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Characterization of novel thrips vector proteins that bind to tomato spotted wilt virus
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Tomato spotted wilt virus (TSWV) is transmitted by thrips species in a persistent, propagative manner. *Frankliniella occidentalis* is the most efficient vector, but at least six other thrips species are competent vectors. The TSWV structural glycoprotein (G_N) plays an important role in viral attachment and possibly entry into insect host cells. However, the insect molecules that interact with G_N during infection and dissemination in thrips vector are still largely unknown. The goals of this study were to characterize TSWV-interacting proteins (TIPs) that interact directly with TSWV G_N and to localize expression of these proteins in thrips tissues of importance along the route of virus dissemination. We identified six novel TIPs from first instar larvae (L1) that share homology to proteins associated with the infection cycle of other vector-borne viruses. Two of the thrips proteins, endocuticular structural glycoprotein (endoCP-G_N) and cyclophilin, were validated to be consistent interactors with G_N in membrane-bound yeast two-hybrid and insect cell co-localization assays. Immunolocalization of these TIPs in L1s revealed robust expression in the midgut and salivary glands of *F. occidentalis*, the key tissues during viral infection, replication and plant-inoculation. In addition, the homologous endoCP-G_N from another thrips vector *F. fusca* was cloned and validated to interact with TSWV G_N. Our findings suggest that the TIPs-G_N interaction occurs in vivo and the TSWV G_N-endoCP-G_N interaction may be conserved among *Frankliniella* vector species of TSWV.

Investigations on the quiescent behavior of *Diaporthe ilicicola* in deciduous holly fruit
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Cut branches of deciduous holly (*Ilex* spp.) carrying brightly colored fruit are commonly used for winter holiday decorations, providing nurseries with a source of revenue in low-income periods of the year. In 2012, a fruit rot disease arose in the US, often leading to significant loss of marketable branches. One of the pathogens, the fungus *Diaporthe ilicicola*, was found to infect flowers during bloom and cause fruit rot symptoms later in the season. The delayed symptom onset suggests that the fungus undergoes a quiescence period until conditions change to favor tissue colonization. We inoculated with *D. ilicicola* during bloom and monitored physiological changes throughout the growing season in the resulting fruit. A strong positive correlation was found between disease incidence and soluble sugar concentrations, and a negative correlation was seen between disease incidence and phenolic compound concentrations. We hypothesize that the observed increase in susceptibility may be facilitated by greater concentrations of available sugars serving as a carbon source for the pathogen, and/or by a decrease in phenolic-based antifungal compounds within the fruit tissue. These results will direct future research aimed to determine the roles of available sugar concentrations within *Ilex* fruits in initiating *D. ilicicola* infection and to identify phenolic antifungal compounds which may inhibit *D. ilicicola* spore germination or infection.

Linking sources and sinks in the long distance aerial dissemination of ubiquitous pathogens
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The few cases where long distance aerial trajectories of plant pathogens from source to sink have been confirmed have specific traits: i) there are a limited number of sources, ii) sinks emerge along paths of air movement, and iii) sink and source populations have low enough genetic diversity to facilitate statistical comparisons of their population structures. Whereas rust fungi have all these traits, ubiquitous, highly diverse pathogens with broad host ranges do not - and this impedes understanding the dimensions of their natural spread. Our objective is to link sources, sinks and the long distance aerial trajectories of dissemination of ubiquitous pathogens. Here we have focused on *Pseudomonas syringae* because of its association with atmospheric processes of the water cycle. Its capacity to incite freezing of cloud water droplets and precipitation fosters its fall-out from long distance travel in air masses. For a sink where we collected *P. syringae* in snowfall - the observatory at Jungfraujoch, CH (46.5475° N, 7.9792° E, 3500 m altitude) - we examined the air mass trajectories of these snowfalls to identify possible sources. From one potential source – ground cover in northern Iceland – we have collected hundreds of strains of *P. syringae*. We present the comparison of the genetic diversity of Icelandic strains with strains from Jungfraujoch and other sites to assess if atmospheric connectivity between sites assures long distance dissemination of *P. syringae*.

Association mapping and transcriptomic analyses reveal genes associated with resistance of *Populus trichocarpa* to Septoria leaf spot
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The ascomycete *Sphaerulina musiva* causes Septoria leaf spot and stem canker of *Populus* and is the most serious pathogen of poplars in North America. Knowledge of genes associated with resistance to leaf spot can be used to select for resistant planting stock. We inoculated and phenotyped 540 distinct *Populus trichocarpa* genotypes in a randomized complete block design to identify loci associated with leaf spot resistance. The leaf spot phenotypes were recorded after two weeks and correlated in a genome-wide association analysis to 8,253,066 SNPs and insertion/deletions. Subsequently, RNAseq was applied to study gene expression in a subset of resistant and susceptible genotypes. We will report the heritability estimates for leaf spot resistance, genes associated with phenotypic variation, and patterns of gene expression in response to *S. musiva* inoculation. The significance of the results for resistance breeding and management of foliar diseases in poplar plantations will be discussed.

***In planta* experimental evolution experiments shed new insights on the mechanism and kinetics of *Xanthomonas* TAL effector host adaptation**
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Phytopathogenic bacteria inject transcription activator-like effectors (TALEs) into plant cells to induce the expression of specific host susceptibility genes. The DNA binding specificity of TALEs is dictated by their tandem repeat regions, which are highly variable between different TALEs. We hypothesize that the unique features of the repeat regions of TALEs allow quick adaptation of the pathogens to variations of host target genes. To test this hypothesis, we utilized experimental evolution approach to mimic host adaptation of *Xanthomonas citri* subsp. *citri* (*Xcc*) *pthA4* mutant carrying “non-adapted” TALE variants on sweet orange. We manufactured three “non-adapted” TALEs that where initially unable to induce the transcription of *CsLOB1,* the canker susceptibility gene, due to the presence of miss-matching repeats compared to the native *CsLOB1* targeting TALE, PthA4. The TALEs were introduced into *XccΔpthA4* and bacteria were repeatedly infected and re-isolated from sweet orange plants for 32 cycles. While initially all strains failed to promote canker development, canker symptoms started to appear between 9-18 infection cycles. Sequence analysis of the adapted TALEs identified four adaptive variants, which displayed deletions and rearrangements within the tandem repeat regions of the TALEs. Reintroduction of these adapted variants into the *XccΔpthA4* restored the ability to induce the expression of *CsLOB1*, promote canker symptoms and colonize sweet orange plants. Our data shed light on the TALE adaptation by *Xanthomonas* within a host-pathogen system.

The morphological and molecular characteristics and population dynamics of *Pratylenchus vulnus* in Taiwan strawberry farm
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Pratylenchus vulnus was discovered in Taiwan during a general microbial population survey in two organic strawberry farms in 2017. The root lesion plant parasitic nematode was extracted from the symptomatic strawberry seedlings and the rhizosphere soil. Morphological measurements and the scanning electron microscope observations of both the adult females and males coincide with descriptions of the species. Molecular analysis of the multiple regions of the ribosome gene and the mitochondria COI gene sequences confirmed the morphological species identification and suggested the population was closely related to *P. vulnus* population reported from Italy. Nematode population density of the two farms were monitored for 14 months along with environmental factors including soil temperature, EC value and pH. The nematode population density was found positively correlating with soil temperature while negatively correlated with soil EC only when there are crops in the field between 20–30°C. Soil pH value only correlated with the

population density when the soil temperature lays below 20°C or above 30°C. To our knowledge, this is the first study of *P. vulnus* population dynamics in strawberry field and the first distribution record of the species in Taiwan.

Extension education using animated illustrations and time-lapse videos of disease progression - Schroth Awardee
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Successful implementation of integrated pest management practices requires an understanding of disease cycles and disease diagnosis. Without this background, growers must rely on spray calendars and often over-use or inappropriately use chemicals to manage diseases. Transmission of this information to growers and agents is often hindered by a dearth of grower-oriented educational materials. Though there are many available disease cycle illustrations, these are generally static, intimidating, difficult to follow, and dated. Reference images and videos used as disease diagnosis aids are often blurry, pixelated, and low-quality. We are currently targeting these problems for gray mold and anthracnose on strawberry and brown rot on peach. We produced a high-quality, jargon-free disease cycle illustration and animation for gray mold on strawberries. To aid in disease diagnosis, we also produced time-lapse videos of gray mold, anthracnose, and brown rot showing every stage of the infection process. The time-lapse images are taken in a studio made to mimic a field setting. We also produced a gallery of professional images for these diseases at the macroscopic and microscopic level. These fresh, intuitive, and scientifically accurate materials can be used by growers to self-educate, by extension educators in grower-training sessions, and by teachers in a classroom setting. The challenges and rewards of creating these materials will be discussed.

Genome-informed recombinase polymerase amplification assay for specific and sensitive detection of *Dickeya* species at point-of-care
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Dickeya species cause soft rot and on black leg diseases on potato, but also responsible for soft rot in many other plant species worldwide. Rapid pathogen detection is essential to facilitate efficient disease management. Our aim in this research was to develop a field deployable recombinase polymerase amplification (RPA) coupled with a lateral flow device (LFD) in order to detect *Dickeya* species from infected plant tissues without the need of DNA isolation. The comparative genomics approach was used to select a unique *mgl*C gene for designing highly specific and robust primers and probe. The RPA assay specificity was validated with 35 strains from all *Dickeya* species and 15 strains from other neighbor genus and species; no false positives or negatives were detected. RPA primers and probe for internal control were also used to enhance the reliability and accuracy of the devloped assay. The detection limits of 1 fg was determined by both sensitivity and spiked sensitivity assays; no inhibitory effect was observed when 1 µl host sap, macerated in TE buffer, was added in each sensitivity reaction. The developed RPA assay is rapid, highly accurate, sensitive and fully field-deployable. It has numerous applications in routine diagnostics, surveillance, biosecurity and disease management.

Effectiveness of fungicide programs against turfgrass fungal disease leaf and sheath spot (mini ring) on Bermuda grass in Hawaii
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Leaf and sheath spot/blight, or mini ring, is a turfgrass fungal disease confirmed in multiple states in USA. The causal pathogen is commonly considered to be Waitea circinata var. zeae, more commonly known as *Rhizoctonia Zeae*. It can be hosted by both cool- and warm-season turfgrasses. This disease usually causes thinned turf areas and/or thinned rings that can be full, half, or quarter circles, with various diameters. Turfgrass under drought stress or routinely irrigated with high salinity water is prone to infestation. Management of this disease on turfgrass is challenging. Some cultural practices can be beneficial, such as raising mowing height; and increasing fertility and using slow-release fertilizers to avoid nutrient deficiencies. This research evaluated 7 fungicide programs and a control, arranged in randomized complete blocks with 4 replicated plots per treatment on Bermuda grass at a golf course in Hawaii. Each plot was 10 ft by 10 ft, with 2-ft buffer area between adjacent plots. Treatments were applied 14 days apart, for a total of 7 times. Data (all on 1-9 scale) on disease severity, turfgrass greenness, and overall turfgrass quality were measured by 3 people prior to treatment and after each fungicide application. Results indicated that active ingredient Azoxystrobin resulted in the best disease suppression and turfgrass quality improvement, and active ingredient Chlorothalonil + Acibenzolar-S-methyl performed the second best. It is recommended that fungicide programs should be used in conjunction with cultural practices to manage this turfgrass disease.

Identification of a new member of the family *Phenuiviridae*, order Bunyavirales, associated with lettuce dieback disease
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Lettuce dieback disease (LDD) causes foliar necrosis, stunting, and death of lettuce plants throughout all western US lettuce production regions. The disease is more prevalent in poorly drained soils and is traditionally known to be caused by either of two viruses from the genus *Tombusvirus*: *Tomato bushy stunt virus* (TBSV) and *Moroccan pepper virus* (MPV). However, in recent years, neither tombusvirus has been found in symptomatic plants collected from the field, nor have degenerate primers detected any divergent tombusviruses from diseased lettuce. To identify additional viruses that may be associated with LDD, lettuce samples were collected from several symptomatic fields and leaves were used to mechanically transmit infectious agents to an array of test plants, resulting in symptoms indicative of a virus but distinct from those caused by TBSV or MPV. High throughput sequencing of RNA extracts from symptomatic lettuce field samples and *Nicotiana benthamiana*test plants consistently identified a virus with 31–36% homology to a virus recently characterized from watermelon, associated with the family *Phenuiviridae*, order *Bunyavirales*. Primers were developed to

a portion of the polymerase protein, and RT-PCR analysis of archived field samples indicates association of the new virus with plants exhibiting lettuce dieback symptoms. Continuing molecular and biological studies are characterizing the genome of the new virus and its involvement in LDD disease development.

Evaluation of sweetpotato germplasm for resistance to pathotypically diverse isolates of *Meloidogyne enterolobii* from the Southeastern United States

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Meloidogyne enterolobii [a.k.a Guava Root Knot Nematode (GRKN)] is an invasive species that has been slowly spreading across the Southeastern United States over the past twenty years. Recent reports of damage caused by this nematode on sweetpotato have raised serious concerns among growers and regulatory agencies because of its high-level of virulence on a wide range of crop varieties that are normally resistant to the endemic root knot nematode species. During recent surveys in South Carolina, we identified multiple isolates of GRKN originating both from field soil, as well as from fresh market sweetpotatoes. These findings in sweetpotato indicate that this clonally propagated crop has the potential to vector the spread of this nematode. Using a GRKN isolate from South Carolina we screened eighty-seven sweetpotato lines from the USDA-GRIN germplasm collections, and successfully identified multiple lines with high levels of resistance to galling and GRKN reproduction. Further screening of this resistant sweetpotato germplasm against additional GRKN isolates identified pathotypic variability, with certain lines providing resistance to only one nematode isolate. Our results provide valuable insight for breeders, farmers, and pathologists who are seeking to manage, monitor, and deploy resistance against GRKN in the field.

Relationship between sudden death syndrome caused by *Fusarium virguliforme* and soybean yield: A meta-analysis

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A total of 57 uniform field experiments conducted in Illinois, Indiana, Iowa, Michigan, Wisconsin, and Ontario Canada from 2013 to 2017 comparing crop protection products against SDS were analyzed using meta-analytic models to summarize the relationship between foliar disease index (FDX) and yield. For each study, correlation and regression analyses were performed separately to determine three effect sizes: Fisher's transformation of correlation coefficients (Zr), intercept (β_0) and slope (β_1). A random- and mixed effect meta-analysis was used to summarize the effect sizes. Study- and location-specific moderator variables; foliar disease level (FDX; low < 10%, high ≥ 10%), date of planting (early = prior to 7 May, optimum = 7 May – May 21, and late = after 21 May) cultivar (susceptible and partially resistant to SDS), study location, and year were used as fixed effects. The overall mean effect sizes of r (the back transformed Zr) was –0.39 and different from zero ($P < 0.001$) indicating yield was negatively correlated with FDX. Zr was affected by disease level ($P < 0.01$) and cultivar ($P < 0.02$) with a greater effect in higher disease levels and with susceptible cultivars. The mean $_0$ was 4121 kg/ha and mean $_1$ was –21 kg/ha/% and were different from zero ($P < 0.01$). For every unit of FDX increase yield will decrease by 0.5%. Study locations and year affected the β_0 while none of the moderator variables significantly affected β_1.

Number of applications and application date affect phosphonate residue in nutmeats of pecan

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Scab (caused by *Venturia effusa*) can cause substantial yield loss in pecan. Phosphonate fungicides have been used since 2011 to manage scab, and remain a valuable class of fungicide due to their efficacy and low risk of resistance development. Phosphonate use can result in residues in produce destined for human or animal consumption. Recently the EU legislated that nut group crops could not exceed a Maximum residue limit (MRL) of 500 ppm. In the USA, it is not known how foliar (canopy) application affects residue in in pecan nut meats. In both 2016 and 2017 we compared how number of sprays applied, and timing of those sprays affected phosphonate residue in nutmeats. Phosphonate residue increased with the number of foliar sprays applied during the season. The relationship was linear, but the rate of accumulation varied showing an increase of up to 57.7 ppm per spray, depending on experiment. The maximum residue recorded in a sample was 928.0 ppm and was from a tree receiving 9 sprays of phosphonate. In spray timing experiments, later sprays tended to result in higher residues, although this was variable. Although up to 9 sprays were tested, 5 are the maximum currently recommended in the state of Georgia. When 5 sprays were applied, the mean phosphonate residues in nutmeats in the three experiments were 83.8, 152.33 and 146.33, respectively. Thus, even with the recommended usage for phosphonate type products the risk of exceeding the EU MRL is small.

Assessing systemic interventions in combating the spread Fall Army Worm (*Spodoptera frugiperda*): Lessons learned in Tanzania and Ghana

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The Fall Armyworm (*Spodoptera frugiperda*), also known as FAW, is a major food crop pest that was first detected in 2016 in Central and Western Africa, subsequently spreading rapidly throughout sub-Saharan Africa. ACDI/VOCA, an organization that implements

food security projects in developing countries, responded through targeted, systemic interventions in agricultural focused projects located in Ghana (ADVANCE II), and Tanzania (NAFAKA II). Both projects launched localized scouting systems to monitor pest spread; developed strategic media campaigns and leveraged private-sector agribusiness providers and extension specialists to raise awareness of FAW; and spearheaded capacity building activities around management practices. Furthermore, Ghana staff facilitated field trials in 2017 and 2018 that examined the efficacy of various pesticides' in reducing maize (*Zea mays*) ear damage. The Ghana project also created a call center for FAW sightings. NAFAKA II enhanced producer connectivity to input service providers and gave small grants to spray service providers and village based agricultural advisors to address FAW emergence. In 2018, seventy-seven and ninety percent of Ghana and Tanzania project participants, respectively, utilized the promoted FAW management practices and services. A comprehensive study composed of a randomized, statistically representative survey of engaged actors, key-informant interviews and stakeholder focus groups revealed the effectiveness of these interventions in managing the onset of a foreign pest such as FAW, including gaps and lessons learned.

Cultivar and environment influence on epiphytic bacterial diversity on wheat seeds

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Seeds are sources of inocula and can facilitate introduction of pathogens into the field. Therefore, it is important to determine seed microbiota to limit spread of plant pathogens. This study aimed to determine the bacterial diversity on wheat seeds as influenced by variety and location in South Dakota. Twenty grams of seed from three replicates of eight wheat varieties from Aurora and Volga were vortexed twice, sonicated for four minutes in a phosphate buffer suspension at pH of 7.4. Fifteen ml of the seed wash were used for DNA extraction and sequencing, while 5 ml were stored for biochemical tests. Sequencing was done at the University of Minnesota genomics laboratory using 16 S rRNA. The resultant sequences were analyzed using Mothur software. Alpha diversity indices such as Chao1, Simpsom, and Shannon determined species richness and evenness, while beta diversity determined diversity between locations and varieties. The taxa identified were *Xylophilus, Sphingomonas, Pseudomonas, Plantibacter, Pedobcater, Neorrhizobium, Microbacterium, Methylobacterium, Massilia, Hymenobacter, Kinecoccus, muciliginabacter, Enterobacteriaceae, Curtobacterium* and *Chrysobacterium*. *Sphingomonas had* a higher relative abundance compared to other genera in both locations while the genus that could harbor potential wheat pathogens was *Pseudomonas*. The varieties differed from each other in genera relative abundance while there was no significant location effect on relative abundance of the genera. Results indicate limited evidence that cultivar and environment influence bacteria diversity.

Association between soil and root microbiomes and within-farm-spatial-variation of soybean yields: II. The fungal perspective

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The within-farm-spatial-variation of yields (WFSVY) has been a key yield limiting factor in soybean growing states including Pennsylvania. The drivers of WFSVY are presently unknown. We hypothesize that the possible differences of soil and root associated fungal communities contribute to WFSVY in soybean. The goal of this study was to investigate the fungal communities of samples obtained from high and low yielding spots at V1 and R8 soybean growth stages (SGS). At each of the eight farms (in Pennsylvania) chosen for this study, bulk soil (BS), rhizosphere soil (RS), and soybean root (R) samples were collected from five high and low yielding spots. DNA extracted from samples were subjected to PCR targeting fungal ITS regions (ITS1F/58A2R primers) and amplicons were sequenced using MiSeq platform. The β-diversity analysis did not distinguish data points based on yield (high/low), farm, or SGS for BS and RS samples, though a clear grouping was observed based on SGS for R samples. For BS at R8, α-diversity was greater in high yield sites than low sites and the opposite trend was observed for R at R8. The relative abundance of Ascomycota and Zygomycota appeared to be different between high and low sites in BS and R at R8 while Chytridiomycota, Zygomycota, and Basidiomycota were different between high and low sites in RS at R8. The global analyses altogether suggested that fungal community differences can potentially contribute to WFSVSY in Pennsylvania.

Pathogenicity and delimitation of a novel *Corinectria* sp. infecting *Picea rubens*

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Red spruce (*Picea rubens*) has been a primary target for restoration in the Appalachian Mountains over the last few decades following more than a century of decline attributed to forest fragmentation and degradation. Additional threats, especially from introduced pathogens and insect pests, could further destabilize these already fragile ecosystems. An undescribed, pathogenic fungus was recently discovered on stressed red spruce in Virginia and West Virginia. Infections were observed as depressed areas along the stems with red perithecia on the surface of the bark, from which the fungus was isolated. Phylogenetic analyses of beta-tubulin and EF1-α DNA sequences indicated the putative pathogen is closely related to but distinct from the former *Neonectria fuckeliana,* now *Corinectria fuckeliana*. The failure of previous surveys to uncover this *Corinectria* sp., even as a secondary pathogen contributing to red spruce decline, raises concerns that this fungus may represent an emerging pathogen. Broad host range studies are warranted as the closely related *C. fuckeliana* is a known pathogen of *Abies*, *Picea*, and *Pinus*. Excised stem assays have confirmed pathogenicity of this *Corinectria* sp. on red spruce as fungus treated wounds had significantly larger ($p < 0.001$) canker areas (5.5 cm ± 0.17 SE) compared to negative controls (1.3 cm ± 0.13 SE). Additional pathogenicity tests are underway. Given this may represent an emerging pathogen, the pathogenicity of this *Corinectria* sp. is of great concern to identify potential threats to red spruce management and restoration efforts.

The use of highthroughput sequencing for detection and characterization of diverse viruses infecting common beans (*Phaseolus vulgaris* L.) in Zambia

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Common bean (*Phaseolus vulgaris* L.) is an important legume crop in Zambia. However, very little is known about the etiological agents of highly prevalent yield-limiting viral diseases across bean fields in Zambia. Field surveys were conducted during 2017/2018 rainy season across 128 fields, and 641 leaf tissue samples showing virus-like symptoms such as mosaic, necrosis, vein-clearing, curling and rugosis were systematically sampled. Total nucleic acid extracts from these samples were pooled into one composite sample which was first ribo-depleted, then used for cDNA library construction. The library was sequenced using the Illumina NextSeq 500 platform. A tBLASTX analysis of the d*e novo* assembled virus-specific highthroughput sequence contigs (ranging in length from 206 to 5207 nt) resulted in the identification of partial and near complete genome sequences of six viruses belonging to three genera in the sample. They include two known potyviruses, two known alphaendornaviruses, a putative novel *Orthotospovirus,* and a putative novel *Begomovirus*. The results represent the first report and molecular characterization of most of these viruses in Zambia. Studies are ongoing to determine the complete genomes of these viruses, their biological properties and prevalence across common bean fields in Zambia.

Surveys for Asian citrus psyllid (*Diaphorina citri*)-infecting viruses and *Candidatus* Liberibacter asiaticus (*C*Las) dispersal in Florida citrus groves

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Candidatus Liberibacter asiaticus (*C*Las), the causal agent of the world's worst disease in citrus, Huanglongbing (HLB), continues to decimate the Florida citrus industry. *C*las' insect vector, the Asian citrus psyllid (ACP; *Diaphorina citri*), shows increased resistance to current insecticides used by Florida citrus growers, and threatens Florida's management strategies for the now endemic vector and disease. This study investigated the incidence of ACP-infecting viruses throughout Florida citrus groves for their potential use as RNA interfering (RNAi) viral vectors, an alternative and biological control option. Every month over a span of two years, ACP populations were sampled from more than 20 different citrus producing counties in Florida. We successfully detected and monitored five previously identified ACP-infecting viruses throughout Florida citrus groves: the *Diaphorina citri-associated C virus* (DcACV), *D. citri flavi*-like *virus*, *D. citri densovirus*, *D. citri reovirus*-like, and *D. citri picorna*-like virus. Quantitative PCR (qPCR) was also used to track *C*Las titers in these ACP samples and to observe any temporal interactions between *C*Las populations and viral incidence. Preliminary results have shown that the DcACV is the most dispersed and prevalent virus in Florida ACP populations, and that the presence of these viruses have not affected the occurrence of *C*Las infection of these ACPs. Using this data, further research may be able to enhance the specificity and deployment of future RNAi control strategies using viruses for elimination of the ACP and HLB in Florida.

Understanding collaboration of genes in the *Citrus tristeza virus* aphid transmission

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Transmission is an essential part of the survival strategy of plant viruses. The most common insect vectors for virus transmission are aphids, leafhoppers, and whitefly. Little is known about the molecular process of vector transmission for many viruses, or how the virus isolate variation affects transmission efficacy. Here we examined the effect of the 5`end genes from different isolates of *Citrus tristeza virus* (CTV) for aphid vector transmission. CTV, which consists of at least 6 different strains, exhibits a variety of different phenotypic characteristics. These strains are VT, T30, T68, T36, T3 and RB, the nucleotide identities change between 80.5–92.4%. The 5`half of the CTV genome is more diverse than 3`end of the genome. To make matters more complex, in addition to these different strains, the rates of aphid transmission are different for different isolates of the strains. CTV encapsidation into long flexuous virions occurs via a complex mechanism involving at least four genes: CPm, CP, Hsp70 homologue and p61. The involvement of the Hsp70h and p61 proteins in aphid transmission has been reported. However, no work has been done to determine if there are replication-associated proteins involved in aphid transmission. We cloned the different 5`end genes from VT, T68, and T30 isolates of CTV into our T36-based infectious clone, which is very poorly transmitted by the aphid vector. Interestingly, interchanging entire 5`half of the genome produced a substantial increase in transmission by the aphid vector, from 0% transmission efficacy to 23.84% with the T68 substitution.

Spore dispersal patterns and apple infection timing by *Colletotrichum fioriniae*, the main bitter rot fungus of apples in Pennsylvania

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Bitter rot of apple has become a major problem in Pennsylvania in recent years. In response to grower requests for management recommendations, research is being conducted to deliver timely science-based management tools, with an initial focus on the characteristics and disease cycles of the causal fungal species. While a state-wide survey of bitter rot apples uncovered six *Colletotrichum* species, over 75% of the infections were caused by *C. fioriniae*. The timing of *C. fioriniae* infections is being investigated by detecting and quantifying *C. fioriniae* inoculum sources and dispersal patterns throughout the season, and by detecting

quiescent *C. fioriniae* infections in immature apples. Spore dispersal was detected in orchards at full-bloom and continued throughout the season. Spores were detected in nearby forests at quantities as high or higher as in heavily infected orchards, and abundant endophytic *C. fioriniae* infections were detected in various asymptomatic forest understory plant leaves. Immature apples from untreated and inoculated trees had some quiescent infections 3 weeks post-bloom, increasing to nearly 100% incidence by 7 weeks post-bloom. We have therefore established that *C. fioriniae* infections can occur early in the growing season in Pennsylvania, which may necessitate season long control efforts. Ongoing research is looking at whether forests are a source of orchard infections, whether early quiescent infections always persist until harvest to cause necrotrophic infections, and whether apples are equally susceptible throughout the growing season.

Dissecting the molecular basis of strain- and temperature-sensitivity to PVY conferred by the potato *Ny_{tbr}* resistance gene
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Potato Virus Y (PVY) is one of the most important plant viruses affecting potato production and enters in the list of the top 10 plant viruses in molecular plant pathology. Here we have investigated the nature of *Ny_{tbr}*-mediated resistance against PVY in potato (*Solanum tuberosum*). The *Ny_{tbr}* gene is strain specific against the common PVY^Obut not the necrotic strains, and we established its sensitivity to increased temperature. While effective in controlling PVY^O at 21°C, elevated growth temperature was sufficient to trigger systemic spread of PVY^O throughout of the *Ny_{tbr}*-bearing potatoes. To shed light on the interplay of elevated temperature and susceptibility, we dissected the earlier step of viral infection in both *Ny_{tbr}*-bearing cultivar Premier Russet (PR) and PVY-susceptible plants. We established that the PVY^O helper component proteinase (HCPro) alone was sufficient to induce a hypersensitive cell death response in PR plants. Remarkably, cell death was enhanced at 28°C, suggesting that at elevated temperature, the HR cell death was not sufficient for restraining PVY^O, and other defenses may have been compromised. We uncovered that the production of callose was part of the *Ny_{tbr}*-mediated response and occurs at a very earlier time point in the signal cascade prior to the appearance of local necrosis. Callose accumulation upon viral infection was delayed up to 24 hours at elevated temperature. We propose that callose deposition is a key component of the *Ny_{tbr}*-resistance response and may also be a target of virulent necrotic PVY strains.

Population density and virulence phenotypes of *Heterodera glycines* associated with soybean fields in Tennessee
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Soybean cyst nematode (SCN), *Heterodera glycines* is responsible for up to $1.5 billion loss each year in the United States, and SCN is a major soybean pest in Tennessee. The use of resistant cultivars is helpful in reducing losses caused by the nematode, however continuous use of resistant cultivars can initiate a selection pressure that can cause SCN population to reproduce on resistant soybean cultivars. This study was conducted with two objectives: (1) to determine SCN population density in soybean fields in Tennessee, and (2) to determine SCN ability to overcome resistance in the resistant soybean cultivars using the HG – *Heterodera glycines* – Type test. To do this, 116 soil samples were collected from soybean fields across 20 counties, and subsamples were processed for the presence and density of SCN. The density was determined by counting the number of eggs present in 100 cc of soil. Forty-seven percent of the samples were infested with SCN, 17, and 10% had densities greater than 500 and 1000 eggs per 100 cc, respectively. The average and maximum SCN densities observed were 1,048 and 4,569 eggs per 100 cc soil, respectively. These results indicate that SCN occurs in high density in many of the fields tested. HG-type test is being conducted to determine the virulence phenotypes of the populations and will be presented. Growers are advised to take appropriate actions to avoid damage by this pathogen in fields where high densities were observed.

Studying the *In vitro* interactions between *Fusarium virguliforme* and soil-borne nematodes using fluorescent microscopy
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Soybean cyst nematode (SCN) and *Fusarium virguliforme* are major threats to soybean production throughout the U.S. Management strategies for both pathogens are similar and include using genetically resistant varieties, diverse crop rotations, and seed treatments containing a nematicide or fungicide. *Fusarium virguliforme* causes soybean sudden death syndrome (SDS) by infecting root tissues, colonizing vascular tissues, and releasing protein effectors and toxins into soybean xylem, inducing foliar SDS symptoms. Though SCN cannot cause SDS, many studies have identified a strong positive correlation between SDS severity and SCN abundance, though the role of SCN in SDS development remains unknown. In this study, we explored the interactions between *F. virguliforme* and the model nematode *Caenorhabditis elegans* and the soybean cyst nematode *Heterodera glycines*. *Fusarium virguliforme* is able to colonize dead and immobilized nematodes, suggesting that *F. virguliforme* is an excellent saprophyte and may be able to use nematodes as a nutrient source, which could increase *F. virguliforme* inoculum. However, colonization of living mobile nematodes was not observed, indicating that *F. virguliforme* is not vectored into soybean roots by nematodes. However, co-infections of soybean roots by both pathogens may affect the expression of *F. virguliforme* effectors, which was also explored in this study. Overall, these results suggest that successful SCN management may also lead to improved SDS management.

Fungicide sensitivity screening for *Corynespora cassiicola* and field evaluations
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Target spot (TS), caused by the fungus *Corynespora cassiicola*, is a foliar disease of cotton and soybean. Over recent years, TS has become a disease of concern. in both production systems. Data for fungicide sensitivity and understanding potential impact on yield is

lacking for *C. cassiicola*. The objective of this study is to conduct fungicide screening to monitor sensitivity in *C. cassiicola* in Tennessee soybean and cotton production. The sensitivity of 18 *C. cassiicola* isolates to 8 technical grade fungicides across multiple fungicide groups (FRAC Groups 1, 3, 7, and 11) was evaluated based on mycelial growth inhibition assays. The EC_{50} of each fungicide was calculated. All active ingredients had EC_{50} > 100 µg/mL with the exception of difenoconazole which EC_{50} varied among isolates from 3 to 40 µg/mL. Field trials were also conducted at 3 locations for soybean and 1 location for cotton. Five fungicide tank mixes were evaluated for control of TS among 3 soybean varieties of differing susceptibility and 6 fungicide products were evaluated among 1 cotton cultivar. While Miravis Top and Quadris Top SBX reduced TS compared to the non-treated check, only Miravis Top significant increased soybean yield. No treatments were significantly different in the cotton trial. Based on the EC_{50} values and lack of control with products not containing difenoconazole one can conclude there is lack of efficacy and/or resistance already in the isolates tested.

The "Fungal Selfie": Integrating a social media collection in higher education courses
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Although social media are an essential component in the communication of younger and next generation scientists, these tools are underutilized in the classroom. Platforms that focus on images, such as Instagram, provide exciting opportunities for students to share their findings and learn from others. Undergraduate and graduate courses aimed at fungal biology are particularly well-suited for this integration. In the fall semester of 2018, we implemented the "Fungal Selfie" project in an undergraduate/graduate "Biology of Fungi" course at Penn State. Students created a free account through the Instagram application and documented their findings of fungal specimens and other mycologically relevant objects in their environment through the use of images and text in a series of searchable posts. For some posts, students were required to collect and identify the specimen. As a result, students were able to apply their familiarity with social media to the skills of fungal identification and characterization that they would typically present in such a course, thus providing a unique dimension encouraging the students to connect concepts of fungal biology to their lives. This approach could be easily adapted to courses that involve field observation of plant diseases. Additionally, this tool has the potential to allow the product of student learning to reach many more people than just the target audience.

Modeling yield losses and risk analysis of fungicide profitability for managing Fusarium head blight in Brazilian spring wheat
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Fusarium head blight (FHB) is a serious disease of wheat that causes economic losses due to both yield reduction and mycotoxin contamination. A review of the literature on fungicide efficacy evaluated in Brazilian field trials was conducted to obtain FHB-yield data and explore their relationship. Thirty-seven studies (10 years and 11 locations) met the criteria for inclusion in the study (FHB index ≥ 5% and max-min range ≥ 4 percent points [p.p.]). Studies were grouped into two production situations: low (*Yl*< 3631 kg ha^{-1}) or high (*Yh* > 3631 kg ha^{-1}) yield, based on the median of maximum yields across trials. Population-average intercepts differed between *Yl* (2,883.6 kg ha^{-1}) and *Yh* (4,419.5 kg ha^{-1}), but their slopes were similar. The calculated relative yield loss (*Dc*, damage coefficient) was 1.05% pp^{-1} and 1.60% pp^{-1} for *Yh* and *Yl*, respectively. A crop model simulated attainable wheat yields (no biotic stresses) for 10 planting dates within each year during a 28-year period including prior (1980–1989) and after (1990–2007) FHB resurgence. The predicted losses, from using FHB predictions to penalize attainable yield, were in agreement with literature reports. The probability of not-offsetting (P_{loss}) the costs (fungicide + application) of one or two sprays of tebuconazole was calculated. For a range of costs (15 to 30 U$/ha) and wheat price (100 to 250 U$/ton) P_{loss} was higher (> 75%) prior to FHB resurge, regardless the number of sprays, than after 1990.

Updates on management of bacterial spot of cucurbits incited by *Xanthomonas cucurbitae*
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Bacterial spot of cucurbits, caused by *Xanthomonas cucurbitae*, is a serious emerging disease in many cucurbit-growing areas of the world. *X. cucurbitae* infects leaves and fruits. Surveys in the North Central Region showed that more than 85% of pumpkin and winter-squash fields had fruits infected with *X. cucurbitae*. In some of the fields, more than 90% of fruits were affected. Screening of more than 500 pumpkin and squash cultivars and accessions did not identify in any lines with reliable resistance to *X. cucurbitae*. In our field trials, *X. cucurbitae* survived for more than 24 months in infected pumpkin leaves and fruits buried at 5 and 15 cm deep. *X. cucurbitae* survived in naturally-infected and artificially inoculated seeds at 4 and 22°C for more than 18 months. Our studies showed that hot-water treatment at 55°C for 15 min or HCl treatment at 0.5% concentration for 40 min eradicated *X. cucurbitae* in pumpkin seeds. We evaluated 27 chemical compounds and biocontrol agents for their efficacy for management of *X. cucurbitae* in pumpkin fields, and found that mancozeb (Manzate PRO Stick), copper hydroxide (Kocide 3000), copper octanoate (Cueva SC), and an extract from *Reynoutria sachalinensis* (Regalia) were the most effective compounds in reducing the severity of bacterial spot in pumpkin leaves and fruits. Effects of crop rotations for management of the disease are being investigated. In addition, we have collected some bacterial isolates from pumpkin plants, which showed antagonism to *X. cucurbitae* in *in vitro* assays.

Modeling spatiotemporal dynamics and time to regional outbreak of soybean rust in southern Brazil
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Soybean rust (*Phakopsora pachyrhizi*) is the disease of greatest concern to soybean growers in Brazil. The fungus has found the right conditions for overwintering and spreading every season throughout all soybean areas of the country. The national 'anti-rust'

consortium has been responsible for the official first report of soybean rust at a given municipality each season. Using publicly available data of the reports for the southernmost states, Paraná (PR) and Rio Grande do Sul (RS) spanning 14 growing seasons (2004/2005 to 2017/2018) we fit models for describing the disease outbreak onset time and spatiotemporal dynamics. The dataset was composed of 3,596 reports in commercial fields located in 258 and 275 locations in PR and RS, respectively. The Kaplan-Meier estimates of the seasonal mean outbreak onset time (relative to sowing date) ranged from 56.6 to 118.4 days (89.3 days, all seasons). The extended K-function analysis showed a stronger evidence of space-time interaction for the first reports in RS than in PR state. The mean nearest-neighbor distances varied across seasons within a state and were most typically greater early in the season, declining afterwards, which suggests that outbreaks initiated at multiple locations and spread locally. The final size of epidemic area, calculated by connecting polygon extreme points, ranged from 50 to 200 ×103 km2 in RS and 50 to 150 × 103 km2 in PR. Further analysis including the effect of covariate on the time to outbreak and epidemic front will be presented.

Prevalence of streptomycin-resistant *Erwinia amylovora* and epiphytic bacteria in Ohio apple orchards
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Fire blight, caused by the bacterium *Erwinia amylovora* (*Ea*), occurs annually and is the most destructive bacterial disease in Ohio apple orchards. Management of fire blight is challenging and requires an integrated approach to minimize losses. Antibiotics are very effective to reduce *Ea* populations during bloom. Streptomycin, oxytetracycline, and kasugamycin are registered in the United States (US) for fire blight management. Streptomycin is the most cost-effective antibiotic and is frequently used in Ohio apple orchards to control fire blight. While streptomycin-resistant (SmR) *Ea* strains have been reported in several northeastern US states, the prevalence and mechanism of SmR in Ohio orchards has not been reported. In 2018, flowers and shoots from 24 orchards with trees known to be infected with *Ea* were collected and a total of 981 putative *Ea* isolates were purified and screened for streptomycin resistance using bioassays. From these 918 isolates, 215 were confirmed as *Ea* using PCR with primers specific to *Ea* plasmid pEA29. Forty-six percent of the strains were SmR, of which 18% had a minimum inhibitory concentration (MIC) value >500 ug/ml and 26% had MIC values >2500 ug/ml. Streptomycin resistance was observed in 67% of the remaining 766 epiphytic isolates. Overall, SmR *Ea* or epiphytic bacteria were recovered from 19 or 21 of the 24 orchards, respectively. The molecular mechanism of streptomycin resistance in all the SmR strains is currently under investigation.

Towards understanding virulence in *Clavibacter michiganensis* subsp. *nebraskensis*
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Clavibacter michiganensis causes bacterial wilt and canker in a variety of crops. It is subdivided into five subspecies according to host plant specificity. In recent years, several virulence factors have been identified in *C. michiganensis* subspecies, however, most of those are identified in *C. michiganensis* subsp. *michiganensis* (Cmm) and *C. michiganensis* subsp. *sepedonicus* (Cms). None of the identified virulence factors have closely related genes in *C. michiganensis* subsp. *nebraskensis* (Cmn), which is a responsible for wilt and blight of maize, and we lack an understanding of molecular mechanisms in the Cmn interaction with maize. The development of molecular resources and techniques in Cmn is a required step towards understanding Cmn pathogenesis. Here, a Cmn transposon mutant library was constructed and screened to identify mutants with reduced or no virulence. Targeted mutagenesis was also performed to knock out virulence factor candidate genes identified from comparative genomics between virulent and non-virulent Cmn isolates.

Drought shapes host response to pathogen infection in *S. bicolor*
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As the effects of global climate change intensify, the interaction of biotic and abiotic stresses increasingly threatens agriculture. The secondary cell wall is a vanguard of resistance to these stresses. *Fusarium thapsinum* (Fusarium stalk rot) and *Macrophomina phaseolina* (charcoal rot) cause internal damage to the stalks of the drought tolerant C4 grass, sorghum (*Sorghum bicolor* (L.) Moench), resulting in reduced transpiration, reduced photosynthesis, and lodging, severely reducing yields. Drought magnifies these losses. The interaction of water limitation with *F. thapsinum* or *M. phaseolina* infection was investigated using two monolignol biosynthetic mutants in sorghum, *bmr6* and *bmr12*. *bmr12* inoculated with each of these pathogens had significantly reduced lesion sizes under water limitation compared to adequate watering and had increased levels of salicylic acid under both conditions, suggesting that drought may prime induction of pathogen resistance. Coexpression analysis under disease and drought reveals a wealth of interacting pathways including cell wall remodeling, phytohormones, spliceosome, proteasome, and amino acid and central metabolism. Coexpression modules are associated with pathogens and soil moisture and interact with day length and plant size, highlighting the tradeoff between growth and defense and its interaction with secondary cell wall biosynthesis and offering promising new engineering targets for plants robust to these interacting stresses.

Cold Shock – Uncovering transcriptional changes driving a model for stress-related disease resistance
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Acute cold Stress-Induced Disease Resistance (SIDR) was first described in the grape powdery mildew (*Erysiphe necator*) pathosystem, and later in powdery mildews of hop and arabidopsis. Exposure of *Vitis vinifera* to brief cold events (*e.g.* 4 hrs at 4C) induced a

transient, quantitative increase in host resistance that peaked 24 hours after the cold event and dissipated within 48 hours. We paired this time course phenotyping of *E. necator* inoculated leaf discs with 3' RNA sequencing to investigate transcriptional changes driving the transient cold SIDR response on *V. vinifera* 'Chardonnay'. Through k-means clustering, pathways analysis in VitisPathways, and molecular network visualization using VitisNet 12X, the data most strongly supported a transient down-regulation of photosynthetic activity as the transcriptional driver of the cold SIDR response. Pathways associated with photosynthesis, including electron transport and antennae proteins were highly down-regulated. Moreover, gene precursors to proteins of photosystem I and II were down-regulated at the peak resistance point, 24 hrs after cold exposure, as were most gene precursors to light harvesting complexes I and II. Cold SIDR has been shown to cause stalling of powdery mildew epidemics in both grapevine and hops. Thus, these results provide gene targets in cold SIDR associated transcriptional pathways that could affect disease management in diverse powdery mildew pathosystems.

Wheat blast dynamics within the plant canopy: A spatio-temporal analysis

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Understanding the spatio-temporal dynamics of wheat blast (WB), a devastating disease caused by the Triticum pathotype *of Magnaporthe oryzae* (MoT), will provide information useful for developing effective WB management strategies. Field experiments were conducted in 2014 at two locations in Bolivia to study the vertical spread of MoT and WB within the canopy of susceptible cultivar Arex, influenced by in-field crop residue as a source of inoculum. WB severity and MoT spore density were quantified following systematic assessments and sampling on leaves and spike, and negative exponential models were fit to the data to quantify disease gradient. The models showed a significant inverse vertical gradient of WB severity and spore density ($P < 0.05$) from lower leaves up to the spike, using data collected at seven sampling dates after emergence. Repeated measures analyses showed significant differences in severity and spore density among plant organs at all seven sampling dates ($P < 0.001$). The effect of crop residue on severity and spore density was statistically significant in the lower canopy but not in the middle or upper canopy. Correlations between leaf layers in the canopy at a given sampling date and between sampling dates within same leaf layers were moderate to strong for both severity and spore density. These results provide invaluable insights into the spread of MoT inoculum and WB from leaves to spikes within the canopy, and the significance of auto-infection at the canopy level for wheat blast development.

Microneedle-based rapid plant DNA extraction: Towards in-field detection of plant pathogens

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Extraction of DNA from infected plant leaves is an important step for molecular diagnosis of plant pathogens. Current laboratory-based plant DNA extraction protocols (e.g., cetyltrimethylammonium bromide (CTAB) extraction) involve multiple steps and require bulky benchtop equipment and skilled technicians. As such, sample preparation becomes a major bottleneck that constrains advanced molecular diagnosis of plant diseases in the laboratory settings. Here, a rapid DNA extraction method has been developed by using polymeric microneedle (MN) patches. This method is minimally invasive, and isolates polymerase chain reaction (PCR) amplifiable DNA within one minute from plant leaves. Furthermore, we used these MN patches to successfully extract *Phytophthora infestans* DNA from both laboratory-inoculated and field samples for late blight disease detection in tomato. For laboratory-inoculated samples, the MN patch-based DNA extraction method achieved 100% detection rate compared to CTAB extraction method when the samples were taken 3 days after inoculation. For field samples, the MN extraction method successfully detected *P. infestans* in all blind samples tested from older lesions. This simple and rapid MN patch-based DNA extraction method could be applicable to extract DNA from other plant pathogens in infected plant material directly in the field, constituting a crucial first step towards the development of an in-field molecular diagnostic platform for plant pathogens.

Improved management efficacy of late leaf spot on peanut through combined application of prothioconazole with fluxapyroxad and pyraclostrobin

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Late leaf spot, caused by *Nothopassalora personata* (syn. *Cercosporidium personatum*), is the most economically important fungal disease affecting peanut foliage in South Carolina and can result in combined management and yield loss costs of greater than $490/ha. An integrated approach towards managing this disease is recommended for greatest efficacy and maximum return. Application of protectant fungicides is a critical part of effective integrated management under commercial production, and their strategic alternation and combination in management programs can provide enhanced control. Trials were conducted in Blackville, SC in 2017 and 2018 to investigate whether combinations of prothioconazole (0.1 kg/ha) with fluxapyroxad (0.05 or 0.1 kg/ha) and pyraclostrobin (0.1 or 0.2 kg/ha) could provide more efficacious management of late leaf spot compared to either product alone. Two applications of 0.1 kg/ha prothioconazole with 0.05 kg/ha fluxapyroxad and 0.1 kg/ha pyraclostrobin resulted in significantly ($P < 0.05$) less (25 to 53%) peanut canopy defoliation compared to the same number of applications of either product (prothioconazole at 0.1 kg/ha or fluxapyroxad and pyraclostrobin respectively at 0.05 and 0.1 kg/ha) applied individually. Increased rate of fluxapyroxad and pyraclostrobin application (0.1 and 0.2 kg/ha, respectively) with 0.1 kg/ha prothioconazole did not improve management relative to their combination at the examined lower rate. Results will be discussed in context of efficacy and treatment cost.

A plasmid gene cluster makes major contributions to *Pantoea ananatis* virulence on onion by conferring tolerance to a reactive sulfur phytoanticipin
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Pantoea ananatis is an unusual bacterial necrotroph causing center rot of onion, a recurrent disease of concern in many onion producing regions. It is predicted to produce an, as of yet, unidentified phosphonate phytotoxin as a primary virulence factor to kill host plant cells. When damaged, onion and other alliaceous plants produce the characteristically pungent phytoanticipin allicin. Allicin is an antimicrobial reactive sulfur compound and oxidant that reacts spontaneously with thiol groups and depletes the reduced glutathione pool. We used comparative genomics of *P. ananatis* strains with variable pathogenicity on onion, to identify four clusters of plasmid-borne genes that strongly correlated with onion virulence. Among these, we found a sub-cluster of 11 contiguous genes enriched for annotated functions in sulfur metabolism and thiol redox homeostasis. We found that engineered deletions lacking these genes reached 100-fold lower populations in onion bulb scales. Natural variant *Pantoea* isolates and engineered mutant strains lacking these genes had major growth defects in red onion juice as well as increased sensitivity to garlic juice and purified allicin. In addition, a nearly identical cluster of plasmid-borne allicin tolerance genes was identified in a sequenced onion pathogenic *Enterobacter cloacae* strain. We hypothesize that the acquisition of allicin tolerance genes by horizontal gene transfer is a common strategy for pathogenic bacteria that aggressively colonize onion bulb tissue.

