and stabilize soils. Due to the increasing demand, many growers in the region are interested in commercial sunn hemp seed production. In fall 2020, we surveyed a commercial sunn hemp seed production field for diseases with sunn hemp plants grown at three different densities. Seed pods from affected plants were chlorotic and discolored, and their interior had white mycelium surrounding shriveled and discolored seeds. Twenty seed pods from five random quadrats were surveyed and rated on a 1–10 ordinal scale of disease severity. There was significantly lower disease incidence and severity at the lowest planting density. Fungi from symptomatic seed pods were isolated on potato dextrose agar, and the isolates were preliminarily identified as *Fusarium* spp. based on colony appearance and macroconidia spore type. Two fungal isolates were used to complete Koch's postulates and all subsequent experiments. Seed pods in two field trials inoculated with the two fungal isolates created similar symptoms to those described from the field. Seeds incubated with fungal conidia from both isolates had significantly slower germination rate compared with the untreated control and had significantly lower overall germination at 5 days post inoculation. Results from our study show that the seed pod disease may limit sunn hemp seed production in south Texas.

Botrytis fruit rot incidence in commercially harvested strawberries throughout California

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Botrytis fruit rot (BFR), caused by *Botrytis cinerea*, creates significant yield losses both pre-harvest and post-harvest. The disease is favored by wet and cool weather conditions which are prevalent during winter months in California, but not during summer months. This provides an opportunity to strategically apply fungicides during wet, cool periods. To understand the relationships between fungicide application practices, weather conditions and disease development, a multi-year project is in progress. In 2020, marketable fruit were collected weekly from 15 farms with conventional or organic practices and 8 cultivars throughout the strawberry production districts. During 4 months of the harvest season, fruit (7.3 kg) were hand-harvested weekly by commercial picking crews and stored for 7 days at 4°C and evaluated for BFR incidence. This study resulted in 262 individual samples with BFR incidence ranging from 0% to 14.5% with a median 0.4%; 12% of the samples exceeded 2% (the USDA limit for US No. 1 Grade). BFR incidence was 1% and 0.8% in conventional and organic farms, respectively. Average BFR incidence was 1.5%, 0.9%, and 0.6% in Ventura, Santa Maria, and Watsonville-Salinas districts, respectively. These preliminary results contribute to a better understanding of BFR epidemics in California. Research in 2021 is focused on comparing the levels of BFR in fruit that were fungicide treated or non-treated in relation to weather conditions over time.

Targeted transcriptomics reveal signatures of large-scale birth and concerted regulation of effector genes in *Radopholus similis*

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The burrowing nematode, *Radopholus similis*, is an economically important pathogen that inflicts damage and yield loss to a wide range of crops. This migratory endoparasite is widely distributed in warmer regions and causes extensive destruction to the root systems of important food crops (e.g., citrus, banana). Here we combined spatially and temporally resolved next-generation sequencing datasets of *R. similis* to select a list of candidates for the identification of effector genes for this species. We confirmed spatial expression of transcripts of 31 candidate effectors within the esophageal glands of *R. similis* by in situ hybridization. These transcripts included homologues of known effectors with diverse molecular functions from other plant-parasitic nematodes, as well as a large set of pioneer effectors specific to *R. similis*. We then combined in situ localization of the effectors and available genomic data to identify a non-coding motif associated with gene expression specific to the nematode esophageal glands. The motif is a putative hallmark of spatial and concerted regulation of these effectors. Our data allowed us to infer plausible evolutionary histories that shaped the effector repertoire of *R. similis*, suggesting a large-scale effector birth in this particular species. Considering the economic damage caused by *R. similis*, this information provides valuable data to elucidate the mode of parasitism of this nematode. It also offers critical suggestions for the potential use of *R. similis*-specific effector genes to control this important pathogen.

Identification of *Phytophthora* species causing Phytophthora root and crown rot on lavender in the United States

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Phytophthora root and crown rot (PRCR) is a serious threat to the lavender industry in the USA. PRCR occurs on various species of lavender (*Lavandula* spp.) and is caused by several species of *Phytophthora*. We have collected over 450 isolates of *Phytophthora* spp. from lavender samples originating in 27 states, with most collected since 2015. Isolates were obtained from samples sent to our lab or to the Clemson University Plant and Pest Diagnostic Clinic. Isolates were recovered by direct isolation or through a standard baiting bioassay. Fifteen isolates, collected between 1992 and 2010, were provided by the Pennsylvania Department of Agriculture. All isolates were examined for morphological and physiological features—including colony appearance on selective medium, sporangium characters, mating type, and mefenoxam sensitivity. A subset of 71 isolates was selected for molecular identification. DNA was extracted by boiling mycelium in 400-μL TE buffer for 10 min or by utilizing a DNeasy Plant Mini Kit. PCR was performed to amplify the internal transcribed spacer and the cytochrome oxidase subunit 1 regions using primer pairs ITS 6 and ITS 4 and OomCox1-levup and OomCox1-levlo, respectively. *P. nicotianae* was the most common species identified, followed by *P. palmivora*. Other species identified include *P. cactorum*, *P. cinnamomi*, *P. citrophthora*, *P. drechsleri*, *P. megasperma*, *P. sansomeana*, and *P. tropicalis*. Our results suggest more species of *Phytophthora* than previously reported are responsible for PRCR on lavender in the USA, but *P. nicotianae* is the primary causal agent.

Non-target fungicidal effects in the corn and soybean microbiome

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There is a desire to study community dynamics under fungicide pressure so that fungicides are used in the most sustainable way possible. Here we examine non-target effects of a foliar fungicide application on fungal and bacterial communities of corn and soybean in both conventional and no-till management systems. Corn and soybean were grown within the Kellogg Biological Research (KBS) Long-Term Ecological Research experiment in Michigan. In 2017, pyraclostrobin (Headline®) at a commercial rate of 877 ml/ha was sprayed onto corn leaves at the V7 growth stage and corn leaves and roots were collected one day before, 9 days after, or 35 days after fungicide application. In 2018, trifloxystrobin and prothioconazole (Delaro®) were sprayed as a combination at a commercial rate of 731 ml/ha onto soybean leaves at the R3 growth stage and soybean leaves and roots were collected one day before, 1 week, or 1 month after fungicide application. Fungicides had a small but significant effect on the phyllosphere fungal composition at least 9 days post fungicide application in corn and up to 30 days in soybean. Intriguingly, basidiomycete yeasts residing in/on the foliage were the fungal guild most affected by the fungicide applications in both crops, and fungal communities in the no-till management were more resilient to fungicide disturbance. The affected yeasts co-occurred with bacteria in the *Sphingomonas*, *Methylobacterium*, and *Hymenobacter*. Indirect effects on the plant microbiome mediated through cooccurring leaf bacteria are currently being investigated.

Population and genetic diversity of *Fusarium oxysporum* f. sp. vasinfectum: The causal agent of Fusarium wilt

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Fusarium wilt is a vascular disease of cotton inflicted by the soil fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (Fov). Symptoms of Fusarium wilt include leaf chlorosis, darkening of the vascular tissue, and wilting which can lead to plant death. While multiple races of Fov have caused Fusarium wilt in the United States for over 100 years, a new race (race 4) was found in California in 2001. Previously restricted to India, race 4 differs from other races by its increased virulence and ability to cause severe disease in the absence of nematodes. Race 4 has since been identified in Texas and New Mexico in 2017 and has the potential to spread to the remaining cotton belt. A total of 130 Fov field isolates collected around Alabama from 2014 to 2016 were characterized using the DNA sequence of two housekeeping genes (translation elongation factor 1 alpha and DNA-directed RNA polymerase II core subunit). Most Alabama isolates grouped in the race 1/2/6 clade as expected, and consistent with previous surveys, no isolates grouped in the race 3/5 clade. Four field isolates grouped with the race 4/7 clade, but upon further analysis with the *TfoI* transposon insertion in the PHO gene,

all isolates were determined not to be California race 4 isolates. Additionally, pulse-field electrophoresis shows that California race 4 isolates have variation in the number and size of small chromosomes which could be associated with host-specific virulence.

Determining limit of detection of high-throughput sequencing diagnostics by including internal controls in e-probes

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High-throughput sequencing (HTS) can be applied to plant pathogen detection with the online platform Microbe Finder (MiFi®). Diagnostic limit of detection (LoD) is a crucial metric of any diagnostic assay. We calculate LoD for HTS diagnostics with a statistical model using pathogen-specific e-probe matches in metagenomic data. The LoD is the estimated Bayes decision boundary for a quadratic discriminant analysis (QDA) based on a total score (an index: sum of score per e-probe where score per e-probe is defined as sum of score per hit; $\sum \#$ of nucleotide per hit/# of nucleotide per e-probe $\times 100\%$). E-probes include non-host internal controls (ICs), *cox 6* and *15*, from *Pistacia vera* and *Malus domestica*, to confirm successful HTS sequencing and determine diagnostic LoD. The QDA equation is not statistically valid with zero variance in the negative samples. ICs provide variance in negative samples but must be carefully designed to avoid overwhelming the target pathogen signal in positive samples. Specific ICs were ranked based on average total scores of healthy controls and the five lowest scores were added to the citrus exocortix viroid (CEVd) e-probes. We calculated the LoD for CEVd at two e-probe lengths, 20 and 30 nt, in citrus HTS data. The CEVd-20nt e-probe set, comprised of 12 e-probes plus four *cox6* and one *cox15* e-probes, had an LoD score of 433 ($\times 10^k$). The CEVd-30nt e-probe set, comprised six of unique viroid probes plus four *cox6* and one *cox15* e-probes, generated a score of 131 ($\times 10^k$). The LoD-based results were consistent with known positive and negative RT-qPCR results.

Investigating genomic differences between two isolates of *Fusarium sambucinum*, the causal agent of Fusarium canker of hop and dry rot of potato

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Fusarium sambucinum is the primary causal agent of two diseases on economically important crops: Fusarium canker of hop (*Humulus lupulus*) and dry rot of potato (*Solanum tuberosum*). While a considerable amount of research has been done on dry rot historically, Fusarium canker has not been extensively studied and is becoming a greater problem in hop production in the Pacific Northwest. Genomic resources for *F. sambucinum* from any host are limited. To give more insight into the causal agent of these diseases, we sequenced, assembled, and annotated the genomes of two isolates of *F. sambucinum*—one derived from a diseased hop stem and one derived from a diseased potato tuber. Both genomes were assembled into five gapless contigs. The hop-derived genome is 38.5 Mb with 13,388 annotated genes and a 99.7% BUSCO score. The potato-derived genome is 38.7 Mb with 13,473 annotated genes and a 97.3% BUSCO score. These genomes will help to address the question of how the isolates differ based on host origin and whether there are differences in gene clusters involved with mycotoxin biosynthesis. Additionally, these resources will provide insights into the gene expression and genetic basis of differences in pathogenicity in these two isolates inoculated on potato and hop, advance our understanding of the epidemiology of the diseases, and enable future genomic studies involving *F. sambucinum*.

Epiphytic colonization and pathogen entry points of the fire blight pathogen *Erwinia amylovora* on apple leaves

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Fire blight, caused by a bacterium *Erwinia amylovora*, is a devastating disease of rosaceous plants such as apples and pears. Infection of actively growing shoots in summer months, often referred to as the shoot blight, not only causes significant yield loss and tree death, but also can lead to regional disease epidemics. However, the disease biology of shoot blight, including the epiphytic colonization, pathogen entry, as well as the conducive conditions are not well characterized. Currently there are debates whether *E. amylovora* enters apple leaves through microinjuries or natural openings. Here, we showed that *E. amylovora* is able to cause shoot blight without artificial injuries, suggesting that infection through natural openings is possible. GUS staining and electron microscopy examination of the epiphytic growth of *E. amylovora* on leaf surfaces post-dipping inoculation suggests *E. amylovora* colonization was highly abundant on trichomes and hydathodes during the initial infection. Infections through natural openings only occur under high temperature and high humidity conditions. Findings from this study suggest the abundance of the susceptible leaf surface structure (e.g., the trichome presence and density) may be an additional breeding target for fire blight resistant plants. Understanding the correlation of environmental factors and shoot infection may contribute to the development of a future disease prediction model for shoot blight.

Collective impact of agronomic practices on the diversity and abundance of pathogenic and beneficial fungal genera in soil

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Farmers use different agronomic practices such as crop rotation (CR), tillage (T), cover crops (CC), and manure application (M) in crop production. Studying their influences on soil microbial communities is important to understand how such practices can be purposefully manipulated to improve soil health. We collected bulk soil samples from 14 farms across Pennsylvania with histories of different agronomic practices. DNA extracted from samples were PCR amplified targeting fungal ITS region (ITS1F/58A2R primers) and subjected to MiSeq sequencing. Impact of individual practices and their combinations on (i) entire taxa; (ii) pathogenic genera ($n = 16$);

and (iii) beneficial genera ($n = 14$) based β -diversity was assessed using PERMANOVA and beta dispersion tests. Results showed that M ($R^2 = 0.062$, $P < 0.001$), CC-CR ($R^2 = 0.095$, $P < 0.001$), and T-CR ($R^2 = 0.062$, $P < 0.01$) have significant effects on entire taxa-based β -diversity. Effect of CC-M-T-CR was significant on both pathogenic and beneficial genera based β -diversity ($P < 0.001$) and explained over 40 and 38% of the total variation, respectively. The impact of the level of each practice and their combinations on mean abundance of pathogenic/beneficial genera was investigated using the generalized linear mixed model approach by considering farm as a random factor. Results revealed that specific combinations of the levels of certain agronomic practices are more useful in decreasing the abundance of some pathogenic fungal genera while increasing the abundance of several beneficial fungal genera in soil at landscape scale.

Quantitative isolation of potential biocontrol agents for control of bacterial wilt

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Biological control could be a sustainable and environmentally friendly method to control bacterial wilt (BW) caused by *Ralstonia solanacearum* species complex (RSSC) in tomato and pepper. Isolation and evaluation of a maximal number of putative biocontrol agents (BCA) increase the chance of discovering an effective BCA. Adaptation of the BCA to the plant and pathogen environment is critical to ensure its field efficacy. Quantitative isolation of potential BCAs from the soil surrounding the roots of tomato and pepper, from an uncultivated organic plot, and from a BW sick plot in the WorldVeg campus in Taiwan were evaluated. Serial dilutions of the soils were cultured on five different media to determine the frequency and diversity of potential BCAs. The soil from the roots of tomato yielded the greatest quantity of bacterial isolates, while the organic fields and BW sick plot exhibit higher numbers of fungal isolates. A greatest diversity of microbial species was found from the samples from the organic field. A total of 125 isolates that include species of the genus *Bacillus*, *Streptomyces*, *Pseudomonas*, *Trichoderma*, and *Penicillium* that are putative BCAs for BW were isolated and selected for co-culturing with *R. pseudosolanacearum* strain PSS4 (=Asian origin, Phylotype I; Biovar 3), and for screening for the reduction of disease incidence and severity in tomato following artificial inoculation with PSS4 in the greenhouse. Although technically demanding and time-consuming, quantitative isolation and screening can maximize the number of potential BCAs against BW identified.

Understanding the disease resistance mechanism through RNA-Seq analysis of SqVYV-resistant watermelon

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Viral watermelon vine decline caused by whitefly-transmitted squash vein yellowing virus (SqVYV) is one of the major viral diseases of watermelon. Recently, a vine-decline resistant watermelon line 392291-VDR was identified after successive screening and pureline selection through six generations from U.S. PI 392291. Inoculated 392291-VDR plants showed the presence of the virus; however, plants remained symptomless. In contrast, inoculated susceptible ‘Crimson Sweet’ (CS) watermelon plants produced typical symptoms of viral watermelon vine decline, had higher virus titers and succumbed to virus infection. A global expression profiling with the help of RNA-seq was used to better understand the disease resistance mechanism. A time course experiment was conducted following the disease progression for the identification of the pathways/candidate genes aiding resistance. From an average of 4.4 billion clean reads with 78.3% of mapping percentage, a total of 5,114 differentially expressed genes (DEGs) was obtained in 392291-VDR and 7984 DEGs in CS with log 2-fold change >1 . Of the total DEGs, 1947 DEGs were upregulated in 392291-VDR compared to 3657 DEGs upregulated in CS. Top gene ontology (GO) defense-associated terms with biological process and molecular function such as “response to stimulus,” “immune system process,” “biological process involved in interspecies interaction,” and “translation regulator activity” were found among the enriched GO terms. These findings will help us mine interesting candidate genes contributing towards SqVYV resistance in watermelon.

Comparison of egg hatching rate and cold shock tolerance of *Meloidogyne enterolobii* to other root-knot nematode species to better inform disease risk

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Meloidogyne enterolobii is a highly virulent root-knot nematode (RKN) species capable of causing significant damage to a broad range of host plants. Current knowledge of this species is limited, especially regarding its biology. Initial reports suggest *M. enterolobii* is more aggressive in symptomology than other RKN species. An experiment was performed to assess whether *M. enterolobii* has a biological advantage in egg hatching rate when compared to *M. incognita* and *M. hapla*. Egg hatch was quantified by inoculating eggs on to hatching screens. Significant differences in egg hatching rate were found between the RKN species. A higher egg hatching rate may suggest a larger initial population translating to greater pathogen pressure, potentially leading to the observed increase in symptomology. The results also suggest the need to monitor for a potential shift in species prevalence and disease caused by *M. enterolobii*. Comparisons among the three root-knot species’ ability to hatch after cold shock treatment was also assessed. Nematode eggs from each species were subjected to cold shock treatments at various temperatures and egg hatch rate following treatment was determined. Our results indicate *M. enterolobii* and *M. incognita* have similar egg hatch cold shock tolerance profiles, suggesting that these two species may share common geographic limitations based on soil temperature. The results may also inform the potential shift in disease risk, as *M. enterolobii* is yet limited in distribution within the continental United States.

The ubiquitous plasmid pEA29 is an essential virulence factor in *Erwinia amylovora*

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Erwinia amylovora is a plant pathogen that causes the disease fire blight on apple and pear trees. Bacterial cells colonize the host vascular tissue and form robust biofilms mainly consisting of exopolysaccharides (EPSs) that contribute to the wilt symptom of fire blight. The nearly ubiquitous plasmid pEA29 of *E. amylovora* is an essential virulence factor, but little is known for the underlying mechanism. Here, we showed that pEA29 was required to produce amylovoran and biofilm formation, and this regulation was dependent on the thiamine biosynthesis operon *thiOSGF*. Carbohydrate and genetic analyses showed that the thiamine-mediated regulation of amylovoran was indirect as cells lacking the *thi* operon produced an EPS that did not contain the key component of amylovoran, glucuronic acid, whereas the transcriptional activity and RNA levels of the amylovoran biosynthesis genes were not changed. Alternatively, addition of exogenous thiamine restored the amylovoran production in the pEA29-cured *E. amylovora*. Deletion of chromosomal thiamine biosynthesis genes affected amylovoran production, implying that a complete thiamine biosynthesis pathway is required for the thiamine-mediated effect on amylovoran. Finally, we determined that an imbalanced tricarboxylic acid (TCA) cycle negatively contributed to amylovoran production, which was restored by addition of thiamine or overexpression of the *thi* operon. In summary, our report proposed that the thiamine biosynthesis pathway enhances bacterial respiration which provides energetic requirements for the biosynthesis of amylovoran.

Improving the toolbox to manage *Phytophthora* diseases of strawberry: Searching for chemical alternatives

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The Florida strawberry production is affected by two economically important diseases, *Phytophthora* crown rot (PhCR) and leather rot (LR), caused primarily by *P. cactorum* and *P. nicotianae*. Although products are available, chemical control is limited to three fungicide classes and issues with resistance have been reported. Assays were conducted to evaluate the effectiveness of different chemical classes for control of PhCR and LR. In *in vivo* assays, differences in sensitivity of the products were observed between the two *Phytophthora* spp. Mandipropamid, fluopicolide, oxathiapiprolin, and cymoxanil completely inhibited mycelial growth of both species at 1 µg/ml. The same fungicides controlled LR and PhCR caused by *P. cactorum*, in detached fruit and greenhouse trials, respectively, including mefenoxam-resistant isolates. However, for PhCR caused by *P. nicotianae* only oxathiapiprolin and cymoxanil were effective in controlling the disease. Cyazofamid, fluazinam, propamocarb, and tebuconazole did not control PhCR caused by either *Phytophthora* spp. Except for tebuconazole, all fungicides tested reduced incidence of LR caused by *P. cactorum* in the detached fruit assay, however fluazinam, cyazofamid, and propamocarb had no effect on *P. nicotianae*. This study sheds light for four fungicides that could potentially be registered to manage both PhCR and LR of strawberry. The registration of fungicides with different modes of action would provide options to fruit and nursery growers for alternation of products that would minimize the risk of fungicide resistance selection.

Organic matter used for anaerobic soil disinfestation influences the organic acids profile of the soil solution

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Anaerobic soil disinfestation (ASD) is of rising interest as a potential organic alternative to chemical soil fumigation. This process exploits repressive conditions to soilborne plant pathogens created by anaerobic fermentation. ASD consists of incorporating organic matter (OM) rich in labile carbon in the soil, followed by tarping and irrigation to saturation. The selection of OM is of particular interest because it can influence the type of organic compounds produced by the soil microbiota and the eventual efficacy of ASD. The objective of the study was to evaluate the effect of different OMs used for ASD on the production of different organic acids presumably toxic to plant pathogens. ASD was conducted in airtight glass containers with water saturated soils amended with either apple pomace, blackberry pruning residues, compost of blackberry/raspberry culture residues, brewers' grains, broccoli culture residues, coffee grounds, maple autumn-shed leaves, molasses, raspberry pruning residues, or sawdust. Concentrations of six organic acids (acetic, propionic, isobutyric, butyric, isovaleric, and valeric) in the soil solutions were then determined by HPLC. Concentrations of acetic acid (0–37.4 mM), butyric acid (0–105 mM), propionic acid (0–5.9 mM), isobutyric acid (0–1.7 mM), isovaleric acid (0–1.2 mM), and valeric acid (0–0.44 mM) varied according to the OM amended in the soil. This study shows that the OM incorporated in the soil influences the profile of organic acids produced during ASD.

RND transporter contributes to *Pseudomonas cannabina* pv. *alisalensis* virulence

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Brassinin is an essential indole phytoalexin in Brassicaceae, including cabbage. Conversely, bacterial pathogens have virulence factors for effluxing or suppressing phytoalexins. *Pseudomonas cannabina* pv. *alisalensis* (*Pcal*) causes bacterial blight of cabbage. To investigate brassinin importance in plant defense and the *Pcal* virulence factors for regulating brassinin biosynthesis, we first investigated whether brassinin biosynthesis related genes are induced during *Pcal* infection. Brassinin biosynthesis related genes showed greater expression after *Pcal* infection. We previously demonstrated that a RND transporter mutant (NU19) showed reduced virulence on cabbage. RND transporter roles to date include extrusion of various toxic compounds. Thus, we assumed that the RND transporter contributes to cause disease by effluxing brassinin. NU19 showed greater sensitivity against antimicrobials including brassinin, suggesting that the RND transporter contributes to brassinin tolerance. We also demonstrated that the RND transporter contributes to other antimicrobials tolerance. Therefore, to further investigate that the RND transporter is also required for infection, we conducted an inoculation assay on multiple host plants, including Japanese radish, broccoli, Chinese cabbage, tomato, and oat.

Interestingly, NU19 exhibited reduced virulence on all dicot plants, but not on oat. Although its contributions were different depending on the host plant, these results indicate that the RND transporter contributes to *Pcal* virulence through effluxing antimicrobials.

Streptomyces actinobacteria as biological control agents of stem canker disease of royal poinciana caused by *Neoscytalidium dimidiatum*

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Streptomyces and non-streptomyces actinobacteria were isolated from royal poinciana rhizosphere in the United Arab Emirates (UAE). Three isolates, *Streptomyces* UAE1, *Streptomyces* UAE2, and *Streptomyces* UAE3, showed strong in vitro antifungal activity against *Neoscytalidium dimidiatum*, the causal agent of stem canker of royal poinciana. The antifungal activity of *Streptomyces* UAE1 and *Streptomyces* UAE2 was due to production of antifungal metabolites and the production of chitinase and β -1,3-glucanase, respectively. *Streptomyces* UAE3 was associated with both mode of actions. Using apple fruit bioassay, the three isolates significantly reduced lesion development on apple fruits inoculated with *N. dimidiatum*. Each of the three isolates exhibited great efficacy in reducing the severity of stem canker when applied 1 week before the application of *N. dimidiatum* (preventive treatment) compared to curative treatment (the same BCAs were applied after the inoculation with *N. dimidiatum*) under greenhouse conditions. The curative effects of the fungicide Cidely Top and *Streptomyces* UAE3 on disease symptoms were not significantly different on royal poinciana. *Streptomyces* UAE3 was superior in reducing disease severity over all other chemical and biological treatments tested. In conclusion, the multiple mode of actions in *Streptomyces* UAE3 can be particularly effective to produce synergistic actions against *N. dimidiatum*. The present study is the first to report the use of microbial antagonist to control stem canker of royal poinciana caused by *N. dimidiatum* under greenhouse conditions.

Genome sequence analysis of *Dolichos yellow mosaic virus* in country beans (*Lablab purpureus*) from Bangladesh

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The sustainability of country bean (*Lablab purpureus* L.) in smallholder farming systems in Bangladesh is seriously affected by yellow mosaic disease. Based on preliminary studies, sequences related to *Dolichos yellow mosaic virus* (DoYMV, genus *Begomovirus*, family *Geminiviridae*) were detected in symptomatic plants. In this study, we characterized the genome of DoYMV and examined its relationships with begomoviruses causing yellow mosaic symptoms in legume crops. Total nucleic acids from symptomatic leaves were subjected to high-throughput Illumina sequencing followed by Sanger sequencing of viral amplicons to assemble the genome sequence of DNA-A and DNA-B components of DoYMV. The complete genome of DoYMV was determined to be 2,670 nucleotides (nt) for DNA-A and variable between 2,723 nt and 2,734 nt for DNA-B. In pairwise comparisons, DNA-A and DNA-B components of DoYMV isolates from Bangladesh showed high similarity with corresponding viral sequences reported from India than to other legume-infecting begomoviruses. In phylogenetic analysis, DNA-A of DoYMV isolates from Bangladesh and India segregated into three clades, with Bangladeshi sequences clustering into one clade. In contrast, DNA-B sequences of DoYMV isolates from Bangladesh and India segregated into two groups, with Bangladeshi sequences distributed in both the clades. Recombination analysis identified three putative recombination events, with two events mapped to DNA-A and one to DNA-B. These results showed the presence of genetically diverse variants of DoYMV in country beans showing yellow mosaic symptoms.

Rapid real-time detection of *Hop latent viroid* in hops and cannabis using isothermal AmplifyRP XRT assay based on recombinase polymerase amplification

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Hop latent viroid (HLVd) was first described in 1988 and is a member of the genus *Cocadviroid* in the family *Pospiviroidae*. HLVd infects hops worldwide and often causes no clear visual symptoms on many hop cultivars but does affect the quality and yield of hops. More recently, HLVd was found to cause a stunting disease with visual symptoms on commercially important cannabis plants. HLVd does not code for any proteins and is composed of a single-stranded circular RNA of approximately 256 nucleotides long without encapsidation. Thus, detection methods for HLVd are limited due to lack of serological and other rapid detection methods. In this report, we describe the development of a rapid, real-time isothermal AmplifyRP® XRT assay that is based on reverse transcription-recombinase polymerase amplification. This AmplifyRP® assay utilizes crude plant extracts as templates and a constant temperature of 42°C for all reactions including recombination, amplification, and reverse transcription to occur in a single PCR tube. The assay results are reported in real time within 25 min using a portable, barcode-guided fluorescence reader AmpliFire®. There is no need for RNA purification and a thermal cycler. This assay can specifically detect HLVd isolates in hops as well as in cannabis and has a detection sensitivity of as low as 20 fg of in vitro HLVd transcripts. It is applicable both in laboratories and in the field. This is the first report on HLVd detection through RT-RPA.

Competitive advantage of *Geosmithia morbida* in low-moisture wood predicts geographical differences in the severity of thousand cankers disease

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Bark beetles vector symbiotic fungi and the success of these mutualisms may be limited by competition with other microbes. The outcome of fungal competition is strongly influenced by the physical and chemical conditions of the wood they inhabit. These conditions are in turn subject to climatic variation. In particular, wood moisture content influences fungal competition and, therefore, could help determine environmental suitability for thousand cankers disease (TCD) caused by *Geosmithia morbida* and its vector *Pityophthorus juglandis*. We conducted competition experiments in *J. nigra* wood that was naturally or artificially colonized by *G. morbida* and other fungi over a range of wood moisture content expected across prevailing U.S. climatic conditions. *G. morbida* outcompeted antagonistic fungi *Clonostachys* and *Trichoderma* spp. at <5% equilibrium moisture content. *Aspergillus* spp. outcompeted *G. morbida* at low moisture in wood from Indiana. We fit a logistic regression model to results of the competition experiments to predict survival of *G. morbida* across the U.S. Expected survival of *G. morbida* was highest in historical TCD epicenters and accounted for the low incidence and severity of TCD in the eastern U.S. Our results also predict that under future climate scenarios, the area impacted by TCD will expand into the native range of *J. nigra*. Given its role in emergent forest health threats, climate change should be a key consideration in the assessment of risks to hardwood resources.

Annotation and comparative genomics of *Coniothyrium glycines*, causal agent of red leaf blotch of soybean

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Coniothyrium glycines causes soybean red leaf blotch, is a damaging fungal pathogen of soybean, and a USDA select agent. Although there are draft genome sequences for three isolates of *C. glycines* available through NCBI, there was no annotation available for *C. glycines*. We developed a method to train the gene prediction model of the AUGUSTUS software using the existing annotations for the related species *Alternaria alternata* as a model organism to predict numbers of genes for *C. glycine* isolates (Cg). Using the *A. alternata* annotation as a model, we predicted 11,437 genes from the Cg-13, 12,202 from the Cg-17, and 11,562 from the Cg-18 genome sequences. We then developed a BLAST analysis pipeline that has allowed us to identify common and unique genes for each of the isolate using comparative genomic analyses. Additional whole genome sequences will allow us to refine this approach to help with the identification of unique and shared genes to develop diagnostic markers for this species of quarantine significance.

Evaluation of the translocation of fungicides and their efficacy for control of brown root rot disease of trees

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Brown root rot (BRR), caused by *Phellinus noxius*, is a devastating disease of over 200 species of woody plants in tropical/subtropical areas. To achieve maximum chemical control of BRR and deter the emergence of fungicide resistance, this study conducted efficacy screening for systemic fungicides with different modes of action. Fourteen fungicides were tested for inhibitory effects in vitro on thirty-nine *P. noxius* isolates from Taiwan, Hong Kong, Malaysia, Australia, and the Pacific Islands, using potato dextrose agar containing 0.1, 1.0, and 10 ppm (mg L⁻¹) of each fungicide. Results showed cyproconazole, epoxiconazole, and tebuconazole (FRAC G1) inhibited colony growth of *P. noxius* by 97.8–99.8% at 1 ppm. Other effective fungicide treatments were cyprodinil (FRAC D1) + fludioxonil (FRAC E2) and mepronil (FRAC C2), which showed growth inhibition of 76.7–100% and 79.1–100% at 10 ppm, respectively. *P. noxius* isolates from American Samoa, which exhibited high resistance to mepronil, carry point mutations in the *SdhC* gene. Upward translocation of six selected fungicides was evaluated in bishop wood (*Bischofia javanica*) seedlings by treating the root tips with 100 ppm of the fungicides, followed by liquid or gas chromatography tandem-mass spectrometry analyses of consecutive segments of root, stem, and leaf tissues at 7- and 21-days post-treatment. Cyproconazole was evenly distributed throughout the plant at the highest concentrations. Further research is needed to determine the optimal dose and application method to achieve effective control of BRR in planta.

Examining shifts in root microbiome of *Juglans nigra* in response to infection by *Geosmithia morbida*, the fungal pathogen in thousand cankers disease

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Thousand cankers disease (TCD) is caused by the fungus *Geosmithia morbida* and threatens *Juglans* spp. and *Pterocarya* spp. trees. Treatment options for TCD are limited. Interactions of *G. morbida* and the *J. nigra* (black walnut) phytobiome are not understood. To address these knowledge gaps, we examined how *G. morbida* infection altered the *J. nigra* phytobiome and characterized host responses to treatment with potential *G. morbida* controls. Phospho-Jet (basal bark spray) and RootShield (soil drench) treatments were compared to water (control) applied to 24 trees/treatment ($N = 72$). One week after treatment, 36 trees (12/treatment) were inoculated with *G. morbida* at 4 heights along the main stem (0 DAI). Remaining trees were inoculated with 4 PDA (only) plugs. Destructive tree sampling at 14, 28, and 56 DAI yielded images of induced cankers and collection of root tissues. Canker area was measured using ImageJ. DNA was extracted from roots, amplified for fungal ITS2 region and bacterial 16S rRNA gene, sequenced on the Illumina MiSeq platform, with sequences processed into amplicon sequence variants using QIIME2. Canker size did not significantly differ between treatments; with a trend in canker size reduction noted for Phospho-Jet treated trees by 56 DAI. We expect microbial communities in infected tree roots to have reduced richness and diversity compared to uninfected tree roots. This study will extend our understanding of thousand cankers disease influence on tree microbiota and health, and may inform strategies for mitigating thousand cankers disease where it has spread.

Bacterial biocontrols in sustainable management of Phytophthora crown and fruit rot in pepper and watermelon

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Vegetable crop production in the southeastern U.S. is severely affected by various eukaryotic pathogens, such as fungi and oomycetes, causing millions of dollar crop losses annually. Among them *Phytophthora* crown and fruit rot caused by *Phytophthora capsici* is the most destructive soilborne oomycete in solanaceous and cucurbit crops. Due to wide host-range and presence of inoculum reservoir throughout the year, the pathogen poses serious threat to almost all vegetables and fruits, in pre- and post-harvest stages, which contributes significant economic losses to the vegetable industry. Due to lack of resistant cultivars, growers depend heavily on fungicides to control the spread of *P. capsici*. However, development of fungicide insensitivity among *P. capsici* isolates are a major concern. One of the best alternative sustainable approaches to prevent fungicide insensitivity in *P. capsici* is the use of environmental bacterial biocontrol with antifungal properties, which can suppress pathogen growth both in field as well as during storage and transport. In the current study, we have identified 40 bacterial isolates from natural environments, which can inhibit *P. capsici* growth on V8 media. The zone of inhibition activity against *P. capsici* ranges at a scale from 1 to 10 based on their antifungal properties. *Bacillus velezensis*, an aerobic PGPR forms the major category with highest antifungal activity. Further in planta experiments on selected antagonistic bacterial strains against *P. capsici* in watermelon fruits and pepper plants are in progress to be presented during the meeting.

The microbial community of winter wheat seed and its potential for biocontrol of the wheat pathogen *Fusarium graminearum*

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The fungal pathogen *Fusarium graminearum*, the causal agent of Fusarium head blight on wheat causes extensive damage and is the source of the mycotoxin deoxynivalenol (DON), which is toxic to humans and livestock. Due to the lack of strongly resistant wheat, manipulating plant-associated microbes to reduce FHB and improve crop yields is an appealing approach to control, but requires a thorough understanding of interactions within the phytobiome. With this in mind, we have used metagenomic techniques paired with the isolation of a range of bacterial and fungal communities present on wheat populations from the Long-term Ecological Research site at Kellogg Biological Station (LTER-KBS) in Battle Creek, MI. Bacterial endophytic strains isolated from kernels were tested for in vitro antagonistic activity against *F. graminearum* along with phenotyping for known plant growth promotion (PGP) activity. Eight bacterial strains were chosen based on successful performance of the inhibition and PGP trials for future study in enhancing wheat growth and disease inhibition. Microbial isolates such as these may serve as a future method of biocontrol to limit the disease and mycotoxin levels in wheat, reducing the need for pesticide applications.

Development and optimization of a universal detection assay for *Little cherry virus-2*

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Little cherry virus-2 (LChV-2) is one of two viral causal agents of little cherry disease (LCD), which is currently affecting cherry and stone fruit production in the Pacific Northwest. Diagnosis of LChV-2 infection is difficult as the characteristic fruit symptoms are only apparent at harvest and can be confused with syndromes caused by other biotic and abiotic factors. In addition, trees can be infected and asymptomatic for several years, allowing for further spread of LChV-2 via mealybug vectors before an accurate diagnosis can be made. With the increased availability of LChV-2 sequences and based on genotypic surveys of LChV-2 isolates in Washington state, we developed a new real-time RT-qPCR assay for LChV-2 detection. The assay was optimized; its sensitivity, selectivity, and exclusivity were assessed and a comparison against previously published LChV-2 assays was performed. It was found that the assay was specific to only LChV-2, able to detect both low and high-titer infections of multiple genotypes and was more sensitive to multiple genotypes than all other extant assays examined. Overall, this work allows for quick identification of both early and established viral infections in symptomatic and asymptomatic trees, as well as enabling out-of-season detection so that infected trees can be identified before fruit ripening. Early detection of disease aids in informed management plans for stone fruit orchards and the quick removal of infected trees.

New viruses associated with an emerging disease of crucifers that threatens the production of crucifers in Hawaii

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In 2019, bok choy (*Brassica rapa* subsp. *chinensis*) and mizuna (*B. rapa* subsp. *japonica*) plants presenting leaf mosaic and deformation were observed on Oahu, Hawaii. High throughput sequencing studies on both dsRNA and ribodepleted total RNA extracted from these plants indicated the presence of three novel viruses that are closely related to members of the genera *Coguvirus*, *Benyvirus*, and *Varicosavirus*. Mechanical inoculation assays on bok choy and mizuna seedlings allowed us to reproduce the symptoms observed in the field. More severe symptoms were observed when two or more viruses were present, suggesting the involvement of a synergistic interaction. Interestingly, the efficiency of one of the three viruses to be transmitted mechanically to bok choy seedlings was influenced by the choice of inoculation buffer. Resting spores consistent with *Olpidium* were observed following Coomassie blue staining of roots from field symptomatic plants. Multiplex PCR for discrimination of *O. brassicae*, *O. bornovanus*, and *O. virulentus*, revealed the presence of only *O. brassicae* in DNA extracted from roots of these plants. Thus, *O. brassicae* represents the most likely fungal vector for the putative new varicosavirus, while a plasmodiophorid is speculated to be a candidate vector for the new benyvirus. No vector is known for coguviruses. Due to the soilborne nature of the virus vectors and the high incidence of symptomatic plants at multiple locations on Oahu, this disease represents a threat to crucifer production in Hawaii. Studies looking for resistance to this new disease are warranted.

Transcriptional control of broad-spectrum defense response genes: Fast and furious

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Single gene resistance against pathogens in economically important crops is insufficient for long-term protection in many cases because pathogens progressively adapt and overcome these resistances. Identifying and understanding quantitative trait loci (QTL) for disease resistance can be a more durable option for crop improvement, and may have the added benefit of providing resistance against diverse pathogens. While rice (*Oryza sativa*) is a highly diverse species, transfer of diversity for QTL to agronomically relevant cultivars has proven difficult by conventional breeding techniques because of the complexity of these traits. Defense response (DR) genes participate in a wide variety of functions and typically underlay disease resistance QTLs. These genes are sometimes found in clusters of multiple duplicated members that show differential activation upon pathogen infection. Rice chromosome 3 contains a tandem repeat of four copies of oxalate oxidase (OXO) genes, with one gene, OXO4 being highly activated during pathogen infection. Evidence suggests that this is the result of polymorphisms found in the promoter region, which controls transcriptional activation of the gene. Using RNAi silencing and genome editing approaches, we demonstrate OXO4s influence as a broad-spectrum DR gene and highlight the importance of specific *cis*-regulatory motifs for fast and strong transcriptional activation during pathogen attacks.

Coinfection of soybean with *Fusarium virguliforme* and the root pathogens *F. acuminatum*, *F. oxysporum*, *F. solani*, and *Clonostachys rosea*

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Many diseases reduce soybean yield in the U.S., with root diseases often resulting in large losses. One important root pathogen of soybean is *Fusarium virguliforme* (Fv), the causal agent of sudden death syndrome (SDS). Although soybean roots from plants displaying SDS symptoms are often infected by multiple fungal pathogens, it is unknown how interactions between Fv and the other pathogens may alter SDS development or soybean growth. In this study, we examined potential interactions between Fv and two test isolates each of *F. acuminatum*, *F. oxysporum*, *F. solani*, and *Clonostachys rosea* in soybean plants in a growth chamber and in vitro to determine their effects on SDS disease development, soybean growth, and fungal growth. The in planta results suggest that coinoculation of soybean with Fv and *C. rosea* and with Fv and *F. solani* can reduce SDS foliar severity (FDS), and that coinoculation of soybean with Fv and *F. oxysporum* and Fv and *F. acuminatum* can increase FDS compared to the Fv-only treatment. Coinoculation of soybean with Fv and the test isolates individually did not increase or decrease root rot or plant biomass compared to infection by Fv alone. The growth of Fv was not significantly affected by the test isolates in vitro; however, the growth of some test isolates was reduced in the presence of Fv. This is the first study to investigate how Fv interacts with some common soybean root pathogens and suggests how these interactions may influence SDS disease development.

***Lysinibacillus*: A poorly characterized genus with potential for agricultural and environmental applications**

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Strains of the genus *Lysinibacillus* have been isolated from many environments but little is known about the ecological niche occupied by its member species. The presence of some *Lysinibacillus* species on plants and in soil and their ability to produce a plethora of metabolites suggest potential to be employed as biocontrol agents. Some *Lysinibacillus* strains are already used for either mosquito control or have been shown to have antifungal activity. Other strains withstand adverse environmental conditions and could be used in bioremediation. One species, *L. parvivoronicapiens* (Lp), has ice nucleation activity (INA), meaning it is able to trigger freezing at relatively warm temperatures and could be used for snow making or cloud seeding. Lp does not contain the INA gene of the well

characterized ice nucleator *Pseudomonas syringae*. Its INA is dependent on a non-ribosomal peptide synthase and a type I polyketide synthase. INA-negative mutants grow to significantly higher cell densities in pure culture implying a fitness cost associated with INA in *Lp*. Gene expression is being compared between mutant and wild-type strains, growth phases, and growth at different temperatures and environments to shed light on the role of INA in the life cycle of *Lp*. Comparative genomic analysis of the genus is being performed to determine evolutionary relationships and infer adaptations of its species to different environments. The investigation of *Lp* in the context of the *Lysinibacillus* genus is expected to open new avenues for the use of the genus in agricultural and environmental applications.

Identification of differentially expressed genes (DEG) of *Fusarium proliferatum* growing at a subinhibitory dose of iprodione

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Hormetic stimulatory responses have been widely reported in economically important plant pathogens. Subinhibitory doses of commonly applied fungicides can enhance fungal growth, conidiation, secondary metabolite biosynthesis and virulence in vivo and in vitro. However, little is known about the molecular mechanisms behind the hormetic response. The main objective of this study was to analyze and compare changes in gene expression in a *Fusarium proliferatum* isolate grown 72 h on solid media supplemented with the maximum growth-stimulating dose of iprodione (0.55 ppm) and a fungicide-free control using Illumina RNA-Seq analysis of three biological replicates per treatment. Illumina sequencing produced around 20M PE reads per each sample. Quality trimmed reads were mapped to the *F. proliferatum* reference genome (Refseq accession: GCF_900067095.1) and differentially expressed genes (DEGs) were identified using the fungicide-free samples as control. One-hundred seventy-seven genes were identified as being differentially expressed, one-hundred seventeen upregulated and sixty downregulated. Functional annotation of the DEGs revealed 57 had signal peptides, 24 were identified as carbohydrate active enzymes, 15 were putative fungal effectors (11 probably secreted in the apoplast), and 4 were predicted secondary metabolism genes. Some upregulated processes included carbohydrate metabolism and detoxification mediated by ABC transporters and cytochrome P450 enzymes. Six upregulated genes were selected based on their putative function and Log2FC to perform a RT-qPCR validation.

Development and integration of the new-age decision support in crop disease protection

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Answers to the frequent questions posed by producers are often available but not easily accessible. Producers have access to large quantities of data, but it is often scattered and does not reach them promptly to inform appropriate decisions. Integrating these data sources into reliable new-age crop protection decision support tools (DST) could provide useful information improving both tactical (in-season) and strategic (between season) decision-making in crop production. The increased availability of open-source software, data storage, and cheap computational resources allows their integration to improve integrated pest management and increase the reliability and sustainability of these DSTs. Our group's objective is to integrate multiple sources of information into an accessible and functional database. The data integration includes 1) environment (weather, climate, and soil characteristics); 2) host (varietal disease resistance levels and hybrid yield performance); and 3) pathogen (disease resistance scores and fungicide efficacy). This database will serve as an open data source for the new-generation disease management advisory platform based on big data and advanced statistical learning techniques by ensuring accurate data digitization, data quality control, and integration. Additionally, new learning resources and tools developed will facilitate the continuing education of a new generation of data-driven and technologically aware plant pathologists.

Development of a real-time PCR assay for the detection of *Erwinia aphidicola*, a recently reported pathogen in the United States

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The bacterium *Erwinia aphidicola*, first found in the gut of the pea aphid (*Acyrtosiphon pisum*), was reported as a plant pathogen in Spain in 2003, causing leaf spot and significant crop losses on beans and peas. In 2017, *E. aphidicola* was found in the U.S. on melon and since has been reported on onion, alfalfa, lupine, and lettuce. We are collaborating with the USDA APHIS-PPQ Beltsville laboratory to develop a real-time PCR assay (rtPCR) for rapid and efficient, species-specific detection of *E. aphidicola*. We compared the genomes of *E. aphidicola* and related strains to design species-specific primer sets in the Uniqprimer software. Four unique primer sets were evaluated by conventional PCR with DNA extracted from bacterial cultures of *E. aphidicola* and related strains. Primer set Ea4 and Ea5 resulted in DNA amplification from *E. aphidicola* and not from the related strains. We then performed rtPCR with SYBR® Green reagents to assess primer efficiencies. PCR fragments were sequenced and we designed TaqMan® probes that are currently under evaluation. The *E. aphidicola* rtPCR assay will be shared with other diagnostic labs and can be used to monitor the pathogen occurrence and spread in the U.S.

Characterization of *Pratylenchus dakotaensis* n. sp. (Nematoda: Pratylenchidae), a new root-lesion nematode species on soybean in North Dakota, USA

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Root-lesion nematodes (RLNs; *Pratylenchus* spp.) are important nematode pests on soybean (*Glycine max* (L.) Merr.). In 2015 and 2016, soil samples collected from a soybean field in North Dakota (ND) contained RLNs from 125 to 2,000/kg of soil. A greenhouse bioassay conducted with the RLN-infested soil on a soybean cultivar Barnes demonstrated that this RLN was able to infect and reproduce on Barnes with an average reproductive factor of 5.02. Morphological measurements and molecular characterization revealed that this nematode species is distinct in both morphology and DNA sequences from other known *Pratylenchus* spp. in the literature. In 2019, additional nematodes were recovered from a greenhouse culture on soybean originally from the same field for further analysis. Males, females, and juveniles of the target nematode were extracted from soil and root samples, and were examined morphologically. Additionally, DNA from single nematodes were extracted and the nucleotide features of D2-D3 region of 28S rDNA, ITS rDNA, and *COXI* gene were characterized. Phylogeny trees were constructed and PCR-RFLP was performed to ascertain the relationship with other *Pratylenchus* spp. Molecular features indicated that it is a new, unnamed *Pratylenchus* sp. that is different from morphologically closely related *Pratylenchus* spp., including *P. convallariae*, *P. pratensis*, *P. fallax*, and *P. flakkensis*. Both morphological and molecular observations indicate that the ND isolate on soybean represents a new root-lesion nematode species which is named and described as *Pratylenchus dakotaensis* n. sp.

Generation of a *Fusarium verticillioides* 7600 near isogenic Mat1-2 line for multi-gene mutant generation

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Fungal genetic systems ideally combine molecular tools for genome manipulation with a sexual reproduction system for informative assortment of combinations of genomic modifications. Critical molecular tools include efficient transformation and capacity for homologous integration, while the assortment approach requires a heterothallic sexual cycle. When employing the sexual cycle to generate multi-mutants, the background genotype variation of the parents may impact phenotypic variation amongst progeny. Here, to mitigate this variation, we generated a Mat1-2 strain that is near isogenic to the sequenced Mat1-1 *Fusarium verticillioides* wild type, FGSC7600. This was accomplished by crossing FGSC7600 as a male to the divergent wild-type strain FGSC7603 as female; followed by six generations of Mat1-2 progeny backcrosses to the FGSC7600 male. We sequenced each generation and mapped recombination. The first 3 crosses generated progeny with near predicted contributor genome content. The parental cross involved 26 crossovers on 9 of the 11 chromosomes, and the F1 had 54% (50% predicted) non-recurrent parent SNP genotype. Cross 2 and 3 progenies had 32% and 12%, (25% and 12.5% predicted), respectively. Inheritance of complete chromosomes without crossover was frequently observed. The final product strain (10/35, Mat1-2) retained 7% of the original Mat1-2 parent SNP genotype and, thus, is about 93% identical to FGSC7600. This is below expected, but still should theoretically yield final double mutants containing about 98% FGSC7600 compared to 75% by our standard two-cross method.

Developing a probe-based real-time quantitative PCR assay to detect *Peronospora belbahrii* using species-specific diagnostic markers

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Basil downy mildew, caused by the obligate oomycete *Peronospora belbahrii* (*Pbel*), is one of the most destructive diseases affecting basil production worldwide. Management can be challenging due to wind-dispersed sporangia and contaminated seed. Therefore, identifying *Pbel* in seed-lots before sale or planting, or in the field before symptoms develop could provide greater precision when deploying disease management strategies. In this study, a probe-based real-time quantitative PCR (qPCR) assay was developed, using two distinct *Pbel*-specific diagnostic markers as templates, with the goal of detecting *Pbel* in leaf tissue and seed samples. Standard curves were generated for both markers based on 12 genomic DNA concentrations (1 fg/μl to 10 ng/μl) and 8 sporangial counts (1 to 1,000). The two markers, M1 and M2, were capable of reliably detecting as low as 500 fg of *Pbel* genomic DNA and as few as 10 sporangia. The qPCR assay was further tested against seed samples collected from a heavily infected basil cultivar experiment. An internal control sequence was included in those reactions at a concentration of 10 aM to detect the presence of PCR inhibitors. In total, 48 seed samples were collected from 8 different cultivars (6 samples per) and tested using this assay. *Pbel* was detected in all cultivars and was quantified through comparison with the standard curves. PCR inhibition was not observed. This assay is valuable for diagnostics, identifying *Pbel*-contaminated seed lots, and detecting airborne sporangia, making it useful for predicting incoming basil downy mildew epidemics.

The colonization of various cover crops by the *Xylaria necrophora*—Causal agent of soybean taproot decline disease

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Taproot decline disease of soybean caused by *Xylaria necrophora* (*Xn*) has been reported as an emergent disease in the southern US. Cover crop planted in between harvesting and growing seasons is likely to improve overall soil health by contributing to the soil organic matter, reducing erosion, suppressing weeds and nematodes. However, it may have adverse effect to serve as potential host of *Xn* allowing overwintering. Species within *Xylaria* are commonly found on plant debris as saprophytes or in living plants and seeds as endophytes. Host range of *Xn* is currently poorly documented. In this study, we investigated the susceptibility of eight cover crop species: wheat, barley, rye, mustard, black oat, vetch, winter pea, blue lupine, along with susceptible soybean cv. Hutcheson. Two *Xn* isolates were tested in vitro and in growth chamber experiments. In vitro experiments, surface sterilized seeds were plated onto media

with and without *Xn* isolates growing, fungal colonization on seedlings was rated after 14 days. Under growth chamber condition, seed cup assays were carried out at 25°C with 12 h of light and 20°C with 12 h of dark cycle for optimal germination. Stand count, plant weight, and root weight data were collected to determine the pathogenicity of *Xn* on different cover crop species. In this study, we developed further understanding of the cover crop susceptibility to *Xn*, long-term survival strategy of *Xn* in soil using cover crops as rotation crop and will help with better cover crop species selection for reducing disease pressure in soybean cropping system.

Inoculation of two Roseau cane varieties with fungi isolated from die-off and non-die-off affected areas

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Roseau cane (*Phragmites australis*) is a wild plant with an extensive root system that is useful in areas vulnerable to soil erosion. In Louisiana, Roseau cane plays an important role in protecting the coastal infrastructure of the lower Mississippi River Delta (MRD). Currently, Roseau cane growing in the MRD is being affected by a die-off. Several biotic and abiotic factors have been associated with this problem. Because plant pathogens can negatively affect the growth of Roseau cane, we isolated foliar fungi from plants growing in die-off-affected and non-affected areas and conducted inoculations to two Roseau cane varieties. Fungi were cultured and identified at the genus level by morphology and molecularly using PCR with primers that amplify the internal transcribed spacer (ITS) region of the fungal ribosomal DNA. Four fungal isolates classified in the genera *Bipolaris*, *Alternaria*, *Nigrospora*, and *Fusarium* were used in detached leaves and whole plant inoculation experiments. The Delta and European Roseau cane varieties that are commonly found in the sampled areas were inoculated. In whole plant inoculation experiments, initial symptoms appeared 4 days after inoculation, and plants developed full foliar symptoms 10 days later. The European variety showed less severe symptoms than the Delta variety, which suggests that it might be more tolerant to plant pathogenic fungi.

Distribution of alleles related to carboxylic acid amide and quinone outside inhibitor resistance in host-adapted clades of *Pseudoperonospora cubensis*

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Pseudoperonospora cubensis, causal agent of cucurbit downy mildew, infects crops within the *Cucurbitaceae*. Downy mildew became the most destructive disease of cucurbits in the eastern United States after a population shift in 2004 resulted in the loss of cucumber host resistance and reduced efficacy of some commercial fungicides. Two host-adapted clades have been described for *P. cubensis* and clade-specific qPCR diagnostic markers were developed for differentiation. TaqMan® qPCR assays for detection of single nucleotide polymorphisms (SNP) that confer resistance to quinone outside inhibitor (QoI) and carboxylic acid amide (CAA) fungicides were also developed and used to genotype 385 isolates from diverse cucurbit hosts in North Carolina. Our results show that a specific SNP for resistance to CAA fungicides was more prevalent in Clade 2 isolates, and interestingly, CAA-sensitive isolates were less genetically differentiated regardless of *P. cubensis* clade. Additionally, wild hosts were capable of harboring *P. cubensis* isolates with resistance SNPs related to QoI and CAA resistance, with the QoI resistance SNP widespread in both clades. The clade assay was implemented into impaction-type spore trapping systems for field use validation in sentinel plots. Collectively, these findings will ultimately guide population-informed management practices for *P. cubensis* by monitoring inoculum levels, delivering information about cucurbit infection risk, and the occurrence of fungicide resistance alleles within a growing season.

Impacts of ground cover and plant variety on anthracnose in tea (*Camellia sinensis*)

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The US imports over 106,000 metric tons of tea annually, valued at approximately \$474 million. With increasing consumer interest in locally sourced foods, and improved harvesting technologies now available, US growers are taking renewed interest in tea production. In order to examine sustainable growing system components for tea production in Florida, we established two research sites in fall of 2018 on grower farms to test impacts of ground cover, tea variety, and shade on plant health and yield; data presented here are from one site. The primary disease concern for tea in the US is anthracnose, typically caused by *Colletotrichum camelliae*. Ground cover and variety impacted anthracnose incidence to different degrees through the first three growing seasons; in August of 2020, effects of variety and cover both remained non-significant at $\alpha = 0.05$. However, in spring of 2021, anthracnose incidence increased and was significantly higher in plants grown with "living mulch" ground covers than in plants under weed barrier cloth. Differences emerged among varieties as well, with lower disease incidence in the 'Fairhope' plants than in 'Red Leaf' and 'Large Leaf.' An unusual series of frost events interspersed with warm weather during the winter of 2020–2021 was a likely factor in the sharp increase of disease between fall and spring ratings, and in varietal differences. Work is ongoing to examine differences in pathogen dynamics among groundcover treatments.

Rapid assays for detection and differentiation of *Xylella fastidiosa* subsp. *fastidiosa* and *X. fastidiosa* subsp. *multiplex* on blueberry

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Xylella fastidiosa (Xf) causes bacterial leaf scorch (BLS) of blueberry in the southeastern United States. Two different subspecies of Xf have been associated with this disease: *X. fastidiosa* subsp. *multiplex* (Xfm) and *X. fastidiosa* subsp. *fastidiosa* (Xff). Originally, Xfm was thought to be the only cause of BLS, but Xff isolates have been reported recently in naturally infected blueberry plantings in

Georgia. Wide-scale investigations into the origins of isolates causing Xf outbreaks require routine methods for subspecies identification of isolates. Unfortunately, sequencing-based methods can be cumbersome, expensive, and relatively low throughput. To rapidly discriminate between the Xfm and Xff subspecies infecting blueberries, we developed a cleaved amplified polymorphic sequence (CAPS) marker and a LAMP assay that specifically target the RNA polymerase sigma-70 factor (*rpoD*) gene sequence of Xfm. The subspecies identities of Xf isolates grown in pure culture and infecting field-grown and inoculated greenhouse-grown blueberries could be reliably differentiated using the CAPS marker. Furthermore, using either the Genie® III system, gel electrophoresis, or SYBR™ Green I DNA staining, our LAMP assay was able to specifically detect Xfm isolates, allowing their differentiation from Xff isolates. Taken together, our results demonstrate that both of our developed methods have the potential to be routinely used alongside existing diagnostic methods to rapidly and reliably differentiate between the Xf subspecies causing BLS in blueberry in the southeastern United States.

Site-specific soil pest management in strawberry and vegetable cropping systems

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California produces 88% of the fresh market strawberries in the U.S. with a value exceeding \$2.2 billion/year. The majority of production is in fields where preplant soil fumigation has been used to manage soilborne pests. With production cost exceeding \$60,000/acre/year in the California central coastal region, pathogen management is essential for economic return for the growers. Multi-year field trials are in progress at five sites reflecting the major production districts of California to evaluate site-specific management of soilborne pathogens. Variable fumigant rates based on disease risk and crop rotation are being evaluated for their impacts on pathogen populations. The objective is to develop a fully integrated management approach for soilborne pathogens that reduces both fumigation rates and disease incidence. qPCR assays are used to quantify and map several lethal soilborne pathogens and assess fumigation rates, which are applied based on a risk assessment of observed pathogen populations/disease severity in the prior season. Weekly/biweekly drone flights capture calibrated remote sensing data with a 5-cm spatial resolution that supports monitoring of plant vigor and disease incidence. Precision yield data are collected with GPS enabled sensors throughout the season to evaluate the effect of fumigation and rotation treatments on yield. An economic analysis of alternative production practices provides information to support grower decision making. Correlations between remote sensing data and yield will be used to develop yield prediction models.

Pathogenicity of *Alternaria*, *Stemphylium*, *Neofusicoccum* and *Lasiodiplodia* species to cannabis (*Cannabis sativa* L., marijuana) plants

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Cannabis (*Cannabis sativa* L., marijuana) plants with stem canker symptoms grown at production sites in British Columbia (BC) and Ontario were sampled and affected tissues were surface-sterilized and plated onto potato dextrose agar. Isolates were identified by colony and spore morphology as well as PCR amplification and sequencing of the ITS1-ITS2 region of rDNA. The most frequently isolated fungi were *Alternaria alternata*, *Neofusicoccum parvum*, and *Lasiodiplodia theobromae*. In addition, *A. alternata* was recovered from cannabis inflorescences in BC and both *A. alternata* and *A. infectoria* were recovered from surface-sterilized hemp seed. *Stemphylium vesicarium* was isolated from hemp seeds for the first time. The pathogenicity of all these fungi to cannabis was confirmed by inoculation of leaves, stems, buds, rooted cuttings and mature plants. Disease ratings varied depending on the tissue type and pathogen, with both *L. theobromae* and *N. parvum* producing extensive stem canker symptoms on mature cannabis plants as well as extensive lesions on leaves. A comparison of the susceptibility of five cannabis strains to foliar infection by *N. parvum* indicated there were significant differences ($P < 0.05$) in lesion size, with some strains showing resistance. This is the first report of *L. theobromae*, *N. parvum*, *A. alternata*, and *S. vesicarium* causing stem canker and leaf spots on cannabis in Canada. The recovery of these fungi adds to the growing list of pathogens that cause infection and loss of quality in cannabis production. Disease management options have not been investigated.

A preliminary study on control of foliar fungal pathogens in North Florida watermelon production using environmental favorability index values

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In North Florida, powdery mildew can affect watermelon and causes severe foliar damage. In spring 2020, a field trial was conducted in North Florida to evaluate the use of MELCAST for timing fungicide applications for managing watermelon powdery mildew. This trial consisted of 6 treatments; grower standard spray program that targeted powdery mildew, grower standard with a secondary fungicide that targeted downy mildew and gummy stem blight, and three model-driven treatments with fungicides being applied when the sum total of the environment favorability index (EFI) values exceeded 30, 35, and 40 or at a 14-day interval, and a water control. The EFI value treatments were sprayed 8 times, and the three control treatments were sprayed 12 times during the trial period. Disease evaluations were made using the Horsfall-Barratt (H-B) scale at approximately 2- to 5-day intervals once the target disease began to

appear, and disease progress was determined using the area under the disease progress curve (AUDPC). Statistical analyses of AUDPC data indicated weekly application of the grower standard with the secondary fungicide provided the best disease control, separating statistically from other treatments ($P = 0.05$). The grower standard program was intended to target powdery mildew, but downy mildew and gummy stem blight disease pressure was high. A study with change in primary fungicide program focusing on downy mildew and adjustment of EFI thresholds is in progress to evaluate the potential of using EFI in North Florida watermelon production.

Genetic variability of *Xanthomonas euvesicatoria* pv. *allii*, associated with leaf blight outbreaks on onion crops at central region of Brazil

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Xanthomonas leaf blight, caused by *Xanthomonas euvesicatoria* pv. *allii* (XEA) has been a yield-limiting onion (*Allium cepa*) disease in certain regions. In Brazil, no remarkable outbreaks of the disease had been described until the recent occurrences observed on onion crops cultivated during the summer periods of 2018 and 2019, under sprinkle irrigation, at the central regions of the country. In order to better understand these occurrences and drive future researches on the disease management, a genetic study on the pathogen has then been initiated, and its results on the variability of BOX-PCR (primer BOX-A1R) patterns are reported. A collection of 30 isolates was obtained from symptomatic onion leaf samples, representing three hybrid cultivars, and *Euphorbia heterophylla* (three isolates), a weed species spontaneously grown among the onion plants in one of the sampled field. Reference isolates IBSBF 1770 (XEA) and EH 2010-60 (*X. euvesicatoria* pv. *perforans*) were included. Eight haplotypes were visually resolved. Ten isolates belonged to the same haplotype of IBSBF 1770. Each cultivar presented a predominant haplotype, even if they had been grown in different places at the same year, the cultivars do not share haplotypes among them, with one exception. These facts lead to a hypothesis of a possible association of the pathogen with the imported seeds of the cultivars used at that time. The isolates of *E. heterophylla* and of onion at the same site shared common haplotypes. Besides, the role of alternative hosts as primary inoculum source should also be addressed.

ValSten1, a new source of resistance to stem rot identified by greenhouse and field evaluation methods

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Peanut stem rot (white mold), caused by the fungus *Athelia rolfsii* (*Sclerotium rolfsii*), is the major soilborne disease in peanut. Peanut has limitations for disease improvement due to its narrow genetic base and the lack of strong stem rot resistance in the cultigen. Wild relatives are sources of new resistance that can be utilized for breeding. In this study, stem rot resistance in wild-derived allotetraploid peanuts was assessed by greenhouse bioassay (GB) and field evaluations (FE). Twelve allotetraploids and two controls, GA-09B (susceptible) and GA-12Y (resistant), were evaluated using GB three times in 2020. Stem cuttings were inoculated with *A. rolfsii* mycelial plugs, lesions were measured at 3, 5, 7, and 9 days after inoculation and the area under the disease progress curve (AUDPC) was calculated. Seven allotetraploids and controls were assessed by FE in 2019 and 2020. Seedlings were transplanted into rows of GA-12Y as interspersed individuals for similar environmental conditions within the canopy. Disease was rated during midseason and at harvest. Lesions of BatSten1 (*A. batizocoi* K9484 × *A. stenosporma* V10309)^{4x}, GregSten1 (*A. gregoryi* V6389 × *A. stenosporma* V10309)^{4x}, ValSten1 (*A. valida* PI 468154 × *A. stenosporma* V10309)^{4x}, and IpaDur3 (*A. ipaensis* KG30076 × *A. duranensis* 30060)^{4x} were significantly smaller than GA-09B and similar to GA-12Y in AUDPC of GB. Consistently, disease of ValSten1 was significantly less than GA-09B and similar to GA-12Y in FE. We conclude that the allotetraploid ValSten1 provides a potential new resource for stem rot resistance breeding.

Development of a system to assess effects of in vitro bacterization on phytobiome assembly in early generation seed potato production

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Manipulation of plant microbiomes is a promising strategy for improving crop health. Current approaches to phytobiome manipulation rely on biopesticides or biofertilizers but these tend to be expensive and inconsistent in performance. We hypothesize that introducing beneficial microbes in early stages of seed potato production will provide greater stability of plant-microbe interaction through the production process and increase crop resilience. To test our hypotheses, we isolated bacteria from plant tissues, inoculated them into micropropagated plantlets, and studied their effects. Individual colonies were selected based on morphological characteristics and a collection of 46 microbes was made. A subset of these strains was used to inoculate nodal cuttings of five-week-old tissue plantlets and, after 6 weeks, half the plantlets were rated for growth or stored for amplicon sequencing. The other half was transferred to a greenhouse. After 12 weeks, greenhouse plants were rated for growth and tissue samples were harvested for amplicon sequencing. Initial observations indicate positive effects of specific strains on root and shoot growth in vitro as well as earlier flowering in the greenhouse. Amplicon sequencing will be used to determine the effect of microbial inoculants on the microbiome and subsequent experiments will investigate the effects of applied microbes over multiple generations. Overall, the data generated will provide information about the impact of in vitro bacterization on phytobiome assembly and its possible use as a new strategy to improve crop health.

Identifying the determinants of aggressiveness in *Fusarium graminearum*

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Fusarium graminearum, a causal agent of Fusarium head blight on wheat, can cause significant yield loss and release mycotoxins that are known to cause diseases in both humans and animals. Levels of aggressiveness vary between *F. graminearum* strains. We hypothesize that the aggressiveness of *F. graminearum* is affected by the resistance level of the wheat source. Isolates of *F. graminearum* were collected from naturally infected wheat lines with different levels of resistance. For this research 31 isolates from highly susceptible and 26 isolates from moderately resistant wheat lines were used. We conducted in vitro phenotyping of these isolates by recording radial growth at 2, 4, 6, 8, and 10 days and sporulation at 10 days. To assess aggressiveness of the isolates, wheat was grown in the greenhouse and inoculated at anthesis. Three disease ratings were taken weekly after inoculations. The harvested kernels will then be used for DON quantification. We also extracted DNA from these isolates and submitted for library preparation and whole-genome resequencing to the University of Illinois' biotechnology center. This data is currently being analyzed and the results will be presented. We expect to better understand the determinants of aggressiveness of individual strains through the combined results of in vitro phenotyping, aggressiveness assays, DON quantification, and whole-genome resequencing. Our results will be useful for managing the deployment of disease-resistant wheat varieties.

Using transcriptome analysis to compare molecular mechanisms for stalk rot resistance in two maize hybrids with contrasting phenotypes

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Fusarium graminearum causes stalk rot in maize, a disease that can result in plant death, reduced yields, and mycotoxin contamination in livestock feed. Maize producers have limited capability to use chemical control for disease management due to costs; thus, the development of resistant varieties is desirable. However, the defense mechanism in maize against *F. graminearum* remains understudied and can be strongly influenced by environmental conditions. Transcriptome analysis of a resistant and a susceptible maize inbred line in response to *F. graminearum* during a time-course infection revealed differential downregulation of gene expression during the first two weeks in a resistant inbred line. Gene ontology enrichment analysis indicated that resistance may be caused by a modulation of gene expression for redox homeostasis, hormone biosynthesis, cytoskeleton activity, cell wall remodeling, and a delayed stress response. We validated RNA-seq data by measuring the expression of ten differentially expressed genes (DEGs) from plant samples grown in greenhouse and growth chamber environments using RT-qPCR, which revealed the effect of environmental conditions on the resistant phenotype and gene expression. This research expands our knowledge of molecular events underlying resistance to stalk rot and the impact of environmental conditions on the disease interaction. Our findings provide important information regarding the genetic basis of maize resistance to *F. graminearum* and are a first step towards developing resistant varieties.

Are the major genes to white pine blister rust controlled by the same orthologous locus in southwestern white pine and limber pine?

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Both southwestern white pine (*Pinus strobiformis*, SWWP) and limber pine (*P. flexilis*) are highly susceptible to the non-native pathogen *Cronartium ribicola*, which causes white pine blister rust (WPBR). Major gene resistance (MGR) has been identified in both species and their genetic loci were designated as *Cr3* and *Cr4* in SWWP and limber pine, respectively. *Cr4* was previously mapped on the *Pinus* consensus linkage group 8 with single nucleotide polymorphism (SNP) markers of functional genes. No genetic mapping has been performed in SWWP. In this study, following inoculation of progeny of one open-pollinated SWWP seed tree (BC8) using basidiospores, assessment of WPBR disease development detected *Cr3*-mediated phenotypic segregation. Resistant (*Cr3*) seedlings were stem-symptom-free while susceptible (*cr3/cr3*) seedlings developed cankers. Needles of individual seedlings were used for genotyping using Sequenom MassARRAY platform with a panel of *Cr4*-linked SNP markers. Association analysis using the general linear model (GLM) revealed that several *Cr4*-linked SNP markers were significantly associated with SWWP phenotypes, implying that *Cr3* and *Cr4* may share the same orthologous locus or loci close to each other. The top-associated SNP markers were developed into TaqMan tools and used to verify their association in SWWP disease screening trials, demonstrating that they were useful for marker-assisted selection (MAS) to distinguish MGR from quantitative disease resistance in SWWP. This is the first demonstration of the molecular basis of a WPBR resistance trait in SWWP.

Identification of the second nuclear donor involved in a somatic hybridization event that led to the Ug99 lineage of *Puccinia graminis* f. sp. *tritici*

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The history of recombination events leading to the formation of different lineages of the wheat stem rust fungus, *Puccinia graminis* f. sp. *tritici*, is not very well understood. Recently, haploid assemblies of two isolates (21-0 and Ug99) demonstrated that the Ug99 lineage was formed through somatic hybridization by transfer of the 21-0 lineage "A" haploid genome and an unknown donor lineage "C" genome (Li et al 2019). Allelic variation of eight conserved genes was used to screen isolates of *P. graminis* f. sp. *tritici* representing different lineages and identified clade II as the likely donor for "C" genome. To confirm, near-complete haplotype assemblies of two isolates, 87KEN3018 (clade II) and 72ETH11-4 (clade I, Ug99 lineage) were constructed using PacBio HiFi sequencing and HiFiasm assembler. The two haplotypes identified from 72ETH11-4 were 85 Mb (25 contigs) and 87 MB (21 contigs) representing genomes "A"

and “C” respectively, whereas 87KEN3018 comprised the “C” haplotype (85 Mb, 24 contigs) and a new haplotype “D” (88 Mb, 57 contigs). For verification of the haplotype assignments of these assemblies, sequence alignments were done against haplotypes “A” and “B” (21-0), and “A” and “C” (Ug99). In addition, pairwise diversity analysis of alleles from 1,299 conserved BUSCO genes further verified that the “C” haplotype in the Ug99 lineage (clade I) was derived from clade II lineage as represented by isolate 87KEN3018. Genomic tools are now available to elucidate the recombination events leading to the evolution of lineages of *P. graminis*.

Deciphering the *Chaetomium globosum* induced defense signaling network in tomato against early blight disease

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Chaetomium globosum is a potential biological control agent against various plant pathogens. While most studies report on the mycoparasitism and antibiosis of *C. globosum* against plant pathogenic fungi, only a few reports on its induced resistance. To gain insights into the induced defense mechanisms of *C. globosum* (Cg-2) against early blight of tomato, the suppression of disease by Cg-2 was evaluated and RNA-seq performed. There was a 30.9% reduction in disease severity in the Cg-2-treated plants. The expression of hormone signaling marker genes was analyzed by qPCR to determine the best time point for RNA sequencing. The transcriptome data revealed that 22,473 differentially expressed genes (DEGs) were expressed in tomato at 12hpi compared to control plants, of which, 922 DEGs had a two-fold up- or downregulation ($P < 0.05$). The KEGG pathway analysis revealed that most of the DEGs represented metabolic pathways, biosynthesis of secondary metabolites, plant-pathogen interaction, chlorophyll metabolism and plant hormone signal transduction. GO analysis revealed that DEGs were mainly related to the binding and catalytic activities, metabolic processes, response to stimulus and biological regulation. The gene modulations in hormone signaling transduction, phenylpropanoid biosynthesis and MPK signaling indicated their involvement. The results revealed the activation of JA and SA signaling pathways indicating the potential involvement of both induced systemic resistance (ISR) and systemic acquired resistance (SAR) in the resistance activated by Cg-2 in tomato against early blight.

Susceptibility to azoles of *F. musae*, agent of crown rot disease of banana

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Crown rot disease is one of the main post-harvest diseases of banana. It is a devastating disease that causes important losses every year. The rot is not visible when the bananas are boxed, and symptoms generally appear only after shipping to banana-consuming countries. *Fusarium musae* is a pathogenic species belonging to the *F. fujikuroi* species complex. It is a novel species firstly described in 2011. It contributes especially in central American countries to crown rot disease. In addition, it has also been found on human patients as a saprophytic agent. Given the potential risk of this novel pathogen to spread from food to clinical settings and the unknown mechanisms of transmission from one host to the other, knowledge on its susceptibility to widely used agricultural azole fungicides was investigated. Eight DMIs used in crop protection (prochloraz, tebuconazole, epoxiconazole, difenoconazole, propiconazole, tetraconazole, flusilazole, and fenbuconazole) were tested using the CLSI protocol methodology on 11 *F. musae* strains collected worldwide from infected bananas. The goal of the work was to define the level of susceptibility of the population to different drugs currently used in agriculture. Overall results show that the agricultural azole fungicides tested need to be applied at higher concentrations to inhibit the grow of *F. musae* compared to its sister species *F. verticillioides*.

Identification of a suppressor of RNA silencing encoded by Grapevine fanleaf virus

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RNA silencing is an effective antiviral defense mechanism utilized by plants to fight virus infections. To counteract this defense response, plant viruses encode viral suppressors of RNA silencing (VSRs). VSRs have been identified in most plant viruses, but it is unclear whether nepoviruses in the family *Secoviridae* possess VSRs, although the coat protein of Tomato ringspot virus, a subgroup C nepovirus, has been demonstrated to interact with Argonaute-1 protein to potentially prevent translation repression. No VSR has been reported yet for subgroup A nepoviruses, including Grapevine fanleaf virus (GFLV). We aim to identify a VSR encoded by GFLV, the causative agent of fanleaf degeneration, which is one of the most devastating viral diseases of grapevine. Following silencing of the enhanced green fluorescent protein (eGFP) in transgenic *Nicotiana benthamiana* expressing eGFP by *Agrobacterium tumefaciens*-mediated delivery of a dsRNA hairpin eGFP construct, chimeric constructs containing individual GFLV proteins or fused GFLV proteins were transiently expressed in eGFP-silenced plants via *A. tumefaciens*-mediated delivery. Local and systemic suppression of eGFP silencing by GFLV proteins was evaluated and quantified using fluorescence imaging, spectrometry, and RT-qPCR with specific primers. In addition, we probed the GFLV genome for the conserved tryptophan-glycine (WG)/glycine-tryptophan (GW) motif, which has been detected in numerous plant VSRs. The identification of a VSR of GFLV will enhance our understanding of virus-host interactions.

First report of oxytetracycline and streptomycin resistance genes in *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot in peach

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Xanthomonas arboricola pv. *pruni* (*Xap*) is a bacterial pathogen causing millions of dollars in annual losses to the southeastern U.S. peach industry. Conventional management strategies rely heavily on copper-based compounds and oxytetracycline (OTC); however, the disease incidence is still high, especially under favorable environmental conditions. During 2017–2020, over 400 *Xap* isolates from symptomatic leaves and fruit were collected from commercial peach orchards in South Carolina and screened for chemical sensitivity on antibiotic-amended media. A small percentage (2.2%) of *Xap* strains with OTC resistance were isolated over multiple years from geographically distinct orchards. Interestingly, the same isolates were also resistant to streptomycin (STR). PCR screens for OTC and STR-resistant candidate genes confirmed the presence of the tetracycline efflux gene *tetC*, the tetracycline repressor gene *tetR*, and the STR resistance operon *strAB*. Both the OTC and STR resistance phenotypes were transferrable from *Xap* to other *Xanthomonas* species via conjugation. When cloned into OTC-sensitive bacteria, the *tetC* and *tetR* genes together conferred OTC resistance levels of the field resistant isolates. A greenhouse experiment revealed that the currently recommended OTC rate was only able to reduce disease incidence and severity caused by the sensitive strains but not by the OTC-resistant strains. The existence of OTC resistance in the natural *Xap* populations of commercial peach orchards must be considered in current bacterial spot management programs.

Detection and initial molecular characterization of two novel emaraviruses co-infecting *Callicarpa* (beautyberry) by high-throughput sequencing

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The genus *Callicarpa* (beautyberry) contains approximately 140 species of perennial deciduous shrubs and small trees. The common name refers to its most well-known ornamental feature: prolific white, lilac, lavender, or purple-colored fruit that covers the shrub in late summer through autumn. Mosaic symptoms were observed on a *C. americana* L. plant growing in Auburn, AL. Total RNA was purified from leaves of this plant, and a custom cDNA library was prepared for paired-end Illumina MiSeq high-throughput sequencing (HTS) following rRNA depletion and NEBNext® Ultra™ RNA library prep kit protocols. Raw sequence reads (3.22 M) were trimmed and de novo assembled into 152,354 contiguous sequences. Contigs were subjected to BLASTX analysis against known viral sequences in GenBank databases and 15 contigs had significant protein homologies with members of the genus *Emaravirus*. Complete or near-complete sequences were obtained for genomic RNAs 1–4 of two distinct and novel emaraviruses. The in silico translated proteins have 18–55% amino acid identities with emaravirus orthologs RdRp (RNA 1), glycoprotein precursor (RNA 2), nucleoprotein (RNA 3), and movement protein (RNA 4). Results of ongoing HTS and RT-PCR analysis (using both sets of *Callicarpa* emaravirus-specific primers) with other *Callicarpa* samples will also be presented. Previously, the only virus reported to infect beautyberry was *Cucumber mosaic virus* (CMV). Tissue from this sample was negative using CMV-specific ImmunoStrips (Agdia, Inc.). To our knowledge, this is the first report of emaravirus infections of *Callicarpa*.

Establishment of Fusarium head blight screening nursery for winter wheat in Idaho

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Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an emerging disease of wheat in the Pacific Northwest. In Idaho, the occurrence of FHB is directly related to the increase in irrigated corn acreage. In addition to yield losses, FHB causes Fusarium-damaged kernels (FDK) and deoxynivalenol (DON) accumulation. Since 2019, an inoculated FHB screening nursery has been established in Kimberly, ID, to evaluate the susceptibility of widely grown and advanced lines of winter wheat. A total of 113 winter wheat lines were evaluated for FHB susceptibility with 49 lines planted in both 2019 and 2020. Plots were planted in randomized complete blocks with two replications. For each plot, disease severity (FHB Index) was assessed at soft dough. Subsamples of harvested grain were sent to a DON testing lab for analyses. All data were analyzed using the GLIMMIX procedure in SAS (v9.4). Under low FHB pressure in 2019, FHB Index and DON ranged from <0.1 to 56.4% and 0.8 to 39.4 ppm, respectively. Under high FHB pressure in 2020, FHB Index and DON ranged from 3.3 to 55.7% and 11.6 to 101.6 ppm, respectively. For both years, LCS Shark, WB1529 and WB1783 consistently had the highest levels of DON while WB4623CLP, OR2X2CL+ and Sequoia had the lowest DON concentrations. Since the majority of wheat varieties are susceptible to FHB, continuous FHB screening is necessary to reduce economic impacts and provide appropriate management recommendations to local growers in southern Idaho.

Transcriptomic analysis of two near-isogenic lines of bell pepper infected with bell pepper endornavirus and Pepper mild mottle virus

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Most bell pepper cultivars grown in the United States are infected with bell pepper endornavirus (BPEV). BPEV is a persistent RNA virus that lacks the genes for coat and movement proteins, and it is transmitted only vertically. The objective of this study was to characterize the transcriptome of two near-isogenic lines of bell pepper cv. Marengo, one infected with BPEV and the other BPEV-free after inoculation with the acute virus pepper mild mottle virus (PMMoV). Total RNA was extracted from BPEV-free, BPEV-infected, BPEV-free + PMMoV-infected, and BPEV-infected + PMMoV-infected plants and used for Illumina mRNA next generation sequencing. Differential gene expression analysis was conducted using the DESeq2 package on R. The results showed over 1,000 differentially expressed genes in plants infected with BPEV. In contrast, more than 3000 genes were differentially expressed in plants infected with PMMoV alone and in plants mixed-infected with BPEV and PMMoV. Gene ontology analysis indicated that most of the identified up regulated genes are involved in defense response to external biotic and abiotic stimulus and interspecies interactions

between organisms. These results suggest that BPEV is actively interacting with the host and with PMMoV. The data generated in this study will be useful to further investigate the molecular and biological interactions between endornaviruses and acute plant viruses.

Fine mapping of *Ur-11*, a unique gene that protects common bean from all but one of the many known virulent races of the bean rust pathogen

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Bean rust, caused by the fungus *Uromyces appendiculatus*, threatens common bean production. Planting rust-resistant varieties offers an effective control of bean rust. Genes with broad resistance to many races are needed to develop varieties with durable resistance because of the large number of races of *U. appendiculatus*. Most of the rust resistance genes in common bean are susceptible to more than 40% of about 100 races maintained in our laboratory. Conversely, the *Ur-11* gene from the common bean landrace PI 181996, confers resistance to all but one of all known races. Inoculating an F₂ population from the Pinto 114 (susceptible) × PI 181996 (resistant) cross with four races of *U. appendiculatus*, confirmed the presence of a single and dominant rust resistance gene in PI 181996. To accurately map the *Ur-11* gene we used a multi-prong approach that included high-throughput SNP genotyping (Illumina SNP chip), SSR and KASP markers, whole genome sequencing, and local haplotype analysis. The *Ur-11* gene was positioned in a 9-kb genomic region flanked by KASP markers SS375 and SS322 on chromosome Pv11. This region contained the PHAVU_011G200900g gene that encodes a nucleotide-binding site with a C-terminal leucine rich-repeat domain, typical of pathogen resistance function. Phenotyping and genotyping 201 common bean cultivars revealed the usefulness of the SS322 KASP marker for molecular breeding of common bean cultivars combining *Ur-11* with other rust resistance genes that together confer broad resistance to rust.

Light intensity and temperature effects on *Sclerotinia sclerotiorum* apothecial development

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Brassica carinata is a non-food oil crop being introduced to the southeast (SE) US as a winter crop. *Sclerotinia stem rot*, caused by *Sclerotinia sclerotiorum*, is the disease of most concern to SE US winter carinata with the potential to substantially reduce yield. Epidemics due to *S. sclerotiorum* are driven by ascospores ejected from apothecia that develop from sclerotia in the soil. We investigated the effect of light intensity in two temperature regimes on apothecia development. Four fungal isolates were collected from winter carinata or canola in the SE US and were conditioned by exposure to 18/6°C day/night (12-h cycle) for 6 weeks while shallowly buried in pots of sterile sand. After conditioning, ten sclerotia were placed on the surface of moist sterile sand in deep Petri dishes (25 mm depth) after which three replicate dishes were placed in each of two growth chambers with different temperature regimes (day/night: 18/6°C and 26/11°C). Three light intensity levels were established using netting: one layer of netting reduced light intensity by 57%, a second layer further reduced intensity by ~20%. Apothecia developing from sclerotia were counted and cumulative numbers of apothecia after 4 weeks were used in data analysis. Isolate and light intensity, but not temperature regime significantly affected the number of apothecia that developed. Apothecia numbers differed significantly for each light intensity level only for the Brewton, AL (south AL) isolate; however, numerically, sclerotia exposed to the lowest light intensity consistently developed greater numbers of apothecia.

Investigating the influence of heavy metals on aster leafhopper fitness and associated symbiotic bacteria

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Aster leafhopper (ALH; *Macrostelus quadrilineatus*) is the vector of the bacterial plant pathogen, aster yellows phytoplasma (AYp; *Candidatus Phytoplasma asteris*). Successful transmission of insect-vectored pathogens requires conditions conducive to both pathogen and vector fitness. Recent studies have elucidated inadvertent negative effects of heavy metal containing fungicides on insects; however, this phenomenon has not been investigated thoroughly in plant pathogen vectors. To better understand how heavy metal inputs regularly applied to AYp-susceptible crops affect the insect, ALH development and fitness was evaluated in a controlled environment. Even-aged, early instar nymphs were released onto oats (*A. sativa*) treated with copper hydroxide or left untreated. Estimates of nymphal development stage and survivorship were monitored until adulthood. Recent studies have demonstrated the importance of bacterial symbionts on insect fitness and pathogen transmission. The ALH is known to have two obligate symbionts, *Sulcia* and *Nasua*, that provide essential amino acids not obtained from the vector's diet, as well as facultative symbionts that could affect pathogen-insect interactions. Developmental effects we observed in the ALH in response to copper hydroxide may be due to the antimicrobial nature of these heavy metal containing compounds. To test this hypothesis, qPCR was used to measure the prevalence and relative abundance of known ALH symbionts in insects exposed in these investigations.

Differential effect of agronomic practices on the diversity and abundance of beneficial bacterial genera in soil

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Soil bacteria play a major role in plant health and soil fertility. Beneficial bacteria improve plant growth by various means such as enhanced nutrient availability and antagonizing harmful microbes. A study was conducted to examine how crop rotation (CR), tillage (T), cover crops (CC), and manure application (M) affect the diversity and abundance of beneficial bacterial genera in soil. Bulk soil samples were collected from 14 farms across Pennsylvania with histories of different agronomic practices. DNA extracted from samples were PCR amplified targeting bacterial 16S rRNA genes (515F/806R) and subjected to MiSeq sequencing. Effect of individual practices and their combinations on entire genera and beneficial genera ($n = 23$) based β -diversity was assessed using PERMANOVA and beta

dispersion tests. Results revealed a significant effect of CC-M-T-CR on entire genera ($R^2 = 0.26$, $P < 0.001$) and beneficial genera ($R^2 = 0.28$, $P < 0.001$). The impact of the level of each practice and their combinations on the mean abundance of individual beneficial genus was explored using a generalized linear mixed model approach. Results revealed an effect ($\alpha = 0.1$) of CC-M-T-CR on *Arthrobacter*, *Bradyrhizobium*, *Bryobacter*, *Microvirga*, *MND1*, *Mycobacterium*, *Pseudonocardia*, and *Sphingomonas*. None of the practices (individually or in combination) had an effect on 11 genera. Overall, results revealed how specific combinations of agronomic practices can be used to augment the abundance of certain beneficial bacterial genera in agricultural fields.

Multiplex real-time PCR assay for detection and discrimination of *Ralstonia solanacearum* R3bv2 from other strains in *R. solanacearum* species complex

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Ralstonia solanacearum R3bv2 strains, listed as a Select Agent in the United States, is a highly destructive pathogen causing bacterial wilt on many crops worldwide. A sensitive, accurate and discriminative tool is critical for monitoring, surveillance, and seed certification for domestic and international regulatory agencies. Unique genes in *R. solanacearum* R3bv2 and other strains in the *R. solanacearum* species complex (RSSC) were identified through comparative genomics. Primers and probes showed 100% specificity with the target strains based on in silico analysis. The assay was validated using 3 sets of primers/probes specific for RSSC, RS R3bv2 and UIC (universal internal control). The RSSC primers were designed to amplify all *Ralstonia* genospecies. The 2nd primer set was specific for RS R3bv2, and UIC was used to monitor potential inhibition during amplification. The specificity of the developed assay was confirmed with 94 bacterial strains ($n = 42$ RS R3bv2, $n = 43$ other RSSC and $n = 9$ other species and endophytes). No false positives or negatives were obtained with any strain in the exclusivity or inclusivity panels. The sensitivity of the developed assay was 1 pg for both pure bacterial genomic DNA and bacterial DNA spiked into plant DNA assays (potato and geranium). The RSSC assay detected and discriminated the target pathogens from plant samples with 100% accuracy with no false positives or negatives (both naturally and artificially infected). The developed assay has potential applications in routine diagnostics, disease management, and agricultural biosecurity.