Elucidating the phoretic blue-stain fungal community of root-feeding beetles in Georgia using a metabarcoding approach
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In recent decades, blue-stain *Grosmannia* species and their root-feeding beetle vectors have been associated with areas of loblolly pine mortality in the Piedmont region of Georgia; however, their potential role in host mortality is unestablished. With this study, we aim to 1) determine the phenology of loblolly pine associated root-feeding beetles in Georgia, 2) assess the blue-stain fungi community associated with these beetles and whether this association varies in stands with differing management practices and across seasons. Root-feeding beetles were live trapped in two loblolly pine stands with differing management histories (regularly prescribed burned or unburned), from May 2017 to October 2018. Across vectors, abundance varied over time and peak catches within a species occurred at similar times in both stands. Preliminary fungal isolations detected *G. profanum*, *G. huntii* and *G. alacris.* While the latter two have been previously associated with loblolly pine, *G. profanum* is unexpected, as the fungus was thought to only occur on hardwoods, and not believed to be beetle vectored. To elucidate the whole blue-stain fungal community associated with each vector species, including fungi potentially overlooked using traditional isolation methods, a subset of beetle vectors has been analyzed with a metabarcoding approach, using the *ß-tubulin* gene as target region. Results of this study will lay the groundwork for future understanding of this system in the Piedmont.

Pipecolic acid confers systemic immunity by inducing formation of free radicals
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Systemic acquired resistance (SAR) is a form of broad-spectrum resistance induced in response to local infections that protects uninfected parts against subsequent secondary infections. SAR signaling requires two parallel branches, one regulated by salicylic acid (SA), and the other by pipecolic acid (Pip), azelaic acid (AzA) and glycerol-3-phosphate (G3P). AzA and G3P function downstream of the free radicals nitric oxide (NO) and reactive oxygen species (ROS). The non-protein amino acid Pip functions by increasing NO/ROS levels. During SAR, SA, Pip, AzA, and G3P accumulate in the infected leaves, but only a small portion of these is transported to distal uninfected leaves. Plants defective in NO, ROS, G3P, or SA biosynthesis accumulate reduced Pip in their distal uninfected tissues although they contain wild-type-like levels of Pip in their infected leaves. This in turn is associated with transcript levels of *AGD2-LIKE DEFENSE RESPONESE PROTEIN1* (*ALD1*), which encodes an rate-limiting enzyme in the Pip biosynthetic pathway. Together, these data indicate that Pip functions upstream of free radicals and de novo synthesis of Pip in distal tissues is dependent on both SA and G3P.

Cyclic-di-GMP regulates autoaggregation and zinc uptake in *Erwinia amylovora*
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Erwinia amylovora is the causal agent of fire blight, which affects apple and pear. *E. amylovora* primarily suppresses host defenses using type III secretion effectors. It then forms biofilms within the xylem vessels, eventually leading to shoot blight. The ubiquitous bacterial second messenger cyclic-di-GMP (c-di-GMP) enables *E. amylovora* to transition between these infective stages by regulating several critical virulence factors including Type III secretion and biofilm formation. Intracellular levels of c-di-GMP are modulated by the targeted hydrolysis action of phosphodiesterases, PdeA, PdeB and PdeC. The combined deletion of *pdeA*, *pdeB* and *pdeC* in *E. amylovora* results in significantly elevated intracellular levels of c-di-GMP, resulting in an autoaggregating cell behavior, that impacts biofilm formation and cell separation post septation. We screened a library of transposon mutants generated in *E. amylovora* Ea1189Δ*pdeABC* for suppression of this autoaggregation phenotype. We identified a peptidoglycan hydrolase encoding gene *eagA* (*Erwinia* aggregation factor A), that is involved in the regulation of autoaggregation under high intracellular levels of c-di-GMP. *eagA,* is located downstream of, and co-regulated with *znuA,* the zinc-binding transporter component of the ZnuABC high-affinity zinc uptake system. Our results suggest that EagA responds to a localized intracellular pool of c-di-GMP generated at elevated Zn^{2+} concentrations by diguanylate cyclase DgcE, and positively regulates the expression of *znuA*. EagA also positively impacts *E. amylovora* virulence in apple shoots.

When is *Pseudomonas syringae* bacteriocin production beneficial?
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Bacteriocins are narrow-spectrum antibacterial protein toxins produced by bacteria to inhibit the growth of closely related species. A fitness benefit of bacteriocin production has been shown for the producing strain in structured environments (e.g. agar plate surface). To explore this observation *in planta*, we performed a series of co-infiltration experiments of bacteriocin-producer *Pseudomonas syringae* pv. *syringae* (*Psy*), or bacteriocin-deficient mutant of *Psy* (*Psy* D_{bac}), against bacteriocin-sensitive *P. syringae* pv. *phaseolicola* (*Pph*). Populations of each strain were measured over an 8-day period. In 1:1 co-infiltrations at day 4, bacteriocin production showed an increase in killing of sensitive cells with a 100- and 10-fold reduction of *Pph* by *Psy* and *Psy* D_{bac}, respectively. By day 6, there was a 100-fold decrease of *Pph* by both strains removing the initial benefit observed at day 4. When starting at a low cell density (1:9), *Psy* achieved a significantly greater population at day 6 compared to *Psy* D_{bac}, showing a bacteriocin production benefit when in the minority. Moreover, *Psy* reduced *Pph* to a larger extent than *Psy* D_{bac} for all sample days, indicating a combination of bacteriocin-mediated antagonism and resource competition between co-colonizing bacteria *in planta*. Our data demonstrates that under specific conditions bacteriocin production can promote *Psy* fitness and reduce the growth of a competing strain.

Whole-genome sequencing of a *Puccinia striiformis* f. sp. *tritici* mutant population identifies avirulence gene candidates
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Stripe rust pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*) threats world wheat production. Resistance to stripe rust is often overcome by pathogen virulence changes. To unravel the genomic basis of variation and identify avirulence genes in *Pst*, 30 mutants were developed using ethyl methanesulfonate (EMS) mutagenesis. The progenitor isolate was sequenced, assembled, and annotated for a high-quality reference genome. The mutants were phenotyped for virulence changes on 18 wheat *Yr* genes for stripe rust resistance and were sequenced. The sequences were mapped to the wild-type reference genome to identify genomic changes. After selecting EMS preferred CG to TA mutations, 217,775 single nucleotide polymorphisms (SNPs) per isolate on average were detected. Among the 30 mutant isolates, 15 changed from avirulent to virulent and the other 15 remained avirulent to *YrSP*. Association analysis identified 109 SNPs significantly ($P < 0.0001$) associated with the avirulent and virulent phenotypes to *YrSP*. Two of the SNPs were responsible for nonsense mutation and 12 SNPs were accountable for missense mutations in the coding regions. Fourteen genes containing non-synonymous mutations were significantly associated to the changes from avirulence to virulence to *YrSP*. The candidates for the *YrSP*-avirulence gene and other avirulence genes are valuable for functional confirmation and characterization to unravel the molecular basis of the wheat-*Pst* interactions.

Increasing accuracy of strawberry powdery mildew prediction via dynamic simulation modeling
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Strawberry powdery mildew (SPM), caused by *Podosphaera aphanis*, is gaining in importance as the production of day-neutral strawberry and resistance to fungicide increase. This disease is difficult to predict because of the wide range of weather conditions favorable to its development. Weather, disease (cv. Seascape), and host data collected at three, five and four sites in 2006, 2007 and 2008, respectively, for a total of 12 epidemics were used in this study. First, temperature-based disease risk index and classification trees approach were evaluated but found unreliable under Eastern Canada weather conditions. Hence, we explored dynamic simulation modelling. The model use published algorithms describing subprocesses of *P. aphanis* life cycle (e.g. sporulation, spore germination, leaf colonization etc.) and assembled knowledge of the interactions among pathogen, strawberry, and weather. The results show an accurate simulation of the trends, shape and amplitude of SPM severity and the relationship between simulated and observed SPM severity was significant in all sites and years ($P \leq 0.001$). The proportion of linear variations in the observed SPM explained by the variation in the simulated disease severity were below 0.70 hence, still need improvement. However, this is the only model that can quantitatively predict the SPM severity and could be combined with the previously developed action threshold to tune fungicide applications strategies.

It's complicated: The *Xanthomonas perforans* population across Florida commercial tomato fields
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Bacterial spot of tomato (BST), caused by *Xanthomonas euvesicatoria, X. gardneri, X. perforans, and X. vesicatoria*, is one of the most economically important diseases of tomato. In Florida, prior surveys determined that *X. perforans* (*Xp*) is the primary cause of BST and detected dramatic shifts within the *Xp* population over the past 30 years despite the lack of a clear selective pressure. These population shifts have adversely impacted the efficacy of control measures and resistance breeding efforts in tomato. We characterized 585 *Xanthomonas* isolates from symptomatic tissue in 70 commercial tomato fields throughout Florida in fall 2017 and found that *Xp* remains the primary cause of BST in Florida. The *Xp* population consisted of three phylogenetic groups and tomato races 3 (8%) and 4 (92%). Further characterization revealed that 99.8% of strains were resistant to copper, 25% of strains were resistant to streptomycin, 51% of strains produced bacteriocins, and effector profiles, that impact host range, were highly variable among the *Xp* strains. Our

results indicate that the composition of the *Xp* population in Florida is complex and varies within and among fields. We will use this strain collection to test for associations between genetic variation and tomato production chain variables.

Pre and post silk emergence weather variables associated with Gibberella ear rot and deoxynivalenol in corn
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The objective of this study was to quantify associations between variables summarizing weather conditions before, during, and after silk emergence (the R1 growth stage) and the severity (SEV) of Gibberella ear rot (GER; *Fusarium graminearum*), one of the most economically important ear diseases of corn in the U.S. Midwest, and its associated mycotoxin, deoxynivalenol (DON). Fifteen hybrids were planted in each of 10 Ohio counties from 2015 to 2018. At R1, ten arbitrarily-selected primary ears per hybrid were tagged and inoculated via the silk channel with a spore suspension of *F. graminearum*. All inoculated ears plus 5-10 non-inoculated ears were hand-harvested at R6 for SEV and DON quantification. Temperature, relative humidity (RH), surface wetness, and rainfall data were recorded at each location and used to generate weather summaries (e.g. daily, daytime, and nighttime averages, totals, and number of hours within certain ranges) for 6 time window lengths (5 to 30 days) relative to R1. Associations between each weather summary by window length combination and SEV and DON were quantified with Spearman rank correlation coefficients. All tested weather summaries were significantly correlated SEV and DON ($P < 0.001$) for at least one time window length, regardless of inoculation. However, the magnitude and direction of these associations varied with window length and starting point relative to R1. These results will be used to identify potential predictors in order to develop risk assessment models for GER and DON contamination.

Characterizing the sweetpotato NLR resistome: Diploid wild relatives genome survey and bait design
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Despite continued implementation of management strategies, the pathogen *Ceratocystis fimbriata* persists as a significant threat to the sweetpotato industry in the United States. The presence of few breeding programs, limited knowledge of the genetic basis of resistance, and hexaploid nature of the crop pose challenges to develop resistant lines. Plants possess an innate immune system with disease resistance *R* genes encoding for proteins that recognize pathogen effectors during infection. An important class of resistance gene contains nucleotide-binding and leucine-rich repeat domains, NLRs. We performed a protein motif-based search of NLRs in the diploid sweetpotato wild relatives *I. trifida* (NSP306) and *I. triloba* (NSP323) and compared the genome annotation with the annotation we conducted using NLR-parser. Our analysis revealed 121 and 117 complete NLRs in *I. trifida* and *I. triloba* respectively, while the original genome annotation had only identified 28 and 35 complete "NBS-LRR" sequences. Identified NLRs were used to design a bait-library to capture the NLR repertoire of sweetpotato using exome capture. Phylogenetic analysis of NLRs in sweetpotato, diploid wild relatives, and potato was also performed after annotating NRLs in genomes of white potato, peanut, cocoa, quinoa, strawberry, and lettuce. The identified NLR genes provide insight into the resistome of sweetpotato and a first step towards identifying *R*genes effective for control of *C. fimbriata*.

Distribution and characteristics of *Xanthomonas* species causing bacterial spot in midwestern processing tomatoes
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Bacterial leaf spot of tomato (BLST) is a disease of both processing and fresh market varieties. Outbreaks cause defoliation and fruit lesions, decreasing yield and marketability. BLST is associated with four species of *Xanthomonas* (*X. vesicatoria*, *X. euvesicatoria*, *X. gardneri* and *X. perforans*). Typically outbreaks of BLST within a geographic location are dominated by one of these species. Temporal shifts of the predominant pathogen within geographic locations have been reported in Florida in 1998 (*X. euvesicatoria* to *X. perforans*) and in Ohio in 2010 (*X. vesicatoria* to *X. gardneri*). Surveys were conducted over six years (2010–2012, 2017–2018) to determine the distribution of *Xanthomonas* spp. in Midwestern tomato fields and the sensitivity of 738 isolated strains to copper and streptomycin sulfate was assessed *in vitro*. *Xanthomonas gardneri* was the predominant species isolated in 2010, 2011, and 2012 (83%, 75%, and 65%, respectively). By 2017, *X. perforans* isolations had increased over previous years and was the predominant species isolated from symptomatic tomato fruits by 2018. Resistance to copper sulfate increased in all strains over the survey period. In 2010, all the recovered strains were sensitive to the highest concentration of copper sulfate (200 mg/ml). By 2018, over 50% of the recovered strains were resistant. Despite the limited use of streptomycin products in Midwestern processing tomatoes, resistance to streptomycin sulfate was commonly observed.

Changes in prevalence of *Potato virus Y* strains in seed potato in Idaho and Washington, 2016–2019
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Potato virus Y (PVY) exists as a complex of strains, with multiple recombinants associated with tuber damage. Prevalence of strains was studied in the Idaho and Washington seed potato winter grow-out (WGO) trials, between 2016 to 2019. Foliar potato samples exhibiting mosaic during the WGO trials were collected and subjected to serological and molecular analyses using monoclonal antibodies and two sets of strain-specific primers probing most common recombinant junctions in PVY recombinants. In Idaho, prevalence of the non-recombinant strain PVY^{O} was stable at around 3%, while the most prevalent strain of PVY was found to be

PVY^{N-Wi}, at approximately 70%, followed by PVY^{NTNa}, at around 20%, with little changes during the period of observation. In the 2019 season, the latter two recombinants, PVY^{N-Wi} and PVY^{NTNa} together, represented more than 90% of all PVY positives found in Idaho seed potato lots. In Washington, a drastic transition occurred, from mostly non-recombinant PVY^{O} and PVY^{N} strains in 2016, to mainly PVY^{N-Wi} and PVY^{NTNa} strains in 2019. The contrasting strain compositions of PVY in Idaho and Washington seed potato may reflect very different production practices between specialty potato cultivars grown in western Washington, and processing potato cultivars grown in Idaho.

Identifying dispersal mechanisms of *Ceratocystis fimbriata*, causal agent of black rot, within sweetpotato storage and packing systems

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Ceratocystis fimbriata is one of the most devastating pathogens of sweetpotato, yet little is known about the pathosystem. To better understand pathogen dispersal in the sweetpotato production system, this study aimed to: i) assess the role of dump-tank water in dispersal and to ii) identify potential insect vectors. In water tests, *C. fimbriata* spore suspensions were diluted (0,5,50,500, & 5000 spores/mL), aged (0,24,48,96, & 144 hrs), or heated/chilled (10, 23, 35, & 45°C) to test for the effects of inoculum density, age, and water temperature on dispersal, respectively. Wounded and non-wounded sweetpotatoes were soaked in water treatments for 20 min, stored at 29°C for a 14-day period, and rated for incidence every other day. To identify insect vectors, sticky traps were placed in sweetpotato storage facilities for one-week and insects were visually identified. Water tests showed temperature had no impact on disease incidence, but incidence did significantly decrease as inoculum concentration decreased and inoculum age increased. The insect survey found that overall, significantly more *Drosophila* species were recovered than any other insect. *D. hydei,* a relatively easy to maintain species which comprised 85% of the samples, was selected as a good candidate for future vector studies. These findings advance our knowledge about the black rot pathosystem, as well as inform integrated management approaches for sweetpotato black rot.

A UBC13-Interacting, RING Type E3 Ligase Acts with BAK1 And BIK1 to regulate plant immunity

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The tomato UBC13-type ubiquitin E2 Enzyme Fni3 modulates plant immunity through K63-linked ubiquitination. However, the molecular basis underlying the modulation remains elusive. We report here that a RING type E3 ligase, Fti1 regulates plant immunity through acting with BAK1 and BIK1. The tomato Fti1 (*Solanum lycopersicum* Fni3 interacting protein 1, SlFti1) and its Arabidopsis homolog, AtFti1 were found to interact with Fni3 and AtUBC13, respectively to catalyze K63-linked ubiquitination. The *SlFti1* and *AtFti1* gene both undergo alternative splicing, generating two transcripts that encode protein isoforms differ solely in the presence of a nuclear localization signal (NLS). The two isoforms of AtFti1 and the isoform that lacks the NLS (AtFti1-2) interacted with BIK1 and BAK1, respectively and the interaction required the N-terminal region of AtFti1. AtFti1-2 ubiquitinated BAK1 but not BIK1 *in vitro*. Meanwhile, both BIK1 and BAK1 phosphorylated AtFti1-2. The BIK1 phosphorylated AtFti1-2 mainly at amino acid S395, leading to compromise of its E3 ligase activity. The null mutant of *AtFti1* displayed reduced sensitivity to flg22 and were more susceptible to infection by *Pseudomonas syringae* pv *tomato* (*Pst*). Consistently, plants overexpressing AtFti1-2 displayed increased accumulation of FLS2 and elevated sensitivity to flg22 and reduced pathogen growth after *Pst* infection. Our study suggested that AtFti1-2 is a positive regulator of FLS2-mediated immunity and BIK1 targets AtFti1-2 as a feedback. Characterization of the role of BAK1-AtFti1 interaction is undergoing.

Using whole-genome sequencing for pathogen identification: *Candidatus* Pectobacterium maceratum, a potato blackleg pathogen in New York State

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Blackleg and soft rot are bacterial diseases of potato that reduce plant emergence and can damage tubers in storage. Beginning in 2014 there has been an increase in blackleg disease incidence in US potato production. To help understand the cause of this disease outbreak, in 2017 we surveyed potato plants with blackleg symptoms from several locations in New York State (NYS). We isolated pathogens from these plants, confirmed their pathogenicity, and used PCR and *dnaX* single gene sequencing to determine the species. We found that *Dickeya dianthicola* and *Pectobacterium parmentieri* were the two most common causal agents in those plants. However, this approach could not resolve species assignments for nine *Pectobacterium* isolates. Here, we used whole-genome sequencing-based methods to determine the species of these nine *Pectobacterium* isolates. We used single gene ortholog analysis, digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) to classify the unknown isolates. We found that eight of the nine isolates were most similar to a newly designated species, *Candidatus* Pectobacterium maceratum; the one remaining isolate was likely *P. carotovorum* subsp. *carotovorum*. *Ca.* P. maceratum is not previously known to exist in the United States and data are consistent with it being a blackleg pathogen in NYS.

Life history of a *Puccinia coronata* form infecting two invasive wetland plants

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In 2017, heavy rust infections on *Frangula alnus* (glossy buckthorn) were observed in the metro area of the Twin Cities (MN). Infections were observed on leaves, leaf petioles and axillary buds that led to a reduction of growth on new shoots, flowers and fruits. *Phalaris arundinacea* (reed canary grass) adjacent to infected *F. alnus* developed uredinia following the release of aeciospores from *F.*

alnus. Experiments were conducted to determine the rust pathogen's life cycle and host specificity by inoculating *P. arundinacea*, as well as other grasses and cereal crops with aeciospores collected from *F. alnus*. Results from preliminary studies indicated that this rust fungus is heteroecious, completing its life cycle by alternating between *R. frangula* and *P. arundinacea*, and that the fungus does not infect oats, barley, wheat, rye, or a number of other grasses except *Phalaris* and *Calamagrostis* spp. Microscopic examination of spore morphologies indicated that this rust fungus belongs to the *Puccinia coronata* complex. Experiments are in progress to determine telial and aecial host specificities and phylogenetic relations with other known forms of *P. coronata.*

Exploring apoplastic sulfate restriction as a component of pattern triggered immunity in *Arabidopsis thaliana*
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Transcriptome analysis of the pathogen *Pseudomonas syringae* pv. tomato DC3000 (DC3K) during early infection and exposure to Pattern Triggered Immunity (PTI) in *Arabidopsis thaliana* provided insights into the earliest transcriptional events corresponding with immune exposure. We observed that the induction of sulfur starvation response genes such as alternative sulfur importers in DC3K is a rapid and specific response to PTI exposure. We confirmed observations that *A. thaliana SULTR1;2*, a high affinity sulfate transporter gene, is induced in leaves during PTI. The objective of our study is to determine if the apoplast becomes sulfate depleted during PTI and thus restricts pathogen access to sulfur. To test our hypothesis, we optimized a method for extracting high purity apoplastic wash fluid from *A. thaliana* free of cytoplasmic contamination. We used ion chromatography analysis to determine that apoplastic fluid from naïve *A. thaliana* contains approximately 2mM sulfate. We also found that DC3K growth *in vitro* is inhibited at sulfate concentrations below 10 μM. In addition, we are conducting mutagenesis of five sulfate transporters in DC3K, to determine whether sulfate uptake is important for growth *in planta*. Taken together, our research suggests that apoplastic sulfate limitation could be a mechanism for PTI. Our findings bridge the gap of plant immunity and nutrient metabolism in the context of plant-microbe interactions.

Deciphering maize chlorotic mottle virus translation initiation: Toward resistance to maize lethal necrosis disease
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Maize chlorotic mottle virus (MCMV) interacts in mixed infections with potyviruses to cause maize lethal necrosis disease (MLND). In 2011 MLND appeared for the first time in East Africa and China. No resistance genes to MCMV are known, thus MLND remains a devastating disease in East Africa. Similar to other members of the *Tombusviridae* family, MCMV RNA lacks the 5' cap required for translation of host mRNAs, and instead harbors a cap-independent translation element (CITE) in the 3' untranslated region (UTR). Because this differs from host translation, here we characterized the MCMV translation mechanism as a potential target for engineered resistance. The MCMV 3' CITE (MTE) both resembles and differs from the well-characterized panicum mosaic virus class of 3' CITE (PTE). We used mutagenesis and chemical probing to show that, like the PTE, the MTE forms a branched stem-loop structure with a purine bulge containing a hypermodifiable G. Unlike PTEs, no pseudoknot involving the purine bulge was evident. Translation and infectivity assays showed that the MTE must base pair to the 5' UTR to facilitate translation initiation and virus replication. Gel shift assays revealed that, like the PTE, the uncapped MTE binds cap-binding translation initiation factor eIF4E. Thus, eIF4E binds the MTE differently than host mRNAs which require a 5' cap for eIF4E binding, and it should be possible to identify eIF4E mutants that bind only capped mRNA and not the MTE. Introducing such mutations into maize eIF4E genes may provide resistance to MCMV.

Transcriptional regulation of miR528 by *OsSPL9* orchestrates antiviral response in rice
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Insect-transmitted viruses constitute a major threat to rice productivity and thus worldwide food security. To tackle this problem, we have been using *Rice stripe virus* (RSV), which is transmitted by small brown planthopper (*Laodelphax striatellus*), as a model to unravel antiviral defense mechanisms in rice that can be harnessed to combat rice virus epidemics. Our previous work revealed a complex interplay between RSV infection and the RNA silencing pathways in rice that determine infection outcomes. We established that defense to *Rice stripe virus* (RSV) invasion entailed a reduction of miR528 accumulation in rice, thus alleviating miR528-mediated degradation of *L-Ascorbate Oxidase* (*AO*) mRNA, bolstering the antiviral activity of AO. These and other reports suggest that many miRNAs act as pivotal modulators of plant antiviral immune responses by regulating the turnover of immunity-associated targets. In particular, the expression of miR528 was dramatically down-regulated. However, it is unknown whether the down-regulation is directly caused by lower transcription. Here we show that this *miR528-AO* defense module is regulated by the transcription factor SPL9. SPL9 displayed high affinity binding to specific motifs within the promoter region of *miR528*. Loss-of-function mutations in *SPL9* correlated with a significant reduction of miR528 but a substantial increase of *AO* mRNA, enhancing rice resistance to RSV. Conversely, transgenic overexpression of *SPL9* stimulated the expression of miR528, hence lowering the level of *AO* mRNA and compromising rice defense to RSV. In conclusion, SPL9-mediated transcriptional activation of miR528 expression adds a novel layer of regulation of the *miR528-AO* antiviral defense.

Peach tree tolerance to *Cytospora leucostoma* infections under water deficit and increased soil pH
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High pH soils and a limited water supply, in combination with the canker pathogen *Cytospora leucostoma,* are major limiting factors to peach fruit production in Colorado growing conditions. Tolerance to *C. leucostoma*, in association with tolerance to abiotic stressors, are important peach cultivar selection criteria for growers. Selection of tolerant peach cultivars could lead to a decrease in *C. leucostoma* incidence and an increase in orchard longevity. Our specific objective was to evaluate differences in tolerance to *C.*

leucostoma infections under water deficit, pH stress and normal conditions among thirteen peach cultivars. Trees displayed increased stress (leaf water potential) under higher soil pH and 40% deficit irrigation in green house conditions. Further, *Cytospora leucostoma* displayed a fitness increase on water deficit and increased soil pH trees. *Cytospora leucostoma* lesion sizes did not differ statistically amongst the different peach cultivars tested, in terms of lesion sizes. These results reinforce the necessity for proper management techniques including proper soil pH management and proper irrigation management in commercial orchards.

Impact of developmental growth on intra-host evolution of Plum pox viral quasispecies in perennial host
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Plum pox virus (PPV), a *potyvirus,* is the most devastating threat to production of stone fruit worldwide. Strains have shown the ability to generate and maintain new variants that display differential pathologies. To further investigate PPV variant dynamics, we analyzed high-resolution sequence data generated from ribosome-associated PPV genomes ('Translatome') isolated from leaves of infected plum trees (*Prunus domestica*) over two growth periods (GP). During each GP, leaf samples were collected at 2, 4, 6, and 12 weeks leaf emergence. Bud samples were also collected pre- and post-vernalization. Translatome associated viral genomes are likely being translated and thus actively contributing to the infection process. Sequences were processed and variants called against the PPV Penn7 genome (EF640935). PPV translation levels were consistent with expected viral infection levels. Results show within GPs, translatome PPV variants occur at proportionately higher levels in newly developing plum tissues with low levels of infection while more mature tissues showing high infection levels display proportionately lower number of variants. Analysis of individual variants revealed distinct groups as some variants were maintained at either high or low levels at all sample times while other variants showed dynamic changes in their levels. Combined, these results suggest that PPV populations undergo cyclical transitions during seasonal bud break and leaf development.

Predicting the impact of root-associated bacterial endophytes through in vitro functional characterization
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Plant root microbiome, a community of bacteria living in close association with plant root, plays a significant role in plant health and productivity. How their functions contribute to plant growth are not well-understood. We hypothesized that phenotyping multiple *in vitro* functions of the culturable root endophytic bacteria could predict their impact on *in planta* growth. 183 bacterial endophytes were isolated from the roots of field-grown domesticated tomato species and its wild cousin. 30 isolates from each genotype were screened for four *in vitro* functional traits: production of auxin and siderophores, phosphate solubilization, and direct antagonism to a soilborne bacterial pathogen of tomato, *Ralstonia solanacearum*. Biosynthesis genes of DAPG or HCN were also screened for their presence in the genome of the isolates. Hierarchical clustering of the *in vitro* functional traits suggested the isolates was clustered into four distinctive groups: auxin producers, phosphorus solubilizer, siderophore producer, and a group with high antagonism against *R. solanacearum*. Auxin producers tended to have low antagonistic traits. Isolates with the DAPG biosynthesis gene also tended to be strong siderophore producers. Two isolates from each of the functional group were then individually inoculated on plants. Preliminary results suggested that isolates with similar *in vitro* functional traits performed similarly in plants. Understanding how *in vitro* functional traits of bacteria relate to their impact on plants potentially offer a powerful strategy for further microbiome modeling studies.

Modeling vector behavioral thermoregulation as an effect on epidemic dynamics
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Organisms' experience of the environment is modulated by physiological and behavioral processes. Arthropod vectors of plant pathogens are able to exploit environmental variation, resulting in survival and development that occur at rates higher than would be expected if the arthropods randomly experience environmental variation; however, most vector phenology models assume uniform and deterministic evironmental experience of arthropods. One mechanism through which arthropods exploit environmental variation is by moving in space to affect their thermal experience, which is the principal abiotic driver of development. Epidemic models of vector-transmitted plant disease are improved by accounting for arthropod behavioral thermoregulation, resulting in better predictions of vector phenology and consequent pathogen transmission. The objective of this study was to incorporate thermal ecology of vectors into predictive models of plant disease epidemics. I empirically model arthropod development as a function of temperature, and temperature as a composite function of the environment and arthropod behavior. The primary result of this study is a framework through which to interpret variation in meteorological data that are becoming increasingly resolved and useful for epidemic modeling, justified by improved predictive performance. A similar preliminary approach to moisture experience is discussed, and applications of the framework to example agricultural vector-borne diseases are demonstrated.

Assessing and optimizing sprayer technologies in commercial eastern Washington state wine grape vineyards
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In-season vineyard pest management relies on proper timing, selection, and application of products. Pertinent research on this topic often focuses on the influence of regional environment or product timing and efficacy. However, the influence of local environmental conditions on performance of application technologies, especially related to deposition and drift, is not fully vetted. Three commercially-available sprayers were optimized and assessed in Washington state in the 2016 and 2017 growing seasons. Spray

deposition and drift data were collected in commercial *Vitis vinifera* vineyards at two growth stages, EL 23 (50% bloom) and 25–30 days later at EL 31 (pea-sized berries) using a fluorescent tracer. Deposition was collected from 5 canopy zones, and drift was collected from the vineyard floor in the first 3 downwind rows from the sprayer and aerially in 0.3 m increments above the canopy in the first 3 downwind rows from the sprayer. All sprayers showed consistent within canopy deposition; the canopy contained between 80.0–98.5% of the total deposition collected. Aerial drift was generally <4% of total deposition collected. Floor drift ranged between 1.5–16.0% of total deposition collected. In both drift cases, most of the deposition were in the row closest to the sprayer, indicating relatively low longer-distance drift with modern spray technologies. Ultimately, this information can be used to optimize sprayer selection for different vineyard sites.

Network meta-analysis of trials evaluating the susceptibility of Florida strawberry cultivars to *Macrophomina phaseolina*
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Macrophomina phaseolina, the causal agent of charcoal rot, is a soilborne pathogen that affects strawberry crowns leading to plant wilt and collapse. Disease management involves a combination of pre-plant fumigation of soil and the use of resistant cultivars. Trials were conducted during nine consecutive seasons (2010–11 to 2018–19) to determine the susceptibility of strawberry cultivars to charcoal rot and the impact of cultivar selection on disease management. Six cultivars grown commercially in Florida were chosen and grouped in "susceptible" [(S) 'Strawberry Festival' and 'Treasure'], "moderately resistant" [(MR) 'Florida Radiance' and 'Florida Beauty'], and "resistant" [(R), Sensation® 'Florida127' and 'Winterstar']. Following primary analysis of the individual trials, a network meta-analysis was conducted to estimate and compare the final disease incidence and the disease progress rate of each susceptibility group. Final disease incidence means recorded for the S, MR, and R groups were, respectively, 53, 12, and 4%, whereas disease progress rate values were 0.07, 0.05, and 0.02 day^{-1}. The adoption of R and MR instead of S cultivar groups reduced disease incidence by 92 and 78%, respectively, whereas the use of R instead of MR reduced disease incidence by 63%. Significant differences were only observed between disease progress rates of R and S groups. Therefore, cultivar resistance is an effective management strategy to reduce charcoal rot incidence.

Understanding microbial communities: Single-gene phylogenies and their resolution
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A significant problem for the future of microbial ecology and plant pathology is the disentanglement and identification of species and sub-specious members within microbial communities. Current technologies can be cumbersome; they rely on assumptions about the community (short-read sequencing and library preparations), can be technologically challenging (MLSA approaches), or do not provide enough resolution between closely related species and sub-specious (16S rRNA sequencing). A possible solution is to identify single-genes that provide species and sub-species level phylogenetic resolution. The phylogenies of *Pseudomonas spp.,* are well established and characterized to sub-specious level, providing a suitable starting point to generate a protocol that can be applied to other genera. Previous research has shown that using a single gene can get sub-species and phylogroup resolution in *Pseudomonas syringae*. Two genes were selected, rpoB and atpD, from already published MLSA phylogenies; when tested on three-species, seven-species, and fifteen-species synthetic communities the genes effectively identified the community composition. Single-gene RAXML phylogenies were created and congruence was estimated against already published MLSA-phylogenies. These results indicate that single-gene phylogenies may provide a framework to observe complex communities without relying on library preparation methods or attempting to pair multiple genes to the same species.

Molecular and cellular interactions of the root lesion nematode (*Pratylenchus penetrans*) with alfalfa (*Medicago sativa* L.)
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The root lesion nematode (RLN) *Pratylenchus penetrans* is a migratory species of plant parasitic nematodes that attacks a wide range of crops including alfalfa, the third most valuable field crop in the U.S. High levels of infection can reduce alfalfa forage yields and the plant's ability to take up water and nutrients. Currently, there are no commercially certified varieties with resistance to RLN. To advance our understanding of the host-pathogen interactions and to gain biological insights into the genetics and genomics of host resistance to RLN, we performed a comprehensive assessment of resistant and susceptible interactions of alfalfa with *P. penetrans* that included root penetration studies, ultrastructural observations and global gene expression profiling of host plants and the nematode. Several gene-candidates associated with alfalfa resistance to *P. penetrans* and nematode parasitism genes encoding nematode effector proteins were identified for potential use in alfalfa breeding programs or development of new nematicides, respectively. The induction of host genes involved in biosynthesis and accumulation of secondary metabolites in the cells, especially of the phenylpropanoid pathway, appears to be critical for alfalfa resistance to *P. penetrans*. We hypothesize that constitutive defenses, such as significant accumulation of tannin-like deposits in root cells of the resistant cultivar, could be a key mechanism of alfalfa resistance against *P. penetrans*.

Recent advances to control soft rot disease
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Pectobacterium sp. are devastating pathogen of potato and ornamentals and there are limited options for management of this pathogen. Ornamental *Ornithogalum* lines were transformed with the antimicrobial peptide *tachyplesin* I to obtain soft rot resistance plants and transformant resistance was correlated with *tachyplesin* I level in transformed plant lines. Furthermore, to identify traits associated with resistance to *Pectobacterium*, sensitive and resistant lines of the *Ornithogalum* were crossed, and *Pectobacterium*-resistant offspring were selected. Resistance levels of the parental and progeny lines were correlated with the level of phenolic content in the plant lines. In addition, plant phenolic molecules were screened for antimicrobial activity and the best performing phenolics, salicylic acid and carvacrol, were studied to understand their mode of action. These two molecules affected the bacterial quorum sensing system, resulting in low synthesis of exoenzymes, thereby reducing virulence. Collectively, these discoveries suggest that plant phenolics are a useful breeding target to develop *Pectobacterium*-resistant plant varieties and that plant phenolics can promote resistance by interfering with regulation of bacterial virulence genes.

Crop rotation sequences affects subsequent soybean yield, soil microbiome, root microbiome and soil health
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Rotation is an important cultural practice that farmers use to manage crop growth and diseases. Diversified crop rotations directly affect plant residue inputs that may enrich specific groups of microbes that form association with the following crop. At two field sites, we observed yield differences in soybean planted after the following four long-term (14 year) crop sequences - continuous corn (CC), corn/corn/soy (CCS), corn/soy/corn (CSC), soy/corn/soy (SCS). Soybean yields were in decreasing order, CC>CCS>CSC>SCS, and the yield differences could not be explained by soil chemical and physical properties. Our goal was to relate soil health indicators and soil microbial community composition with the differences in soybean yields. Soil protein, permanganate-oxidizable carbon (POX-C), and beta-glucosidase activity were measured for soil health. After 14 yr of the rotation regimes, soil protein was higher in CC plots compared with other rotations. POX-C was also greater in CC plots. Cyanobacteria were associated with higher POX-C and protein levels. Three taxa of bacteria and one taxa of fungi were associated with higher levels of beta-glucosidase and correlated with the lower soybean yield observed in the SCS treatment. We also found differential abundance of specific bacterial and fungal OTUs to be informative on predicting yield differences, especially fungal pathogens from genera Macrophomina and Corynespora at one location. Additionally, random forest classifier was effective at identifying informative OTUs that were associated with rotational treatments and soybean yields.

Deciphering evidence of plant parasitic nematode dispersal and biogeographic patterns derived from DNA barcoding
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The distribution of plant parasitic nematodes is largely a result of dispersal capabilities, human activities and historical biogeographic processes. Given high-resolution taxonomic approaches like DNA barcoding, we can more precisely evaluate hypotheses intended to explain observed patterns of nematode distributions. Some commonly evoked, but seldom tested patterns include: cosmopolitan distributions, centers of origin, latitudinal gradients, and areas of diversity and endemicity. We have been able to evaluate some of these patterns and their associated processes by phylogenetic and phylogeographic analyses using mtDNA COI sequences. Nematodes in the families Pratylenchidae, Meloidogynidae, and Heteroderidae which have enhanced dispersal capabilities due to their feeding behavior and association with major agronomic crops generally exhibit weak distribution patterns characterized by multiple long distance dispersal events. Nematodes in the family Criconematidae which typically have limited dispersal capabilities and relatively few associations with agronomic crops, exhibit strong distribution patterns in North America consistent with the recolonization of plant communities following the retreat of the Wisconsin Glacier 18,000 years ago. One surprising pattern emerging from an analysis of Criconematidae, is that of biome conservatism, wherein species tend to retain their ancestral ecology over millions of years and shifts between biomes are rare. A better understanding of this process may help predict future nematode distribution in a dramatically changing climate.

Susceptibility of sweetpotato varieties to *Meloidogyne enterolobii* and its distribution in eastern North Carolina
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Meloidogyne enterolobii is an aggressive root-knot nematode species that has been detected in North Carolina within the last decade. In collaboration with the NCDA Nematode Assay Lab, root-knot nematode samples from sweetpotato, soybean, and tobacco grown in the eastern-half of North Carolina were analyzed using PCR to assay for the presence of *M. enterolobii*. This species has been detected in Columbus, Sampson, Harnett, Johnston, Wayne, Greene, Wilson, and Nash counties. Potential resistance to *M. enterolobii* in sixty selected sweetpotato [*Ipomoea batatas* (L.) Lam.] genotypes was evaluated in greenhouse experiments. 10,000 eggs of *M.* enterolobii were inoculated on each sweetpotato genotype grown in a 3:1 sand to soil mixture. Nine weeks after inoculation, roots were weighed, and nematode eggs were extracted from roots. Genotype susceptibility to *M. enterolobii* was assessed as the number of nematode eggs per gram of root. Susceptible genotypes included Covington averaging 3,730 eggs per gram of root, Beauregard at 4,263 eggs/gram root, NCDM04-001 at 19,613 eggs/gram root, and HiDry at 9,925 eggs/gram root. Resistant sweetpotato genotypes included Tanzania at 1.81 eggs/gram root, Murasaki-29 at 2.98 eggs/gram root, Bwanjule at 5.06 eggs/gram root, Dimbuka-Bukulula at 11.30 eggs/gram root, Jewel at 1.91 eggs/gram root, Centennial at 2.87 eggs/gram root, and Tib-11 with 10.77 eggs/gram root. We are collaborating with NC State sweetpotato breeders to incorporate the observed resistance to *M. enterolobii* into commercial sweetpotato cultivars.

Endophytes and tree health: Disease anatomy and microbial ecology of the *Neonectria ditissima* pathosystem (European apple canker)
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The fungus *Neonectria ditissima*, the causal agent of European apple canker, may cause asymptomatic infections of plant tissues that can lay dormant up to three years. This hinders the effective management of cankers. During the asymptomatic stage, *N. ditissima* is speculated to behave inside trees like an endophyte, colonizing plant tissues at a distance from the initial infection/entry site. The extent of its potential internal colonization and its interactions with other apple endophytes have not been investigated, but could have an impact on disease management. Answering these questions could help to develop sampling strategies for diagnostics, or to exploit endophytes for biological control. In this study we first quantified the extent of internal colonization prior to visual symptoms. Using real time qPCR it was possible to track the spread of *N. ditissima* in the host, and we found that the pathogen does not extensively colonize plant tissues, being predominantly localized at the entry site. Currently, we are using high-throughput next generation sequencing techniques to characterize the endophytic species profiles associated with different apple cultivars, either resistant or susceptible to canker. Our aim is to assess whether the cultivar response to canker is related to its endophytes, and if so how different endophytic species correlate with disease resistance.

Anaerobic soil disinfestation: Manipulating the Florida soil microbiome
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Anaerobic soil disinfestation (ASD) is a pre-plant, non-fumigant soil treatment, used to manage soil-borne pathogens, nematodes and weeds. The primary mechanism behind ASD is the manipulation of the microbiome by creating an anaerobic environment and providing labile carbon. In Florida, feed grade molasses, the carbon, and composted broiler litter are incorporated into the soil, which is then covered with a plastic mulch and watered to field capacity. ASD has been applied in both annual and perennial crops including: tomato, pepper, cucumber, strawberry, fresh cut flowers, and citrus. Plants are transplanted three-weeks post application. By combining molecular techniques and temporal sampling, shifts in the microbiome were observed during ASD treatment. Within 24 hrs. of ASD application, the microbiome shifts to an anaerobic population and the pH decreases. Throughout the treatment the microbial population is in flux. Changes in the microbiome are highly correlated to the ebb and flow of organic acids detected in ASD treated soil. ASD has an expeditious and long-term effect on the microbiome. An ASD experiment on citrus, consisted of planting three different rootstocks in ASD treated and non-treated soil. Two years post ASD application, the soil bacterial populations were significantly influenced by the non-treated rootstocks. However, rootstock had little to no influence in the ASD treated soil. While all the citrus trees tested positive for Huanglongbing, ASD treated trees had greater trunk and stem diameters, canopy, and yield compared to the non-treated trees.

Weather-based epidemiological models for Alternaria blight of oilseed brassicas in India
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Alternaria blight is the most widespread and destructive disease of oilseed *Brassica* across the globe. In India, it is mainly caused by *Alternaria brassicae* (Berk.) Sacc. which infects all aboveground part of crop and produces grey color spots. Although total destruction of crop due to disease is rare, yet yield loss can reach up to 47% with reduction in seed quality *viz.,* seed size and viability. The knowledge of probable attack of disease in advance may be very useful to farmer to take timely and appropriate protection measure to reduce the loss. Weather plays an important role in disease development. A well-tested weather-based model can be an effective tool for disease forewarning. In this study, weather-based forewarning model was developed for crop age at peak severity and maximum severity (%) of the disease on leaf and pod (used as dependent variable) for three location in India *viz.,* New Delhi, Hisar (Haryana) and Mohanpur (West Bengal). Historical disease data (2004–14) and weather data (Temp., RH, rainfall and bright sunshine hours) were utilized as independent variable for model development and their validation for two subsequent year (2014–16). Validation of the prediction model for crop age at peak severity and maximum severity (%) of the disease proved the efficiency of the targeted forecasts. On this basis, advisory to farmers could be issued at least 2–3 week in advance with information for timely application of fungicide to manage *Alternaria* blight

Machine learning-based early rice disease detection using spectral profiles
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Local and landscape-level detection of plant diseases can be a laborious and time-consuming process. Once disease symptoms are widespread, options for management may be limited, particularly in developing countries that lack resources. Therefore, methods capable of detecting diseases before the onset of symptoms, and in a relatively inexpensive manner, would be useful for more proactive and targeted disease management. The objective of this study was to evaluate the applicability of near-infrared (NIR) and Raman spectroscopy, combined with machine learning, for early detection of rice sheath blight (ShB), a devastating disease affecting rice production. To test this approach, we collected NIR and Raman spectra from leaves of the ShB-susceptible rice cultivar, Lemont, and inoculated the base of the stems with agar blocks containing the fungus *Rhizoctonia solani*, the causal agent of ShB. Spectra were

collected from asymptomatic rice as early as one day post-inoculation. Machine learning, including support vector machine analysis, was then used to build and evaluate the accuracy of disease predictive models. Our results suggest that machine learning can be used to diagnose infected but asymptomatic rice plants based on spectral profiles collected from plants in early stages of disease development. This technique holds promise for application in the field, although field testing is needed to validate and refine disease predictive models.

Stronger together: Coculture screen for specialized metabolites in the rhizosphere
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To address food security, agricultural yields must increase to match the growing human population partially by keeping plant disease at bay. Currently, there is a strong push to develop more sustainable agricultural practices, one of which is using 'biological control' rather than chemical pesticides. In plant pathology, this term applies to the use of microbial antagonists to suppress diseases; organisms that suppress pathogens are referred to as biological control agents (BCAs). Many groups have attempted to identify single strains that can act as BCAs, but bacteria often live as members of complex microbial communities where an important component of their local environment is other microbial cells. In order to communicate with these neighboring microbes, many bacteria secrete specialized (or secondary) metabolites. Most of the biosynthetic gene clusters that produce specialized metabolites are inactive during normal laboratory growth, and thus are hidden from discovery during traditional screens. However, coculture has been shown in numerous cases to elicit production of otherwise cryptic metabolites. Our long-term goal is to identify functional microbial consortia and novel bioactive compounds to rationally manipulate microbial communities and improve the environment. Therefore, we are using coculture screening coupled with MALDI-TOF to stimulate the production of these hidden metabolites and screen for their potential activity against plant pathogens.

What is the role of auxin in root-mediated resistance to *Ralstonia solanacearum*?
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Bacterial Wilt disease is caused by soilborne plant pathogen *Ralstonia solanacearum*, which infects economically important crops such as tomatoes, potatoes, peppers, and tobacco. *R. solanacearum* enters via natural openings in plant roots, invades the xylem tissue, and produces large amounts of exopolysaccharides (EPS) that block water transport, resulting in wilted plants. In tomato, resistance response to *R. solanacearum* is quantitative and is the result of many genes, however, the molecular basis still remains to be studied. Studies with grafting experiments have shown a significant reduction of Bacterial Wilt. These studies have demonstrated that resistance mechanisms in root are key to plant response. Salicylic acid and jasmonic acid are two plant hormones that play important roles in plant defense responses. Auxin is involved in almost every plant growth process, but recent studies have shown its participation in plant-microbe interactions. However, the role of this hormone in tomato root-mediated resistance to *R. solanacearum*is unknown. Previous work has shown that a tomato auxin transport mutant *diageotropica* (*dgt 1-1*), is resistant to *R. solanacearum.* RNA-sequencing data from inoculated plants has indicated that auxin-related genes like *PIN1*, *ARFs* and *IAA/AUX*s are downregulated in roots of resistant tomato plant. Preliminary results have identified another auxin transport mutant and an auxin signaling mutant with delayed symptom development. Understanding the role of auxin in defense responses to *R. solanacearum* in tomato is important for Solanaceae crop improvement.

The phytovirome: Viral communities in soil and plants
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Our group uses metagenomics and metatranscriptomics to characterize viral communities in soil and plants and their contributions to microbial ecology, nutrient cycling, and plant health in natural and agricultural ecosystems. Here we report the recovery of thousands of previously unknown viral population sequences from these ecosystems. For example, we recovered 3,488 viral populations from 16 near-surface soil samples from eight agricultural tomato plots at two time points: pre-planting in April 2018 and at harvest in August 2018. Significant differences in viral community composition were observed between pre-planting and harvest samples and between viral size-fraction metagenomes (viromes) and bulk soil metagenomes. Nearly all viral populations recovered in bulk soil metagenomes were also recovered in viromes, and the vast majority of viral diversity was recovered in viromes alone, highlighting the utility of purifying viral particles prior to DNA extraction. A significant shift in viral community composition between pre-planting and harvest samples suggests the potential for recruitment of a distinct virome to rhizosphere soils. Separately, high dsRNA yields from oak leaves and conifer needles suggest the potential for thriving viral communities in and on these plants, which we will explore through metatranscriptomics. Together, these results highlight the vast untapped diversity of the phytovirome.

With what resolution does *Phytophthora infestans* distinguish among substrates?
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Phytophthora infestans distinguishes artificial growth medium from a living host by consistent differential expression of thousands of genes that lead to large differences in phenotype. For example, sporangia produced in pure culture (plate sporangia) are less aggressive and produce more necrosis than do sporangia from an infected tomato leaflet (leaflet sporangia). We analyzed gene expression in different genotypes of *P. infestans* to test if this pathogen distinguished among different genotypes of tomatoes. We found no significant

differentially expressed genes (DEGs) between sporangia produced on susceptible tomato cultivar Jetstar versus susceptible tomato cultivar Rutgers. This was true for each of two different genotypes of *P. infestans* (US23 and US24). In contrast there were 352 significant DEGs when the gene expression of US23 growing on the tomato cultivars was compared to that of US24 on the tomato cultivars. US23 is a good pathogen of both potatoes and tomatoes and grows biotrophically on tomatoes. In contrast US24 is primarily a pathogen of potatoes and produces more necrosis on tomatoes than does US23. The gene expression differences are consistent with these phenotypes. Eight crinklers were significantly up-regulated in US24 compared to US23. Crinklers are so named because of an initial association with a crinkling and necrosis phenotype. Seven of these eight crinklers were also up-regulated in one comparison of plate sporangia to leaflet sporangia. A next goal is to determine if *P. infestans* distinguishes between potatoes and tomatoes.

Effect of anaerobic digestates on the suppression of plant-parasitic nematodes: Do the substrate and the digestion process influence the effect?
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Novel chemical-independent approaches for suppression of plant parasitic nematodes are urgently needed. Anaerobic liquid digestates from biogas production have demonstrated suppressive capacity of plant-parasitic nematodes and may serve as bio-fertilizers. In this study, effects of substrates and fermentation conditions on digestate's properties were investigated. In different experimental contexts, drench-applied digestates, derived either from food waste or dairy manure, processed mesophilically or thermophilically, were tested for suppressive potential of plant-parasitic nematodes. In radish bioassays, root penetration by *Heterodera schachtii* J2 was reduced when exposed to thermophilic digestates, and was 10-times less than the control in food waste digestate at 2 ml/35 ml soil. In a watermelon bioassay with *Meloidogyne incognita*, and a pot experiment with peach and *Pratylenchus vulnus*, thermophilic digestates improved plant growth more than mesophilic digestates. In the watermelon test, nematode suppression was stronger in thermophilic than mesophilic digestates, and higher in food waste rather than manure substrate. In a microplot trial with bell peppers and *M. incognita*, both digestates had some negative effects on plant growth. In summary, thermophilic fermentation and the food waste were the most effective process and substrate, respectively. Further adjustments are necessary to identify application rates that suppress nematodes without negatively effecting plants.

Citrus miraculin-like protein hijacks viral movement-associated protein to facilitate defense against *Citrus tristeza virus*
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Citrus Tristeza Virus (CTV), which colonizes phloem-associated cells in the infected citrus trees, continuously causes severe losses of the citrus crop worldwide. Dissecting the host immune response against virus infection would provide essential insights to develop effective management strategies. Recently, we found that the p33 protein encoded in the CTV genome is a viral effector and one of the pathogenicity determinants. In this work, p33 was used as a molecular bait to explore virus interactions with the host immunity. We found that citrus miraculin-like protein 2 (MLP2), a member of the Kunitz-type soybean trypsin inhibitor family, interacts with the viral p33 protein. Upon expression, MLP2 localizes to the endoplasmic reticulum and the Golgi apparatus and induces cellular stress and accumulation of the reactive oxygen species. Interestingly, the oxidation-related function of MLP2 relies on its N-terminal signal peptide, but not the Kunitz motif. Binding of MLP2 interrupts the cellular distribution of p33 whose proper function is necessary for the effective virus movement throughout the host. The overexpression of MLP2 from the virus dramatically reduced the infectivity of CTV in a plant host. Altogether, our data demonstrate that citrus MLP2 hijacks the viral movement-associated p33 protein and induces cellular oxidative stress to mount a defense against CTV infection.

Soybean and corn hosts unveil transcriptional plasticity of *Fusarium virguliforme*
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In the United States, soybean sudden death syndrome is caused by the soil borne ascomycete *Fusarium virguliforme.* Recent studies revealed that *F. virguliforme* colonizes additional crop species, including corn; specifically, and with significance to our work, corn is an asymptomatic host of *F. virguliforme*. This asymptomatic interaction provides a unique application to uncover novel pathogen responses that are important for both endophytic and pathogenic processes on two different hosts. A comprehensive analysis of *F. virguliforme* transcriptomes from colonized soybean and corn over a 14-day time course uncovered a nearly complete network rewiring, with less than 8% average gene co-expression module overlap upon colonizing the different hosts. Fungal modules were uniquely enriched for catabolic processes of amino acids and sugars within soybean, yet co-expression clustering was enriched for upregulation of primary metabolism processes on corn. Differential gene expression highlighted a significant upregulation of carbohydrate-active enzymes on soybean when compared to corn as *F. virguliforme* shifted from biotrophic to necrotrophic lifestyle over 14 days. Interestingly, toxin production was similar upon both hosts. This analysis reveals the ability of a fungal pathogen on soybean to rewire a transcriptional program to asymptomatically colonize a corn, rotational crop of soybean, potentially enabling persistence in agroecosystems.

Effector profiles of *Xanthomonas perforans* and *X. euvesicatoria* provide insights into the evolution of host range and virulence in BLS pathogens
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Xanthomonas perforans (*Xp*) and *X. euvesicatoria* (*Xe*) cause bacterial spot disease (BLS) on tomato and pepper. Symptoms include necrotic lesions on leaves, sepals, and fruits, incurring major crop yield and economic losses worldwide. *Xp* and *Xe* have high genome plasticity and recent findings demonstrate a high rate of recombination among them. *Xp*, which was historically restricted to tomato, has recently been found causing bacterial spot of pepper. The complex host-pathogen interactions of BLS *Xanthomonas,* known to depend largely on type III effectors (T3E), are not yet fully understood. The content and distribution of T3E were analyzed in a worldwide sample of 269 *Xp* and 131 *Xe* strains. The influence of demographic processes, such as geographic location, host and year of collection, on the structure of the effector profiles were examined. Using this broad sample, core and non-core effectors common and unique to each species are described, expanding current knowledge. This is a first step towards identifying the complex demographic processes influencing the distribution of the effectors, hence, the evolution of host range and virulence in BLS strains.

Targeted mutagenesis of basil candidate susceptibility gene *DMR1* using CRISPR/Cas9

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Production of sweet basil is in jeopardy due to obligate-biotrophic oomycete *Peronospora belbahrii* causing basil downy mildew (BDM). Emerging biotechnology tools promises to broaden disease resistance and accelerate molecular understanding of basil-*P. belbahrii* interactions. The CRISPR/Cas9-mediated gene-editing has revolutionized crop improvement and functional genomics. The efficacy of this robust genomic tool in tetraploid sweet basil is being tested to knockout a potential susceptibility (S) gene. Genes exploited by pathogens in facilitating infection in plants are considered as plant S genes. *ObDMR1*, basil homolog of *Arabidopsis DMR1* (Downy Mildew Resistance 1) is a S gene that upon knockout confers nearly complete resistance against *Arabidopsis* downy mildew pathogen *Hyaloperonospora arabidopsidis*, was chosen for mutagenesis. Two binary constructs having one sgRNA and two sgRNAs respectively were generated targeting *DMR1* sequence encoding the N-terminal half of the protein. A total of 41 transgenic lines were obtained via *Agrobacterium*-mediated stable transformation exhibiting no apparent phenotypic aberration. Mutation analysis of 24 first-generation transgenic lines carrying one sgRNA verified 29.16% chimeric plants and 20.83% with indels. Also, a mutation frequency of 11.76% was obtained from 17 transgenic plants carrying two sgRNAs. Mutants with all four homeoalleles mutated in second-generation will be subjected to pathogen inoculation assay. This study demonstrates the feasibility of CRISPR/Cas9 in basil functional genomics and to develop BDM resistant varieties.

Comparing populations of plant-parasitic nematodes in a corn-soybean rotation in Ohio

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Corn-soybean rotation is very common in several Midwestern US states, but little is known about the effect of this practice on vermiform plant-parasitic nematode (PPN) communities. In 2016, a commercial field in Wayne County, Ohio with a history of PPNs was planted to corn and the following year to soybean. Soil samples were collected between 16 and 67 days after planting (DAP) at 10 cm increments to a depth of 70 cm. Total PPN population was higher under corn at all sampling intervals. Both Shannon and Simpson's diversity indices were significantly higher in corn at the 0–10 cm depth compared to soybean. The vertical distribution of three PPNs species was also examined. *Pratylenchus crenatus* predominantly inhabited the upper 40 cm and occurred at significantly higher densities between 42 and 54 DAP, particularly in the root fraction for corn. In soybean, *P. crenatus* predominantly inhabited the upper 30 cm and occurred at significantly higher densities at 34 DAP, particularly in the soil mineral fraction. The highest population densities of *Helicotylenchus pseudorobustus* were found in the upper 10 cm in soybean and in the top 40 cm in corn. For *Paratylenchus neoamblycephalus*, population densities were highest in the upper 20 cm for both corn and soybean. Our results showed that PPN populations varied with sampling depth, growth stage, and crop, suggesting that these factors should be taken into consideration when developing a sampling protocol for vermiform PPN.

Temporal dynamics of Wheat Spike Blast and disease quantification using Multispectral Aerial Imagery

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Wheat blast is a devastating disease caused by the Triticum pathotype of *Magnaporthe oryzae* (MoT). Studies have shown that MoT inoculum and blast symptoms can spread from leaves to spikes, suggesting that wheat blast might follow a polycyclic pattern. There is increasing need to study the temporal dynamics of MoT and wheat spike blast (SB). In addition, early detection and accurate identification of SB are critical for implementing adequate management strategies. Two field experiments were conducted in Bolivia to characterize the change in SB severity and incidence over time and determine if multispectral imagery can be used to accurately assess SB intensity. Disease progress curves and regression models were fitted to describe SB epidemics. The logistic population growth model best described SB incidence and severity change over time in both inoculated and non-inoculated plots from both locations

(R^2=0.87-0.98; MSE=0.24-0.94). Lin's concordance correlation coefficients were estimated to measure agreement between visual and digital estimates of SB intensity and to estimate accuracy, precision, and bias. Multispectral aerial imagery accurately (ρ_c = 0.65-0.91) and precisely (r = 0.1-0.95) estimated SB incidence and severity in inoculated and non-inoculated plots at both locations. Our findings suggest that SB might follow a polycyclic pattern and that SB incidence and severity can be quantified using aerial imagery. Higher resolution imagery of plants showing initial SB symptoms would likely increase electronic sensor-based disease detection and quantification in the field.

Perfect storms: Tropical cyclones and their effects on pathogen invasion and saturation

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Tropical cyclones affect agriculture by disrupting infrastructure, directly damaging plants, altering pathogen-vector community dynamics, and dispersing pathogens and pests between distant regions. Understanding the spread of pathogens is critical for improved monitoring and risk assessment, and for preventing and responding to new outbreaks. We analyzed historic cyclone path data from the Atlantic basin to understand which countries are connected by storms and which areas are most frequently affected. We found that cyclones connected countries in three main groups: Central America, the Caribbean islands, and greater Atlantic countries. These connections suggest that pathogen spread due to storms may be more common within a region. We also found that parts of the southeastern US were more frequently affected by cyclones compared with Caribbean countries, although this may be offset by the increased severity of storms affecting islands. We modeled the impact of reduced host availability and increased vector activity using an epidemiological model of laurel wilt disease. We found that the impacts of increased vector activity were more important than reduced host availability for determining disease incidence, likely due to the aggregation of avocado groves in south Florida. This model suggests how the effects of storms may be pathosystem-specific, and that spread of pathogens between regions may be aggregated due to storm path.

Studies on plant-parasitic nematodes in sugarbeet fields and host status of sugarbeet and rotational crops for *Paratrichodorus allius*

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The Red River Valley of North Dakota and Minnesota is one of the major sugarbeet production regions in the United States. However, no comprehensive study has been carried out for plant-parasitic nematodes (PPN) in the region thus far. Hence, a field survey was conducted during 2016 and 2017 in sugarbeet production fields across 13 counties. The major genera of PPN identified from 217 soil samples were *Heterodera*, *Helicotylenchus, Tylenchorhynchus, Paratylenchus, Pratylenchus*, *Paratrichodorus, Hoplolaimus,* and *Xiphinema.* Eight of them were identified at the species level using species-specific PCR assays and/or amplification and sequencing of the ribosomal rDNA. The cyst nematodes analyzed from 31 samples were *Heterodera glycines*, and *H. schachtii*, a nematode known to cause damage to sugarbeet, was not identified. Among identified species, stubby root nematode, *Paratrichodorus allius*, is one of the important nematode pests for sugarbeet production worldwide. With little information on the host range of this nematode, a second study was conducted to determine the host status of sugarbeet and their rotational crops for *P. allius* under greenhouse conditions. The results indicated that almost all the sugarbeet and rotational crops supported the reproduction of *P. allius* in two trials with reproductive factors greater than one. This research is a critical first step towards developing effective pest management strategies in the infested sugarbeet fields to improve their productivity.

Screening method for selecting *Cercospora* cf. *flagellaris* target genes for silencing via dsRNA application

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Cercospora leaf blight (CLB) of soybean (*Glycine max* L. Merr.) is one of the most important soybean diseases in Louisiana, primarily caused by *Cercospora* cf. *flagellaris*. *C.* cf. *flagellaris* produces a toxin, cercosporin, which is an important virulence factor. So far, there are no known CLB resistant cultivars, and substantial fungicide resistance has developed in the pathogen. Consequently, alternative methods are needed to manage CLB. One possibility is RNA interference via topical application of dsRNA, with the critical step being the selection of the target genes, whose silencing will effectively reduce fungal growth or cercosporin production. We have developed a method to test the effectiveness of candidate genes in suppressing toxin production by silencing the genes known to be involved in the production of cercosporin (*CTB1*, *CTB8*), as well as several genes recently associated with cercosporin accumulation. The desired gene fragments were cloned into a vector that enables dsRNA production upon overexpression in *Escherichia coli* cells. Purified dsRNA was combined with complete media broth, inoculated with macerated mycelia of *C.* cf. *flagellaris* and incubated until cercosporin was produced in the control treatments. Several of the tested dsRNAs demonstrated efficacy at various concentrations in reducing cercosporin production in culture. This method will now allow the identification of the most potent dsRNAs for potential CLB management under field conditions.

Soil treatment options for nursery applications against plant-parasitic nematodes

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Nematode-free stock is paramount for production of perennial crops. California's nursery certification regulations mandate sampling or prescribed cultivation patterns plus pre-plant soil treatments. Because methyl bromide is being phased out, and use of 1,3-dichloropropene (1,3-D) is restricted, alternative treatments to prevent *Pratylenchus vulnus* infection of nursery stock were compared to a 1,3-D control. In fall 2015, replicate microplots were drenched with one of four low-volume chemicals or three biocidal materials. In spring 2016, additional microplots of sandy or sandy loam soils were drenched with selected treatments based on the 2015 results. In fall 2016 in field plots, anaerobic soil disinfestation (ASD): incorporating easily decomposable C-sources (here: rice bran, molasses, tomato pomace or mustard seed meal), covering soil by plastic tarp and heavy watering, was evaluated. Peach rootstock 'Nemaguard' was planted. In the first growing season of each experiment, nematode survival was determined by nematode counts in soil and roots of the saplings. In all experiments, Salibro (experimental nematicide, Corteva), especially at high rates, ASD regardless of substrate, and one of the large-volume materials kept nematode numbers close to detection limits. Promising methods for cleaning soils from nematodes were identified but further optimizing is required to ensure the reliable high-level efficacy for the nursery industry.

Role of *avrBsT* in dispersal of *Xanthomonas perforans* and severity of bacterial spot of tomato

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Bacterial spot caused by *Xanthomonas perforans* (*Xp*) is an economically important disease in tomato. Previous studies have shown that more than 70% of recently isolated *Xp* strains have acquired and retained an effector gene a*vrBsT*, which has been reported as a fitness factor. In order to elucidate how *avrBsT* confers fitness to *Xp,* we conducted a study with the objectives of quantifying pathogen dispersal and predicting the prevalence and movement of *Xp* strains in relation to the *avrBsT* gene. We compared movement of two wild-type Xp strains, GEV872 and GEV1001, which carry *avrBsT*, with their respective *avrBsT*-mutants when co-inoculated in the field. We developed a model to quantify the spread of *Xp* with or without *avrBsT* over spatial and temporal dimensions. The model was obtained using binomial distribution for the absence or presence of each strain in samples collected over the tomato growing season. Weather variables and the lag period were accounted for in the model. According to the model, wild-type strains spread faster than mutant strains (~4 vs. ~1.25 meters/week). Preliminary results show that plants infected with wild-type strains exhibit higher disease severity than those with mutant strains. Based on the model, we developed an application to predict and visualize the spatio-temporal distribution of *Xp* with and without the *avrBsT* gene. Our results show that the presence of a single gene can affect pathogen movement and significantly alter bacterial population dynamics.

Examining the effectiveness of *in vitro* and *in planta* assay in assessing biocontrol activity of *Pseudomonas* strains against *Agrobacterium rhizogenes*

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A well-known method used in the identification of potent bacterial antagonists is to test *in vitro* activity by plating potential antagonist with the pathogen and evaluate the inhibition of pathogen growth (i.e. zone clearing assays). However, we hypothesized that relying on this method might miss other potential antagonists that do not rely on antibiotics production. In this study, we examined the ability of 52 different Pseudomonas strains to limit the formation of hairy root disease (HRD) caused by *Agrobacterium rhizogenes*. HRD is a problem on hydroponic grown tomatoes and cucumbers in Europe and North America. Our *Pseudomonas* collection was screened in both *in vitro* (zone clearing assays) and *in planta* (reduced disease formation) assays. Our *in vitro* studies identified fourteen Pseudomonas strains that exhibited zone clearing activity. These strains were then tested *in planta* from which we identified two that were effective in reducing disease incidence in tomato under soil and hydroponic conditions. We also screened the remaining collection *in planta* on tomato aiming to identify strains that may have been missed in the *in vitro* study. From this study we identified five additional strains that were effective in reducing disease incidence on soil (hydroponic tests are underway). In conclusion, *in vitro*, screening is helpful to identify potential strains, however, many other strains with other potential modes of actions could be lost during the process.

Improving sting nematode management in North Carolina by investigation nematicide application timing

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Management of sting nematode (*Belonolaimus longicaudatus*) on putting green turf was centered around a single nematicide until 2011. Nemacur was a highly efficacious nematicide until its voluntary removal from the market, and its loss presented a major challenge for nematode management on golf courses. Very few products were available for use, and those that were did not reduce sting nematode populations. Two new products were released, but little was known about the best timing for application in order to maximize sting nematode management. The objective of this study was to investigate application timing for abamectin (Avid or Divanem) and fluopyram (Indemnify) on reducing sting nematode populations and improving turfgrass quality. The study was conducted from 2014–2018 at a golf course in Raleigh, NC on a putting green that was severely infested with sting nematodes. Up to 4 Avid applications were applied from March to August, and there were no differences in the timings in relation to turf quality or nematode populations. Indemnify was studied starting upon its release in 2016. Applications were made from April to July, and all application timings reduced nematode populations. However, fluopyram applications in May were most effective at reducing nematode populations below 5 nematodes per 500 cc soil in August indicating summer long population reduction can best be achieved with this application timing.

Interactive Extension: Using grower surveys and real time polls to identify management strategies and needs in organic tomato late blight control
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Effective control of late blight, caused by *Phytophthora infestans*, requires integration of cultural, genetic, and chemical strategies. For organic growers, management must adhere to strict standards. Few commercial resistant tomato varieties are available and copper-based fungicides are a last resort due to concerns about its environmental impact. To identify the potential for adoption of non-copper fungicide alternatives in integrated disease management, a survey of 48 Wisconsin growers was conducted in 2018. Worker safety (57% of 23) and knowledge of application were extremely important factors (61% of 23) in influencing management strategies. More than one third (36%) of 22 indicated that they had used copper; just half of the respondents used non-copper fungicides. We investigated this further by polling organic growers at the Midwest Organic & Sustainable Education Service conference in Feb. 2019. More than half of the growers (26% of 47) confirmed their greatest limitation in using organic-approved non-copper fungicides was uncertainty in effectiveness. Just 6% of 47 were limited by cost or worker and consumer safety. More than half (50% of 21) indicated they were unsatisfied, unsure, or experienced variable satisfaction with non-copper organic approved fungicides. These responses suggested that enhancing knowledge of organic-approved fungicide effectiveness may improve integrated management of tomato late blight in the Upper-Midwest. Live polling technologies can be an exciting approach to obtain data about grower needs and facilitate two-way learning in Extension.

Nationwide assessment of leadership development for graduate students in agricultural plant sciences
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Leadership development is a universally important goal across the agricultural plant science disciplines. Although previous studies identified a need for leadership skills, less is known about leadership skill development programming in graduate programs. To address this, we constructed a mixed-method study to assess current graduate students, and early-, mid-, and late-career scientists about their leadership experiences. We sent an online survey to 6,728 agricultural plant scientists that were U.S.-based members of the APS, Entomological Society of America, and the tri societies (ASA-CSSA-SSSA). There were a total of 1,086 completed responses. Most participants reported employment in doctoral institutions (42.7%), followed by industry (20.3%), and government (17.7%). Among experiences identified important for their leadership role(s), 95.7% of graduate students and 90.0% of scientists identified ‘observing other leaders’ as top most significant by both groups. The second most identified among graduate students was ‘mentoring students in research’ and among scientists was ‘job experiences’. The third most identified by graduate students was ‘mentorship from graduate advisor’ and among scientists, ‘mentoring students in field or lab research’. Respondents ranked the top three leadership skills for success in their leadership roles as: ‘effective listening’, ‘ability to identify and analyze problems’, and ‘well-developed ethic, integrity, and loyalty’. Data will be used to identify priorities for leadership skill development and opportunities for graduate student training.

Development of a full-length clone of sugar beet cyst nematode virus 1 and its successful introduction into soybean cyst nematode
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Sugar beet cyst nematode (SBCN; *Heterodera schachtii*) and soybean cyst nematode (SCN; *H. glycines*) are major pests of sugar beet and soybean, respectively. Sugar beet cyst nematode virus 1 (SBCNV1) is a single-stranded RNA virus recently discovered from eggs and second-stage juveniles (J2) of SBCN that may have the potential to reduce crop losses caused by the two species of nematodes. To establish a reverse-genetic system for SBCNV1, a full-length cDNA copy of the virus genome was amplified from SBCN total RNA using virus-specific primers that added a T7 RNA polymerase promoter and a long poly(A) tail to the 5’ and 3’ termini, respectively, and cloned into a modified pBR322 vector. The SBCNV1 genome was *in vitro* transcribed from the linearized recombinant plasmid in the presence of cap analog, precipitated onto gold particles, and bombarded into SCN J2 using a PDS-1000/He gene gun. Three weeks post inoculation, SBCNV1 infections were detected in mature SCN males using TaqMan qRT-PCR and nested RT-PCR virus-specific assays. These preliminary results indicate the successful inoculation of SCN with SBCNV1 and the infectivity of SBCNV1 in SCN cells. To our knowledge, this may represent the first reverse-genetic system developed for a virus infecting a plant parasitic nematode, and the first biolistic inoculation of nematodes with nucleic acids of a single-stranded RNA virus.

Big data solutions for smallholder farmers in Southeast Asia: Machine learning for real-time forecasting of cassava mosaic disease risk
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Cassava mosaic viruses, cause of cassava mosaic disease (CMD), are a major threat to global cassava yields. *Sri Lankan cassava mosaic virus* (SLCMV) was first reported in Cambodia in 2015 and more recently in Vietnam and China, and with symptoms reported in Thailand. Risk of dispersal of this pathogen is twofold; it is vectored locally by whiteflies and also through the movement of infected cassava planting material, in the form of stakes. Stakes are transported long distances with regular movement across national boundaries. We present a computational framework for integrating disease incidence, climate, weather, land use, and social data layers

using machine learning methods to predict and map regional CMD establishment risk. Model outcomes were used in scenario analyses with multilayer network models to predict the locations which should be targeted for surveillance and clean seed deployment. Data for parameterizing the stake trade network include household surveys conducted in Cambodia and Vietnam. Interestingly, locations that were optimal for surveillance were not necessarily optimal for the deployment of clean seed material, a practical finding for epidemic mitigation in the region. Additionally, we discuss risk pathways that may be important for the introduction of future disease threats for the region. The methods presented here provide a framework for modeling emerging pathogens and pests across systems.

Unsupervised learning for efficient detection of plant disease through low altitude remote sensing
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Low altitude remote sensing (LARS) offers promise for economical, precise, high-throughput detection of agronomically relevant phenomena including disease. A major limitation to realizing this promise has been that analytical techniques have not developed at the pace with which LARS data quality and volume have increased. In this presentation, the unsupervised learning technique of mixture modeling is discussed as an efficient and effective means to utilize LARS-sourced data for disease detection in plant pathosystems. Benefits of mixture modeling include obviating some calibration requirements, and enabling the use of image data captured during unstructured vehicle flight plans. This technique was successful in physical plant phenotyping, and is being developed for detection of diseases of turf grass. Specifically, detection will begin with the causal agent of large patch, *Rhizoctonia solani*, and by relating symptoms of the pathogen to observable characteristics in the data.

One lab-one protocol: Synergetic effect of 5'AT-rich flap to harmonize qPCR protocols for easy, sensitive and cost-effective diagnostics
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Accurate and rapid diagnostics streamline efficient disease management and can minimize losses due to diseases. In diagnostic labs, hundreds of protocols are in place to detect different pathogens from different hosts. In this research, our aim was to devise a single qPCR protocol that can be used for different pathogens. Customized non-complementary AT-rich flap sequences were added at the 5' position of each primer to improve the reaction thermodynamics, and then developed TaqMan qPCR protocols that worked under the same condition for *Clavibacter michiganensis*, *C. michiganensis* ssp. *nebraskensis*, *C. michiganensis* subsp. *michiganensis*, *Dickeya*, *D. dianthicola*, *D. solani*, *Pectobacterium*, *P. parmentieri* and a universal internal control (UIC). The primer and probe sets for these pathogens can be mixed and matched based on the user's preference without the need for further validation with exclusivity and inclusivity panels. Also, the inclusion of 5' AT-rich sequences enhanced the qPCR reaction efficiency from 0.82 (M = –3.83) and 0.91 (M = –3.54) to 1.04 (optimum slope value; M = –3.23) for both *C. michiganensis* and *C. michiganensis* ssp. *nebraskensis*, respectively; an increase of 10-fold sensitivity was also obtained with *C. michiganensis*. No false positives or negatives were detected. The proposed methodology will improve existing protocols and reduce the labor, processing time and reagent consumption; overall the diagnostic cost per sample will be reduced.

Development of novel diagnostic and management tools for citrus greening using peptidomics
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The molecular interactions between the Asian citrus psyllid and '*Candidatus* Liberibacter asiaticus' (CLas) is a critical area of citrus greening research. In this work, we used high resolution mass spectrometry to measure the collection of small, native proteins found in the psyllid (the peptidome), and discovered evidence for multiple classes of bioactive peptides that are up- or down-regulated in CLas-infected psyllids as compared to non-infected psyllids. Psyllid peptides with strong sequence and structural homology to insect neuropeptides were identified, and remarkably, 10 of the 13 neuropeptide precursors identified were down-regulated in CLas-infected insects. Oral delivery of two neuropeptide analogs to the psyllid from artificial diets induced wing deformities and high levels of mortality in adult psyllids. In addition, a series of candidate peptide biomarkers that predict the CLas-infection state of psyllids were identified. A total of 15 candidate biomarkers, which were found consistently up-regulated in or unique to CLas-infected insects, resemble known antimicrobial peptides (AMPs). These candidate AMPs have been synthesized and will be tested for activity against a panel of bacteria, including the only culturable species of Liberibacter, *L. crescens*. Taken together, the information obtained in this work not only yields new insights into psyllid physiology and multitrophic interactions, but may also lead to the development of novel diagnostic and management tools for citrus greening disease.

Classical breeding and gene-silencing: Multi-pronged strategies to address incomplete resistance to Sclerotinia stem rot in soybean (*Glycine max*)
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Soybean (*Glycine max*) resistance to *Sclerotinia sclerotiorum* is incomplete, and yield losses from Sclerotinia stem rot (SSR) can exceed 300 kg/ha for every 10% increase in severity. We aim to enhance resistance to SSR using classical breeding and modern RNAi methods. Newer soybean germplasm, identified through selections in the greenhouse and field nurseries, has served as an unprecedented source of high physiological resistance to SSR. Crosses integrating this germplasm with susceptible, high-yielding lines has been

recently accomplished. Additionally, the pathogenic success of *S. sclerotiorum* requires the secretion of oxalic acid (OA). Virus-induced gene silencing (VIGS) was used to target OA biogenesis in *S. sclerotiorum*. A sequence corresponding to the fungal *oxaloacetate acetylhydrolase* (*Ssoah1*) was cloned into a BPMV vector and introduced into *G. max*. Plants containing BPMV vectors targeting S*soah*1 had significantly enhanced resistance to *S. sclerotiorum* (P=0.0035) and significantly lower expression of *Ssoah1* (P=0.0003), compared to empty-vector control plants, 48 hours post inoculation. Our study also evaluated exogenous applications and uptake of dsRNA by *S. sclerotiorum* to limit OA production. These results provide evidence supporting host-induced gene silencing and exogenous applications of dsRNA targeting virulence factors as viable strategies to control SSR. Combining classical breeding with RNAi technology may offer superior resistance to SSR in *G. max*.

Virus prevalence in Ohio wheat fields is associated with cropping practices
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Ohio is a leading producer of soft red winter wheat in the U.S. Viruses are known to impact wheat production; however, the prevalence of wheat-infecting viruses in Ohio has been under-reported. Samples were collected from 103 wheat fields in Ohio in 2012, 2016, and 2017, and subjected to RT-PCR or ELISA for virus identification. Cropping practices for each location were recorded regarding the presence of nearby volunteer wheat, nearby corn fields, and the previous crop used in the rotation. Barley yellow dwarf virus (BYDV), Cereal yellow dwarf virus (CYDV), Wheat spindle streak mosaic virus (WSSMV), Wheat streak mosaic virus (WSMV), Brome mosaic virus (BMV), and Cocksfoot mottle virus (CfMV) were among the most prevalent, being detected in 67, 63, 55, 28, 26, and 17% of the samples, respectively. Logistic regression analyses indicated that the presence of BYDV, WSMV, WSSMV, and CfMV were associated with the presence of volunteer wheat, BYDV and CfMV with monocots as the previous crop, and BMV with the presence of nearby corn fields ($p < 0.10$). Runs and odds ratio analysis indicated that BMV, BYDV-PAV, CYDV-RPV, WSMV, and WSSMV were spatially clustered in some fields, but only WSSMV was significantly more likely to be clustered than WSMV ($p < 0.05$). These results indicate that known and previously unreported viruses may impact wheat production in Ohio. The associations between virus presence and cropping practices and evidence of clustering occurring at multiple spatial scales provide insight into virus biology and potential management strategies.

Integrated management of turnip scab caused by *Streptomyces* spp.
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Turnip scab is caused by *Streptomyces* spp., soil-inhabiting filamentous bacteria. Turnip production for fresh market or processing in Michigan comprises approximately 429 acres. Growers reported turnips yield $3,000-$6,000/A in gross revenue in 2015 and 2016. Losses due to diseases cost growers significant revenue. Recently, turnip production in Michigan has been affected by *Streptomyces* spp. Therefore, the objective of this study was to develop integrated management tools for turnip scab. An experimental research trial in a commercial grower field was established with the turnip variety “Purple Top White Globe” to evaluate different rates of fumigant (chloropicrin) and two commercially available biological products. Turnip seed was planted at 1” depth into four rows by 100-ft plots and replicated four times with a randomized complete block design. Six treatments; fumigant with three different rates, two biological treatments were tested and compared to the non-treated control. Treatments were applied pre-planting and at planting. Two rows of turnip per plot were hand harvested 70 days after planting. The number of total and marketable turnips was calculated. Disease severity was measured as an index. No treatments were significantly different from the non-treated control in number of total roots. Two different rates of fumigant had significantly higher total and marketable yield compared to the non-treated control.

The role of multi-site fungicides for controlling *Cercospora beticola* and managing fungicide resistance
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Cercospora beticola causes Cercospora leaf spot (CLS), the most destructive foliar disease of sugar beet worldwide. Growers combine improved genetics, cultural practices, and fungicides to manage CLS. *C. beticola* has a history of developing resistance to several classes of fungicides. In 2016, widespread QoI resistance resulted in a CLS epidemic and over $200 million reduction in revenue for growers in North Dakota, Minnesota and Michigan. Since 2016, field studies were conducted in the USA to evaluate the efficacy of fungicides for controlling a *C. beticola* population resistant to QoI fungicides and with reduced sensitivity to demethylation inhibitors (DMI). Results indicated that QoI fungicide treatments resulted in disease severity that was not significantly different from the non-treated check, and DMI fungicides were becoming less efficacious. The efficacy of DMI fungicides improved when used in mixtures with multi-site fungicides, such as triphenyltin hydroxide, copper products, and mancozeb. Results also showed that the use of only multi-site fungicides in a rotation program provided season-long control of CLS. Deploying only multi-site fungicides for CLS control may be a strategy to reduce *C. beticola* populations that are resistant to site specific fungicides.

A group plot experiment to incorporate agricultural field research training into summer undergraduate internships
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The design and execution of field plot experiments are sought-after skills in the agricultural workforce, yet are not often introduced to undergraduate student researchers. In the 13 national summer internship programs advertised in plant health areas for the summer of 2015, only 15 of 109 student projects reported a fieldwork component. To help address this deficiency, we designed a group field project to fit into a 9-week summer program funded by the USDA at SCSU and the CT Agricultural Experiment Station over three summers. In addition to conducting individual mentored research projects, ten undergraduate interns per summer researched the effects of nanoscale nutrients on the growth and disease susceptibility of chrysanthemum. Interns attended programmatic workshops in experimental and plot design, planting and inoculation, data collection, and analysis. Plot setup and weekly data collection tasks were divided evenly among interns, while experimental design and analysis activities were performed by partners in a large group setting. Students presented their research to the general public in a plot display at the station's annual Plant Science Day. Successes, challenges, and outcomes from student surveys will be presented, along with recommendations for conducting group field research with undergraduates.

A hitchhiker bacterium on the *Rhizoctonia* highway

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Bacterial-fungal interactions are endemic phenomena in the rhizosphere, where bacterial partners may utilize the fungal mycelium for reaching to new niches. Previously in our lab, an *Enterobacter* sp. strain, named *Enterobacter* sp. En-Cren, was isolated from the hyphae of the soil pathogen fungus *Rhizoctonia solani* AG2-2IIIB (Rs-cren), the causal agent of turfgrass brown patch disease. Endohyphal En-cren can be released from damaged hyphae as a free-living bacterium and rapidly migrate along the outside of hyphae. While most identified bacterial hitchhikers of the fungal highway are flagellum-dependent, En-cren does not require flagellum to move along the fungal hyphae of Rs-cren. To identify the determinant factors for the hyphal motility, we created an En-cren mutant library using transposon Tn5. Mutants were screened and identified for loss of ability to migrate along fungal hyphae. Complementation and functional gene characterization are undergoing. This study will contribute to the knowledge of bacterial-fungal interaction and bacteria distribution in the mycorrhizosphere.

A smartphone-based volatile sensor platform for noninvasive detection of plant pathogens

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Plant disease diagnosis conventionally relies on molecular assays that are complicated, time consuming, and constrained to centralized laboratories. Here, we developed a cost-effective, field-deployable, and smartphone-based volatile organic compound (VOC) sensor platform that allows noninvasive diagnosis of plant pathogen infections by monitoring characteristic leaf volatile emissions. This integrated system incorporates a paper-based chemical sensor array (test strip) to detect key plant volatiles at the ppm level within one minute of reaction. The colorimetric test strip was then digitally imaged and analyzed by a custom-developed smartphone reader device. Using this platform, we demonstrate the multiplexed detection and classification of 10 individual plant volatiles, including (*E*)-2-hexenal, one of the major VOC markers of late blight. In addition, diagnosis of tomato late blight (*P. infestans*) as early as 2 days after inoculation was demonstrated with this handheld device, which is much earlier than the manifestation of visible symptoms. Finally, we demonstrate a detection accuracy of 95% for *P. infestans* diagnosis in both lab-inoculated and field-collected infected tomato leaves in blind pilot tests.

Identification of putative effector genes in the causal agent of spinach Fusarium wilt, *Fusarium oxysporum* f. sp. *spinaciae*

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The only region suitable for spinach seed production in the United States is the maritime Pacific Northwest, where mild, dry summers with long day length are conducive to high yields of quality seed. Fusarium wilt of spinach, caused by *Fusarium oxysporum* f. sp. *spinaciae* (*Fos*), is the primary biotic limitation to spinach seed production in this region. Host-specificity of other *F. oxysporum* ff. spp. is associated with unique combinations of effector genes, but it is unclear which genes control pathogenicity to spinach in *Fos*. Nine *Fos* isolates and five non-pathogenic, spinach-associated (NPS) isolates were characterized phenotypically using pathogenicity tests on two spinach inbreds. These same isolates were characterized genotypically with effector gene profiles predicted from whole genome sequences based on Illumina (n = 13) and PacBio (n = 1 *Fos* isolate) platforms. *Fos* isolates separated into two groups, both of which were distinct phenotypically and genotypically from NPS isolates, based on disease severity on two inbred spinach lines and predicted effector gene profiles, respectively. The association of predicted effector genes and two phenotypic groups of *Fos* has not been described previously. Characterization of the predicted effector genes and other genomic regions of *Fos* will aid in understanding mechanisms of pathogenicity, developing molecular tools for rapid detection of this pathogen, and breeding cultivars with increased resistance to Fusarium wilt.

The influence of crop production practices, and temperature on the abundance of *Pythium* and *Phytophthora* in soybean

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In Ohio, soil-borne diseases, especially those caused by *Pythium* and *Phytophthora*, can be a limiting factor for soybean production. Reproduction and infection are dependent on soil edaphic factors, soil moisture levels, and temperature. However, how these influence which species of *Pythium* and *Phytophthora* are favored is poorly understood. This study used an amplicon sequencing approach with

oomycete specific primers (ITS6/ITS7) paired with direct isolation methods of soils baited at 15 and 25°C to determine which communities were favored in five research farm fields with different production practices over five years. *Pythium* and *Phytophthora* communities differed between fields where continuous soybean was grown compared to continuous corn and to those with a soybean, corn and wheat rotation. The species *Pythium sylvaticum* and *Pythium ultimum* were the most recovered species using direct isolation methods. These were also abundant when using amplicon sequencing. Additionally, based on OTU abundance, four different *Pythium* species were significantly more abundant at 15°C compared to 25°C. This study reports how oomycete communities are shaped based on agricultural practices and how the abundance of these species can change when exposed to different temperatures.

Comparative genomics of *Pectobacterium* species revealed remarkable heterogeneity in pathogenicity determinants, antimicrobial compounds and CRISPR Cas

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Pectobacterium species, pectolytic necrotrophs, are responsible for devastating soft rot and blackleg diseases which lead to significant economic losses mainly on potato worldwide. The aim of this study was to reveal unknown genomic biology of all known species within the genus *Pectobacterium* through comparative genomic analyses. Numerous pseudogenes in *P. carotovorum* ssp. *odoriferum* suggest a low coding capacity and genomic degradation. Genome atlases showed distinct DNA structures and highlighted suspicious high transcription zones, possibly modulated by the nucleosome positioning. Orthologous analysis determined a pan and core genome sizes of 9,296 and 2,414 genes, respectively. Remarkable divergences were observed in gene clusters encoding pathogenicity determinants and antimicrobial compounds. Three distinct CRISPR-Cas systems, IF, IE and IIIA, were identified, with some species presenting both subtypes IF and IE. Additionally, a novel cellobiose phosphotransferase system was found exclusively in *P. parmenteri,* and an unreported T5SS is conserved in almost all species. Several virulence determinants, bactericide clusters and even a complete IIIA CRISPR-Cas system were predicted, confirming the horizontal gene transfer among the species. Our findings enhance understanding of how different species employ diverse mechanisms during the disease process and other important cellular functions.

Deciphering early transcriptome changes during *Sw-5* NLR immune receptor-mediated resistance to TSWV

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Tomato spotted wilt tospovirus (TSWV) is a member of the order *Bunyaviriale*, family *Peribunyaviridae* and genus *Tospovirus*. Two crops, highly damaged by TSWV, fresh tomato and pepper, represent 18% of the value of all U.S. vegetables. The virus is transmitted by several thrips species, the most important being Frankliniella occidentalis, the western flower thrips. There are several resistance genes for TSWV, *Sw-5* being the most commonly deployed in tomato because it mediates resistance against multiple tospovirus species. The *Sw-5* gene encodes an intracellular nucleotide binding leucine rich repeat (NLR) class of immune receptor that is critical in recognition of the TSWV movement protein, NSm, resulting in a hypersensitive response and containment of virus to the infection site. Our goal is to understand early transcriptional changes that occur during *Sw-5*-mediated resistance. We used RNA-seq to identify early transcriptional changes during Sw-5 mediated resistance against TSWV by comparing mechanical and thrips inoculated susceptible (*Sw-5*-/-, Santa Clara) and resistant (*Sw-5*+/+, CNPH-LAM147) tomato isolines. We will discuss our results on differentially expressed genes (DEGs) in resistant plants and their potential function in *Sw-5*-mediated resistance.

Transmission and functional role of carrot endophyte communities

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The important role of endophyte communities in plant health is becoming increasingly recognized, yet information is still lacking about their source and functional role in early stages of plant development. Thus, we sought to determine whether endophyte communities are being vertically transmitted to offspring via seed and if so, begin to elucidate their functional role. Endophyte present in surface sterilized carrot seed and seedlings of nine genotypes were isolated using a culture dependent approach. Endophytes were detected in all plant extracts and abundance increased from seed to seedling, suggesting that vertical transmission of endophytes does occur in carrots and could differentially affect processes at various stages of plant development. Taxonomic identification of individual endophytes using their 16s rRNA or ITS partially sequenced regions, indicated that *Pantoea spp*. and *Xanthomonas spp*. were the most abundant bacterial genera in seeds and seedlings respectively, while *Alternaria spp*. was the most dominate fungal genera observed in both plant stages. *In-vitro* assays indicated that several isolates can produce siderophores and IAA, fix nitrogen and solubilize phosphate. This indicates that these communities could play a beneficial role in early carrot development and possibly promote processes that the carrot is unable to fulfill on its own. Running a pathogenicity test for detected isolates and transcriptomic profiling of host in the future can further support our findings and help to understand endophytes effect on carrot growth under real world situations.

Requirements for *in vitro* pseudothecia development and ascospore maturation of *Venturia effusa*

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Pecan scab, caused by *Venturia effusa*, is considered the most destructive disease of pecan in the southeastern United States. Current control practices require the use of fungicide sprays targeted at the asexual cycle addressing the polycyclic nature of this pathogen. However, identification and characterization of mating type idiomorphs led to successful crosses producing pseudothecia *in vitro*, suggesting the sexual stage may play a role in disease development. To investigate factors influencing the development of pseudothecia, three isolates of each mating type were crossed pairwise on oatmeal agar (totaling eight crosses). To assess pseudothecial production

and maturation, the crosses were exposed to different temperatures (4, 8, 12, 16, 22C), incubation periods (2, 3, 4 mo), and photoperiods (0, 12h) at 24C following incubation. The number of pseudothecia were quantified, and their maturation assessed under the different environmental conditions. There were significant effects of temperature, month, and cross on production of pseudothecia. Although pseudothecia developed at all temperatures, greatest numbers developed at ≤10C. Pseudothecia remained immature at 22C suggesting a cold period is required for ascospore maturation. The results indicate the likely environmental conditions to identify pseudothecia in the field, and potential times for ascospore release.

Determining the environmental parameters that influence growth of the soybean root rot pathogen, *Fusarium proliferatum*
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Soybean root rot is a devastating disease throughout the U.S. and Canada that reduces yield due to decreased absorption of plant nutrients. This disease is caused by *Fusarium* species and other common soilborne fungi. Recently, *Fusarium proliferatum* (FPR) has gained attention as a contributor to seedling disease and root rot in soybean. However, little is known about the relative aggressiveness of FPR as a root rot pathogen, especially pertaining to the environmental and ecological parameters influencing the ability of this species to cause disease. Fifty-two seedborne and seedling-associated FPR isolates from Kansas were collected and characterized using *in vitro* techniques to determine the optimum pH, temperature, and water potential tolerance ranges for fungal growth and pathogenicity. FPR isolates were characterized morphologically and by PCR to confirm species assignment. Thirteen of the seedling isolates were assayed using a rolled-towel pathogenicity test at 25–27°C and a pH of ~6.5. Seedling isolates reduced germination by 18% and reduced root length by 52% compared to mock-inoculated controls. Similarly, seedborne FPR isolates were shown to be pathogenic in both rolled-towel and greenhouse pot assays. In addition, niche width experiments that measure the variety of carbon sources used are in progress. The results provide foundational information that further studies can build upon to develop better field management practices against root rot in soybeans.

Physiological changes in conidia of the fungal pathogen *Neofusicoccum mediterraneum* when exposed to desiccation
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Neofusicoccum mediterraneum is an important Botryosphaeriaceae fungal pathogen that impacts pistachio production in California, with crop losses reported from 40–100%. When conidia of *N. mediterraneum* are not continuously exposed to moisture, their germination process is interrupted, and the one-cell hyaline conidia become brownish black, develop thickened cell walls and septa within the cell. It is hypothesized that these physiological changes in the conidia allows the fungus to adapt to and survive adverse climatic conditions making them solar and drought tolerant. The objective of this research was to determine if this physical alteration of the conidia is induced by desiccation. Fungal colonies were grown on low nutrient pistachio leaf agar medium for two to three weeks. A 1×10^5/ml conidial suspension was placed onto a semi permeable cellophane sheet in a glass petri dish and exposed to air from a laminar flow hood for 20 minutes. The conidia were then exposed to desiccation periods of 1, 2, and 3 hours. After the desiccation period, the cellophane sheets were placed on water agar and allowed to rest for 24 hours before conidial transformations were observed. There was a significant difference among treatments ($P < 0.05$), where 55, 77 and 87% of the conidia were converted as above at 1, 2, and 3 hours, respectively. These results suggest that desiccation can induce changes in *N. mediterraneum* conidia making them tolerant and able to survive adverse conditions.

Relationship between soil fungal densities and soil chemical properties in Pennsylvania
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Soil borne diseases cause significant yield losses in many economically important crops in the United States. Species of *Fusarium*, *Pythium*, and *Phytophthora* and *Rhizoctonia solani* are among the most common soil borne fungal pathogens. The objective of this study was to investigate the relationship between selected soil chemical properties and population densities of different fungi in soil. Soil samples were systematically collected from 22 locations in Pennsylvania covering 17 counties. Enumeration of fungal colonies was conducted using soil serial dilutions on selective media (*Fusarium* = Nash and Snyder; *Pythium* = P5ARP; *Phytophthora* = P5ARP + hymexazol; *Rhizoctonia* = Ko and Hora). Organic matter (OM), cation exchange capacity (CEC), pH, and nutrients (P, K, Mg, Ca, Zn, Cu, S) of soil samples were determined. Pearson correlation analysis showed a significant relationship between soil *Fusarium* density (CFU/g) and S ($r = 0.74$, $P < 0.0001$). *Pythium* density was significantly correlated with K ($r = 0.71$, $P = 0.0002$) and CEC ($r = 0.58$, $P = 0.0051$). Neither *Rhizoctonia* nor *Phytophthora* densities were significantly correlated with any of the measured properties. Therefore, despite their importance for enhanced crop production, S and K have the potential to increase inoculum densities of soil borne *Fusarium* and *Pythium* species respectively and could indirectly promote crop's susceptibility to soil borne diseases caused by these fungi.

Association of selected biological and chemical properties of soil with within-farm-spatial-variation of soybean yields in Pennsylvania
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The within-farm-spatial-variation of soybean yields has been a major obstacle to maximize yields in Pennsylvania and other soybean growing states. The underlying causes behind such yield variations are currently unknown. The objective of this study was to investigate whether variation in selected biological and chemical properties of soil contribute to spatial heterogeneity of soybean yields. Fourteen locations in Pennsylvania were chosen for this study. Bulk soil samples were collected from five historically high and low yielding sites per location at V1 growth stage. The plant pathogenic nematode counts (lesion, stunt, spiral, stubby root, dagger, ring, lance, and pin), fungal counts (*Fusarium*, *Pythium*, and *Phytophthora* species and *Rhizoctonia solani*), organic matter, cation exchange capacity, pH, and nutrients (P, K, Mg, Ca, Zn, Cu, S) of soil samples were determined. Based on ANOVA, none of the measured variables was significantly different between high and low yielding sites. The principle component analysis (PCA) revealed that first two PCs contribute to 46% of the total observed variation in the data set. However, this variance maximizing data point distribution failed to distinctly cluster high and low yielding sites in the PC space. Findings suggested that the underlying biological and chemical causes behind within-farm spatial-heterogeneity of soybean yields in Pennsylvania is more complex and beyond the scope of the variables investigated in this study.