Development of serological diagnostic assays for boxwood blight disease

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Boxwood plants have been the backbone of many historical gardens and landscape sites. They are also important nursery crops; the annual wholesale value in the USA is at least \$10 million. Boxwood crops and plantings have been devastated by the boxwood blight disease, caused by *Calonectria henricotiae* (*Che*) and *C. pseudonaviculata* (*Cps*), since its discovery in the UK in 1990s and first report in the USA in 2011. To date, this disease has been found in all temperate continents and 30 U.S. states. Because it has no known cure, early detection and prompt removal of infected plants are the key to successful management of the disease. Diagnosis of boxwood blight currently relies on symptoms, pathogen isolation and identification by morphological characteristics. There is an urgent need for rapid and sensitive diagnostic assays for point of care detection of the pathogens on asymptomatic plants. In the present study, we are developing antibody-based assays to detect *Che* and *Cps*. Based on proteomics data, we identified a high-abundance secreted protein that is unique to *Che* and *Cps*. Rabbit polyclonal antibodies were raised against two individual partial sequences of the protein target. Using Western blot analyses, we found these antibodies to be reactive and specific to the target protein in extracts of *Che*- and *Cps*-infected boxwood leaves, but did not react against *Pseudonectria buxi*-infected or healthy leaves. Additional evaluations including enzyme-linked immunosorbent assay and Western blot screens are underway to determine the specificity and sensitivity of these antibodies.

Movement of grapevine red blotch virus within the three-cornered alfalfa hopper and transstadial transmission

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Grapevine red blotch is an emerging disease that affects the grapevine industry by altering fruit quality, reducing vine growth, and lowering a vineyard's overall profitability. Grapevine red blotch virus (GRBV) from the genus *Grablovirus*, in the family *Geminiviridae* is the causal agent of the disease. GRBV is transmitted by *Spissistilus festinus*, the three-cornered alfalfa hopper via circulative, nonpropagative transmission with an extended acquisition access period. A comprehensive analysis of how GRBV and *S. festinus* specifically interact is important for understanding disease spread in vineyards and developing integrated disease management strategies. The aim of our research is to characterize transmission attributes of GRBV by *S. festinus*, particularly virus movement within the vector and through molts. We used time course experiments to quantify the virus titer by qPCR in dissected guts, hemolymph, and heads with salivary glands and identified the epithelial cell layers of the gut and salivary glands as potential barriers of GRBV movement within the insect vector. We also designed assays to monitor sequential virus transmission by *S. festinus* nymphs through adulthood and documented transstadial transmission. Our findings lead to the development of a model of the GRBV pathway in *S. festinus* following acquisition on infected plants, in different *S. festinus* developmental stages, as well as in progeny over time.

Inoculation of stigma-colonizing microbes to apple stigmas alters microbiome structure and reduces the occurrence of fire blight disease

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Flowers secrete nutrient-rich exudates that support the growth of an assemblage of microorganisms, including both beneficial and pathogenic members, most of which belong to the phylum Proteobacteria. Given the potential role of microbiome in plant health, manipulating the microbiome to promote growth of beneficial members holds promise in controlling plant diseases. In this study, we inoculated four bacterial strains that were originally isolated from apple stigmas, alone or in mixtures of increasing complexity, onto apple flowers. We tested whether such treatments would influence fire blight occurrence, a disease caused by *Erwinia amylovora*, and whether we could detect a shift in the structure of the microbiome due to the treatments. We show that various inoculations did influence the occurrence of fire blight in different levels dependent upon specific bacterial strains. Treatments using different strains or strain mixtures predominantly resulted in increased representation of the inoculated strains, suggesting that disease suppression was due to an alteration of the stigma microbiome structure. Compared with treatments using single strains, a *Pantoea*–*Pseudomonas* strain mixture produced a homogeneous microbiome structure with less interflower variability. Findings from this study suggest that the microbiome on the flower stigma can be manipulated through microbial inoculation. Due to flowers' short life span yet important role in plant disease infection, even a short-term influence on microbiome composition may result in significant decreases in disease susceptibility.

Identification of Tobacco streak virus and a novel allexivirus infecting *Liriope muscari* (lily turf)

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Total RNA was extracted from a combined leaf sample from two plants of *Liriope muscari* cv. Royal Purple, and one of cv. Monroe White showing leaf mottling. To determine the cause, RNA was DNase treated, rRNA depleted and cDNA prepared for Illumina NextSeq500 by SeqMatic (Fremont, CA) to yield 75 bp single end reads. 15,010,111 reads were obtained, of which 1,147,377 were assembled into three contigs of 3,495 nt (328,139 reads, 7,113× coverage), 2,896 nt (85,128 reads, 2,226×), and 2,239 nt (728,110 reads, 24,623×), respectively, with >99% nt identity to RNAs 1, 2, and 3 of soybean isolates of tobacco streak virus (TSV), comparable to the 3,491 nt, 2,926 nt, and 2,205 nt of RNAs 1–3 of the NCBI RefSeq, suggesting that almost the full sequence of each TSV RNA was obtained. A further 12,536 reads were assembled into an 8,777 nt contig (108× coverage) with closest affinity to *Arachis pintoi* virus, recently accepted by ICTV as a species of the genus *Allexivirus*, with several other allexiviruses infecting hosts other than *Allium* spp. The degree of genome coverage to various allexiviruses did not exceed 47%, with a maximum of 69.12% nt identity over the region of the genome covered. Six ORFs were detected using ORF Finder (NCBI), representing respectively Rep protein of 1,735 amino acids (aa), TGB1 (236 aa), TGB2 (114 aa), TGB3 (59 aa), '40K protein' (368 aa), CP (247 aa). The degree of coverage (~62–100%) and range of aa identities (<52–71%) for each ORF suggest that the allexivirus from *L. muscari* is a new species of the genus, while TSV has not previously been reported from this host.

Distributions of two aflatoxin biocontrol product active ingredients in southern U.S. corn

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Biocontrol utilizing non-aflatoxigenic strains of *Aspergillus flavus* is effective for reducing aflatoxin in crops. Two aflatoxin biocontrol products are currently registered by the U.S. EPA: Afla-Guard, for use in peanuts, tree nuts, and corn in the continental U.S., and AF36 Prevail, for use in a limited number of states in cotton (AZ, TX, CA); pistachio, almond, and fig (CA, AZ, TX, NM); and corn (AZ, TX). Corn growers in states not included on the AF36 Prevail label have expressed a desire to have this product as an additional option for aflatoxin control. Thus, objectives of this study were to 1) determine distributions of biocontrol strain genotypes in southern U.S. corn; and 2) document areas where AF36 is endemic as a basis for expanding the AF36 Prevail label. In 2014–2017, corn was collected from grain elevators throughout the southern U.S. *Aspergillus flavus* was isolated and genotyped with 17 SSR markers to determine frequencies of AF36 and Afla-Guard haplotypes. Afla-Guard was dominant across the Mid-South (mean = 52.5% of isolates) suggesting it is regularly applied in the region, but it occurred less frequently in southeastern states (mean = 1.4%). AF36 was detected at a low frequency (mean = 1.7%) throughout the southern U.S. Thus, AF36 is widely distributed in corn from southern states where it is not in commercial use. This indicates AF36 is endemic and adapted to southern corn production areas. Expansion of the AF36 Prevail label to include additional states will provide growers with another tool for aflatoxin mitigation.

Development of a species-specific diagnostic assay for *Ceratocystis fimbriata* using a high-quality genome assembly and comparative genomic analysis

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Ceratocystis fimbriata, causal agent of black rot, is known to be one of the most destructive pathogens of sweetpotato and can lead to annual financial losses of over \$150 million. Such losses are possible in part because *C. fimbriata* can infect sweetpotato at all stages of production, from seed roots to postharvest storage and packing. Accurate detection of *C. fimbriata* during production would allow for more efficient deployment of management tactics, serving as a great benefit for disease control efforts. To develop such a diagnostic assay, PacBio SMRT DNA and Illumina NextSeq DNA and RNA reads of *C. fimbriata* were assembled and annotated using CANU and MAKER. The resulting 31.8 MB assembly was near-chromosome level, with 19 contigs, 6,485 predicted genes, and a BUSCO completion score of 98.6%. Additional Illumina MiSeq DNA reads from *C. manginecans*, *C. platani*, and a second *C. fimbriata* isolate

were then mapped to the assembled genome using BOWTIE. The number of reads mapped to all predicted genes were counted using HTSeq, which identified 148 genes present only within *C. fimbriata*. Of these genes, 3 were single-copy and 10 were highly multi-copy (>100 BLAST hits), as determined through a BLAST alignment. Primers designed in the 200-bp flanking region of each exon are being validated against a diverse DNA panel containing *Ceratomyces* species, sweetpotato pathogens, and plant hosts. Once validated these genetic markers will serve as valuable diagnostic tools with multiple applications including the detection of *C. fimbriata* in seed, soil, and wash water.

Analytical chemistry as a tool to discover potato soft rot and black leg disease resistance

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Soft rot and black leg disease, caused by *Pectobacterium*, are one of the major threats to cultivated potato (*Solanum tuberosum*). The disease is spread worldwide and curative measures does not exist. Recently, resistance was identified in *Solanum chacoense* M6, however past efforts to identify genetic loci tied to resistance failed. We extracted metabolites from *S. chacoense* M6 and found that they inhibited quorum sensing, motility, and exoenzyme activity in *Pectobacterium* and that they also caused filamentous growth in bacterial cells. To identify the metabolites affecting bacterial virulence, we used analytical chemistry methods to compare metabolites from *S. chacoense* M6 with the susceptible *S. tuberosum* DM1. We identified several candidate metabolites from multiple chemical classes that may contribute to resistance, including alkaloids, phenolic amines, phenols, amines, and peptides. We initiated screening of nearly 100 recombinant inbred lines from an *S. chacoense* M6 $\times$ *S. tuberosum* DM1 cross for their potential to inhibit *Pectobacterium* quorum sensing. Initial results suggest involvement of three separate chromosomal regions. The combination of this genetic approach with further metabolomic analysis and genomic analysis may allow identification of specific genes and metabolites that inhibit *Pectobacterium* phenotypes tied to virulence.

Genome-wide association studies of Fusarium wilt resistance in wild spinach, *Spinacia turkestanica*

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Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *spinaciae* (*Fos*), is responsible for major losses in spinach (*Spinacia oleracea*) seed production in the Pacific Northwest USA. Wild spinach, *S. turkestanica*, has been used as a source of resistance to spinach diseases. Genotypes of *S. turkestanica* (68) and *S. oleracea* (16) were evaluated for reactions to *Fos* at moderate and high levels of inoculum of a mix of three isolates. The area under the disease progress curve (AUDPC) for genotypes planted in moderate- and high-risk soils ranged from 0 to 11.0 and 1.5 to 13.3, respectively, with an AUDPC of >5.5 considered susceptible. Single nucleotide polymorphism (SNP) markers ($n = 7,065$) identified with genotyping-by-sequencing were used for genome wide association studies (GWAS) with a general linear model in which population structure was used as a covariate in TASSEL 5. Population structure analysis revealed three subpopulations: *S. oleracea* (*Q1*), *S. turkestanica* (*Q2*), and *S. turkestanica* (*Q3*), comprising 10, 30, and 30% of the genotypes, respectively, with 31% of the genotypes showing admixture. Nineteen SNPs were associated significantly with AUDPC ratings at the moderate inoculum level ($R^2 = 11.7$ to 27.7%, $-\log P = 3.0$ to 5.49) and seven SNPs were associated significantly with AUDPC ratings at the high inoculum level ($R^2 = 5.3$ to 9.7%, $-\log P = 3.0$ to 3.86). A major QTL was detected on chromosome 6, and minor QTL on each of chromosomes 1 and 3. These SNPs can be used to develop high-throughput markers to breed for resistance to Fusarium wilt in spinach.

Screening grain crops for resistance/susceptibility to *Meloidogyne enterolobii*

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Guava root-knot nematode (GRKN = *Meloidogyne enterolobii*) is now considered one of the most important *Meloidogyne* species because of its high reproduction rate and ability to overcome root-knot resistance genes of important crops. This nematode has a broad host range and is known to cause significant damage and economic losses on several crops worldwide. *M. enterolobii* was first transported to Louisiana in sweetpotato seed roots shipped from North Carolina in March 2018. There have been several reports of crops that are considered either susceptible or resistant to GRKN. However, reports on resistance/susceptibility are conflicting within a crop. There is limited information about the reaction of important grain crops grown in Louisiana to *M. enterolobii*. We therefore assessed the reaction of soybean, corn, and sorghum varieties to a population of GRKN derived from the infected seed from NC. Screening of grain crop varieties was conducted in two consecutive years in a greenhouse following a special protocol for containment of GRKN. Low levels of GRKN reproduction occurred on two of the five soybean varieties tested in the first year and reproduction levels were much lower than on Rutgers tomato, the susceptible standard. However, the reproduction levels on soybeans was considerably greater in the second screening with all soybean varieties showing severe root galling, a typical symptom caused by *M. enterolobii*. Corn and sorghum varieties had high levels of resistance to GRKN and could potentially be used in crop rotation as a management option for this nematode.

Virulence enhancement and registration of a bioherbicide for *Striga hermonthica* (witchweed): Flipping the traditional role of plant pathology

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Striga hermonthica (witchweed) is a critical obstacle for crop production on more than 40 million farms across Sub-Saharan Africa. *Striga* infestations reduce maize and sorghum yields by 20–80%. *Fusarium oxysporum* f. sp. *strigae* is a host specific pathogen of *Striga*

with insufficient virulence to function as an efficacious bioherbicide. Its virulence can be enhanced considerably by selection of strains that excrete the amino acids L-leucine, and L-tyrosine. These amino acids inhibit amino acid biosynthesis in *Striga*, but not maize. Methionine is converted by soil microbes to ethylene, a seed germination stimulant. As the soil bank of seeds is problematic, excretion of methionine was selected to trigger germination. Amino acid excreting strains were field tested to control *Striga* with average maize yield increases on smallholder farms from 42 to 56%. In the control of *Striga*, the primary inoculum was produced on toothpicks (wooden dowels), hence the name, the Toothpick Project. These are delivered to farmer's groups to manufacture a village-level inoculum with cooked rice. This secondary inoculum is co-planted with the maize, resulting in reduced *Striga* counts and increased yield. Registration for commercial use of Kichawi Kill™ as a bioherbicide for *Striga* biocontrol was issued in Kenya in February 2021. *F. oxysporum* is a pathogen of numerous weeds, and most if not all plants can be inhibited by at least one amino acid. Therefore, the opportunity exists to use this virulence enhancement of host specific fungi as weed biocontrol agents.

Management of pre- and post-emergence damping-off of soybean caused by *Calonectria ilicicola* using fungicide seed treatments

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Calonectria ilicicola is a soilborne fungus known to cause red crown rot in soybean and to cause Cylindrocladium black rot in peanuts. Symptoms include pre- and post-emergence damping off early in the season, tap root rot, and reddening of the crown area that often becomes encrusted with red-orange perithecia later in the season. The objective of this greenhouse experiment was to determine if fungicide seed treatments could reduce the level of pre- and post-emergence damping off in soybean. Two greenhouse trials used six different seed treatments and a non-treated control. All treatments were inoculated with a slurry of the fungus poured over the seeds at planting. Data on germination were recorded 7, 14, and 21 days after planting. On day 25, the roots were washed, rated, and placed into a dryer for a week. Seed treatments of fluopyram, pydifumetofen, or sedaxne, had the highest stand counts, the lowest rating of root rot, and highest root weights, while other fungicide treatments had little or no difference from the non-fungicide treated seed. Overall, fluopyram, pydifumetofen, or sedaxne, had increased seed germination of over 75.2% compared to the non-fungicide treated seed. The results of this experiment show that seed treatments provide adequate control of pre- and post-emergence damping off and could be useful to soybean growers that encounter stand establishment problems due to *C. ilicicola*.

Analysis and improvement of *Plasmopara* genus- and species-specific diagnostic markers for detection in vineyards

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Early and rapid detection of pathogens within agricultural entities is a critical first step in disease management. Within vineyards, downy mildew (DM) of grapes, caused by *Plasmopara viticola*, is a rampant polycyclic disease. The oomycete pathogen overwinters in leaf litter as oospores and causes infections throughout the season through zoospore secondary inoculum spread. Control of DM is accomplished using chemical control as well as cultural practices, but resistance to numerous fungicides has developed. To understand the inoculum load and mutations found throughout a growing season, molecular detection methods using quantitative polymerase chain reaction (qPCR) and TaqMan probes are used. These tools allow for pathogen quantification as well as low-inoculum detection within a field for asymptomatic diagnosis. In this study, genus- and species-specific primers and probes were studied and developed to optimally detect *P. viticola* within vineyards from impaction style spore traps. Two previously designed markers utilizing unique gene regions were compared to determine the most sensitive and accurate method for *P. viticola* detection. Additionally, an improved species-specific molecular marker was developed. Primers and probes were validated against 75 *Plasmopara* spp. isolates as well as species within the Peronosporales order. The development of these diagnostic tools plays a crucial part in integrative disease management as they can be used in combination with cultural practices for increased control of recurrent and resistant populations of DM in vineyards.

Dip application of sanitizers in managing *Calonectria pseudonaviculata* on boxwood greenery

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Calonectria pseudonaviculata, which causes 'boxwood blight', is constantly threatening the boxwood greenery market. It often causes significant economic loss to all parties (growers-retailer) in the postharvest chain. The objective of this study was to evaluate the efficacy of sanitizers for managing postharvest boxwood blight disease on boxwood greenery. 'Green Velvet' boxwood greenery (10–15 cm long) were harvested and inoculated with a conidial suspension of *C. pseudonaviculata* adjusted to 1×10^5 conidia/ml. Boxwood greenery was then placed in a plastic container with a tight-fitting lid and a wet paper towel to create enough moisture and promote sporulation of inoculum. After 3 days of incubation at 25°C, the boxwood greenery was dipped in a treatment solution for 10 s. The positive control received sterilized distilled water whereas the negative control was only treated with conidial suspension. The cut ends of the boxwood greenery were submerged in 50-ml centrifuge tubes filled with tap water; leaves and stems were aerially displayed. Boxwood blight severity was rated using a 0-100% scale every 2 days for 1 week. The treatments including Simple Green D Pro 5 (15.6 ml/l), KleenGrow (15.6 ml/l), Vinegar (50%), sodium hypochlorite (10%), Virkon (2%), and GreenShield (55.9 ml/l) were the most effective in reducing the postharvest boxwood blight disease severity and progress on harvested boxwood greenery. The findings of this study will assist growers and allow for better management of boxwood blight disease in boxwood greenery.

ImageJ analyses of blueberry selections infected with *Colletotrichum fioriniae*

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Anthraxnose fruit rot of blueberry caused by the fungus *Colletotrichum fioriniae* causes substantial damage to fruit structure and quality, causing significant losses in highbush blueberries in Michigan. Analyzing physical decomposition is critical to understanding

the process of plant-pathogen interactions caused by this fruit rot. ImageJ analysis was conducted on 15 blueberry selections showing both high and low incidence of anthracnose. Blueberry selections were imaged pre-infection and post-inoculation to compare fruit rot incidence, berry size, calyx size and ratio of berry to calyx. Blueberry selections were placed in pans, photographed and inoculated with a concentration of 1.0×10^6 spores/ml. Pans were covered in plastic wrap to limit spore dispersal and increase humidity. After 14 days post-inoculation, berries were photographed again to capture disease incidence and berry size changes. Susceptible and resistant selections were observed. Blueberry selections with the highest disease incidence had the most noticeable variations in berry size and calyx size. In general, there was a direct correlation between anthracnose fruit rot levels and alteration/decrease of blueberry fruit and calyx size. This study ultimately gives us insight into the effects of infection and fruit quality after harvest.

Evaluating early blight fungicide program efficacy and return on investment in Wisconsin potato systems

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Early blight (EB) caused by the fungus *Alternaria solani* is a perennial foliar disease of potato (*Solanum tuberosum*) that negatively impacts production in Wisconsin and other temperate regions. The disease causes premature defoliation of most commercial cultivars, ultimately leading to losses in yield and tuber quality. Conventional potato growers in Wisconsin rely on routine fungicide applications for effective EB control. There are many fungicides currently registered for managing EB, with additional in various stages of development. New registrations can provide enhanced control, but cost must be considered for positive return. Thus, we conducted a trial with a randomized complete block design and 4 replications on ‘Russet Burbank’ potato at the UW Hancock Agricultural Research Station to evaluate the efficacy and return on investment (ROI) of 20 fungicide programs, including at-planting and weekly foliar treatments, for EB control. EB severity was assessed six times using the Horsfall-Barratt (0–11) rating scale. The area under the disease progress curve (AUDPC) was determined and converted into relative AUDPC (RAUDPC). Compared to the non-treated control, all 20 treatments resulted in significantly reduced EB severity, and 15 had significantly increased marketable yield. A ROI analysis was then conducted using current market data. These ROI and disease control analyses provide growers with valuable information to guide selection of cost-effective and efficacious EB fungicide programs with consideration to fungicide resistance management and farm economics.

Building a teaching case study about grapevine phylloxera, planting decisions, and area-wide pest management

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Case studies are teaching tools to help students engage on a personal level with real-world challenges faced by growers. Writing a successful case study for publication and classroom use requires building outward creatively from a central question with multiple plausible answers. In order to accurately reflect real-world conditions and provide enough information to support students’ decision-making, fact-finding and expert input are necessary. “The phylloxera challenge: Planting a new grape vineyard in Victoria, Australia” is a case study that was developed to incorporate issues related to the spread of the insect pest phylloxera, resistant grape cultivars, rootstock grafting, area-wide pest management, and grape and wine quality. The greatest challenge in writing the case study was avoiding overly lengthy or detailed explanation, while providing enough background for students of varying academic experience to make informed decisions. The case was presented in Iowa State University course Understanding Grape and Wine Science (Hort/FSHN 276). Course delivery was virtual but synchronous (via Webex), and the “breakout session” feature was used for small group discussions. The case study elicited a broad range of discussion and perspectives. Feedback from students (via Qualtrics) indicated that the case improved their understanding of vineyard management, phylloxera as a vineyard pest, and strategies for controlling phylloxera in at-risk grape growing regions. Successful use of the case study as a virtual-format teaching tool is an encouraging option for active learning.

Greenhouse screening and identification of sunflower germplasm with resistance to Sclerotinia basal stalk rot

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Basal stalk rot (BSR) of cultivated sunflower (*Helianthus annuus* L.) is caused by necrotrophic fungal pathogen *Sclerotinia sclerotiorum* (Lib) de Bary. It is an economically vital disease limiting sunflower production in the Northern Great Plains region of the USA. Improvement of host plant resistance is an important goal for sunflower breeding because producers have limited options for managing this disease. However, resistance to BSR is quantitative and generally complex, hindering efforts to develop a sunflower hybrid with a high level of resistance. This study aims to evaluate genotype $\times$ isolate interactions under greenhouse trials; 16 inbred lines that are significantly more resistant than moderate susceptible inbred check variety are selected from greenhouse BSR evaluation of 60 cultivated sunflower lines (based on results obtained from field and nursery trails). Sunflower plants (5 weeks old) are inoculated by multiple fungus isolates and evaluated for 28 days based on terminal wilt and desiccation of the plant. The results suggest that one inbred line displayed significantly high-level resistance to the isolates. Future works will include molecular marker evaluation of these inbred lines with QTL markers developed from a RIL population derived from a cross between HA 441 and RHA 439 to develop a novel QTL for physiological resistance and genetic mapping.

National Plant Diagnostic Network Protocols and Validation Committee: Best practice guidelines in plant disease diagnostics

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Accurate and early diagnosis of plant diseases remains the cornerstone of a successful disease management program. Early detection and diagnosis of pests and diseases play a vital role in eradicating and preventing their spread. The National Plant Diagnostic Network's (NPDN) Protocols and Validation Committee compiled best practice guidelines (BPG) that focus on specimen collection, transport to the lab, processing, morphological identification, microbiological testing, interpretation of results, and reporting of results. Molecular-based identification methods were not included in this current version due to the complex nature of testing and interpretation of results. Many factors can determine the outcome of a disease diagnosis effort. BPG at each step ensure quick turnaround times and reliable results. While both macroscopic and microscopic features of symptoms and signs are considered for correct identification of a pathogen, BPGs that are consistent with the recommendations made by this committee should lead to most desirable outcomes. New diagnosticians and plant pathologists who detect and identify pathogens will benefit from these BPGs. We aim to provide a more profound understanding of the diagnostic process and its challenges. Researchers who describe or characterize new diseases or develop new detection methods under BPGs will consider method limitations in real-life situations encountered in plant diagnostic laboratories. This process will provide more realistic expectations to NPDN stakeholders and customers.

Using UAS-based data and classical epidemiology methods to quantify tar spot intensity in small field plots

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Tar spot of corn is an emerging, and yield-reducing disease in the U.S. We hypothesized that unmanned aircraft systems (UAS)-based data can provide capability to quantify tar spot intensity reliably in small plots, making shifts towards efficiency. In 2020, we collected multispectral UAS data, and visual estimation of tar spot severity (%) over the cropping season in four field environments in Indiana. We performed correlation analyses (r) between human visual severity and phenotypic information derived from the UAS data. The UAS phenotypic features (UASPF) analyzed were canopy cover (CC), canopy volume (CV), excess greenness (ExG), modified soil adjust vegetation index (MSAVI), normalized difference vegetation index (NDVI), and soil adjust vegetation index (SAVI). The best correlation between human visual severity and UASPF occurred in the middle canopy. Values were: MSAVI (−0.85 to −0.92), NDVI (−0.84 to −0.91), SAVI (−0.84 to −0.91), and CC (−0.64 to −0.77). Correlation of area under the disease progress curve (AUC) using visual severity and UASPF were: MSAVI (0.51 to 0.75), NDVI (0.51 to 0.74), SAVI (0.50 to 0.74), and CC (0.42 to 0.67). EXG and CV showed poor correlations. The ANOVA of AUC for visual severity and UASPF from individual experiments showed differences among treatments (<0.0001). Treatment ranking based on visual estimations was comparable to those obtained with MSAVI, NDVI, and SAVI. Although we conclude that UAS-based data can support tar spot disease quantification and adequately serve for treatment ranking in small plots, further studies are needed.

QScout: A plugin tool suite for georeferencing and analyzing field-scouted and remote-sensing data

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Field scouting is a critical component of integrated disease management and many research methodologies in plant pathology, including validating plant health metrics remotely derived from aerial or satellite imagery. However, using scouting data with remotely sensed field imagery is often hampered by the time-consuming task of georeferencing data with a geographic information systems (GIS) program. We developed the QScout tool suite for processing raster data with scouting data in QGIS, an open-source GIS program, to specifically link non-georeferenced field scouted data to high-resolution aerial/satellite imagery, and to more broadly reduce novice users' barrier to entry in using remote sensing for plant pathology research and extension. The central features of QScout are Drop Pins and Locate Pins in Field plugins to easily link scouted data to remote sensing imagery. QScout also includes Value Grabber and Grid Aggregator plugins, which transfer raster data into pins and aggregate data from pins into a grid respectively. The final tools, Drop, Grab, and Aggregate and Locate, Grab, and Aggregate, are plugins that combine the four core plugin functionalities. The interface allows novice GIS users to effectively make use of field scouted observations with remote imagery and can improve data organization, analysis, and identification of locations of interest for further scouting or targeted management. QScout is publicly available as a GitHub repository (<https://github.com/GoldLabGrapeSPEC/QScout>).

New strategies for engineering resistance against cotton bacterial blight

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Cotton bacterial blight (CBB), caused by *Xanthomonas citri* pv. *malvacearum* (*Xcm*), is a highly impactful bacterial disease of cotton. In 2011, CBB re-emerged in the United States causing severe yield losses. Here we describe the development of two strategies that have potential for enhancing cotton resistance to CBB. The first strategy is to enhance plant immune defense via recognition of bacterial

EffTu as a pathogen associated molecular pattern (PAMP) by introducing the *AtEFR* pattern recognition receptor (PRR) construct into cotton. This strategy has shown promise in tomato, wheat and rice plants. The second strategy is designed to disrupt susceptibility gene expression by *Xcm* transcription-activator-like (TAL) effectors. These effectors have a modular structure and use a transcription factor binding motif to interact with the γ subunit of the TFIIA. Transcriptional suppression of TFIIA γ in sweet orange, tomato and pepper has resulted in increased resistance to their respective *Xanthomonas* pathogens. It has been observed in rice with the *xa5* recessive resistance gene that by having the 39th amino acid mutated from valine to glutamic acid, TAL effector recruitment is impaired, without detrimental effects on the host. We aim to use the CRISPR-Cas9 system to inactivate endogenous *GhTFIIA* γ while simultaneously introducing the *GhTFIIA* γ^{V39E} transgene. Generation of *AtEFR* expressing and TFIIA γ modified cotton lines is underway. After generation of the above described lines, they will be tested for resistance to *Xcm*.

Inoculum reduction strategies for improved *Cercospora* leaf spot management in sugarbeets

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Cercospora leaf spot (CLS), caused by fungal pathogen *Cercospora beticola*, is a major foliar disease of sugarbeet. One important factor in management of CLS is overwintering inoculum, especially following high disease pressure. In this study, methods to reduce inoculum survival were investigated. Three fall treatments, specifically plowing, foliar heat treatment, and foliar desiccation, were compared to a non-treated control. After treatment application, leaf samples were evaluated at harvest, 45-, 90-, and 135-days post-harvest (dph) to determine *C. beticola* viability. The following season, susceptible beets were planted in the same plots, disease ratings were recorded, and sentinel beet lesions were evaluated to determine inoculum levels. In 2019, treatment differences were detected in lesion sporulation ($P < 0.0001$) and lesion viability ($P < 0.05$) in at harvest samples with reductions in the heat-treated samples. In 2020, reduced numbers of CLS lesions were observed on sentinel beets in heat treated plots from May 26 to June 1 ($P < 0.05$) and June 2–9 ($P < 0.01$). The heat treatment reduced the area under the disease progress curve for the season following treatment application ($P < 0.01$). In 2020, lesion sporulation was again reduced in heat-treated samples at harvest ($P < 0.0001$), 45 ($P < 0.01$), and 90 dph ($P < 0.05$). Differences in leaf degradation were detected 90 ($P < 0.02$) and 135 dph ($P < 0.002$). Use of a foliar burner has the potential to significantly reduce inoculum levels and aid in CLS management; impacts on yield and sugar continue to be evaluated.

Postharvest decay control of plums using orange oil

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Postharvest decay of stone fruit causes up to 75% losses. A major contributor to this problem is grey mold caused by the ascomycete fungal pathogen *Botrytis cinerea*. The aim of this study is to establish whether orange oil is a viable alternative to conventional synthetic fungicides for postharvest control of grey mold. Orange oil is generally regarded as safe and has been shown to effectively inhibit fungal growth in vitro for several postharvest pathogens (*Aspergillus* spp., *B. cinerea*, *Penicillium* spp., and *Rhizopus* spp.). D-Limonene, one of the active compounds in citrus oils is a known antimicrobial compound and likely to be the key contributor to the fungistatic effects of orange oil. The efficacy of orange oil as aqueous dip application (0.05%, 0.5%, 1.0% v/v for 30 s) was tested on plums (cvs. Angelino, African Delight and Letitia) either applied preventatively or curatively on fruit that was wound-inoculated with *B. cinerea* conidiospores. The incidence and severity of grey mold decay was evaluated 7 and 12 days after inoculation. Incidence was reduced by 46% when orange oil was applied at 1% in curative application. Grey mold decay severity was reduced up to 80% as curative application at 1%. In this study plum fruit trial data confirmed efficacy of orange oil as potential postharvest decay treatment.

Meta-analytic modeling of the severity-yield relationships in soybean frogeye leaf spot epidemics

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Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is an economically important foliar soybean disease in the United States. A meta-analytic approach including 39 multi-state fungicide trials conducted from 2015 to 2019 across eight states (AL, AR, IL, IA, KY, LA, MS, and TN) was used to assess the relationship between FLS severity and soybean yield. Correlation and regression analyses were performed separately to determine Fisher's transformation of correlation coefficients (Z_r), intercept (β_0) and slope (β_1). Growing season, disease pressure (low = ≤ 20 and high = $> 20\%$ severity in the non-treated) and baseline yield (low = $\leq 3,626$ and high = $> 3,626$ kg ha⁻¹ yield in the non-treated) were included as moderators. The Pearson's r , obtained from back-transforming the Z_r estimated by an overall random-effects model, showed a significant negative linear relationship between FLS severity and yield ($r = -0.57$; $P < 0.0001$). A random-coefficients model estimated a slope of -20.2 kg ha⁻¹%⁻¹ for a mean attainable yield of 3,614 kg ha⁻¹. Based on the overall mean (95% CI) of the intercept and slope estimated by the random-coefficients model, the overall relative damage coefficient was estimated to be 0.55%⁻¹ (0.37–0.78), indicating that a percent increase in FLS severity would result in a 0.55% yield reduction. The Z_r

was affected by disease pressure ($P = 0.0005$), but not by growing season ($P = 0.0891$) and baseline yield ($P = 0.6986$). The current results highlight the potential impact of FLS on soybean yield if not well managed and may be helpful in disease management decisions.

Genetic variation of rice kernel smut populations in the United States

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Rice, *Oryza sativa*, is the second leading cereal crop in the world and is among the most important field crops in the US, valued at approximately \$2.5 billion. Kernel smut (*Tilletia horrida* Tak.), once considered as a minor disease, is now an emerging, economically important disease in the US. In this study, we investigated genetic diversity of 63 isolates of *T. horrida* collected from various rice-growing regions in the U.S. Genomic DNA were isolated and four genomic regions, translation elongation factor 1- α (*EF-1 α*), the largest subunit of RNA polymerase II (*RPB1*), ITS regions of the rRNA (ITS), and D1/D2 domains of the large subunit (LSU) rRNA, were amplified and sequenced. Multi-locus sequence analyses were performed on these isolates. Two gene sequence sets that consisted of all four genes concatenated together and only ITS region gene sequences, were used to conduct three different phylogeny analyses: maximum-likelihood, neighbor-joining, and minimum evolution. Results of multi-gene analysis revealed four clades of the US populations, with 33 out of 63 isolates clustering grouped together. Furthermore, ITS region-based analysis revealed three clades of *T. horrida* with a majority of the isolates clustering along with the 22 isolates from eight different countries grouped together. Future studies will be focused on understanding polymorphisms among these population and the impact of these genetic variations in kernel smut pathogenicity and disease management.

Population diversity and differentiation of *Puccinia striiformis* f. sp. *hordei* on barley in comparison with *P. striiformis* f. sp. *tritici* on wheat

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Barley stripe rust is a relatively new disease to the United States. The pathogen, *Puccinia striiformis* f. sp. *hordei* (*Psh*), was first reported in Texas in 1991 and has spread north and westwards and mainly caused epidemics in the western United States. To study the diversity, differentiation, and dynamics of the *Psh* populations, 167 *Psh* isolates collected from 2011 to 2017 were identified as 154 multi-locus genotypes (MLGs) using 14 simple sequence repeat markers. These isolates were analyzed together with 10 *P. striiformis* f. sp. *tritici* (*Pst*) isolates representing the 10 sub-molecular groups that were recently identified for the wheat stripe rust pathogen in the same period in the United States. The *Psh* and *Pst* isolates were separated into two major molecular groups (MGs) with MG1 that was further separated into four sub-groups (MG1-1, MG1-2, MG1-3, and MG1-4). The four sub-groups of MG1 all contained isolates collected from barley, wheat, and grasses with the 10 representative *Pst* isolates included in MG1-2, MG1-3, and MG1-4, indicating possible genetic recombination between *Psh* and *Pst*. The isolates in MG1 were clearly separated from those in MG2 that contained isolates only from barley. Strong gene flow was detected among some of the epidemiological regions. During the 7 years, the *Psh* populations of 2011 and 2012 were more distant from the other yearly populations, suggesting that greater population changes occurred between 2010 and 2013. These results are useful for understanding the pathogen evolution and for managing stripe rust.

Comparative genomics and association analysis of 264 isolates of *Aspergillus* spp. from Georgia field soils and Mississippi corn

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While the mechanisms involved in aflatoxin production in *Aspergillus* spp. are well understood, less is known about its upstream regulation and what factors influence plant pathogenicity-related traits such as conidiation, sclerotia formation, and environmental stress tolerance. To identify these regulatory mechanisms, large-scale whole genome re-sequencing (WGRS) was used for 264 diverse *Aspergillus* spp. isolates: 100 from corn plants from Mississippi, and 164 from crop field soils in Georgia. Genetic polymorphisms between these data and the *A. flavus* AF13 reference genome were called including SNPs and InDels using BWA and SAMtools. A total of 43,356 SNPs and 1,080 InDels distributed across eight chromosomes of *A. flavus* were obtained after using $MAF \geq 0.01$. Most number of SNPs (8,780) were identified on chromosome 1 and the least number (3,259) on chromosome 7. Out of 1,080 InDels, 565 were insertions and 515 were deletions. The maximum number of InDels (207) were identified on chromosome 5 and a minimum of 72 was identified on chromosome 7. SNPs and InDels were distributed with an average density of 1.15 SNPs/Kb and 0.03 InDels/Kb. A total of 30,639 transitions and 12,717 transversions were observed. These markers will be used for a genome wide association study (GWAS) and subsequent comparative analyses to identify novel aflatoxin and pathogenicity regulators. These identified regulators will shed light on the gene networks coordinating these traits and inform selection criteria in corn and peanut breeding programs.

Prediction of potato diseases using soil pathogen population density and soil physiochemical properties

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Soilborne diseases are a major challenge to potato production through the Midwest. In particular, Verticillium wilt and potato early dying, caused by *Verticillium dahliae*, and common scab, caused by *Streptomyces scabies*, cause substantial crop and economic loss. Molecular techniques have enabled quick quantification of pathogens in the soil. However, it is unclear whether those measurements can predict disease severity and provide management guidance. Our goals are (1) to determine the population density of *V. dahliae* and

S. scabies in potato fields of Wisconsin, and (2) to assess the prediction power of soil pathogen density in estimating potato disease severity. We collected 129 soils from 43 commercial potato fields across central to north Wisconsin in the fall of 2018 prior to planting potatoes, and measured soil *V. dahliae* and *S. scabies* population densities using quantitative PCR. Soil physiochemical properties were also measured. We returned to the sampling location during harvest to collect potatoes, with which the infection of *V. dahliae* and severity of common scab were assessed. We found little correlation between soil pathogen population density and disease severity in potato plants. Instead, soil organic matter content and micronutrient level are better predictors for common scab severity. This study provides novel insights for developing disease prediction tools for potato management in Wisconsin.

Survey for potential causal agents of sugarberry dieback and mortality (SBDM) in southeastern US using molecular tools

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Sugarberry (*Celtis laevigata*) is an ecologically important species that occurs in natural forests and urban areas. Widespread mortality of this species has been observed in areas of the southeastern US since 2010, and the disease is referred to as sugarberry dieback and mortality (SBDM). Symptoms of SBDM include leaf chlorosis, early leaf drop, and progressive crown dieback, as well as brown discoloration of the phloem in the stem and roots. Similar symptoms have been reported in European hackberry (*C. australis*) decline, in which phytoplasmas are involved, and in acute oak decline, which is caused by bacteria. Hence, we hypothesized that bacteria or phytoplasmas might be the primary causal agents of SBDM. Given the limitations of standard culturing techniques for isolating and identifying fastidious organisms such as phytoplasmas, a metabarcoding approach was used. Root and stem samples were obtained from symptomatic and asymptomatic sugarberry trees in North Augusta (SC) where SBDM is widespread, and the prokaryotic communities of trees were evaluated using next generation sequencing of the 16S region. Results confirmed the presence of phytoplasmas in root samples of one tree, which prompted us to focus on this group of pathogens. We have subsequently optimized a nested PCR protocol and identified additional trees with multiple species of phytoplasmas, including one that is new to the geographic area, and that has not been previously reported in the *Celtis* genus. To validate our findings, we expanded the survey to multiple sites and seasons.

Phylogenetic history and global population dynamics of wheat powdery mildew

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The globally distributed causal agent of powdery mildew on wheat, *Blumeria graminis* f. sp. *tritici* (Bgt), is one of the most rapidly adapting plant pathogens. To understand the global genetic structure of the Bgt population, reduced-representation sequencing was performed on a collection of 654 Bgt isolates from 15 countries on six continents. Phylogenetic tree inference, Structure, and principal component analyses revealed that the global Bgt population was genetically differentiated into five larger subpopulations: Fertile Crescent/Asia, Europe, Brazil, South Africa, and U.S.A./Canada/Australia. Isolates from Turkey formed a genetic crossroads, showing relatedness to those from Israel and Egypt as well as those from Russia, China, and Nepal. Samples from Brazil and South Africa stood out as genetically distinct populations, and intriguingly the Brazilian isolates clustered far from other subpopulations and showed little genetic similarity to either the U.S. or the European samples. Samples from the U.S., Canada and Australia clustered together, indicating shared population history, with U.S. and Canadian isolates forming a single clade. While European samples clustered with each other as expected, they shared a pattern of admixture with Turkish and Chinese samples, indicating likely common ancestry. Further analysis of migration patterns will help illuminate which populations exchange migrants, which can aid with selection of resistance genes for country-specific breeding programs.

Biological fungicide efficacy against hemp powdery mildew and Botrytis gray mold and the effect on the hemp cannabinoid profiles

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Fungicides can be an important tool for growers to manage crop diseases. To date there are few published studies on the efficacy of control products against hemp diseases. This study aimed to compare the efficacy of several products for use on hemp to control powdery mildew (caused by *Golovinomyces spadicus*) and gray mold (caused by *Botrytis cinerea*). Products tested had active ingredients including *Bacillus amilquifaciens*, *B. mycoides*, potassium silicate, and azoxystrobin. This study also explored the effect of both fungicide application and pathogen infection on cannabinoid levels in hemp. The fungicides tested in this study were effective in controlling disease pressure caused by powdery mildew but were not effective against *Botrytis* gray mold. Pathogen infection with either powdery mildew or *Botrytis* gray mold did not affect the profile of several cannabinoids including Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), and cannabichrome (CBC). However, some biological fungicide treatments did impact the levels of some cannabinoids in hemp.

Assessment of fungicide resistant *Plasmopara viticola* populations in vineyards of the Great Lakes region

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Downy mildew, caused by an oomycete, *Plasmopara viticola*, is one of the most devastating diseases of grapevines. This disease is commonly managed by carboxylic acid amides (CAA), and quinone outside inhibitors (QoI) fungicides. The occurrence of *P. viticola*

resistant populations to CAA and QoI fungicides have been already reported in Europe, India, Brazil, China, and the U.S. due to their repetitive use. Single point-mutations including G1105S (glycine to serine) at PvCesA3 and G143A (glycine to alanine) at Cyt b genes are mainly responsible for CAA and QoI resistance in *P. viticola* populations. Therefore, a survey was conducted in 21 vineyards across three states including Michigan, Indiana, and New York. A total of 96 leaf samples were collected and leaf discs with *P. viticola* were cut and stored in petri plates at -20°C . The collected samples were tested for the detection of these point mutations using PCR and amplified products were sequenced. If the mutations are present, then probe-based TaqMan detection tools will be developed. These rapid detection techniques will provide researchers and diagnostic laboratories with tools to monitor the buildup of CAA and QoI resistance in *P. viticola* populations and aid growers in timely decision for disease management.

Characterization of outer membrane proteome of *Candidatus Liberibacter* species

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Huanglongbing (HLB) is currently the most devastating citrus disease in the world. The causal agent of HLB is the phloem-colonizing bacteria *Candidatus Liberibacter* spp. So far, seven *Ca. Liberibacter* species have been reported including HLB causal agents *Ca. L. asiaticus* (CLAs), *Ca. L. africanus*, and *Ca. L. americanus*, *Ca. L. solanacearum* (syn. *Ca. L. psyllauros*) causing diseases on many solanaceous plants and on carrot, celery, parsley, and parsnip, *Ca. L. europaeus* infecting scotch broom, *L. crescens*, and *Ca. L. brunswickensis*. We hypothesize that outer membrane proteins (OMPs) of *Ca. Liberibacter* spp. are involved in interactions with components of sieve elements and companion cells, thus contributing to HLB disease development. Here we used an integrated approach to investigate OMPs of *Ca. Liberibacter* spp. We analyzed the genomes of 47 *Liberibacter* strains and predicted their putative OMPs. We also conducted Yeast-2-hybrid assay to identify putative targets of OMPs of *Ca. L. asiaticus* (CLAs). The putative functions of OMPs will be discussed.