Huanglongbing-irrigation pH interaction affects disease progression
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Huanglongbing (HLB), associated with '*Candidatus* Liberibacter asiaticus', is the greatest limitation in citrus production with root loss as one of the first symptoms. Field observations indicated irrigation water quality and soil pH greatly affect fibrous root health signifying the possibility of a pH-HLB interaction. An experiment was set with the objective to evaluate the effect of irrigation water pH on performance of healthy and HLB-affected citrus plants. Healthy and HLB-affected 'Midsweet' sweet orange grafted on Kuharske citrange arranged in a randomized complete block design with 7 replicates and irrigated every 4–5 days with phosphate buffers at pH 5.8, 6.4, 7.0 and 8.0 were monitored for 6 weeks. HLB-affected plants at pH 5.8 showed greater increase in plant height with 100% survival rate compared to highest death rate at pH 8.0. Soil media of HLB-affected plants had higher electrical conductivity (EC) than healthy plants at pH 8.0 indicating poor nutrient uptake by roots, not just nutrient unavailability. At pH 8.0, HLB-affected plants had more leaf drop leaving just 0.25g leaf fresh weight on the plant compared to 15g on healthy plants. Among all treatments, HLB-affected plants at pH 8.0 had the most limited root growth and development pattern, leading to lowest root dry weight. In contrast, at pH 5.8, HLB-affected plants had the same root dry weight as healthy plants. HLB symptoms were more prominent at pH 8.0 suggesting irrigation water pH management as a potential option for minimizing HLB effects.

Impact of crop rotation on genetic diversity of *Fusarium* isolates causing Fusarium head blight in Pennsylvania
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Pennsylvania small grain production is marked by diverse crop rotations. However, there is a lack of information regarding the interactions between different management strategies and how they impact *Fusarium* spp. composition and Fusarium head blight (FHB) occurrence. During the summer of 2018, 184 field samples of small grains (wheat, spelt and oat) were collected from 9 counties in Pennsylvania using a standardized sampling procedure. Information about the field including previous crop and FHB index were also recorded. In the laboratory, isolation of *Fusarium* strains were made from FHB-symptomatic spikelets. These were surface sterilized with a 5% sodium hypochlorite solution and plated on potato dextrose agar (PDA) media. After 4 days, fragments of mycelia typical of *Fusarium* spp. were transferred to specific nutrition-poor agar (SNA) to obtain pure cultures from a single spore. The genomic DNA extraction will be conducted to amplify the partial sequencing of the translation elongation factor 1 alpha (TEF-1α) gene and confirm species identity. PCR reactions targeting Tri3 and Tri12 genes will determine trichothecene types (3-ADON, 15-ADON and NIV) among isolates. The genetic structure of isolates will be measured using the variable number tandem repeat (VNTR) markers. This new knowledge will help us better understand the influence of crop rotation on the *Fusarium* species composition in small grains and the potential for FHB and mycotoxin development.

Effect of light-sensing genes on growth and pathogenicity of *Zymoseptoria tritici*
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The ascomycete fungus *Zymoseptoria tritici* (synonym: *Mycosphaerella graminicola*) is the causal agent of the major wheat foliar disease Septoria tritici blotch. Despite its importance as a pathogen, very little is known about how *Z. tritici* senses and responds to light. Light is a major environmental cue for fungi, but also is a major stressor, causing DNA damage and oxidative stress within fungal cells. Fungi use light as a cue to produce photoprotective pigments and proteins, and to control many aspects of growth and reproduction, such as asexual versus sexual sporulation, and the production of mycotoxins. A previous RNA sequencing experiment identified many *Z. tritici* genes that respond to different wavelengths of light. Multiple putative light-sensing genes in *Z. tritici* as well as those that showed differential responses to light were knocked out using *Agrobacterium tumefaciens*-mediated transformation. Phenotypes to be tested include changes in growth morphology and spore production under a variety of conditions, such as the effect of high and low temperatures, rich and nutrient-poor media and changes in pathogenicity to wheat. Environmental stresses to be tested include the effects of light and dark, varying levels of oxidative stress and different osmotic conditions. These experiments should help to reveal the effects of light on basic biological processes and the pathogenicity of *Z. tritici* to wheat.

Influence of Physical and Chemical Soil Variables on Intensity of Fusarium Wilt of Blackberry Caused by *Fusarium oxysporum* Species Complex
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Mexico, is the leading producer of blackberries in the world with a yearly production of more than 240,000 tons. Fusarium wilt of blackberry, caused by Fusarium oxysporum species complex, is currently the most important disease of this crop because more than 2500 ha have been lost. The objective of this study was to determine the influence of soil physical and chemical variables on the intensity of the disease. During November 2017, 31 commercial plots of blackberry cv 'Tupy' were sampled in Los Reyes Valley, Michoacán, Mexico. At each site, a composite sample of soil was taken for further nutritional analysis. The incidence and disease severity was also estimated in each plot, and its geographical position, altitude, and the type of irrigation system used were recorded. In the laboratory, the quantification of CFU g-1 of soil per sample was estimated on Fusarium selective media. With the recorded variables, a Redundancy Analysis (RDA) was performed. The RDA analysis indicated that the variables that explained most of the variation in the incidence and severity of Fusarium wilt of blackberry were: the content of copper and magnesium (RDA1), the organic matter and sodium content (RDA2) and the electrical conductivity, boron and iron content (RDA3). Organic matter, iron content magnesium, and copper content were also correlated with the UFC density in the soil as well. These studies will contribute to reducing the risk factors that influence the intensity of Fusarium wilt of blackberry epidemics in Mexico.

***Pythium sylvaticum* delays soybean emergence and increase seed loss at high soil moisture**
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Pythium sylvaticum is an Oomycete causing damping-off of soybeans in the Midwest of the United States. A growth chamber experiment was performed to determine the effect of soil moisture and inoculation with *P. sylvaticum* on the rate of soybean emergence and percent seed loss. Soybeans were planted in cups filled with vermiculite and either a layer of *P. sylvaticum*-infested millet or sterile millet, used as a control. Soil moisture was kept at 10% volumetric water content (VWC), 22% VWC, or 40% VWC to simulate water deficient, field capacity, and saturated soil conditions, respectively. The cups were placed in a growth chamber at 18°C with 12 hours of daylight. Emergence was recorded daily for 21 days. Days to 50% emergence and percent seed loss (seeds not emerged) were calculated. In the absence *P. sylvaticum*, 50% emergence occurred after 6.6 days at 22% VWC and was slightly delayed at 10 and 40% VWC. Percent seed loss was minimal (10% seed loss) under optimal moisture conditions (22% VWC), and increased slightly at 10 and 40% VWC. The presence of the pathogen delayed emergence at all soil moistures and under saturated conditions (40% VWC), 50% emergence was not achieved by 21 days. In the presence of the pathogen, the increase in soil moisture also increased seed loss, which reached a maximum of 66% under at 40% VWC. These data on the effect of soil moisture on soybean emergence and damping-off caused by *P. sylvaticum* will contribute to efforts to develop a model for soybean seedling disease in the Midwest.

Influence of time of sudden death syndrome foliar symptom onset on SDS intensity, soybean yield, and yield components
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Sudden death syndrome (SDS), caused by *Fusarium virguliforme*, causes substantial yield losses in soybean. However, relationships between components of disease progress, including time of disease onset, and soybean yield are poorly understood. Individual soybean plants and 1.5 × 3 m quadrats were monitored in commercial fields and experimental plots to quantify the impact of SDS foliar symptom onset on SDS intensity, soybean yield components and yield, in Iowa. The day of year when SDS foliar symptoms were first detected (onset time) and progress of SDS incidence and severity were recorded weekly. Individual soybean plants and quadrats were harvested at the end of each season. Linear regression showed that SDS onset time had a strong negative relationship with SDS intensity and a strong positive relationship with soybean yield. In individual plants, SDS onset time explained 81 to 96% of the variation in final disease severity, while at the quadrat level, it explained 84 to 99% and 88 to 92% of the variation in final SDS severity and incidence, respectively. SDS onset explained 51 to 96% of the variation in number of pods, number of seeds and total seed weight on individual plants, and 84 to 95% of the variation in seed yield in quadrats. For each day SDS onset was delayed, soybean yield increased by 6.7 to 67.3 Kg/ha. This new quantitative information improves understanding of the impact of SDS on soybean yield. Further experiments are needed to determine how this relationship is affected by site-specific factors.

Effects of statistical distributions of diseased leaves and numbers of classes in disease scales on the accuracy of estimates of mean disease severity
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In agricultural research, different ordinal scales of measurement are often used to estimate disease severity. However, the characteristics of the distribution of diseased leaves in the population and the number of classes in a disease scale often affect the accuracy of the resulting estimates of mean disease severity. The purposes of this study are to compare various interval-scale estimates to nearest percent estimates and to further investigate the effects of the number of classes in certain disease scales. A simulation method was employed to execute the study. The criterion for comparison was the mean squared error for each of the different scales used for estimation. The results of this study indicate that, when preparing numeric category scales for rating disease severity, scales with grades of ≥7 are preferable. Moreover, linear category scales with sensitivity to low disease severity are preferable to nonlinear category scales

for assessing disease severity. For practical application, to take one example, pear scab is one of the most notorious diseases around the world. However, so far no objective criteria for assessing pear scab severity have been identified in the literature. Therefore, real data from the field was used to verify the simulation results. We believe that the results of our study will be helpful in improving the accuracy of disease severity estimates in plant epidemiology and related areas of research.

Modeling pathogen distribution in strawberry production fields with the goal of developing precision fumigation strategies
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In an effort to develop a fumigation strategy based on the need for controlling the pathogens present rather than a uniform application of a high rate of fumigant a study was undertaken to validate sampling approaches for obtaining accurate pathogen risk factors. We modeled the distribution of *Verticillium dhaliae* microsclerotia (MS) prior to fumigation in a 10 acre field in Watsonville CA. 96 sample points were arranged in a grid, and samples were collected via pooling scoops of surface soil directly over the sample "point" and scoops 5ft N, E, S, W of the point. This sampling strategy was compared with a finer strategy, where the NESW subsamples were kept separated, and additional subsamples 5ft further out were also collected. Total DNA was extracted from 0.5g soil samples, and a TaqMan qPCR assay determined the approximate number of MS/g of soil from the resulting Ct values. Counts of pre fumigated propagules ranged from ~20 MS/g, to over 300 MS/g of each pooled soil sample, and occasionally varied similarly between the subsampled samples. Eight additional samples were revisited at five timepoints following fumigation, and both *V. dhaliae* DNA and MS were observed, suggesting residual DNA and propagules have the potential to persist in the soil following fumigation. These observations will be overlaid with remote sensing data from drone flights and yield monitoring to find correlations between productivity, pathogen presence and disease incidence.

Biology, epidemiology and detection of oak wilt in Michigan
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Oak wilt, caused by the fungal pathogen, *Bretziella fagacearum* (Bf) has the capacity to kill red oaks (group *Lobatae*) within 6 weeks of infection and its presence across 24 states in the US has resulted in the death of thousands of red oak trees. Bf is vectored by nitidulid beetles when spore-laden insects visit fresh wounds on healthy trees. To determine the high-risk periods of infection, we are examining the periods and the duration of spore production, contaminated insect activity, and tree susceptibility. Additionally, we are working to improve and optimize molecular detection methods and conduct a population genomic study to understand how Bf has spread across the US. Three field sites in the lower peninsula of Michigan were surveyed at two-week intervals from April to November 2018 for the presence of sporulating mycelial mats, spore carrying nitidulid beetles, and symptomatic trees. Mature red oak trees have been inoculated and monitored twice a month at each site throughout this time period. Results to-date indicate that sporulating mats are produced during the spring, summer, and fall. Peak periods of insect vector activity overlap with spore availability in late spring and early summer. Inoculated trees succumb to infection until early October. Results of the on-going field and lab experiments will be used to determine the key risk periods of Bf transmission, which may influence the timing of activities such as pruning that cause tree wounding.

Modeling *Huanglongbing* dispersal gradients in Brazilian epidemics
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Huanglongbing (HLB) is the main citrus disease worldwide. In the absence of curative or genetic control measures, HLB management is performed with use of healthy seedlings, eradication of symptomatic trees and chemical control of the vector, *Diaphorina citri*. Despite the management, incidence continues to increase in São Paulo state, Brazil. The difficulty of managing HLB is frequently related to the movement of infective vectors from inoculum sources to orchards where management is carried out (primary spread). The objective of this study was to model HLB dispersion gradient of epidemics in São Paulo, from orchards edges. For this, power law and exponential models were fitted to data of 21 rectangular orchards (~25 ha and ~13.800 trees each), in São Paulo state. The sweet orange orchards were planted, with healthy seedlings, between 2010 and 2012 and managed with at least four HLB symptoms inspection and trees eradication per year and from one to three chemical sprays per month for *D. citri* control. The gradient models were tested by non-linear regression to the proportion of trees eradicated due HLB symptoms, every 20 m, in relation to the edges of the orchard (concentrically). HLB incidence ranged, on the 21 orchards, from 0.7 to 23.4% of eradicated trees. Both models fitted to data, but the power law presented better fitting than the exponential law for 20 of the 21 orchards. The results confirm the primary spread of HLB in orchards under strict management.
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Temporal dispersal of brown and orange rust spores of sugarcane in Florida
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Brown rust (caused by *Puccinia melanocephala*) and orange rust (caused by *P. kuehnii*) are two economically important fungal diseases of sugarcane. Temporal dispersal of air-borne spores of these two diseases was determined in 2014–2015 in six different locations of the Everglades Agricultural Area (EAA). Real time qPCR assays specific to each pathogen were used to quantify the number of spores captured twice a week on glass slides using wind vane passive traps. Rust spore counts varied according to location and sampling week but spores of *P. melanocephala* and *P. kuehnii* were captured every month in at least one location. Data from all locations combined, the highest amounts of brown rust spores were collected from April to June in 2014, and in April, July, and August 2015. The highest

counts of orange rust spores were obtained in June-July 2014 and 2015, in November 2014, and in November-December 2015. Rust inoculum appeared to be produced all year long in the EAA but atmospheric spore concentrations varied for each disease throughout the crop season. The number of spores collected per month was higher for *P. kuehnii* than for *P. melanocephala* during 9 and 11 of 12 months in 2014 and 2015, respectively, suggesting that the orange rust pathogen may have a higher sporulation capacity than the brown rust pathogen. Temporal variation data of spore dispersal could be used for development of forecasting models to predict sugarcane rust epidemics in Florida.

Phytophthora canker affecting CCN51 clones on high productivity cacao farms in Ecuador
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Phytophthora spp. cause the greatest losses in cacao (est. 800K MT/year), producing disease on both pods and stems. Management practices and site characteristics influence disease development by affecting pathogen growth and survival. In Ecuador, high levels of canker disease were reported on CCN51, a widely planted clone. However, the epidemiology of canker infection in the field is poorly understood. The objective of this study was to identify variables affecting canker development and establish a baseline for incidence on commercial farms. Data on productivity and disease incidence were collected every 2–3 weeks, on 25 randomly selected trees on each of the 12 plots. The pathogen was isolated from stem and fruit lesions and identified as *Phytophthora palmivora* based on ITS and COX1 sequences. Canker incidence ranged from 0 to 28% of trees, with more infections observed during the dry season (Jun-Aug). Cankers reach up to 2 m in vertical length but do not cause mortality. No canker was observed on trees of the Nacional background genotype, supporting the hypothesis that CCN51 is relatively more susceptible. Pod infection was greatest from Jan-Mar, with 1.2–6.9% of pods infected per tree. No relationship was found between pod infection and canker. In addition, planting density did not appear to affect disease development, with the most densely planted lot (1800 plants/ha) having the lowest incidences of pod rot and canker. In addition to providing valuable baseline disease data for CCN51 in South America, this study identifies contributing factors for canker development.

Bioclimatic models predict areas of current and future suitable habitat for *Armillaria* spp. in western Oregon and Washington
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Six *Armillaria* species were found in western Oregon and Washington: *A. solidipes*, *A. sinapina*, *A. gallica*, *A. nabsnona*, *A. cepistipes*, and *A. altimontana*. Of these, *A. solidipes* is known as an aggressive root disease pathogen that causes significant growth loss and mortality in conifers. Under certain environmental, host, and plant community conditions, *A. solidipes* was found in a non-pathogenic state. In this situation, *A. solidipes* can be difficult to distinguish from other *Armillaria* spp. on the basis of signs and symptoms. Ecological roles of *A. gallica*, *A. sinapina*, *A. nabsnona*, *A. cepistipes,* and *A. altimontana* have been generally characterized as primarily saprophytic to weakly pathogenic. However, recent observations suggest that these species can also be associated with deteriorating forest health, which frequently results in tree mortality. In particular, *A. nabsnona* and *A. cepistipes* both appear to be more common in the region than was previously known, and both were found in association with a combination of dead, dying, and/or apparently diseased hardwoods. In this study, we used DNA-based methods to confirm species identification and utilized location-specific climate data for bioclimatic modeling to predict where *Armillaria* spp. are likely to occur under changing climates. Models are presented for contemporary and future time periods.

Growth stage vs. forecasting model based fungicide application to manage Phomopsis stem canker of sunflower (*Helianthus annuus*)
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Phomopsis stem canker is a yield-limiting disease affecting sunflower (*Helianthus annuus*) in the United States. In 2018, a logistic regression based model was developed to predict Phomopsis stem canker. Upon validation using data sets not used for model development, the model predicted Phomopsis stem canker in 100% of the cases (sensitivity) and no disease in 92% of the cases (specificity). The model was converted to a risk map and hosted at iPIPE. To validate the risk map, field trials were set up at a total of five locations in Nebraska (1 field), North Dakota (2 fields) and South Dakota (2 fields). The study design was a randomized complete block on a susceptible hybrid with 10 treatments containing Headline and a non-treated check. Of the 10 treatments, seven included growth stage based applications [V8 (late vegetative), R1 (bud initiation), R5 (flowering), V8+R1, V8+R5, R1+R5, and V8+R1+R5] and three included forecasting model based applications. Disease severity was evaluated at growth stages between when flowering was complete (R6) through to physiological maturity (R9) stage using a 0-4 scale. While no disease was observed in North Dakota, disease was observed in Nebraska and South Dakota. Results from the Nebraska and South Dakota trials indicate that although significant differences in yield were not observed among treatments, greater yield was observed when the fungicide was sprayed at R1 or using the model compared to non-treated check.

Effect of temperature and wetness duration on temporal rate of Sclerotinia stem rot disease development in canola
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Sclerotinia stem rot (SSR) caused *by Sclerotinia sclerotiorum* is endemic to North Dakota canola production areas. Development of ascosporic epidemics caused by *S. sclerotiorum* occurs in three stages: apothecia formation, flower/leaf colonization, and stem colonization. The objective of this study was to model the second stage as function of leaf wetness duration and incubation temperature. The study was conducted twice in growth chambers using a randomized complete block design with three replications. Fresh canola flowers were dry-inoculated with *S. sclerotiorum* ascospores and placed on leaves of individual plants. Each plant constituted a replication and received ten inoculated flowers. Inoculated plants were incubated at 10, 25, or 30°C with 6, 10, or 18 h of wetness daily for six days. The number of foliar lesions formed on each plant was recorded daily and expressed as percentage. Results of fitting five temporal models of disease progress to this data will be presented. Modeling the temporal progress of disease development as a function of leaf wetness duration and temperature will improve our understanding of how SSR epidemics progress in field conditions.

Impact of relative humidity, temperature, photoperiod and plant age on infections of *Sphaerulina* leaf spot and stem canker of wild lowbush blueberry

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Lowbush blueberry, a valuable horticultural crop managed as a wild plant, is prone to leaf spot and stem canker [*Sphaerulina amelanchier*] which causes premature defoliation in fruiting fields and affects yields and fruit quality. In this study, we investigated the impact of relative humidity (RH), temperature, photoperiod and plant age on disease development and progress under controlled conditions. With an increase in the accumulated periods of high RH (>95%), leaf infections increased on the inoculated plants with a 4-day exposure showing high severity. Exposure to high RH (>95%) was more important than wet periods for leaf infections as inoculated plants kept in a high RH chamber for 3 days showed a high leaf spot severity after 3 weeks. Inoculated blueberry leaves kept in a Petri dish chamber under free water or high RH (≥97.5%), or inoculated plants kept in a high RH humid chamber, showed 70.5–90.5% spore germination after 3 days. Leaf spot severity on the inoculated plants increased over time reaching maximum after 30 days. Inoculated plants kept for 3 days under a 14-h-photoperiod showed high leaf infections, increased spore germination (51.56%) and increased mycelium penetration (19.56%) after 4 weeks of inoculation. Inoculated plants showed very high leaf infections at 20 and 25°C and leaf spot severity increased with an increase in exposure time at high RH (>95%). Incubation temperatures of 20 and 25°C also showed high leaf spot severity under continuous high RH (>95%). Leaf spot severity was 2.69-fold higher on 4-week-old plants compared with 8-week-old plants.

A compartmental (SEIR) model for predicting Fusarium head blight epidemics in wheat

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A few dozen models have been developed for predicting the risk or intensity of Fusarium head blight (FHB) in wheat, mostly commonly by correlating weather variables with a disease variable. These statistical models have played an important role in disease management but are not suitable for understanding which and how different factors affect the behaviour of the epidemics. A few mechanistic FHB models, which link equations that describe epidemic processes (infection risk, inoculum risk, etc.), allow to estimate a daily risk which accumulates during the season. We developed a compartmental model, also known as SEIR (Susceptible, Exposed, Infectious, Removed), for simulating FHB epidemics. A series of ordinary differential equations are linked to estimate the amount of susceptible host sites (spikelets), based on degree-day accumulation, which move through the other three compartments. Two temperature-driven rates drive the amount of latently infected sites: rate of infection (anthers) and a rate of lesion expansion (spikelets within a wheat head). A time-decay model corrects for new infections by taking a spore cloud density into account. Model outputs resembled the typical FHB progress curves. The structure and expansions (host resistance and fungicide effects) of the model as well as a sensitivity analysis will be presented.

Post-anthesis Rainfall effects on the Efficacy of Tebuconazole+Prothioconazole against Fusarium head blight and Deoxynivalenol in wheat

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The influence of persistent post-anthesis rainfall on the efficacy of tebuconazole+prothioconazole (TEBU+PROT) against Fusarium head blight (FHB) and deoxynivalenol (DON) was investigated. Separate plots of susceptible (S), moderately susceptible (MS), and moderately resistant (MR) cultivars were treated at anthesis (TR) or left untreated (CK), inoculated with a spore suspension of *Fusarium graminearum*, and then subjected to one of three simulated rainfall regimes: R1) rain during the first 10 days after anthesis, R2) rain during the second 10 days after anthesis, and R3) no rainfall. FHB index (IND) and DON were quantified, and efficacy was estimated as percent control (C) of both responses relative to CK under each rainfall regime. Relative to the susceptible CK (S_CK), C for both IND and DON was highest under all rainfall regimes when the fungicide was applied to the MS or MR cultivar (MS_TR and MR_TR). Post-anthesis rainfall had a greater effect on efficacy against DON than against IND, with considerably lower C values for R2 than R1 and R3. For instance, relative to S_CK, C for DON with MR_TR was 74% for R3, 68% for R1, and only 42% for R2. Similarly, within a given resistance class, C for DON was comparable between R1 and R3 (57-61%), but much lower for R2 (23-42%). Persistent rainfall shortly after anthesis had little effect on the efficacy of TEBU+PROT against IND and DON, but efficacy against DON was severely compromised by persistent late-season rainfall.

Risk factors for bacterial diseases of banana in Haiti and the Caribbean

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Banana and plantain are important components of the diet in Haiti and are subject to yield loss from several plant pathogens. The introduction of the emerging pathogen *Xanthomonas campestris pv. musacearum* (Xcm) would threaten food security for many farmers. We evaluated several risk factors that are potentially important for the spread of Xcm into Haiti and the Caribbean region. We found that the risk of introduction of *Xcm* is very high because it has been informally reported in some neighboring regions causing important economic losses in banana production. *Xcm* may be transmitted through banana and plantain trade between these neighboring regions and Haiti. We also used cropland connectivity analysis and found that a high disease risk due to spread between plantings of banana and plantain and other species that share the same pathogens. Plantings that are closer together, and that have denser production of banana and plantain, have a greater risk of spread. Haiti has particularly dense plantings of banana and plantain and has suitable weather conditions for the disease, placing the country at high risk of pathogen introduction and establishment. We evaluate how these system components interact to create higher and lower disease risk regions for banana and plantain in Haiti.

Discovery of tunicamycin-related biosynthetic gene clusters in three *Rathayibacter* species, including one endemic to the Northwest U.S. (*R. agropyri*)

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Rathayibacter toxicus, an APHIS-listed Select Agent, produces a neurotoxigenic glycolipid similar to tunicamycin. Endemic to Australia, *R. toxicus* is obligately vectored by Anguinid seed gall nematodes to the developing seedheads of forage grasses, where tunicamycin is synthesized and subsequently consumed by foraging livestock. This results in the only known naturally occurring tunicamycin-associated disease, annual ryegrass toxicity of livestock. To investigate whether a tunicamycin-related biosynthetic gene cluster (TGC) was present in other *Rathayibacter*, all available genome sequences were analyzed. Novel TGCs were identified in *R. agropyri, R. iranicus,* and the undescribed South African *Rathayibacter* species "*EV*". The putative TGCs shared conserved tunicamycin-related genes essential for toxin production, but differ in gene order, orientation, and number. All tested *R. toxicus, R. agropyri, R. iranicus,* and *R. EV* strains possess a putative TGC. Therefore, all three species appear to be capable of synthesizing tunicamycin-like toxins; although it remains to be determined whether and under what conditions toxin is actually produced. Exploration of tunicamycin sensitivity and genetic variation within the TGC of each species is ongoing. The identification of multiple TGCs highlights the conserved nature of tunicamycin-like antibiotics within the *Rathayibacter* genus.

Cell-length heterogeneity: A population-level solution to growth/virulence trade-offs in the plant pathogen *Dickeya dadantii*

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Necrotrophic plant pathogens acquire nutrients from dead plant cells, which requires the disintegration of the plant cell wall and tissue structures by the pathogen. Infected plants lose tissue integrity as a result, exposing the nutrient rich, decayed tissues to the environment. One challenge for the necrotrophs to successfully cause secondary infection is to effectively utilize nutrients released from hosts towards building up a large population before other saprophytes come. In this study, we observed that the necrotrophic pathogen *Dickeya dadantii* exhibited heterogeneity in bacterial cell length in an isogenic population during infection of potato tuber. While some cells were regular rod-shape (<10 μm), the rest elongated into filamentous cells (>10μm). Short cells expressed all necessary virulence factors and motility, whereas filamentous cells did not engage in virulence, were non-mobile and more sensitive to environmental stress. However, compared to the short cells, the filamentous cells displayed upregulated metabolic genes and increased growth, which may benefit the pathogens to build up a large population necessary for the secondary infection. The segregation of the two subpopulations was dependent on differential expression of the alarmone guanosine tetraphosphate (ppGpp). When exposed to fresh tuber tissues, filamentous cells quickly transformed to short virulent cells. The pathogen adaptation of cell length heterogeneity identified here presents a model for how some necrotrophs balance virulence and vegetative growth to maximize fitness during infection.

Role of lipid droplets in self-resistance of *Cercospora* cf. *flagellaris* to cercosporin

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Cercospora cf. *flagellaris,* the causal agent of Cercospora leaf blight (CLB), is a yield-limiting pathogen of soybean in Louisiana and many other Gulf states. *C.* cf. *flagellaris* produces a perylenequinone toxin, cercosporin, largely responsible for the leaf-blighting symptoms of CLB. This toxin absorbs visible light, generating reactive oxygen species (ROS) that damages cell membranes and releases nutrients to the fungus. In a perylenequinone producing endolichenic fungus, lipid droplets (organelles containing neutral lipids) trap the toxin preventing ROS production. Lipid droplet-deficient yeast mutants show an increase in susceptibility to certain fungicides. We investigate if *C.* cf. *flagellaris* traps cercosporin in lipid droplets for self-resistance to the toxin. In two independent runs, three isolates of *C.* cf. *flagellaris* were grown in liquid culture and after five days the amount of cercosporin and total lipids were measured. Results showed, on average, isolates that produced high (23.5 and 37 μg/ml) and low (12 μg/ml) cercosporin, had high (24.75 and 33.83 g/L) and low (10.8 g/L) amounts of total lipids, respectively. The experiment will be expanded to include other *Cercospora* spp., known to differ in cercosporin production. Confocal microscopy will be used to determine co-localization of cercosporin in lipid droplets. If lipid droplets trap cercosporin, this could provide new possibilities to control CLB. Fungicides that inhibit lipid synthesis will be tested against *C.* cf. *flagellaris* with the final goal of translating our findings into practical CLB management.

Determination of secondary metabolites and their role in root infection by soybean fungi, *Macrophomina phaseolina* and other pathogens

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Macrophomina phaseolina (Tassi) Goid. causes a variety of diseases, including charcoal rot, crown rot, seed rot, wilt, and many others in over 500 commercially-important plants, including soybeans, trees, vegetables and ornamentals. *M. phaseolina* primarily infects plants from the soil reservoir through the roots, although it may also be seed-borne. *M. phaseolina* has been reported to produce several secondary metabolites, including asperlin, phomenone, phomalactone, phaseolinone, phaseolinic acid, patulin, and (-)-botryodiplodin, one or more of which may play a role in facilitating the root infection process. A previously-developed in-culture colorimetric assay for the mycotoxin (-)-botryodiplodin was used to screen *M. phaseolina* isolates and other soybean fungal pathogens for their ability to produce the toxin. Isolates of *M. phaseolina* negative for (-)-botryodiplodin production in this assay (20–25%) were cultured in potato dextrose broth medium for 7 days, then the culture medium filter-sterilized, mixed 1:1 with hydroponic medium and tested for 72 hours for root toxicity with soybean seedlings in hydroponic culture. 50% of (-)-botryodiplodin-negative isolates exhibited potent phytotoxic responses ranging from prevention of lateral root production to complete seedling death. Culture media from phytotoxic isolates are being examined further by bioassay-guided fractionation and LC/MS, GC/MS, and HP/LC analysis to identify production of secondary metabolites that may play a role in facilitating soybean root infection by *M. phaseolina* and other fungi.

Production of cell wall degrading enzymes by pathogenic *Streptomyces* isolated from turnip

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Plant pathogenic *Streptomyces* spp grow both inter- and intracellularly. Thus it is likely that these pathogens produce cell wall and other polymer degrading enzymes as part of their process of pathogenesis. Likely candidate enzymes include pectinases and cellulases. In this study, pathogenic species of *Streptomyces* spp., isolated from turnip showing symptoms of scab, were screened for their ability to grow on media containing pectin, polygalacturonic acid, cellulose, or glucose as sole carbon sources, and for production of enzymes that are capable of degrading these polymers. Enzyme production was determined by plate assays and the release of reducing sugars. Isoforms of the cell wall degrading enzymes were analyzed by gel electrophoresis with substrate overlays. The production of cell wall degrading enzymes in infected tissues was also assessed. The production of enzymes was compared to virulence of the isolates on turnip tissue discs. This is the first report of cell wall and related enzyme production by *Streptomyces* pathogenic on turnip.

X-ray Microscopy and Spectroscopy of Brown Spots of *Quercus acutissima*

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Brown spots on sawtooth oak (*Q. acutissima*) leaves were investigated for elemental composition using analytical electron and X-ray microscopy. The spots were approximately 1 mm in diameter and had a yellow halo. Leaf fragments with spots were directly mounted to a loader and observed using X-ray microscopy. Three-dimensional reconstructions showed that grain-like structures were mostly concentrated in the spots as well as along the leaf veins. Field emission scanning electron microscopy revealed no distinct surface differences between the spots and asymptomatic leaf regions. *In situ* X-ray microanalysis showed a homogeneous distribution of calcium and potassium on the asymptomatic leaf regions. However, a notable increase in calcium concentration was detected in the spots. Based on the elemental composition and distribution in leaves, the grain-like structures were assumed to be calcium oxalate. These results suggest that the necrotic leaf tissues may have undergone the elemental relocation in response to infection. Noninvasive X-ray microscopy can provide insight into the pathological changes in plants.

Silverleaf effects on plum fruit yield and quality

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Silverleaf, caused by the basidiomycete *Chondrostereum purpureum,* is an important disease which affects fruit trees producing wood necrosis and foliar silvering, due to the activity of an endoPG enzyme. It deteriorates plant physiological conditions by altering photosynthesis, reducing fruit quality and yield, like in apple (60%) and blueberry (40%). The objectives were to assess the effects of endoPG on fruit yield and quality in a commercial plum orchard. A 4-year old orchard of *Prunus domestica* cv. Angeleno was assessed by evaluating 20 symptomatic and 20 asymptomatic plants. The presence of *C. purpureum* was determined based on pathogen isolation on culture media, ELISA test, and PCR. Physiological parameters such as water potential, fluorescence, chlorophyll content and stomatal conductance were measured. Moreover, total yield, caliber, soluble solids, titratable acidity, firmness, colour and defects on fruit were evaluated. The results indicate the fungus was present in all the trees, however, in symptomatic ones the endoPG levels in foliage was greater. All the physiological parameters were altered in symptomatic plants, being water uptake and chlorophyll content the most affected ones. Fruit yield reduction in symptomatic trees was 51% compared to asymptomatic ones, being caliber, cover colour and firmness the most affected traits. Consequently, endoPG level is determinant in the yield and fruit quality reduction in plum.

Histology of plant-microbe interaction at the *Populus trichocarpa-Sphaerulina musiva* interface
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The ascomycete *Sphaerulina musiva* causes Septoria leaf spot and stem canker of *Populus* and is the most serious poplar pathogen in North America. The disease has a severe effect on the naïve host *Populus trichocarpa*. Despite the pathogen's impact, it is unclear whether *S. musiva* is a necrotroph or a hemibiotroph. Knowledge of the nutrient acquisition strategy would open novel avenues to explore the host-microbe interaction in *Populus*. We studied the cellular interaction of *P. trichocarpa* genotypes and *S. musiva* isolates with different levels of susceptibility and virulence. We investigated whether *S. musiva* forms intracellular structures in intact *P. trichocarpa* cells for nutrient acquisition. Inoculated stem samples were collected three weeks post inoculation for histological analysis. We report differences in host colonization and formation of potential intracellular feeding structures affected by host susceptibility and pathogen virulence. The results are discussed in the context of plant-fungal interaction along the necrotroph-hemibiotroph-biotroph continuum.

Bacteriocin evolution and regulation in *Pseudomonas syringae*
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Bacteria encode numerous antagonistic traits, including narrow-spectrum proteinaceous toxins, termed bacteriocins. Although bacteriocins are ubiquitous across phylogenetically diverse bacteria, in most cases, we lack sufficient understanding of their specific adaptive benefit. In particular, the patterns and processes underpinning the evolution of bacteriocins, as well as the environmental signals that stimulate their expression, are largely unknown. The goal of this study is to try to understand the evolutionary relationships and environmental conditions that contribute to regulation of bacteriocins in *Pseudomonas syringae. P. syringae* has served as a model plant epiphyte and pathogen for several decades, providing both a deep understanding of its ecology and evolution, as well as a developed molecular toolbox that can be used to assess environmental gene regulation. To address the question of evolution, this study will first use bioinformatic techniques to map out regions of bacteriocins in known *P. syringae* and possible evolutionary relationships. To address the question of regulation, the model phage-like bacteriocin from *P. syringae*, B728a, will be utilized to measure bacteriocin expression in combination with a fluorescent protein transcriptional reporter in various agriculturally important phyllosphere conditions. This study will combine knowledge of evolution and the environment to have a more comprehensive grasp of the role bacteriocins play to address questions regarding agriculturally important interactions in the phyllosphere.

Biological control agent, *Rhizobium vitis* strain ARK-1 suppresses expression of the essential and non-essential *vir* genes of tumorigenic *R. vitis*
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Crown gall of grapevine caused by tumorigenic (Ti) *Rhizobium* (=*Agrobacterium*) *vitis* is the most important bacterial disease of grapevine throughout the world. Recently, a nonpathogenic *R. vitis* strain ARK-1 was identified as a potential biological control agent. By soaking grapevine seedling roots with a cell suspension of ARK-1 prior to planting in the field, ARK-1 treatment significantly ($P < 0.05$) reduced the number of plants with crown gall symptoms. To gain insights into the virulence suppression mechanism of ARK-1, we co-inoculated potted grapevine shoots with ARK-1 and a Ti strain of *R. vitis* at a 1:1 ratio, and examined the expression of four essential virulence genes (*virA*, *virD2*, *virE2,* and *virG*) and one non-essential gene (*virD3*) of the Ti strain at the wound site. The expression level of the five tested virulence genes in grapevines treated with ARK-1 and a Ti at one day after inoculation was significantly lower ($P < 0.05$) than that of Ti-only treatment. We also demonstrated the lack of ability by ARK-1 to catabolize a plant-derived metabolite acetosyringone, which induces the entire *vir* regulon in Ti strains. These results suggest that the reduction in symptom development by ARK-1 treatment may be due to a direct interaction between ARK-1 and Ti strains that affects the expression of a broad range of *vir* genes.

Using nematophagous fungus Esteya vermicola to control the disastrous pine wilt disease
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Pine wilt disease induced by the pinewood nematode, *Bursaphelenchus xylophilus*, is a great threat to pine trees in many country. Once this nematode transferred from the vector, *Monochamus alternatus* to twig of pine branch, pine tree gets wilted very quickly within 3 months. Despite of enormous investments and efforts, pine wilt disease is still spreading all over the world. Since 2006, We started our research to find "natural enemy" in order to save pine trees. This field test evaluated the protective effects of the nematophagous fungus "*Esteya coreanus*" on the large pine trees of Mt. Wora, South Korea. When pine trees were treated with *Esteya coreanus,* 110 days before normal pinewood nematode (PWN) infection, ~50% of the trees survived for six years. When pine trees were treated with *Esteya coreanus* one week after artificial normal PWN infection, 40% of the trees were saved. In contrast, all of the control trees were dead in the first year. Although it has been more than six years since the beginning of this experiment, *Esteya coreanus* was successfully detected using a PCR method with two pairs of specific primers in pine trees. In addition, one of the city in China, Heyuan, had invited us to demonstrate this invention with more than 2,000 trees. It successfully ended with the official result from their government, and this data shows ~60% in cure/prevention effects. These results suggest that *Esteya coreanus* possesses great potential as a biocontrol agent to combat the disastrous pine wilt disease.

Virulence enhancement of weed pathogens: Efficacious herbicidal fungi by selection of increased amino acid excretion
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Biological control of weeds is achieved by enhancing the virulence of weed-pathogenic fungi through selection of strains that over-excrete amino acids that cause metabolic disorders in plants. Amino acid synthesis pathways in plants have evolved to economize the metabolic cost of biosynthesis. Misregulation by one amino acid can lead to deficient biosynthesis of two other amino acids in the same pathway. We exploited this metabolic misregulation in several plant pathogens, including our application for *Striga hermonthica*, the weed causing the most significant crop loss in Africa. We determined that leucine, threonine, and tyrosine each inhibit *Striga*. Using *Fusarium oxysporum* fsp. *strigae* endemic to Kenya, we selected strains for elevated leucine- and tyrosine-excretion to increase the pathogen's virulence. These strains were embedded on toothpicks and sealed in sterile drinking straws. To grow an on-farm inoculum, farmers incubated the strains in cooked rice for three days, at which point they planted ca 0.5g of the inoculum with each maize seed hill. In 500 paired plots (using hybrid seed and fertilizer) crop yield increased an average of 56% in the long season and 42% in the short season ($p < 0.0001$, pair-wise t-test). This village-based fresh inoculum is host-specific, inexpensive, safe, effective, and well-received by farmers. We are now assessing the efficacy of virulence-enhanced pathogens for other weeds.

Isolation, characterization, and evaluation of potential biocontrol bacterial strains for managing bacterial spot disease of pumpkin
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Bacterial spot disease caused by *Xanthomonas cucurbitae* is among the most important foliar and fruit diseases of cucurbits worldwide. Given the absence of resistant cultivars, along with the limited effects of chemical treatments, we determined whether potential biocontrol agents could be effectively used to manage the disease. More than 2000 bacterial isolates associated with bacterial spot symptomatic fruits and leaves were collected and screened *in vitro* for their antagonistic activity against *X. cucurbitae* ATCC 23378. Of these, 241 isolates inhibited the growth of *X. cucurbitae* in a disc diffusion assay. These antagonistic isolates were preliminarily identified using 16s rRNA primers, and the species identity of promising strains was later determined using six housekeeping genes in a MLST analysis. Based on the *in vitro* assays, we found that the most effective isolates were *Bacillus amyloliquefaciens*, *Pantoea agglomerans*, and *Pseudomonas putida*, with all of these species having previously described biocontrol functions against other disease-causing bacteria in plants. In a greenhouse experiment, some of the isolates reduced bacterial spot disease symptoms and showed growth promoting effects on 'Howden' pumpkin (*Cucurbita pepo*). Our results suggest that some of these biocontrol agents may provide effective disease control, with the added advantage of plant growth promotion, when used under field conditions.

Characteristics of *Aureobasidium pullulans* strains exhibiting biocontrol activity against fruit decay pathogens from different parts of the world
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The yeast-like fungus, *A. pullulans*, is a commonly occurring natural saprophyte on wide range of plants. This fungus, isolated from fruit and leaves in different parts of the world, exhibited strong biocontrol activity against postharvest decays on various fruit, and was developed into a few commercial products. Knowledge of common characteristics of these strains may be very useful in future selection of the best antagonists of this species. Thus, we obtained 20 of these strains and characterized them for growth at 37 and 0°C, tolerance to 50°C, osmotic tolerance, biofilm formation, activity of specific enzymes, and production of siderophores. All strains grew at 0°C, which is very important to control decays at low storage temperature, and none grew at 37°C, which eliminates concern for human safety. Only 2 strains survived for 24 h at 50°C, while 18 survived for 2 h. Osmotolerance varies; however, all strains grew on medium with 14% NaCl and 16 even with 18% NaCl. Tolerance to high temperature and osmoticum enables compatibility with postharvest practices. Small differences were observed in enzymatic activity, although some strains didn't produce chitinase (7), xylanase (10), or urease (10). Siderophore productions on CAS agar was generally low, except for one strain that was high and one that produced no detectable siderophores. Ability to form biofilm vary widely between the strains. Merits and demerits of the presented characteristics will be discussed.

Identification and screening of bacteria for the biocontrol of *Xanthomonas hortorum* pv. *carotae* in carrot seed crops
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Xanthomonas hortorum pv. *carotae* (*Xhc*) causes bacterial blight of carrots and carrot seed crops. *Xhc* can colonize plants epiphytically without causing symptoms, but still resulting in infested seed. A biocontrol agent that inhibits the growth of *Xhc* on plants in the field could reduce pathogen populations and subsequent seed lot infestation. Nine bacterial isolates originating from carrot seeds or plants and exhibiting biocontrol potential *in vitro* were identified using 16S DNA sequences and screened for the ability to inhibit *Xhc* growth on carrot plants in the greenhouse. Amplification of the partial 16S rDNA gene using primer pair 1627R/161492R resulted in products 627-1404 bp in size. BLASTn results from the GenBank Database identified four *Pantoea* spp., three *Pseudomonas* spp., one

Enterobacter sp., and one *Flavobacterium* sp. A significant ($P < 0.0001$) effect of bacterial isolate on *Xhc* growth was observed on carrot leaves using viability quantitative PCR. Negative binomial regression parameter estimates for *Pseudomonas* isolates Pss 422 and 17-049 were negative and significantly less than zero ($P \leq 0.0001$), indicating that these isolates reduced *Xhc* colonization on carrot foliage. In contrast, the parameter estimate for isolate 16-297 was positive and significant (P=0.045), suggesting that the isolate increased *Xhc* growth on carrot leaves. Further testing is needed to determine the potential for biocontrol of bacterial blight, especially under field conditions.

Mycoparasitism and Host-Specificity of *Sphaerellopsis* Species on Rust Fungi
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Rust fungi are major pathogens in important crops. Although fungicides are almost the only and the most common option to control these pathogens, they are not practical in forestry and unacceptable in management of organic crops. The fungus *Sphaerellopsis* has been found worldwide on different rust species associated with degradation of rust sori and prevention of rust spore dissemination. To better understand the relationship between *Sphaerellopsis* and rust species, this study focuses on determining the phylogenetic relationships of the mycoparasite to examine for evidence of host-specificity and create a baseline for further studies in rust biocontrol. Samples used in this study were taken from rust specimens at the Arthur Fungarium collected in different continents. DNA was extracted to amplify gene regions including ITS and LSU. The sequence data were used for phylogenetic analysis. The results showed levels of host-specificity within the *Sphaerellopsis* species that infect rusts in the genera *Melampsora*, *Coleosporium*, and *Puccinia* which raise the possibility that this mycoparasite might have potential as a biological control of rusts where fungicide application is not possible.

The use of proteases for the management of plant diseases
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Biological control of microorganisms using enzymes has the potential to become a key component of IPM and resistance management strategies. A serine protease was found to significantly reduce disease severity caused by oomycete and bacterial pathogens. Using commercial enzymes from Novozymes' diversity collection, *in-planta* greenhouse assays were conducted to determine the efficacy of serine proteases against *Phytothphora infestans*, ***Pseudoperonospora cubensis,*** *Plasmopara viticola* and *Pseudomonas syringae*. Under greenhouse conditions, the enzyme effectively reduced disease severity (>85%) of late blight of tomato and downy mildew of grapes and cucumber. In addition, the enzyme effectively reduced disease severity (>75%) of bacterial speck. Using microscopy and laboratory assays, we elucidated the mode of action in which the protease reduced disease. For *P. infestans* and *P. viticola*, zoospores were either lysed or germination was inhibited on the foliar surface and, for *P. syringae*, biofilm formation was disrupted. This enzyme persists on the foliar surface for 16 days and is UV stable for up to 7 days. When applied at suggested application rates, there were no phytotoxicity effects. The protease is active under a wide range of temperatures and pH levels, with the optimum temperature for activity between 40–60°C. This suggests that the effective concentration may vary in different climates. This serine protease has the potential to offer growers an additional tool for disease and resistance management of oomycetes and bacterial pathogens.

Evaluation of a strain of *Bacillus subtilis* for management of Phytophthora blight of bell pepper
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Phytophthora blight, caused by *Phytophthora capsici*, is the most economically significant disease of bell pepper in the US. Over the past several decades, isolates of *Phytophthora capsici* exhibiting resistance to mefenoxam have been reported. This threat of resistance coupled with an increased market for organically grown crops has led to interest in disease management through biological control. In this project, we evaluated a strain (AFS032321) of *Bacillus subtilis* that is antagonistic *in vitro* to *Phytophthora* spp. on Phytophthora blight of bell pepper in greenhouse and field conditions. A wettable powder formulation of the strain was applied as a soil drench at transplant prior to pathogen inoculation. In the greenhouse, 30 and 40 lb/acre applications of *B. subtilis* strain AFS032321 significantly reduced disease severity and the area under the disease progress curve (AUDPC) from 30.7 in controls to 20.5 and 15.5, respectively. In the field, *B. subtilis* strain AFS032321 significantly reduced disease comparable to the labeled rate of mefenoxam on a susceptible pepper variety "Red Knight" and AUDPC from 37.9 in the control to 28.7 and 25.6 respectively. Ongoing research includes investigating timing of biocontrol application and the effect of soil type on management efficacy and the potential of *B. subtilis* strain AFS032321 to manage mefenoxam-resistant isolates of *P. capsici*.

Biological control of sudden decline syndrome of date palm caused by *Fusarium solani* using ACC deaminase producing actinobacteria in the UAE
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Thirty three actinobacterial isolates were isolated from date palm rhizosphere in the United Arab Emirates (UAE). These were screened for their abilities to produce chitinase, β-1,3-glucanase and antifungal metabolites active against *Fusarium solani* the causal agent of sudden decline syndrome (SDS) of date palm in the UAE. Only eight isolates showed *in vitro* exceptional antifungal metabolites and *chitinolytic* activity and caused the lysis of *F. solani* hyphae. These eight isolates were subsequently tested for their ability to produce 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, the immediate precursor of the stress hormone ethylene. Under greenhouse conditions, the ACC deaminase-producing ($ACCD^+$) isolates were significantly more effective in reducing the incidence of SDS

compared to ACC deaminase-non-producing ($ACCD^-$) isolates. The application of these $ACCD^+$ isolates resulted in the reduction of the *in planta* levels of ACC compared with date palm seedlings treated with the $ACCD^-$ isolates. The results clearly showed that $ACCD^+$ isolates could replace Cidely® Top which is the currently recommended fungicide for the management of SDS of date palms in the UAE. The results showed the potential to enhance most, if not all, of the biocontrol agent's performance by including the ACC deaminase ability into the biocontrol strains. This is the first study to demonstrate the superiority of antagonistic actinobacteria to enhance their effectiveness as biocontrol agents by their ability to produce ACC deaminase, antifungal metabolites and cell-wall degrading enzymes.

Biocontrol of spruce decline: Comparison of interactions between *Diaporthe* spp. and fungal endophytes from Colorado blue spruce (*Picea pungens*)
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Colorado blue spruce (CBS; *Picea pungens*) is affected by spruce decline throughout the Lower Peninsula of Michigan. *Diaporthe* spp. are associated with cankers visible in the inner bark on declining trees exhibiting needle loss and branch dieback. The presence of antagonistic endophytes associated with branches may play an important role in management of spruce decline that could be integrated into current management practices. The objective of this research was to identify potential biological control agents inhibiting the growth of *Diaporthe* spp. associated with declining trees. Endophytic fungi were isolated from surface sterilized sapwood and inner bark tissues cut from the branches of symptomatic and asymptomatic CBS and identified via DNA barcoding of the ITSrDNA region. A total of 43 endophytic fungi were isolated and evaluated against *Diaporthe* spp. via *in vitro* dual culture and culture filtrate assays. Out of the 43 endophytic fungi isolated, 14 showed mycelial growth inhibition greater than 50% in dual culture assays with *Diaporthe* spp. The fungi that demonstrated substantial antagonistic activity against *Diaporthe* spp. are possible candidates for biocontrol agents. *Eppicocum nigrum* was identified as a promising biocontrol agent, producing potential antifungal compounds against *Diaporthe* spp. Further studies will focus on the validation of biocontrol efficacy of candidate fungi through colonization and interaction assays *in planta*.

Evaluation of biocontrol agents against *Fusarium virguliforme*: Potential tools for soybean sudden death syndrome management
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Sudden death syndrome (SDS) caused by *Fusarium virguliforme* is among the most important diseases affecting soybean yield in the USA. The exploitation of biocontrol agents (BCAs) can be a valuable resource to suppress *F. virguliforme* populations. *Trichoderma* spp. are known to be antagonistic against a broad range of phytopathogens, however their activity against *F. virguliforme* has not been fully explored. Therefore, the objective of this study was to screen native *Trichoderma* isolates against *F. virguliforme in-vitro* and evaluate the performance of highly antagonistic *Trichoderma* isolates under semi-field conditions (microplot) using two application methods; seed-coating and *in-furrow.* A total of 41 *Trichoderma* isolates were screened in a dual plate assay. The most effective isolates belonged to the *T. harzianum* species and were able to inhibit *F. virguliforme* radial growth by up to 92%. Three selected isolates were tested in microplots. The isolates showed a protective effect in both application methods by significantly reducing root rot and disease index 30 days after planting, compared to the plants inoculated with only *F. virguliforme.* Further, a qPCR assay showed an increased extent of *Trichoderma* colonization of soybean roots when the BCAs were delivered *in-furrow*. Our results highlight the potential of native *Trichoderma* isolates to inhibit *F. virguliforme* growth and reduce SDS severity on soybean.

Biocontrol activity and mechanism perspective of a novel *Bacillus thuringiensis* strain
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Plants are constantly challenged by different stresses. Failure to overcome stresses could lead to a lack of plant fitness and significant yield losses. Bacteria in the genus of *Bacillus* are ubiquitous in nature and are well known for their ecological, clinical or industrial significance. Many *Bacillus* strains have been reported to promote plant growth and inhibit plant disease development. However, the detailed biocontrol mechanisms of *Bacillus* remain unclear. Here we isolated one novel *Bacillus thuringiensis* strain XY44 from switchgrass plants. This strain can promote plant growth and induce resistance to plant pathogens. The preliminary data also show that strain XY44 can inhibit the growth of *Botrytis cinerea* and several other fungal pathogens by in-vitro plate assay. Further plate co-assay tests indicate that volatile metabolite(s) produced by strain XY44 slow the growth and cause abnormal development of *B. cinerea*. Future research aims to investigate how strain XY44 interacts with the fungal plant pathogen *B. cinerea* and *Arabidopsis*/tomato plants to inhibit the pathogen growth and activate the plant disease resistance. An integrative biochemical, genetic, genomic, and molecular approach will be applied to the related study. The outcomes will lead to a deeper understanding of the related mechanisms and provide insights for developing more efficient and sustainable approaches for enhancing the related plant disease control for increasing plant health and yield. This will also reduce the chemical applications which are harmful to the environment and human health.

Understanding Niche Clearing as a Means to Improve Establishment and Efficacy of Applied Bacterial Biological Controls
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Introduced biological control agents (BCAs) are available for use in turf but remain under-utilized due to inconsistent efficacy in the field. By disrupting existing microbial communities, it may be possible to improve the survival and efficacy of introduced BCAs. To better understand introduced BCA survival, we examined applications on perennial ryegrass (*Lolium perenne* L. cv. "Express II")

maintained in a greenhouse. The trial utilized a split-split plot design with a factorial treatment arrangement to assess combinations of hydrogen peroxide with BCAs (*Bacillus subtilis* QST-713, *B. subtilis* Exp. W9, *Pseudomonas chlororaphis* AFS009). Five weeks after trial initiation, half of the pots were inoculated with *Rhizoctonia solani* L. Foliage samples were collected at 0, 2, 4, 7, 10, and 13 days post application for 4 sequential applications. Leaf washings were plated onto selective media to assess bacterial populations (*Pseudomonas* spp., *Bacillus* spp., and general bacteria). While brown patch progressed rapidly across all inoculated pots, pots treated with hydrogen peroxide survived 48 h longer. The first application of *B. subtilis* QST-713 significantly increased general bacterial populations compared to all other treatments, 0 days after treatment (DAT). Higher *Bacillus* and *Pseudomonas* populations were observed in hydrogen peroxide treated pots, 4 and 7 days after the third application. Although populations of BCAs were not high enough to reduce brown patch on this date. Initial data show hydrogen peroxide may help establishment of BCAs, but additional research is needed.

***Rhizophagus intraradices* Reduces Disease Severity of Sudden Death Syndrome on Soybean**
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There is increasing interest to incorporate arbuscular mycorrhizal fungi (AMF) into agricultural production because of the benefits they provide, including protection against pathogens and pests. Sudden death syndrome (SDS) of soybean is a devastating disease caused by the soil-borne pathogen *Fusarium virguliforme.* Multiple management methods are needed to control SDS. There is little known about the relationship between *F. virguliforme* and AMF. The overall goal of this study was to determine if the AMF species *Rhizophagus intraradices* could reduce SDS severity of soybean. Fourteen soybean genotypes were evaluated for their response to *F. virguliforme* and with or without *R. intraradices* in a greenhouse experiment. On average, there was a 45% reduction in foliar SDS severity and a 27% reduction in relative *F. virguliforme* quantities in roots across all genotypes in mycorrhizal plants compared to non-mycorrhizal plants. Root weight of mycorrhizal-inoculated plants was significantly increased by 56% compared to non-mycorrhizal plants. A nutrient analysis of root tissues showed a 75% increase in mycorrhizal roots of potassium and increases in phosphorus, sulfur, boron, and aluminum compared to non-mycorrhizal roots. This study showed that *R. intraradices* considerably reduced SDS severity and *F. virguliforme* colonization while simultaneously increasing growth and nutrient uptake of plants. This potential of *R. intraradices* and possibly other AMF species may be an effective strategy for use in a multi-management approach to control SDS.

Characterization of antifungal activity of soybean endophytic bacterium *Burkholderia pyrrocinia* strain MS455
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Strain MS455 is an endophytic bacterium isolated from a soybean plant growing in a charcoal rot disease patch in Mississippi. It possesses a broad-spectrum antimicrobial activity against plant pathogenic bacteria and fungi including the charcoal rot disease pathogen *Macrophomina phaseolina*. The bacterial strain was preliminarily identified to be member of *Burkholderia pyrrocinia*. Random mutagenesis revealed that a nonribosomal peptide synthetase gene is required for MS455 antifungal activity. The complete genome of strain MS455 was obtained by PacBio sequencing. Genomic analysis revealed that the MS455 genome consists of three chromosomes and one plasmid. The gene targeted by transposon in a mutant was localized in a 57-kb gene cluster which consists of fifteen open reading frames (ORFs). ORF15, located at the right border of the gene cluster, was predicted to encode a LuxR-type transcriptional regulator that is associated with regulation of production of antifungal activity. Site-specific mutagenesis and complementation of the ORF15 gene are under way to characterize its function in antifungal activity. This research will provide critical clues for development of biologically-based approaches for plant disease management.

Biological control activity of *Bacillus* sp. RAB14R against rice blast disease and its formulation to increase shelf life
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Rice blast fungus, *Pyricularia oryzae* Cavara, is an important disease of rice. *Bacillus* sp. RAB14R was isolated as a highly antagonistic bacterial stain against *P. oryzae*. Bacterial suspension of *Bacillus* sp. RAB14R was sprayed to rice plants at their tillering and heading stages. In the tillering stage of the rice cultivar M202, a highly susceptible cultivar, all forms of *Bacillus* sp. RAB14R failed to suppress leaf blast. However, the antagonistic bacterium suppressed the disease significantly for a less susceptible rice cultivar CL151, in spite that its disease suppression activity was less than that of fungicide Stratego and there were no significant difference among the different forms of the bacterium. Same pattern was also observed in panicle blast, where *Bacillus* sp. RAB14R significantly suppressed the symptom development in CL151. Furthermore, long-term survival of *Bacillus* sp. RAB14R was tested in four different storage conditions: 1) freeze-dried with 5% sucrose, or 2) with 0.85% NaCl and then stored at 4°C, and 3) mixed with talcum powder and stored at 4°C, or 4) at room temperature. Survival rates of RAB14R at the four different conditions were evaluated throughout a 13-month period. After 13 months, freeze-dried bacteria with 5% sucrose showed the highest percentage of viability (~ 20%) among the storage conditions tested, while the bacterium formulated with talcum powder showed less than 10% of survival rate regardless of the storage temperatures.

Biological control of black Sigatoka in bananas and plantains in Sub-Saharan Africa
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Black Sigatoka, also known as black leaf streak, caused by *Mycosphaerella fijiensis* (M. Morelet) (anamorph: *Pseudocercospora fijiensis*), is regarded as the most damaging and economically important disease of banana and plantain (*Musa* spp.) worldwide. Chemical control and resistant cultivars remain the two dominant strategies to control this disease. However, most synthetic fungicides used are hazardous to the environment, pose a risk of resistance development in the pathogen population, and are not easily accessible to small-holder farmers especially in Sub-Saharan Africa. Other alternative management strategies that are easily accessible and less costly to farmers are needed. The discovery of biological products that can effectively control black Sigatoka may result not only in improved integrated pest management programs for the management of this disease, but also reduction of the high risk of fungicide resistance in *M. fijiensis* populations. At AgBiome, we identify microbial-based solutions for agricultural pathogens and pests by leveraging a diverse and growing collection of fully sequenced microbes from the plant-soil microbiome. We selected over 400 microbial strains randomly or prioritized by genomic features from our collection and screened them in a laboratory-based assay and several gave promising results after three rounds of testing. Seven strains that were most effective are being further evaluated in Nigeria under greenhouse and field trials.

Biological control of sorghum anthracnose in Sub-Saharan Africa

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Sorghum anthracnose, caused by *Colletotrichum sublineolum* Hann. Kabát et Bub., is one of the most destructive foliar diseases of cultivated sorghum [*Sorghum bicolor* (L.), Moench] in Africa. The current available management strategy includes use of resistant cultivars and synthetic fungicides. However, high variability in the *C. sublineolum* population frequently overcomes host resistance, yet fungicides are expensive especially to smallholder farmers, besides being hazardous to the environment. Therefore, alternative methods for managing this disease that are easily accessible and less costly to farmers are needed. Biological control of agricultural pests is a promising solution in many areas, posing no risk of exposure compared to synthetic pesticides. AgBiome undertakes research aimed at identifying microbial-based solutions for agricultural pests by leveraging a diverse and growing collection of fully sequenced microbes from the plant-soil microbiome. Accordingly, bioinformatic tools were deployed to prioritize strains with diverse activity and, in parallel, strains were chosen randomly from our collection. Laboratory studies using a detached-leaf disk assay on sorghum, cultivar. 12-GS9016-KS585, with over 400 strains gave promising results. Several strains that were most effective in the lab against *C. sublineolum* are being further evaluated in Uganda under greenhouse and field trials.

Genetic factors influencing the efficacy and persistence of biocontrol of aflatoxin contamination in maize

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A large acreage of maize grown in the southern United States is chronically at risk of aflatoxin contamination caused by *Aspergillus flavus*. Biocontrol treatments using atoxigenic strains of *A. flavus* can reduce contamination by up to 95%. Commercial biocontrol strains are of the mating type *MAT1-2* and are applied annually for effective performance. Currently, there is interest in the development of sustainable biocontrol approaches to successfully reduce aflatoxin contamination. First, we examined the efficacy of RMb10, a novel biocontrol strain of the *MAT1-1* type, in replicated large-scale field trials in AL, MS, NC, and TX from 2016 to 2018. Second, we investigated the genetic factors that may influence the persistence of biocontrol strains in the field. Isolates (n = 300) of *A. flavus* were collected from specific maize fields in MS, NC, and TX. These fields had 3 to 7 years history of annual Afla-Guard treatment, but applications were terminated when harvested grain exhibited no aflatoxin contamination in subsequent seasons. In both experiments, isolates were screened for colony morphology, sclerotia production, mating type, and aflatoxin cluster composition, and then subjected to multi-locus sequencing using five loci (*amdS*, *aflM*, *aflW*, *mfs*, and *trpC*). Results of this study will provide insights into the population genetic structure of fields with reduced contamination and inform future biocontrol strategies.

Bee Vectoring Technology using *Clonostachys rosea* as biological control for Botrytis fruit rot on strawberry in Florida

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Botrytis fruit rot (BFR), caused by *Botrytis cinerea,* is a major disease of strawberry. The intensive use of fungicides for its management has resulted in resistance to several fungicide groups. Therefore, the need to incorporate alternative strategies to manage BFR. In this study, we evaluated the efficacy of the Bee Vectoring Technology (BVT) using *Clonostachys rosea* as a biological control. The technology consists in the use of bumblebees to carry *C. rosea* to strawberry flowers where it competes with *B. cinerea*. Three experiments were conducted at two commercial farms in Central Florida during the 2016–17, 2017–18, and 2018–19 seasons. Treatments consisted of non-treated control; fungicide program; BVT; and BVT + fungicide program. Fruit were harvested twice a week throughout the season to determine BFR incidence. Postharvest activity of *C. rosea* was quantified by incubating fruit from selected harvests. The BVT + fungicide program, but not BVT alone, reduced BFR incidence compared to the non-treated control in all three seasons. For postharvest evaluation, BVT + fungicide program also reduced BFR incidence but only during the first season. Furthermore, strawberry yields tended to be higher in the fungicide and BVT + fungicide treatments. Thus, the BVT technology may be used in combination with fungicides to reduce BFR. Additional research is needed to address the potential yield benefits of using pollinators and the economics of the technology.

The potential of herbal essential oils to control pulse crop pathogens
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The cultivation of chickpea, lentil, and dry peas (pulse crops) are on the rise in the United States, from 1.4M acres in 2013 to 2.5M acres in 2018. Many major diseases of these pulse crops are fungal in origin. These diseases reduce seed quality, crop establishment, and potential yield. Currently, these diseases are controlled primarily by chemical fungicides where resistant varieties are not available. The market for organically produced chickpea and pea is growing with different uses leading to an increase in demand. In the current study, 38 commercially available essential oils were screened to evaluate their *in vitro* inhibitory effects on *Aphanomyces euteiches, Botrytis cinerea*, *Collectotrichum truncatum, Didymella pisi*, *D. rabiei*, *D. lentis*, *Fusarium avenaceum*, *Pythium ultimum*, *Stemphyllium globosum*, and *Sclerotinia sclerotiorum*. Essential oil-infused agar toxicity method was used to determine the percent fungal growth inhibition, and essential oils with >60% pathogen inhibition across 10 pathogens were evaluated for inhibition of spore germination and the fungicidal/fungistatic effects of their volatile in inverted petri-plate assay. Palmarosa, oregano, clove, cinnamon, lemongrass, citronella, and thyme oils showed 100% growth inhibition of the fungi at concentrations ranging from 1/1000 to 1/4000. These essential oils also inhibited spore germination by 22–100%. The oils were either fungistatic or fungicidal. The herbal oils with efficacy *in vitro* are currently being investigated for use *in vivo* for potential use in organic and conventional production systems.

Characterization of *Trichoderma* sp. isolates for use in biological control of plant pathogens
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Trichoderma sp. is a fungus found in the soil and works as a biofungicide due their parasitism, antibiosis, and competition. Some species are resistance inductors of plant diseases. The objective of this study was to evaluate the mycelial growth and sporulation of 22 isolates of *Trichoderma* sp. collected from regions in Brazil to be used as biological control of different pathogens. Each isolate grew under a 12-hour photoperiod at 25°C. The mycelial growth was also compared in PDA and oatmeal-agar media, and the colony diameter was measured daily for 3 days. Sporulation was measured with a hemocytometer by counting conidia produced after five days. Mycelial growth was faster in PDA than oatmeal-agar media. Five isolates had significant differences on mycelial growth (A4, A5, A6, A8 and A20), but A5 showed the highest growth. There were also significant differences among isolates for conidia production, and A5 presented the highest value. Isolate A3 presented the lowest mycelial growth and sporulation. Therefore, the A5 *Trichoderma* sp. isolate could be a potential bio control agent against plant pathogens because of its rapid growth and conidia production in culture.

Discovering biological control strategies to control Bacterial Panicle Blight of Rice
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Bacterial panicle blight (BPB) of rice, caused by *Burkholderia glumae,* affects rice production worldwide. Currently, there are no rice varieties that are completely resistant to the disease, and chemical control methods are not available or effective. Thus, in the absence of completely resistant cultivars or chemical methods of control, an alternative strategy is to identify sources of biological control. The objectives of this study are: 1) to identify bacterial strains that could be used as biological control agents against *B. glumae* and 2) to dissect their mechanism of control. Ten laboratory strains were initially screened using a disc diffusion assay, and two of them (*Pseudomonas fluorescens* and *Burkholderia cenocepacia*) significantly inhibited the growth of *B. glumae in vitro.* Moreover, co-inoculation of *P. fluorescens* and *B. cenocepacia* with *B. glumae* also reduced disease symptoms in rice. To dissect the mechanism behind the growth inhibition of *B. glumae* by *P. fluorescens* and *B. cenocepacia,* cell-free secreted fractions from *P. fluorescens* and *B. cenocepacia* were obtained and used to evaluate the growth of *B. glumae.* Cell-free secreted fractions in amended culture media reduced the growth of *B. glumae in vitro* in comparison with non-amended controls. Moreover, when these cell-free secreted fractions were mixed with *B. glumae,* they hindered the ability of this bacteria to cause disease in rice. These results suggest that these strains of *P. fluorescens* and *B. cenocepacia* are promising agents to control BPB.

Identification of beneficial *Pseudomonas* strains for the biocontrol of *Botrytis cinerea* in floriculture crops
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Botrytis cinerea is one of the most common fungal pathogens found in the greenhouse industry worldwide. This pathogen is increasingly resistant to conventional fungicides, which has increased the need for alternative management methods. The objective of this study was to use *in vitro* and *in planta* assays to evaluate 60 *Pseudomonas* strains for their biocontrol activity against *B. cinerea.* A dual culture assay was utilized in which one *Pseudomonas* strain was plated on PDA medium with *B. cinerea*. The growth reduction of *B. cinerea* compared to a negative control plate, containing no bacteria, and the zone of inhibition were measured. The *in vitro* screen identified 20 strains with growth reduction activity against *B. cinerea*. All 60 *Pseudomonas* strains were also evaluated in a greenhouse experiment. The individual strains were sprayed on the leaves and growing media of *Petunia × hybrida* 'Carpet Red Bright' plants biweekly for six weeks prior to inoculation with *B. cinerea* (1×10^4 conidia/ml). All open flowers were tagged before *B. cinerea* inoculation and then rated daily for disease severity. A disease severity index was calculated based on the daily ratings. The *in vitro* and *in planta* assays were used to select seven top performing bacteria. The bacterial strains identified in this study can be used in future work to develop additional biocontrol options for the effective management of *B. cinerea* in floriculture crops.

Plant metabolite-based resistance to *Oidium neolycopersici* in organic- and high tunnel-grown tomato
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Powdery mildew of tomato, caused by *Oidium neolycopersici,* can result in significant yield loss in high tunnel production. Induction of plant defenses with non-synthetic materials may be an avenue for disease management in organic systems. To this end, a fermentation by-product, which contains multiple naturally occurring plant defense inducers, was assayed to determine its effect against powdery mildew in a high tunnel tomato production study. Treatments included Nordox (copper hydroxide), and Companion (*Bacillus subtilis* GB03) as grower standards, along with a filtered and unfiltered version of the fermentation by-product. High tunnel-grown tomato plants were inoculated with *O. neolycopersici* (3.7×10^4 conidia/ml) and foliar symptoms were rated. The fermentation by-product significantly reduced ($P < 0.05$) powdery mildew compared to the inoculated control and Companion, and was similar to the protectant standard Nordox. These results suggest that the fermentation by-product reduces powdery mildew similar to a protectant-based grower standard. Molecular analyses suggest that the plant defense inducers present in the fermentation by-product confer resistance by priming for plant defense. Together, these results suggest that fermentation-by product can be as effective as currently marketed disease management products, and its efficacy is derived at least partially by induced resistance.

Potential of Beneficial *Trichoderma* isolates in Alleviating Drought Stress in Tomato
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Tomato (*Solanum lycopersicum*) is the world's second most important vegetable crop. Currently, global productivity of tomato is challenged by drought due to inadequate irrigation and erratic rainfall. Research in *Trichoderma*, a naturally occurring beneficial fungi suggests its role in abiotic stress tolerance. However, the efficacy of colonization and beneficial effects can be isolate specific and depend on plant genotype. The goal of this project is to examine the potentiality of *Trichoderma* isolates primarily native to Nepal to ameliorate drought stress in tomato. Forty-one *Trichoderma* isolates, collected from diverse agroecological regions of Nepal, two Ohio isolates and *T. harzianum*-T22 were selected for this study. An in-vitro assay was undertaken to identify *Trichoderma* isolates with potential to survive in low moisture environments induced by polyethylene glycol in Potato Dextrose Agar plates. Fourteen isolates were found to be desiccation tolerant and were able to grow up-to -8.5 Mpa. Twenty-four isolates were selected and tested for drought tolerance under greenhouse condition. Four isolates (T16, T31, T33, and T-37) resulted in reduced wilting symptoms under drought and recover faster after irrigation. Plants treated with these isolates had 25–30% greater shoot biomass compared to control. Isolates collected from drier areas of Nepal were able to promote growth under drought stress as compared to wet areas, indicating a potential adaptation response.

Beneficial microorganisms can enhance greenhouse floriculture plant performance under reduced fertility
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Beneficial microorganisms provide greenhouse growers with a tool to enhance plant nutrient uptake, allowing them to reduce fertilizer inputs during floriculture crop production. The objective of this study was to determine if high quality plants could be produced at reduced fertilizer rates by treating with beneficial microorganisms. The first experiment tested two microbe-containing commercial products (MycoApply Endo and Cease) on *Viola* × *wittrockiana* 'Delta Pure Rose' (pansy) transplants fertilized with 0, 0.25, 0.5, 0.75, or 1.0X water soluble 15-5-15 Ca-Mg for 6 weeks, where 1.0X was equivalent to 150 $mg \cdot L^{-1}$ N. Plant performance was evaluated by determining flower number, rating the visual quality of the roots and shoots, and measuring final dry weights of roots and shoots. Under no fertilizer (0X) conditions, MycoApply Endo and Cease treatments resulted in plants with greater flower numbers and higher shoot dry weight than the untreated control. Quality ratings of plants treated with MycoApply Endo and Cease were also higher at 0.5X. The second experiment tested the ability of three *Pseudomonas* strains to enhance plant performance and nutrient uptake of pansy plants grown with 25 $mg \cdot L^{-1}$ N fertilizer. Shoot dry weight and nutrient content (N, P, K) was higher in plants treated with each of the *Pseudomonas* strains compared to the untreated control. A reduced fertilizer regime can produce high quality floriculture plants when beneficial microbes are utilized.

***Verticillium nonalfalfae* as a Potential Biological Control Agent for the Invasive *Toona sinensis* Tree in Pennsylvania**
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The tree-of-heaven (*Ailanthus altissima*) is a highly invasive tree species in urban, agricultural, and forest settings in the United States and Europe. For the past decade, the native soil-borne fungus *Verticillium nonalfalfae* (strain VnAa140) has been utilized in the eastern U.S. as a biological control agent against Ailanthus with great success. In 2014, the invasive spotted lanternfly (*Lycorma delicatula*), which feeds preferentially on Ailanthus, was found on this host in Pennsylvania. Following this discovery, there has been a renewed interest in utilizing *V. nonalfalfae* as a biocontrol against Ailanthus to help combat infestation of spotted lanternfly. Another invasive tree species also present in the U.S., the toon tree (*Toona sinensis*), is a known reproductive host for spotted lanternfly. However, there is no report of *V. nonalfalfae* causing Verticillium wilt on toon, and with the threat of this invasive insect increasing, *V. nonalfalfae* should be tested on toon trees. In July of 2018, a prelimnary experiment was conducted on 100 toon trees were inoculated with *V. nonalfalfae* while also using Ailanthus trees as a positive control. At seven weeks post-inoculation toon trees exhibited severe symptoms of wilting, vascular discoloration, stunting, and low levels of mortality. Continued research using *V. nonalfalfae* as a biocontrol for both Ailanthus and toon trees may represent a useful tool in mitigating the spread of the highly invasive spotted lanternfly.

Enhanced hypovirus transmission by engineered super donor strains of the chestnut blight fungus, *Cryphonectria parasitica*, in a forest setting
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Horizontal transmission of virulence attenuating hypoviruses of *Cryphonectria parasitica* is restricted by an allorecognition system termed vegetative incompatibility (*vic*). A super donor formulation of two engineered *C. parasitica* strains (SD328/SD82) with gene disruptions at four of six *vic* loci transmitted hypovirus to strains in the laboratory independent of *vic* genotype. We now report the transmission of hypovirus by the SD328/82 formulation to a diverse, natural *C. parasitica* population infecting American chestnut in a forest setting. Hypovirulent (HV) isolates were recovered from 94% of cankers treated with the hypovirus-infected SD328/82 formulation compared to 51% of cankers treated with a hypovirus-infected EU5/6 formulation (strains having the same *vic* genotypes as SD strains but lacking *vic* gene disruptions). Overall, the SD328/82 formulation transmitted hypovirus into more divergent *vic* genotypes compared to the EU5/6 formulation. These results demonstrate the SD328/82 formulation can serve as an enhanced hypovirus vector for highly divergent *C. parasitica* populations. Ongoing studies are focused on optimizing delivery of SD strains including several application methods and hypoviruses to further reduce canker expansion in a forest setting.

Biocontrol potential of *Streptomyces* isolates on fungal pathogens (*Helminthosporium solani, Pythium ultimum*) of potatoes
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Pythium leak, caused by *Pythium ultimum*, and silver scurf, caused by *Helminthosporium solani,* are fungal pathogens that significantly reduce yields and lower quality for the potato industry. They are not controlled effectively by current methods. *Streptomyces* spp. are recognized as producers of antimicrobial and antifungal secondary metabolites. Previous studies have lacked analysis of more than two or three isolates of *Streptomyces*. By looking at more than 70 isolates, we seek to set a foundation to determine what *Streptomyces* make a viable biological control and what secondary products are effective control mechanisms. We have conducted bioassays on *Streptomyces* isolates against *P. ultimum* and against *H. solani*. The bioassays are conducted by placing *Streptomyces* in the center of a Petri dish. Plugs of *P. ultimum* and *H. solani* are placed around the *Streptomyces*. The growth of the fungi toward the *Streptomyces* is then measured, and compared, to the growth away from the *Streptomyces*. Several isolates of *Streptomyces* significantly inhibited *P. ultimum* growth with ME02-6988A causing inhibition at 67%. More *Streptomyces* isolates caused significant inhibition of *H. solani*. The range of percent inhibition ranges between 14–59% with strain ID-05-47c causing inhibition at 59%. More research should be done in determining what secondary metabolites are causing this inhibition and what genes are encoding for the secondary metabolites.

A Bacterial Isolate Shows Promise as a Biofungicide for Sclerotinia Stem Rot Disease on Canola
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Sclerotinia stem rot, caused by the pathogen *Sclerotinia sclerotiorum*, poses a serious threat to canola production worldwide. Due to lack of strong genetic resistance to Sclerotinia, biocontrol is the best alternative and an environmentally friendly strategy to manage the disease. The aim of this research was to test the efficacy of a bacterial isolate (PENSV20) in inhibiting the germination of Sclerotinia ascospores and to understand the induced defence mechanisms involved. A highly susceptible canola cultivar 'Westar' was used for our experiments. Five treatments, (1)'Westar' × blank solution, (2)'Westar' × Sclerotinia alone, (3)'Westar' × PENSV20 alone, (4)'Westar' × PENSV20 × Sclerotinia, and (5)'Westar' × Sclerotinia × PENSV20, with 3 biological replicates per treatment were performed. For treatments 4 and 5, PENSV20 was applied 24 hours, before and after sclerotinia spray, respectively. At 5 days post treatment, the phenotypic assessment showed severe infection on 'Westar' × Sclerotinia plants while no signs of infection were observed on 'Westar' × PENSV20× Sclerotinia and very limited stem infection on 'Westar' × Sclerotinia × PENSV20. RNA samples from leaf tissue are being sequenced to determine the group of genes and pathways involved in defence mechanisms in the plant globally. The level of expression of significantly differentially expressed genes over different time points will be quantified using qPCR. This work will greatly contribute toward the management of sclerotinia stem rot on canola and potentially on other crops infected by *Sclerotinia sclerotiorum.*

Verticillium klebahnii* and *V. isaacii* isolates exhibit host-dependent biocontrol activity against Verticillium wilt caused by *V. dahliae
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The soilborne fungal pathogen *Verticillium dahliae* causes vascular wilt on a number of economically important crops that results in major yield losses. This pathogen is notoriously difficult to manage as it produces long-lived microsclerotia and there is little or no host resistance. A single nonpathogenic isolate each of *V. klebahnii* (PD659) and *V. isaacii* (PD660) were evaluated as potential biocontrol agents (BAs) in eggplant, lettuce, and tomato by pre, post or co-inoculation with virulent isolates of *V. dahliae*. Initial studies demonstrated biocontrol efficacy of BAs similar to the reference BA-*Talaromyces flavus* (NRRL 15936) across all hosts (α= 0.05). Modified treatments using isolates from the three hosts- Sm113 from eggplant, Ls17 from lettuce, and Le1811 from tomato

demonstrated significant biocontrol efficacy in eggplant ($P < 0.001$) and tomato ($P < 0.001$) but not in lettuce ($P > 0.301$), suggesting host-dependent efficacy of these BAs. Competitive exclusion might be a possible biocontrol mechanism as indicated by the limited root colonization by the pathogen in the presence of introduced BAs relative to the pathogen alone. Quantitative expression analysis of defense regulating genes in tomato in the presence or absence of BAs is in progress. Integrated use of nonpathogenic *V. klebahnii* or *V. isaacii* isolates reduce Verticillium wilt incidence and severity in a host-dependent manner.

In vitro* control of *Colletotrichum acutatum* isolates from citrus using extract from brown alga *Dictyota dichotoma
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Citrus postbloom fruit drop (PFD), caused by *Colletotrichum acutatum*, is a sporadic disease in Brazilian orchards, however can cause yield losses up to 85%, resulting in frequent fungicide applications. This situation can decrease commercial products efficacy, cause environmental impacts and have harmful effect on human. Additionally, alternative control of PFD is absent in commercial orchards. The aim of this work was to evaluate the effect of dichlorometane/methanol (2:1 v/v) extract of *Dictyota dichotoma* in the control of *C. acutatum* germination *in vitro*. Discriminatory dose of *D. dichotoma* (100 or 1000 $\mu g.mL^{-1}$) or fungicides (10 $\mu g.mL^{-1}$ of trifloxystrobin or triazole) were amended into PDA medium. PDA without extract and fungicides served as control. Aliquots of conidia suspension were deposited on plates with the treatments, in a total of 9 repetitions per treatment. The plates were randomly incubated at 23°C under light for 9 hours and spore germination was calculated. The extract in the highest concentration resulted in inhibition of 97.4% of *C. acutatum* germination, which was statistically the same obtained by 10 $\mu g.mL^{-1}$ of trifloxystrobin. The seaweed extract in a concentration of 100 $\mu g.mL^{-1}$ resulted in a lower inhibition in conidia germination than that achieved with triazole, although its effect was better than the control. These previous results demonstrated the potential of seaweed extracts to be used as an alternative control in the management of PFD.

Influence of biological agents on the production of *Magnaporthe oryzae´s* conidia in upland rice, under field conditions
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The inclusion of a biological agent in the integrated management of rice blast (*Magnaporthe oryzae*) enables to suppress disease severity, protect and promote the expression of host genetic resistance and reduce the number of fungicide applications. We compared leaf blast severity (LBS) suppression by *Burkholderia pyrrocini* (BRM 32113), *Pseudomonas fluorences* (BRM 32111) and tricyclazole, and monitored conidia production in each treatment. In randomized blocks and four replicates, 16 treatments combined the application by seed (TS), spraying at vegetative stage (VS-15 days after planting-dap) and reproductive stage (RS-65 dap). We evaluated LBS at 24, 31, 38 and 52 (dap) using a scale of notes and conidia production rom the same sampled leaves. All treatments suppressed LBS and decrease conidia production by TS, VS or in combination between TS and VS. BRM 32111 suppressed LBS by 64 and 75% at 24 and 31dap, and decrease conidia production by 92 and 86%, at 38 and 52 dap, respectively; And triciclazole suppressed by 95%, at 38 and 52 dap, and decrease conidia production by 95% at 24 and 31dap, respectively.

Effects of chitin on antibiotic and lytic enzyme gene expression by the biocontrol bacterium *Lysobacter enzymogenes*
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Lysobacter enzymogenes C3 is a biocontrol agent that can inhibit fungal diseases by producing beta-1,3-glucanases, chitinases, and the antibiotic HSAF. Lytic enzyme production by C3 can be enhanced by supplying chitin, but the influence of chitin on HSAF production is unknown. Previously, we used qRT-PCR to demonstrate that the PKS-NRPS gene (PKS) essential for HSAF synthesis and the structural genes for glucanase C (gluC) and chitinase A (chiA) are expressed by C3 in soybean rhizospheres at different levels and times following C3 seed treatment. One objective of this study was to determine the effects of chitin on antibiotic and lytic enzyme gene expression by C3 in vitro. When cultured in a chitin-supplemented mineral salts medium, C3 exhibited peak expression of chiA and gluC after 24 hours, while high PKS gene expression occurred at 12 hours. Another objective was to determine if soil amendment with chitin can affect the expression of antibiotic and lytic enzyme genes by C3 in bulk soil and in soybean roots. C3 in soil expressed PKS, chiA, and gluC when chitin was added but not when chitin was withheld. In contrast, chitin soil amendment caused little or no change in gene expression by C3 in roots compared with no chitin amendment. Our results suggest that in nutrient-deficient environments such as bulk soil, C3 expression of biocontrol genes is highly responsive to the addition of chitin, whereas in the rhizosphere, exogenous chitin has a lesser effect on gene expression by C3.

Dissecting the role of oxidative stress in host-*Aspergillus flavus* interactions using genomics and genetic engineering
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Dissecting the connection between drought stress and aflatoxin contamination in maize and peanut has led to the observation that drought tolerant, aflatoxin contamination-resistant plants tend to accumulate less reactive oxygen species (ROS) in their tissues under stress conditions. Oxidative stress also leads to exacerbated aflatoxin production to varying degrees among diverse isolates of *Aspergillus flavus*. To take advantage of this observation and investigate this system as a potential avenue of mitigating aflatoxin contamination, a combination of genetic engineering and genomics has been employed. Genetic engineering approaches were done including transgenic overexpression and CRISPR-Cas9-mediated silencing for antioxidant genes in maize and peanut. Genotypic and phenotypic evaluation of effects on plant ROS accumulation, drought tolerance, and aflatoxin resistance is ongoing. In addition, to explore stress-linked mechanisms in *A. flavus* related to host resistance, a pair of reference genomes for *A. flavus* isolates with contrasting growth behavior, stress tolerance, and aflatoxin production were *de novo* assembled using Pacbio long-read sequencing and Bionano optical mapping. These represent the first pseudomolecule-level genomes produced for this important pathogen. Comparative analyses with other re-sequenced, diverse isolates as well as examining the effects of oxidative stress on early signaling events will also be discussed.

Engineering high affinity protein ligands to plant pathogens and pests via continuous evolution
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Crop yield losses due to pathogens and pests are a major threat for food security and sustainable agriculture worldwide. GM approaches and resistant varieties have been widely used to control plant pests and pathogens. However, pathogen and insect resistance against GM traits and resistant varieties are becoming serious issues.
Studies in other laboratories have identified many potential target proteins whose inhibition would improve crop yields. Our business Innatrix is developing continuous laboratory evolution technology for the production of high-affinity, high specificity protein ligands, which we expect to serve as inhibitors. An advantage of our methodology is that it can produce new inhibitors quickly (given the sequence of the resistant target) once resistance occurs. We have created our own evolution selection system (patent pending) using E. coli and M13 phage. Innatrix has also invented a proprietary apparatus named the EvoStat™. It is an automated platform for protein engineering via continuous laboratory evolution. The EvoStat™ provides advanced control of cultural conditions, reporting, recording, simulations, state of the evolution, and remote access, which enables us to easily explore the evolution of ligands to different targets or many evolution conditions in parallel.

Transgenic Arabidopsis expressing a modified plant thionin enhanced resistance to Fusarium graminearum
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In our previous studies, we showed that transgenic tobacco and citrus plants expressing a modified thionin (Mthionin) markedly enhanced resistance to disease incited by bacterial pathogens including Pseudomonas syringae tabaci, Xanthomonas citri and Candidatus Liberibacter asiaticus. The fungal pathogen Fusarium graminearum causes Fusarium head blight (FHB) on wheat and barley. FHB causes yield reductions and contamination of grain with trichothecene mycotoxins. Fusarium graminearum can infect the model plant Arabidopsis. The aim of this study is to investigate whether overexpression of the Mthionin in Arabidopsis can increase resistance to F. graminearum. Transgenic Arabidopsis plants expressing the Mthionin were generated by Agrobacterium-mediated floral dip transformation. Expression of the transgene was evaluated by reverse transcriptase quantitative PCR analysis. Detached leaves expressing the Mthionin displayed less severe disease symptoms compared to transgenic controls expressing GUS. When infiltrated with a F. graminearum strain expressing GFP, leaves expressing the Mthionin reduced fungal spore germination and growth compared with leaves expressing GUS. Microbiome profiling demonstrated broad similarity in microbiome structure between transgenic Arabidopsis expressing the Mthionin or GUS, although a small number of taxa differed significantly in relative abundance among transformants. Our data indicate that the Mthionin is a promising candidate for producing transgenic crops with increased FHB resistance and ultimately reduced mycotoxin contamination.

CRISPR-*LbCas12a (LbCpf1)*-mediated modification of citrus
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Recently, CRISPR-Cas12a (Cpf1) from *Prevotella* and *Francisella* was engineered to modify plant genomes. In this report, we employed CRISPR-LbCas12a (LbCpf1), which is derived from *Lachnospiraceae bacterium ND2006*, to edit a citrus genome for the first time. First, LbCas12a was used to modify the *CsPDS* gene successfully in Duncan grapefruit via Xcc-facilitated agroinfiltration. Next, LbCas12a driven by either the 35S or Yao promoter was used to edit the PthA4 effector binding elements in the promoter (EBE_{PthA4}-CsLOBP) of *CsLOB1*. A single crRNA was selected to target a conserved region of both Type I and Type II CsLOBPs, since the protospacer adjacent motif (PAM) of LbCas12a (TTTV) allows crRNA to act on the conserved region of these two types of CsLOBP. *CsLOB1* is the canker susceptibility gene, and it is induced by the corresponding pathogenicity factor PthA4 in *Xanthomonas citri* by binding to EBE_{PthA4}-CsLOBP. A total of seven 35S-LbCas12a-transformed Duncan plants were generated, and they were designated as #D_{35S}1 to #D_{35S}7, and ten Yao-LbCas12a-transformed Duncan plants were created and designated as #D_{Yao}1 to #D_{Yao}10. LbCas12a-directed EBE_{PthA4}-CsLOBP modifications were observed in three 35S-LbCas12a-transformed Duncan plants (#D_{35S}1, #D_{35S}4 and #D_{35S}7). However, no LbCas12a-mediated indels were observed in the Yao-LbCas12a-transformed plants. Notably, transgenic line #D_{35S}4, which contains the highest mutation rate, alleviates XccΔpthA4:dCsLOB1.4 infection. Therefore, CRISPR-LbCas12a can readily be used as a powerful tool for citrus genome editing.

Genome editing of citrus using the CRISPR-Ribonucleoprotein complexes
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Genetic improvement of citrus using conventional breeding is a lengthy and challenging process due to the complex reproductive biology of citrus, including sexual incompatibility, highly heterozygous nature, nucellar seedlings, male or female sterility and a long juvenile phase. Biotechnology approaches such as CRISPR-mediated genome editing have the potential to accelerate the citrus improvement process. Heritable citrus genome modifications have previously been conducted via transgenically expressing Cas9/sgRNA *in planta*. Genetically modified plants generated via the transgenic expression of Cas9/sgRNA contain foreign DNA sequences, requiring a rigorous deregulation process before commercialization. Non-transgenic genome editing of plant can be conducted by transient expression of the CRISPR-Ribonucleoprotein (RNP) complexes in embryogenic plant cells. Here, we expressed, isolated and directly delivered three types of Cpf1 proteins from *Francisella novicida* (Fncpf1), *Lachnospiraceae bacterium* (Lbcpf1), and *Acidaminococcus* spp (Ascpf1) with the corresponding CRISPR RNAs (crRNA) using polyethylene glycol-mediated transformation and biolistic delivery methods. crRNAs targeting the canker susceptibility gene *CsLOB1*were used. Our results showed that Fncpf1 had the highest activity and is compatible and active with the Ascpf1 and Lbcpf1 crRNAs. Our results indicate that CRISPR-Cpf1 RNPs direct delivery is a potent method to generate foreign DNA-free genome edited citrus.

Improvement of CRISPR/Cas9 genome editing efficacy in citrus
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Citrus is one of the top fruit crops in the world. Citrus breeding is critical to improve fruit quality and to overcome the disease challenges such as Huanglongbing. However, conventional citrus breeding is difficult, inefficient and time-consuming because of long juvenile period, heterozygosity, sexual incompatibility and polyembryony in citrus. CRISPR/Cas9 mediated genome editing is a promising tool with great potentials. CRISPR/Cas9 mediated genome editing has been successfully used to modify citrus genome. However, the efficacy of CRISPR/Cas9 mediated genome editing of citrus needs to be improved. In previous study, Arabidopsis U6 and 35S promoters have been used to drive the expression of sgRNA. Here, we identified the U6 promoters in citrus and tested their efficacies in CRISPR/Cas9 mediated genome editing in citrus compared with the heterogenous U6 promoter (AtU6). Our results showed that the endogenous promoter has higher genome editing efficiency in citrus.

CRISPR/Cas9-enabled multiplex genome editing and locus-dependent DNA repair in *Magnaporthe oryzae*
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*Magnaporthe oryzae*is the causal agent of rice and wheat blast diseases and poses a major threat to rice and wheat production worldwide. To facilitate functional discovery of the fungal genes and subsequent elucidation of its virulence mechanisms, it is important to develop and improve CRISPR/Cas9 tools and methods for genome editing and targeted mutagenesis in *M. oryzae*. In this study, we successfully performed CRISPR/Cas9-mediated multiplex genome editing in the rice blast fungus using the polycistronic tRNA-gRNA (PTG) strategy. Upon creation of double stranded breaks (DSBs) by Cas9, targeted gene mutation in *M. oryzae*was achieved by either non-homologous end joining (NHEJ) or homology-directed repair (HDR) depending on the targeted loci. In absence of donor templates, Cas9-induced DSBs frequently triggered genomic rearrangement, leading to the loss of PCR-based amplification. By providing donor templates, however, HDR-mediated precise genome editing was achieved at the efficiency up to 100% in PCR positive transformants when targeting a single locus. The PTG-based multiplex genome editing via HDR also successfully led to the generation of double and triple gene mutants. The resulting CRISPR/Cas9 tools and strategies from this study are expected to aid in the efficient genome editing and functional genomics analysis for *M. oryzae*and other fungal species.

Editing of plant eIF4E genes for potyviral resistance
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Many positive-sense, single-stranded RNA viruses carry a viral protein (VPg) covalently linked to the 5'-terminus of their genomic RNA. VPg is functionally analogous to the cap of mRNA and serves as the binding site for the eukaryotic translation initiation factor 4E (eIF4Es, eIF(iso)4Es, and nCBPs). This interaction is a prerequisite for translation of viral genomic RNA, and therefore viral infection in host cells. To demonstrate the feasibility of engineering viral resistance by disrupting VPg-eIF4E interactions, we employed RNAi and CRISPR technologies to systematically knockdown and knockout the eIF4E family of genes in *Nicotiana benthamiana*, a ubiquitous host for many plant viruses. Seven eIF4E genes were cloned and the corresponding proteins were identified from *N. benthamiana*. Potential interactions of the eIF4E proteins with VPgs of selected potyviruses were screened by the yeast two-hybrid assay. RNAi and CRISPR constructs targeting the eF4E genes were designed and transformed to *N. benthamiana* to create knockdown and knockout lines of specific eIF4E genes, respectively. Significant reductions in the transcript level of the targeted eIF4E gene were observed in independent RNAi lines. In the CRSPR lines, random insertion and deletions were found around the eIF4E-coding sequences targeted by the CRSPR gRNAs. The RNAi knockdown lines and the CRSPR knockout lines are currently being characterized and evaluated for their resistance to potyviruses.

A New Source of Silicon for Blast Control on Rice
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Blast, caused by *Pyricularia oryzae*, is the major disease affecting rice production worldwide. This study investigated the potential of a new silicon (Si) source to control rice blast. Plants were grown in a Si-deficient soil that received the application of calcium silicate slag (CSL) (1.5 g/kg of soil), wollastonite (W) (1.5 g/kg of soil; Goianira, Goías, Brazil), and a mixture of calcium carbonate and magnesium carbonate (control). The amount of calcium (Ca) and magnesium (Mg) were equilibrated among treatments. Blast severity was evaluated at 24, 48, 72, 96, 120, and 144 hours after inoculation on the leaves of each plant and values were used to calculate the area under blast progress curve (AUBPC). Foliar Si concentration was significantly high for CSL and W treatments (1.63 and 2.02 dag/kg, respectively) compared to control (0.45 dag/kg). There was no significant difference among treatments for foliar Ca and Mg concentrations. Many necrotic lesions with intense chlorosis and coalescence were noticed on the leaves of plants from the control treatment. The number and size of the necrotic lesions were reduced, and there were fewer lesions coalescence and much less chlorosis on the leaves of plants from CSL and W treatments. The AUBPC was significantly reduced by 73 and 48%, respectively, for CSL and W treatments in comparison to control. Silicon from Goianira´s wollastonite showed great potential to complement rice resistance against blast.

Quantifying Control Efficacy of Fungicides Commonly Applied for Potato Early Blight Management
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Early blight is economically important foliar disease of potato. Due to lack of resistant potato cultivars, fungicides are applied extensively to obtain adequate control of this disease. To manage early blight, standard protectant fungicides and single-site-mode of action “specialty” fungicides are commonly applied, either alone or incorporated into a fungicide rotation program. Control efficacy at two crop growth stages and the overall tuber yield response to standard and specialty were assessed using network meta-analytic models. Control efficacy of both fungicides was moderate to very high (>30 to 75%) compared to the nontreated check. For both potato growth stages, specialty fungicides performed better than standard protectant fungicides. Furthermore, control efficacy of both fungicides was higher (3–9%) at late bulking and tuber maturation when compared to early bulking crop growth stage. Specialty fungicide programs increased overall tuber yields by 4% and 9% over standard fungicides and nontreated check, respectively. Based on the meta-analysis more precise fungicide use recommendations and fungicide programs can be developed for early blight management.