Improving *Fusarium* training materials: A needs assessment of diagnosticians working with *Fusarium*-associated diseases of specialty crops

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Plant disease diagnosticians provide the important first step in determining the most appropriate management response to disease. An accurate and timely diagnosis ensures that growers can respond to a disease using the right tools at the right time. *Fusarium*-associated diseases, particularly in specialty crops, can be challenging to diagnose. Their ubiquity in the soil and decomposing plant material and evolving taxonomy justifies a need for continued education on best practices for working with and identifying *Fusarium*-associated plant diseases among diagnosticians. Determining current practices and education needs is the first necessary step in adapting and developing workshops, trainings, and other resources that support *Fusarium* identification, detection, and diagnosis. It is critical to keep the needs and goals of practicing diagnosticians in mind when developing the most relevant research and resources. This information is not only important to those regularly offering *Fusarium* workshops, but also to plant pathology research claiming diagnostic implications. Therefore, we are conducting a survey for members of the National Plant Diagnostic Network (NPDN) to determine current practices, training needs, and desired resources in *Fusarium* diagnostics. The survey, administered online, assesses these factors, using a combination of questionnaire types such as open-ended and close-ended questions, indices and scales. The results of the survey will reveal situational logic used when making an identification and resource preferences among diagnosticians.

In the search of soybean cyst nematode in Pennsylvania

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Soybean cyst nematode (SCN, *Heterodera glycines*) is the most important pathogen in soybean in the U.S. In Pennsylvania, the pathogen's distribution remains unknown, with only one positive county (Lancaster) of the 67 counties. To determine if SCN's distribution is increasing, we established a free testing program in 2019. To date, a total of 264 samples from 38 counties were tested for SCN and other plant parasitic nematodes. Participants also provided a field history form with the submitted sample. Just 21% of stakeholders indicated they had sampled or scouted for SCN in the past. Twenty-two percent indicated that they use a resistant variety; however, just 13% of those farmers were aware of the SCN source of resistance. Nine samples from four counties have tested positive for SCN, with a maximum of 125 eggs per 250 cm³ of soil. In each of those situations, we coordinate with the Pennsylvania Department of Agriculture to re-sample and test the positive fields. York County has been officially added to the list of counties where at least one field has tested positive for SCN. Other nematodes found in the samples include lesion, stunt, spiral, dagger, pin, stubby root, ring, and root knot nematodes. Lesion nematode was most frequently detected (83%), followed by spiral and lance nematodes, in 72% and 50% of the samples, respectively. We will integrate stakeholder-provided information about agronomic practices, common weeds, and diseases present to improve management recommendations for SCN and other plant parasitic nematodes in the Commonwealth.

Farming system effects on the root rot pathogen complex of pea (*Pisum sativum*) and faba bean (*Vicia faba*) in Germany

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The decline in the area planted to legumes in Germany over the past several decades has become an important part of agricultural policy debates. In practice, however, the expansion of grain legume cultivation faces many challenges and is restricted by highly fluctuating or low yields often caused by soilborne pathogens. We will present findings from a 4-year survey (2016–2019) on root health status, diversity and frequency of pathogens associated with roots of organically and conventionally managed pea and faba bean fields over a north-south transect in Germany. We examined 4,590 roots from 110 faba bean and 113 spring and winter pea fields. Root rot incidence was on a low to moderate level. The dominant pathogens isolated were *Didymella pinodella* (40% colonized roots), *Fusarium redolens*, *F. oxysporum* (30%), *F. avenaceum* (25%), and *F. solani* (22%). The isolation frequencies of the single species were strongly influenced by the host plant and the management system. For example, *F. redolens* and *D. pinodella* were considerably more important in organic than in conventional faba beans while *F. avenaceum* was more important in conventionally grown plants. Roots of spring peas grown in both management systems were colonized at similar frequencies with all major pathogens, whereas in organically grown winter pea roots, *Fusarium* was found at particularly low frequencies and *D. pinodella* at very high rates. The effects of abiotic soil properties and frequency of legumes in the rotation on the occurrence of major pathogens will also be discussed.

Fungicide efficacy against hemp powdery mildew in the greenhouse

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Hemp powdery mildew, caused by the fungus *Golovinomyces* sp., is an important disease of hemp (*Cannabis sativa* L.) in greenhouses and high tunnels, and information on fungicide efficacy against the disease is lacking. In two greenhouse trials, the efficacy of 18 fungicide treatments were tested on hemp. The cultivars ‘BaOx2’ and ‘Sweetened’ were used in trials 1 and 2, respectively. Trials were arranged in a randomized complete block design with four replicates. Each experimental unit consisted of one hemp plant in a one-gallon pot. Plants were inoculated with a 2×10^5 /ml suspension of *Golovinomyces* sp. conidia. Fourteen treatments were applied as protectants, 1 or 5 days before inoculation, and four treatments were applied as curatives, 1 or 7 days after inoculation. Additional applications, two applications for the protectants and one for the curatives, were made at 7-day intervals after the first application. Applications were made to achieve thorough coverage using hand-held pump sprayers. Powdery mildew incidence and severity were rated for all treatments three times at 7-day intervals. All the treatments reduced powdery mildew disease index compared to untreated plants. The protectant treatments, including AG2b, Cinerrate, Defguard, Exile, Luna Experience, MilStop, Regalia, Sil-Matrix, Sulfur, TPOS, TPCS, and TP, showed excellent efficacy that resulted in 85% to 100% disease reduction. The curative treatments, Lyt2, Lyt3, Lyt4, and Regalia, showed moderately high efficacy that resulted in 73% to 82% disease reduction.

A Sentinel planting approach to risk assessment of invasive tree pathogens and phytophagous insects

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Invasive alien tree pests (IATP) are among the greatest threats to forest ecosystems worldwide, having the potential to alter forest structure and cause species removal. Prevention and early detection of IATPs are the most effective management strategies in mitigating their impact. It is thus imperative that potential invaders are accurately and rapidly identified. Predictions of which species will become invasive if introduced into a potential sink environment is often based upon those with documented histories of establishment. An inherent problem, however, is that many of the IATP that become established are not known to be invasive beforehand and are sometimes not even known to science. Thus, they are often missed during risk assessment exercises. Sentinel plantings can address this issue via direct establishment and monitoring of target plants in potential source environments with exposure to native insects and pathogens. In this study, we established twin plantations of European and Chinese tree species in Columbus, Ohio, and Portsmouth, New Hampshire, and monitored sites for signs and symptoms of insect and pathogen attack. Fungi were isolated from symptomatic plant tissue and identified via ITS sequencing and morphological traits. Observed insects were collected in ethanol and identified via morphology and DNA barcoding. Established relationships between host tree species with identified organisms were determined using existing literature. Knowledge of these relationships can be informative to the countries of origin of the target tree species during risk assessment.

Identification of a minimal region of *Triticum mosaic virus* coat protein required for superinfection exclusion activity

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Superinfection exclusion (SIE) is defined as the phenomenon where prior infection of cells by a virus prevents subsequent infection by the same or closely related viruses. The SIE phenomenon has been illustrated in various viral systems infecting humans, plants, and animals. In this study, we identified the minimal region of *Triticum mosaic virus* (TriMV) coat protein (CP) required to exhibit SIE activity. TriMV is a single-stranded positive-sense RNA virus that belongs to the genus *Poacevirus* in the family *Potyviridae*. TriMV CP has been reported to be an SIE elicitor. A series of deletions were introduced in TriMV CP, which comprises amino acids (aa) 3 to 40, 41 to 80, 81 to 130, 131 to 170, 171 to 210, 211 to 250, and 251 to 294. These mutants were inserted into the *Wheat streak mosaic virus* (WSMV) genome between P1 and HC-Pro cistrons. Wheat seedlings were infected with in vitro transcripts of WSMV containing TriMV CP deletions. Crude sap extracted from these infected wheat leaves was inoculated to wheat seedlings at the single leaf stage. At 10 dpi, the upper non-inoculated third leaf was challenge inoculated with crude sap of TriMV-GFP. Examination for local and systemic infection at 5 and 9 days post-challenge inoculation, respectively, showed that WSMV expressing TriMV CP deletions 41 to 80, 81 to

130, 131 to 170, 171 to 210, and 211 to 250 efficiently permitted the infection by TriMV-GFP, whereas TriMV CP43-40 and CP4251-291 prevented superinfection by TriMV-GFP. These observations demonstrate that TriMV CP aa 41 to 250 is the region required for SIE activity.

Characterization of Cowpea mild mottle virus infecting vegetable legumes in Bangladesh and Nepal

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Cowpea mild mottle virus (CPMMV; genus *Carlavirus* family *Betaflexiviridae*) is emerging as a damaging problem to legume crops in many countries. During survey of farmers' fields between 2015 and 2018 growing seasons, we collected leaves showing yellow mosaic, vein banding and leaf distortion symptoms from country beans (CB, *Lablab purpureus*) and yard-long beans (YLB, *Vigna unguiculata* ssp. *sesquipedalis*) in Bangladesh and from CB in Nepal. Total RNA preparations from symptomatic samples that tested positive for CPMMV in DAS-ELISA and RT-PCR assays were subjected to high-throughput Illumina sequencing. Sanger sequencing of viral amplicons was used to fill internal gaps in viral genome sequences. Near-full genome sequences, varying in size between 8,058 and 8,066 nucleotides (nt), were obtained for five virus isolates, three from CB and one from YLB from Bangladesh and one from CB from Nepal. Pairwise nt sequence comparisons indicated that CPMMV isolates from CB and YLB are genetically divergent but closely related to corresponding viral sequences reported from other countries. In coat protein-based phylogenetic analysis, CPMMV isolates from Bangladesh and Nepal clustered with virus isolates from India than with virus isolates reported from Africa and the Americas. In grow-out tests, CB seeds obtained from commercial sources in Bangladesh showed seed-to-seedling transmission varying between 4.8% and 19%, depending on the seed source. This is the first report of the occurrence of CPMMV in two important food legume crops cultivated by smallholder farmers in Bangladesh and Nepal.

Letting it flow: Ecological stoichiometry in the southern pine beetle (*Dendroctonus frontalis*) system

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A critical pest of southeastern pine plantations is *Dendroctonus frontalis*, the southern pine beetle (SPB), which can cause significant economic losses to the forestry industry. SPB is associated with three fungal species: an antagonistic species, *Ophiostoma minus*, and two mutualists, *Ceratocystopsis ranaculosus* and *Entomocorticium cobbii*, which are nutritional symbionts for SPB larvae. An understanding of the interactions between these fungal species and the beetle has been elusive, especially since SPB feed on phloem of freshly killed trees, which has made in vitro testing difficult. In this project, we used ecological stoichiometry to describe the flow of elements and energy within the SPB galleries, following an approach that has recently proved successful in unraveling complex interactions in similar bark beetle-fungi systems. We hypothesize that *O. minus* is antagonistic to SPB larvae due to its lesser ability to concentrate elements such as nitrogen, carbon, and phosphorous than the mutualists which are of key importance in larval development. This research clarifies how the three fungi function in the SPB gallery system and helps in further dissecting the role of *O. minus*, which has been considered as either mutualist or antagonist, depending on perspective. The project derives relevance both from the economic and ecological importance of SPB and from its ability to broaden our ecological understanding of bark beetle associated fungi.

Selection of fungicides for the management of *Botrytis cinerea* blossom infection in lemons

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Blossom infection by *Botrytis cinerea* causes rind distortion and cracking in developing and mature lemons and can lead to significant downgrading of harvested fruit. The aims of this study were to evaluate the efficacy of fungicides in reducing blossom infections in lemon orchards previously not exposed to fungicides, and to determine the fungicide sensitivity of *B. cinerea* isolates collected from blossoms in these orchards. Of the fungicides tested, benomyl had the highest efficacy, reducing the incidence of blossom infection by 54% compared to the untreated control. Azoxystrobin and fenhexamid reduced the incidence by 33 and 31% respectively, whereas iprodione reduced incidence by 3%. *B. cinerea* from blossoms collected in the untreated orchards revealed that 13% of the isolates were highly resistant to benomyl, with 40% resistance detected in two individual orchards, whereas 70 and 98% were highly sensitive to azoxystrobin and fenhexamid with no resistant isolates being detected to these two fungicides. All isolates were sensitive to iprodione. Considering both fungicide sensitivity and efficacy results, it is concluded that benomyl and iprodione not be recommended as blossom application in lemons for *B. cinerea* control. Azoxystrobin and fenhexamid were found to be effective in reducing blossom infection by *B. cinerea* in lemons and currently pose a low risk for loss of sensitivity, and dosage rates of these two fungicides should be further investigated to improve their efficacy.

Environmental components may play a role in the occurrence of culturable fungi on watermelon in Texas

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The relationship between environmental conditions and fungal microbe occurrence in Texas watermelon production is not well understood. Environmental data was collected from weather stations within 8 km of five Texas watermelon production fields surveyed for fungal pathogens. The environmental component data collected were: humidity, dew point, temperature, air pressure, wind speed, wind gust, and precipitation from 6 weeks prior through sampling dates. Data was processed using Principle Components Analysis to

determine the most influential components and which locations were the most unique. Data of individual environmental components were compared across locations using Tukey Comparison tests. PCA indicated humidity and dew point were the most influential components for all locations. Three of the five locations did not have significant differences among their environments, while Maverick and Glasscock counties were significantly different overall from the other three counties. Glasscock county was significantly different in six of the seven environmental components, which was the most of all locations sampled. Glasscock also had the lowest total recovery of fungi, and in combination with environmental data, it can be inferred that environmental components had a role in this outcome. Maverick county had the highest recovery of *Fusarium* spp., and it can be inferred that the differences in humidity, dew point, and precipitation may have played a role in prevalence. More research is needed to confirm the extent of environmental components may have on pathogen occurrence.

***Bacillus* secondary metabolites as potential biocontrol agents against plant pathogenic oomycetes**

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Crop production is significantly disrupted by a variety of oomycetes. The overuse of fungicides causes environmental pollution and resistance. This warrants exploration for alternative treatments using biocontrol agents. To search for anti-oomycete secondary metabolites (SMs) as biocontrol agents, we carried out a comprehensive genome mining and antibiosis assay of 293 strains of diverse *Bacillus* species. Our analyses predicted a total of 3,078 biosynthetic gene clusters (BGCs), in which 1,486 (48%) were predicted to encode production of known SMs. To identify *Bacillus* strains that may be producing anti-oomycete SMs, we tested each *Bacillus* strain for its ability to inhibit growth of *Phytophthora nicotianae*, a causative agent of the black shank of tobacco. The results revealed 51 (17%) strains as strong antagonists, 37 (13%) strains as weak antagonists, and 205 (70%) strains as non-inhibitors. A comparative analysis of *Bacillus* strains producing known SMs and an antibiosis response against *P. nicotianae* revealed that some BGCs are highly conserved in strongly antagonistic *Bacillus* strains, including lichenysin, surfactin, fengycin, bacillibactin, bacilysin, and difficidin. In contrast, these SMs were less frequently observed in non-inhibitory *Bacillus* strains. Acid-methanolic extracts from *Bacillus* strains strongly antagonistic to *P. nicotianae* revealed characteristic UV-visible absorption features attributed to the presence of distinct secondary metabolites. Isolation and in vitro analyses of these extracts as well as their LC-MS identifications are presently ongoing.

Managing *Neopestalotiopsis* spp. in Florida strawberry using chemical control and cultivar selection

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Recently, a new disease caused by a more aggressive species of *Neopestalotiopsis* has affected fruit and leaves of numerous commercial strawberry fields in Florida, causing severe losses. Along with the failure of most fungicides registered for strawberry, the current cultivars are susceptible to the pathogen. Thus, *Neopestalotiopsis* spp. isolates were screened in vitro for their sensitivity to 30 fungicides, and the most effective products were evaluated in the field. Commercial cultivars, UF advanced selections, and wild species accessions and selections with a wider range of genetic diversity were also assessed. In in vitro trials, mycelial growth inhibition was evaluated by placing isolate plugs on PDA amended with fungicides. Non-amended PDA was used as a control. Strawberry plants, in both chemical and cultivar field trials, were inoculated with a mixed spore suspension (5×10^4 spores/ml) of three isolates. In the efficacy trial, products were sprayed weekly according to label rates, whereas fungicides were not applied in the cultivar trial. Disease on leaves and fruit was evaluated throughout the season. Fluazinam, chlorothalonil, thiram, fludioxonil+cyprodinil, and difenoconazole reduced disease incidence and increased yield over the control. 'Florida Beauty' and 'Florida Brilliance' were the most susceptible cultivars, whereas 'Strawberry Festival', 'Treasure', the new white-fruited UF release Pearl®, along with several selections and wild species accessions were less susceptible. Our findings are relevant for developing an integrated disease management approach.

Hormone imbalances during plant defense response—Verticillium wilt as a case study

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Hormone signaling is essential for the overall maintenance of plant health. While plant hormones like jasmonic acid (JA), salicylic acid (SA), ethylene (ETH), and abscisic acid (ABA) are primarily involved in plant stress responses, other hormones like auxin (AUX), gibberellic acid (GA), cytokinins (CK), and brassinosteroids (BR) are mainly involved in regulating plant growth. Sustaining an elevated defense response comes at the cost of resources required for plant growth and development. In contrast, an inability to synthesize defense-related metabolites and enzymes during pathogen attack leaves the plant exposed to opportunistic pests. Multiple hormone signaling pathways play a role in defense against various pathogens, and hormone imbalances during pathogen infection underlie the allocation of resources that may either contribute to elevated defense response or drive plant growth and development, compromising defense. Here we present the importance of such hormonal imbalances that underlie defense, growth, and transition to flowering in response to the fungus *Verticillium dahliae*, that causes Verticillium wilt. Our results indicate that *V. dahliae* infection led to a significant reduction in the JA levels with a concomitant increase in the GA₄ leading to early flowering in the wild-type Col-0. Finally, we provide evidence that altering the GA levels in plants using multiple approaches led to altered resistance to *V. dahliae*. Our

results provide a model on the impacts of pathogen-mediated hormonal imbalances while uncovering a new role for GA in regulating plant defense.

Evaluation of copper sulfate pentahydrate on reducing bacterial leaf streak severity in hard red spring wheat

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Bacterial leaf streak (BLS) of wheat caused by the pathogen *Xanthomonas translucens* pv. *undulosa* (*Xtu*) is one of the most important foliar diseases in hard red spring wheat (HRSW) in North Dakota (ND). Yield loss can be variable depending on disease onset, variety resistance, and environmental conditions. Host resistance is the most important BLS management tool, yet resistance may not be readily available in high-yielding and high-quality HRSW varieties. This has led to question the use of copper-based products to help suppress BLS and protect yield. The objective of this research was to evaluate the use of copper sulfate pentahydrate (CSP) on reducing BLS severity and protecting HRSW yield. A field experiment conducted in 2020 was arranged in a split-plot with a randomized complete block design and four replications. Three treatments served as the main plots (non-treated control-non-inoculated, non-treated control-inoculated, and CSP-inoculated at flag leaf). Five HRSW varieties varying in resistance served as sub-plots. Artificial inoculation was conducted twice with a *Xtu* suspension. Data collection included measurements of disease severity, canopy location of disease, yield and test weight. A high level of disease occurred with significant differences in yield and BLS severity being observed among varieties. The non-inoculated treatment resulted in the highest yield and lowest BLS severity. The application of CSP did not provide adequate BLS suppression and did not protect yield. Results suggest that future field research is needed on the use of CSP in HRSW production.

Detection and distribution of *Cronartium ribicola* and *Cronartium comandrae* hybrids in Colorado and Wyoming forests, USA

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White pine blister rust caused by the invasive fungus *Cronartium ribicola* (Cr) is the most serious diseases threatening 5-needle pines. Comandra blister rust caused by the native fungus *C. comandrae* (Cc) is an important disease threatening 2-needle pines. In 2007, a hybrid of these two rust species (CrxCc) was reported in southwestern Alberta, Canada. Surveys were conducted in five national forests in Colorado and Wyoming, USA in 2019–2020, to assess the presence and distribution of CrxCc. In total, 299 and 583 aecia from 64 lodgepole (*P. contorta* ssp. *latifolia*) and 150 limber (*P. flexilis*) pines were collected from a total of 23 sites. Aeciospores collected from 35 trees (16 lodgepole and 19 limber) had intermediate morphology and contained heterozygous SNPs in the *DCON10* region, suggesting these aecia were formed as a result of hybridization between Cr and Cc. CrxCc aeciospores were found in five lodgepole pine and nine limber pine sites. Germination assays confirmed that CrxCc aeciospores are viable and formed a germination tube after 48 h of incubation both on agar slides and host tissue. There is current ongoing investigation to determine whether CrxCc aeciospores are pathogenic and capable of inducing urediniospore production. Overall, these results suggest that CrxCc hybrids are present in multiple forests of Colorado and Wyoming, USA. The implications of the CrxCc hybrids expanding into the large range of susceptible pines could have cascading impacts.

GFP as a marker to study the colonization of CRISPR/Cas9 based mutant *Leptosphaeria maculans* isolate in *Brassica napus*

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Blackleg disease caused by *Leptosphaeria maculans* is a devastating disease in canola plants. CRISPR/Cas9 mediated gene editing is a promising method to knock out genes to understand their function. However, sometimes this method gives rise to non-target mutations. We have a CRISPR/Cas9 created *L. maculans* strain, mutated from JN3 with a well-sequenced genome. The mutation is a non-target mutation, yet it has inhibited the mutant (mJN3) from developing the blackleg disease in canola. To the best of our knowledge, this is the first *L. maculans* isolate that can not cause blackleg disease in the susceptible canola variety Westar. This study aims to tag the JN3 and mJN3 isolates with the green fluorescent protein (GFP) to see their differences in plant tissue invasion. The pRM-eGFP vector with the *egfp* gene was transferred into *Agrobacterium tumefaciens* cells by electroporation. The transformed *A. tumefaciens* cells and *L. maculans* spores were co-cultured to obtain fungal transformants. The fungal transformants that express *egfp* were confirmed by fluorescence microscopy and PCR. The stability of the selected transformants was tested for five generations by subculture. The selected JN3gfp and mJN3gfp strains will be inoculated into canola cotyledons to observe their differences in plant tissue colonization through fluorescence microscopy. The results will help expand our knowledge of the *L. maculans* infection process, and the method can be used to tag different *L. maculans* strains with GFP in the future.

An assessment of foliar fungicide use for soybean disease management in Nebraska

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Fungicide resistance is a growing concern in Nebraska, yet little is known about how the decision to apply fungicides is currently made. To address this, we designed a survey to assess the perceived importance of soybean diseases, reasons for foliar fungicide applications, and sources of information used. This survey had 10 multiple-choice and short answer questions. Participants were recruited at row crop-related meetings. Results of 618 participants from 78 counties showed the most important soybean diseases were sudden death syndrome (19.0%), white mold (16.5%), and frogeye leaf spot (12.0%), while crop rotation (23.7%), herbicides (21.4%), and foliar fungicides (13.3%) was the most cited crop management strategies. More than half (64.3%) reported that they used or recommended

foliar fungicides for soybean in the last 5 years. Participants that did not apply or recommend fungicides considered soybean cyst nematode to be more important than frogeye leaf spot. Factors most reported as an influence on their disease management decisions were the amount of disease (23.2%), fungicide cost (21.6%), and crop market value (18.4%). The most cited source of information used to make disease management decisions was recommendations from the local agricultural co-operative service provider (14.9%). Whether participants considered applying foliar fungicides or not, the ranking for factors and sources of information used remained the same. These results will be used to customize future educational outreach materials to inform farmers and their affiliates about fungicide use.

Pathogen diversity, cultivar susceptibility, and cold storage of brown rot disease on chestnut in Michigan

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Brown rot, caused by *Gnomoniopsis smithogilvyi* (*G.s.*), is a pre- and post-harvest disease found globally, that affects up to 91% of chestnuts at harvest. Brown rot symptoms, characterized by soft, brown lesions on the kernel, increase over time in storage. To evaluate the effects of cold storage and host cultivar on symptom incidence and severity, three cultivars of chestnut in Michigan (cv. ‘Colossal’, ‘Labor Day’, and ‘Benton Harbor’) were evaluated for symptom incidence and severity at different time points after harvest in fall 2019 and 2020 using inoculated (with *G.s.*) and intact nuts (natural infection). To determine the diversity of fungi associated with nut rot in Michigan 1,249 nuts were collected in 2020 from a local co-op processing line prior to quality checks and were rated for nut rot incidence and severity. Intact and inoculated Colossal nuts exhibited the highest incidence and severity of symptoms. Nuts stored in the cold for 1–2 months exhibited lower symptom incidence and severity when compared to nuts left at room temperature. Fungi isolated from nuts were categorized into 17 morphological groups, with 2% of the 236 cultures isolated identified as *G.s.* in 2020. These results indicate that brown rot in chestnut may be caused by more than a single fungal species and that cold storage suppresses symptom incidence and severity. Future research will focus on completing Koch’s postulates on a sub-set of the 17 fungal groups isolated from rotted nuts.

The transcriptional regulator MucR from ‘*Candidatus Liberibacter asiaticus*’ mediates exopolysaccharides synthesis

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Huanglongbing (HLB), caused by non-culturable bacteria *Candidatus Liberibacter asiaticus* (Las), is the most devastating disease of citrus worldwide. Las has a much smaller genome size than its relative *Sinorhizobium meliloti* and has very few regulatory genes. Interestingly, Las encodes a transcriptional regulator MucR that has been reported to regulate the expression of transporter genes, control exopolysaccharide synthesis, and be involved in resistance to oxidative, detergent, and saline stresses and cell envelope modifications. We hypothesize that characterization of MucR of Las will help us understand the biology of Las. In this study, we used *S. meliloti* to investigate the function of MucR. MucR represses the *exp* genes for galactoglucan (EPS II) synthesis and activates the *exo* genes for succinoglycan (EPS I) synthesis in *S. meliloti*. Mutation of *mucR* resulted in increased production of EPS II and reduced synthesis of EPS I. The Las homologous gene *mucRLas* complemented *mucR* mutant of *S. meliloti* and restored exopolysaccharides production. Furthermore, we investigated *rem*, an OmpR-like DNA-binding response regulator, which is under the control of MucR and positively controls the swimming motility in *S. meliloti*. The *rem* mutant of *S. meliloti* has reduced motility, which was complemented by *remSme*, but was not fully complemented by the homologous gene *remLas*. Next, we will characterize targets of Rem from class II flagellar genes in both *S. meliloti* and Las, and investigate regulation and their implication in Las movement in plants and in psyllids.

Identifying target control periods to optimize southern blight management in California specialty potato production

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California grows potatoes year round, and it is the nation’s largest producer of spring-marketed potatoes. Southern blight (SB), caused by *Athelia (Sclerotium) rolfsii*, is a heat-driven disease that results in tuber rot and substantial yield losses pre- and post-harvest. With increasing spring and summer temperatures, effective SB management is increasingly important for potato growers throughout the Central Valley; presently, in-season chemical management strategies are inadequate for control. In field studies over three years, SB activity was detected up to 35 days pre-harvest and disease incidence increased 2- to 3-fold between desiccation and harvest. Using these new target periods in subsequent trials we observed Quadris® and ammonium bicarbonate applications reduced tuber rots when applied nine days before harvest. To enable SB risk prediction and effective chemical use, we generated a growth response curve and identified sclerotia germination thresholds below 12°C and above 35°C. These thresholds were validated using naturally infested field soil. We are using this to develop a degree day model to predict germination in response to thermal time; this model will be validated in a field setting. These studies will be used to develop grower recommendations for risk-based fungicide tools and complementary methods to enable effective SB management in potatoes.

Opportunities for an improved potato late blight management in the Republic of Ireland

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Potato late blight (PLB), caused by oomycete *Phytophthora infestans* (Mont) De Bary, is arguably the most important disease of potato in terms of economic losses and environmental and economic impact of the disease management in Ireland and globally. The Irish Rules model was proposed in the 1950s and is operationally used by the national meteorological agency, Met Éireann, to aid in identifying periods of blight conducive weather. Changes in the pathogen population and potato production systems prompted the need to evaluate the model criteria and decision thresholds. The evaluation and subsequent re-calibration of the model using historical disease and weather data led to a significant improvement in diagnostic performance. The modified Irish Rules (MIR) was further evaluated under field conditions to showcase and empower the use of integrated pest management tools to reliably inform the standard potato late blight management practice under growing conditions and contemporary *P. infestans* population in the Republic of Ireland. An extensive evaluation in combination with varietal resistance has shown that the MIR model represents an optimum risk modelling compromise due to the uncertainty of the model input variables, which currently demand simpler modelling approaches, such as the MIR. Seasonal fluctuations in the population structure of the pathogen and the arrival of EU_36_A2 in the Republic of Ireland, require routine population monitoring to ensure that potato late blight control strategies remain effective.

Annotation of the *Xanthomonas perforans* metabolome identifies novel metabolites that provide insights into the ecology of the pathogen

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The genus *Xanthomonas* comprise some of the most devastating plant pathogens. While previous studies hypothesized *Xanthomonas* as reservoirs of novel metabolites, progress in uncovering the metabolites has been slow. In this study, global metabolomics profiling of *X. perforans*, a bacterial spot of tomato pathogen, was carried out using Thermo Q-Exactive Orbitrap mass spectrometer. Samples included wild type reference strain Xp91-118 treated with carvacrol at 32 mg/L for 1 h and 6 h, and an untreated control. Mass-to-charge and retention time were initially searched against the in-house metabolomics database at the University of Florida Southeast Center for Integrated Metabolomics (SECIM). Unidentified metabolites were searched against Human Metabolome database (www.hmdb.ca) and METLIN (<https://metlin.scripps.edu/>) for putative identification. We detected novel metabolites, some of which are first time reports in a microbial or *Xanthomonas* system. One of these annotations corresponded to methoxybrassinin A which has only been previously reported in plant systems, especially members of the Brassicaceae. Similarly, maculosin, a host-specific phytotoxin initially identified in *Alternaria alternata*; β -carbolines harman, norharman, and an unidentified β -carboline; and unsaturated Y-hydroxynitrile glucoside sarmentosin were found in significant amounts in *X. perforans*. Some of these metabolites represent unique ecological lifestyles. The convergence of metabolites in *X. perforans* may provide additional insights into the ecology and evolution of this important pathogen.

Suppression of coffee rust with metalloid and metal oxide nanoparticles

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The use of balanced nutrients should be considered as a priority plan in the management of diseases. Nanomaterial is an efficient way to deliver micronutrients compared to conventional forms. In the present research, an in vivo experiment were carried out with the objective of verifying the nanoparticle's effect of metal oxides and metalloids in reducing the severity of coffee rust. To obtain the inoculum, urediniospores of *H. vastatrix* from naturally infected leaves were collected from coffee plants, and their viability was assessed. The greenhouse trial was designed to examined NPs of B, ZnO, MnO, CuO, MoO₃, Ag, CeO₂, TiO₂, AlO, and NiO on coffee rust. Suspensions of NP (500 mg l⁻¹) were applied to seedlings coffee. There were five replicates and plants were arranged on plastic bags as a 12 treatments $\times$ 2 inoculum levels (inoculated with *H. vastatrix* or not inoculated) factorial randomized complete block design. After five weeks, plants were harvested for biochemical analysis. The severity of the disease increased over time, varying from 0.8 to 10%, reaching a maximum of 9.98% at 66 days after inoculation experiment. Eight nanoparticles were effective in reducing the AUDPC coffee rust, but the suppression was the greatest for silver and copper (93.0 and 75.0%, respectively). There was a significant difference for the total soluble phenol and lignin variables, plants with the zinc nanoparticle had the highest concentration of lignin. For total soluble phenol, copper and boron increased the concentration of this compound by 22.61 and 27.3%, respectively.

Screening of antimicrobial peptides against bacterial leaf streak disease of wheat through *Wheat streak mosaic virus* as a transient expression vector

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Bacterial leaf streak (BLS) disease is an emerging threat to wheat in the Great Plains region of the USA. BLS disease can reduce wheat yield up to 40%, and there is no effective chemical control for the management of this disease. In this study, we engineered sequences encoding several plant-based antimicrobial peptides (AMP) into the *Wheat streak mosaic virus* (WSMV) genome between the P1 and HC-Pro cistrons. Wheat seedlings were inoculated with in vitro generated transcripts of WSMV harboring AMP sequences, followed by an examination of antimicrobial activity against *Xanthomonas translucens* pv. *undulosa*, the causal agent of BLS disease of wheat. Out of nine AMPs screened for antimicrobial activity against *X. translucens* pv. *undulosa*, Ace AMP1 found in onion, and Defensins TK AMP D1 and D3 and Ta PDF 13 found in wheat, showed a statistically significant reduction in lesion size caused by the bacterium in wheat. Wheat plants expressing Ace AMP1, Defensins TK AMP D1 and D3, and Ta PDF 13, had lesion sizes of 1.3 cm, 1.5 cm, 1.4 cm, and 2.2 cm, respectively in BLS-infected wheat leaves, compared to WSMV-infected (8.3 cm) and mock-inoculated (9.1 cm)

wheat. RT-PCR analyses from wheat plants infected with WSMV harboring AMPs at 21 days post-inoculation suggested that WSMV stably retained inserted AMP sequences in planta. This study suggests that WSMV can be used as an efficient and high-throughput transient expression vector to screen a large number of AMPs for antimicrobial activity.

Characterization of *Pseudomonas quasicaespiana*, *P. magnolii*, and *P. californiensis*, three novel species causing disease in ornamentals in California

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Six bacterial strains were isolated from circular, black necrotic lesions surrounded by yellow haloes present on hydrangea, achillea, delphinium and magnolia in California. Isolations were made on King's medium B (KMB) and bacterial colonies appeared as white, mucoid, and round. Phylogenetic analysis based on multilocus sequencing using 16S rRNA, *rpoB*, *rpoD*, and *gyrB* genes placed the bacteria into three distinct groups within *Pseudomonas* with the strains being most closely related to *P. viridiflava*, *P. cichorii*, or *P. caespiana*. Phenotypic analysis included the following tests: fatty acid methyl ester, LOPAT, fluorescence, BIOLOG, and transmission electron microscopy. Finally, whole genome sequencing of the six strains was done and the sequences were compared with reference *Pseudomonas* spp. based on average nucleotide identity (ANI). The first group consisting of 3 strains had 95.6 to 96.9% ANI; the second group consisting of 2 strains had 100% ANI; and the final group included only 1 strain. Comparisons of the three groups with publicly available genomes of *Pseudomonas* species revealed that the three groups had less than 95% ANI with all pseudomonads. Combining genomic and phenotypic analyses, we conclude that these strains belong to new species and the names proposed are *Pseudomonas quasicaespiana* sp. nov., *P. magnolii* sp. nov., and *P. californiensis* sp. nov., based on either their close phylogenetic relation with *P. caespiana*, host of isolation, or geographic location of isolation, respectively.

Occurrence of bitter rot of apple in Illinois orchards

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Bitter rot, caused by *Colletotrichum* spp., is one of the most important diseases of apple in the world, and is the most difficult summer apple disease to manage in Illinois. In the past five years, outbreaks of bitter rot were experienced in most Illinois apple orchards, causing up to 100% yield losses in some apple cultivars. Our surveys in 2019 and 2020 showed that the incidence of bitter rot in apple orchards in Illinois ranged from 0 to 63% in 2019, and 0 to 100% in 2020. Bitter rot was observed in most orchards in central and southern Illinois, while most orchards in the northern part of the state were bitter rot-free. Incidence of fruit with bitter rot in 'Empire' and 'Honeycrisp' apples was higher than other apple cultivars. Bitter rot incidence was higher in 2020 compared to 2019 in the orchards where mummified fruits and dead wood from previous years were not collected. Infected fruits were collected from apple cultivars Braeburn, Empire, Fuji, Gala, Golden Delicious, Granny Smith, Honeycrisp, Jonagold, Jonathan, and McIntosh in 28 orchards throughout the state. Infected tissues of fruits were cultured on potato dextrose agar (PDA) medium, and the pathogens were isolated. Identification of pathogen species was based on the morphological characteristics and molecular identity of the isolated fungi. Identified species belonged to the *Colletotrichum gloeosporioides* and *C. acutatum* species complexes. More than one species of the pathogen was identified in some orchards.

Reanalyzing the genetic diversity of global *Ralstonia* complex strains to assign sequevars to unclassified strains

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The *Ralstonia* species complex ("*Ralstonia*" hereafter) is a major group of bacterial phytopathogens that cause bacterial wilt and attack a plethora of crops around the world. This study aims to develop a centralized database of the extensive diversity of *Ralstonia* strains and determine the taxonomy of previously isolated strains with unknown sequevars. To do this we created a database that has catalogued metadata on 2,328 individual *Ralstonia* strains. The metadata indicates the taxonomic classification (Phylotype-Sequevar) with genome or gene NCBI accession numbers, isolation host, isolation location, and isolation year, when known. 181 strains from nine papers were published with "unknown" or "possibly novel" sequevars. Using the NCBI accession numbers of the strains with unknown sequevars, we gathered the *egl* sequences in FASTA format. To analyze the genetic relationships of these unknowns, we used the alignment software MEGAX to perform a Muscle alignment and construct phylogenetic trees. The unknown strains were compared to 125 reference strains representing each of the previously determined 60 sequevars. Within the 181 strains that we classified, we confirmed and identified several novel sequevars. Our meta-analysis highlights the unappreciated genetic diversity of *Ralstonia* that remains to be discovered and classified.

Harnessing the microbiome of improved potato cropping systems and management practices

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Soilborne diseases and inadequate soil health can severely limit potato production and result in economic losses for growers. Incorporation of disease suppression and soil health management practices, such as longer rotations, cover crops and green manures, organic amendments, and reduced tillage, into improved cropping systems can mitigate disease pressure and improve production and sustainability. Investigating microbial communities and their functions under these different cropping systems is essential in understanding and harnessing beneficial microbes to suppress potato diseases and increase crop yield. In this study, long-term field trials (established in 2004) assessed different 3-year potato cropping systems focused on management goals of soil conservation (SC),

soil improvement (SI), and disease suppression (DS) compared to a 2-year standard rotation (SQ) and a non-rotation control (PP). Here, we combined a longitudinal taxonomic analysis of bacterial and fungal communities using high throughput amplicon sequencing, community-level functional and trophic reconstruction, and abiotic factors assessment to reveal the principles governing microbial community assembly favoring potato disease suppression and increased yield. Our results showed significant shifts in microbial communities' structure and composition among potato cropping systems, suggesting management practices can be manipulated to promote keystone taxa playing roles in potato soil health and crop yield.

Efficacy of pre-harvest application of plant product and antagonistic yeast on *Alternaria alternata*-induced fruit rot of tomato

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Tomato (*Solanum lycopersicum* Mill) is the most important vegetable crop in Oman, grown across the entire country. Fruit rot caused by the filamentous fungus *Alternaria alternata* is one of the major constraints in tomato production in Oman. The fungus invades quiescently in unripe fruits in the field and starts to grow aggressively when the fruit begins to ripe during packaging, transportation, and storage. In this study, the efficacy of pre-harvest application of antagonistic yeast *Meyerozyma guilliermondii* strain SQUCC-33Y isolated from the phyllosphere of tomato and *Tamarindus indica* leaf extract showing antifungal activity towards *A. alternata*, in the management of *A. alternata*-induced fruit rot of tomato was evaluated under greenhouse and field conditions. The plants were sprayed with the plant extract or biocontrol agent starting from fruit set until harvest stage at weekly intervals. The results showed that *M. guilliermondii* treatment significantly reduced the post-harvest *Alternaria* fruit rot infection, maintained the level of lycopene and titratable acidity of the harvested fruits, and did not impair the surface color of the fruits during postharvest storage. Field application of *M. guilliermondii* reduced the fruit rot severity by 52% over control after 20 days of storage at room temperature. However, aqueous extract of *T. indica* did not significantly reduce the postharvest *Alternaria* fruit rot infection. The antagonistic yeast *M. guilliermondii* strain SQUCC-33Y has the potential to be used as a biofungicide for the management of fruit rot of tomato.

Catching the right mutation-enhanced disease resistance mutants in wheat protect against stripe rust and other pathogens

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We have previously phenotyped over 2,000 EMS-mutagenized lines of tetraploid elite wheat *Triticum turgidum* cv Kronos for enhanced disease resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) under field conditions. The identified 35 enhanced disease resistance (EDR) lines were tested for heritability of the phenotypes for several years showing robust resistance across environments and against variable pathogen races. Our EDR mutants can be grouped into distinct phenotypic categories, from bright green without any cell death response to increasing levels of running cell death, that in a few EDR lines manifests in the absence of infection and is reminiscent of lesion mimic mutants described in other species. Such cell death phenotypes are indicative of elevated levels of immune response, which prompted us to evaluate EDR mutants for potential tradeoffs. Specifically, we examined whether elevated disease resistance against an obligate biotroph had a yield penalty and we were intrigued to find that many lines performed better than control plants. To clone underlying mutations, we established a mapping by sequencing pipeline as well as sequenced a draft genome of Kronos, which we are currently improving with long-read sequencing. Overall, we demonstrated that dominant enhanced resistance without visible tradeoffs can be derived through traditional mutagenesis. Once we understand the types of genes and mutations underlying EDR, these alleles can be targeted with genome editing in other elite wheat varieties as well as in other crop species.

Use of the normalized difference vegetation index (NDVI) to determine fungicide efficacy in sunflower

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Foliar fungicides belonging to DMI (Fungicide Resistance Action Committee [FRAC] 3), QoI (FRAC 11), and SDHI (FRAC 7) groups are labeled for use in sunflower (*Helianthus annuus* L.) against fungal diseases, such as Phomopsis stem canker, that can compromise yield in the U.S. Northern Great Plains. To determine fungicide efficacy, the potential of vegetation indices, which are commonly used to assess vegetation growth, greenness, and biomass accumulation, has not been explored in sunflower. This study aims at evaluating the use of Normalized Difference Vegetation Index (NDVI) obtained from a handheld active sensor (GreenSeeker) to determine fungicide efficacy. To achieve the objective, a field trial was established using an oilseed hybrid susceptible to Phomopsis stem canker in a randomized complete block design with four replications at Brookings, SD, in 2020. Seven fungicides were applied at two rates, using a Hi-Boy Sprayer, at the floral bud formation (R1) growth stage. A no-fungicide control was included in the trial. For each plot, NDVI values were obtained 4 days before (R1 growth stage) and 9 days after (R5 growth stage, flowering) fungicide application. No disease was observed between R1 and R5 growth stages. The Analysis of variance (ANOVA) results showed no significant effect of treatments on NDVI values and yield. These data support previous research findings that fungicides have little or no effect on crop health under field conditions in the absence of disease. This study suggests that applying fungicides under low disease pressure might not be economical.

Mapping the abundance and diversity of *Pseudomonas syringae* in a river throughout a watershed in a Mediterranean fruit and vegetable production region

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Surface waters provide irrigation, influence soil fertility and humidity, and capture run-off that alters their chemistry and microbiology. In stark contrast with knowledge about human and animal pathogens in water, little is known about plant pathogens in the surface waters that flow through a watershed. With sufficient knowledge bases about plant pathogens in surface waters, it would be possible to create applications—similar to those to assess risks of river water for human health—to estimate risks for plant health resulting from irrigation with river water. We have mapped the abundance and diversity of *Pseudomonas syringae* (*Ps*) in a 270-km stretch of the 300-km reach of the Durance River in Southern France. Situated in a fruit and vegetable production region, this river drains >14,000 km² of which 20% is agricultural production. Water collected at 21 sites in fall, winter, spring and summer over a 2-year period resulted in ca. 5,000 putative isolates of *Ps* whose identification was confirmed by partial sequences of the citrate synthase gene. *Ps* was detected in nearly all samples from the fall, winter and spring, whereas populations were below the detectable level in ca. 25% of summer samples at sites almost exclusively in the southern half of the watershed. Population densities ranged from 10³–10⁵ cells /L with *Ps* phylogroups 2, 13, 10, 7, and 1 detected most frequently (in descending order of frequency) and harboring novel phylogroups. Current work aims to reveal the most risky lines of *Ps* for local crops and their origins and trajectories throughout the basin.