Asian Soybean Rust Control on Soybean Sprayed with a New Source of Soluble Silicon
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Asian soybean rust (ASR) is the most important disease affecting soybean production worldwide. This study investigated the efficiency of a new source of soluble silicon for ASR control. The treatments used were: plants sprayed with deionized water (control), fungicide (F) (Opera, Basf S.A.), Alpha (also known as “Silício Forte”) (45 g/L) (Verde Agritech Plc, England), and Alpha (45 g/L) + F. Plants (V4 growth stage) were inoculated with *Phakopsora pachyrhizi* at 24 hours after spraying. At nine days after inoculation, the first, second, and third leaves of each plant were used to evaluate ASR severity, to determine the total chlorophyll (Chl) *a*+*b* and carotenoids concentrations, and to obtain the variable-to-maximum Chl fluorescence ratio parameter (F_v/F_m) related to Chl *a* fluorescence. There were significant decreases of 99, 42, and 99.5% on ASR severity, respectively, for F, Alpha, and Alpha + F treatments in comparison to control. The total Chl *a*+*b* concentration was significantly high by 172, 91, and 142% and the concentration of carotenoids was significantly high by 89, 50, and 67%, respectively, for F, Alpha, and Alpha + F treatments in comparison to control. The F_v/F_m was significantly higher for F, Alpha, and Alpha + F treatments (0.81, 0.77, and 0.80, respectively) in comparison to control (0.74). The spray of Alpha was efficient to reduce ASR symptoms and to result in less photosynthetic impairments imposed by fungal infection.

Zinc oxide as an ultraviolet light protectant for natamycin in pre-havest management of gray mold and powdery mildew of strawberry
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The biofungicide natamycin is sensitive to ultraviolet light and degrades quickly in sunlight, limiting its field use. Zinc oxide (ZnO) and ferulic acid were evaluated as pre-mixtures with natamycin (provided by registrant) or in tank mixtures with natamycin against the pre-harvest pathogens *Botrytis cinerea* (gray mold) and *Podosphaera aphanis* (powdery mildew) of strawberry. In a field study with two strawberry cultivars, 4 weekly applications of natamycin 500 µg/ml + ferulic acid (50,000 µg/ml) or + ZnO (50,000 µg/ml) treatments were done. Fruit were harvested 7 days after the last application, inoculated with *B. cinerea*, and evaluated for gray mold incidence after 5 days. For both cultivars, the natamycin-ZnO formulation was significantly more effective than the natamycin-ferulic acid formulation or natamycin alone. In a greenhouse study with powdery mildew, natamycin 1000 µg/ml or a natamycin 1000 µg/ml + ZnO 10,000 µg/ml tank mixture were applied to plants. Inoculum was provided by diseased plants placed among experimental plants. After 18 days, leaves were rated for disease severity (based on % diseased area). The natamycin-ZnO treatment was significantly more effective than natamycin alone. Natamycin and ZnO are naturally occurring compounds and efficacious in mixtures. Because viable resistance in filamentous fungi has not been reported to natamycin after decades of use in the food industry, this mixture may be important in fungicide resistance management.

The efficacy of MgO bactericide against bacterial spot disease of tomato is particle size-dependent
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For many years management of *Xanthomonas perforans*, the causal agent of bacterial leaf spot (BLS) of tomato, with copper bactericides has been impractical because of the presence of only copper (Cu)-tolerant strains. Over the past decade, micron-sized particles (>100 nm size) and nano-sized particles (1-100 nm size) of magnesium oxide (MgO) particles were shown to have antimicrobial activity against several mammalian pathogens. In this study, three sizes of MgO were evaluated against *X. perforans*. The three different sized particles, 20nm, 0.3µm, and 0.6µm of MgO, all had high bactericidal activity against *X. perforans in vitro*. In field experiments, disease severity was significantly reduced by applications of the 20 nm sized particles at 200 µg/mL compared to the untreated control (UT), whereas the grower's standard (Cu-EBDC), and the micron-sized (0.3µm, 0.6µm) particles of MgO treatmentswere not significantly different from UT ($p = 0.05$). In addition, fruits from MgO treated plots did not significantly alter levels of Mg, Cu, Ca, K, Mn, P and S compared to fruit in the UT. In current study, newly formulated Mg nano-materials significantly reduced disease severity to as low as 100 µg/mL compared to Cu bactericide treated plots in greenhouse and in the field ($p = 0.05$). Based on these findings, Mg nano-materials could be an effective alternative to the current grower standard.

Efficacy of fungicides and biotic agents for the control of basil downy mildew in Illinois

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Basil downy mildew (*Peronospora belbahrii*) is a pathogen of sweet basil (*Ocimum basilicum*). While the disease is well controlled by fungicides, it is imperative to develop a management plan to keep the pathogen from developing fungicide resistance. Several fungicides were sprayed on basil weekly in varying combinations, application rates, and sequences over the course of one field season, and the treatment effects were evaluated on three different dates. The treatments included mixtures and different rotations of azoxystrobin, potassium phosphite, mandipropamid, cyazofamid, oxathiapiprolin, experimental compound A18269SE, dimethomorph, zoxamide + mancozeb, fluazinam, fluopicolide, mefenoxam + copper hydroxide, fenamidone, mancozeb, and ametoctradin + dimethomorph. All fungicide treatments significantly lowered the area under the disease progress curve (AUDPC) values compared to the untreated control. Cyazofamid and mandipropamid were both utilized in three different fungicide regimes that resulted in the lowest AUDPC values. Two novel biological control organisms (*Bacillus amyloliquefaciens* AS 43.3 and *Papiliotrema flavescens* OH 182.9 3C) were also tested for efficacy on basil downy mildew in greenhouse experiments. Azoxystrobin, the positive control, was effective against the pathogen, but the biological control organisms did not reduce disease severity. More experiments are needed to identify effective biological control organisms for basil downy mildew.

Evaluation of SAR Inducers for the Management of Bacterial Spot on Fresh-Market Tomatoes

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Bacterial spot, *Xanthomonas* spp., is one of the most damaging diseases of tomato worldwide. Due to limited disease management options, growers rely heavily on copper-based bactericides, that are often ineffective due to the presence of copper-tolerant *Xanthomonas* strains. This study was undertaken to evaluate the efficacy of three systemic acquired resistance (SAR) inducers, Leap™ ES (*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351), LifeGard™ WG (*B. mycoides* isolate J), and Actigard® 50WG (acibenzolar-S-methyl), alone or in combination with Harbour® (streptomycin) or Kocide® 3000 (copper hydroxide) as potential alternatives to the grower standard, copper-mancozeb, under greenhouse and/or field conditions. In two separate greenhouse studies, tomato plants treated with either Actigard® 50WG at 0.4 g/L alone, or in combination with Harbour® at 1.2 g/L or Kocide® 3000 at 2.1 g/L, significantly reduced bacterial spot disease severity when compared to the grower standard and water controls ($P < 0.05$). Under field conditions, LifeGard™ WG at 0.3 g/L significantly reduced bacterial spot disease severity when compared to the nontreated control without a significant impact on yield ($P < 0.05$). However, mixing LifeGard™ WG or Leap™ ES with Kocide® 3000 did not improve disease control under greenhouse or field conditions. This study highlights the potential of several different SAR inducing products for the management of bacterial spot of tomato.

Chemical control of sudden decline syndrome of date palm caused by *Fusarium solani* in the United Arab Emirates

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Date palm (*Phoenix dactylifera* L.) trees in the UAE suffer from serious disease called sudden decline syndrome (SDS) or *Fusairum* wilt which is characterized by whitening on one side of the rachis, progressing from the base to the apex of the leaf until the whole leaf dies. The internal disease symptoms is characterized by reddish roots and highly colored vascular bundles causing wilting and death of the tree. Three *Fusarium* spp. (*F. oxysporum, F. proliferatum* and *F. solani*) were isolated from infected root tissues, and it was found that *F. solani* was the main causal agent of SDS on date palm in the UAE. These three species were identified based on their cultural, morphological characteristics and in addition the internal transcribed spacer regions and large subunit of the ribosomal RNA (ITS/LSU rRNA) gene complex of the pathogens was further sequenced. Pathogenicity tests confirmed that the main causal agent of SDS on date palm in the UAE was *F. solani*. Under greenhouse conditions the application of Cidely® Top (difenoconazole and cyflufenamid) significantly reduced the fungal mycelial growth *in vitro* and reduced SDS development on date palm seedlings pre-inoculated with *F. solani*. Affected plants that were treated with Cidely® Top clearly showed vegetative growth recovery and developed healthy root

system comparable to control plants with no prior artificial infection. This current study is the first report confirming that the fungicide Cidely® Top is strongly effective against SDS and can be used to control this disease on date palm trees.

Efficacy of seed treatment fungicides for the management of seed-borne Ascochyta blight disease of chickpea under high disease pressure

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Ascochyta Blight (AB) of chickpea caused by *Ascochyta rabiei* is one of the most damaging disease of chickpea worldwide. The recent development of resistance to promising fungicides used in managing AB intensified grower concerns and threaten optimum production. This field study evaluated the efficacy of nine modes of action from six fungicide classes - fludioxonil, sedaxane, thiabendazole, penflufen, prothioconazole, fluxapyroxad, pyraclostrobin, and mefentrifluconazole formulations at recommended rates for the control of seed-borne *A. rabiei*. The study used naturally infested chickpea seeds produced in Montana. The trial received additional foliar fungicide sprays against AB due to high disease pressure. Data collected at 2, 4, 6 and 20 weeks after planting were analyzed using SAS VS 9.4. All nine seed treatment fungicides performed better than the untreated control in terms of vigor, plant number and yield with the exception of AB disease ($P = 0.4118$). Ascochyta lesions were positively correlated with vigor ($r = 0.57$), and yield ($r = 0.65$) but negative for plant number ($r = -0.0026$). This first-year study provided information on the importance of seed treatment fungicides in the management of AB disease. The second-year study is in progress.

Reduction of Shoot Blight Initiation of Fire Blight Cankers on Apple Wood by Post-infection Applications of Prohexadione-calcium

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The 2016 fire blight epidemic in northern New York led to $16 million damage and significant tree losses in high-density orchards. Part of the destructive effects of fire blight relate to canker formation on apple wood, usually originating from shoot blight symptoms. To reduce or prevent shoot blight severity, we evaluated 12 post-infection spray programs on mature 'Royal Cort' trees after the artificial inoculation of shoots with *Erwinia amylovora*, using prohexadione-calcium (PCA) alone or combined with acibenzolar-S-methyl (ASM) sprayed dilute to drip, in an experiment repeated annually for two years. The greatest reduction of shoot blight severity, of a 72.5 and 78.8%, in the first and second year, respectively, was achieved with the highest PCA rate (247.1 mg/L) applied twice, 14 days apart, followed by the same treatment, but applied once (53.5 and 89.5%). The greatest control of canker initiation on perennial wood was achieved with a single 247.1 mg/L PCA application which reduced canker initiation for 71.5% the first year and a 100% in the second year. Three applications of 123.6 mg/L PCA + 25 mg/L ASM, each 14 days apart, reduced canker initiation a 78.6 and 83.5% on years 1 and 2, respectively. As cankers can kill dwarf trees, serve as inoculum source and overwintering reservoirs for the pathogen, our most effective post-infection programs might help in reducing or preventing canker development, thus minimizing tree losses after fire blight outbreaks.

***In planta* antibacterial activity of oxytetracycline against *Candidatus* Liberibacter asiaticus causing citrus HLB disease**

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Oxytetracycline (OTC) is currently being used to control citrus Huanglongbing (HLB) in Florida by foliar spray. However, the antimicrobial activity of OTC against the HLB pathogen *Candidatus* Liberibacter asiaticus (Las) remains poorly understood due to the difficulty to culture this bacterium. We investigated the antibacterial activity of OTC against Las in greenhouse and field experiments. Citrus trees infected by Las were treated with OTC via trunk injection. Las titers and OTC residues in leaf samples were determined using qPCR and HPLC assays respectively, following OTC treatment. Greenhouse experiments showed that OTC injection at 0.05g/tree resulted in a decline of Las populations to undetectable level (Ct value ≥ 36.0) from 7 to 30 days post treatment (DPT), with observed levels of OTC residues at 0.7-0.8 ppm. Field experiments revealed that at an injection rate of 2.0 g/tree, OTC resulted in the decline of Las titers to the undetectable level from 7 to 28 DPT, with observed levels of OTC residues at 0.9-1.2 ppm. In both greenhouse and field trials, nonlinear regression analyses indicated that the levels of suppression of Las population were positively associated with OTC residue levels in leaves. These results suggested that the threshold for OTC to eliminate Las from HLB diseased trees could be ≥ 0.9 ppm in leaf tissues under field conditions. These findings may be useful for citrus growers in developing an effective HLB management program with OTC.

Evaluation of management strategies for Fusarium crown and root rot in Michigan asparagus

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Michigan is the second largest producer of asparagus nationally, generating 41% of the country's production in 2018. Fusarium crown and root rot, caused by *Fusarium* spp. including *F. proliferatum* **and** *F. oxysporum*, is a major limiting factor for asparagus production in the state resulting in reduced yield and field longevity. Our objective was to evaluate biocontrol and fungicide products for their ability to protect asparagus crowns against crown and root rot. A greenhouse study included soil drenches of EndoMaxx (*Glomus intraradices/Glomus mosseae/Glomus aggregatum/Glomus etunicatum*), Actinovate (*Streptomyces lydicus* WYEC 108), Mycostop (*Streptomyces griseoviridis*), and the industry standard Cannonball (fludioxonil) on inoculated asparagus seedlings. Significant differences were observed as Cannonball resulted in increased fern weight compared to Actinovate and EndoMaxx. A non-inoculated field study compared 12 soil treatments applied to a seedling crown nursery. One-year old crowns were weighed and rated for size

(1=low root mass; 4=high root mass). Presidio SC (fluopicolide) alternated with Topsin (thiophanate-methyl) and Miravis (pydiflumetofen) produced the most #4 crowns and the highest per crown weight in the first and second year of the trial, respectively. The results of these studies can be used to develop disease management strategies for asparagus seed beds which could improve the quality of crowns used to establish production fields.

Evaluation of SP2700 antiviral activities in three model patho-systems
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SP2700 (Trade name: Ninja™) is a biochemical product extracted from soil bacterial fermentation. This compound activates the innate plant defense system and demonstrates antiviral properties. It has been used extensively in plant viral disease management outside of the US. In this study, SP2700's curative antiviral property was examined using three model patho-systems: 1) *Tobacco mosaic virus* (TMV) on tobacco (*Nicotiana tabacum* var. Hicks MR) as a local lesion host, 2) *Tomato bushy stunt virus* (TBSV) on pinto bean (*Phaseolus vulgaris* cv. Pinto) as a local lesion host, 3) TBSV with a green fluorescent protein (GFP) reporter gene on *Nicotiana benthamiana* as a susceptible systemic host. Inoculations with purified TMV virions via a soft brush or TBSV RNA transcripts via rubbing were conducted 1 hour before the application of the product. A half-leaf assay was used for these studies where SP2700 was brushed onto one half of the leaves and water to the other half leaves serving as the control. SP2700 treated half leaves had consistently less TMV local lesions compared to the control with reduction from 76% to 49% on older leaves and 89% to 20% on young leaves based on 4 experiments. A similar effect was observed with TBSV on pinto half leaves. Surprisingly, fewer fluorescent foci were observed in the TBSV-GFP SP2700 treated half leaves despite the total susceptibility of *N. benthamiana* to TBSV systemic infection. This demonstrates that SP2700 is able to decrease viral infections using a non-specific mechanism and shows great promise as an antiviral compound.

Sensitivity of baseline and exposed *Podosphaera leucotricha* populations from apple to fungicides from three chemical groups
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Powdery mildew, caused by *Podosphaera leucotricha*, is among the foremost biotic challenges faced by the apple industry in the US Pacific Northwest. Site-specific fungicides, including demethylation inhibitors (DMIs), quinone outside inhibitors (QoIs) and succinate dehydrogenase inhibitors (SDHIs) have been used for decades in conventional apple orchards and some decreased efficacy has been observed recently. In 2018, sixty *P. leucotricha* isolates were collected from two organic (*n=26*) and three conventional (*n=34*) apple orchards in Washington State. Sensitivity to trifloxystrobin (TRI, QoI), boscalid (BOS, SDHI) and triflumizole (TRIF, DMI) was assessed on detached leaves sprayed with four doses of each fungicide and a non-treated control. Relative growth, measured after 14 days, was used to determine EC_{50} values and variation factors (VFs) within the tested populations. VFs for TRI, BOS and TRIF were 23, 4, and 9 in the baseline populations versus 43, 4, and 15 in the exposed populations, respectively. Overall, these results indicate some variability in sensitivity levels in *P. leucotricha* populations, especially for TRI both in the baseline and the exposed populations which may suggest population drifts. Further studies that include a larger population size are needed to make general conclusions about potential shifts toward reduced sensitivity.

Comparative performance of fungicides, biofungicides, host plant defense inducers, and fertilizer in suppression of Phytophthora root rot in boxwood
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Phytophthora root caused by *Phytophthora nicotianae* can affect all growth stages of field or container grown boxwood plants. Management of *Phytophthora* requires an integrated approach. The efficacy of fungicides, biofungicides, host plant defense inducers, and fertilizer against Phytophthora root rot of boxwood was assessed in field and greenhouse trials. Plots/pots were inoculated with *P. nicotianae* grown on rice grains. 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 field trial, the treatments most effective in reducing Phytophthora disease severity were Orkestra Intrinsic, Subdue MAXX, Segovis, fertilizer ON-Gard alone and the RootShield *PLUS*$^{+}$+ fertilizer ON-Gard application program and disease severity for those treatments were not significantly different than the non-treated, non-inoculated control. All of the treatments except Subdue MAXX and Aliette resulted in a significantly greater plant height compared to the non-treated, inoculated control. At the end of the greenhouse trial, the treatments most effective in reducing Phytophthora disease severity were Orkestra Intrinsic, Empress Intrinsic and Orvego and disease severity for those treatments were not significantly different than the non-treated, non-inoculated control. There were no significant differences in plant growth between treated and non-treated control plants.

Etiology, biology and management of almond trunk and scaffold canker diseases in California
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Canker diseases are the main cause of tree mortality in almond orchards in California. Pruning wounds serve as the primary infection court for fungal pathogens. Protection of wounds using fungicides and sealers has been broadly employed to control canker diseases of woody crops. The aims of this study were to identify the principal fungal pathogens associated with almond trunk and scaffold canker diseases (TSCD), assess pathogenicity, and investigate management strategies through understanding of pruning wound susceptibility

and pruning wound protectant efficacy. From 2015–2018 over 120 almond orchards with TSCD were surveyed. Isolations revealed numerous fungal species associated with cankers including Botryosphaeriaceae spp., *Ceratocystis destructans*, *Cytospora* spp., *Diaporthe* spp., Diatrypaceae spp., and *Collophorina* spp. Cankers caused by Botryosphaeriaceae spp. and *C. destructans* were the most prevalent in California. Field trials were conducted to evaluate pruning wound susceptibility according to the month of pruning. Fungi were inoculated on pruning wounds made between September and January and varying in age from 0- to 8-weeks-old. In the pruning wound protection trials, twenty-six products were tested against eight canker-causing fungi. Results show that pruning wound susceptibility was lowest for January pruning. Susceptibility of wounds decreased after two weeks following pruning. Thiophanate-methyl and a *Trichoderma* biocontrol agent were the most efficacious products for protection of pruning wounds against the various canker pathogens.

The influences of infection timing and postharvest fungicide applications on perennation of the downy mildew pathogen in hop
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Downy mildew of hop, caused by the biotrophic pathogen *Pseudoperonospora humuli*, overwinters in buds and crowns of hop plants as systemic mycelia within the plant. The impact of infection timing on pathogen survival and subsequent disease development in the next year is not well understood. To describe this process, batches of hop plants were inoculated monthly from June to October and the following year emerging shoots were rated for downy mildew. In 2017, plants inoculated in October had significantly less disease than plants inoculated in earlier months. In separate experiments, plots in commercial hop yards and experimental plots were treated with fungicides up to 4 times weekly following harvest. Experimental plots treated with fungicides had a slight decrease in downy mildew infection in the following year as compared to nontreated plots. Taken together, the early findings of this research indicate that infection leading to overwintering can occur over a broad period of time, but overwintering of the pathogen is reduced when infection is delayed until after September. Experiments are being repeated in 2018 and 2019 to validate these findings and refine management strategies for the disease.

Optimizing use of new products for management of root-knot nematode, *Meloidogyne incognita*, on carrots
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Two field trials evaluated new products for management of root-knot nematode. Products were tested either alone, or in combination with other products. Trials included an untreated control and were conducted in a randomized complete block design with 5 replicates per treatment. Twenty-three treatments in the first trial included: fluopyram (Velum, Bayer); TECA Fulvic, TECA Nemat-X, and TECA Root from IG Agrosolutions; fluensulfone (Nimitz, Adama); fluazaindolizine (Salibro, DuPont); metam potassium (K-pam HL, AMVAC); QL Agri (*Quillaja*, Desert King); FBS (soil organic acids, Fbsciences); Cedroz (geraniol and thymol, Eastman Chemical Company); and CrossLink (tergatol, amino acids, Beem Biologics). Seventeen treatments in the second trial included: fluensulfone, fluopyram, *Paecilomyces lilacinus* (BioAct, Bayer); *Paecilomyces lilacinus* (MeloCon, Certis); fluensulfone; ISO (*Guayule* extract), SA Propel (soil organic acids), and Focus (bacterial complex) from Beem Biologics; Groactive (*Yucca* and *Quillaja*, Desert King); and 1,3-dichloropropene (Telone II, Dow AgroSciences). All products evaluated increased yields compared to the untreated control. Compared to individual products alone, yields increased with combinations of Velum plus TECA products; of QL Agri plus FBS; of K-pam plus Cedroz; of Velum with BioAct, and of ISO with SA Propel.

Fungicide applications prevent *Colletotrichum acutatum* secondary conidiation from appressoria and conidiogenous hyphae on citrus leaves
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Colletotrichum acutatum causes postbloom fruit drop of citrus (PFD), an important disease in the tropics that may result in significant yield losses during rainy seasons. PFD epidemics are initiated by conidia produced from quiescent appressoria when bloom occurs. The appressoria germinate on leaves and produce a small number of conidia when stimulated by substances that are washed-off from flowers or by sucrose. Conidia that successfully infect flowers produce acervuli on which copious conidia are formed. Our objective was to investigate new management approaches to control PFD that focus on reducing inoculum density or preventing the production of secondary conidia from hyphae or appressoria. We tested the effect of a multi-site fungicide (ferbam) applied to leaves with or without a sucrose stimulant. Two experiments assessed the effect of ferbam (1.8 $g.L^{-1}$) on conidiogenous hyphae and appressoria. Two other experiments assessed the effect of ferbam on melanized appressoria. The number of conidia, appressoria, and colony forming units (CFU) was counted. Ferbam hindered secondary conidiation in all experiments. The number of conidia and CFU in treatments that received the fungicide application was, respectively, 49–98% and 82–100% lower than in those on which sucrose was applied. Ferbam applications during periods of secondary conidiation stimulation suppress inoculum production and might be applied as a new strategy to manage PFD in citrus orchards.

Management of black leaf spot of rose with fungicides
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Black spot (*Diplocarpon rosae*) is a destructive leaf disease of rose in the landscape and nursery production. The efficacy of fungicides against black spot of rose were assessed on rose ‘Queen Elizabeth’ and ‘Louis Philippe’. Six single-plant replications per treatment were arranged under 45% shade in a randomized complete block design. Treatments were Orkestra Intrinsic SC, Mural 45WG, Eagle 20EW

and Broadform SC (4 and 8 fl oz/100 gal). Severity of black spot resulting from natural infections was determined during the experiment and expressed as the percentage of foliage area affected. All of the treatments significantly reduced black spot severity and area under the disease progress curve (AUDPC) throughout the experiment compared to the non-treated control for both cultivars. The treatments that most effectively reduced black spot severity were the high and low rates of Broadform, Eagle and Mural for 'Queen Elizabeth' and the treatments that most effectively reduced black spot severity were the high rate of Broadform, Eagle and Mural for 'Louise Philippe'. There were no significant differences in disease progress between fungicide treatments for 'Queen Elizabeth'. Treatment with the high and low rates of Broadform, Mural and Eagle were more effective in reducing AUDPC in 'Louis Philippe' than Orkestra Intrinsic. Non-treated control 'Queen Elizabeth' plants were not commercially acceptable due to the level of disease at the end of the trial.

Residual effect of fungicides applied for flower protection and control of sclerotinia stem rot of soybean

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Boscalid, picoxystrobin, tetraconazole, and thiophanate methyl are fungicides labeled in the US to manage sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, with application recommended at bloom to prevent flower infection. Since not all flowers open at the time of application, we aimed to characterize residual activity of these fungicides over time. Soybean 'Williams 82' was grown under controlled conditions and fungicides were applied at bloom with 187 L ha^{-1} associated with nonionic surfactant (0.25% v/v), plus the control. Entire plants were collected between 0 and 10 days after fungicide application. Leaves were removed and one to three mature flowers per plant were inoculated with 10µl water with 5,000 ascospores of an isolate of *S. sclerotiorum*. Plants were incubated in moist chambers at room temperature and disease severity was evaluated daily at 6 to 15 days after inoculation and number of sclerotia counted. AUDPC of disease severity showed all applications reduced disease compared to the control at each day after application. To compare residual activity over time, AUDPC was converted to percent inhibition and analyzed with linear regression. Boscalid and tetraconazole showed evidence of systemicity due to sustained disease suppression over time compared to thiophanate methyl. Sclerotia were reduced after all fungicide applications, although results were inconsistent. Results suggest picoxystrobin reduced formation of sclerotia at 6 days after fungicide application ($p < 0.10$), which may be important for long-term disease management.

Possible synergistic effect of prohexadione-calcium and acibenzolar-S-methyl in managing shoot blight of apple

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Fire blight, an economically important disease of pome fruit, has multiple stages of infection including shoot blight. The causal agent, *Erwinia amylovora*, can infect apple shoots from internal movement via infected flowers or from external infection via bacterial cells disseminated by wind and rain. Young apple trees are susceptible to fire blight infection, and in combination with high-density orchards, shoot blight management is a growing concern. Two products, prohexadione-calcium (Pro-Ca) and acibenzolar-S-methyl (ASM), are used to reduce shoot blight on older trees. However, Pro-Ca causes shoot stunting, which is detrimental for growing trees. This study seeks to determine if low rates of Pro-Ca and ASM can reduce incidence of shoot blight without shoot stunting. During shoot growth, two young 'Gala' apple plantings were treated with low rates of Pro-Ca and ASM. Rates of Pro-Ca ranged from .07-.56 kg/ha and ASM at .07-.14 kg/ha; both as stand-alone and tandem treatments. Each experiment was inoculated with *E. amylovora* strain Ea110 to ensure disease development. Shoots were measured weekly for growth and lesion length after inoculation. Scanning electron microscopy was utilized to measure plant cell wall widths. Disease incidence with Pro-Ca at .14 kg/ha + ASM at .07 kg/ha was significantly lower than with either product alone. Lesion length was similar with a high rate of Pro-Ca alone, but with no significant impact on shoot length. Our results suggest that low rates of Pro-Ca and ASM may work synergistically to reduce incidence of shoot blight on young apple trees.

Comparative analysis of two fungicide sensitivity tests for *Helminthosporium solani*

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Helminthosporium solani is a pathogenic fungus which causes silver scurf (SS), a potato-specific disease often found in temperate regions. SS causes cosmetic defects in potato seed tubers which negatively impact the crop's marketability and storability. MI growers must implement strategies to manage the long-term storage of their product and mitigate this persistent disease. Thus, effective fungicides may control SS and extend the storage period for potatoes. This study had two main goals. First, determine the in vitro sensitivity of *H. solani* to three classes of fungicides: DMI fungicides [difenoconazole (DFZ)], SDHI fungicides [Solatenol (STL) and sedaxane (SDX)], and phenylpyrrole fungicides [fludioxonil (FDL)]. Second, compare the accuracy and precision of the spiral gradient dilution method to a standard tenfold agar plate dilution method. Tubers with symptoms of SS were sampled from potato storages from the United States. A total of 32 isolates have been collected. The relative growth and germination of spores for all mono-conidial *H. solani* isolates are being recorded to determine isolates sensitivity to the fungicides listed above. Preliminary results from screening of a limited number of isolates (n=8) collected from ID, WI and MI indicted that EC_{50} values ranged from 0.2 to 2.6 for STL and 1.6 to 3.5 for SDX. Spiral gradient and agar plate dilution to estimate the effective fungicide concentration at which 50% of the fungal growth is inhibited (EC_{50}) in vitro for all isolates are ongoing.

SP2700-a novel antiviral and antifungal plant activator

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SP2700 (Trade name: Ninja™) is a biochemical pesticide that manages a multitude of viruses in high value agricultural crops such as tomato, peppers, and cucurbits. It improves host resistance through inducing plant natural defense systems, both systemic acquired resistance (SAR) and induced systemic resistance (ISR), as well as through direct inhibition of the viruses. SP2700 is produced through fermentation of a naturally occurring soil bacterium originally identified in China. In addition to managing plant viral diseases, SP2700 also has shown efficacy against several fungal diseases such as powdery mildew, diseases arising from soil-borne pathogens, rusts and those caused by *Alternaria* spp. in various crops. Furthermore, it has also been identified as a biological "efficacy enhancer" when used as an additive or in rotation with more conventional pest management programs. Being naturally occurring, SP2700 has excellent toxicity profiles and degrades quickly in natural environments. From 2013–2018 numerous field and greenhouse trials were conducted, which will be reviewed during this presentation. SP2700 offers a novel approach for plant viral and fungal disease management for both US conventional and organic agriculture.

Baseline Sensitivity of *Alternaria alternata* and *A. arborescens* to Natamycin and Control of Alternaria Rot on Blueberry Fruit
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Alternaria rot caused by *Alternaria alternata* and *A. arborescens* is a major postharvest disease of blueberries in California. Natamycin, a food additive and biochemical fungicide, was evaluated for its potential as a postharvest treatment to control Alternaria rot. The baseline sensitivities of *A. alternata* and *A. arborescens* were established. The effective concentrations of natamycin for 50% inhibition of conidia germination relative to control (EC_{50}) of 34 *A. alternata* isolates ranged from 0.640 to 1.032 µg/ml with a mean of 0.778 ± 0.097 µg/ml and from 2.660 to 4.330 µg/ml with a mean of 3.390 ± 0.435 µg/ml for reduction of mycelial growth. EC_{50} values of natamycin for 34 *A. arborescens* isolates ranged from 0.540 to 1.335 µg/ml with a mean of 0.950 ± 0.162 µg/ml for inhibition of conidial germination, and from 0.610 to 3.430 µg/ml with a mean of 1.920 ± 0.637 µg/ml for mycelial growth. Control tests on detached blueberry fruit inoculated with four *Alternaria* isolates showed that even a low concentration of natamycin (230 µg/ml) significantly reduced disease incidence and severity of Alternaria rot, regardless of blueberry variety, application method or incubation condition. Our results suggest that natamycin can be an effective postharvest treatment for control of postharvest Alternaria rot in blueberry.

Novel Boron Containing Chemistry for Management of Plant Pathogenic Fungi
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Boron containing compounds have a proven track record in human health for therapeutic treatment of fungal diseases. Building off that framework, Boragen has developed a platform for boron-based crop protection chemistry. A novel fungicide, BAG8, which inhibits fungal protein biosynthesis, has demonstrated the ability to effectively control a broad spectrum of agronomically relevant plant pathogens. Minimum inhibitory concentration (MIC) was determined for 20 separate plant pathogenic fungi with results ranging from 0.02-5.0 parts per million (ppm). Additionally, BAG8 was screened against 24 isolates of *Alternaria solani* collected from tomato growers in North Carolina, with resistance to azoxystrobin and/or boscalid. *In vitro* screening of these isolates revealed the high intrinsic activity of BAG8 across all isolates with or without resistance, demonstrating a novel mode of action. Broad spectrum activity has been confirmed through continued greenhouse and field trialing on a wide array of pathosystems.

Evaluation of chemical treatments and cultivars on the incidence of powdery scab and *Potato mop-top virus* in potato
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Potato mop-top virus (PMTV), a necrotic virus of potato, and its vector *Spongospora subterranea* (Ss), which causes powdery scab, are growing threats to North American potato production. These two soil-borne pathogens are limiting the production of healthy tubers and are negatively impacting seed and fresh market potato exports. The biphasic life cycle of Ss and the durability and viability of its resting spores makes disease management particularly difficult. We investigated the effects of two chemicals, Omega®500F and FOLI-R-PLUS RIDEZ™, on powdery scab and PMTV and on the reduction of Ss inoculum in naturally infested fields and potting mix using different potato cultivars. There was no significant difference related to chemical treatments on the amount of Ss inoculum in the soil or potting mix nor on PMTV incidence. Ss soil inoculum in fields varied across farms and among potato cultivars. Russet cultivars were more tolerant to powdery scab than the yellow- or red-skinned cultivars. However, PMTV incidences on tubers collected from Classic Russet and Russet Norkotah 8 ranged from 0 to 80%, even though the tubers lacked powdery scab lesions. In addition, 93% of plants were infected by PMTV in the greenhouse, indicating PMTV transmission was high under optimal conditions for powdery scab disease development. These results expand our current understanding of powdery scab and PMTV and aid in development of integrated management practices for these important diseases.

Evaluation of a single seedling treatment with nanoscale nutrients to control Fusarium wilt symptoms of chrysanthemum
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Wilt caused by *Fusarium oxysporum* f. sp. *chrysanthemi* is one of the most significant disease problems to affect chrysanthemum (*Chrysanthemum morifolium* Ramat.). Typical control measures include sanitation and fungicide drenches, but effectiveness of these options are limited by long-term survival of the pathogen in soil. Nanoparticle formulations of copper, manganese, or zinc were recently found to control development of fungal wilt in eggplant and cucurbits. In 2017 and 2018, rooted chrysanthemum transplants were

exposed to a single spray treatment of metallic nanoparticles (NP) (500 µg/ml) or water in the greenhouse a week before planting. Seedlings were potted in *Fusarium*-infested or noninfested potting soil and arranged on landscape fabric in the field. Plant growth and photosynthesis was measured weekly, and disease ratings and dry weights were collected three months after planting. In 2017 and 2018, plants treated with NP Cu were larger than controls, regardless of disease. Estimates of disease in NP Cu-treated plants were less than the inoculated controls in both years and comparable to fungicides. NP Mn and Zn were less effective. The results indicate that nanoparticle treatments may be a useful tool for reducing fungicide use while maximizing production in floriculture. Planting and data collection was performed as a group project by undergraduates participating in a NIFA-funded REEU program, the Summer Undergraduate Fellows in Plant Health and Protection.

Higher rates of phosphonate improve efficacy against scab on pecan

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Venturia effusa causes scab on pecan in the southeastern USA. In susceptible cultivars, frequent fungicide sprays is the only option to control the disease. Resistance to some fungicides has made management challenging. Understanding how rate affects disease control is important to ensure that control is maximized. We compared the effect of different rates of phosphonate products in reducing scab in six field experiments from 2015 to 2018 at two locations in Georgia. Cv. Desirable was used in four experiments, and cv. Wichita was included in two years at one location. Four rates of ProPhyt® (2.3, 3.5, 5.3 and 7.0 L/Ha), three rates of Rampart® (2.3, 4.7 and 7.0 L/Ha), or two rates of K-phite® 7LP (2.3 and 7.0 L/Ha) were tested. Also depending on experiment, 5 to 9 applications of phosphonate product were made. Incidence of scab was invariably high on fruit in the control trees, and most often high on treated trees. Treatment with the higher rates of phosphonate product most often significantly or numerically reduced severity of scab compared to the lower rates of the products applied on foliage and fruit. In 2015 fruit weight was increased where phosphonate application resulted in reduced disease, but in 2016 the effect was unclear due to a late and mild epidemic of scab likely having limited impact on the fruit size. Our results demonstrate that higher rates contribute to more efficacious control of this yield-limiting disease.

Tractor speed and concentration of active ingredient affect the incidence and severity of scab on peach fruit

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Peach scab (caused by *Venturia carpophila*) lesions on fruit result in market downgrade and monetary losses. Regular fungicide applications are required to reduce infection. Total spray volume, as affected by tractor speed at constant rate of active ingredient (a.i.) was investigated as a variable affecting scab control. In 2015 and 2016, trees were sprayed with fungicide at petal fall and shuck split, followed by cover sprays at 7–10 day intervals until harvest. Speeds were 3.2, 4.8 and 6.4 kph (spraying 1403, 935 and 702 L/ha, respectively). All speeds (different spray volumes, but equal a.i. ha) reduced incidence and severity of scab compared to the control. Mean incidence at 3.2, 4.8 and 6.4 kph in 2015 and 2016 was 61.9, 59.4 and 38.2%, and 65.1, 52.4 and 39.5% of fruit scabbed, respectively. Lesions per fruit (LPF) were reduced in 2015 from 12.3 and 20.0 LPF at 3.2 and 4.8 kph to 7.4 LPF at 6.4 kph. In 2016 there were 43.0, 14.7 and 32.8 LPF at 3.2, 4.8 and 6.4 kph, respectively. LPF did not differ between speeds of 6.4 kph and 3.2 or 4.8 kph, but LPF was lower at 4.8 compared to 3.2 kph. Spray date and speed affected spray coverage, with least coverage at 6.4 kph in July when the canopy was full. There was a negative linear relationship between speed and spray coverage. However, concentration of a.i. in low volume early-season sprays may provide some benefit, even though reduced coverage results at higher speeds.

Sensitivity of *Rhizoctonia solani* isolates from soybean and sugar beet to selected SDHI and QoI fungicides

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Rhizoctonia solani can cause significant production losses to sugar beet and soybean. Disease management relies heavily on fungicides. The objective of this study was to determine the sensitivity of *R. solani* to sedaxane, penthiopyrad, and pyraclostrobin. Thirty-five isolates of *R. solani* collected during 1986 - 2018 from soybean and sugar beet in Minnesota and North Dakota were selected for this study. Isolates were subjected to poison plate assays with fungicide concentrations of 0, 0.01, 0.1, 1 and 10 ug/ml in potato dextrose agar. For assessing sensitivity to pyraclostrobin, salicylhydroxamic acid (SHAM) was added to the medium at 100 ug/ml. The fungicide concentration that suppressed 50% radial growth of mycelium compared to the growth on non-amended plates (EC50) was estimated for each isolate using ‘drc’ package in R. The EC50 values for sedaxane, penthiopyrad and pyraclostrobin ranged between 0.04 to 0.34, 0.04 to 0.32 and 0.04 to 0.85 ug/ml with means of 0.12, 0.16 and 0.22 ug/ml, respectively. The EC50 values of penthiopyrad and pyraclostrobin varied significantly among the isolates, but not for sedaxane. The EC50 values of penthiopyrad were significantly higher for isolates from soybean compared to the isolates from sugar beet. The three fungicides are effective for suppressing the pathogen growth in vitro, however *R. solani* isolates appear to be more sensitive to SDHI fungicides than QoI fungicides.

Population structure and fungicide sensitivity of *Phytophthora capsici* in Tennessee

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Phytophthora blight of cucurbits and pepper, caused by the oomycete *Phytophthora capsici*, is one of the most destructive diseases vegetable growers face and management is extremely difficult. Little is known about fungicide sensitivity and population structure of *P. capsici* in Tennessee. In 2018, 87 single-zoospore isolates were obtained from infected fruit, stems or crowns collected from four different field sites throughout eastern and middle TN. All 87 isolates were tested against six fungicides using radial growth assays. Mefenoxam was tested at 10 µg/ml and 100 µg/mll. Five out of 87 isolates were found to be resistant and grew above 40% of the growth of the non-amended control at 10 µg/ml and 100 µg/ml mefenoxam. Dimethomorph was tested at five concentrations ranging from 0 µg/ml to 1 µg/ml and no resistance was found. EC50 values ranged from 0.21 µg/ml to 0.60 µg/ml. Fluopicolide was tested at five concentrations ranging from 0 µg/ml to 5 µg/ml. Thirty-six out of 87 isolates were resistant, with EC50 values >5 µg/ml. Sensitive isolates had EC50 values ranging from 0.16 µg/ml to 3.1 µg/ml. Mandipropamid, cyazofomid and oxathiapiprolin testing is currently underway. All isolates were genotyped using 45 single nucleotide polymorphisms (SNPs) to explore population structure in TN. Information gained through this study will improve management recommendations, and will further our understanding of how *P. capsici* is distributed and proliferated throughout TN.

Comparison of conventional and organic fungicides, application intervals, and cultivars to manage tomato early blight in Tennessee
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Early blight, caused by *Alternaria tomatophila*, is a serious disease of tomato and fungicides are often applied on a calendar-based schedule for management. The objective of this study was to evaluate conventional and organic fungicide programs under two application intervals for management of tomato early blight. Three cultivars reported to have varying levels of early blight susceptibility were tested: 'Mountain Glory' (susceptible), 'Defiant PhR' (intermediate resistance), and 'Mountain Merit' (intermediate resistance). The average number of days between fungicide applications were 8 and 17 for standard-timing and half-frequency intervals, respectively. The conventional program rotated Inspire Super, Manzate, and Fontelis. The organic program included Double Nickel mixed with Cueva. Cultivars did not differ regarding disease or yield. Conventional and organic programs reduced the area under disease progress curve (AUDPC) compared to the non-treated control (NTC), and conventional AUDPC was lower than organic. The conventional program resulted in higher yields than the organic program. In the organic program final disease severity was approximately 90% for both the standard-timing and half-frequency interval. In the conventional program the standard-timing interval resulted in lower final disease severity (14%) compared to the half-frequency interval (42%). These results will inform early blight management recommendations for Southeastern tomato producers.

Efficacy and baseline of succinate dehydrogenase inhibitor (SDHI) fungicides for management of Colletotrichum crown rot of strawberries
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The current management of Colletotrichum crown rot (CCR) of strawberry, caused by *Colletotrichum gloeosporioides*, relies on the use of a few fungicide classes. Since resistance to QoI fungicides was recently detected, alternative fungicide groups are needed to control the disease. Our objective was to evaluate the efficacy of succinate dehydrogenase inhibitor (SDHI) fungicides in managing CCR. Twenty-plant plots of cultivar Strawberry Festival were inoculated 4 weeks after planting. Five SDHI fungicides, fluopyram, isofetamid, penthiopyrad, fluxapyroxad, and benzovindiflupyr, were either applied 2 days before or one day after inoculation (DAI). SDHI treatments were compared with a non-treated control and with the most common fungicides used on CCR management, i.e. thiophanate-methyl, pyraclostrobin, and captan. CCR incidence was evaluated once a week by counting the number of wilted and dead plants. Benzovindiflupyr applied 1 DAI was effective in reducing plant mortality and disease development. Captan was the most effective when applied preventively. The baseline of 100 *C. gloeosporioides* isolates was determined in vitro using a spiral gradient dilution assay. The EC_{50} for benzovindiflypyr and penthiopyrad varied from 0.08-1.11 and 0.45-3.17 µg/mL, respectively, whereas the other SDHI fungicides did not inhibit fungal growth. Our results indicate that benzovindiflupyr could serve as an alternative to manage CCR in Florida if the product is registered.

Improved seed treatment efficacy by nanoencapsulation of fungicides for the control of *Fusarium graminearum*
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Fusarium graminearum is an important soilborne and seedborne pathogen that causes reduced emergence and seedling blight of row crops. The use of seed-applied fungicides is one major management strategy; however, it is constrained by several factors including rapid dissipation of active ingredients, limiting uptake in plant tissues, and absorption by target organisms. Our research investigates improving the efficacy of seed-applied active ingredients through the use of polyanhydride polymers. These polymers have been extensively studied in other systems to facilitate time-release of active compounds. The surface-erosion mechanism of polyanhydride particles promotes a sustainable and predictable release of active ingredients encapsulated in polyanhydride nanoparticles. We hypothesized that nanoencapsulation of commonly-used fungicides could prolong the release due to the slow degradation rate of this nanoparticle coating and hence, improving control of *F. graminearum*. In-vitro efficiency assays using systemic and contact seed treatment fungicides, thiabendazole and fludioxonil, were conducted. For some active ingredients, encapsulated formulations resulted in lower disease severity, and an increase in healthy biomass compared to the standard formulation.

Analysis of fungicide efficacy on *Macrophomina phaseolina* isolates from West Tennessee
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Macrophomina phaseolina, causal agent of charcoal rot in soybean (*Glycine max*) and corn (*Zea mays*), is a prevalent soilborne fungal pathogen occurring across the United States and is most common in the southern and midwestern states. The fungus produces overwintering structures, microsclerotia, that allow it to persist in debris and soil, making no-till fields particularly at risk of harboring inoculum across growing seasons and susceptible crops. Isolates of *M. phaseolina* were collected from soil and soybean root debris samples submitted by farmers in West Tennessee across the 2017–2018 growing seasons. A subset of these isolates were screened for fungicide sensitivity to one or more active ingredients in the following fungicide classes: demethylation inhibitor (DMI), methyl benzimidazole carbamate (MBC), quinone outside inhibitor (QoI), and succinate dehydrogenase inhibitor (SDHI). Colony radial growth was also observed for virulence analysis. Fungicide screening results, colonial growth analyses, and overall distribution and abundance across sampled areas will be presented.

Effects of seed storage conditions and chemical disinfestation treatments on the survival of *Xanthomonas vasicola* pv. *vasculorum* on maize seed
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To evaluate the effects of seed storage conditions on the survival of *Xanthomonas vasicola* pv. *vasculorum* (*Xvv*), the causal agent of bacterial leaf streak on corn, artificially-contaminated seeds (soaked in bacterial suspension of 10^5 and 10^6 CFU/ml) were place in two different environments: refrigerated storage (10°C and 50% RH) and "warehouse" conditions. Seeds were sampled every two months for one year. In a second set of experiments, artificially-contaminated seeds were immersed in disinfectant solutions of sodium hypochlorite, hydrochloric acid and hydrogen peroxide. Bacterial survival was monitored by dilution plating onto semi-selective agar. Suspect colonies of *Xvv* were subcultured onto non-selective medium and confirmed by PCR. In addition, effects of the treatments on seed germination and seedling growth rate were evaluated. After one year, *Xvv* survived on maize seed storage under both storage conditions but the viability of the initial bacterial population declined in both environments, showing a marked reduction especially under warehouse conditions. In the latter, *Xvv* survival was noticeably reduced from an initial 4.77 to 2.68 log CFU/ml after 4 months, after which it remained generally stable. The different chemical disinfestation treatments effectively decontaminated the seeds, specifically the seed treatment with 1% sodium hypochlorite for one minute, which also did not affect germination or seedling growth.

Fungicide sensitivity and efficacy profiling of Georgia *Plasmopara viticola* isolates causing grapevine downy mildew
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Georgia vineyards provide an ideal environment for downy mildew epidemics. The causal oomycete, *Plasmopara viticola* (Pv), can cause near 100% crop loss and vine death if left unmanaged. Management relies on multiple fungicide applications, often leading to resistance development. Although fungicide-resistant populations of Pv were identified in other viticultural regions, no study has evaluated fungicide sensitivities in Georgia. Pathogen samples were collected throughout Georgia and subjected to leaf disk bioassay with seven concentrations of three fungicide classes: (1) a quinone outside inhibitor (QoI), azoxystrobin, (2) a carboxylic acid amide (CAA), mandipropamid, and (3) a phenylamide (PA), mefenoxam. In addition, previously identified point mutations conferring QoI (G143A) and CAA (G1105S) resistance were evaluated utilizing polymerase chain reaction. Furthermore, fungicide efficacy trials with eleven treatments were conducted at three vineyards in North Georgia. Results from bioassays and mutation testing confirmed QoI resistance in 94.4% of Georgia vineyards. All samples were sensitive to both CAA and PA fungicides. Field efficacy trials showed reduced efficacy of QoI fungicides, with disease levels statistically equivalent to those of untreated vines. Based on the frequency of QoI resistance and field trial failures observed where QoIs were applied, QoIs are no longer effective for downy mildew disease management in most Georgia vineyards.

Evaluation of at-plant treatments for control of Rhizoctonia diseases of potato in Wisconsin
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Field trials were conducted in 2017 and 2018 to evaluate seed- and in-furrow-applied fungicides for the control of Rhizoctonia seedling damping-off and tuber black scurf of potato at the University of Wisconsin Hancock Agricultural Research Station, WI. Cut and suberized 'Russet Burbank' seed pieces were planted in early May in each year. Plots were harvested, graded, and twenty tubers were randomly selected from each plot and visually evaluated for black scurf symptoms. There was high variability in results between the 2 years. Disease incidence in 2017 was less than that of 2018. In 2017, disease incidence for all treatments was not significantly different from the control. The seed-applied fungicides resulted in decreased emergence when compared to the non-treated control. In 2018, all seed-applied treatments resulted in significantly lower disease incidence on tubers when compared to the non-treated control. For the in-furrow-applied treatments, only Quadris 2.08SC, Vertisan EC1.67, Elatus 45WG, Priaxor 4.17SC and Regalia 5SC resulted in significantly lower disease incidence on tubers when compared to the non-treated control. Disease incidence with the biofungicide (Regalia 5SC) was not significantly different from that of the conventional fungicides. Despite the variability between years, results indicated that the seed-applied fungicides, Quadris 2.08SC, Vertisan EC1.67, Elatus 45WG, Priaxor 4.17SC and Regalia 5SC were effective against Rhizoctonia diseases of potato.

Developing cost-effective, sustainable fungicide programs for managing downy mildew on cucumber in Florida
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In Florida, managing cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is a weekly ordeal. Developing effective fungicide rotations is crucial for successful CDM management while minimizing the threat of fungicide resistance. Based on prior

studies, programs were developed using Ranman, Presidio, Previcur, Zampro, Omega, Orondis, Elumin, Zing, and Curzate in weekly rotations with Bravo Weatherstik, compared to an untreated control and a weekly application of Bravo alone for CDM management and yield. The objective was to compare fungicide program cost relative to CDM management and yield. For both trials, various fungicide programs that alternated either Orondis, Omega, Ranman, Zing!, or Zampro with Bravo Weatherstik, provided significantly lower levels of CDM, compared to a weekly application of Bravo Weatherstik. Many of these fungicide programs differed drastically in their overall program cost and will provide growers with potential savings for CDM management. Results and conclusions will be used to improve spray recommendations for the management of CDM in Florida.

Impact of metalaxyl, ethaboxam, and mefenoxam on in-vitro growth rate of *Pythium* isolates from Pennsylvania
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Pythium species are among the most important soil borne pathogens that can limit the yields of many economically important crops. Fungicide seed treatments are widely used to control these pathogens. Selection of appropriate fungicides with proper concentration is pivotal for efficient control. The goal of this study was to determine the efficacy of selected fungicide active ingredients on in-vitro growth of *Pythium* isolates obtained from Pennsylvania. One hundred and fifty-three putative *Pythium* isolates were recovered from soil samples collected from 7 counties using selective medium P5ARP. Each isolate was grown in PDA amended with 0, 10, 100, and 1000 ppm concentrations of Metalaxyl, Ethaboxam and Mefanoxam. Colony diameter was measured at different time points to compute the colony growth rate. Analysis of variance showed significant isolate × concentration interaction for all tested fungicides. Among tested isolates, 70.6%, 88.2% and 64.7% were insensitive (non-significant growth rate difference compared to counterpart control = 0 ppm) to Metalaxyl, Ethaboxam and Mefanoxam respectively. 13.1%, 4.6% and 9.8% of the isolates were respectively sensitive to Metalaxyl, Ethaboxam and Mefanoxam only at 1000 ppm. Findings showed that the majority of the tested isolates were unaffected by the different fungicide active ingredients even at higher concentrations and that they cannot be used as an efficient means (i.e. seed treatments) of preventing yield losses.

The effect of EMS (ethyl methanesulfonate) induced mutations on altering virulence of the spinach downy mildew pathogen, *Peronospora effusa*
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Downy mildew of spinach, caused by *Peronospora effusa*, is a major limitation for spinach production in the U.S. There are 17 reported races of *P. effusa* and many new races have emerged in recent years. Examination of the mechanism of evolution of new races that overcome deployed resistance is critical to continue using disease resistance as an effective management strategy. The objective of this study was to develop a mutagenesis protocol with EMS for *P. effusa* and select isolates with an altered virulence phenotype compared to the parental wildtype isolate. Sporangia of isolate UA0510C (race Pfs 13) were exposed to various concentrations of EMS and then used to inoculate a near isogenic line (NIL1) that is resistant to race 13. Untreated sporangia served as a control. None of the 1200 NIL1 plants evaluated showed infection when inoculated with untreated sporangia. However, three infected NIL1 plants were observed when the EMS treated sporangia were used for inoculation. The symptoms were observed on NIL1 plants 23–25 days after inoculation. The three putative mutants recovered had an altered virulence pattern compared to the parental isolate when inoculated onto 20 differential spinach lines. The mutants also were different from each other based on the disease phenotyping. Sequence variation between the wild-type isolate and the mutants with altered virulence phenotypes are being examined.

Effect of Apron Maxx seed treatment on soybean seedling diseases, seedling vigor, and yields in Pennsylvania
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Fungicide seed treatments have extensively been used to control soil borne diseases in the United States. However, reports on fungicide seed treatment-associated positive yield responses are variable in crops such as soybean. The objective of this study was to investigate the impact of Apron Maxx (Mefenoxam + Fludioxonil) on seedling diseases, seedling vigor, and yields of soybean grown in Pennsylvania. On-farm field and small plot trials were conducted in seven counties (Bradford, Armstrong, Lancaster, McKean, Centre, Somerset, and Tioga). At each location, plots were arranged in randomized complete block design. At R1 growth stage, 15 seedlings from each plot (Apron or control) were carefully uprooted to quantify the incidence of root rots. Seedling height (SH), tap root length (TRL), root/shoot weight (RW/SW: dry basis) were measured as seedling vigor indicators. Test weight (lbs/bu) and yield (bu/ac) were measured at harvest. Surprisingly, root rots were absent in both Apron and control plots at all locations. Non-significant differences were observed between control and Apron for SH, TRL, RW, or SW at all locations. Apron did not significantly increase test weight or yield compared to control at all locations. Overall, findings of this study showed that Apron is unable to positively impact soybean seedling vigor and yields. Findings would help Pennsylvania soybean farmers to make rational decision making on the use of fungicide seed treatments to maximize profits.

Strategies for reducing seed rot and seedling damping-off of alfalfa (*Medicago sativa*)
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Alfalfa seeds are often planted when conditions are ideal for infection by seed rot and damping-off (SRDO) pathogens. Treatment of seed with ApronXL (mefanoxam) does not control all SRDO pathogens and does not protect older plants from root rot caused by these pathogens. The EC_{50}values of eight commercial fungicides registered for use on soybean seeds was determined for SRDO pathogens *Pythium sylvaticum*, *P. ultimum*var. *ultimum*, *P. paroecandrum*, *Aphanomyces euteiches*, *Phytophthora medicaginis*, *Fusarium oxysporum*, *F. verticillioides*, and *F. incarnatum-equiseti* using agar plate-based assays. Evergol Energy (metalaxyl, penflufen and prothioconazole) had the widest activity against the SRDO pathogens. Fungicides with metalaxyl and ipconazole had low EC_{50} values for all pathogens except *A. euteiches*. Ethaboxam had low EC_{50}values for all pathogens except *Fusarium* species. The strobilurin fungicides had moderate to high EC_{50} values for all pathogens. Most diseases of alfalfa are managed using resistant cultivars. Selection for resistance to three *Pythium* strains was done using an agar plate assay. Seedlings with moderate levels of resistance were rescued from the plates and adult plants used for intercrossing. One or two cycles of selection resulted in >50% resistant plants in the three germplasm sources used. Fungicide seed treatments in combination with resistance to pathogens would provide effective disease control for seedlings and adult plants.

Sensitivity of *Sclerotinia sclerotiorum* collected from the Mid-Atlantic region of the United States to six fungicide active ingredients
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Sclerotinia sclerotiorum causes white mold that can lead to high yield loss on lima bean in the Mid-Atlantic region. Control of the disease largely relies on use of fungicides. However, no studies have examined the regional population of *S. sclerotiorum* for sensitivity to labeled active ingredients (a.i.'s). *In-vitro* bioassays were conducted to determine the sensitivity of 40 *S. sclerotiorum* isolates to the a.i.'s boscalid, fludioxonil, cyprodinil, thiophanate-methyl, prothioconazole, and fluazinam. Each fungicide was diluted in dimethyl sulfoxide (DMSO) and amended to potato dextrose agar (PDA) at two concentrations. Three, 5-mm diameter plugs of *S. sclerotiorum* were transferred to 9-cm diameter plates amended with fungicide. The diameter of the radial growth was measured daily for 3 days. Data from each assay was analyzed separately. All fungicides significantly affected ($P < 0.0001$) the radial growth of the isolates compared to DMSO+PDA control. The most effective a.i.'s in inhibiting mycelial growth were fludioxonil, which inhibited growth by 97% and 99% at 0.5 and 1.0 µg a.i./ml, and thiophanate-methyl, which inhibited growth by 88% and 94% at 5.0 and 10.0 µg a.i./ml, respectively, when compared to the control. Thiophanate-methyl (P=0.0122), but not other fungicide a.i.'s, inhibited radial growth differently among isolates, indicating that resistance to thiophanate-methyl may exist in the Mid-Atlantic *S. sclerotiorum* population.

Syngenta® Seedcare™ Introduces a New Sudden Death Syndrome and Nematode Solution for US Soybean
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Sudden Death Syndrome (SDS) is among the top soybean yield reducing diseases in the United States. First discovered in Arkansas in 1971, SDS has recently spread to become a much greater disease problem beyond the Mid-South region. Encouraged by cooler, wetter conditions early, *Fusarium virguliforme*, the pathogen that causes SDS, infects young developing root systems. Plant parasitic nematodes, especially Soybean Cyst Nematode, exacerbate SDS infection, symptomology and yield loss. Juvenile nematodes enter developing roots damaging them and leaving them more susceptible to *Fusarium virguliforme* colonization as well as plant stress caused by the nematode and SDS. The Syngenta Crop Protection Seedcare business expects a new seed-applied molecule, Adepidyn®, to be registered by the US EPA in Q3 2019. Adepidyn is effective against SDS by protecting against *Fusarium virguliforme* and providing soybean roots protection against important plant parasitic nematodes. In multi-year field tests, Saltro®, with the active ingredient Adepidyn, delivered a statistically significant (P = 0.1) +3 Bu/Ac yield advantage over the leading competitor under heavy SDS. Additionally, adepidyn has proven extremely safe in terms of seed and plant safety, exhibiting no negative plant response or phytotoxicity symptomology exhibited by the leading SDS solution. In the near future, Saltro will provide soybean growers a strong tool for both SDS and nematode protection.

Where does your fungicide go? Fungicide fate following various mowing and irrigation treatments
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Irrigation is typically applied immediately following fungicide applications when targeting root diseases in golf course putting greens. However, the fate of fungicides following post-application mowing and irrigation is not well documented. The objective of this study was to determine the influence of post-application mowing and irrigation timing on fungicide amounts in turfgrass clippings and the soil profile. Single fungicide applications of pyraclostrobin, triadimefon, and penthiopyrad were applied and 0.64 cm of post-application irrigation was applied either immediately or 6 hours following fungicide application. Turfgrass clippings were collected at 0, 1, or 3 days after treatment (DAT). Soil cores were collected 0, 1, 3, 7, and 14 DAT and dissected into remaining above ground vegetation (RAV), 0-2.54cm, 2.54-5.08cm, and 5.08-7.62cm depths. All samples were homogenized and fungicide residue was analyzed using liquid chromatography mass spectrophotometry. Fungicide recovery as percent of applied ranged from 90-93%, 92-99%, and 92-95% at 0 DAT for pyraclostrobin, triadimefon, and penthiopyrad, respectively. Only a minor amount of fungicide (0.19-2.31%) was removed with turfgrass clippings regardless of mowing and irrigation treatment. Fungicide was detected in the 5.08 to 7.62-cm depth at 14 DAT only when irrigated immediately. Less penthiopyrad was detected in the RAV and total fungicide recovery was greater through 5 DAT compared to pyraclostrobin and triadimefon. More penthiopyrad was detected in the 0-2.54cm depth at 1 DAT compared to pyraclostrobin and triadimefon.

Impact of soybean maturity on the yield response to one versus two fungicide applications targeting white mold
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White mold, caused by the fungal pathogen *Sclerotinia sclerotiorum*, can be a damaging disease of soybeans when cool, wet weather occurs during bloom, and fungicides are often employed to manage the disease. White mold primarily infects soybeans through senesced blossoms, and the period of maximum susceptibility to white mold is extended in soybean varieties with longer maturity, which have a longer bloom period. To test whether a second fungicide application is more likely to be needed in longer maturity varieties, 0, 1, and 2 applications of 0.39 kg ha^{-1} Endura WG (boscalid, 70% by weight; BASF Corp.) were evaluated across 38 varieties from 0.05 to 1.0 maturity in Carrington, ND and 25 varieties from 0.1 to 1.3 maturity in Oakes, ND in 2018. Experimental design was a randomized complete block with a split-plot arrangement and fungicide as the sub-factor. Fungicides were applied at late R1 to full R2 growth stage and 10 to 12 days later. In plots receiving no fungicide, white mold severity index averaged 24% in Carrington and 10% in Oakes. Fungicides reduced white mold by an average of 17% with a single application and 56% with two sequential applications, and the yield increase from two fungicide applications (versus one application) was 105, 250, 402, and 516 kg ha^{-1} for varieties of 0.05-0.09, 0.1-0.3, 0.4-0.6, and 0.7-1.0 maturity in Carrington and 45, 164, 220 and 304 kg ha^{-1} for varieties of 0.1-0.3, 0.4-0.6, 0.7-0.9 and 1.1-1.3 maturity in Oakes. The results suggest the yield gain conferred by a second fungicide application may increase with soybean maturity.

In vitro evaluation of fungicides for the management of citrus brown wood rot in Arizona
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Brown wood rot is a chronic fungal disease of citrus occurring mostly on mature trees in most citrus-growing areas of the world. In Arizona, Brown wood rot has caused extensive destruction in mature lemon plantings in Yuma County. A newly described species, *Fomitopsis meliae*, was the major causative agents among several fungi implicated in brown wood rot. Isolates of *F. meliae* were recovered from the infected wood samples and *in vitro* studies were conducted to evaluate efficacy of various fungicides against these fungal isolates and develop baseline sensitivities to these fungicides. Agar media were amended with fungicide compound at various concentrations. A total of 25 fungicides with various modes of actions were evaluated with 12 isolates. EC_{50} and EC_{90} values were calculated using drc package, R Project. There were ten fungicides showing excellent activities with EC_{50} values less than 0.5 ppm. Any fungicide containing propiconazole, difenoconazole, benzovindiflupyr, fludioxonil, azoxystrobin, and trifloxystrobin were highly effective and showed complete inhibition of all the isolates evaluated at concentrations as low as 5 ppm. Other fungicides showed variable responses among the isolates and were not as effective as the ones mentioned above.

Control of blue mold of apples by Academy application via thermal fogging
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Academy™ fogging formulation is a new Syngenta fungicide for the control of storage rots of pome fruits. A mixture of fludioxonil and difenoconazole, (FRAC code 12 and 3, respectively), applied via thermal fogging, provides outstanding control against storage diseases such as gray mold, bull's eye rot and blue mold. The causal agent of blue mold is *Penicillium expansum*, which is the most common pathogen of apples in storage. This project aimed to evaluate crop safety of Academy on apples, assess the residue levels of its active ingredients, and to confirm its efficacy against *Penicillium expansum*. Apples were placed in a storage shipping container within storage bins arranged in an RCDB experimental design with three replicates. The treatments were three different rates of Academy compared to difenoconazole solo and an untreated inoculated check. Treated fruits were left sealed for 8–24 hours. After treatment, a subsample of fruits underwent residue testing while the rest of the fruits were kept for nine days at 23°C and 100% humidity after inoculation. Disease ratings took place at six, seven and nine days after treatment. Results showed that Academy is safe on apples, and it can reduce the severity of blue mold. Academy is an additional option to support a growing trend towards thermal fogging in the pome market. Academy applied via thermal fogging offers the advantage of excellent control of postharvest decay, plus the added benefits of a reduced amount of fungicide needed, and virtually no leftover disposable waste to manage.

Zinc nanoparticles offer systemic efficacy against '*Candidatus* Liberibacter asiaticus' improving yield
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Huanglongbing (HLB), caused by the phloem-limited bacterium '*Candidatus* Liberibacter asiaticus' (CLas), is the yield limiting factor in many citrus producing regions. Because of the difficulty to reach phloem tissue with chemical treatments, a 2-3 nm zinc oxide-based nanoparticle, Zinkicide, was designed for systemic activity with traditional foliar spray or soil drench application. A canker bioassay demonstrated systemic efficacy. Greenhouse trials have confirmed significant reduction in CLas populations by an average 2.54 log in 20 weeks when applied at a biweekly schedule to maintain an effective dose in the phloem. Multiple field trials on grapefruit and sweet orange were carried out for multiple years on trees of varied age and size. Surprisingly, when the nanoparticle synthesis was reformulated to tolerate impurities in agricultural grade starting materials, nanoparticle size became smaller and field efficacy improved leading to yield increases up to 73%. Young grapefruit trees have responded better than mature sweet orange trees. Dilution effects, preventing effective doses existing systemically, are the likely cause of this apparent tree size dependent efficacy. Hurricane Irma provided a unique opportunity to observe that Zinkicide reduced leaf loosening, leading to much less leaf drop damage from hurricane force winds in young non-bearing trees. Zinkicide nanoparticles show promise as a management tool for young plantings to reach profitability.

Using essential oils as an alternative to chemical fungicides in the control of Early Blight disease of tomatoes
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Fungal diseases account for more than 80% of total diseases that affect crops annually. Early blight, one of the most important diseases of tomato, is caused by the fungal pathogen *Alternaria solani*. The disease is hard to prevent and leads to significant yield losses. Chemical fungicides remain the only way to control it. Nonetheless, their excessive use has led to the appearance of more aggressive strains of *A. solani* in addition to various environmental and health issues. There is therefore an urgent need to find safe and durable alternatives to control early blight. Essential oils are plant compounds that have strong antimicrobial properties. In this project, we tested the ability of peppermint oil, thyme oil, Eucalyptus oil, cardamom oil, coriander oil and clove oil to inhibit the growth of *A. solani in vitro* and control early blight disease in tomato plants. The data collected showed that all six essential oils had strong antifungal activity against *A. solani*. Since thyme oil and peppermint oil displayed the strongest antifungal activity, we sprayed either oil on six weeks tomato plants before inoculating them with spores of *A. solani*. We then assessed the severity of early blight disease in treated and control plants. The results showed that the plants treated with either peppermint oil or thyme oils were resistant to *A. solani* and did not develop early blight disease symptoms. These data indicate that these two oils can be used as an alternative to chemical fungicides in controlling early blight disease.

Efficacy of fungicides containing mefentrifluconazole to manage Frogeye leaf spot of soybean
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The use of foliar fungicides is critical in the successful management of *Cercospora sojina*, the causal agent of Frogeye leaf spot (FLS) in soybean. Most cultivars do not have resistance to this pathogen. In 2012, strobilurin resistant isolates of this pathogen were identified in Illinois and other states underscoring the need of fungicides with multiple modes of action. Mefentrifluconazole (BASF Corporation) is a newly developed DMI fungicide with activity against *C. sojina* and other pathogens of corn and soybean. The focus of this research was to evaluate the efficacy of mefentrifluconazole and other fungicides to manage FLS and protect soybean yield. Field trials were established in 2017 and 2018 to evaluate strobilurins and mixes containing strobilurin, triazole and succinate dehydrogenase inhibitor (SDHI) fungicides. The research sites were naturally infested with strobilurin resistant isolates. Fungicide treatments were applied at the soybean growth stages R3, R5 or R3 followed by R5. Assessments were foliar disease severity, stem and pod quality, seed quality and soybean yield. The treatment containing azoxystrobin and difenoconazole (Quadris Top SBX) and the treatment containing mefentrifluconazole, pyraclostrobin and fluxapyroxad (Revytek) significantly reduced disease severity up to 70 days after application. Soybean yield in Revytek treatments applied at R3, R5 and R3 & R5 were higher than all other treatments and were 10.6%, 11.8% and 16.8% higher than the yield in non-treated plots. This research will provide growers with information to better manage FLS.

Increasing Efficiency and Efficacy of the National Cottonseed Treatment Trial
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The goal of the National Cottonseed Treatment program is to analyze seed treatment efficacy and stand persistence, in relation to pathogens present in the soil of cotton plots across the United States Cotton Belt. For the past 23 years, this goal has been accomplished by plating 100 seedlings, from 10 to 12 trial locations, on selective medias and Potato Dextrose Agar, for morphological identification of *Thielaviopsis basicola*, *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani*. As well as soil dilution platings for *Thielaviopsis basicola* and *Pythium* spp. colony forming unit evaluation, soil baiting for *Rhizoctonia solani*, and the consideration of node counts, disease severity ratings, and seed germination testing. However, when the research team at the West Tennessee Research and Education Center Plant Health Diagnostics Lab took over this trial for the 2018 growing season, it was apparent that through executing the protocol and analyzing the data collected, that changes were needed in order to enhance the sampling process, and to make the data generated from this program more reliable. These changes included the removal of soil dilution platings and the discontinuation of *Thielaviopsis basicola* selective media and Potato Dextrose Agar, the introduction of a selective media for *Fusarium* spp., and use of immunostrips for genus confirmation. This presentation will discuss the data collected from the 2018 growing season, the changes made to the protocol, and preliminary data from the 2019 growing season.

Sensitivity of *Colletotrichum acutatum*, causal agent of anthracnose fruit rot of strawberry to fungicides alternative to quinone-outside inhibitors
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Colletotrichum nymphaeae and *C. fioriniae* belong to the *C. acutatum* species complex and cause anthracnose fruit rot (AFR) of strawberry. Quinone-outside inhibitor fungicides were frequently used for AFR management, however resistant populations were recently reported in Florida strawberry fields. Therefore, alternative modes of action, such as the succinate dehydrogenase inhibitors (SDHIs) and uncouplers of oxidative phosphorylation (OxPhos), were investigated to provide tools for disease management. Isolates collected from diverse strawberry fields in the U.S. were challenged with discriminatory doses of the SDHIs fluopyram, fluxapyroxad, isofetamid, penthiopyrad, and benzovindiflupyr, and the OxPhos fluazinam. Mycelial growth inhibition for each fungicide was evaluated using the spiral gradient dilution (SGD) method. Mean EC_{50} values for *C. nymphaeae* (n=90) were 0.14 µg/ml, 34.3 µg/ml and 0.20 µg/ml for benzovindiflupyr, penthiopyrad, and fluazinam, respectively. Mean EC_{50} values for *C. fioriniae* (n=12) were 0.86 µg/ml, 3.42 µg/ml and 0.06 µg/ml for benzovindiflupyr, penthiopyrad and fluazinam, respectively. Disease incidence was reduced on detached fruit inoculated and treated with these fungicides, as well as in field trials, indicating that the products are effective in controlling AFR. The SDHI benzovindiflupyr may be used as an alternative for disease management in strawberry fields while OxPhos fluazinam may be used in nurseries, upon registration.

Evaluation of antimicrobial effects and plant uptake of novel antimicrobial formulation MS3T to control surface and systemic pathogens in citrus

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MS3T (multifunctional surface/sub-surface/systemic therapeutic) is a novel plant nutrient-based antimicrobial formulation developed for controlling citrus bacterial pathogens '*Candidatus* Liberibacter asiaticus' (*C*Las) and *Xanthomonas citri* (Xc). MS3T components quaternary ammonia (DDAC) and Zn chelate (TSOL) are designed to control plant surface pathogens and systemic pathogens respectively. This study investigated the antimicrobial effects of MS3T and its components in vitro on *C*Las and Xc surrogate *X. alfalfae* (Xa) and *E. coli* (Ec). Ec and Xa were grown in LB and NB with serial dilutions of MS3T, $Zn(NO_3)_2$ and DDAC to determine Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Systemic movement of TSOL in citrus plants after foliar application was evaluated using atomic absorption spectroscopy (AAS). Phytotoxicity of MS3T was evaluated in tomato seedlings as a model system since citrus seedlings are less susceptible to phytotoxicity. The MIC of $Zn(NO_3)_2$ and DDAC against Xa is 16ppm and 0.5ppm whereas MBC is 32ppm and 1ppm respectively. The MIC of MS3T for Xa is 1.5ppm and 6ppm DDAC and Zn. The MIC of $Zn(NO_3)_2$ and DDAC for Ec is 128ppm and 4ppm whereas MBC is 512ppm and 4ppm respectively. The MIC of MS3T for Ec is 6ppm and 24ppm DDAC and Zn. No phytotoxicity was observed in tomato seedlings after MS3T foliar application. AAS analysis revealed that TSOL is uptaken by citrus leaves and transported to roots through phloem.

Evaluation of Isotianil, Isotianil + Fosetyl-Al and *Bacillus subtilis* QST713 on Coffee Leaf Rust and Brown Eye Spot in Coffee nursery plants

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Resistance induction is an alternative to explore within the integrated management of Coffee Leaf Rust (CLR, *Hemileia vastatrix*) and Brown Eye Spot (BES, *Cercospora coffeicola*) diseases, which cause severe defoliation at nursery stage, and yield losses at productive stage. This work evaluated Isotianil, Isotianil + Fosetyl–Al, and *Bacillus subtilis* QST713 (Serenade®) from Bayer S.A., to control CLR and BES in *Coffea arabica* cv. Caturra 6 months old plants at nursery stage, under different environments and pathogen inoculum pressure. Initially, products were applied in three doses every 2, 4 and 6 weeks and compared against a reference inducer Acibenzolar-S-methyl (ASM), a fungicide Cyproconazole, checks applied with water, and a non-applied control. *In vitro* tests for *H. vastatrix* urediniospores showed that Isotianil + Fosetyl–Al and Serenade® totally inhibited germination, Isotianil allowed 48.3% germination and ASM 9.6%, in contrast to 83% in the water check control. No effect on diseases incidence, but severity reduction were obtained with Isotianil + Fosetyl–Al for BES, and Serenade® and Isotianil for CLR, when applied every two weeks. Following experiments showed increases on foliar area and biomass as fresh and dry weight, and lower defoliation; however, shaded environments conditioned disease reduction and plant responses. Time course assays and disease distribution indicated that resistance induction was mainly local within a short time response (one week), limiting application of these inducers from the coffee farmer and commercial management perspectives.

Evaluation of host plant defense inducers for the management of boxwood blight in North Carolina

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Boxwood blight, a devastating disease caused by *Calonectria pseudonaviculata* (*Cps*) has rapidly emerged as one of the most serious problems for the boxwood industry since it was reported in the United States in 2011. Concerns of frequent fungicide applications in landscapes for disease management have led us to find other management tools. The aim of this research was to evaluate the effectiveness of host plant defense inducers; Actigard® (acibenzolar-S-methyl), Lifegard® (*Bacillus mycoides* isolate J), and ProPhyt® (Potassium phosphite) under different application frequencies and methods. Trials were conducted at two locations: A container pad under artificial inoculum conditions and an established landscape planting with a history of boxwood blight. At each location, two cultivars that vary in their susceptibility to boxwood blight; *Buxus sinica* 'Justin Brouwer' (JB) and *B. sempervirens x B. microphylla* 'Green Velvet' (GV) were evaluated. At both locations and for both cultivars, drench applications of Actigard® provided significantly greater control against boxwood blight, compared to the untreated program ($p < 0.05$). Current and future research is focusing on the expression of genes that encode pathogenesis-related (PR) proteins following applications of host plant defense inducers and integration of these tools into conventional fungicide programs for the management of boxwood blight.

Effectiveness of fungicide programs for control of potato early blight in Central Wisconsin, 2018

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Early blight caused by the fungus *Alternaria solani* is a perennial disease of potato (*Solanum tuberosum*) that affects potato production in Wisconsin. The disease can cause premature defoliation of susceptible cultivars, ultimately leading to losses in tuber quality and yield. Conventional potato growers in Wisconsin rely on routine fungicide applications for effective early blight control. There are many fungicides currently registered for managing early blight of potato in Wisconsin, with additional fungicides in various stages of development. Thus, we conducted a field trial with a randomized complete block design and 4 replications on 'Russet Burbank', an industry standard cultivar, at the UW-Hancock Agricultural Research Station to evaluate the effectiveness of 31 fungicide programs, including at-planting and weekly foliar treatments, for disease control. Early blight severity was assessed 6 times throughout the growing season using the Horsfall-Barratt rating scale. All data were analyzed using ANOVA (α=0.05) and Fisher's LSD at α=0.05. Compared to corresponding values of the non-treated control, 30 of the 31 treatments resulted in significantly less disease severity (measured by Relative Area Under the Disease Progress Curve values) and 6 resulted in significantly greater smaller-sized ('B) tubers.

Treatments including in-furrow applications of the fungicide fluopyram were shown to be the most effective in managing early blight. These results are useful to growers in designing disease control programs with consideration to fungicide resistance management.

Discovery of small molecule growth inhibitors to control bacterial leaf spot (BLS) disease of tomato
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Bacterial leaf spot (BLS) is a disease of tomatoes caused by *Xanthomonas gardneri* (*Xg*), *X. perforans* (*Xp*), *X. vesicatoria* (*Xv*) and *X. euvesicatoria* (*Xev*). BLS leads to tremendous losses to the tomato industry and is difficult to manage due to epiphytic survival of *Xanthomonas,* emergence of copper and streptomycin resistant strains, lack of resistant tomato cultivars and effective bactericides against *Xanthomonas*. Objective of this study was to identify novel small molecule (SM) growth inhibitors against *Xanthomonas* spp. Total 17 out of 4,182 SMs completely inhibited the growth of *Xg*, *Xp*, *Xv* and *Xev* at 100 micromolar. Of these, 8 SMs were effective against other BLS isolates (n=119), including copper and streptomycin resistant isolates. These SMs had minimal impact on plant pathogenic (n=22) and beneficial bacteria (n=12) and displayed no toxicity on tomato plant tissues. Electron micrography suggested that SMs treatment disrupted the cell membrane of *Xanthomonas*. Further, these SMs reduced *Xg* population in infested seeds (up to 1.5 log) and infected seedlings (up to 3.5 log CFU/plant; P value less than 0.05) when used as a curative treatment at 200 and 500 micromolar of SMs, respectively. Similar trend was observed after real-time bioluminescent imaging of plants infected with engineered bioluminescent *Xg* (BL-*Xg*) after treatment with SMs (P value less than 0.05). In summary, we have identified 8 novel SMs showing antimicrobial activity against BLS of tomato and our ongoing studies are focused on testing against other *Xanthomonas* spp., and in naturally infected seeds.

Effects of two-treatment fungicide programs on grain yield and quality of Fusarium head blight-affected Wheat
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The combination of a single anthesis application of a triazole fungicide (prothioconazole [PROT], metconazole [METC], tebuconazole [TEBU], or PROT+TEBU) with a moderately resistant (MR) cultivar has been shown to be more efficacious than fungicide or resistance alone for managing Fusarium head blight (FHB) and deoxynivalenol (DON). However, this approach is often not effective enough to prevent grain yield and quality losses when conditions favor FHB and DON. Data were collected from 54 field experiments across 16 states to evaluate the benefits of two-treatment programs (T2; METC or PROT at anthesis followed by TEBU 4–6 days later and PROT+TEBU at anthesis followed by METC 4–6 days after) for FHB management in cultivars with different levels of resistance. Anthesis applications of PROT+TEBU (T1) and non-treated checks were included as references. For each resistance x fungicide program combination, percent reduction of Fusarium damaged kernels (FDK) and DON, and increases in yield (YLD) and test weight (TW) were estimated relative to the susceptible non-treated check. Price discounts based on grain quality were also estimated for each management combination. Combining T2 with a moderately susceptible (MS) or MR cultivar reduced FDK and DON by up to 80% and increased YLD and TW by up to 1.6 MT/ha and 59 kg/m^3, respectively. Depending on the level of FHB, T2 was more effective than T1 on MS compared to MR cultivars. However, total price discounts were consistently lower when T2 was combined with an MR cultivar ($4-9/MT) than with an MS ($5-14/MT) or S ($7-40/MT) cultivar.

Sensitivity of *Fusarium graminearum* in Michigan to fungicide pydiflumetofen, a component of Miravis formulations
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In North America, *Fusarium* species including *F. graminearum* are a major concern due to mycotoxin contamination of wheat grain. Historically triazole products have been the sole option to manage head scab in wheat. Recently a new SDHI active ingredient, pydiflumetofen (Adepidyn™, Syngenta), received registration in the U.S. and will be marketed in the 2019 growing season as Miravis Ace for head scab control. We investigated the *in-vitro* sensitivity and determined baseline sensitivity of Michigan populations of *Fusarium graminearum* to the new chemistry pydiflumetofen. Isolates were collected as part of a survey of Michigan wheat and corn to determine the *Fusarium* species present and fungicide sensitivity of the population. Sensitivity was determined by mycelial growth assays for 97 isolates, and spore germination assays conducted on a subset of 22 isolates. Effective concentration that reduced pathogen by 50% (EC_{50}) and 90% (EC_{90}) were calculated. EC_{50} values from spore germination (mean 0.30 µg/ml) were significantly greater than EC_{50} values from mycelial growth (mean 0.069 µg/ml), however EC_{90} values for spore germination were significantly lower than mycelial growth values. We found isolates less sensitive to triazoles (EC_{50} from 1-4 µg/ml) were still quite sensitive to pydiflumetofen

(<0.1 µg/ml). These studies provide us with phenotypic data that can be used as a baseline for future monitoring and indicate *F. graminearum* populations in Michigan are very sensitive to the new chemistry pydiflumetofen *in-vitro*.

Small molecule screening against the secreted "*Ca.* L. asiaticus" peroxiredoxin, LasBCP, reveals promising chemicals to prevent infection of citrus

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Huanglongbing (HLB) or citrus greening is currently the most devastating bacterial disease of citrus, worldwide. The first host defense response to Ca. L. asiaticus (Las) infection is the oxidative (H_2O_2) burst. LasBCP (CLIBASIA_RS00445) is non-classically secreted and the gene is conserved in all pathogenic Liberibacters, highly expressed in plant but not expressed in psyllid hosts. When expressed in the culturable *L. crescens,* LasBCP confers 214-fold more resistance to *tert*-butyl hydroperoxide (*t*BOOH) than wild type. When transiently expressed in tobacco, LasBCP suppressed H_2O_2-mediated transcriptional activation of *RbohB* and protected against *t*BOOH-induced peroxidative degradation of lipid membranes, preventing the hypersensitive cell death response. Therefore, LasBCP constitutes a secreted effector protein that likely allows Liberibacter colonization of plant hosts. Denaturing this protein during the initial phases of plant host infection would hypothetically prevent or delay colonization of plants. Two Prestwick combinatorial small molecule libraries representing 1,600 different small molecules were screened for efficacy of thermal denaturation via differential scanning fluorimetry and independently for enzyme inactivation using the Quantitiative Peroxide Assay. Results from both assays revealed 28 chemicals that denatured peroxiredoxin. Based on commercial potential (including cost and potential environmental impact), three chemicals were selected for further evaluation and field trials as preventative treatments for HLB.

Effect of foliar spray with Eucalyptus extracts on disease incidence and yield loss to spot blotch in Wheat Bhakkar 2001 under green house condition

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The efficacy of foliar spray with flowering buds of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* extracts on the yield performance of wheat (Bhakkar 2001) artificially infected by *Bipolaris sorokiniana* was evaluated in green house. The foliar aerosol of 3 crude extract at 1, 5 and 10% concentrations were applied under 3 schedules. Untreated and unsprayed controls were also managed. The morphological study after treatments applications reveals that the plants height and leaves length/width decline as the severity of disease rises. The negative relationships were found between disease severity, number of grains/spike and 1000 grains weight. Maximum decline in number of grains/spike (16-20) was recorded in unsprayed control. Treatment of *E. torelliana* significantly increased plant population, grain yield and grain weight, but did not found effective in minimizing black point incidence. Alternatively, foliar sprays with *E. camaldulensis* ethanol ingredients, under all the spray schedules except spray III schedule significantly minimized the disease severity. Among the different spray schedules, spray I schedule with a 10 days gawk spraying at 15, 25, 35, 45 and 55 DAI appeared to be most effective, that was statistically comparable to spray II plan with a 15 days gawk spraying at 15,30,45 and 60 DAI in reducing black point incidence and increasing yield quantity. From economical point of view spray II schedule that including four squirt applications was found best accompanied by Spray III schedule that made up of only three spray applications applied at 15, 35 and 55 DAI.

Preliminary screening of resistance inducers to control Coffee Leaf Rust (*Hemileia vastatrix* Berk. & Br.) at nursery stage in Colombia

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Coffee Leaf Rust (CLR) is still the most important coffee disease worldwide, causing severe defoliation, yield and quality losses up to 50% in susceptible varieties. Considering that, 19% out of 887,000 hectares planted in Colombia are CLR susceptible, additional alternatives to chemical fungicides for integrated management are required. Thus, several products considered as resistance inducers were tested in 6 months old *Coffea arabica* cv. Caturra plants at nursery stage, which were based on Acibenzolar-S-methyl, Harpin protein, Salicylic acid, Potassium phosphite, Copper peptidate, Glucosamine, Phosphated sugars, Potassium silicate, citrus essential oils, and two naturals. They were applied once at 15, 30 and 45 days before CLR inoculation in three doses, within a factorial and randomized design. CLR severity was quantified 30 days after inoculation, and ANOVA analysis and Tukey tests (α=0.05) were conducted. All products and doses significantly affected CLR urediniospores germination at *in vitro* assays, the two naturals and Glucosamine caused the lowest inhibition, and Salycilic acid, Harpin protein, Acibenzolar-S-methyl and Potassium phosphite inhibited between 100% and 84.3% germination. All products had significant impacts on CLR severity averages, ranging from 2.97% for Potassium phosphite to 1.08% for Acibenzolar-S-methyl, in comparison to 8.45% for the water negative control. Most resistance inducers reduced CLR severity when applied at recommended or high doses at short time intervals, and varied in local and systemic responses depending on the product´s nature.

Baseline sensitivity of *Cercospora sojina* isolates to adepidyn causing frog eye leaf spot on soybeans

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Frog eye leaf spot caused by *Cercospora sojina* is a soybean disease that can be controlled with foliar fungicides. Adepidyn® is the active ingredient of the fungicide Miravis® and is the first member of a new chemical group among the succinate dehydrogenase inhibitor fungicides (SDHI, FRAC Group 7), the N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides. The common name for adepidyn is pydiflumetofen. Adepidyn has high binding properties to the complex II enzymes that provide a strong fungicidal potency combined

with the control of a wide range of plant pathogen species. The sensitivity of 35 isolates of *C. sojina* to the fungicide adepidyn was determined using a mycelial inhibition growth assay. Isolates were obtained from symptomatic lesions from several soybean production areas in the USA. Sensitivity tests were conducted *in vitro* using V8 agar media amended with adepidyn. Mycelial growth inhibition was quantified using the following adepidyn concentrations: 0, 0.001, 0.01, 0.1, 1, 10 and 100 mg.ai/L. Baseline sensitivity distributions (ED50 values) of *C. sojina* isolates ranged from 0.0158 to 0.7405 mg.ai/L with a geometric mean of 0.1763 mg.ai/L and a range of 47X. Baseline sensitivity will be used in resistant monitoring studies focusing on early detection of resistant isolates.

Baseline sensitivity distribution of *Bremia lactucae* isolates to the fungicide oxathiapiprolin

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The sensitivity of 26 isolates of *Bremia lactucae* to the fungicide oxathiapiprolin was determined using an *in vivo* assay. Oxathiapiprolin (FRAC code 49) is the active ingredient in Orondis® fungicide, which provides exceptional control of diseases caused by downy mildews and *Phytophthora* species. It is active against mycelial growth, sporangial germination, and zoospore release and germination. Oxathiapiprolin inhibits the oxysterol binding protein, a member of the OSBP related protein family of lipid transfer proteins. Lipid-binding proteins are implicated in many cellular processes related with oxysterol, including signaling, vesicular trafficking, lipid metabolism, and non-vesicular sterol transport. The risk of resistance development is considered to be medium to high and comprehensive resistance management should be implemented when used in the field. The baseline sensitivity for *Bremia lactucae* was established using 26 isolates never exposed to oxathiapiprolin. Isolates were obtained from lettuce infected samples collected in California, Florida and Arizona. Sensitivity tests were conducted on 3–4 week-old lettuce plants sprayed with oxathiapiprolin and an untreated control. Disease severity was quantified for oxathiapiprolin at concentrations of: 0, 0.00001, 0.0001, 0.001, 0.01, 0.1 and 1 mg.ai/L. Baseline sensitivity distributions (ED50 values) of *B. lactucae* isolates ranged from 0.000115 to 0.003318 mg.ai/L with a geometric mean of 0.000758 mg.ai/L and a range of 29X. Baseline results can be used as part of a fungicide resistance monitoring program for *B. lactucae*.

The effects of reducing chlorothalonil use in fungicide spray programs for melon diseases

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Fungicides are applied to nearly 80% of U.S. melon acreage to manage the numerous foliar and fruit diseases that threaten yield. Chlorothalonil (ex.Bravo) is the most widely used fungicide but has been associated with negative effects on human and bee health. We designed alternative fungicide spray programs to examine impact of reducing use of Bravo on watermelon, muskmelon, and honeydew melon in 2016, 2017, and 2018. Bravo was replaced in the tank-mix of weekly sprays of targeted fungicides, with either a polyoxin D zinc salt (Oso) or an extract of *Reynoutria sachalinensis* (Regalia). Powdery mildew (PM-*Podosphaera xanthii*) and gummy stem blight (GSB-*Stagonosporopsis* spp.) were the most prevalent diseases to occur in the three years. Where Bravo was replaced by Oso alternated with Regalia within a program, PM severity was not significantly different ($p=0.08$, Wilcoxon Test) than where Bravo was used (2% and 5%, respectively compared with 35% severity in the untreated control in honeydew melon in 2018). Similarly, for GSB in muskmelon in 2017, the program with Oso and Regalia and the program with Bravo resulted in statistically similar severities ($p=0.20$, Wilcoxon Test) 8% and 7%, respectively, compared to the untreated control at 37%. Bravo, Regalia, and Oso were also applied as standalone treatments and all had higher disease severity than the Bravo tank mixed with targeted fungicides ($p < 0.05$, Wilcoxon Test). Therefore, replacement of Bravo is possible, without loss of efficacy, when Oso is alternated with Regalia as a tank-mix with targeted fungicides to manage PM and GSB.

Management of *Phakopsora pachyryzi* in Brazil

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Phakopsora pachyryzi is the causal agent of soybean rust (SBR). SBR is a major soybean disease in Brazil, damaging yield and increasing production cost, as a result of the four fungicide sprays. However intensive use of single site fungicides has lead to development of resistance. Due to the lack of new chemicals for SBR control, disease management programs must be improve with timing and intervals between sprays, spray rate volume, and use of multisite fungicides. To quantify each factor to improve fungicide efficacy 3 experiments were designed using a fungicide mix of Trifloxistrobin (60g p.c./ha) + Prothioconazole (70g p.c./ha), sprayed four times. Designed in a complete backs randomized. The 1st experiment started sprays at stage R1 and R2 and had 12 and 18 days of intervals to each timing. The 2nd experiment evaluated five fungicide spray rate (40, 80, 120, 160, 200 L/ha). The 3rd experiment evaluated the multisite fungicide Mancozeb (1500g p.c./ha) and Chlorothalonil (1080g p.c./ha). Disease severity and grain yield were evaluated. Data were analyzed using SASMIAgri ($p < 0.05$) and separate by Tukey. Timing and intervals showed yield decrease when spray time was delayed and had 18 day intervals. Fungicide spray rate of 160L/ha had greater yield and less disease severity. Also tank mixture with multisite fungicides increase about 5 bags/ha in yield. These results contribute to SBR program management to avoid further development of resistance.

Bacterial Spot of Peach: Chemical Sensitivity in Strains from Cankers, Leaves and Fruit

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Bacterial spot control on peaches in the Southeastern United States is caused by *Xanthomonas arboricola* pv. *pruni* (XAP) and has been a problem to manage for many years. In susceptible cultivars copper applications starting at delayed dormancy up to preharvest season

is recommended. In addition, many growers also apply oxytetracycline for bacterial spot management. But even with best efforts, in years with frequent precipitation or high humidity after bloom the disease is very hard to control. It is unclear whether resistance development in XAP contributes to a lack of disease management success. The objective of this study was to collect XAP from cankers, leaves and fruit and investigate sensitivity to bactericides copper and oxytetracycline. Strains were collected from four farms located in the major peach producing areas of South Carolina which have experienced an increase in incidence of bacterial spot over the past years. XAP isolated from cankers, leaves, and fruit were assessed for sensitivity to copper and oxytetracycline using a spiral plater gradient. The bacterial strains from the various samples taken throughout the year illustrated high variability in bactericide sensitivity. The number of strains resistant to copper varied depending on the farm and spray history. The results demonstrate how diverse the sensitivity of XAP can be within an orchard block at different times of the growing season.

Untangling the effect of late season soybean diseases on yield
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Brown spot, caused by *Septoria glycines* is a prevalent foliar disease in soybean production areas. Application of fungicides after flowering reduces the disease severity at the end of the season, yet the yield responses are not consistent among locations and years. To start determining the effects of late-season foliar diseases on soybeans, we focused our attention on *S. glycines*. Our goal was to determine the effects of different levels of *Septoria* brown spot on yield. Different levels of disease severity were obtained in the field by weekly application of chlorothalonil for three, six, and nine times after disease inoculation. Fungicide treatments had a significant effect on vertical progress and chlorotic area with no statistically significant effect on yield. However, the yield was negatively correlated with vertical progress of the disease. Vertical progress was the best linear predictor of yield. Based on such model, when the vertical progress of brown spot at R6 increased by 10%, the yield decreased by 142.13 kg/ha. A variance component analysis showed that location explained the largest variation, illustrating the significant effect of local environmental conditions on the disease. Power analyses indicated that at least 8 locations are needed to detect an effect of 403.5 kg/ha yield increase. Our results provide useful information to improve the experimental design for future experiments addressing the yield constraint by late-season soybean diseases.

Assessment of the Relationship Between Bacteria Leaf Spot Severity and Crop Yield of Fresh Market and Processing Tomato
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Tomato bacterial leaf spot (BLS) can reduce fruit quality and yield and existing management strategies are not effective. Furthermore, no economic threshold level has been established for the disease. Field trials were conducted to assess the relationship between disease severity on yield and the economic impact of BLS on the fresh market (FM) and processing tomato industries. Processing and FM tomatoes were evaluated independently. Each trial included a non-inoculated plot treated with an industry standard chemical spray program; plots inoculated with a high or low concentration of *Xanthomonas gardneri* (FM) or *X. perforans* (processing) and no chemical treatments and; a non-inoculated, non-treated control plot. For both trials, disease severity was lowest in the plots managed using a standard chemical spray program with no inoculum and highest in the plots with a high level of inoculum but no chemical treatment ($p < 0.0001$). For FM tomato, marketable yield was significantly lower in the plots that were inoculated with a high concentration of *X. gardneri* ($p < 0.0001$). No difference was observed in marketable yield among the processing tomato treatments ($p = 0.761$). Production costs per ton of FM tomato were 2.1 times higher for plots with a high level inoculum compared to plots that were chemically treated but not inoculated ($p < 0.0001$). There was no difference in production costs per ton of processing tomato ($p = 0.761$). A negative correlation ($p < 0.0001$; $r = -0.621$) was observed between marketable yield and disease severity in the FM tomato trial only.

Field sampling of kernel smut in rice and its effect on yield and quality
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Kernel smut, *Tilletia barclayana*, is a rice disease that has been present in the Sacramento Valley of California since the mid 1980s. In recent years, the disease has become prominent in warm areas of the Valley. The objective of this research was to develop a method to evaluate the disease level in the field, and quantify losses due to kernel smut infection. In 2017 and 2018, affected rice fields of three common varieties were sampled at maturity. In each field, thirty, 1-square yard quadrant samples were hand harvested. From each sample, three sub-samples of ten tillers were randomly selected and inspected for kernel smut. Each quadrant sample was threshed, and number of smutted kernels, and grain, milling and head rice yield determined. To determine the number of smutted kernels in the grain, three 25 g rice sub-samples were soaked in a 0.27 M KOH solution for 24 hours to clear the hulls and make counting easier. The proportion of infected panicles predicted the number of smutted kernels per 25 g of grain. Number of smutted kernels did not affect grain yield. Milling yield was affected by number of smutted kernels in all varieties in both years, with percentage milling yield reduction rates ranging from 0.05 to 0.25. Head rice yield was only affected by number of smutted kernels in in 2018, with average reduction rates in percentage milling yield of 0.085. The results provide a method to evaluate kernel smut in the field and show that kernel smut can significantly affect the quality of rice.

Color- and metric-based k-means clustering (CM-KMC) for quantification of rice sheath blight infection
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Accurate quantification of disease severity is an essential element in breeding for disease resistance. Currently existing approaches in measuring severities of major rice diseases, such as rice sheath blight (ShB), greatly rely on visual-based ratings which are subjective and time-consuming. In our study, we developed an algorithm which takes into account the color similarity and spatial proximity of necrotic ShB disease lesions. Using red-green-blue (RGB) images of ShB-infected rice plants as input, we segmented the RGB images utilizing our algorithm followed by image identification. Quantification of ShB disease severity in ShB-resistant and susceptible cultivars, Jasmine 85 and Lemont, respectively, verified the performance our algorithm. The outcomes of this study will be useful in providing accurate measurements of disease severity for ShB resistance screening programs and will be foundational in the development of automated platforms for ShB detection and quantification in infecting rice samples.