Identification of *Fusarium fujikuroi* as a new root rot pathogen of soybean in Indiana

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Identifying new pathogens in local environments is an integral part of disease management. A screening for emergent seedling pathogens of soybean (*Glycine max* L.) grown at Purdue's ACRE farm was conducted in summer of 2020. Two isolates of *Fusarium fujikuroi* (Sawada) Wollenw. were recovered from seedlings showing dry brown root discoloration and necrotic cotyledons, symptoms typical of seedling infection by *Fusarium* spp. Cultures were obtained from surface sterilized root tissue on water agar and semi-selective media and purified by single-spore isolation. Morphological examinations of the cultures and spores were consistent with species descriptions of *F. fujikuroi*. The ITS, mtSSU, and tef1- α molecular barcodes were sequenced and matched 100% to accessions of *F. fujikuroi* deposited in GenBank. To complete Koch's postulates, sterilized sand-cornmeal media was inoculated either with plugs of a 7-day old isolate on CMA or sterile CMA plug controls. Soybean seeds (cv. Williams) were planted directly on the media, covered, and grown in a greenhouse for 14 days. All seedlings grown on infested media showed symptoms of root rot consistent with field symptoms while controls were healthy. *F. fujikuroi* was recovered from infected plants but not controls and identified as above. Some infected seedlings showed stem elongation as a symptom of infection compared to their matched controls, a first for this host. This is the first report of *F. fujikuroi* infecting soybean in the state of Indiana, warranting assessments of disease risk in this newly reported geographic distribution.

Evaluating the dynamic risk-based survey model for HLB in Southern California

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The key to control of citrus huanglongbing (HLB) is early detection. HLB is one of the most devastating diseases affecting citrus caused by the bacterium *Candidatus Liberibacter asiaticus* (CLAs) and spread by the Asian citrus psyllid (ACP). Since 2012, HLB has manifested in urban neighborhoods in Southern California and has begun to spread further into surrounding areas posing a threat to commercial citrus. Therefore, we developed a risk-based surveillance model that predicts high-risk areas to target for survey on a 1-square-mile scale for the entire state of California. Within the risk-based model framework, numerous factors and model components change over time over time as the epidemic evolves. By April 2021, the predictive model assisted in detecting more than 2,600 CLAs+ trees and psyllids. Through a retrospective longitude analysis for HLB finds, we evaluated the prediction power of each risk factor (including introduction risk, spatiotemporal ACP population development, citrus transportation corridors, previous HLB confirmations, etc.) in the model. In general, introduction risk plays an important role in the early epidemic phase, while established ACP populations and transportation corridors have strong correlations with subsequent HLB spread. Since ACP/HLB dispersal are dynamic processes, it is necessary to update/refine each model component promptly to ensure up-to-date, accurate representation of risk, so as to improve evidence-based decision-making for ACP/HLB management.

Foliar fungicide application timing for optimal control of blackleg in winter canola in northern Idaho

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The fungal pathogen *Leptosphaeria maculans* is the causal agent of blackleg disease of canola (*Brassica napus*). Worldwide, blackleg is one of the most destructive canola diseases and it is an emerging problem in seed production for the Idaho oilseed industry. Researchers and growers have limited knowledge of how environmental conditions impact the pathogen's development and distribution in the Inland Pacific Northwest (IPNW) of the U.S.A. Currently, no specific guidelines are available for timing of fungicide application for optimal control of blackleg in winter canola. Winter canola field trials were established at three sites in northern Idaho and subjected to foliar fungicide (Priaxor®) applications in the fall, spring, or both. Disease incidence in the no-fungicide control plots was low at Genesee (13%) and Moscow (25%), but moderate at Grangeville (61%). Disease was significantly reduced by either a fall (3–28%) or spring (1–16%) fungicide application, but the lowest disease incidence occurred when fungicides were applied in both the fall and spring (1–5%). While no yield response to fungicide application was observed, reducing the incidence and severity of blackleg is crucial to the region's seed production industry. Using Burkard volumetric spore traps deployed adjacent to the trials, ascospores were observed to be released between April and June under temperatures of 7–14°C. This research will contribute to the development of grower guidelines for best management practices against blackleg of canola in the rainfed region of the IPNW.

The use of thermotherapy to reduce *Neopestalotiopsis* spp. inoculum on strawberry transplants

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The lack of resistant cultivars and effective chemical alternatives to control the recent outbreaks of Pestalotia leaf spot of strawberry in Florida has affected the yield of several commercial fields. In addition, nursery transplants can harbor the causal agent, *Neopestalotiopsis* spp., which represent a major threat to the industry. Thus, the efficacy of thermotherapy to reduce inoculum on naturally infested and artificially inoculated strawberry transplants was evaluated. Before planting, naturally infested plug plants ('Florida 127'), and inoculated and non-inoculated transplants of 'Florida Brilliance' and 'Florida 127' were exposed to preheat at 37°C for 1-h f.b. heat treatment with aerated steam at 44°C for 4 h. Disease incidence and severity on leaves were evaluated 30 days after treatment. On naturally infested plants, disease incidence and severity were reduced by 50 and 82%, respectively. On inoculated plants, incidence was not significantly different among treatments for 'Florida 127', but was reduced by 40% on the heat-treated, inoculated 'Florida Brilliance' transplants. Furthermore, reduction in disease severity ranged from 45 to 50% on inoculated strawberry transplants of both cultivars. The tested thermotherapy protocol can reduce *Neopestalotiopsis* spp. inoculum and might be integrated to a disease management program to control Pestalotia leaf spot of strawberry. This practice might be a good alternative for nurseries before the final multiplication cycle and delivery of strawberry transplants for commercial production.

Utilizing random mutagenesis to identify wheat susceptibility genes

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Wheat leaf rust, caused by *Puccinia triticina*, has historically been a major disease of wheat, and continues to be important today. Wheat breeding programs have successfully introduced leaf rust resistance genes into commercial wheat. However, *P. triticina* has overcome many of the resistance genes deployed to date. Maintaining wheat's resistance to leaf rust thus requires continual development and introgression of new resistance genes. An alternative method of combating plant disease is to reduce the susceptibility of the host to infection. By potentially interfering with host-pathogen compatibility, susceptibility (S) genes potentially provide a more durable defense against infection. The susceptible wheat variety 'Thatcher' was mutagenized using ethyl methanesulfonate (EMS) and surviving plants were scored for reduced disease development when challenged with leaf rust. From these plants, three candidate lines have been obtained that show reduced pustule size and quantity compared to the susceptible parent. Altered plant responses to infections are being evaluated using RNAseq based expression analysis of the three mutant wheat lines after infection to leaf rust. Fungal infection is also being evaluated by microscopy. Results from this work will be presented.

Validation of real-time and conventional PCR assays for the detection of the exotic plant pathogen *Phytophthora kernoviae*

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Phytophthora kernoviae is a highly aggressive pathogen that causes stem lesions and bleeding cankers on beech, Rhododendron, and several trees and shrubs including species belonging to the *Quercus*, *Magnolia* and *Pieris* genera. Since its discovery in the U.K. in 2003, *P. kernoviae* has been identified in New Zealand, Chile, and Argentina. A 2018 survey in Chile revealed the presence of *P. chilensis* nom. prov. and *P. pseudokernoviae* nom. prov., two closely related species to *P. kernoviae*. Therefore, it was essential to evaluate robust diagnostic systems for these pathogens. In this project, we evaluated and validated existing *P. kernoviae* real-time PCR screening assays on the Bio-Rad CFX96 platform. We also developed and validated conventional PCR (cPCR) primers targeting the ITS region of the three species followed by sequence analysis. Assay validation involved determining the sensitivity, specificity and selectivity. We determined the limit of detection for the real-time PCR *P. kernoviae* assay to be 500 ag for ITS1 and 5 fg for ITS2. The detection sensitivity of the cPCR assay was determined to be 500 ag (~670 bp amplicon). Moreover, the primers were specific and selective in detecting *P. kernoviae* from several closely related species and hosts, thereby further validating the assays. The detection of *P. kernoviae* and related species is essential to safeguard U.S. agriculture and natural resources from the introduction and establishment of pathogens, which could cause diseases of economic and environmental impact.

Identifying the virulence factors of emerging corn leaf streak pathogen *Xanthomonas vasicola* pv. *vasculorum*

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Bacterial leaf streak of corn (BLS) has rapidly spread to most corn producing regions in the U.S. in recent years. The pathogen has been identified as *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*). We used in vivo selection and high-throughput sequencing of transposon libraries (Tn-seq) to characterize potential virulence factors in *Xvv*. A library of approximately 300,000 individual *Xvv* mutants was generated with a Mariner transposon containing *MmeI* restriction sites on both ends. Digestion of the mutant gDNA with *MmeI*, a type-IIS restriction-modification enzyme that cuts dsDNA 20 bp away from the recognition site, yields fragments of the inserted transposons with 16 bp of adjacent *Xvv* genome on each end. The method enables high-throughput sequencing to identify Tn insertion sites in the population. The *Xvv* mutant library was inoculated into maize at 10⁸ CFU/ml on 3-week-old plants. Samples were collected 1 week after inoculation and used as inoculum for the second round of inoculation on corn. After two rounds of inoculation and selection in the host, the Tn insertion profile of the rescued bacteria was compared to the profile of the original population. The profiles will be discussed in relationship to candidate virulence genes of this emerging pathogen. At the same time, individual *Xvv* mutants of several potential

virulence-related genes have been made using classic reverse genetics methods. These results will be analyzed and compared in parallel, to provide directions for future disease management.

Mechanistic understanding of Xcc-facilitated agroinfiltration to improve the efficacy of transient gene expression in citrus leaves

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Transient gene expression in plant tissues/cells through agroinfiltration is widely used in plant genetic study and plant biotechnology. However, many economically important plant species such as citrus are highly recalcitrant to the agroinfiltration. Citrus is one of the economically important top fruit crops in the world. In our previous study, *Xanthomonas citri* subsp. *citri* (Xcc)-facilitated agroinfiltration has significantly improved the transformation efficacy. In this study, we aimed to understand the mechanism underneath the higher efficacy of Xcc-facilitated agroinfiltration. To conduct this study, we tested Xcc wild-type, *pthA4* mutant and T2SS mutant and how they affect the β -glucuronidase (GUS) expression. We measured the expression of defense related genes and cell wall degrading enzyme genes at different time points. We also further optimized the agroinfiltration. Our study unravels the mechanistic understanding of Xcc-facilitated agroinfiltration and provides valuable information to further improve transient expression in citrus leaves via agroinfiltration.

Investigation of host specificities of various forms of *Puccinia coronata* var. *avenae*, a pathogen of oats and turfgrass species

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In North America, crown rust is an emerging disease of Kentucky bluegrass (*Poa pratensis*), a major cool season turfgrass. Sequences of the ITS region place the causal organism within *Puccinia coronata* var. *avenae*, making the pathogen closely related to *P. coronata* var. *avenae* f. sp. *avenae*, which causes crown rust of oats, and formae speciales that affect *Festuca* and *Lolium*. We hypothesized that common turfgrasses may be able to host *P. c.* f. sp. *avenae*, playing a role in oats crown rust epidemiology. The objectives of this research were: 1) To compare the host specificity of crown rust isolates derived from *P. pratensis*, *P. palustris*, and oats through inoculation onto various species of *Festuca*, *Lolium*, and *Poa*; and 2) to assess rust infection in turfgrass research plots of *P. pratensis* and *L. perenne*. Preliminary findings support the separation of *P. c.* var. *avenae* into formae speciales. One isolate derived from oats produced infection only on a single *Poa* *pratensis* accession, while another isolate from oats did not produce infection on any of the turfgrasses. The two isolates derived from *Poa* infected only *Poa* species including *P. pratensis*, *P. glauca*, *P. attenuata*, *P. compressa*, *P. palustris*, and *P. trivialis*. Field evaluations of rust infection on *P. pratensis* and *L. perenne* showed a diversity of resistance phenotypes. Continued studies will test oat susceptibility to isolates derived from turfgrasses.

Molecular detection of azole-resistant isolates of *Aspergillus fumigatus* directly from air, soil, and plant debris in agricultural environments

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Azoles fungicides are widely used in agriculture due to their high efficacy and relatively slow level of resistance development. Antifungal resistance is a major concern for both crop production and human health since there are few alternatives for the treatment of pathogenic fungi once resistance appears. *Aspergillus fumigatus*, the causal agent of the deadly human mycosis aspergillosis developed resistance mutations that have been linked to agricultural azole use. The mutations are the tandem repeats (TR), TR₃₄/L98H, and TR₄₆/Y121F/T289A in the *cyp51A* gene. These are associated with treatment failure and mortality rates over 80% in immunocompromised and immunocompetent patients. Studying resistance in the environment is demanding and time-consuming since it is necessary to isolate the fungus, then test antibiotic susceptibility through techniques like the minimum inhibitory concentration (MIC) assay, followed by the sequencing of *cyp51A* to describe the mutations. Here, we validated two nested-PCR protocols targeting the environmental mutations directly in DNA obtained from air, plant debris, soil, and compost samples. Both techniques have a 100% specificity and detect one cell of the fungus in 1g of sample. The PCR products were confirmed as *A. fumigatus*. The assays were applied in 33 environmental samples where *A. fumigatus* was detected in 17 (51.5%) samples and from those 9 (27.3%) had the TR₄₆ mutation. The nested PCR assays used here are a more efficient way to identify TR mutations linked to the development and spread of pan-azole-resistance in the environmental.

Investigating interactions of crop rotations, varieties, and fungicides when managing frogeye leaf spot in soybean

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Frogeye leaf spot (FLS), caused by *Cercospora sojina*, damages soybean leaves by forming lesions, that lead to defoliation. Management of FLS includes using resistant varieties, crop rotation, and fungicides. The objective of this project was to investigate the dynamics of these management tactics. Field trials with a 3-way factorial design and four replications were conducted across 2017–2020; two different location, Milan (continuous soybean) and Jackson (crop rotation system); 3 varieties that ranged in susceptibility to FLS; and 5 different fungicides and non-treated, control. Disease severity was rated at R6 and yield was recovered. Means were separate using Tukey ($P < 0.05$). Using disease severity from controls of susceptible varieties FLS resulted in a total severity of 19% for 2020, 40% for 2019, 10% for 2018 and 28% for 2017. Interactions between varieties, fungicides, and locations for 2018, 2019, and 2020 seasons showed greater FLS severity under continuous soybean during 2019, while the crop rotation (corn-soybean rotation) system increased yield across all season, although this varied according to the variety level of susceptibility. Susceptible varieties had the greatest FLS, but summarized yield results across fungicide treatments had the greatest or equal yield when compared to the

resistant variety in three of the four years. Therefore, fungicide treatments were able to manage FLS and protect yield. This study shows the importance on using integrated practices in disease management. The complexities of the interactions will be further discuss in the presentation.

Zinkicide® reduces *Xylella fastidiosa* populations and leaf scorch symptoms in tobacco and blueberry

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Xylem-limited pathogen *Xylella fastidiosa* is of global importance due to its economic impact on fruit crops such as grapes, blueberry, citrus, and recently olives. There is growing interest in employing nano-formulations to manage plant diseases. The goal of this study was to evaluate the efficacy of a novel ZnO-based nano-formulation, Zinkicide® TMN110 (ZnK), against *X. fastidiosa*. In vitro assays revealed that ZnK minimum bactericidal concentration against *X. fastidiosa* was 50 ppm. Further antimicrobial activity assessment of ZnK under flow conditions using microfluidic chambers indicated inhibition of biofilm formation and bactericidal effect against planktonic cells. Additionally, virulence assays were performed in the model tobacco and blueberry plants under greenhouse conditions. Initial experiments showed that the maximum concentration of ZnK added as a drench treatment not causing phytotoxic effects was four doses of 500 ppm on both tobacco and blueberry. Then, non-phytotoxic concentrations of ZnK applied by soil drench in weekly doses to tobacco infected with *X. fastidiosa* showed reduction in the bacterial population of ~3 log₁₀ units, and disease severity was reduced drastically compared to the control. Likewise, ZnK non-phytotoxic concentration applied to blueberry infected with *X. fastidiosa* showed reduction in the bacterial population of ~1 to 2 log₁₀ units, and disease severity was reduced compared to the control. This study highlights the potential role of ZnK to manage plant vascular diseases.

Regular rainfall reduces efficacy of fungicides at controlling *Cercospora beticola* in sugar beet

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Cercospora beticola causes Cercospora leaf spot (CLS), the most damaging foliar disease of sugar beet. Growers use an integrated system, including fungicides to manage CLS. *C. beticola* has developed resistance and/or reduced sensitivity to several fungicide chemistries worldwide. Field studies conducted near Foxhome, MN, US, indicated that multiple applications of mixtures of multi-site fungicides or multi-site with site-specific fungicides in a rotation program at 10- to 14-day intervals provided effective control of *C. beticola*, even on more susceptible varieties, when fungicide applications were followed by several days of dry conditions. However, when fungicide treatments applied to more susceptible varieties were soon followed by heavy and regular rainfall, disease control became less effective, as measured by disease severity, by late August. It was observed that new improved, but not yet approved, CLS resistant varieties with and without fungicide treatments, resulted in less disease severity and acceptable recoverable sucrose in the presence of the pathogen in the same environment. The availability of improved CLS tolerant varieties that can provide acceptable economic returns, even in the absence of fungicides, will reduce the risk of losing 40 to 50% of potential yield when fungicides are rendered ineffective because of adverse weather conditions.

A preliminary metabolomic analysis of potential mycotoxigenic *Fusarium* species infecting sweetpotato

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Fusarium root rot, primarily caused by *Fusarium solani*, is a major postharvest disease of sweetpotatoes that renders infected roots unmarketable and inedible. Beyond direct crop losses, the threat of phytotoxin and mycotoxin contamination exists. In response to fungal infection, sweetpotatoes can produce biologically active stress-metabolites, notably two 15-carbon (15C) furanoterpenoid compounds. These compounds are hepatotoxic, but *F. solani* has shown to reduce them further into four 9-carbon (9C) compounds that are acutely toxic to livestock. Recently, several additional *Fusarium* species that are capable of producing mycotoxins were observed infecting sweetpotato; however, the ability of these species to induce phytotoxin production is unknown. This study sought to characterize metabolites produced in *Fusarium*-infected sweetpotatoes to help inform future studies. Sweetpotatoes (cv. Covington) were wounded, inoculated with one of five different *Fusarium* isolates (including *F. acuminatum*, *F. denticulatum*, and *F. proliferatum*), and incubated at 25°C for 28 days. Infected and healthy tissues were removed and metabolites extracted using a standard methanol-based protocol. The resulting solution was diluted 1:10 and analyzed using high resolution-liquid chromatography/mass spectrometry. Preliminary results suggest that ipomeamarone (15°C) and ipomeanol (9°C) were present in the inoculated sweetpotatoes for each of the species tested. These data provide insight into the metabolites present during the *Fusarium*-sweetpotato interaction and allow for future targeted analyses.

Managing the spread of *Tomato brown rugose fruit virus* and *Cucumber green mottle mosaic virus* using chemical disinfectants

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Tobamoviruses causes severe production loss of economically important crops. These viruses are transmitted via physical contact or through seeds. The virulent nature of two emerging Tobamoviruses: *Tomato brown rugose fruit virus* (ToBRFV) and *Cucumber green mottle mosaic virus* (CGMMV) have threatened several economically important vegetable crops in the Solanaceae (tomato, pepper) and Cucurbitaceae (watermelon, cucumber) worldwide. With the lack of a disease resistant cultivar, preventive measures are the best solution to manage the spread of these viruses. In the present study, we focused our efforts in evaluating 16 chemical disinfectants for

their effectiveness against mechanical transmission of ToBRFV on tomato and CGMMV on watermelon. Results showed that six disinfectants (Lactoferrin, Virocid, Clorox, Virkon S, Virex, and SP2700) under appropriate concentrations generated significant and broad-spectrum activities against the spread of both ToBRFV and CGMMV. In conclusion, identification of common disinfectants against two emerging tobamoviruses in two different pathosystems suggest their potential broader effects against other viruses.

Genetic diversity and population structure of *Alternaria* species infecting tomato and potato in North Carolina and Wisconsin

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Early blight caused by *Alternaria linariae* or *A. solani*, and leaf blight, caused by *A. alternata*, are destructive diseases of tomato and potato in North Carolina and Wisconsin. We collected 214 isolates of *A. alternata* ($n = 61$), *A. linariae* ($n = 96$), and *A. solani* ($n = 57$) and used 220 single nucleotide polymorphisms derived from DNA sequences of 10 microsatellites to analyze the population genetic structure and infer the reproductive mode of the populations. High genetic variation and genotypic diversity were observed in all the populations analyzed. Chi-square tests of the frequencies of mating-type genes and linkage disequilibrium analysis revealed evidence of both clonal expansion and recombination. Pairwise comparison of population differentiation (F_{ST}) and hierarchical analyses of molecular variance showed that most genetic differentiation was within individuals in a population, confirming a lack of population subdivision within species. *A. linariae* populations harbored more multilocus genotypes (MLGs) than *A. alternata* and *A. solani* populations and shared the same MLG between populations. Our results provide new insight into the genetic structure of *Alternaria* spp. and may facilitate the development of resistance breeding strategies for *Alternaria* in tomato and potato.

Patch management of clubroot on canola with a non-traditional product under the influence of lime and without lime

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Clubroot on canola caused by *Plasmodiophora brassicae* infects root hairs forming large distorted galls on developing roots resulting in serious economic losses in terms of yield. The pathogen *P. brassicae* is proven to be managed with certain soil amendments of lime, beet lime, wood ash and fungicides on canola in North Dakota for a few years in research plots. However, these products were used in high rates. Research is ongoing for alternative products that are less expensive to manage patches of clubroot in the field. The current objective was to evaluate the efficacy of a non-traditional product at various rates under the influence of lime and without lime. A field trial with split-plot arrangement was designed with lime and without lime as main plots and various rates of the non-traditional product in sub-plots. Soil samples were collected before and after the application of treatment for pH assessments and to determine soil population of clubroot resting spores. There were no significant differences in clubroot disease index between the main plot treatments of lime and without lime. However, there were statistically significant differences among the sub-plot treatments were found indicating all of the non-traditional product rates tested were effective in managing clubroot when compared to the non-treated control. Likewise, differences were found among the rates tested. The average *P. brassicae* resting spores were 13.5 million spores per gram of soil and there were significant differences in the pH in the lime applied treatments to that of non-applied treatments.

***Colletotrichum* species associated with internal rot of pulp in apparently healthy avocado fruits cv. Hass**

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Avocado fruits available to the consumer on shelves have acceptable quality for the consumer. However, rot that turns pulp light brown to blackish occurs to some ripe-for-consumption fruits. The estimated losses approximate 60%. This study identified the fungi associated with health and rotting pulp in 30 apparently healthy fruits and 11 peduncles from a Mexican supermarket. We randomly analyzed 10 (replicates) fruits at 0 (stone-firmness), 5 (ripe-for-consumption), and 10 (advanced maturity) days after sampling. After fruit disinfection, pulp samples, healthy and rotten, from under the peduncle scar, vascular bundles, under the seed, or peduncles were cultured in 2% malt extract agar under near-UV light. From these cultures, 13 monosporic isolates were selected from a pool for molecular characterization by ITS rDNA, TUB2, and ACT. *Colletotrichum*, a known plant and fruit pathogen, consistently developed in 36% of the sampled fruits. *C. karstii* was isolated from 20 to 100% of the tissue from vascular bundles in advanced maturity samples and peduncles. Fruits at stone-firmness and different maturity stages contained *C. siamense* from 20 to 60%. *Colletotrichum* sp.¹ was isolated from vascular bundle tissue in stone-firmness and advanced maturity (AM) fruits, below the seed tissue (AM), and peduncles from 20 to 100%. *Colletotrichum* sp.² was isolated from 20 to 60% of the tissue at stone-firmness and every maturity stage, from every tissue type, and from peduncles. Future research should avoid establishing *Colletotrichum* in the pulp and peduncles during fruit formation to reduce losses to the consumer.

The *Ralstonia solanacearum* Type II secretion system is a hotspot for mutations that confer resistance to the phage phiAPI

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Ralstonia solanacearum (Rs) causes devastating bacterial wilt disease on a wide range of plant hosts, including economically important crops like tomato, potato, and banana. As a soil dwelling organism that can live for long periods in infested fields or waterways, Rs can

be exceptionally difficult to manage. One promising avenue for controlling bacterial wilt disease is the use of *Rs*-infecting phages to kill bacteria in the environment. Due to its lytic life cycle and ability to infect a wide range of *Rs* strains, the phikmvvirus phiAP1 may be useful as a biocontrol agent. However, *Rs* can evolve resistance to phiAP1. Here, we examine how *Rs* can evade phiAP1 infection using a library of spontaneous bacteriophage insensitive mutants (BIMs). Many BIMs gained mutation in the *Rs* Type II secretion system, an important *Rs* virulence factor. We found that phiAP1 uses the action of the T2SS during infection and that mutating the T2SS to prevent phiAP1 infection generally imposes a severe virulence defect. However, we also found mutations outside of the T2SS that conferred resistance, suggesting that phiAP1 should ideally be used alongside other phages with different targets in order to maintain its controlling effects. Understanding how phiAP1 infects *Rs* and how *Rs* can resist phiAP1 will allow us to develop more durable biocontrol strategies.

Evaluating fungicide programs for management of tar spot in Indiana corn

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Tar spot is a foliar disease of corn caused by *Phyllachora maydis*. In 2015 *P. maydis* was first documented in the U.S. in Indiana and Illinois. Since then, *P. maydis* has been confirmed in ten states and Ontario, Canada. In this study, we assessed the efficacy of two foliar fungicides (Veltyma 3.34 S at 7.0 fl oz/A and Lucento 7.17 SC at 5.0 fl oz/A) in different timing programs. These programs were a single application made at growth stages V8, tassell (VT), and milk (R3), and at first detection (FD); and two application programs that included a second application made 3 weeks after the first treatment (WAT) at timings indicated previously. Tar spot was rated by visually assessing the percentage of stroma and symptomatic tissues during the season, which were used to calculate area under the disease progress curves (AUDPC). Percent green canopy and yield were also collected. A single application of Veltyma applied at the FD, VT and a double application at V8 fb 3 WAT were the best programs that resulted in the lowest AUDPC of tar spot. All other programs significantly reduced tar spot AUDPC over the nontreated control, except those with a single application of either Veltyma or Lucento at V8, Veltyma at R3, and both applied at R3 followed by application 3 WAT. All programs significantly increased the percentage of stay green canopy over the nontreated control, but no difference was detected for yield.

Performance of fungicides, biofungicides, fertilizers, and host plant defense inducers in managing Phytophthora root rot of boxwood in greenhouse

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Phytophthora root rot, caused by *Phytophthora nicotianae*, is a major limitation to boxwood production and can affect all growth stages of container grown boxwood. In greenhouse trials, we evaluated the efficacy of chemical fungicides, biofungicides, host plant defense inducers, and fertilizers for the management of Phytophthora root rot. The experiment was carried out in a randomized design with five replications and the experiment was carried out twice, in 2019 and 2020. Rooted cuttings of boxwood cv. Green Velvet were transplanted in 1-gal pots with nursery mix. Pots were inoculated with *P. nicotianae* grown on rice grains; 4 grains per pot were used. Plant growth data were recorded, and roots were assessed for disease severity using a scale of 0–100% roots affected. At the end of the 2019 trial, all treatments suppressed Phytophthora disease severity, though Empress Intrinsic was the most effective one. At the end of the 2020 trial, all treatments reduced root rot severity when compared to the inoculated, non-treated plants. Treatments most effective in reducing Phytophthora disease severity were Segovis, Empress Intrinsic, and Orvego. There were no significant differences in plant height increase, width increase, total plant fresh weight and total plant root weight between treated and non-treated control plants in both trials.

Investigating microbial communities associated with source irrigation and wet taro field water using amplicon Oxford Nanopore MinION sequencing

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Irrigation water is known as a potential source of contamination of plant and food-borne human pathogens, and contaminated water could play an important role in contaminating soil and plants in agricultural settings. Thus, we investigated microbial communities associated with irrigation water in taro fields on Oahu, Hawai'i. Samples were collected randomly along source irrigation ditches and stagnant taro field water. We aimed to uncover bacterial consortia associated with source irrigation ditches and field water and to identify community differences and contaminating pathogens. Collected samples were passed through a 0.22-µm MCE membrane; gDNA was extracted and amplified to the full length 16S rRNA gene region and subsequently sequenced in replicate using Oxford Nanopore Technologies (ONT) MinION platform. An average of 88-k and 703-k reads from source and field water, respectively, were analyzed and classified using the EPI2ME and BusyBee web server implementing bootstrapped supervised binning. Samples were dominated by bacteria in the phyla Proteobacteria and Bacteroidetes. Dominant plant pathogenic bacterial genera were *Dickeya* and *Pectobacterium* with traces of *Erwinia*, *Pantoea*, *Xanthomonas*, and *Ralstonia*. The average cumulative reads of *E. coli*, the indicator of fecal pollution, increased from 98 at the source to 2,453 in stagnated field water. Discovery and description of microbial communities associated with different water systems are essential for development of better disease management strategies and production of safer fresh produce.

Resistance of *Clarithreedia* spp. to demethylation inhibitor fungicides

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Demethylation inhibitor fungicides (DMIs) are commonly used to suppress dollar spot in turfgrass. Repeated use of DMIs has led to development of pathogen populations exhibiting reduced sensitivity. The objectives of this study were 1) to determine discriminatory doses for nine commercially available DMIs; and 2) to evaluate differences among *Claviceps* spp. isolates of varying resistance to propiconazole across determined discriminatory doses. Fifty isolates were screened across five concentrations of fungicide-amended potato dextrose agar to determine discriminatory doses. Six isolates of known sensitivity to propiconazole (i.e., low, moderate, and high) were screened across discriminatory doses for each DMI. The trials were arranged as completely randomized designs with three replications. Mycelial growth was measured after 48 h of incubation on a lab bench at $23 \pm 2^\circ\text{C}$. Preliminary data resulted in discriminatory doses of $0.01 \mu\text{g a.i. mL}^{-1}$ PDA for mefenftrifluconazole, propiconazole and myclobutanil, $10 \mu\text{g a.i. mL}^{-1}$ PDA for metconazole and $1 \mu\text{g a.i. mL}^{-1}$ PDA for triticonazole, triadimefon, tebuconazole and flutriafol. Triticonazole had the greatest reduction in mycelial growth of the least sensitive isolates and was similar to flutriafol and prothioconazole. Across all isolates, triadimefon resulted in the least suppression of growth. Initial results suggest differences in dollar spot control may exist across DMIs. The experiment will be repeated to confirm results. In addition, trials are underway to assess differences in dollar spot control among DMIs under field conditions.

The microbiome of sugarcane and its interaction with the vascular pathogen *Leifsonia xyli* subsp. *xyli*

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The restricted niche of the xylem provides ample opportunities for microbial interactions which may define the outcome of the colonization process of the host by a pathogen. The composition and functionality of the microbiomes of a resistant and a susceptible sugarcane variety grown in the greenhouse were analyzed after inoculation with Lxx. Stalks, roots, and rhizosphere were collected 60 days after inoculation. The composition of the prokaryotic communities was analyzed by sequencing the V3-V4 region of the 16S rRNA and their metagenome was predicted using PICRUSt. Bacterial abundances were higher in the roots and rhizosphere of both genotypes. *Proteobacteria* and *Actinobacteria* were the most abundant, regardless of the inoculation treatment or compartment, followed by *Acidobacteria* in the stalks and rhizosphere and *Bacteroidetes* in the roots. Lxx-inoculation shifted the abundance of most phyla in the stalks and roots in both varieties. Most of the abundant families detected in the stalks of inoculated plants consisted of oligotrophs, i.e., slow-growing organisms with low metabolism but with a high capacity to survive in nutritionally poor environments. Families with reduced abundances in the roots and rhizosphere of inoculated plants included nitrogen fixing and plant growth promoting species which may contribute to the poor growth of diseased plants. Predicted metabolic pathways that stimulate Lxx growth (e.g., amino acids) were overrepresented in inoculated plants and conversely plant immunity stimulating pathways were underrepresented (e.g., thiamine).

Characterization of multiple virus-like particles found in a historical sample of maize from Minnesota

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Historical samples of corn collected in Jackson (MN) in the 2000s were investigated. Plants samples showed virus-like symptoms including stunting and proliferation of leaves below the tassel. Different sections of the plant stored frozen at -80°C were submitted to a partial purification protocol for the enrichment of virus-like particles. Transmission electron microscopy of partially purified extracts revealed the presence of diverse spherical and filamentous virus-like particles. Two different sizes of spherical particles were observed of 30 and 50 nm in diameter, filamentous particles were approximately 15 and 20 nm in diameter. The genomic characterization of virus-like particles was done by high-throughput sequencing (HTS) of virion-associated nucleic acids (VANA). Among the viral sequences detected were aphid lethal paralysis virus (99%, aa), sclerophthora macrospora virus-A (96–99%, aa), sclerophthora macrospora virus-B (97–99%, aa), rhopalosiphum padi virus (94%, aa), maize yellow mosaic virus (97%, aa). The role of each one of these viruses reported on the disease symptoms is being investigated after this preliminary work. These new sequencing technologies can be used to characterize and rapidly identify viruses and will improve detection and discovery of plant viruses as well as retroactive analysis of stored plant tissues.

Characterizing and monitoring disease occurrence of *Pythium* wilt of lettuce in Salinas Valley

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Pythium wilt is currently considered the most pressing soilborne disease affecting the lettuce production in the Salinas Valley. In 2020, an unprecedented and devastating outbreak of this *Pythium* wilt, with numerous growers reporting up to 100% crop loss in 2020. Yet, little is known about this disease which has been severely understudied. In this project, we aimed to enhance understanding of the *Pythium* wilt affecting lettuce in the Salinas Valley, including factors that influenced the geographical distribution, establishment and development of the disease. Over 55 commercial fields were surveyed, collecting samples of symptomatic plants, as well as associated metadata. Observed symptoms on symptomatic plants included stunted and wilted plants, with characteristic yellowing of the outer leaves collapsing onto the ground, while younger leaves often remain upright until complete death of the plant. Severe root rot was commonly observed, with characteristic necrotic lesions on root tips, fine feeder roots and, on tap roots, though rotting was usually

limited to the root cortex. Over 130 *Pythium* spp. isolates were collected from infected plants. Isolates were characterized morphologically, and molecular identification is underway. This project offers novel information about the biology *Pythium* wilt of lettuce and lays the ground for future studies focusing on designing accurate diseases diagnostic tools, and identifying best management practices.

Discovery and characterization of multiple disease resistance against three bacterial pathogens in tomato

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Tomato (*Solanum lycopersicum*) is one of the most widespread vegetable crops in the world. It is afflicted by many different pathogens, including the foliar bacterial diseases speck (caused by *Pseudomonas syringae* pv. *tomato*, *Pst*) and spot (*Xanthomonas* sp.) and a vascular disease that causes canker/wilt (*Clavibacter michiganensis* subsp. *michiganensis*, *Cmm*). The search for resistance against these pathogens has focused on NLR-triggered immunity (NTI) genes, though they are often limited to a strain-host specific relationship. Therefore, discovering new sources of multiple disease resistance is important to enhance control against these and other emerging pathogens. We previously screened 216 tomato accessions and discovered several with increased resistance to *Pst* that seemed to be governed by a pattern-triggered immune (PTI) response. We tested 21 of these accessions in field trials across several locations on the East Coast for their response to inoculation with *Pst*, *X. cynarae* pv. *gardneri* (*Xcg*), and *Cmm*, and found 15 accessions with increased resistance to more than one of these pathogens. To investigate the genetic bases of the responses, we generated F₂ populations and tested individual plants with multiple pathogens. Early evidence suggests that in some cases the resistance is controlled by a single locus. Our current efforts are focused on mapping the gene(s) involved in the resistance and determining their functions and molecular mechanisms in multiple disease resistance.

Population structure and genetic diversity in Virginia populations of *Parastagonospora nodorum* infecting wheat

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Parastagonospora nodorum is a necrotrophic pathogen that causes Stagonospora nodorum blotch (SNB) in wheat, and interactions between host sensitivity genes and pathogen necrotrophic effectors (NE) influence disease development. Wheat varieties grown in Virginia vary in susceptibility to SNB, but impacts of wheat genetic backgrounds on diversity of *P. nodorum* in the region are not known. The objective of this study was to characterize genetic diversity in *P. nodorum* populations in Virginia and determine if host genetic backgrounds and/or environments influence population structure. A total of 319 isolates were collected from five wheat varieties varying in susceptibility to SNB at seven locations over 2 years. Isolates were genotyped using multi-locus simple sequence repeat (SSR) markers, and NE genes and mating type genes were amplified using gene-specific PCR primers. Fungal populations were genetically diverse across varieties and locations, and frequencies of the two mating type idiomorphs were equal suggesting that the *P. nodorum* population is undergoing random mating. Three major NE were detected in the Virginia population of *P. nodorum*, but the frequency of *SnTox1* (64%) was greater than the frequency of *SnTox3* (24%) or *SnToxA* (0.6%). Implications of genetic diversity and structure of *P. nodorum* populations for SNB management in wheat will be discussed.

Interactive science communication of plant disease epidemiology for the classroom and extension

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Plant disease epidemiology incorporates research across many disciplines such as agronomy, ecology, economics, and sociology. The diversity and the sheer number of studies can pose a challenge for both growers and students to grasp the connectedness of seemingly unrelated research in epidemiology. The purpose of educating growers on epidemiology is to offer them a toolbox of information they can later use to support their decision-making for disease management. With students, the goal is to enable them to relate course content and their research to the broader epidemiological scope of their pathosystem. Our objective is to create a self-guided interactive program to teach growers and students plant disease epidemiology. Our model system is *Sclerotinia sclerotiorum*, the causal agent of white mold. A presentation platform containing short videos, figures, case studies, and activities will be designed to cover specific learning modules. Data from our studies, as well as published results from other research groups, will be used to build the broad narrative of white mold disease epidemiology. The program will be implemented at grower workshops and a graduate-level epidemiology course at Penn State. A pre- and post-lesson survey will be conducted to evaluate the impact of this teaching platform. This tool has the potential to be adapted for other pathosystems and become an accessible online teaching tool for extension and the classroom.

Covering soybean leaves with cellulose nanofiber changes leaf surface hydrophobicity and confers resistance against *Phakopsora pachyrhizi*

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Asian soybean rust (ASR) caused by *Phakopsora pachyrhizi*, an obligate biotrophic fungal pathogen, is the most devastating soybean production disease worldwide. Currently, timely fungicide application is the only means to control ASR in the field. We investigated cellulose nanofiber (CNF) application on ASR disease management. CNF-treated leaves showed reduced lesion number after *P.*

pachyrhizi inoculation compared to control leaves, indicating that covering soybean leaves with CNF confers *P. pachyrhizi* resistance. We also demonstrated that formation of *P. pachyrhizi* pre-infection structures including germ-tubes and appressoria, and also gene expression related to these formations, such as *chitin synthases* (*CHSs*), were significantly suppressed in CNF-treated soybean leaves compared to control leaves. Moreover, contact angle measurement revealed that CNF converts soybean leaf surface properties from hydrophobic to hydrophilic. These results suggest that CNF can change soybean leaf surface hydrophobicity, conferring resistance against *P. pachyrhizi*, based on the reduced expression of *CHSs*, as well as reduced formation of pre-infection structures. This is the first study to investigate CNF application to control field disease.

Grape powdery mildew spray programs affect non-target fungi populations differentially in California vineyards

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Powdery mildew (PM) causes severe loss of table grape production worldwide. To control the causal agent, *Erysiphe necator*, fungicide spray programs have been largely applied in the grape vineyards. However, frequent sprays might also have an effect on populations of non-target fungi. For example, several PM fungicides are also used to control *Botrytis cinerea* that causes postharvest gray mold in table grapes. Therefore, this study aims to investigate the effects of different PM fungicide spray programs on non-target fungi populations and distributions in grape vineyards. We studied two fungi species, *Botrytis* and *Alternaria*, collected from three grape vineyards in California: one field with six PM spray programs (treatments) and two fields with one PM spray program for each. Single-spore cultured *Botrytis* and *Alternaria* isolates were obtained from the leaf or rachis samples collected from each treatment between the bloom and the fruit set growth stages. The isolates were evaluated and analyzed for 1) the fungicide resistance phenotypes for three common botryticides, pyraclostrobin (QoI; FRAC 11), boscalid, and fluopyram (both SDHI; FRAC 7); 2) colony type for morphological characterization; and (3) phylogenetic analysis for species identification. The results showed that both *Botrytis* and *Alternaria* isolates displayed differential populations in their fungicide resistance phenotypes under different treatments, suggesting PM spray programs have varying effects on non-target species such as *Botrytis* and *Alternaria* species.

Race structure of necrotrophic effectors in the Kansas *Pyrenophora tritici-repentis* population

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Tan spot (*Pyrenophora tritici-repentis*) is a common disease in Kansas wheat fields that can manifest with multiple phenotypes due to the presence or absence of at least three necrotrophic effectors, *Ptr ToxA*, *ToxB*, and *ToxC*. The primary necrotrophic effector is produced by the gene *Ptr ToxA*. This gene product causes a hypersensitive response in the host which leads to leaf tissue necrosis and enables invasion of the pathogen. *ToxB* and *ToxC* both cause chlorosis in the host. These genes have been found in all possible combinations, but the US pathogen population primarily carries *Ptr ToxA* and *ToxC*. The objective of this research is to characterize Kansas isolates of the pathogen for their necrotrophic effector race distribution. A representative sample of Kansas isolates were collected by surveying counties throughout the state's wheat production region in 2019 and 2020. Once in pure culture, isolate race was determined by screening on a differential set of three wheat lines that are each susceptible to only one of the necrotrophic effectors. A molecular assay to amplify the genes for *Ptr ToxA* and *ToxB* was also conducted to verify the screening results. These experiments identified that the primary necrotrophic effectors present in the state are *Ptr ToxA* and *ToxC*. Knowledge of which necrotrophic effectors are present in a region can help pathologists and breeders make decisions about which isolates of the pathogen to breed against. These results also indicate that breeders should consider removing the *Tsn1* susceptibility gene from upcoming wheat varieties.

Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2020

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In 2020, stripe rust of barley, caused by *Puccinia striiformis* f. sp. *hordei* (Psh), occurred in California, Idaho, Oregon, and Washington, and stripe rust of wheat, caused by *P. striiformis* f. sp. *tritici* (Pst), occurred in 14 states. For monitoring virulence changes of the pathogens, stripe rust samples were tested on 18 wheat and 12 barley differentials to identify Pst and Psh races, respectively. From 49 Psh isolates, 10 races were detected including one new race, PSH-97 (virulent on differentials Topper, Abed Binder 12, and Bancroft). Races PSH-33 (virulent on differentials Topper and Abed Binder 12) and PSH-46 (virulent on Topper, Abed Binder 12, and Trumpf) were predominant with frequencies of 38.8% and 18.4%, respectively. From 301 Pst isolates, 19 races were identified, including two new races, PSTv-325 (virulent on Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrSP, YrTr1, and YrExp2) and PSTv-326 (virulent on Yr1, Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr24, Yr27, Yr32, Yr43, Yr44, YrTr1, and YrExp2). The predominant races were PSTv-37 (54.2%), PSTv-39 (18.6%), and PSTv-47 (5.6%). PSTv-37 was detected throughout the country, while PSTv-39 and PSTv-47 were almost restricted in the western U.S. Virulence frequencies to Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrTr1, and YrExp2 were high (88.7–98.0%); and to Yr1, Yr10, Yr24, Yr32, YrSP, and Yr76 were low (6.0–24.3%). No virulence was detected to either Yr5 or Yr15. The results are useful for breeding resistant cultivars and control of stripe rust.

Virulence enhancement and registration of a bioherbicide for *Striga hermonthica* (witchweed): Flipping the traditional role of plant pathology

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Striga hermonthica (witchweed) is a critical obstacle for crop production on more than 40 million farms across Sub-Saharan Africa. *Striga* infestations reduce maize and sorghum yields by 20–80%. *Fusarium oxysporum* f. sp. *strigae* is a host specific pathogen of *Striga* with insufficient virulence to function as an efficacious bioherbicide. Its virulence can be enhanced considerably by selection of strains that excrete the amino acids L-leucine, and L-tyrosine. These amino acids inhibit amino acid biosynthesis in *Striga*, but not maize. Methionine is converted by soil microbes to ethylene, a seed germination stimulant. As the soil bank of seeds is problematic, excretion of methionine was selected to trigger germination. Amino acid excreting strains were field tested to control *Striga* with average maize yield increases on smallholder farms from 42 to 56%. In the control of *Striga*, the primary inoculum was produced on toothpicks (wooden dowels), hence the name, the Toothpick Project. These are delivered to farmer's groups to manufacture a village-level inoculum with cooked rice. This secondary inoculum is co-planted with the maize, resulting in reduced *Striga* counts and increased yield. Registration for commercial use of Kichawi Kill™ as a bioherbicide for *Striga* biocontrol was issued in Kenya in February 2021. *F. oxysporum* is a pathogen of numerous weeds, and most if not all plants can be inhibited by at least one amino acid. Therefore, the opportunity exists to use this virulence enhancement of host specific fungi as weed biocontrol agents.

Diverse viruses and *Hop latent viroid* are present in greenhouse-grown cannabis (*Cannabis sativa* L., marijuana) plants in Canada

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The diversity and prevalence of viruses and viroids in cannabis (*Cannabis sativa* L., marijuana) plants was assessed in 2020 and 2021. Samples (5,285 in total) submitted to two diagnostic labs by growers from nine Canadian provinces, and 85 samples collected from commercial producers, were assayed using ELISA and RT-PCR. Results revealed *Hop latent viroid* (HLVd) was present in 51.6% of samples from five provinces. Among six viruses detected, *Tobacco mosaic virus* was the most widespread, found in 4.8% of the samples from seven provinces. *Tomato bushy stunt virus* (TBSV) and *Tobacco streak virus* (TSV) were present in 2-2.3% of the samples from three provinces; these viruses represent first reports of infection on cannabis. *Alfalfa mosaic virus* (AMV) and *Arabidopsis mosaic virus* (ArMV) were present in just two samples from two provinces in 2021. *Lettuce chlorosis virus* was not detected. Symptoms of mosaic, yellowing, and line patterns were evident on some samples while the remainder were asymptomatic. Cuttings taken from symptomatic plants harbored the same viruses/viroid as the original source plant, determined by RT-PCR, and plants showed corresponding symptoms. Variability in virus detection was associated with leaf age, position of leaf on the plant, and handling and storage of leaves after collection. Viruses and HLVd appear to have been spreading during cycles of vegetative propagation of cannabis plants and have remained undetected until recently. Next generation sequencing may identify additional viruses, viroids, or phytoplasmas not reported here.

Emerging plant diseases threaten global food security

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Plant disease outbreaks are increasing and threaten food security for the vulnerable in many areas of the world. The United Nations declared 2020 the International Year of Plant Health. Now a global human pandemic is threatening the health of millions on our planet. A stable, nutritious food supply will be needed to lift people out of poverty and improve health outcomes due to the COVID-19 pandemic. Plant diseases are spreading and exacerbated by climate change, transmission with global food trade networks, pathogen spillover, and evolution of new pathogen lineages. Emerging plant diseases threaten many food crops including coffee, cassava, wheat, banana, and potatoes. Plant pathogens cause global losses estimated to be as high as \$33 billion per year. The risk of introduction of pathogens through trade requires continued monitoring and improved diagnostic capabilities at our borders. Faster and more reliable methods of detection could greatly reduce this cost by reducing time to detection and thus time to action. In order to tackle these challenges, a new set of tools are needed that include global disease surveillance, improved detection technologies that include sensors, rapid pathogen genotyping, data mining and risk modeling to prevent future outbreaks. An ambitious research agenda that helps mitigate plant disease pandemics must include collaboration of landscape ecologists, bioinformaticians, engineers, and plant pathologists. Examples of tools and their use for risk assessment will be discussed.

Evolution and regulation of a large effector family of *Pyricularia oryzae*

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We previously identified a gene encoding a suppressor of plant cell death in *Pyricularia oryzae* (Syn. *Magnaporthe oryzae*). Here we report the gene is one of a 21-member gene family and we characterize sequence diversity in different populations. Within the rice pathogen population, nucleotide diversity is low, however, the majority of gene family members display presence-absence polymorphism or other null alleles. Gene family allelic diversity is greater between host-adapted populations and we thus named them host adapted genes (*HAGs*). Multiple copies of *HAGs* were found in some genome assemblies and sequence divergence between the alleles in two cases suggested they were the result of repeat-induced point mutagenesis. Transfer of family members between populations and novel *HAG* haplotypes resulting from apparent recombination were observed. *HAG* family transcripts were induced in planta and a subset of *HAGs* are dependent on a key regulator of pathogenesis, *PMK1*. We also found differential intron splicing for some *HAGs* that would prevent ex planta protein expression. For some genes, spliced transcript was expressed in anti-phase with an overlapping antisense transcript. Characterization of *HAG* expression patterns and allelic diversity reveal novel mechanisms for *HAG*

regulation and mechanisms generating sequence diversity and novel allele combinations. This evidence of strong in planta-specific expression and selection operating on the *HAG* family is suggestive of a role in parasitism.

Comparative analyses of effector and CAZyme profiles in binucleate *Rhizoctonia* isolates

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Binucleate *Rhizoctonia* isolates belong to the family Ceratobasidiaceae and can be pathogenic in crops of economic importance or saprobes and mycorrhizal symbionts associated with orchids. Isolates within the two major classification groups, multinucleate and binucleate, differ in genome sizes, host specificity, and number of secreted proteins, suggesting differences in pathogenesis or plant associations. We present the comparative analyses of the profile of secreted effector proteins and carbohydrate active enzymes of binucleate *Rhizoctonia* isolates collected from horticultural and field crops. Our aim is to investigate the interactions of these isolates with different hosts and understand their pathogenicity mechanisms. Based on draft genomes assembled de novo from Nanopore sequences, proteins were predicted and functionally annotated. This was followed by the prediction of the secretome based on the presence of signal peptides, presence or absence of transmembrane domains, absence of mitochondrial target sequences, and identification of domains of interest. Between 7.8 and 9.2% of the proteins are secreted by our tested isolates, which corresponds to around 1,600 proteins, and from these potential apoplastic and cytoplasmic effectors were identified. CAZymes were directly predicted from the proteome based on homology search. Enrichment of genes belonging to the glycoside hydrolases family was observed in all isolates. These results contribute to the understanding of host range and virulence differences between isolates within the *Rhizoctonia* species complex.

Exploring ultraviolet C irradiation as a control for oomycete pathogens of stored potato

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Storage diseases of potato are notoriously difficult to control and can cause substantial loss of raw product. Control methods for most storage diseases are limited to a few fungicides applied directly to the tubers post-harvest. For nearly a century the germicidal effect of Ultraviolet C (UVC) irradiation has been well known. The advent of new UVC-emitting technologies, along with a reduction in cost, has led to the adoption of UVC as a sanitizing step in many food production industries. UVC has not yet been implemented in potato production. An initial screen of potato pathogens for susceptibility to UVC irradiation in vitro showed a trend of non-pigmented pathogens exhibiting greater sensitivity to the irradiation. Oomycete pathogens, in particular, were most sensitive. Storage trials were conducted with Russet Burbank, Dark Red Norland, and Snowden varieties to evaluate UVC irradiation in limiting disease on tubers post inoculation. Tubers inoculated with *Phytophthora erythroseptica*, and then irradiated with UVC resulted in a nearly 50% decrease in disease incidence compared to the untreated control. Trials are underway to evaluate efficacy against the oomycetes *P. infestans* and *Pythium ultimum*. Trials were also conducted to evaluate the impact of UVC irradiation on tuber physiology. Metrics including sugar content, fry color, greening, and germination rate were evaluated with no apparent differences between UVC irradiated and non-treated controls.

Using RNA interference to control dollar spot disease caused by *Clariireedia jacksonii* on creeping bentgrass

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Dollar spot is the most common turfgrass disease and is caused by the fungus *Clariireedia jacksonii*. Fungicides are widely used for dollar spot suppression but there is increasing interest in alternative management strategies due to concerns over fungicide cost, development of fungicide resistance, and environmental contamination. RNA interference (RNAi) is an alternative and novel management strategy that has been used to control plant pathogens in recent years by silencing virulence genes essential for oxalic acid production, chitin synthesis, and programmed cell death. However, RNAi has not been researched on dollar spot disease and there is limited research on the role of oxalic acid in *C. jacksonii* pathogenicity and virulence. We hypothesize that there are one or more virulence genes in *C. jacksonii* that can be targeted using exogenously applied RNAi applications and suppress dollar spot development. To test this hypothesis, we will synthesize RNA to silence oxalic acid production, chitin synthase, and programmed cell death genes in *C. jacksonii*. The efficacy of the gene-silencing products will then be tested in vitro and as an exogenous application on creeping bentgrass grown in the growth chamber. The optimal rates and reapplication intervals using RNAi products for dollar spot control will be assessed in the field following optimization in the laboratory and growth chamber. Using RNAi to silence virulence genes of *C. jacksonii* is expected to provide novel dollar spot management strategies on turfgrass and reduce fungicide usage in the future.

Characterization of *Sclerotinia sclerotiorum* from U.S. soybean and dry bean and from different regions and climates using AmpSeq

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Sclerotinia sclerotiorum is mostly clonal, however recombination may increase in warm and humid climates, like Mexico, where distinct populations were identified previously. To investigate these differences further, we used a hierarchical sampling of 183 *S.*

sclerotiorum isolates from dry bean and soybean. An AmpSeq primer array was developed to amplify and sequence variants in SSRs, genes conferring fungicide resistance, SNPs, and putative virulence loci. Included were all previously developed primers for SSR loci, which produced amplicons <500 bp. Primers for fungicide resistance developed previously were available for FRAC groups 1, 7, and 11. If amplicons were ≥500 bp, additional primers were designed via SnapGene. Polymorphic SNPs and virulence regions were identified *de novo* using whole-genome sequences of five *S. sclerotiorum* strains characterized previously. Primers were designed to produce amplicons <500 bp using FastPCR for multiplex PCR and any remaining primers the software could not design were created in Primer3Plus. Primers were validated using in silico PCR on the *S. sclerotiorum* reference genome. For PCR1 of AmpSeq, linkers were concatenated to the 5' end of the F and R primers and then synthesized in batch by IDT oPools. Following PCR1, amplicons will be sent to Cornell U. for PCR2 sample barcoding and sequencing. Raw sequence reads will be demultiplexed by HaplotypR and filtered using methods described previously. Variants will be analyzed using VcfR/poppR to determine differences and provide deeper insight into factors shaping *S. sclerotiorum* populations.

Unique group of *Xanthomonas perforans* isolated from pepper in Taiwan

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Xanthomonas perforans (Xp), *X. euvesicatoria* (Xe), *X. gardneri*, and *X. vesicatoria* (Xv) are bacterial pathogens that cause bacterial leaf spot in pepper (BSP) and tomato (BST). Taiwan has 2,497 ha of pepper production. Population shifts in xanthomonads associated with BST have been reported in Taiwan in recent decades. In Taiwan, from 1989 to 1999, 5% of BST strains were identified as Xp, whereas between 2010 and 2016, this shifted to 99%. However, BSP strains, from 2010 to 2016, only 8% were Xp. Given that population shifts have been reported on tomato in Taiwan and that BST and BSP xanthomonads are known to develop resistance to two important bactericides used in management, streptomycin and copper, continued monitoring of the populations for population shifts, copper and antibiotic tolerance is critical in devising appropriate management strategies. In this study, we conducted whole genome sequencing of 84 xanthomonads isolated, from pepper over years (1989 to 2019) across Taiwan for population study. This study showed that in case of xanthomonads isolated from BSP in 2007 ($n = 1$) 100%, 2016 ($n = 1$) 11.7%, 2019 ($n = 23$), 34% were Xp, indicating a possible shift in xanthomonad population in pepper as well. Phylogenetic analysis using core genomes of 10 Xp strains revealed a distinct group of Xp, closer to a unique BST strain (NI1) isolated from Nigeria. NI1 was previously determined to be a novel lineage of strains associated with recombination. NMDS analysis of the pangenome of all the Xp strains, strongly supported the closeness of these unique Taiwan strains to NI1.

Seasonal dynamics of conidia production of *Venturia effusa* from lesions of scab on pecan shoots

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Scab (caused by *Venturia effusa*) is the most important disease of pecan in the southeastern U.S. The role and characteristics of conidia production from lesions on the shoots is poorly understood but may be important to infection of young leaves and fruit early in the season. The objective was to characterize the seasonal trends of spore production from scab lesions on pecan shoots. The experiment was conducted on cvs. Wichita, Cherokee, and Apache in orchards in Georgia, the U.S. Samples of shoots with scab lesions developing each season were collected every 2 weeks for 12-month periods starting in late May-June of each season from 2018 through 2020. The number of lesions/shoot section varied with sample (14.2 to 861.0 lesions/section) but conidia production showed a similar pattern each season on all cultivars with most conidia per lesion produced on new, young lesions formed from late June to September (up to 31,690 conidia/lesion, depending on season and cultivar). Generally fewer conidia were produced from October to May (up to 4,384 conidia/lesion, depending on season and cultivar, but most often much less). There was evidence of a slight increase in spore production on the previous season's shoot lesions in the spring (March-April) coinciding with the period of pecan bud break and leaf development, but the increase was small. These results provide further evidence for a role of conidia produced from overwintering shoot lesions as a source of infection for newly emerged foliage early in the season.

Cell wall degrading enzymes associated with *Rhizoctonia solani* can increase sugar beet root damage in the presence of *Leuconostoc mesenteroides*

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Rhizoctonia solani mediated crown and root rot in sugar beet is a major yield constraint in the U.S. and worldwide. The overall damage of sugar beet roots is significantly increased when *R. solani* AG2-2 IIIB is in close contact with the bacterium *Leuconostoc mesenteroides* in comparison to either alone. We hypothesized that the lack of plant cell wall degrading enzymes in *L. mesenteroides* and provision of such enzymes by *R. solani* when closely associated, is the major cause of increased root damage. In planta sugar beet root inoculation with or without the presence of cell wall degrading enzymes revealed greater ($P < 0.0001$) rot when *L. mesenteroides* was combined with cell wall degrading enzymes such as cellulase (49 mm of rot), polygalacturonase (48 mm), and pectin lyase (35 mm) versus these enzymes (0–11 mm), *R. solani* (13 mm), and *L. mesenteroides* (22 mm) individually. The enzyme data was further supported by carbohydrate analysis of the infected root samples. Higher degradation of complex cell wall carbohydrates was reflected by the increase in simpler sugars such as glucose + galactose (407–1,033%), and fructose (161–411%) in the *R. solani* + *L. mesenteroides* infected roots in comparison to mock control or *L. mesenteroides* only infected samples. Gene expression data in *R. solani* when interacting with *L. mesenteroides*, corroborated well with the enzyme and carbohydrate studies. The data suggest future

targeting of *R. solani* cell wall degrading enzymes could be an effective strategy to mitigate sugar beet root damage during its interaction with *L. mesenteroides*.

Genetic and genomic analysis of race determination and resistance to *Verticillium dahliae* in tomato

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Host resistance is the principal strategy to manage *Verticillium dahliae* of tomatoes. Two effectors that condition host resistance to Verticillium wilt, Ave1 (race 1) and Av2 (race 2), have been characterized. While the race 1 resistance gene has been characterized, the race 2 resistance gene is unknown. Race 2 resistance appears to be controlled by a single dominant locus. F2 analysis of 3 tomato hybrid rootstocks resistant to race 2, Aibou, Bowman, and Ganbarune-Karis, yielded a 3:1 ratio of resistant to susceptible progeny. Subsequent analysis of F3 and F4 progeny confirmed the single dominant nature of the race 2 resistance gene. Whole genome sequence analysis of race 1, 2, and 3 *V. dahliae* isolates ($n = 18$) from tomatoes in California, North Carolina in the USA, and different prefectures in Japan revealed four highly clonal phylogenetically distinct lineages. Loss of the race 1 and 2 effectors resulted from segment deletions from chromosomes 5 and 4, respectively. Within lineage group 4, the loss of the race 1 effector appears to have been followed by sequential loss of the race 2 effector. In contrast, isolates in group 1 have lost the race 2 effector, but not the race 1 effector, indicating separate race 2 effector deletion events. Further analysis of these regions revealed higher levels of chromosomal rearrangement than non-race effector containing regions. These data indicate that race determinant genes are highly prone to chromosomal restructuring that drastically impact host-pathogen evolution.

Characterization of North Dakota spring wheat germplasm for resistance to rust diseases caused by *Puccinia* species

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Puccinia graminis f. sp. *tritici* (*Pgt*) and *P. striiformis* f. sp. *tritici* (*Pst*), causal agents of stem and stripe rust, respectively, are devastating fungal pathogens that cause severe yield losses in wheat (*Triticum aestivum* L.). Although stripe rust incidence is low in North Dakota, *Pst* isolates that can survive at higher temperature have been reported in the United States. To identify new and existing sources of resistance against rust diseases, ~400 North Dakota hard red spring wheat germplasm and breeding lines were phenotypically characterized for adult plant and seedling responses to *Pgt* and *Pst*. The seedling screening was conducted at the 2-leaf stage with different *Pgt* and *Pst* races that are common in Northern Great Plains. Seedling data suggest that most of the tested germplasm is resistant (67%) to *Pgt* but 48% of the entries susceptible to *Pst*. Field trials are being established at different locations in North Dakota and Washington to evaluate adult plant resistance to stripe rust. These lines were genotyped by wheat 90K SNP bead chip array for genome-wide association studies (GWAS). Genomic locations associated with resistance to rust diseases will be identified using the association genetics approach. The current study will provide a road map of existing and new sources of rust resistance present in ND spring wheat germplasm. Results of phenotypic evaluations and GWAS analysis will be presented.

Screening *Cucurbita* germplasm for resistance to *Xanthomonas cucurbitae*, incitant of cucurbit bacterial spot

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Bacterial spot of cucurbits, caused by *Xanthomonas cucurbitae*, is an emerging disease of cucurbits. This study was conducted to identify *Cucurbita* spp. resistant to *X. cucurbitae*. First, we developed a reliable method for inoculating plants with *X. cucurbitae*. Then, we evaluated *X. cucurbitae* isolates collected from Illinois, Michigan, Kansas, Ohio, and Wisconsin to determine the most virulent isolates for screening cucurbit cultivars and accessions. Finally, we screened 81 commercial cultivars of gourds, pumpkins, and squashes, and 300 cucurbit accessions for their resistance to *X. cucurbitae* in greenhouse and field conditions. In the greenhouse study, all commercial cultivars and most of the accessions developed typical symptoms of the bacterial spot disease, while some of the accessions either did not develop symptoms of the disease or severity of the symptoms was low. In the field studies, infection of leaves and fruits was caused by both natural inoculum and spray-inoculation of plants with a suspension of the *X. cucurbitae* isolate from Illinois. All pumpkin cultivars developed the disease. Among 300 accessions tested, 9 and 21 accessions were classified as resistant and less resistant to *X. cucurbitae*, respectively. Resistant and less resistant accessions belong to the species *Cucurbita maxima*, *C. maxima* subsp. *maxima*, *C. maxima* subsp. *andreana*, and *C. okechobeensis* subsp. *martinezii*. This is the first report of potential resistance of cucurbits to *X. cucurbitae*.

The effect of tissue type and fungicides on the fruit microbiome of highbush blueberry

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In highbush blueberry (*Vaccinium corymbosum*), post-harvest rots are caused by fungi that can trigger losses of greater than 90% post-harvest. These rots are typically managed by use of fungicides. Understanding the microbiome of the fruit surface and how it may be altered by fungicide use may provide insights into post-harvest rot management. Five fungicide spray programs were evaluated for their influence on the fungal microbiome of blueberry fruit. Two programs used biological fungicides, two used azoxystrobin, and one used a series of sprays to simulate a commercially standard spray program. The microbiome of the fruit pulp was much more variable than the skin microbiome. Fungicide use had a strong impact on the fruit skin microbiome and a moderate impact on the pulp microbiome ($R^2 = 0.493$ and 0.207 respectively, $P < 0.001$). Compared to the untreated controls, each fungicide spray program had a strong and significant effect on the microbiome of the fruit skin ($R^2 = 0.59–0.67$, $P < 0.001$). In the pulp, three of the treatments had moderate effects in

comparison to the control ($R^2 = 0.14\text{--}0.18$, $P < 0.001$). The untreated controls had a high prevalence of *Peltaster fructicola*, which was absent in most treated samples. Species within the genus *Sporobolomyces* were abundant across treatments. There was a high prevalence of the genus *Alternaria*, a post-harvest rot, regardless of treatment. Also, *Botrytis cinerea* populations were enriched in the pulp of fruits treated with synthetic fungicides, highlighting the need for further understanding of fungicide resistance in agricultural microbiomes.

Seedling reaction to leaf rust in spelt wheat (*Triticum spelta* L.) collection

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Spelt wheat (*Triticum spelta* L.) is an ancient cereal that is gaining small grain growers' attention under an organic setup due to its high protein content, high fiber, and other health benefits. Spelt wheat, like common wheat, also succumbs to leaf diseases, including leaf rust. Leaf rust is a significant yield-limiting disease of wheat worldwide, especially in the U.S. Great Plains region. Identification of disease resistant spelt genotypes could be valuable for spelt production under organic management. In this study, 108 spelt accessions (National Small Grain Collection) from 20 countries were screened against leaf rust at the seedling stage in the greenhouse. The genotypes exhibited varying response ranging from susceptible ($n = 49$), to moderately susceptible (39), moderately resistant (17), and resistant (3). All 108 genotypes were also characterized for tan spot (race 1 and race 5) and spot blotch. Spelt accessions resistant to leaf rust and leaf spots will be further evaluated for the agronomic traits in the field and results will be shared with the growers to assist in selection of superior spelt genotypes.

Root-knot nematode survey of Asian vegetable farms in Hillsborough County, Florida

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Root-knot nematodes (RKNs) are the most economically important plant-parasitic nematodes with broad host ranges that include many Asian vegetables. Sixteen RKN species have been reported in the Florida. There are over 40 Asian vegetables grown on more than 4,000 ha in Florida and the industry is growing rapidly. No information on RKNs is available from Asian vegetables in Florida, and most growers are unaware of their existence. During June 2020–January 2021, a RKN survey was conducted on seven Asian vegetable farms in Hillsborough County, FL. RKNs were present on all farms. Severely galled roots were found on luffa, sweet potato, basil, pumpkin, bitter melon, Malabar spinach, amaranth, perilla, jute, golden egg, and Indian cream cobra melon. RKN species were identified through primers TRNAH/MRH106 and MORF/MTHIS, followed by restricting PCR products of TRNAH/MRH106 using *HinfI* and *MnII* enzymes. The RKN species were confirmed by species-specific primers Far/Rar, Me-F/Me-R, and MI-F/MI-R. Four RKN species were found, *Meloidogyne arenaria*, *M. enterolobii*, *M. haplanaria*, and *M. incognita*. *M. enterolobii* was found in six out of seven farms on sweet potato, Thai basil, pumpkin, luffa, jute, Malabar spinach, golden egg and perilla. Our survey indicated that RKNs are likely cause significant yield loss in Asian vegetable production in Florida. Especially the concern of high prevalence of *M. enterolobii*, as it breaks down the RKN-resistance of several crops, and is currently considered a major threat to the sweetpotato and vegetable industry in the southeastern U.S.

A transcriptomic analysis of wheat lines that are resistant and susceptible to tan spot (*Pyrenophora tritici-repentis*) disease

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Tan spot (TS), causal agent *Pyrenophora tritici-repentis* (*Ptr*), is a major threat to wheat production due to the lack of resistant cultivars. In our previous work, we identified MAGIC population parental lines exhibiting TS resistance and susceptibility, namely Robigus and Hereward, respectively. To understand the mechanisms underlying these phenotypes, we performed RNA-seq analysis of leaves before and during *Ptr* interaction. Principal component analysis suggested that infection of Hereward resulted in major shifts in the transcriptome but only minor changes were observed in the resistant line. When comparing mock- and *Ptr*-inoculated samples, differentially expressed genes (DEGs) were identified with DESeq2, leading to the targeting of 15,193 DEGs. Functional annotation showed the pathways enzyme classification, solute transport, RNA biosynthesis, protein modification and homeostasis represented 49.5% of DEGs in Robigus. Cellular metabolism pathways were induced, as well as vesicle trafficking, actin polymerization and cellulose. The upregulation of these cell wall related genes along with microscopic data suggested that barrier defense is a major feature of TS resistance in Robigus. Conversely, photosynthesis was the top fifth pathway in Hereward, totaling 389 repressed genes (12.63%). Photosynthesis collapse was linked to the activation of oligosaccharide metabolism and suppression of glycolysis, TCA cycle and amino acids degradation. This may reflect mobilization of host nutrients to *Ptr*. Our observations could inform wheat-breeding programs targeting TS resistance.

Two-year survey of *Diaporthe* species in soybean across Delaware and Maryland

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The *Diaporthe* disease complex in soybean (*Glycine max* (L.) Merrill) can cause significant quality and yield losses. Soybean diseases caused by the multiple *Diaporthe* species involved include stem canker, pod and stem blight, and Phomopsis/Diaporthe seed decay. Surveys were conducted across Delaware and Maryland in 2019 and 2020 to determine which species are most prevalent in the region. Survey locations included 60 fields in 2019 and 45 in 2020. Symptomatic plants identified through field scouting were brought back to the lab for fungal isolation from stem pieces. Four *Diaporthe* species, *D. longicolla*, *D. aspalathi*, *D. sojae*, and *D. ueckerae*, were identified across the two seasons. Among the 24 *Diaporthe* isolates collected in 2019, 88% were *D. longicolla*, 8% *D. aspalathi*, and

4% *D. ueckerae*. Environmental conditions were highly conducive for *Diaporthe* infection in 2020, with 175 isolates recovered. In 2020, 98% of isolates were identified as *D. longicolla*, 1% *D. ueckerae*, and 1% *D. sojae*. Although *D. longicolla* is the causal agent of Phomopsis/Diaporthe seed decay, all isolates in this survey originated from cankered or blighted stem tissues, indicating its role in soybean stem diseases prior to seed decay and confirming association of this species with stem zone lines in the Mid Atlantic. *D. ueckerae* was recently reported on soybean in the U.S. for the first time and this survey documented the first identification of this species in MD.

Biofilm formation in the filamentous fungal plant pathogen *Fusarium graminearum*

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Biofilms are known to play important roles in bacterial pathogens of plants and animals, where the formations help protect cells from defense responses and antimicrobial treatments. Although biofilms in bacterial plant pathogens are well studied, their role in filamentous fungal plant pathogens is virtually unexplored. *Fusarium graminearum* is a filamentous fungal pathogen that causes Fusarium head blight (FHB) in cereal crops, which can result in devastating crop losses. We are characterizing the formation of biofilms in *Fusarium graminearum* in vitro, which provides a basis for moving this work to plants. We hypothesize that biofilms impact many steps in the disease cycle of FHB, from initial plant infection, to colonization, to overwintering on crop residues in the field. *F. graminearum* grows as fluffy hyphal colonies on most media; however, we observed an altered morphology, with predominantly short, bulbous hyphae under some conditions. We have mapped the full development of *F. graminearum* biofilms in vitro, adhered to a polystyrene surface, from the initial adhesion to dispersal of propagules and senescence of biofilms, and are investigating the conditions that promote biofilm formation. Additionally, we have identified components of the complex extracellular polymeric matrix, which is an important protective component of biofilms. From this work, we hope to provide a better understanding of the disease cycle of *F. graminearum*.

Report of *Mycoleptodiscus terrestris* as a new soybean root rot pathogen in Indiana

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Screenings of soybean (*Glycine max* L.) seedlings grown at Purdue's ACRE farm were conducted in the summers of 2019 and 2020. Four isolates of *Mycoleptodiscus terrestris* (Gerd.) Ostaz. were recovered from seedlings showing reddish-brown girdled lesions on the crown, root rot, and discolored vascular tissue. Fungal cultures were obtained from surface sterilized root tissue on water agar then semi-selective media and purified by hyphal tipping. Morphological examinations of the cultures were consistent with species descriptions of *M. terrestris*. The ITS region and TEF1- α gene were sequenced and matched 99–100% to accessions of *M. terrestris* in GenBank. To complete Koch's postulates, modified cotton seed meal broth was inoculated with plugs of a 7-day old isolate on PDA or sterile plugs of PDA and incubated for 2 weeks. Soybean seeds (cv. Williams) were grown for 1 week before being inoculated by root dip in each treatment for 30 min and replanted under indoor light racks. After 7 days, all seedlings dipped in the infested broth showed symptoms of root rot consistent with field symptoms while controls were healthy. *M. terrestris* was recovered from infected plants but not controls and identified as above. This is the first report of *M. terrestris* infecting soybean in the state of Indiana. *M. terrestris* has been studied and developed for biocontrol of invasive aquatic weeds; however, introduction of this pathogen into soybean production areas may increase disease risk.

Phylogenetic characterization and genealogy of strains in the *Ralstonia solanacearum* species complex associated with ironwood decline in Guam

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The bacterial wilt pathogen, *Ralstonia solanacearum* (*Rs*) was first detected in wood drill shavings from declining ironwood trees of Guam in 2010. Extensive surveys in 2015 and 2019 confirmed the island-wide presence of *Rs* associated with ironwood. Whereas a majority of ironwood *Rs* strains were later reclassified as Phylotype I, *R. pseudosolanacearum* (*Rps*), two strains were Phylotype II and later confirmed to be *Rs* through *dnaA* gene sequences. Carbon source utilization and chemical sensitivity patterns for all ironwood *Rps* strains were similar. The genetic relatedness between the Guam ironwood strains and other *Ralstonia* strains worldwide was examined in the effort to trace their ancestral genotypes. Three housekeeping and two virulence-related genes were used to infer the diversity, evolutionary relationships, and genealogy of representative ironwood strains. The *Rps* population in Guam was considered to be highly clonal based on low nucleotide diversity and lack of any significant evidence of recombination in individual gene sequences. Our analysis showed that the potential origin of ironwood Phylotype I *Rps* strains was in the geographical region of northern and eastern Asia, Indonesia, or northern Australia, whereas the geographical region around Central America, southeastern USA, northern Latin America, and Caribbean may be the potential origin of Phylotype II *Rs* strains. The predominant ironwood *Rps* population in Guam recently diverged from a common ancestor as shown by a Timetree analysis.

Evaluation of cover crops in reducing *Spongopora subterranea* inoculum

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Spongopora subterranea is a soilborne obligate biotrophic plasmodiophorid that causes powdery scab on potato tubers and gall formation on roots. Powdery scab tuber lesions are filled with resting spores (sporosori) and reduce potato quality and marketability. *S. subterranea* also vectors Potato mop-top virus, which causes necrotic arcs in tuber flesh. Currently, there are no effective chemical

methods to manage *S. subterranea*, which has a wide host range in addition to potato. However, cover crops may be a useful management tool for this pathogen. To determine whether cover crops may alter *S. subterranea* population levels in the soil, 18 cover crops, including legumes, grains, and mustards were assessed. For each species, five plants were grown in individual pot inoculated with 10 sporosori/g of potting mix and compared with five plants grown in non-inoculated potting mix. After harvest, plant roots were stained using trypan blue and examined by microscopy. *S. subterranea* level in the potting mix was determined with qPCR. Plasmodium-like structures were present in roots of all plants species tested, but sporosori were only observed in potato. The pathogen appeared unable to complete its life cycle on 12 of 18 cover crops based on low post-harvest *S. subterranea* levels. A follow-up experiment was conducted by inoculating the potting mix with 40 sporosori/g to better detect the pathogen. The results from this experiment will be used to guide in-field cover crop experiments and to advise farmers on cover crops that may reduce the inoculum levels of *S. subterranea* in the soil.

Occurrence of *Pythium myriotylum* and *Globisporangium* spp. on potted hydrangea with different susceptibility among the host plant cultivars

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Potted hydrangea (*Hydrangea macrophylla*) is economically important with sales reaching 1,773 million JPY (2,284 million potted plants) through 2019 in Japan. New high value-added cultivars have also increased farmers' profits recent years. Since 2014, severe stem and root rot has been found on potted rooted cuttings of hydrangea grown in commercial greenhouses in Shimane Prefecture, Japan. Water-soaked and dark-brown lesions initially appeared at the base of stems of the young plants. The symptoms gradually enlarged upward and whole plants were killed within 3 weeks. Severe discoloration and abscission of leaves was followed by plant death. *Pythium* and *Globisporangium* spp. that were consistently isolated from the plants were identified as *Pythium myriotylum*, *G. splendens*, *G. spinosum*, and an undescribed species of *Globisporangium* based on morphological analysis and rDNA-internal transcribed spacer and *cox1* gene sequences. All four species reproduced stem and root rot on inoculated potted hydrangea. Plant cultivars significantly differed in their susceptibility to the pathogens. The cv. Mangekyo was the most susceptible to all the pathogens. Mangekyo makes up more than half of the potted hydrangea production in Shimane Prefecture, so it is important to the local economy. Pathological characteristics and control methods of the causal agents will be discussed.

Development of race specific primers to molecularly distinguish between race 0 and race 1 of *Phytophthora nicotianae*

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Phytophthora nicotianae is a polycyclic hemibiotroph and is the causal agent of black shank disease in tobacco. Presently, it is not possible to distinguish between races of *P. nicotianae* in situ, and current diagnostic methods for race determination are time consuming and require greenhouse screening assays with varieties of tobacco containing known resistance genes. The goal of this research is to develop a race specific molecular assay to save researchers and growers time, money, and resources. The genomes of representative *P. nicotianae* isolates from race 0 and race 1 populations were sequenced, annotated, and aligned. RNA for five representative isolates of each race were sequenced to aid in genome alignment and annotation. Diagnostic PCR primers will be designed from the annotated genomes and transcriptomes to distinguish between race 0 and race 1 by utilizing differential SNPs. Primers will be tested from known race 0 and race 1 isolates from North Carolina. An updated survey of race structure in North Carolina will be conducted utilizing the developed primer sets.

Role of the soybean ionome in cultivar resistance to *Cercospora* leaf blight

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Cercospora leaf blight (CLB) of soybean, caused predominantly by *Cercospora* cf. *flagellaris* and *C. cf. sigesbeckiae*, has been a major production constraint in Louisiana and other states of the Gulf South. A toxin, cercosporin, produced by the pathogen is considered largely responsible for CLB symptoms. Commercial soybean cultivars with some CLB resistance are now available, although considerable symptom variation is observed across years and locations. Previous studies have indicated that certain mineral ions can affect cercosporin production and toxicity. In other plant diseases the host ionome (all mineral nutrients and trace elements of an organism) is known to affect disease resistance. We explored the importance of the soybean ionome in CLB susceptibility and resistance. Leaves from twenty-five soybean cultivars resistant, intermediate, and susceptible to CLB were collected from two locations in Louisiana and subjected to nutrient analysis (to determine the concentration of 14 elements). Compositional data analysis indicated that Ca, K, Mg, and N were the major determinants of ionome variability across cultivars. The resistant cultivars appeared to have higher ratios of Al to other elements compared to susceptible and intermediate varieties. Cercosporin concentration will also be assessed in these cultivars to correlate toxin concentration with resistance. The study will be repeated in 2021 and ionomics will be explored as a novel tool for resistance screening and nutrient management against CLB.

Identification and pathogenic potential of fungi associated with stored potato minitubers

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The potato minituber production process allows colonization by fungi with potential to influence the tuber's overall health and microbiome as well as that of daughter plants. We hypothesized that specific minituber-associated fungi can significantly impact plant health and sought to identify those fungi most commonly isolated from stored minitubers. To isolate and identify the fungi, sections of surface sterilized tubers of six commercial potato varieties were plated on antibiotic amended PDA and hyphal tips were subcultured until pure cultures were obtained. The fungi were categorized into morphological groups and Sanger sequencing was performed. Based on morphological identification, several morphotypes were present in multiple tuber varieties but none were present in all six. Further, each variety hosted a unique fungal assemblage. These results indicate a strong variety-specific effect on minituber fungal community despite common growth and storage conditions. In subsequent studies, the fungi will be inoculated onto sliced potato tubers to determine their pathogenic potential. This study provides us with important insights on the fungi associated with stored potato minitubers, their variety specificity, and whether these fungi are of agronomic concern for sustainable potato production.

The color of the light at the end of the tunnel: TaqMan probes reveal molecular detection test results for thousand cankers disease

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Thousand canker disease (TCD), a complex disease system, which affects walnut, butternut, and wingnut trees, is caused by the fungal pathogen *Geosmithia morbida* that is vectored by the bark beetle *Pityophthorus juglandis*. TCD can spread rapidly, resulting in widespread tree decline and death and severe economic and ecological losses. Traditional detection methods using fungal isolation can take weeks to confirm presence and identity of the pathogen. Our group previously developed a molecular detection tool to confirm the presence of the pathogen and/or vector, yet this method required technical competency, specialized laboratory equipment, and was costly. This report explains our use of TaqMan technology to develop a quick, inexpensive method to detect and confirm TCD infection. The improved protocol uses TaqMan probes that are designed within the flanking regions of microsatellite loci to signal both pathogen and vector presence. We simplified test interpretation by using a conventional gel and a blue light (440–460 nm) flashlight to visualize DNA presence/absence results in infected host plant tissue samples. Our tested visualization methods detected DNA from both TCD-associated fungal and insect samples. Regulatory agents and diagnostic laboratories that adopt this rapid detection method can reduce time needed to make regulatory decisions and restrict movement of infested plant material. Our methodology is also being adapted for detection of other tree diseases in complex host/vector systems.

Using spore traps in conjunction with unmanned aerial vehicles for the detection of spores associated with plant disease

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Many important sugar beet foliar crop pathogens such as *Alternaria alternata*, *Botrytis cinerea*, and *Erysiphe betae* are dispersed via airborne spores. In Idaho, these pathogens are typically managed on calendar-based schedules. Fungicide timing can be optimized if growers know when spores are present. The aim of this study was to evaluate different spore trapping technologies to determine which are most suited to the detection of *A. alternata*, *B. cinerea*, and *E. betae* spores. Two types of trap were evaluated in the study, a ground-based Burkard multi-vial cyclone sampler and the Sporenado, which was used at both ground level and attached to a Multicopter UAV (DJI M600) or DJI Phantom 4. Flights of 3 min each were conducted at altitudes of either 5 or 25 m above a sugar beet field, replicated three times, while spore trapping was conducted at ground level simultaneously using both types of spore traps. DNA was extracted from the spore material using the Wizard® Magnetic DNA Purification System for Food in conjunction with a KingFisher ML and species-specific real-time PCR assays were used to detect the DNA of each pathogen. On a per minute basis, detection using the Sporenado at either height was significantly more efficient than ground-based spore trapping. However, sampling over longer time periods at ground level allowed for significantly more robust detection of all spores. Trapping technologies used in conjunction with a sensitive real-time PCR assays is a useful tool for the early detection of pathogens prior to the observation of visual symptoms in the field.

Role of small RNA in the development and cross-kingdom interaction of *Diaphorina citri* with its endosymbionts and *Candidatus Liberibacter asiaticus*

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Candidatus Liberibacter asiaticus (CLAs), the causal agent of citrus greening, inhabits a complicated niche in its vector, *Diaphorina citri*. CLAs transmission relies on interactions with both *D. citri* and the vector's bacterial endosymbionts. Small non-coding (nc) RNAs regulate pathogen and endosymbiont interactions in insects. To study the role of small ncRNAs in the interaction between *D. citri* and its microbial population, we sequenced the small ncRNAs from CLAs-/+ nymph and adult *D. citri*. We identified 52 novel candidate *D. citri* micro (mi) RNAs. Statistical analysis showed a total of 17 miRNAs were differentially expressed between CLAs-nymph vs CLAs-adults. Of those, just five miRNAs were changed between CLAs+ nymph vs CLAs+ adults showing CLAs infection may have an impact on the expression of miRNAs associated with *D. citri* development. Expression of four miRNAs were significantly changed between CLAs- and CLAs+ adult *D. citri*, while just one miRNA was significantly downregulated in CLAs+ nymphs as compared to CLAs-

nymphs. We found the let-7 miRNA just in nymphs, a well-known miRNA key to development of *Drosophila* and nematodes, suggesting *D. citri* may use the let-7-dependent pathway for its development. We found that 30–50% of the total ncRNAs aligned to tRNA of *Proffittella* (*D. citri* endosymbiont), suggesting that *Proffittella* may use tRNA derived fragments for the interaction with *D. citri*. We applied dual in silico target prediction and parallel transcriptome analysis to find the possible role of ncRNA in CLAs infection, development, and endosymbiosis.

Candidatus Liberibacter asiaticus-encoded BCP peroxiredoxin suppresses LPS-mediated defense signaling and nitrosative stress in planta

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The lipopolysaccharides (LPS) of Gram-negative bacteria are surface-exposed pathogen-associated molecular patterns (PAMPs) that trigger both nitrosative and oxidative bursts in animal and plant cells during host invasion. *Liberibacter crescens* (Lcr) is a nonpathogenic surrogate for functional genomic studies of the uncultured *Ca. L. asiaticus* (Las) associated with citrus greening disease. Pretreatment of tobacco leaves with purified Lcr LPS reduced growth of inoculated *Xanthomonas perforans* and abolished the hypersensitive response, thus confirming a role of Lcr LPS in activation of systemic acquired resistance (SAR). Lcr LPS also elicited rapid NO burst of in suspension cultured tobacco cells. Pharmacological inhibitor assays confirmed Arg-utilizing NO synthase activity as the primary source of NO generation elicited by Lcr LPS. LPS treatment resulted in biological markers of NO-mediated SAR activation, including an increase in the glutathione pool, callose deposition and activation of the salicylic acid and azelaic acid (AzA) signaling networks. Transient expression of Las BCP peroxiredoxin in tobacco compromised AzA signaling, a prerequisite for LPS-triggered SAR. Western blot analyses revealed that Las BCP peroxiredoxin prevented peroxynitrite-mediated tyrosine nitration in tobacco. Las BCP peroxiredoxin thus attenuates NO-mediated SAR signaling and scavenges peroxynitrite radicals, likely making it a critical effector needed for repetitive cycles of Las acquisition and transmission by fecund psyllids throughout the limited flush period in citrus hosts.

Opportunities in the field of plant diagnostics

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Like many other plant diagnostic laboratories, the Plant Diagnostic Clinic (PDC) at Colorado State University provides services beyond disease diagnostics and pathogen testing. Research, teaching, and outreach are also part of its mission. The PDC offers internships for graduate students in the M.S. in Pest Management program at CSU. In 2019–2020, student-led projects addressed needs of the PDC in pathogen detection, extension, and outreach. The first project improved the detection of *Pectobacterium parmentieri*, a bacterium that causes soft rot of potato. A species-specific, real-time PCR assay that detects *P. parmentieri* was developed and optimized. The second project addressed the need for updated hemp disease resources. We prepared an extension publication focused on *Fusarium* diseases on hemp, commonly found in samples submitted to the PDC. The third project aimed to improve communication with Hispanic communities in Colorado. Colorado census data were collected and analyzed to find relationships between population categories. The data analyses revealed important factors impacting access to resources. For example, we found that in counties where a higher percentage of the population spoke languages other than English, there were fewer households with at least one computer. These results will aid in developing a distribution plan for Spanish resources produced by the PDC and improve the impact of CSU extension. Training in a diagnostic laboratory can provide early career plant pathologists with experience and skills in all aspects of the land-grant mission.

A decision support simulation model for analyzing management of citrus Huanglongbing and its vector in Southern California

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Huanglongbing (HLB), spread by the Asian citrus psyllid (ACP), is a devastating disease affecting many citrus-producing regions worldwide. In Southern California, ACP populations have become increasingly established and more than 2,200 HLB+ trees have been detected and removed from residential properties. Moreover, ACP/HLB dynamics vary across California citrus landscapes requiring different strategies for sustainable, effective control and preserving commercial citrus. Therefore, we developed a spatially explicit agent-based model to investigate spread, evaluate epidemic risk, and analyze dynamic management programs on real-world citrus landscapes. Through scenario-based simulations, we investigated ACP/HLB mitigation performance in Southern California landscapes to guide spatiotemporal management strategies for cost-effective approaches as the epidemic evolves. Results indicate that existing strategies are not sufficiently effective at deterring further ACP in urban epicenters, but reductions in the delimitation response protocols are possible without significant losses in HLB mitigation efficacy. Additionally, smart risk-based surveillance strategies, insecticide treatments, and targeted management efforts including fast removal timeframes in residential buffers are critical strategies in delaying invasions of HLB into commercial citrus.

Field occurrence and overwintering of oospores of *Pseudoperonospora cubensis* in the eastern United States

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Cucurbit downy mildew caused by *Pseudoperonospora cubensis* is a devastating disease of cucurbits globally. In the US, the pathogen overwinters in areas below 30 degrees latitude where hosts are present year-round. These areas are thought to provide primary inoculum

for infection in areas that experience harsh winters. Oospore formation by isolates under controlled conditions has raised the possibility of oospores as an alternative source of initial inoculum. This study was conducted to provide a comprehensive assessment of occurrence of oospores in the field and document their survival during winter months in locations that experience harsh winters. About 1,600 diseased cucumber or cantaloupe leaves from fields in Rocky Mount, NC, and Charleston, SC, were collected in fall 2018, 2019, and 2020, and microscopically examined for oospores. About 5% of sampled leaves had oospores with a mean of 570 oospores per cm² of infected leaf. To generate oospores for survival assays, two isolates of opposite mating type were crossed on detached cucumber leaves and infested leaves were placed in the field in Rocky Mount, NC, in November of each year. Oospore survival was assessed monthly from January to end of April using the plasmolysis method. Survival oospores during winter decreased over time from 40% to 22% at the end of the monitoring period in April. Occurrence and survival of oospores in the field suggest that oospores could serve as a source of initial inoculum in areas that experience harsh winters in the US.

Geographic disease risk analysis for major crops of Central America and Mexico: Regional analyses and global structures

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The spread of emerging and re-emerging pathogens and pests is an important threat to crop production. The connectivity of croplands, the trade routes of crop commodities, and climatic suitability can facilitate the dispersal of diseases and pests. Integrating this data in geographic risk assessments can help to inform strategies to prevent epidemic invasion and saturation. Here, two geographic factors are analyzed, cropland connectivity and international trade, for 10 crops—avocado, banana, beans, cacao, coffee, maize, pepper, potato, tomato, and wheat—both globally and in a more detailed regional analysis of Central America and Mexico. Cropland and trade hubs (nodes connected to many other locations) and bridge nodes (connecting parts of the network that might otherwise be separated) are highlighted. For example, regions with high cropland connectivity for avocado are in Portugal and central Mexico, and for beans are in central India. Regions with important potential bridge cropland nodes include central Mexico for tomato, central and east Africa for coffee and banana and plantain, and central Africa for cacao and avocado. In the regional analysis, important locations with high cropland connectivity are at the border between Guatemala and Honduras for coffee, western Mexico for avocado, and eastern Mexico for maize and cacao. Using these maps of risk for planning in advance can inform phytosanitary strategies in the region, especially for dealing with extreme weather associated with El Niño and La Niña phenomena and recovery from emergencies such as hurricane damage.

Characterization of pathogens and foliar disease interactions impacting sugarbeet storage

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In Michigan, sugarbeets (*Beta vulgaris*) are harvested and stored for up to 200 days prior to processing. Rotting of the beets during storage; however, can reduce sugar content and overall profit. Fungal mycelium and pectinase by-products also clog filters and slow the extraction process. Michigan Sugar Company estimates delaying onset of storage rot by one month could save the industry \$1 million per year. In 2019 and 2020, a preliminary survey identified *Penicillium* spp. (55%), *Botrytis cinerea* (12%), and *Fusarium* spp. (11%) as the most frequently isolated pathogens in MI piling grounds; these observations were consistent with previous findings from North Dakota and Germany. *Geotrichum* spp., not previously found on sugarbeets in MI, was isolated from piled roots in 2020. In-season diseases such as Cercospora leaf spot (CLS) also may affect storability, though these impacts are debated. In 2020, post-harvest trials were conducted to evaluate root susceptibility to *F. graminearum*, *B. cinerea*, *P. vulpinum*, and *Geotrichum* spp. in beets with high or low in-season CLS severity. This study also examined the effect of CLS on post-harvest respiration rate. No significant difference in respiration rate was observed between beets with high and low CLS levels 60 DPH ($P > 0.05$); these findings are consistent with results from ND. CLS level also did not significantly impact rot development in a CLS-susceptible beet variety 60 DPH ($P > 0.05$); however, additional trials are ongoing. This study will help improve understanding and management of sugarbeet storage rot.

Divergent selection in wild rice for resistance and susceptibility to foliar diseases to decrease reliance on fungicides

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Cultivated wild rice (*Zizania palustris*) is grown in man-made paddies and commercialized for its nutritious grain and subtle flavor. The University of Minnesota breeding program has developed several varieties, and some have been improved for foliar disease resistance exhibiting delayed disease development. In the field this type of resistance is overwhelmed by pathogens if no fungicides are used. Toward understanding plant defenses, we conducted two cycles of mass phenotypic recurrent selection to increase the frequency of resistant (R) and susceptible (S) alleles to generate two divergent populations from the original cultivar Itasca-C12, coupled with genotyping-by-sequencing (GBS) and bulk segregant analysis. The selection differentials varied from 1.1 for the R selections to 7.0 in

the S selection in 2020, while the selection intensity ranged from 0.32 to 0.74 in R selections from 2019 and 2020, respectively. The genetic gain estimated for R and S selections were 3.43% and 4.26%, respectively, indicating modest but steady progress from selection. GBS identified 12 differential SNP patterns between R and S plants in 2019 samples, including some associated with putative defense response genes. Plants carrying R alleles will be incorporated into a new cultivar to reduce dependency of fungicides, while those with S alleles will provide opportunities to study crop vulnerability and its association with DNA markers used for negative selection. This research can facilitate and accelerate selection of plants using associated SNPs and identify disease resistance mechanisms.

Characterization of the spatial and temporal distribution of *Cucurbit leaf crumple virus* in yellow squash planted in southern Georgia

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A 2-year study was conducted to characterize the spatio-temporal distribution of *Cucurbit leaf crumple virus* (CuLCrV; a whitefly-transmitted begomovirus) in experimental and commercial yellow squash (*Cucurbita pepo*) fields in southern Georgia. In the fall of 2019 and 2020, field trials and surveys were conducted. Field trials comprised of 30 rows of squash (variety: Gentry) were planted in Tifton, GA. Each row was partitioned into 10 0.91 m × 3.05 m quadrats resulting in a total of 300 quadrats. Six commercial squash fields in Tift, Worth, and Colquitt Counties were surveyed. Ten rows of plants per field were partitioned into 70 1.83 m × 9.14 m quadrats. Plants in each quadrat were visually assessed for CuLCrV symptoms at a 7-day (for field trials) or at a 14-day interval (commercial fields). Results from “ordinary runs analysis” conducted early in the season found a typically random distribution of plants symptomatic for CuLCrV. As the season progressed, plant symptomatic for CuLCrV became aggregated ($Z < -1.64$). Spatial distribution in commercial fields was determined from Moran’s I and index of aggregation (I_a) values. Moran’s I and I_a values increased over time signifying a shift from random to aggregated patterns (Moran’s $I > 0$ and $P < 0.05$, $I_a > 1$ and $P < 0.05$). Heat maps generated from I_a values had a noticeable edge effect on the spatial distribution of CuLCrV in field trials. An understanding of the edge effect on CuLCrV incidence and the shift from random to aggregated distribution are considerations for refining current management strategies.

Ascorbic acid rescues plant health in the presence of pathogen-induced oxidative stress

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Charcoal rot, caused by the soilborne necrotrophic fungus *Macrophomina phaseolina* (MP), is a prevalent soybean disease that causes significant yield reduction when the air and soil temperature ranges between 28 and 35°C under limited soil moisture conditions. It is hypothesized that MP induces oxidative stress-mediated senescence in plants via the accumulation of reactive oxygen species (ROS) that contribute to basal defense-related signal transduction or oxidative damage in plant cellular components depending upon their concentration. ROS accumulation leads to cellular imbalances, damage, and host tissue senescence. MP is a necrotroph that takes advantage of this cell senescence mechanism as it depends on necrotic tissue for nutrition. The objective of this study was to determine if ascorbic acid, a ROS scavenging molecule, could quench MP-induced H₂O₂ generation in the soybean-MP pathosystem. In vitro sensitivity tests showed that MP isolates were sensitive to ascorbic acid at EC₅₀ of 10.5–14.3 mM. In planta cut-stem assays demonstrated that pre-treatment with 10 mM of either the reduced or oxidized forms of ascorbic acid significantly decreased lesion length caused by MP. Further, quantification of H₂O₂ from ascorbic acid-pre-treated tissue followed by MP inoculation showed significantly less ROS accumulation than the inoculated control or the mock-inoculated control. This result suggests that MP not only induces H₂O₂ upon host infection, but that ascorbic acid is a ROS scavenger that limits MP-induced ROS-mediated senescence.

Survey and management of diseases impacting potato storage in Michigan

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Potato (*Solanum tuberosum*) is the fourth most produced crop in the world. Michigan is the eighth largest potato producer in the United States and produced an estimated market value \$200 million in 2019. Year-round demand for fresh market and processed goods requires potato storages to maintain tuber quality for 5–8 months. Losses are incurred each year due to postharvest disease and shrinkage. Approximately 22,300,000 cwt (11%) was lost in the 2019–2020 storage season. In 2019, a survey of postharvest diseases was performed in six counties. Tubers were collected during harvest and examined for disease before, during, and after storage. Destructive sampling was performed on samples and potential pathogenic isolates were cultured and morphologically characterized. Putative pathogens were recovered from 84% of at-harvest tuber samples ($n = 682$) and 90% of postharvest tuber samples ($n = 673$). Identified species of concern include bacterial soft rot (*Pectobacterium* spp. and *Dickeya* spp.), Fusarium dry rot (*Fusarium* spp.), leak (*Pythium ultimum*), late blight (*Phytophthora infestans*), and pink rot (*Phytophthora erythroseptica*). In 2020, key diseases were used to evaluate potato varieties and breeding germplasm (38 entries) for postharvest disease resistance. Varieties that exhibit blemish and postharvest disease resistance will be evaluated in future studies to help reduce storage losses. In addition, characterization of isolates that represent major pathogen genera is ongoing and will help inform and direct future management studies.

Characterizing the resistance response of *Solanum sisymbriifolium* elicited by root-knot nematode infection

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Root-knot nematodes (RKN) can infect potatoes and negatively affect potato production. There are no commercially available RKN resistant potato cultivars so other control methods warrant investigation. Therefore, we started to research the trap crop called litchi tomato, *Solanum sisymbriifolium*. Previous research showed that *S. sisymbriifolium* is resistant to the potato cyst nematode (PCN), *Globodera pallida* and various species of root-knot nematodes (RKN). We hypothesize that *S. sisymbriifolium* resistance is conferred by a hypersensitive response after the RKN infect the roots. We have infected *S. sisymbriifolium* with RKNs *Meloidogyne chitwoodi* and *M. hapla* and used acid fuchsin staining to visualize the nematodes in situ. We have also evaluated the amount of reactive oxygen species produced in roots during nematode infection. Additionally, time course assays have been completed creating a timeline of infection. We have discovered that *S. sisymbriifolium* is resistant to *M. chitwoodi* and *M. hapla*, but there are subtle differences in their ability to infect the roots. Investigations into the effects of root exudates of litchi on the activity of RKN juveniles will be completed to enhance characterization of the overall resistance response.

Pan-genomic approaches to predicting virulence factors in *Pantoea ananatis* affecting *Allium porrum* and *Allium fistulosum*

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Pantoea ananatis is a part of a *Pantoea* species-complex that causes center-rot of onions as well as infecting other *Allium* crops (leeks, chives, Japanese bunching onions [JBO], garlic). This pathogen lacks a type II, III, or IV secretion systems but relies on a chromosomal phosphonate biosynthetic gene-cluster and the plasmid-borne onion-virulence region for disease in onion. Pathogenicity and virulence factors associated with other *Allium* species remain unknown. We used phenotype-dependent genome-wide association (GWAS) and phenotype-independent gene-pair coincidence (GPC) analyses on 108 *P. ananatis* strains inoculated on leek and JBO to compare with onions. Greenhouse studies showed that these strains were highly aggressive on JBO as opposed to leek. Within the coincident gene clusters, several phenotype-dependent genes can be found such as a VirB4 associative gene network and the *alt-A* dissociative gene network. Our results indicate that *P. ananatis* may utilize a repertoire of common and unique genes to infect different *Allium* spp.

Pydiflumetofen sensitivity of *Fusarium virguliforme* isolates causing sudden death on soybeans

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Pydiflumetofen (ADEPIDYN™) was recently registered as a seed treatment to manage soybean sudden death syndrome caused by *Fusarium virguliforme*. Pydiflumetofen is a member of the succinate dehydrogenase inhibitor class of fungicides (FRAC Code 7). It has a high binding affinity to the complex II enzyme and efficacy against a broad range of fungal plant pathogens. The pydiflumetofen sensitivity of 130 isolates of *F. virguliforme* was determined using a mycelial growth inhibition assay. Isolates originated from symptomatic root tissue showing typical disease symptoms. The root tissue samples came from Illinois, Iowa, Kentucky, Michigan, and Mississippi. Sensitivity tests were conducted in vitro using agar medium amended with pydiflumetofen at the following concentrations: 0, 0.01, 0.1, 1, 10, 0.01, and 100 mg a.i./L. The distribution of sensitivities (EC₅₀ values) of *F. virguliforme* isolates to pydiflumetofen ranged from 0.011 to 0.4602 mg a.i./L, with a geometric mean of 0.0537 mg a.i./L and a range of 41.8x. The results obtained validate the pathogen sensitivity to pydiflumetofen, and the baseline sensitivity distribution will be used in future monitoring studies looking for shifts in sensitivity of *F. virguliforme* to pydiflumetofen.

A wild relationship: Distribution and genetic diversity of ilarviruses in wild *Prunus* spp.

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Prunus necrotic ringspot virus (PNRSV) and *Prune dwarf virus* (PDV) are members of the *Ilarvirus* genus in the family *Bromoviridae*. PNRSV and PDV are prevalent in peach orchards in the southeastern region of the United States. These viruses have the potential, both individually and in co-infections, to negatively impact peach production by degrading tree health and lowering fruit yield. Although the main cause of spread is via dissemination of infected propagation material, both viruses can also spread horizontally (tree to tree) through infected pollen, potentially assisted by pollinators. PNRSV and PDV have additional hosts in the Rosaceae, but the contribution of alternative hosts to ilarvirus epidemiology in peaches is not well understood. We sampled wild *Prunus* spp. near peach orchards in South Carolina and Georgia for the presence of PNRSV and PDV by RT-PCR. We then conducted genetic analyses on the virus isolates recovered from wild *Prunus* spp. and the nearby peach orchards. Samples included in this study are predominantly from *P. serotina*. PNRSV was detected in 12% of trees sampled, and PDV was not detected in our wild *Prunus* collections. Pollen was also collected from infected wild *Prunus* spp. to assess the potential for virus movement between forest edges and peach orchards. Phenological observations of bloom and genomic comparisons of virus isolates from forest edges and orchards will help to inform the risk of movement of pollen-borne ilarviruses in and around peach orchards.

Detection of oak wilt eDNA in insect traps in eastern Canada using metagenomics and qPCR

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Oak wilt is caused by the fungus *Bretziella fagacearum* which develops in the outer sapwood of oak trees. Being one of the most problematic forest diseases in North America, the causal pathogen is present in 28 eastern states of the USA, including infection centers along the Canadian border. Despite significant importation of oak logs from northeastern states, the disease has never been reported in

Canada. To determine the risk of introducing the disease in Canada, we sampled oak wilt insect vectors collected from southern Ontario and from Quebec sawmills importing US red oak logs. Using a qPCR assay, we tested for the presence of *B. fagacearum* in these samples. *B. fagacearum* DNA was detected in some Quebec and Ontario insect trap samples collected in 2019, while all Canadian samples collected in 2020 were negative. Metabarcoding of insects and fungi from insect trap samples collected in 2019, using both Illumina and Ion Torrent HTS technologies, supported the presence of the *B. fagacearum* DNA in one Ontario sample and provided partial information about the spatiotemporal distribution of known oak wilt insect vectors in eastern Canada. While environmental DNA detection of *B. fagacearum* is not absolute proof of active oak wilt disease and does not confirm any establishment or first report of the disease in the country, it may serve as a tool for early warning and help in promoting public awareness, guide field surveys by territory regulation authorities, and increase biological knowledge about the pathogen and its potential vectors.

Resistance to tar spot of maize accessions by remote sensing in Honduras

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Tar spot (TS) is one of the most important diseases of maize causing defoliation and yield reduction. It is caused by the ascomycete *Phyllachora maydis*. In Honduras, the use of fungicides is costly for small farmers who contribute 45% of the total maize production with landraces and improved seeds. Although in other countries there are resistant varieties, in Honduras the resistance of native germplasm has not been evaluated, in different environments, using remote sensing (RS) techniques. The objective of present study was to evaluate the resistance of maize varieties to TS through conventional phenotyping and RS. In 2020 and 2021, TS severity was evaluated in 32 maize varieties and 10 multispectral vegetation indexes were analyzed. A strong inverse correlation ($R^2 = -0.92$) of the area under the disease progress curve (AUDPC) and the rate of disease increase ($R^2 = -0.88$) with performance was observed. The NDVI, NAVI, RDVI, GNDVI, RNDVI, and MCARI1 indices showed the highest reflectance relationship with AUDPC, and the DICTA-96 variety was determined as to be the variety with the high resistant to TS. The results of this study allow to start a plant breeding process to confer the resistance found to other varieties of interest that are susceptible to TS and to replicate the RS technique in other foliar diseases to reduce the time and cost of conventional phenotyping.

Metagenomics and long-read nanopore sequencing for rapid and culture-free plant pathogen surveillance

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The ONT MinION™ has made it possible to sequence entire metagenomes rapidly and at a relatively low cost. Through the use of appropriate classification tools, any pathogen can now be detected without culturing or developing pathogen-specific probes. This also allows for fast, precise, and sensitive detection of plant pathogens, which can be used for surveillance to prevent epidemics. In multiple studies, we have successfully used the MinION™ to detect and identify bacterial and fungal pathogens from tomato, boxwood, and grapevine plant samples. Both, assembled and unassembled reads, gave consistent results in terms of pathogen identification. In a project on Pierce's disease of grapevine, we assembled a metagenome-assembled genome (MAG) with a length almost identical to the *Xylella fastidiosa* subsp. *fastidiosa* (*Xff*) genome. Using the MAG, the most similar genome was identified among all publicly available *Xff* genomes. By building a core genome tree, it was possible to identify the likely geographic source of the Pierce's disease outbreak in Virginia. This demonstrates the strength of metagenomic sequencing for pathogen detection and identification. However, there are challenges that still need to be overcome, including more comprehensive pathogen databases, the reduction of sequencing errors, and the development of bioinformatics tools for a better differentiation and precision in the identification of closely related strains and of more user-friendly tools. These improvements should allow the use of metagenomics in routine diagnostics at plant disease clinics.

Molecular characterization of fungal isolates associated with mango quick wilt and its chemical management

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Mango is the most important fruit due to its excellent flavor, taste and fragrance. Mango quick wilt (MQW) is a potential threat to successful mango production that has recently appeared in Pakistan. Survey of the districts of southern Punjab on spatio-variational analysis revealed Khanewal 83.21%, Multan 75%, Muzafergarh 67.86%, Rahim Yar Khan 71.54% were highly effected with MQW. Sampling of 68 disease samples were conducted for isolation of fungal isolates, 50 identical isolates with 73% isolation frequency were recovered from carrot baits. Morphological and molecular studies of three gene regions, Internal transcribed spacer, translational elongation factor and beta-tubulin, revealed the fungus is *Ceratocystis manginecans*, which consisted of 1,833-bp characters, with 100% pairwise identity of four isolates and closely nested at bootstrap 98% with already described *C. manginecans* from Oman and Pakistan in a *C. fimbriata* subsp. clade with a close relation to *C. coacoqfinesta*. *C. manginecans* was recovered from inoculated mango seedlings in pathogenicity test results. Chemical evaluation with Carbendazim, Topsin-M, Difenconazole, and Benomyl on different concentrations under in vitro, greenhouse, and field experiments revealed that Carbendazim was significant in all three experiments, followed by Topsin-M + Carbendazim at concentrations of 500 ppm and 750 ppm.

Identifying optimal wavelengths to detect peanut infection with *Athelia rolfsii* using a hyperspectral sensor and machine learning

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Stem rot of peanut, caused by *Athelia rolfsii*, is one of the most important soilborne diseases in peanut production worldwide. Current scouting methods based on the detection of signs or symptoms of the disease by human raters are labor-intensive and time-consuming. A recent study demonstrated it was possible to distinguish between healthy and *A. rolfsii* infected peanut using a handheld hyperspectral sensor. However, only two wavelengths of the spectrum were used in the analysis, and they were selected based on visual inspection of the spectra collected from leaves of healthy and diseased peanut plants. To more precisely identify important wavelengths that are signatures of peanut infection with *A. rolfsii*, a machine learning approach was used to further analyze spectral reflectance data. Spectra were categorized into five classes based on visual disease symptoms. One feature extraction and five feature selection methods from the scikit-learn machine-learning library were evaluated for the wavelength selection. Recursive feature elimination (RFE) methods outperformed the univariate feature selection and SelectFromModel methods. The top 10 wavelengths identified by the RFE methods performed as well as all bands for the five-class classification, with an overall accuracy of ~70%. Wavelengths around 505, 690, and 884 nm were repeatedly selected by different methods. Results may have applications for automated stem rot detection in peanut fields using optical sensors, and the methodology can be adapted to identify spectral signatures of disease in other plant-pathogen systems.

Assessment of the direct quantification of peach viruses by reverse transcription droplet digital PCR

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Digital PCR is a new generation of quantitative polymerase chain reaction (qPCR), which can be applied to absolute quantification of target nucleic acid without the need of external standard based on the partitioning of individual analyte molecules into many replicate reactions. In this study, we used reverse transcription droplet digital polymerase chain reaction (RT-ddPCR) to establish the detection and absolute quantification of two major viruses infecting peaches. The quantitative linearity, accuracy, and sensitivity of RT-ddPCR for detection of PLMVd and PPV were comparatively assessed to those of reverse-transcription real-time quantitative polymerase chain reaction (RT-qPCR). The specificity assay shows no amplification in major other peach viruses and negative control. Our results also indicated that RT-ddPCR assay is 2- and 10-fold more sensitive, respectively than RT-qPCR and could therefore, be used to detect viruses in field samples. Furthermore, the levels of transcripts determined using RT-ddPCR and RT-qPCR showed a high degree of linearity and quantitative correlation. In summary, the established RT-ddPCR assay represents a promising alternative method for the precise quantitative detection of peach viruses; it would be particularly applicable for diagnosing peach viruses in plant quarantine inspection and virus-free certification program. To the best of our knowledge, this is the first study of the development of RT-ddPCR and RT-qPCR assays for ultra-sensitive detection of peach viruses.

Efficacy of phytoextracts against yellow mosaic disease in *Vigna mungo*

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Yellow mosaic disease (YMD) is one of the most destructive viral diseases causing nearly 100% loss in leguminous crops including *Vigna mungo* L. YMD is caused by begomoviruses in the family *Geminiviridae* and are vectored by whiteflies (*Bemisia tabaci*). Many chemical-based insecticides are available to manage whiteflies, but can also have adverse effects on human health. The objective of this study was to evaluate various plant extracts against YMD caused by *Mungbean yellow mosaic India virus* (MYMIV). This study was performed during rainy seasons of 2016 and 2017 using urdbean cv. T9. Experiments were arranged in a randomized complete block design with three replicates. Phytoextracts were prepared using respective plant parts in a ratio of 1:1 (w/v) with distilled water. Stock filtrates were diluted to 10, 15, and 20% in distilled water and served as three treatment levels. Extracts from *Zingiber officinale* (rhizome), *Azadirachta indica* (leaf), *Datura stramonium* (leaf), *Allium cepa* (bulb), *Allium sativum* (bulb), and a water-only control were sprayed at 20 and 45 days after sowing. All treatments were effective in viral suppression with the 20% *D. stramonium* extract being the most effective in reducing YMD incidence, severity and whitefly counts and in improving seed yield to 299.5 kg/ha in 2016 and 272.17 kg/ha in 2017. Thus, the phytoextract from *D. stramonium* is a sustainable treatment to manage YMD by growers.

The structured coalescent with ancestral recombination model and its application to the fungal pathogen *Aspergillus flavus*

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Knowledge about the demographic history of a fungal pathogen including their movement between populations is necessary to understand and control disease spread. Phylogeographic methods can help reveal the migration of pathogen lineages between populations. However, due to recombination, the phylogenetic relationships among sampled lineages can vary across the genome and obscure inferences about pathogen movement. We, therefore, developed a new method, called the structured coalescent with ancestral recombination (SCAR), which extends structured coalescent models to include recombination. The SCAR model allows for recombination and migration rates to be estimated while reconstructing pathogen movement from an ancestral recombination graph (ARG), which we infer using ARGweaver. After testing on simulated genome sequence data, we applied this framework to the recombining fungal pathogen *Aspergillus flavus*, which produces carcinogenic aflatoxins that contaminate corn, peanut, and other seed

crops. While predominantly asexual, *A. flavus* also reproduces sexually and undergoes recombination, which leads to a complex mosaic of phylogenetic and migration histories across the genome. Applying the SCAR model to *A. flavus* lineages sampled across the United States, we explore patterns of recombination and migration across the *A. flavus* genome.

Molecular quantification of *Fusarium oxysporum* f. sp. *fragariae* in soil

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Informed management decisions with early pathogen detection is a main line of defense against soilborne pathogens and their respective diseases. Early detection is especially important for soilborne pathogens that have few control measures following disease incidence. While some fungal pathogens such as *Verticillium dahliae* (*Vd*) and *Macrophomina phaseolina* (*Mps*) can already be detected from soil DNA at inoculum levels approaching their limit of pathogenicity, currently available methods do not achieve this for *Fusarium oxysporum* f. sp. *fragariae* (*Fof*). Factors hampering molecular-based detection and quantification of *Fof* include a uninucleate long-term resting chlamydospore, a qPCR TaqMan assay targeting a single copy gene, and the lack of effective DNA extraction methods that overcomes PCR inhibitors commonly found in soil/environmental samples. Here, we describe a new technique to extract DNA from larger masses of soils, compared to commercial kits, and a cleanup process that is effective and automated. *Fof* could be detected through qPCR at counts below 10 CFU/g of soil. In some instances, soil samples were positive using molecular detection and below the limit of detection for *Fof* in plating assays. Compared to the MP FastPrep Soil kit that extracts DNA from 0.5 g of soil, our method yields an average of 2.5× more DNA from 15.0 g of soil. The application of our method is extended to *Vd* and *Mps* and increases the sensitivity of qPCR assays for those pathogens, thereby opening the possibility of pooling multiple soil samples and reducing costs for pathogen detection.

Spatiotemporal dynamics of in planta *Candidatus Liberibacter asiaticus* movement, host response, and huanglongbing disease development

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Candidatus Liberibacter asiaticus (CLAs), a fastidious α -proteobacterium vectored by Asian citrus psyllids (ACP), causes huanglongbing (HLB), the most devastating citrus disease worldwide. The detailed events of CLAs infection, in planta movement, systemic progression of disease and impact on host response remain poorly understood. We investigated the CLAs movement and colonization in different tissues post-ACP transmission to the young flush of sweet orange. The qPCR based spatiotemporal CLAs titer estimation suggests the infected young leaves as primary site for colonization and then movement to the roots followed by young leaves post-ACPs feeding. CLAs was also detected in source tissues (mature leaves not fed on by ACPs), indicating active movement into mature leaves. Furthermore, we monitored the CLAs movement towards young leaves in HLB positive sweet oranges from different age groups and environmental conditions. Transmission electron microscopy (TEM) and qPCR assays were used to analyze CLAs movement from infected branch to young flush in correlation with developing sieve pores. We also conducted RNA-Seq and qRT-PCR based expression analysis of young leaves of HLB positive tree with and without detectable CLAs. In sum, our study shed light on the movement and biology of CLAs and how CLAs affects young flush and leads to disease development.

Roles of potential inoculum sources in the epidemiology of spinach downy mildew

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Downy mildew, caused by *Peronospora effusa*, causes severe economic losses in spinach. Use of resistant spinach cultivars remains an effective management strategy, particularly for organic production, yet new *P. effusa* races continue to emerge that overcome resistance. The roles of potential inoculum sources in the disease cycle are not clearly understood, in part due to the difficulty in studying the obligate pathogen. While the sporangiospores are known to initiate disease via airborne dispersal, their survival in the environment is likely limited. We found sporangiospores to remain infectious for up to 4 days, but not beyond 8 days on detached and desiccated spinach leaves, indicating that they can remain virulent for short periods of time. Oospores produced during the sexual cycle have been found in leaves of various infected spinach cultivars as well as numerous seed lots and potentially persist in soil. Based on plasmolysis tests and direct observation of germination, viable oospores were recovered from both leaves and seed, although at a low percentage. The germinating oospores observed from spinach seed lots dated up to five years in age, suggesting that their long-term survival may contribute as a potential inoculum source. However, direct infection by oospores on spinach plants has not been shown, and replication of seed transmission via oospores has proven difficult. Current efforts are focused on factors behind oospore germination and their ability to infect spinach plants and initiate disease.

Association of *Diaphorina citri* flavi-like virus and *Candidatus Liberibacter asiaticus* in the Asian citrus psyllid

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Candidatus Liberibacter asiaticus (CLAs) associated with huanglongbing (HLB), also known as citrus greening disease, is devastating Florida's citrus industry. CLAs is transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama. Currently there is no cure for HLB, and disease control heavily relies on vector management by chemical and biological approaches. ACP harbors various insect

specific viruses from several distinct taxa. Availability of an efficient viral vector using ACP viruses might offer another biocontrol agent. Toward that goal, we used high throughput sequencing, RT-PCR, and florescent in situ hybridization techniques and show the presence of *Diaphorina citri flavi-like virus* (DcFLV) in the midgut of the ACP, where it co-localized with CLas bacteria. DcFLV was found in different organs and tissues of the ACP such as salivary glands, filter chamber, head, eggs, and honeydew suggesting systemic infection of the ACP by DcFLV. Additionally, we show the vertical transmission of DcFLV from female to offspring of the ACP. Finally, positive correlation between DcFLV and CLas in the field-collected ACP populations from Florida suggests probable association between DcFLV and CLas in the ACP.

Teaching plant pathology during a pandemic? A semi-virtual approach for plant pathogenic bacteria labs

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Like the rest of the world, the COVID-19 pandemic sent us home and left us wondering how to teach a plant pathology laboratory course remotely. The students would be unable to attend physically, and even if we found a way to connect with them from the lab, how could we make it engaging? Using a semi-virtual approach, we performed lab experiments that the students could watch and participate with in real-time. This lab focused on molecular identification of bacterial soft-rot pathogens of potato. The students had access to a step-by-step interactive timeline where they could experience each step prior to the lab session. Steps taking a significant amount of time were summarized in short and engaging videos the students could watch before the class. During the class, we walked small groups of students through the molecular identification using a "cooking-class" approach. This allowed them to participate actively and observe the process through the end. After which, TAs worked with the students on sequence data using free bioinformatic tools to draw their own conclusions. Transforming an in-person laboratory to a semi-virtual environment with remote participation was truly challenging. We hope that our approach can help and inspire other educators on active remote learning experiences in the future.

Effects of seed-treatment chemicals on red rot of sugarcane in billet planting system

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Red rot of sugarcane, caused by *Colletotrichum falcatum*, is an important constraint to crop production. The disease primarily affects seed-cane in Louisiana and is more severe when stalk sections or billets are planted rather than whole stalks. Seed-cane treatment with a mixture of fungicides azoxystrobin and propiconazole alone and in combination with systemic insecticide thiamethoxam has improved stand establishment and increased yields obtained from planting of billets, with consistently beneficial results obtained using the fungicide/insecticide combination. However, there has been no scientific investigation to determine how these benefits are accomplished. A greenhouse experiment was conducted with three-node billets of cultivar HoCP 96-540 to determine the effect of dip application of the azoxystrobin and propiconazole mixture and/or thiamethoxam on stalk rot symptom development. Billets were planted in non-sterile sugarcane field soil and inoculated with *C. falcatum* and planted in steamed field soil. Fungicide treatment alone reduced stalk rot severity while insecticide alone did not. Treatment with the combination of fungicides and insecticide promoted greater reductions in red rot severity than fungicides alone in inoculated billets and billets exposed to natural inoculum. Node rot was reduced, and this could be a factor contributing to improved stand establishment. These are preliminary results expected to be replicated during fall 2021. This research will provide scientific support for management recommendations using these chemicals.

The role of hydrophobins in *Penicillium expansum* biology and blue mold disease

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Blue mold, a post-harvest disease of apples and pears, is caused by the filamentous ascomycete *Penicillium expansum*. This necrotrophic pathogen also produces the mycotoxin patulin which upon presence in processed food products, poses a threat to food safety. Research on this pathosystem has largely focused on the genetic regulation of patulin and decontamination strategies. There is a growing body of work focusing on *P. expansum* molecular biology and virulence strategies. Previously, the carbon catabolite repressor CreA was described as a *P. expansum* virulence factor. We used an RNA-seq dataset from that study to investigate potential genes that may contribute to virulence in the *P. expansum*-apple pathosystem. There was one hydrophobin encoding gene significantly differentially downregulated in the $\Delta creA$ mutant when compared to WT in in vivo conditions. Hydrophobins are small fungal specific proteins that provide hydrophobicity to filamentous fungi. These proteins have also been shown to be involved in virulence of other pathosystems. We have identified seven putative hydrophobin encoding genes in the *P. expansum* genome. In order to study these genes and their role in fungal biology and virulence, we have generated single deletion mutants and a quintuple deletion mutant to account for functional redundancy. These strains are being compared to the WT strain in different aspects of fungal physiology including conidiation, germination, radial growth, resistance to mechanical stress, and in vivo virulence assays.

Genome-scale analysis of *Fusarium oxysporum* f. sp. *cubense* race 1 and tropical race 4 isolates from Colombia

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Fusarium wilt (FW) is caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). The substitution of the highly susceptible cultivar (Gros Michel) to *Foc* race 1 (R1) by the resistant cultivar (Cavendish), mitigated the devastation in banana production caused by *Foc* R1.

Currently, Cavendish represents the source of 99% of banana exports. However, the new isolate of *Foc* TR4 is capable to invade and cause FW on a wide range of other banana varieties (including Cavendish). *Foc* TR4 was recently reported in Colombia and Peru. In this study, three *Foc* TR4 isolates from La Guajira Department and one *Foc* R1 isolate from Uraba region in Colombia, were sequenced Oxford nanopore technology. Nanopore reads of the three *Foc* TR4 isolates were assembled de novo. The resulted draft genomes have a median size of 48.145 Mbp, 37 contigs, and an N50 of 4.74 Mbp. A genome-scale phylogenetic analysis was performed comparing Colombian and the previously reported *Foc* TR4 genomes. Initially, we compared the Colombian *Foc* TR4 genomes against three high-quality reference genomes (UK0001, FO_II5 and *Foc*TR4_58). Additionally, we included a wider range of sequences. We focused on genomic differences to determine (i) genetic diversity of the *Foc* TR4 isolates in Colombia compared with worldwide isolates; (ii) pathogenic differences at a genomic level between *Foc* R1 and TR4 isolates; and finally (iii) to identify new specific molecular markers for *Foc* TR4 detection. We were able to identify new potential molecular markers for *Foc* TR4 detection and genomic regions, which seem to be unique to the Colombian isolates.

Soil nutrients and texture affect the diversity and abundance of pathogenic and beneficial fungal genera in soil

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Soil nutrients and texture are directly related to plant health. They can also affect the soil microbiome. In particular, understanding the impact of soil nutrients on pathogenic and beneficial microbial taxa can be useful when deciding optimum soil fertility levels for crop production. We collected bulk soil samples from 14 farms across Pennsylvania. Subsamples were used to assess the soil nutrients (P, K, Mg, Ca, S, Zn, Cu), pH, CEC, organic matter (OM), and texture (sand/silt/clay). Based on each attribute, samples were classified into three groups: high, medium, and low (ex: high/medium/low P sample). DNA extracted from the same soil samples were PCR amplified targeting fungal ITS region (ITS1F/58A2R primers) and sequenced (MiSeq). Impact of each soil attribute on (i) pathogenic ($n = 16$) and (ii) beneficial ($n = 14$) genera based β -diversity was assessed using PERMANOVA and beta dispersion tests. P, K, pH, sand, and silt had significant effects ($\alpha = 0.1$) on pathogenic genera-based β -diversity. All soil attributes except Ca and Zn had significant effects on beneficial genera-based β -diversity. Negative correlations were observed between a number of soil attributes and pathogenic fungal genera (ex: K and *Fusarium*, *Microdochium*; silt and *Corynespora*, *Microdochium*, *Neonectria*, *Periconia*; Zn and *Fusarium*, *Ilyonectria*). Positive correlations were observed between certain soil attributes and beneficial fungal genera (ex: P and *Metarhizium*, *Chaetomium*; clay and *Preussia*). Results, taken together, indicated how fungi-based soil health can be improved through soil nutrient management.

First evidence of viral infections on *Opuntia* sp. and *Hylocereus* sp. in Costa Rica

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Cactaceae is a large and morphologically diverse family with more than 2,000 perennial plant species. Some members of this family are economically important as ornamentals, human food, and for their medicinal properties. Cacti could be infected with different plant viruses affecting development and growth. There is no information about the viruses infecting cactus species in Costa Rica and a severe mosaic symptom was observed during 2018 in ornamental *Opuntia* spp. in parks. Therefore, we focused our research on surveying *Opuntia* spp. and dragon fruit (*Hylocereus* spp.) to test for some of the main viruses previously reported in these cacti genera. Dragon fruit is of economic importance due to the recent government incentive for its production in Costa Rica. Using RT-PCR and general primers, 17 *Opuntia* and 22 *Hylocereus* samples from different localities in Costa Rica were analyzed to detect members in genera *Potex*-, *Tobamo*-, *Poty*- and *Orthotospo*-virus, and also *Cucumber mosaic virus* (CMV). We found 16 *Opuntia* samples infected with *Opuntia* virus X (OpVX), three of those samples were also coinfecting with rattail cactus necrosis-associated virus (RCNaV), and six *Hylocereus* samples with zygocactus virus X (ZyVX). Mechanical inoculation assays on different indicator plants using an *Opuntia* sample coinfecting with OpVX and RCNaV were made; however, only RCNaV was successfully transmitted. Knowledge of the virus species currently present in cacti in Costa Rica could decrease the chance of its dispersion by deploying appropriate management strategies for cacti species in the country.

Changes in the fungal microbial community on an invasive grass after foliar fungicide application

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Fungal phytobiomes include communities of endophytes, pathogens, and saprophytes associated with plants. Fungicide applications can be used to manage some foliar diseases, but their effects on microbial community composition remain relatively understudied. The application of fungicides may have significant impacts on microbial composition, interactions, and community functions. We compared fungal communities in fungicide treated and control plots by sampling leaves of the invasive annual grass *Microstegium vimineum* growing in natural areas. The communities were characterized at three timepoints during the 2019 growing season using Illumina amplicon sequencing of the ITS2 region. Our results showed substantial shifts in the fungal communities associated with *M. vimineum* leaves between timepoints. Fungal composition in the July samples was determined to be more variable than the early or late August samples. Plant pathogenic taxa were most prominent in early August. Fungicide treatment affected fungal composition at two of the three timepoints, most strongly in early August. Fungicide reduced the relative abundance of OTUs assigned to the *Pleosporales*, but increased the relative abundance of OTUs assigned to the *Nectriaceae*. Despite substantial changes in fungal communities, the relative abundance of plant pathogens did not change between treated and untreated plots. Together, our results suggest that the application of a fungicide changes foliar fungal communities while maintaining functional group composition.

Insights into the transmission of grapevine red blotch virus by the three-cornered alfalfa hopper on natural and experimental virus hosts

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Little is known on the transmission biology of grapevine red blotch virus (GRBV) from the genus *Grabovirus* in the family *Geminiviridae* by *Spissistilus festinus*, the three-cornered alfalfa hopper. Our research aims to understand transmission factors that contribute to GRBV spread in the vineyard. We documented GRBV transmission by *S. festinus* from infected *Vitis vinifera* 'Cabernet franc' to excised healthy *V. vinifera* 'Cabernet franc' leaves (10%, 4 of 39). Similarly, using *Phaseolus vulgaris* as a pseudo-systemic host of the virus and a feeding host of the insect, we demonstrated efficient GRBV transmission by *S. festinus* from infected snap bean plants to excised snap bean trifoliates (90%, 27 of 30). Interestingly, the acquisition access period was extended when feeding on infected *V. vinifera* (10 days) compared to infected *P. vulgaris* (6 days). The development of a reliable transmission assay for GRBV lead to additional studies regarding latency and retention periods, as well as comparative investigations of GRBV transmission efficiency in greenhouse and vineyard settings. The atypically long acquisition access period on *V. vinifera*, the natural host of the virus, and snap bean, an experimental host of the virus, compared with other geminiviruses, indicates the need for continued research regarding the increasingly unique attributes of GRBV transmission biology by *S. festinus*. Such information is crucial for proper disease management strategies of both GRBV and *S. festinus* in the vineyard.

Heterogeneity in landscapes and practices influence fungicide resistance

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Management of *Zymoseptoria tritici*/*Mycosphaerella graminicola*, a major fungal pathogen of wheat, depends on careful selection of fungicides and a resistant cultivar of wheat. We developed a model that allows us to explore the role of heterogeneous use of fungicides and cultivars in a regional wheat landscape to evaluate resistance management strategies. Ideal strategies often assume cooperation amongst growers or unrealistically assume a farm is isolated from the rest of the regional wheat crop. We used this hypothetical model of asexual and sexual stages of the pathogen with regional dispersal of ascospores to study how (1) single-use and combinations of fungicides; (2) novel and traditional fungicides; and (3) resistant and susceptible cultivars determine the evolution of resistance to fungicides under realistic scenarios. In each modeled scenario, five types of management practices were simultaneously used over multiple years in the region.

Identification of new races and pathogenic variants of coffee leaf rust (*Hemileia vastatrix*) in Colombia

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Coffee leaf rust (CLR) is the most important disease for coffee worldwide, causing 30 to 80% losses. The main management strategy is planting resistant varieties and by 2020 Colombia reached 83% of its area planted with composite resistant varieties Colombia, Castillo®, Cenicafe 1 and Tabi, developed by FNC CENICAFÉ. However, this great achievement increases selection pressure to the pathogen to mutate. To identify CLR physiological races, 29 single-pustule isolates were collected on commercial varieties and contrasting germplasm by its origin, and nine aggressive populations from resistant varieties components at research stations in Tolima, Antioquia, Quindío and Caldas departments. Urediniospores suspensions were inoculated on whole detached leaves of C1FC (Portugal) differential clones having *Sb1* to 9 and ? resistance genes alone or combined. In single pustule isolates were identified races II and XXII known in Colombia, XXIII and XXIX were confirmed, and I, XVI, XXVI, XXVIII, XXXIX and similar to XLII were new. Six pathogenic variants with virulence genotypes new for Colombia were found: v12456, v23456, v245679?, v12345679?, v2356789? and possibly v23456789?. In population inoculum from individual components, new race XXXIX was also found and three new complex genotypes: v5679?, v12456789? and possible v123456789?. These results confirm CLR high ability to adapt and co-evolve on coffee with diverse genetic resistance at different environments, allowing a better understanding of coffee-*H. vastatrix* interactions to prevent economic impacts on Colombia's coffee farmers.

Molecular identification of *Colletotrichum* spp. causing bitter rot disease of apples in Illinois

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Bitter rot, caused by *Colletotrichum* spp., is one of the most important diseases in apple-growing areas of the world, and is the most difficult summer apple disease to manage in Illinois. Studies have shown that there is considerable genetic and molecular variability within isolates of *Colletotrichum* spp. The accurate identification of *Colletotrichum* spp. within the species complex is therefore critical since species exhibit differences in pathogenicity and sensitivity to fungicides. A species-specific diagnosis is very important for developing an effective disease management strategy. Orchard surveys were conducted in Illinois in the 2019 and 2020 seasons and infected apples were collected from 10 different cultivars in 28 orchards. The pathogens were isolated using potato dextrose agar (PDA). After performing DNA extraction from conidia, PCR amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene using the primer GDF1 was done. Sequencing and subsequent blast in NCBI GenBank positively identified the isolates as *C. fioriniae*, which reside within the *C. acutatum* species complex, and *C. siamense*, belonging to the *C. gloeosporioides* species complex. Preliminary analysis showed that *C. fioriniae* is the most common species causing bitter rot in Illinois apple orchards, comprising more than 60% of the isolates. Both species were found in the same orchard, and in some cases, on the same tree.

***Bacillus* and *Streptomyces* VOC suppression of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 (Fov4) virulence on pima cotton (*Gossypium barbadense*)**

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Emergence of a highly virulent *Fusarium oxysporum* f. sp. *vasinfectum* race 4 (Fov4) with aggressiveness towards pima cotton (*Gossypium barbadense*) has raised significant concern for cotton growers and producers. This disease also revealed challenges in soilborne cotton disease management strategies which rely heavily on crop resistance and chemical controls. An alternative management tool is to identify and develop Fov4 antagonistic bacteria as biocontrol agents. In our preliminary screening for antagonistic bacteria from soil samples, we identified a unique combination of *Bacillus* Rz 141 and *Streptomyces* HC 658 isolates which displayed a mutualistic relationship capable of altering Fov4 growth. Notably, our experimental design placed Fov4 between each isolate where colonies were not in direct physical contact with the other isolate. These observations led us to hypothesize that bacterial volatile organic compounds (VOCs) impact the growth and virulence of Fov4. Ensuring physical separation, I-plate cultures showed Rz 141 had a VOC inhibition of 24%. Similarly in Q-plate cultures of Rz 141 with HC 658, VOC inhibition slightly increased to 28% with some loss of Fov4 pigmentation. Pathogenicity assays where Fov4-infected cotton was exposed to VOCs from physically separated Rz 141 and HC 658 showed VOCs can suppress Fov4 infection and colonization of cotton xylem. Our results provide evidence that rhizosphere bacteria can use VOCs as a communication tool to impact fungal physiology and virulence, and ultimately Fov4-cotton interactions without direct physical contact.

Contour-based detection and quantification of a pathogen of corn using RGB imagery

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The recent emergence and epidemics of tar spot of corn across the US (Midwest and Florida) presented the catastrophic potential of this disease to US corn production and amplified the need to better understand its pathobiology. However, despite the increase of research projects related to tar spot management and its well-known pathogen, *Phyllachora maydis*, improved disease quantification method is limited. Here, we developed the stromata contour detection algorithm (SCDA)-based detection of tar spot stromata (digital measurements) using red-green-blue (RGB) acquired under a controlled environment. The accuracy of SCDA was assessed by comparing its performance (stromata counts) to that of two independent human raters' estimations using image J (visual estimates). With the use of Cohen's weighted kappa coefficient (κ), we observed adequate agreements of stromata counts for both between SCDA and human rater A ($\kappa = 0.83$) and human rater B ($\kappa = 0.83$), as well as between the human raters ($\kappa = 0.95$). Our study demonstrates the potential of RGB image-based method to assist the limitations of the labor-intensive yet subjectivity-prone visual-based quantification and allow high-throughput data analysis, facilitating large-scale tar spot experiments. Moving forward, the applicability of this method to quantify tar spot in RGB images acquired under uncontrolled/field conditions and its potential to assess the temporal and spatial change in stromata development is currently being investigated.

Exploring the genomes of *P. syringae* sensu stricto and *P. viridiflava* isolates causing bacterial stem blight of alfalfa in the United States

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Alfalfa (*Medicago sativa*) is vulnerable to frost damage upon dormancy breakage in the spring. Significant injury due to frost has been observed in alfalfa stands in the Intermountain Western United States, resulting in reduced yields up to 50% in the first cutting. Bacterial stem blight (BSB) of alfalfa is often associated with frost injury due to the ice-nucleation activity of the causal pathogen group that promotes frost formation and damages stems. Bacteria residing in *Pseudomonas syringae* phylogroup 2B (PG2b) and *P. viridiflava* phylogroup 7 (PG7) are attributed as the causal pathogens of this disease. Characteristic BSB disease symptoms leaf and stem necrosis, wilting, and stunting. Presented here is a comparative analysis of the genomic content of PG2b-grouping and PG7-grouping isolates causing BSB on alfalfa in the United States. Twelve genomes of *P. syringae* PG2b-grouping isolates and three genomes of *P. viridiflava* PG8-grouping isolates were sequenced using Oxford nanopore long read sequencing in combination with Illumina short read sequencing. Phenotypic data, including disease scores and ice-nucleation scores, were collected and associated with each isolate. The isolates in this analysis exhibit differences in level of virulence, ice nucleation capability, and geographic location of isolation. Genomic content was compared between isolates exhibiting differing properties, such as the number of shared genes between isolates and SNPs in genes relevant to pathogenicity such as the ice nucleation gene, *ina*, and Type III secretion system-related genes.

Understanding microbial communities' composition for management practice improvement in lettuce hydroponic systems in Ohio

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Microbial communities of plants grown in hydroponic systems have not been deeply studied in contrast with the efforts to understand those in soil and rhizosphere. The microbial diversity in hydroponic systems is lower than in soil, and this controlled system potentially could enable enhanced manipulation of microbial interactions to increase productivity. In 2018, we surveyed four lettuce hydroponic production sites in Ohio and analyzed the diversity of bacteria, fungi, and protozoa through amplicon metabarcoding of ribosomal markers (16S, ITS1 and SSU) from roots and nutrient solution. We compared two different setup systems—nutrient film technique

(NFT) and deep water culture (DWC)—finding that bacterial, fungal, and protozoan community structure in water and roots varied between setup systems. Also, comparison of bacteria genera across different sites showed that management differences (e.g., water source) are a potentially big driver of diversity. We continue to analyze the microbial communities of hydroponic lettuce production in Ohio through amplicon sequencing survey of nine commercial facilities from 23 to 13,150 m² in size using NFT or DWC setups. By studying the microbial composition of shoot, root, water, and operational surfaces (e.g., floor or benches), we will identify critical points for crop protection and food safety, with the aim to optimize management and production practices. Deciphering the microbiome is a great opportunity to develop specific and accurate biofertilizers, biostimulants or to use resident microbes as biocontrol agents for plant health.

Rapid detection of dollar spot caused by *Clarireedia jacksonii* and *C. monteithiana* using probe based loop-mediated isothermal amplification

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Rapid detection of dollar spot caused by *Clarireedia* spp. is required for an effective control and for reducing turfgrass management costs. Turfgrass is a vast industry where many sectors have low tolerance for disease symptoms. There is a vital need for a fast, accurate disease diagnosis. Dollar spot of cool-season grasses (*C. jacksonii*) and warm-season grasses (*C. monteithiana*) in the United States, cause symptoms of sunken, necrotic spots that can kill turfgrass plants to the soil surface. This study was performed to develop a rapid pathogen detection method for *C. jacksonii* and *C. monteithiana* using probe-based loop-mediated isothermal amplification (LAMP). LAMP primers were created targeting the calmodulin gene region (CaM) and were designed to be specific to *Clarireedia* spp. When first LAMP assay validation results did not yield specificity, therefore a loop backward (LB) probe for fluorescence resonance energy transfer (FRET) was designed and added, increasing the specificity. The LAMP assay was temperature-optimized and tested for specificity and sensitivity. The assay worked best at 72°C and had no cross amplification to other tested species. The LAMP assay had a sensitivity limit of 0.05 ng/μL. The resulting assay can be used to detect *C. jacksonii* and *C. monteithiana* in sample DNA in under 20 min. This assay can be used directly for diagnosticians or trained turfgrass managers to rapidly and specifically identify the causal agent of dollar spot. Future work will include developing a field-ready protocol using these LAMP primers and procedures.

Biological control of bacterial leaf streak of corn by *Bacillus* strains that trigger induced systemic resistance

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Bacterial leaf streak of corn, caused by *Xanthomonas vasicola* pv. *vasculorum* (Xvv), is an emerging disease in North America. Sources of host resistance are being identified, but cultural and chemical control measures are currently unavailable. The objective of this study was to determine whether there is potential for using plant-growth promoting strains of *Bacillus* spp. to provide biological control of bacterial leaf streak. In two greenhouse experiments, *B. pumilus* R190 and *B. subtilis* 6051 were applied separately as cell suspensions to corn seed prior to planting, and untreated seed was the control. The plants were grown in pots to the late V3 to early V4 stage when they were inoculated with Xvv. Pathogen inoculation in one experiment was performed by spraying leaves with a suspension of Xvv, while leaves were swabbed with bacterial cells in the other experiment. In both experiments, seed treatment with *B. pumilus* R190 reduced the severity of bacterial leaf streak by over 50% compared to the untreated control. Disease severity was reduced by seed treatment with *B. subtilis* 6051 in one experiment, but not the other. The reduction in the foliar disease through the application of bacteria to seed was an indication that induced systemic resistance was involved as a control mechanism. Furthermore, this is the first demonstration that plant-growth promoting bacteria have potential as biological control agents to suppress a bacterial disease in corn.

Type III effector AvrXacE1 of *Xanthomonas citri* pv. *citri* suppresses plant immunity

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Citrus cancer is caused by *Xanthomonas citri* pv. *citri* (Xcc) and affects most commercial citrus varieties and causes significant economic loss. Effectors secreted by type III secretion system (T3SS) play important roles in infecting hosts including suppressing plant immunity or assisting acquisition of nutrients from plant. However the functions of most Xcc T3SS effectors remain poorly understood. Here, we investigated AvrXacE1. AvrXacE1 has N-myristoylation site and is conserved among Xcc varieties. In a previous study, the avrXacE1 mutant caused lesions with larger necrotic areas relative to the wild-type strain when infiltrated in citrus leaves. Transient expression of *avrXacE1* in *N. benthamiana* leaves decreased ROS production triggered by Flg22. Mutation of N-myristoylation site (AvrXacE1G2A) led total inhibition of Flg22-triggered ROS. Both AvrXacE1G2A and AvrXacE1 induced ion leakage in *N. benthamiana* cells, but AvrXacE1G2A caused dramatically more ion leakage. At 3-days post-inoculation, cell death was observed in AvrXacE1G2A-expressing leaf region. However, AvrXacE1G2A-induced cell death was not accompanied with increased expression of PR genes (PR1, PR1 and PR5). On the contrary, AvrXacE1G2A inhibited *Agrobacterium*-induced expression of PR genes (PR1, PR1 and PR5), indicating that AvrXacE1G2A-activated cell death doesn't belong to traditional defense response. The molecular mechanism of AvrXacE1 suppressing plant immunity will be further discussed.

Detection of endogenous and episomal *Rubus yellow net virus* in different raspberry cultivars

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Rubus yellow net virus (RYNV), a badnavirus, can be present in endogenous (inactive) and/or episomal (infectious and active) forms. We sequenced the genome of 25 raspberry cultivars and found a diverse array of fragmented RYNV DNA in 76% of material. Sequences were re-assembled to create at least six near full-length RYNV lineages. None of the newly discovered lineages has an intact genome and no badnavirus particles were observed using electron microscopy with antibody against *Sugarcane bacilliform virus*. We developed SYBR Green PCR assays to genotype the new lineages as well as the episomal RYNV (GenBank accession number: NC_026238), and confirmed that the new lineages are endogenous. Endogenous RYNVs are very widespread in commercial raspberries, and having a fool-proof diagnostic test will facilitate safe movement of planting stock across borders.

The effects and mechanisms on abiotic and biotic stress tolerance of Chinese cabbages induced by *Bacillus mycoides*

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Bacillus mycoides (BM) is a rhizosphere-associated bacterium with plant growth-promoting property. In agriculture, applications of BM have been reported to have beneficial effects on plant against biotic and abiotic stresses. This research is to evaluate the beneficial effects of BM (BM02 isolate) on Chinese cabbage under biotic and abiotic stresses. First of all, the interactions between BM02 and various stresses were assessed. Secondly, BM02-treated Chinese cabbages were subjected to different stresses and the effects of BM treatment under these stresses were evaluated. Lastly, defense-related genes expressions of BM02-treated Chinese cabbage with and without pathogen inoculation were analyzed to uncover the mechanisms of BM02 beneficial effects. BM02 showed higher adaptability in mild salinity and higher temperature, and demonstrated significant inhibition on the growth of *Fusarium oxysporum* f. sp. *rapae* (F-43). BM02 treatments also enhanced tolerance to salt stress during Chinese cabbage seed germination. Additionally, Chinese cabbage seedlings showed decreased wilt disease index during F-43 infection after BM02 treatments. Genes expressions of Chinese cabbage seedlings demonstrated that salicylic acid and jasmonic acid signaling-related genes were induced by BM02 pre-treatments after F-43 inoculation and differences in gene expressions were observed in different Chinese cabbage lines. In conclusion, BM02 could promote the growth, enhances stress tolerance, and showed biocontrol potential to Fusarium yellows in Chinese cabbage.

***Pythium* species found in Pennsylvania soybean production systems**

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Pythium is an important pathogen which, in conjunction with other seedling disease pathogens, causes an estimated annual soybean yield loss of 0.5–3% in Pennsylvania (PA). This study focuses on identifying *Pythium* species in PA soybean production systems, particularly to compare species variation between Southeastern PA where soybean has historically been grown and studied and Northern and Western PA. Bulk soil samples were collected in 2018 from fields located in 17 PA counties at different soybean growth stages or production periods (V1, R1, R6, R8, and post-harvest). Putative *Pythium* isolates were identified using P5ARP selective media, which yielded over 300 isolates that were then grown on PDA with cellophane to collect mycelium for DNA extraction. PCR amplification targeting the internal transcribed spacer region (ITS-1 and ITS-4) was completed prior to PCR product purification and Sanger sequencing is being done at the PSU Genomics Core Facility. Sequence reads are used to obtain a consensus sequence to input into the BLASTn search algorithm for *Pythium* species identification, which will enable the comparison of *Pythium* species by region. Overall, while regional variation in *Pythium* species makeup is expected, *P. sylvaticum* is hypothesized to be the most common species found in PA soybean production systems. Ultimately, this project lays the groundwork for further research that identifies best management strategies tailored to soybean farmers by region.

Bradyrhizobium japonicum* IRAT FA3 alters *Arabidopsis* root architecture via regulation of auxin efflux transporters *PIN2*, *PIN3*, *PIN7*, and *ABCB19

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Beneficial rhizobacteria can stimulate changes in plant root development. Phytohormones production and distribution play a major role in plant root architecture. We found that the plant growth promoting rhizobacterium (PGPR) *Bradyrhizobium japonicum* IRAT FA3 produces auxin in culture and increases auxin levels in plants. Using RNA-sequencing of wild type *Arabidopsis thaliana* plants and loss-of-function mutants that had been co-cultivated with *B. japonicum*, and confocal microscopy we showed that this PGPR influenced specific auxin efflux transporters to alter root morphology. Co-cultivation of the bacterium with loss-of-function auxin efflux transport mutants revealed that *B. japonicum* requires *PIN3*, *PIN7*, and *ABCB19* to increase lateral root development and utilizes *PIN2* to reduce primary root length. The absence of *B. japonicum*-stimulated lateral root growth, in the form of accelerated lateral root primordia development through emergence, in single *pin3*, *pin7*, or *abcb19* mutants suggested independent roles for *PIN3*, *PIN7*, and *ABCB19* during the plant-microbe interaction. Gene expression profiling of host transcripts in control and *B. japonicum*-inoculated roots of the wild-type *A. thaliana* accession Col-0 confirmed upregulation of *PIN2*, *PIN3*, *PIN7*, and *ABCB19* with *B. japonicum* and identified genes potentially contributing to a diverse array of auxin-related responses. Our work demonstrates *B. japonicum*'s influence over host transcriptional reprogramming during plant interaction with this beneficial microbe and the subsequent alterations to root system architecture.

Overexpression of a phenylpropanoid master regulator impacts responses to stalk rots in sorghum

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Sorghum (*Sorghum bicolor*) is a drought tolerant C4 grain crop grown throughout the world for food, feed, and fuel. In spite of its drought tolerance, sorghum is susceptible to stalk rots caused by *Macrophomina phaseolina* (charcoal rot) and *Fusarium thapsinum* (Fusarium stalk rot), especially under drought conditions. Phenylpropanoid biosynthesis is involved in plant responses to biotic and abiotic stresses; therefore, altering phenylpropanoid biosynthesis may affect immune responses. Previous research showed that lines overexpressing SbMyb60, a positive activator of phenylpropanoid metabolism, grown under normal growth conditions, had altered central metabolism, response to oxidative stress and components of the immune system as compared with the wild-type. In contrast, overexpression of the phenylpropanoid enzyme, caffeoyl coenzyme A 3-O-methyl transferase (SbCCoAOMT) resulted in few differentially expressed genes compared to the wild-type. In the current study, when infected with *F. thapsinum* or *M. phaseolina*, SbMyb60 overexpression lines had shorter lesions than the wild-type under well-watered conditions. SbCCoAOMT overexpression lines had shorter lesions in response to *M. phaseolina*, but only under water deficit, whereas they were susceptible to *F. thapsinum* under both conditions. Transcriptomics of *F. thapsinum*-infected SbMyb60 overexpression lines indicated activation of genes involved in phytohormone signaling, protein turnover, spliceosome, and responses to other stresses.

Caliciopsis canker disease, an emerging native disease, on *Pinus strobus* in Michigan

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Occasional mortality and branch flagging on understory *Pinus strobus* L. have been reported in northern Michigan, USA, since 2006. Disease signs and symptoms were similar to those of Caliciopsis canker disease, an emerging native disease caused by *Caliciopsis pinea* Peck currently affecting *P. strobus* in New England and Appalachia, USA. This disease is characterized by reddish, sunken cankers on *P. strobus* that eventually coalesce and girdle branches and stems. Our goals were to 1) ascertain the distribution of *Caliciopsis* in Michigan, 2) identify the species of *Caliciopsis* present, and 3) compare the virulence of *Caliciopsis* spp. on *P. strobus*. From 2017 to 2019, we surveyed *P. strobus* trees and collected samples of *Caliciopsis* fruiting bodies in forests across Michigan. To determine the species of *Caliciopsis* present at each site, the ITSrDNA gene region was sequenced from single hyphae isolates cultured from the *Caliciopsis* fruiting bodies. Phylogenetic analyses indicated that *C. pinea* and a novel *Caliciopsis* species were present on *P. strobus*. *Caliciopsis* was present in 35 of 70 surveyed stands. Pathogenicity trials on *P. strobus* seedlings and excised stems indicate the novel *Caliciopsis* species can cause Caliciopsis canker disease. Future research will focus on epidemiology of *Caliciopsis* spp. in affected *P. strobus* stands, additional host risk assessments, and describing the novel species. Assessing distribution and virulence of *Caliciopsis* spp. is critical for informing efforts to mitigate pathogen impact.

Three *gab* genes facilitate shifting the cyclic flux of the TCA cycle to an alternative non-cyclic pathway via GABA shunt in HLB-infected citrus

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Huanglongbing (HLB), caused by '*Candidatus Liberibacter asiaticus*', is a devastating citrus disease worldwide. The tricarboxylic acid (TCA) cycle is an iconic metabolic hub in all aerobic organisms. Under biotic and abiotic stress, plants might shift the cyclic flux from the TCA cycle to an alternative non-cyclic pathway via γ -Aminobutyric acid (GABA) shunt. Herein, we used integrative metabolomics, fluxomics, transcriptomics, and bioinformatics to understand how '*Ca. L. asiaticus*' manipulates the conversion of α -ketoglutarate to succinate outside the TCA cycle. Infection with '*Ca. L. asiaticus*' reduced α -ketoglutarate but enhanced the succinate and GABA levels. In silico analysis showed that the citrus genome possesses three putative genes that connect the GABA-shunt with the TCA cycle. These genes include amino-acid permease (*gabP*), GABA transaminase (*gabT*), and succinate-semialdehyde (SSAD) dehydrogenase (*gabD*). *gabP* is an amino acid transporter that transfers the cytosolic GABA into the mitochondria. Once GABA is transferred into the mitochondria, it is catalyzed by *gabT* to produce SSAD. Finally, *gabD* catalyzes SSAD to succinate. Interestingly, the transcript levels of all these genes were increased due to '*Ca. L. asiaticus*' infection and exogenous GABA application. Moreover, the D₆-based fluxomics showed that exogenous D₆-GABA was quickly metabolized to D₄-succinate in treated plants. Collectively, our findings suggest that *gabP*, *gabT*, and *gabD* contribute to the non-cyclic flux toward succinate via GABA Shunt rather than from α -ketoglutarate directly via the TCA cycle.

The National Plant Diagnostic Network's Professional Development Program: An opportunity for research application, education, and outreach

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To meet the challenges of safeguarding agriculture, forests and natural resources, it is critical for plant diagnosticians to continuously update their knowledge and the methods used in their labs. Since its establishment, the National Plant Diagnostic Network (NPDN) has regularly provided opportunities for the professional development (PD) of its members. Although most diagnosticians participate in PD activities and contribute to their development, the resources generated through these efforts were not often widely distributed nor updated in a sustainable manner. To address this, the 2019 NPDN Strategic Plan included the formation of a PD committee and program. The NPDN PD Program is currently under development and aims to provide resources for diagnosticians, researchers, students, agricultural professionals, and other members of the plant pathology community to strengthen their knowledge and skills. PD resources will include topics related to diagnostics and detection fundamentals and methods, laboratory information management, materials for new diagnostician onboarding, trainings, workshops, and meetings. A robust and sustainable PD program will increase our

ability to respond to ongoing disease/pest problems and emerging threats to plant systems, and positively impact our communities. The development of the PD Program creates new opportunities for NPDN members and labs to collaborate with researchers and educators to develop PD, extension, and outreach resources that will not only benefit diagnosticians, but also the wider plant pathology community.

Viral determinants of evolution and host adaptation

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Within a given environment, plant-virus infections involve complex interactions between virus, vector, and host factors. Viruses generate genetic diversity through mutations, recombination, and reassortment. Selection pressure acts on these variants through host and vector interactions, resulting in changes in the virus genome. To maximize the flexibility and function of their genome, viruses have a variety of genome structures and translation methods. Segmented genomes make up 60% of plant virus families with high degrees of reassortment and recombination consistently driving the evolution of new species and strains. Symptoms have been traced to specific proteins, such as silencing suppressors, movement proteins, and coat proteins. Single amino acid mutations in these proteins can alter pathogenicity or symptom development. Further, resistance breaking strains and host range expansions are linked to genome-level changes. This indicates that viral determinants are either gained or lost by specific viruses, likely correlated with specific mutations or the gain of new proteins. Therefore, viral determinants of adaptation and evolution have a genetic basis that can be identified by comparing and contrasting viral genomes across and within virus families. This can be done by examining DNA and RNA variation in connection with the function and structure of viral factors. Studying genome-wide variation has the potential to provide novel insights into the mechanisms driving virus evolution and the development of new viral properties, such as adaptation to new hosts.

Exploring the responses of elite wheat germplasm to the bacterial wheat streak pathogen

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Xanthomonas translucens, the causal agent of bacterial leaf streak (BLS) in wheat, is a rising pathogen that is present in all of wheat-growing regions of the world. There is currently no commercial resistance available that is effective against BLS, and because it can be seed-transmitted epidemics may become more common. *X. translucens* infects during humid and warm environmental conditions, so there is the potential for BLS to become more problematic in future years due to climate change and therefore there is a great need for BLS disease resistance. Here, we screened 50 elite winter wheat germplasm lines from the Colorado wheat breeding program and 12 commercial varieties for resistance and susceptibility against 2 2018 Colorado *X. translucens* isolates from barley and wheat. We found that these two isolates displayed very different symptoms between the wheat varieties, suggesting that there are differences in both the host responses between wheat varieties and the pathogen virulence between isolates. We plan to develop markers to discover resistance/susceptibility loci that can be incorporated (resistance) or eliminated (susceptibility) from the Colorado wheat breeding program, map the genes involved in the host response, and determine the molecular mechanisms governing the resistance/susceptibility. These findings may hold future promise for discovering resistance against BLS in wheat.

Genetic resistance to *Bean leafroll virus* in pea genetic resources

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Bean leafroll virus (BLRV) is a major pathogen impacting pea (*Pisum sativum* L.) production in the US Pacific Northwest region. Leaves at the top of symptomatic plants are often yellow, distorted and plants are stunted. Pods are underdeveloped or do not form and seed quality and yield are negatively impacted. The virus is transmitted by aphids in a persistent manner. The most effective and economical management approach for this disease is the use of host resistance. The pea aphid, *Acyrtosiphon pisum* Harris, is the primary vector and was used in the current research to phenotype genetic resistance to BLRV of 279 pea accessions from the USDA National Plant Germplasm System (NPGS), Pea Single Plant Collection, under greenhouse conditions. Four currently grown US commercial cultivars were also evaluated. Twenty-three accessions were determined to have complete resistance to BLRV of the lines evaluated based on negative PCR results. These resistant accessions originated from 17 countries spanning four continents, Africa (1), Asia (7), Europe (11), North America (3), and South America (1), indicating the potential for unique sources of host resistance. Previous research determined the recessive gene *lrv* was responsible for BLRV resistance in pea, but this gene has not been successfully mapped. A genome-wide association study will be conducted on the BLRV-screened lines that have previously been genotyped and a mapping population developed to identify molecular markers that can be used in marker-assisted selection to identify pea germplasm with resistance to BLRV.

Dynamic shifts in foliar fungal and plant diversity controls on grassland productivity through the growing season

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Foliar fungi and plant diversity are important mediators of plant biomass in grasslands. However, potential seasonal variation (early versus late season) in the impacts of fungi and diversity on plant productivity has significant consequences for the provisioning of energy and carbon to grassland ecosystems, yet remains poorly understood. Here we measure how foliar fungal and diversity controls on plant biomass production vary in significance across the growing season and their impacts on plant biomass phenology (timing of maximum biomass) using 8 years of biweekly NDVI measures in a grassland experiment that manipulates plant species richness and foliar fungi through the use of pesticides. We found that the foliar fungicide treatment increased biomass most late in the season, whereas biomass differences between high diversity plots and monocultures were greatest early in the season. The impact of foliar fungi on biomass production also varied with plant diversity, with the greatest effects observed in high diversity plots. Finally, more diverse plots and plots containing foliar fungi reached maximum biomass earlier than monocultures and plots treated with foliar fungicide.

Taken together, these results highlight the significant role that foliar fungi play in dynamically reducing the availability of plant biomass through the growing season, effectively controlling the delivery of energy and carbon to support the other microbial and animal communities that rely on grassland productivity.

A survey of *Botrytis* inoculum source and fungicide resistance in Willamette Valley, Oregon, vineyards

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Bunch rot (*Botrytis* spp.) is an important grape pathogen that can greatly affect crop quality. Vineyards with bunch rot issues rely heavily on fungicide applications, leading to an increased risk of resistance emergence. In 2020, nine sites from eight different vineyards in the Willamette Valley, Oregon were sampled for *Botrytis* from June to September. Grape clusters, vineyard floor grapevine debris, and nearby wild blackberries were collected, incubated, and visually assessed for *Botrytis*. Bunch rot incidence on clusters throughout the field season ranged from about 3% to over 30%. *Botrytis* isolates generated were screened for fungicide resistance to Benomyl (FRAC 1), Iprodione (FRAC 2), Difenconazole (FRAC 3), Boscalid (FRAC 7), Cyprodinil (FRAC 9), Trifloxystrobin (FRAC 11), and Fenhexamid (FRAC 17). Some level of tolerance to all fungicide classes was seen, with tolerance to more than one fungicide class observed in 25% of the 200 isolates tested. *Botrytis* incidence on vineyard floor debris decreased as the season progressed. For field sites where wild blackberries were present, *Botrytis* on blackberry flower parts and berries was found at low levels throughout the season, and at one site incidence increased dramatically at the end of the season near harvest after significant rainfall. These results suggest that fungicide resistance levels of vineyard *Botrytis* are of concern and should continue to be monitored, along with the seasonal and environmental changes of *Botrytis* inoculum to better time applications of fungicides and other integrated pest management tools.

Knockout of *pil* genes decreases virulence of the plant pathogenic bacterium *Xylella fastidiosa*

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Xylella fastidiosa (Xf) is a xylem-limited, gram-negative bacterium that has an economic impact on important crops worldwide, including grapes, citrus and more recently olives. Type IV pili (TFP) are responsible for the movement of this bacterium through twitching motility, which, together with biofilm formation, is a major virulence factor of Xf that helps colonizing host plants. In this study, a subset of *pil* genes was deleted in Xf by site-directed mutagenesis and their virulence was assessed in tobacco plants. The obtained mutants presented different movement phenotypes, including higher twitching motility ($\Delta pilA1$ and $\Delta pilS$), lower twitching motility ($\Delta pilA2$) and non-motile ($\Delta pilA1pilA2$, $\Delta pilQ$, and $\Delta pilR$). However, regardless of their movement phenotype, all analyzed mutants presented lower virulence when inoculated in tobacco plants. The only exception was $\Delta pilA2$, which caused similar disease incidence and severity to the wild type (WT) in inoculated plants. However, only WT-inoculated plants consistently presented abundant leaf scorch symptoms, while plants inoculated with the mutants presented minor symptoms. In addition, when analyzing the overall Xf population by qPCR in basal and top leaves, only $\Delta pilA1pilA2$ showed a slight defect in colonizing the top leaf of the host plant. Overall, results suggest that proper functioning and regulation of TFP are required for full symptom development caused by Xf.

Phenotypic analysis of maize and maize-teosinte resistance to *Ustilago maydis*

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The corn smut fungus *Ustilago maydis* (*U. maydis*) is responsible for significant corn yield losses infecting all aerial parts and locally inducing tumors. The lack of resistant maize cultivars necessitates the identification of new sources of resistance. The near-isogenic lines (NILs) obtained from the cross between maize (B73) and teosinte (*Zea parviglumis*) have shown some evidence of resistance to corn smut. However, little is known about the varietal effects of *U. maydis* on the tassel and ear-gall development. Repeated experiments in a controlled environment were carried out to investigate the resistance of four maize genotypes and two maize-teosinte NILs (B73, H95, Golden Bantam, Mo17, NIL1, and NIL2) to treatment levels of *U. maydis*. Ears and tassels of the six genotypes were inoculated with 10^6 cells/ml of *U. maydis* at four different treatment levels (1 ml, 2 ml, 3 ml, and 5 ml) and phenotypically evaluated for: (1) disease incidence; (2) disease severity; (3) gall size; (4) gall number; (5) gall weight; and (6) the area under the disease progress curve. The area under disease progress curve scores was calculated to compare the resistance between maize genotypes and treatment levels. The two NILs (tassels and ears) were more resistant to *U. maydis* than the four maize inbred lines including the B73 NIL parent. The phenotypic results support the genetic data and indicated that the two maize-teosinte NILs carry the genes that are associated with resistance to *U. maydis* and these genes were derived from the teosinte parent.

Deep-sequencing of viruses and viroids in Michigan hop yards: Predominance of named 'latent' species typically associated with asymptomatic infection

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Hop (*Humulus lupulus* L.) is grown globally for beer production and in demand for craft-brewing. As in other perennial crops, viruses and viroids can accumulate in hop, but their impact on yield and quality is incompletely understood. This gap leaves growers guessing about management, particularly for asymptomatic infections. A key first step is to characterize the viruses and viroids present and the variant distribution. To that end, we deep-sequenced virus-enriched RNA (bulk by locale) from 125 commercial hop plants from 13

areas in Michigan, the fourth largest US hop producer. Virus reads were dominated by sequences most similar to four hop virus species: two that often cause symptoms—*Hop mosaic virus* (HpMV) and *Apple mosaic virus* (ApMV)—and two that do not—*Hop latent virus* (HpLV) and *American hop latent virus* (AHLV). On average, the reads mapped to the latent virus group were 3× more abundant (35.5% of total reads) than those mapped to the symptomatic group (11.5%), with both groups found at most sites. Phylogenetic analysis found variants similar to those from both the Pacific Northwest and Europe. Two plant viroids were also detected: hop latent viroid in all 13 areas and hop stunt viroid in only two. The ubiquity of typically asymptomatic latent viruses and viroids highlights the need to better understand their influence on crop yield and quality. Anecdotal reports suggest that asymptomatic infections might enhance flavor profiles, and research indicates that other viruses may improve host stress tolerance, but the extent of such benefits in hop remains unknown.

Efficacy of *Sclerotinia sclerotiorum* targeted biofungicides *Bacillus amyloliquefaciens* and *Coniothyrium minitans* in soybean

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Sclerotinia sclerotiorum (Lib.) de Bary is a soilborne pathogen of soybean that causes white mold. *S. sclerotiorum* can produce significant yield losses under cool and wet environmental conditions. Current white mold management strategies include variety selection, planting population, tillage, and synthetic fungicides. However, it is hypothesized that *S. sclerotiorum* is at risk of developing resistance to several synthetic fungicides. Biofungicides can be applied in place of synthetic fungicides to provide an alternative mode of action. Contans WG (*Coniothyrium minitans*) and Double Nickel LC (*Bacillus amyloliquefaciens*) are two biofungicides currently available and labeled to suppress or control white mold in soybean. We know that *C. minitans* and *B. amyloliquefaciens* can interact directly with *S. sclerotiorum* leading to disease control. However, limited research has been done to confirm the effectiveness of the commercial products Contans WG and Double Nickel LC in the greenhouse. The goal of this project was to confirm the ability of *C. minitans* and *B. amyloliquefaciens* to control *S. sclerotiorum* under laboratory conditions, and determine the efficacy of Contans WG and Double Nickel LC under greenhouse conditions. Using amended media, we confirmed that *B. amyloliquefaciens* can control the mycelial growth of *S. sclerotiorum*. Through a modified soil plate assay, we confirmed that *C. minitans* can degrade the sclerotia of *S. sclerotiorum*. Efficacy results from the greenhouse will also be discussed.

Cross-cutting investigations shed light on the cause of a decade-old disease syndrome in tree nut

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An idiopathic disease syndrome known as abnormal vertical growth (AVG) threatens macadamia, a commercially important tree nut. Yields of AVG-affected trees have reduced by over 70% for decades, and the only control method has been to remove the trees, resulting in a long-term reduction in farm productivity. The syndrome spreads at exceptionally slow rate of approximately 2% per annum and the disease trees are highly aggregated, suggesting the involvement of a pathogen. The disease symptoms suggest that the putative pathogen modulates plant hormone production. Using specific molecular diagnostic assays, we have dismissed some existing hypotheses that AVG is caused by environmental factors alone, phytoplasmas, an endogenous geminivirus and *Bacillus megaterium*. Instead, metabarcoding techniques revealed a consistent association between a *Serratia* species (Enterobacteriales) in the roots and the disease symptoms, providing circumstantial evidence that this is the cause of the syndrome. The bacterial species is known to modulate plant hormone balance, thus may cause epinasty, alter tree architecture and reduce flowering as expressed in the AVG syndrome. This study has helped to focus future research but it is still essential to inoculate healthy trees with this bacterium to see if AVG symptoms can be reproduced.

Determining the detection threshold when pooling samples for rapid detection of QoI resistance in *Cercospora soja*

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We recently developed a rapid, in-field assay to detect QoI fungicide resistance in *Cercospora soja*, causal agent of frogeye leaf spot. In this assay, a single reaction can identify up to three mutations (G143A, F129L, and G137R) in the *cyt b* gene, allowing direct detection from an infected leaf sample in 2–3 h. Our next goal is to analyze a larger number of leaf samples per reaction, to reduce the cost per sample and enable a broader survey at a single time. Thus, we propose pooling lesions from multiple leaf samples of known sensitivity into the same reaction and determination of the detection threshold. We will use two isolates, one with the G143A mutation (the only reported mutation in *C. soja*) and one without (wild-type), to inoculate soybean leaves and produce leaf lesions. Leaf punches of individual lesions will be used in reactions with increasing ratios of sensitive to resistant leaf samples and used to determine the maximum number of samples that can be pooled together and still identify a sample with fungicide resistance. Complementary to this, we will evaluate the threshold of detection using two DNA extraction methods, a proprietary chemical DNA extraction for in-field use and a standard spin column extraction performed in the lab. Finally, since one outcome is to be able to offer a diagnostic service for samples from throughout the U.S., we will validate the assay using QoI-resistant *C. soja* isolates that represent different races reported in the U.S. Rapid detection of fungicide resistance will allow timely response by producers to mitigate yield loss.

The effect of *Septoria glycines* and fungicide application on soybean phyllosphere microbiome

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Septoria brown spot, caused by *Septoria glycines*, is the most prevalent soybean disease in Illinois. We hypothesize that the colonization of *Septoria* has an impact on the phyllosphere fungi. Leaf samples were collected from three soybean lines at V4, R3, and R5 stages. Plots were treated with fungicide and inoculation of *S. glycines*. We sequenced fungal ITS and partial LSU region using oxford

nanopore technology. The alignment of amplicons to Unite and RDP databases produced 3,342 operational taxonomic units (OTUs). The cultivars had a significant effect on the microbiome communities at the V4 stage. OTUs for *Gibberella*, *Alternaria*, *Pleosporales*, and *Cladosporium* were the most abundant in all samples. Other abundant OTUs identified in some samples were *Didymella*, *Colletotrichum*, *Bipolaris*, and *Septoria*. The α and β diversity analysis indicated that inoculation with *S. glycines* did not significantly affect the phyllosphere fungal community. At R5 stage, the fungicide application was an important factor that shaped the fungal communities. However, the abundance of the *Septoria* OTU did not significantly decrease in the fungicide-treated samples. The fungicide treatment significantly reduced the proportion of most fungi but increased *Bipolaris* and *Diaporthe*. Understanding the dynamics of the phyllosphere microbiome is necessary to disentangle the late-season disease complex and provide information for disease control.

LsGRP1^C inducing resistance of strawberry against anthracnose

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Antimicrobial peptides (AMPs) are a class of small peptide, mostly 12–50 amino acids, with antimicrobial activity against bacteria, fungi, etc. LsGRP1 (*Lilium* ‘Star Gazer’ glycine-rich protein 1) is a defense protein of *Lilium* ‘Star Gazer’. It is known that the cysteine-rich C-terminal region, LsGRP1^C, exhibits antimicrobial activity by inhibiting spore germination of many plant pathogenic fungi and conducting lethal effect on different kinds of bacteria. In situ treatment of LsGRP1^C significantly reduce plant tissue necrosis caused by *Colletotrichum siamense* ML133 on the leaves and runners of strawberry. Evans blue staining showed that the death of ML133 infection structure occurred on strawberry leaves with LsGRP1^C treatment. Treatment with LsGRP1^C on the main leaf of a fully developed trifoliate and simultaneously performing fungal inoculation on untreated leaves of same trifoliate reduced the necrotic area incited by ML133. By challenge with flg22, the LsGRP1^C-treated and untreated leaves of the trifoliate increasing callose depositions were observed, indicating that LsGRP1^C combats strawberry anthracnose via an ability of inducing resistance in strawberry.

When microbes go to the spa: Effect of steaming on soil microbiome and chemistry as a phytosanitary practice

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Plant nurseries and land managers are interested in managing soilborne pathogens. Justification: Soilborne pathogens can move in soil between nurseries and can be introduced into the environment through outplantings; this is a major threat to plant health. Previous research shows that steaming soils at high temperatures can kill pathogens (e.g., *Phytophthora* spp.); however, few studies investigate how steaming affects soil microbiota and plant nutrients. Our objectives were (1) to investigate how the soil microbial community identity and function shift after steaming and (2) to measure how steaming influences soil chemistry. Methods: We conducted an experiment in the fall/winter of 2019–2020 where nursery soil underwent a steaming event within a rectangular plot (2.04 × 3.55 × 0.25 m) located outdoors at the National Ornamentals Research Site at Dominican University of California (NORS-DUC). Soil samples were collected at five time points before and after steaming. Thirty soil samples were collected for DNA extraction and 18 for chemical analysis. High throughput sequencing was used to identify the soil microbiome. Initial results show marked shifts in microbial composition and chemistry over time. Notably, plant micronutrients levels fluctuated post-steaming; for example, soil iron levels dropped 24% 1-day post-treatment. Results will contribute to phytopathology by identifying key fungal and bacterial microbes present after steaming and will inform nurseries on how to optimize their soil fertility amendments to support plant health.

Hyperspectral imaging of cranberry fruit for rapid evaluation of breeding accessions

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Breeding and selection of woody perennial crops is time consuming and labor intensive. Our program typically evaluates over 1,000 plots each year during the selection process. One of the key parameters measured is fruit rot, since losses to fruit rot are high in the northeast growing regions. We also evaluate fruit for color, insect damage, size, etc. To date, cranberry fruit samples are either collected from the field and manually sorted for fruit rot determination or estimated visually in the field. Although visual estimations are useful, quantitative data are more desirable. Using hyperspectral imaging (600–1,700 nm), pre-sorted fruit (rotten, sound, spotted, poor color, etc.) were assayed. We then processed the images using automated fruit recognition, separating detritus from the image, and used machine learning to train the system for classification of individual fruit across varied parameters. Several methods were tested, including random forest and neural network classification algorithms. The data and processing algorithm generated will greatly speed in-lab fruit evaluation. In addition, wavelengths that hold the most importance for classification of each fruit characteristic will be used to streamline further algorithm and system development for in-field data collection using less costly multispectral imaging sensors.

Utilizing grower partnerships to validate and promote laser-guided intelligent spray technology for apple disease and pest management

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Implementation of new precision technologies in agriculture is impeded by high cost, perceived risk, and equipment complexity. On-farm demonstration trials with early adopters and industry influencers can increase the adoption of new technologies. Intelligent spray technology is a target-oriented spray control system with a decade worth of research and development. This technology promises reduced pesticide use and spray drift without compromising disease and insect pest control. A demonstration trial that was conducted on

a commercial apple farm in Ohio in 2020 consisted of two side-by-side 2.5 A (1.01 ha) plots sprayed with either conventional or intelligent orchard sprayer for comparison. The grower used their standard fungicide and insecticide program for application timing. Percent marketable yield exceeded 95% in both plots. However, the intelligent sprayer used 280 L/ha less pesticide volume, representing a 42% reduction in pesticide use, and achieved the same level of disease and insect pest control. A science-based outreach program to promote the grower adoption was also implemented, which includes a website (smartapplespray.plantpath.iastate.edu), social media account (Twitter: @ISU_OSUApples), blog posts, technical support videos (YouTube: Smarter Spray for Apples), and grower testimonials. Four additional on-farm trials are planned in Ohio and Iowa with the goal of increasing the adoption of intelligent spray technology in Midwest apple integrated pest management programs.

An updated global *ToxA* haplotype network with evolutionary model for this gene in necrotrophic fungal pathogens

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ToxA is the first proteinaceous necrotrophic effector to be identified in fungal plant pathogens. *ToxA* was originally identified in *Pyrenophora tritici-repentis* (*Ptr*) in 1989, and subsequently detected in other necrotic fungal pathogens. Here we have identified a novel *ToxA* haplotype in *P. nodorum* and analyzed *ToxA* sequences previously published to provide an updated global *ToxA* haplotype network. Interestingly, hypothetical ToxA-like proteins were also identified in the fungal genome databases in species from Dothideomycetes and Sordariomycetes. The distribution of *ToxA* and ToxA-like necrotic effectors in the filamentous fungal plant pathogens seems to be more complex than we thought. The discovery of *ToxA*-like genes in large number of ascomycetes raises the hypothesis that an unknown fungal (or non-fungal) ancestor might be the *ToxA* donor to *P. nodorum* and other pathogens. We have used these information to propose an evolutionary model for *ToxA* in necrotrophic fungal pathogens.

Developing zebra chip disease resistant potato by modulating salicylic acid receptor activity

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Zebra chip (ZC), an emerging disease of potato (*Solanum tuberosum* L.) is caused by the fastidious, phloem-limited bacterium, *Candidatus Liberibacter solanacearum* (CLso). It is transmitted from plant to plant by the tomato/potato psyllid (*Bactericera cockerelli* Šulc.). In plant immunity, systemic acquired resistance (SAR) response is mediated by NON-EXPRESSOR OF PATHOGENESIS RELATED (NPR) proteins, which are transcriptional co-factors and receptors of the defense hormone, salicylic acid. While NPR1 is an activator of SAR in Arabidopsis, its homologs, NPR3 and NPR4, function as negative SAR regulators. In this study, we show that either heterologous expression of *NPR1* or CRISPR/CAS9-based genome editing of endogenous potato *NPR3* can impart resistance to zebra chip disease. A heterologous *NPR1* gene from tomato (*S. lycopersicum* L.) was identified by phylogenetic analysis and overexpressed in potato. Similarly, a potato ortholog of *AtNPR3* was identified (*StNPR3*) and edited by CRISPR/CAS9 technology. Expression analysis of pathogenesis-related (*PR*) genes indicates enhanced SAR responses in the transgenic plants. Greenhouse experiments, no-choice feeding bioassay with CLso-carrying psyllid on *StNPR1-OE* plants, and with *StNPR3*-edited plants showed enhanced ZC disease resistance and significant reduction in CLso titers, when compared to non-transgenic plants. In conclusion, we demonstrate that genetic manipulation of salicylic acid receptor activity of NPR genes in potato is a viable strategy for developing ZC disease resistance.