Influence of carbon input on anaerobic soil disinfestation efficacy for control of Fusarium wilt in strawberry
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Fusarium wilt caused by *Fusarium oxysporum* f. sp. *fragariae* (*Fof*) is a major threat to strawberry production in coastal California. The disease was historically controlled with chemical fumigants; however, the phase-out of methyl bromide has increased demand for alternative management strategies. Anaerobic soil disinfestation (ASD) is one such method that has proven effective in the management of soil-borne pathogens in certain systems. The objective of this study was to evaluate the efficacy of ASD treatments utilizing different carbon inputs in managing Fusarium wilt of strawberry. Soils artificially infested with *Fof* were amended with either rice bran (4.9 tons ha^{-1}), ground orchard grass (10 tons ha^{-1}), or ground wheat grass (10 tons ha^{-1}) in combination with ASD and compared to no-amendment and aerobic controls. Susceptible strawberry crowns (cv Albion) were planted in soils post-ASD treatment and grown for a maximum of 61 days. The addition of carbon inputs to soil without subsequent ASD treatment amplified disease progression and resulted in a higher density of *Fof* in the soil and strawberry crowns than those treated with ASD. Strawberry plants grown in soils treated with ASD exhibited a slower rate of disease progression, however no treatment provided sufficient disease control.

Impacts of anaerobic soil disinfestation carbon source on dynamics of the soil metabolome and microbiome
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Anaerobic soil disinfestation (ASD) is a pre-plant soil treatment which entails soil incorporation of a labile carbon source, saturation of soil pore spaces with water and subsequent tarping. ASD induces a shift in soil microbiome composition but the relationship between carbon input type and effects on subsequent trajectory of the microbiome and metabolome structure has not been assessed. With the appropriate carbon source, ASD has proven effective in control of specific soil-borne diseases in strawberry, such as Verticillium wilt caused by *Verticillium dahliae*. However, no such combination has consistently controlled Fusarium wilt in strawberry, caused by *Fusarium oxysporum* f. sp. *fragariae* (*Fof*). The impacts of ASD using different carbon amendments on the soil microbiome and metabolome were characterized over a time-course study conducted in pasteurized and native soils artificially infested with *Fof*. Carbon inputs tested were ground orchard grass 10 tons ha^{-1}; rice bran 4.9 tons ha^{-1}; ground wheat grass 10 tons ha^{-1} and compared to no amendment controls. Sampling was performed on days 0, 1, 3, 7, 15, and 21 during ASD and days 24, 28 and 31 post-ASD. DNA extracted from soil was used in quantification of *Fof* by RT-qPCR. To define potential mechanisms of disease suppression, the soil metabolome was characterized using LC-MS and GC-MS, the soil microbiome was described by amplicon sequencing, and resulting profiles were were analyzed relative to pathogen density.

Aerated steam by the 'Plant Sauna' eradicates powdery mildew from strawberry transplants
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The 'Plant Sauna' is a closed container with an even distribution of aerated steam at a set temperature. It was originally developed in Norway to disinfest greenhouse equipment but is now being adapted to treat strawberry (*Fragaria* × *ananassa*) planting material for diseases and pests. Aerated steam from the 'Plant Sauna' has successfully been used to eliminate or significantly reduce important diseases in transplants of strawberry. Powdery mildew (*Podosphaera aphanis*) on strawberry transplants may rapidly reach epidemic levels after planting, and such plants may carry fungicide resistant strains from nurseries. The standard steam treatment of strawberry planting material developed in Florida is a 1-hour pre-treatment at 37°C to increase heat tolerance of the plants, followed by 1 hour at ambient temperature (20–25°C), and then 4 hours at 44°C. In repeated experiments with strawberry plug plants infected with powdery mildew, the disease was completely eradicated. Two hours at 44°C was also sufficient to kill the pathogen. Following the treatments, plants were kept for 21 days under conditions conducive for powdery mildew. There was no regrowth of the pathogen on leaves infested prior to the treatments, and no disease appeared on newly developed foliage. No negative effects on yields following steam treatments have so far been observed. Experiments are continued to pinpoint doses (temperature × time) necessary to manage powdery mildew on strawberry transplants.

The effects of sanitizers on *Calonectria pseudonaviculata* and *C. henricotiae* conidia and microsclerotia viability
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The boxwood blight pathogens *Calonectria pseudonaviculata* (Cps), and *C. henricotiae* (Che), produce microsclerotia (ms) on and in leaf tissue and conidia in sticky masses that can be spread. We evaluated the effects of sanitizers on conidia, excised ms or all Cps life stages in 4-mm-d leaf disks. Leaf disks and excised ms were exposed to sanitizers or water for 3 to 180 min (disks), or 0.5 to 30 min (ms), wicked dry and placed onto ½ PDA to determine viability. Conidia were loaded onto a filter, exposed to alcohol concentrations for between 2 s to 2 min and rinsed with water. Filters were backwashed with air to transfer conidia onto water agar and the percent germination was counted after 24 h. Cps in leaf disks was killed within 10–12 min for 70% ethanol and between 2-3 h for 0.525% NaOCl. Chlorophenol did not eliminate Cps from tissue. Individual ms were killed by 70% ethanol in 4 min. In contrast, ms exposed to 0.525% NaOCl or 0.05% chlorophenol for 30 min retained viability. Exposing conidia of Cps and Che to 10%, 25%, 40% and 70% ethanol demonstrated no germination after 20 s in 25% ethanol or 5 s for 40 and 70% ethanol. Conidia of Cps and Che exposed to 70% isopropanol were also dead within 5 s. A dose-response study for ethanol and isopropanol estimated 99% mortality from exposure to 63.6% alcohol for 2 s, with no difference between the alcohols. Ethanol and isopropanol can be used to quickly and inexpensively disinfest tools and contaminated surfaces.

Effect of biofumigation with solarization in suppressing Phytophthora root and crown rot on field-grown boxwood
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Phytophthora root and crown rot, caused by *Phytophthora nicotianae*, is one of the major limitations to boxwood production. In this study, we evaluated the efficacy of biofumigants with solarization for controlling Phytophthora root and crown rot of boxwood. Ground beds were inoculated with *P. nicotianae* grown on rice grains and inoculated/non-biofumigated non-solarized, non-inoculated/non-biofumigated non-solarized beds were used as controls. Treatments were yellow mustard 'White gold', turnip 'Purple top forage', arugula 'Astro', mighty mustard® 'Pacific Gold', rape 'Dwarf essex', mustard green 'Amara', brown mustard 'Kodiak', biofumigant Dominus (170 and 340 lb/A), mustard meal (968 lb/A) and only solarization. All biofumigant incorporated as well as solarization alone beds were covered with polyethylene film for 30 days. After the 30-day period, boxwood plants were transplanted into the ground beds. At the end of the experiment, plant growth data were recorded, and roots were assessed for disease severity using a scale of 0–100% roots affected. All treatments except both rates of Dominus and turnip 'Purple top forage' significantly reduced disease severity compared to the inoculated/non-biofumigated non-solarized control. Mustard green 'Amara' incorporation with solarization significantly increased the total fresh weight compared to other treatments and controls. Phytotoxicity were not observed in any of the boxwood plants.

Management of Soil-borne Strawberry Diseases with Alternative Fumigation Methods
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Black root rot complex and crown rot of strawberry caused by soil-borne fungi limit sustainable strawberry production in the Northeast especially in perennial matted row systems. Many organic and small growers who can't use chemical fumigants due to new regulations and potential health hazards need alternative management options. This study was conducted by growing strawberry plug plants with beneficial microbes inoculated or regular planting mix followed by transplanting in fruiting field plots that either were bio-fumigated with mustard cover crop, anaerobically disinfested or left untreated. Different combinations of plug plants and field plot treatments were used to determine efficacy of individual treatment or synergistic effect of combination treatments. Plug plants were transplanted in pre-treated plastic mulched raised beds and grown following a typical organically-recommended production method. Plug plants grown on inoculated planting mix showed enhanced plant vigor in fruiting field compared with non-treated plants. Bio-stimulant/beneficial microbe treated plants had significantly ($P \leq 0.045$) higher fruit yield with higher weight/fruit and better fruit quality containing enhanced amounts of antioxidants than non-treated plants. Antioxidants that were enhanced included total phenolics, vitamin-C, flavonoids and anthocyanins. Results indicated that alternative fumigation methods could provide soil-borne disease suppression and improve yield.

The effect of three-year crop rotation on soybean production
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Crop rotation is a common agricultural practice for soybean production to suppress soil-borne pathogens and improve production. Our research is exploring benefits from 3-yr rotation of soybean, corn and wheat compared with traditional 2-yr rotation of soybean and corn. The field experiments were established in 2013 at Northwest Agricultural Research Station (NWARS) and Western Agricultural Research Station (WARS) of the Ohio Agricultural Research and Development Center. The soybean seedling establishment, biomass and yield were collected from rotation fields. Since 2016, after one round of 3-yr rotation, NWARS soybean yield followed a pattern of yield increase. Specifically, in 2018, the 3-yr rotation soybean from NWARS had 34% greater seedling root biomass and yielded 5% more compared to the 2-yr rotation. However, in WARS, 3-yr rotation did not affect soybean production compared with 2-yr rotation. Thus, the 3-yr rotation effects on plant biomass and yield differed between locations. The research will continue to test NWARS and WARS soil quality and rhizosphere bacterial community from both soybean rotation fields. The correlations of the measured soil and microbial parameters with soybean production in 3-yr rotation will be analyzed. We predict that the 3-yr rotation will have more

diversity of soil bacterial community compared with the 2-yr rotation, regardless of site, and soil quality is higher in soybean 3-yr rotation field compared with 2-yr rotation.

Determining the viability of *Candidatus* Liberibacter asiaticus (CLas) via RNA-based assay and grafting results
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Florida citrus production has declined 70% in recent years, due to Huanglongbing (HLB), caused by CLas. Thermotherapy on HLB-affected trees was proposed as a short-term management solution to maintain field productivity. With the hypothesis that thermotherapy could eliminate CLas from affected branches, the objectives were to show which time-temperature combinations killed CLas in woody tissues. Just hardened, rounded Valencia twigs, collected from HLB-affected field trees, were treated in a steam chamber at 50°C-60s, 55°C-0s, 55°C-30s, 55°C-60s, 55°C-90s, 55°C-120s, 60°C-30s and an untreated control (UTC). Three independent repetitions of 13 branches/treatment were treated, grafted onto healthy rootstocks, and tested for CLas DNA after 6, 9, and 12 months. For the expression analysis of CLas, 3 branches/treatment were used, bark samples were collected, and stored at -80°C for RNA extraction. At 12 months after grafting, leaf samples from branches treated at 55°C-90s, and 55°C-120s had significantly lower CLas titers ($p \leq 0.0001$), when detected, possibly remnants of degrading DNA. Additionally, CLas *16S rRNA* expression significantly decreased at 55°C-90s, and 60°C-30s (7.5 and 11.5-fold change, respectively; $p \leq 0.0001$) in samples 5 days post-treatment. Heat injury, not direct CLas kill, could explain limited changes in transcriptional activity; however, failed recovery and eventual death of CLas could result in no detection in most grafts at severe treatments.

Effect of deficit irrigation on development of Fusarium wilt in processing tomato
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Fusarium wilt of tomato, caused by *Fusarium oxysporum* f. sp. *lycopersici* race 3 (Fol3), is a major driver of yield losses in California processing tomatoes. In response to decreasing water availability, it is becoming more common to deficit irrigate processing tomatoes (a water stress-tolerant crop); however, this change in irrigation practice may influence Fusarium wilt (FW) risk. The effect of deficit irrigation on FW risk was examined in a 2018 field trial with four irrigation treatments [0, 25, 75, and 100% evapotranspiration (ET)] using a highly susceptible cultivar. In this preliminary study, there was a linear increase in incidence of mid-season FW, as water inputs decreased. By harvest, incidence increased in all deficit irrigation treatments compared to 100% ET. In a greenhouse trial, we evaluated whether the use of a FW-tolerant cultivar could mitigate effects of deficit irrigation on disease risk. When comparing full irrigation [45% soil volumetric water content (VWC)] to mild (35% VWC) and moderate (28% VWC) deficit in UC Mix, deficit irrigation did not influence disease development under variable inoculum densities (IDs) (0, 10^2, 10^4, or 10^6 spores/mL drench) of Fol3. Analysis of effects on colonization and IDs in substrate are underway. These results suggest that deficit irrigation influences disease risk, but that the use of FW-tolerant cultivars may allow growers to manage this disease while taking maximum possible advantage of deficit irrigation.

Thermotherapy via aerated steam: A safe practice to strawberry with benefits in disease control
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A thermotherapy protocol via aerated steam, including a 1 h treatment at 37°C, a 1 h cool-down, and a 4 h treatment at 44°C, has been confirmed to effectively reduce or eliminate pathogens from strawberry nursery stock. However, its practical adoption is hindered in part by the fear of potential harm to strawberry plants. In this study, we conducted field trials to evaluate the effect of the current thermotherapy protocol on plant quality in several strawberry cultivars: Florida Beauty, Florida Brilliance, Florida Radiance, Florida 127, Strawberry Festival, and Ventana. Within each cultivar, numbers of new leaves and runners on steam-treated plants were equivalent to those on nontreated controls. Similar results were observed for flowering onset, except that steam-treated 'Strawberry Festival' showed significantly earlier flowering than the nontreated control. Fruit yields of steam-treated 'Florida Beauty' and 'Strawberry Festival' were higher than their corresponding nontreated controls. Nearly all the strawberry plants, regardless of steam treatment, remained vigorous throughout the study, whereas 52% of nontreated 'Florida Radiance' collapsed, which was attributed to infection by *Phytophthora* spp. likely occurring in nurseries. Overall, our results indicated that thermotherapy via aerated steam is a safe practice for managing latent pathogens on strawberry nursery stock and can be integrated into the existing production practices to supply clean planting stock.

Effect of thermotherapy for ratoon stunt on leaf scald control
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New sugarcane fields covered 1.2 million hectares in Brazil in 2018. These fields were formed by vegetative propagation, which is also the major inoculum for both ratoon stunt (RSD) and leaf scald (LS), two systemic diseases. RSD is caused by *Leifsonia xyli* subsp. *xyli,* a fastidious bacterium with no characteristic symptom, whereas LS caused by *Xanthomonas albilineans* (Xa) has a latent infection, a symptomless stage of the disease. Once the dissemination of these diseases into new fields is an invisible process, the use of hot water treatment (HWT) of seedpieces is a major strategy of disease control. Numerous studies are available on thermotherapy for RSD control, the most recommended in Brazil are 50°C for 2h and 52°C for 30min. The prescribed HWT for LS is 50°C for 3h. There is no information on LS control by the thermotherapy used for RSD. Therefore, the present work examined the effect of the HWT for RSD on the LS suppression. Two cultivars LS-contaminated with different titers of Xa were submitted to 50°C/2 h and 52°C/30min and

compared to untreated control. DNA was extracted from vascular bundles of 194, 215, 230 days old canes and PCR test carried out employing primers PGBL1/PGBL2. Our data showed that the use of HWT had a beneficial effect to control Xa. Also, the combination 50°C/2h were more effective for controlling the disease if compared with 52°C/30min, suggesting that HWT employed for RSD is also effective for LS, which was not documented previously.
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Multi-site field assessment of bio-renovation methods to control apple replant disease
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Commercial scale orchard trials were conducted in the north, central and south districts of the primary apple production region in Washington State to evaluate bio-renovation approaches for the management of replant disease. Treatments examined included anaerobic soil disinfestation using Triticale or Timothy hay (ASD; 8 tons/A), Brassicaceae seed meal (BSM; 1.6 lbs per tree-row-foot rate, 1:1 formulation of *Brassica juncea* and *Sinapis alba*) amendment, grower standard soil fumigation (1,3-dichloropropene/chloropicrin), and no-treatment control in randomized complete block design. Soil microbial profiling was conducted at three weeks post-treatment. Anaerobic soil conditions were effectively attained in response to ASD at the north and central sites, but not the south site trial. Significant changes in soil microbial profiles were observed in response to ASD and BSM treatments. In bioassays conducted in orchard soils collected post-treatment, BSM effectively suppressed seedling root populations of *Pratylenchus penetrans* at all field sites. In addition, BSM, but not fumigation, suppressed lesion nematode root densities recovered from 'Cosmic Crisp'/G.41 at the end of the first growing season at the central site. Treatment effects on fungal/oomycete root infection detected in seedling bioassays were variable, with *Rhizoctonia* root infection reduced by ASD and BSM at the central and south sites, respectively.

Resistance to Non-Race 1 *Verticillium dahliae* in Tomatoes Suppresses Necrosis and Chlorosis Symptoms in Infected Plants
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In 2017 and 2018 field trials were conducted in Mills River, NC to evaluate grafted and non-grafted tomatoes with reported resistance to non-race 1 *Verticillium dahliae*. In 2017 'NC-7GEM' (7-1K), had significantly lower AUDPC scores than the susceptible 'Red Defender' (RD). 7-1K also had less disease than non-fumigated RD. However, when 7-1K was used as a rootstock for RD, it did not prevent the susceptible scion from wilt symptoms. The susceptible RD scion grafted on to 7-1K was just as susceptible as non-grafted and self-grafted RD. When the trial was repeated in 2018, similar results were found. In 2018 similar results were found, but we included 7-1K grafted onto RD, and showed that even with a susceptible rootstock, the 7-1K prevented disease symptoms in the foliage. In 2017 yields from RD/7-1K, RD/'Maxifort', and RD were 48,876, 70,285, and 58,323 lb/A respectively; in 2018 the yields were 37,717, 37,439, and 26,830 lb/A respectively. These results indicate that vigorous rootstocks such as 'Maxifort' play a bigger role in boosting yields in *V. dahliae* infested fields than resistant rootstocks such as 7-1K. In both years non-grafted 7-1K had the lowest AUDPC. *V. dahliae* can be isolated from symptomless 7-1K, indicating the resistance may prevent symptoms caused by the pathogen, but not growth. 7-1K has been developed into 'NC-7GEM', as an inbred parent line for future varieties. These results imply that a combination of vigorous rootstocks, and resistant scions or standalone varieties would be the optimal way to manage non-race 1 *V. dahliae*.

Synergy of Anaerobic Soil Disinfestation (ASD) and *Trichoderma* in Rhizoctonia Root Rot Suppression
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We evaluated potential synergy between ASD and *Trichoderma* spp. in suppression of Rhizoctonia root rot (RRR) in radish. A split plot design with three replications was used; main plots were *T. harzianum* T22, *T. asperellum* SM1785 and a non-*Trichoderma* control. Subplots were ASD carbon sources wheat bran, molasses, chicken manure, and mustard greens and two non-amended controls: anaerobic (covered and flooded) and aerobic (not covered or flooded). Carbon sources and *R. solani* inoculant were mixed with soil, which was placed in pots and flooded, followed by drenching *Trichoderma* spore suspensions and sealing the pots in Ziploc bags. After three weeks, bags were removed, soil aired for one week and radish 'SSR-RR-27' seeded. RRR severity was lowest in radish plants grown in ASD-treated soil amended with wheat bran, molasses or mustard greens across all *Trichoderma* treatments. Disease severity was lower in plants treated with SM1785 than with T22 or the non-*Trichoderma* control across all ASD treatments, and in radish grown in ASD-treated soil amended with wheat bran plus SM1785 compared to ASD-wheat bran or SM1785 alone. *Trichoderma* populations were significantly reduced compared to the controls only by ASD-mustard greens. ASD-wheat bran and SM1785 acted synergistically to reduce disease pressure. The efficacy of ASD amended with most carbon sources against soilborne pathogens may be improved by addition of one or more ASD-compatible *Trichoderma* isolates.

Autonomous UV-C application on tabletop strawberries to control mildew: Optimization and compatibility with IPM
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UV-C light is a promising technique that can tackle current issues of powdery mildew management in strawberry: chemical resistance and fruit residue. A prototype robot is used to apply UV-C on tabletop strawberries and a strategy for mildew control is being developed. The side-effect of UV-C is also assessed on crop yield, other diseases, pests and beneficials to ultimately integrate UV-C in integrated pest managment. Results reveal that nighttime application has an increased efficiency compared to daytime application.

Three applications a week throughout the cultivation resulted in sufficient disease control. Side-effects of UV-C are both positive and negative as spider mite and beneficial mites (*A. limonicus*) are harmed by UV-C with a more substantial suppression at night. No other side-effects were revealed thus far. Integration of UV-C in IPM will be challenging but if we succeed, it will be a big step forward to sustainable strawberry production.

Evaluation of UVC for suppression of powdery mildew and other diseases of strawberry in open field production

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Open fields of cv Florida Beauty and Florida127 strawberries were exposed to ultraviolet-C (UVC) doses ranging from 68 to 169 J/m2 once or twice weekly. Applications began 1 hr after sunset and were completed within 6 hrs of sunrise using a tractor-drawn array of Osram 55W 83V G55T8/OF G13 germicidal lamps (peak irradiance 254 nm, FWHM < 5 nm), backed by polished reflectors. Lamps over each row were arranged in hemicylindrical arrays, each containing 10 lamps, yielding a total array length of 180 cm. At ground speeds of 2.3 to 5.6 kph, plants were under the lamp array for 1.2 to 2.9 s. In repeated trials, UVC at 85 to 169 J/m2 applied once per week suppressed incidence of powdery mildew (*Podosphaera aphanis*) on fruit as well as, or more than, the best-performing fungicide treatments. There were no deleterious effects on yield of harvestable fruit. UVC doses of 68 J/m2 reduced foliar disease severity of powdery mildew by 53% when applied once a week, and by 70% when applied twice per week. Under severe conditions, higher UV dose and shorter intervals between treatments generally, but not always, resulted in greater disease suppression. No UVC treatment provided more than 10% suppression of anthracnose fruit rot (*Colletotrichum acutatum*) and Botrytis fruit rot (*Botrytis cinerea*). The UVC technology is being adapted to a variety of tractor-drawn and robotic platforms to suppress powdery mildews and arthopod pests in diverse cropping systems.

Optimizing nighttime UV-C doses and application intervals for control of cucumber powdery mildew

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Fungicide resistant isolates of the cucumber powdery mildew pathogen (*Podosphaera xanthii*) are prevalent, and many commercially important cultivars are highly susceptible. Germicidal ultraviolet (UV-C) has shown exceptional efficacy against a broad range of powdery mildews in prior reports in greenhouse production systems. We conducted a study to optimize the UV-C application interval for control of cucumber powdery mildew in preparation for larger-scale commercial field trials. Differential effects of daytime versus nighttime UV-C doses (at 72 and 144 J/m^2), and specific nighttime doses (7.2, 26, 70 J/m^2) at four application intervals (from nightly to every 8th night) were studied. Consistent with previous reports, nighttime application of UV-C allows lower doses to achieve disease suppression. At constant doses, shorter intervals were uniformly more effective than longer intervals in suppressing *P. xanthii*, but leaf area decreased at 70 J/m^2 dose when applied every night. Our results suggest that applications of 70 J/m^2 every 4th to 8th night will effectively suppress cucumber powdery mildew without leaf injury. These results provide guidance for end-users in defining dose and application intervals for future field efficacy trials.

Red and blue LEDs used for horticulture lighting can suppress sporulation of *Peronospora belbahrii*, the causal organism of basil downy mildew

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Continuous broad- as well as narrow-band spectra have been shown to suppress sporulation of many downy mildew pathogens. However, the suppressing effects of intermittent light at night, to save electric energy, using commonly available narrow-band blue (λ_{max} = 458 nm) and red LED (λ_{max} = 670 nm) horticultural lighting systems have never been demonstrated. Sporulation inhibition of *Peronospora belbahrii* (the casual pathogen of basil downy mildew) was studied using blue and red LEDs at two irradiances (12 and 60 µmol /m^2/s) for two intermittent-lighting protocols (three 1.3-h light pulses and one 4-h light pulse), and a continuous 10-h lighting protocol, as well as a control. A nighttime dose of 2.20 mol /m^2/night of continuous blue or red light was able to inhibit basil downy mildew sporulation by nearly 100%. For doses (0.17 – 0.87 mol/m^2/night) that did not completely suppress sporulation, continuous light exposures through the night were always more effective than the tested intermittent light exposures. In general, at equal intermittent doses between 0.17 – 0.87 mol/m^2/night that did not completely suppress sporulation, red light was slightly more inhibitory than blue light.

Enabling water use adaptation in containerized annuals: Using substrate to reduce disease risks associated with deficit irrigation

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As growers face increasing constraints on water use, understanding disease risks associated with deficit irrigation is paramount. Previous studies with *Phytopthora capsici* root and crown rot in tomato indicate that DI increases latent infection and disease risks. This work evaluates how different substrates influence DI-associated disease risk in containerized annuals, with a focus on an annual vegetable (tomato) and floricultural crop (chrysanthemums). We hypothesized that substrates with greater water holding capacity (*e.g.,* peat) would present a lower disease risk under DI due to less plant water stress. We evaluated three substrates: Bark, Fiber, and Peat; and two irrigation schemes: “Well-Watered” and “Deficit”. *Phytopthora capsici* alone increased root necrosis in all treatments (*P* <

0.001), as did *P. capsici* and Deficit together ($P < 0.001$). Comparing measurements among substrates, disease risk was lowest in Bark, intermediate in Peat and greatest in Fiber ($P < 0.05$). Similar results were observed in studies with Chrysanthemum and *Phytopythium helicoides*, suggesting that substrate may influence the risk of DI. More work is needed to elucidate the details of that relationship and translate to field soils.

Genomic alterations in *Sclerotinia sclerotiorum* after sub-lethal fungicide exposure
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Fungicide resistance has been reported for 203 phytopathogenic fungi and poses a serious threat to fungicide efficacy. Understanding the genomic basis of resistance emergence can help to delay evolution of resistance. Sub-lethal fungicide dose exposure may stress plant pathogens to generate novel mutations, some of which may confer adaptive traits like fungicide resistance or aggressiveness. Previously, we exposed five progenitor isolates of *S. sclerotiorum* to sub-lethal doses of four fungicides (azoxystrobin, boscalid, iprodione, and thiophanate-methyl) for twelve consecutive generations. In this study, we sequenced pre- and post-exposure isolates (55 in total) and aligned the reads to the reference genome. We found that frequency of mutations did not increase with sub-lethal fungicide exposure but differed significantly according to the progenitor isolate (P-value < 0.05). About 47% of the genetic variation was explained by variation among isolates and 1% variation by sub-lethal fungicide exposure. High within-species variance may have masked the effect of fungicide exposure using the reference genome alignment. To address this, we will use *de novo* genome assembly tools for further analyses. Since sub-lethal fungicide exposure may confer traits like aggressiveness, greenhouse assays will be conducted to evaluate changes in aggressiveness. Results will expand our understanding about the role of sub-lethal fungicide dose in resistance emergence, which may be useful in designing strategies to delay resistance evolution and retain the efficacy of currently used fungicides.

A closer look into fungicide resistance: How does application rate affect the development of SDHI resistance?
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Fungicide resistance is problematic in plant pathogens where management relies heavily on the use of fungicides. Resistance development often results in a lack of effective management and henceforth crop loss. With the advent of next generation succinate dehydrogenase inhibitors (SDHIs), it is essential to incorporate sustainable resistant management practices to prevent future loss of this fungicide class. To understand programs that best reduce resistance risk in the field, the effect of fungicide application rate on the development of resistance was tested in three pathosystems with a history of resistance: *Venturia inaequalis,* the causal agent of apple scab on apple, *Stemphylium vesicarium,* the causal agent of *Stemphylium* blight on onion, and *Botrytis cinerea,* the causal agent of gray mold on grapes. Selection was carried out through repeated fungicide applications of high or low rates of the following SDHI fungicides: fluxapyroxad, fluopyram, and benzovindiflupyr. After selection, fungicide sensitivity was tested via *in vitro* growth inhibition assays. Current results in apple and onion suggest use of lower application rates may result in an increased frequency of isolates with reduced sensitivity. Further, these outcomes support that managing overall populations size may be the first step in managing fungicide resistance emergence. Sensitivity testing and selection with *B. cinerea* on grape is currently ongoing.

Fungicide resistance is associated with transposable elements and variable genome size in the apple scab fungus, *Venturia inaequalis*
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Apple scab, caused by *Venturia inaequalis,* is an economically important fungal pathogen that greatly reduces fresh market yields. Due to a lack of durable resistance in commercially relevant cultivars, fungicides remain essential for disease management. Sequencing, detailed annotation, and analysis of four North American *V. inaequalis* genomes of baseline sensitive and multiple fungicide resistance phenotypes have been completed. The assembled genomes ranged in size from 39.9 Mb to 61.5 Mb. The predicted gene models through GeneMark-ES and AUGUSTUS were annotated using multiple approaches including InterproScan 5.1, SignalP 4.1, Phobius, CAZy and antiSMASH. The number of scaffolds greater than 500bp ranged from 619-7555 and contained 12225-12628 unique genes based on GlimmerHMM, implemented within QUAST. Genome completeness was determined with the BUSCO v3 *Ascomycota* odb9 database including 1315 targets resulting in 1259-1282 benchmarking universal single copy orthologs. Initial gene annotations through a Diamond blastP search of the UniProt database version 2018_09 resulted in 581-601 valid annotations. An analysis of the predicted secretome with SignalP resulted in 1381-1401 annotations and the number of transposable elements detected ranged from 48 to 153. The differences observed herein provide opportunities for further investigation into the mechanisms involved in fungicide resistance as transposons may be driving observed variations in genome size and/or enabling fungicide resistance in the field under various selection pressures.

Copper-resistant strains of *Xanthomonas gardneri* and *X. perforans* from Illinois tomato fields
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During 2017–2018, 159 *Xanthomonas* isolates were collected from symptomatic leaf and fruit samples from commercial tomato fields in Illinois. Among the 159 isolates, 102, 56, and 1 isolates were identified as *X. gardneri, X. perforans,* and *X. euvesicatoria,*

respectively based on Sanger sequencing using *hrp* gene primers (RST65 and RST69). All the *Xanthomonas* isolates were grown on media containing 0.08 mM copper sulfate ($CuSO_4$) to screen for copper resistance. Isolates that grew on the copper media were further evaluated using PCR to confirm the presence of two copper-resistance genes, *copA* and *copM*. Based on our phenotyping and genotyping analyses, 17 *X. gardneri* and 13 *X. perforans* isolates were identified as copper resistant. The efficacy of commonly used chemicals for management bacterial spot of tomato in Illinois are being investigated against a subset of these identified copper-resistant isolates. Commonly used chemicals for management of *Xanthomonas* spp. on tomatoes in Illinois are copper hydroxide (Kocide-3000), copper octanoate (Cueva), copper sulfate pentahydrate (Phyton 27AG), mancozeb (Manzate PRO Stick), quinoxyfen (Quintec 250SC), and an extract of *Reynoutria sachalinensis* (Regalia). The findings of this study are expected to help to develop effective management of bacterial spot of tomatoes.

Competitiveness of Multifungicide Resistant *Botrytis cinerea* Isolates Under Field Conditions
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Botrytis cinerea isolates with resistance to multiple chemical classes of fungicides pose a great threat to the continuous use of site-specific fungicides. A better understanding of competitiveness of *Botrytis* isolates with multifungicide resistance will allow us to improve resistance management strategies. In the present study, a mixture of spore suspensions of sensitive *B. cinerea* isolates (0CCR), isolates with five chemical classes resistances (5CCR), or 7 CCR was artificially inoculated on blackberry plants at 20 to 30% bloom in 2016. *Botrytis* isolates were recovered from symptomatic or asymptomatic flowers and fruit from each treatment over the course of three years (2016 to 2018). No fungicide was applied during the three experimental years. In 2016, 0CCR, 5CCR and 7CCR isolates were collected from the 0CCR, 5CCR and 7CCR treatments, respectively, but a wide range of phenotypes (i.e. 0, 2, 3, and 4CCR) were also collected from the uninoculated control plants. Multiple CCR phenotypes were observed in each treatment in the following two years. 7CCR isolates were isolated in all of the three experimental years. Sequence analysis of mrr1 gene showed 7CCR isolates isolated in year 3 had the same genotype with those 7CCR isolates used for the inoculation in year 1. These findings indicate that *B. cinerea* isolates with multifungicide resistance may be as competitive as other phenotypes including isolates sensitive to fungicides in the absence of fungicide selection.

Fungicide resistance in *Penicillium expansum* from pome fruit in the U.S. Pacific Northwest
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Blue mold caused by *Penicillium expansum* and *Penicillium* spp. is the most important postharvest pathogen of apple and pear in the U.S. Pacific Northwest and worldwide. Its management is achieved through sanitation and application of fungicides, from three chemical groups, i.e. methyl-benzimidazole carbamate (MBC), phenylpyrrole (PP), and anilinopyrimidine (AP), applied at harvest. We conducted two years of fungicide resistance monitoring and assessed the sensitivity of more than 6,000 *P. expansum* isolates to the MBC-thiabendazole (TBZ), the PP-fludioxonil (FDL), and the AP-pyrimethanil (PYR). Sensitivity was assessed using germination and germ tube length on agar media amended with discriminatory doses of each fungicide. Overall, resistance frequencies ranged from 15% to 45% for TBZ and 12 to 40% for PYR. About 5 to 12% or isolates tested showed reduced sensitivity to FDL. The majority (80%) of the isolates tested was either sensitive to all fungicides or resistant to one fungicide only. Our results indicate that the three postharvest fungicides can still be part of the overall disease management program including as long as they are rotated yearly. However, because pome fruit are stored for up to a year, further investigation is needed to ensure that the relatively low resistance frequencies observed do no cause a significant reduction in the efficacy of fungicides over the long storage period.

Species identification and fungicide resistance profiling of *Botrytis* isolates from Maryland vineyards
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Botrytis bunch rot of grapes, caused by *Botrytis* spp., is a significant disease in the Mid-Atlantic United States and the pathogen is notorious for acquiring resistance to multiple chemical classes of fungicides. In the fall of 2018, 92 *Botrytis* isolates were collected from symptomatic grape berries in six Maryland vineyards. A subset of 20 isolates were identified to be *Botrytis cinerea* based on sequences of the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene. In addition, all 92 isolates were subjected to a resistance profile kit described previously. Frequencies of resistance to the following fungicides were: 0% to adepidyn, fludioxonil, and isofetamid, 1% to benzovindiflupyr, 2% to penthiopyrad, 13% to boscalid, 27% to iprodione, 41% to fenhexamid, 66% to cyprodinil, 84% to thiophanate-methyl, and 98% to pyraclostrobin. These isolates were resistant to one (4%), two (15%), three (35%), four (38%), or five (8%) different chemical classes. Mutations were found in the following target genes of resistant isolates: I365N and I365S in *bos1,* E198A in *β-tubulin*, G143A in *cytB*, F412I/S in *erg27*, and H272R/Y and P225F in *sdhB*. Resistance frequencies were largely consistent among the six vineyards for all fungicides except for cyprodinil, fenhexamid, and iprodione, presumably due to diverse spray programs. Use of phenylpyrroles and newer succinate dehydrogenase inhibitors needs to be limited to maintain their efficacy.

Molecular and bioassay evidence of fungicide resistance of *Podosphaera clandestina*
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Synthetic fungicides with a single site of action are generally more prone to encounter resistance by fungal pathogens than their multi-site counterparts. Early identification of target mutations in response to continued use of similar material is critical to the longevity of fungicide tools. A suite of 14 foliar *Podosphaera clandestina* isolates representing various growing regions of the Pacific Northwest (PNW) was subjected to high throughput Illumina sequencing. The ITS sequences from all isolates confirmed the identity of *P.*

clandestina, incitant of sweet cherry powdery mildew. The data were used to identify the fungicide target genes, Cytochrome b (COB, FRAC 11) and, CYP/ ERG11 (FRAC 3). The deduced amino acid sequences from the Illumina contigs were screened for the presence of target mutations. The results indicated the presence of COB target mutation at position 143 (G143A/R) in samples across PNW. But, no target mutations were found for CYP/ ERG11 gene in all of the samples analyzed. A pilot-scale bioassay on one of the isolates using eight fungicides revealed resistance to three different FRAC groups. Overall, studies confirmed that the fungicide resistance is factual and more information is needed to strategically address this situation. The information from such studies will likely avoid a widespread problem of fungicide resistance and poor management of powdery mildew on cherries. Additionally, replacing compromised fungicides with newer chemistries offers better disease management and promotes the economic sustainability of the cherry orchards across the PNW.

In vitro* insensitivity of *Rhizoctonia solani* to the natural product UK-2A associated with L198F variant at the Qi target site of cytochrome *b

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The amino acid composition of the cytochrome *b* gene of 38 fungal and 12 oomycete species from the NCBI database was analyzed to identify key polymorphic sites at the Qi center of the proton-motive Q-cycle. The objective was to determine whether amino acid substitutions reflect a significant variation at the Qi target site among true fungi and oomycetes. The analysis was directed mainly to 3 amino acid changes N31K, G37C and L198F, which were reported elsewhere to be associated with low sensitivity of *Saccharomyces cerevisiae* mutants to the Qi inhibitors UK-2A, antimycin A and fenpicoxamid (Inatreq™ active). The fungal and oomycete species analyzed contained wild type amino acid variants at the 31, 37 and 198 positions except *Rhizoctonia solani* which carried the amino acid change L198F. Sequencing of the *R. solani* cytochrome *b* gene confirmed the presence of phenylalanine at the 198 position. An *in vitro* sensitivity test showed that this *R. solani* isolate was fully resistant to UK-2A, but remained sensitive to azoxystrobin, fluxapyroxad and epoxiconazole. The relationship between the change L198F and the low sensitivity of *R. solani* to UK-2A remains to be determined.

Impact of *CYP51* gene copy number and expression on DMI fungicide resistance in *Blumeria graminis* f.sp. *tritici*

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Wheat powdery mildew, caused by *Blumeria graminis* f.sp. *tritici* (*Bgt*), is notorious for quickly becoming resistant to fungicides. Rapid adaptation to early demethylation inhibitor (DMI) fungicides was observed in European fields after intensive DMI use in the early 1980's. These old European *Bgt* isolates with reduced DMI sensitivity were found to contain a Y136F mutation in CYP51, the 14α-demethylase protein targeted by DMI fungicides. Recent fungicide tests of United States (U.S.) *Bgt* isolates have revealed reduced DMI sensitivity in the eastern U.S., where wheat powdery mildew occurs most regularly, as compared to the Plains states. The same Y136F mutation was found in U.S. isolates, but was not highly correlated with reduced DMI sensitivity. Recently sampled DMI-resistant United Kingdom (U.K.) isolates with at least double the reduction in sensitivity were found to have multiple *CYP51* gene copies and increased *CYP51* gene expression. Some U.K. isolates and several U.S. isolates were found to possess both wildtype and mutant *CYP51* alleles; however, the U.K. mutant alleles had a double mutation of Y136F and S509T, while U.S. mutant alleles had only the Y136F mutation. The S509T mutation was not found in U.S. isolates, indicating that S509T is specific to DMI resistance in European *Bgt*. To determine the underlying cause of the reduced DMI sensitivity observed in U.S. *Bgt*, evaluation of *CYP51* gene copy number and expression in U.S. isolates is currently underway.

***In vitro* fitness comparisons among *Cercospora nicotianae* of varying sensitivity to azoxystrobin**

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The frogeye leaf spot pathogen of tobacco, *Cercospora nicotianae,* has three levels of sensitivity to azoxystrobin, the only synthetic, systemic fungicide currently labeled for tobacco fungal leaf spot management. Single nucleotide polymorphisms result in F129L and G143A mutations in the *C. nicotianae* cytochrome b gene, which confer moderate and high resistance, respectively. In some pathosystems, mutant pathogen individuals are less biologically fit than wild type individuals. To establish if there was a fitness cost in *C. nicotianae* with the mutant phenotypes, *in vitro* comparisons were made. Fitness was determined from conidial viability and mycelial growth rate in potato dextrose agar (PDA). In the mycelial growth assay, isolates were grown for 14 days and perpendicular diameters were measured. Conidial viability was measured by germination counts after 20-28 h incubation on PDA. Average values by sensitivity grouping were compared using one-way ANOVA. Growth rates and conidial viability for the wild type, moderate, and highly resistant groups averaged 105.67, 112.10, and 120.33 mm^2/day, and 92.8, 94.8, and 94.1% germination, respectively. There were no statistically significant differences, suggesting no *in vitro* fitness cost associated with reduced sensitivities to azoxystrobin. This lack of fitness cost suggests populations composed of resistant *C. nicotianae* individuals may not rapidly return to wild type sensitivity levels in the absence of azoxystrobin use.

Sequence analysis of cytochrome *b* gene in *Venturia nashicola* isolates with reduced sensitivity to QoI fungicides

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Scab is the most serious disease of Japanese pear and the control of this disease largely relies on fungicides. As a result, the isolates of *Venturia nashicola* resistant to DMI fungicides are distributed in the pathogen populations. In this study, the sensitivity of *V. nashicola* to azoxystrobin was monitored because QoI fungicides carrying high risk for resistance development are also sprayed. Monoconidial isolates were cultured on PDA plates supplemented with a series of concentration of azoxystrobin plus 1 mM *n*-propyl gallate. The mean and maximum EC_{50} values of azoxystrobin was 0.3 µg/ml and 2.2µg/ml, respectively, for baseline isolates never received fungicide selection pressure. In contrast, the EC_{50} values for 20 out of 162 isolates sampled from commercial pear orchards in Oita and Fukuoka prefectures exceeded the threshold concentration of 2.2 µg/ml and maximum value was 77.2 µg/ml indicating the distribution of isolates with reduced azoxystrobin sensitivity in the field. The nucleotide sequence of cytochrome *b* gene in these isolates was analyzed by PCR and direct sequencing. Surprisingly, the sequence of all 40 isolates analyzed was GCT at codon 143 and deduced amino acid was alanine rather than glycine in both sensitive isolates and those with reduced azoxystrobin sensitivity. The sequence was confirmed by RFLP analysis when the PCR products were cut with the restriction enzyme *Fnu*4HI. To know the potential risk of these isolates for disease control, inoculation tests will be conducted using fungicide treated pear trees.

Fungicide Sensitivity of 395 *Sclerotinia sclerotiorum* Isolates from Dry Bean and Soybean
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Fungicide applications are integral to reduce yield loss from *Sclerotinia sclerotiorum*on dry bean and soybean. Increasing use in production may lead to resistance and few studies have investigated this. We sought to determine if there is a difference in fungicide sensitivity betweenisolates from dry bean and soybean and those exposed to intensive, moderate, and no fungicide, in fungicide field trials (FFT), farmer fields (FF), and white mold nurseries (WMN), respectively. We also sought to determine if reduced sensitivity is linked to use of fungicides with a single active ingredient. Initially, fungicide sensitivity was assessed to thiophanate methyl (MBC), tetraconazole (DMI), boscalid (SDHI), and picoxystrobin (QoI) using serial dilution for 42 isolates and a dose-response curve was fit to estimate the EC_{50}. Linear regression of % mycelial growth at each concentration vs. logEC_{50} was used to identify the best predictor of EC_{50}, used as the discriminatory concentration (DC). Thiophanate methyl had a qualitative DC of 10 ppm thatclassified as sensitive or resistant. Others were 2.0 ppm for tetraconazole, 0.2 ppm for boscalid, and 0.01 ppm for picoxystrobin. These concentrations will be used to assess sensitivity to all four fungicides for an additional 165 isolates (1-3 per field) to compare isolates grouped by source (FF, FFT, WMN) and also by host (dry bean and soybean). An additional 188 isolates (5-10 per field) from fields with applications of single-site fungicides will be used to assess ifthere is reduced sensitivity to fungicide used in each field.

Detection and fungicide sensitivity surveillance of wild rice (*Zizania palustris*) pathogens
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Fungal brown spot caused by *Bipolaris oryzae*, and spot blotch caused by *B. sorokiniana*, are commonly found in cultivated wild rice paddies. Strobilurin-based fungicides are effective in reducing these diseases in the upper plant canopy. After three years of fungicide applications *Bipolaris* spp. populations decreased to low levels in wild rice plants early in the season, although the populations increased later in the year. Phenotypic identification of diseases based on small lesions early in the season is difficult and often misleading. To aid in an accurate and rapid detection of *Bipolaris* spp. pathogens, we developed SYBR Green-based PCR assays with primers designed from ITS1 and the *B*-tubulin genes. The assays discriminate both *Bipolaris* spp., and other fungal species. To maintain control of foliar diseases during the wild rice growing season, azoxystrobin-based fungicides are needed. Even though fungicide resistance is unlikely to develop in *B. oryzae* due to an intron after the G143 codon of the cytochrome *b* gene, changes toward reduced sensitivity could still occur in codons 129 and 137. To monitor changes in fungicide sensitivity, we developed a PCR assay based on TaqMan technology to detect changes from phenylalanine to leucine in position 129, while nucleotide changes in positions G143 and G137 will be monitored with SYBR Green-based assays. These assays will aid in understanding the epidemiology of wild rice pathogens and timely application of fungicides.

Occurrence of quinone outside inhibitor (QoI) resistance in Virginia populations of *Parastagonospora nodorum* infecting wheat
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Stagonospora nodorum blotch (SNB) of wheat, caused by *Parastagonospora nodorum,* is managed using cultural practices, resistant varieties, and foliar fungicides. Frequent fungicide use can select for fungicide resistance, making certain chemistries less effective; this may in part explain increasing severity of SNB in the mid-Atlantic U.S. Quinone outside inhibitor (QoI) resistance has been documented for a diversity of fungi, but it has not been reported for *P. nodorum* in the U.S. The objectives of this study were to 1) evaluate QoI sensitivity of *P. nodorum* from Virginia wheat fields, 2) screen *P. nodorum* for QoI target site mutations in the cytochrome b gene (cytb), and 3) develop a molecular assay to detect target site mutations associated with QoI resistance. Sensitivity of 16 isolates to pyraclostrobin and azoxystrobin was evaluated with radial growth assays, and cytb was sequenced. One isolate was insensitive to both fungicides, and it had the G143A mutation in cytb. For azoxystrobin, 9 isolates without target site mutations had reduced sensitivity. Additional isolates (N=74) were sequenced, and 7 had the G143A mutation. A pyrosequencing assay targeting G143A was developed as a rapid method to screen *P. nodorum* for the QoI resistance-conferring mutation. This is the first report of QoI resistant *P. nodorum* in the U.S. Overall resistance frequency was low, but resistance management practices are needed to maintain the efficacy of fungicides for SNB control.

Development of a rapid isothermal assays to detect QoI resistance in *Erysiphe necator* the causal agent of grape powdery mildew
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Quinone outside inhibitor fungicides (QoIs) are frequently applied for management of grape powdery mildew. However, repetitive use of QoIs can lead to development of resistance in *E. necator* populations as the result of a single nucleotide polymorphism (SNP). Currently, several allelic discrimination assays are available for this SNP, all of which require DNA extraction and costly laboratory instruments. Two isothermal approaches were designed to detect the SNP associated with QoI resistance, namely a recombinase polymerase amplification (RPA) assay and a loop-mediated isothermal amplification (LAMP) assay. These techniques are inexpensive, rapid, field-portable and fit for crude DNA. The RPA assay was sensitive to 1pg of crude DNA but was unable to discriminate the SNP. For LAMP, a set of four primers were designed to amplify the target DNA containing the SNP. The LAMP assay was able to discriminate this SNP and was optimized to improve the sensitivity and specificity by modifying, 1) primer concentration, 2) reagents, and 3) reaction temperatures. Results were compared with other allelic discrimination assays and amplifications were observed using multiple platforms (e.g. portable reader units and a qPCR machine). This assay was validated by testing outgroups of related powdery mildew species and QoI resistant and sensitive isolates of *E. necator* collected from different locations around the US. This technique provides growers with another tool to rapidly monitor the buildup of QoI resistance in *E. necator* populations.

Investigating fungicide sensitivity shifts in North American *Zymoseptoria tritici* field populations
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The fungal pathogen *Zymoseptoria tritici* causes worldwide Septoria tritici blotch (STB) in wheat. Under high disease pressure, control of STB is heavily dependent on foliar sprays, and, consequently, resistance shifts have been reported to different classes of fungicides (i.e. MBC, QoI, DMI and SDHI). The fungicide sensitivity status of two North American populations, sampled in Oregon and Michigan, were assessed and compared to South American, New Zealand and European field populations. Sequence analysis of *CYP51*, the azole target protein sterol 14α-demethylase encoding gene, indicates that resistance is evolving independently in the North American populations in comparison to European populations, albeit much slower, whereas European isolates appear to have migrated to South America and New Zealand. The Oregon and Michigan populations showed a similar sensitivity range for the SDHI bixafen. However, there were differences in fluopyram sensitivity with 61% of the Oregon strains and only 2% of Michigan strains having EC_{50} values >1 ppm. No mutations were found in *Sdh*B, C and D, indicating the presence of a non-target site resistance mechanism. QoI resistance due to cytochrome b G143A is present in 68% of the Oregon strains, whereas 25% of the Michigan population is insensitive to azoxystrobin. No MBC resistant isolates were found within the Michigan population, while 22% of Oregon isolates are insensitive to carbendazim and carried beta-tubulin E198A.

Azoxystrobin sensitivity testing in *Rhizoctonia solani* AG2-2 populations affecting Michigan sugar beet
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Rhizoctonia root and crown rot (RRCR) is caused by *Rhizoctonia solani* AG 2-2 and continues to be a major pest of sugar beets. In Michigan, azoxystrobin (Quadris) is widely applied to manage RRCR. Azoxystrobin, a quinone outside inhibitor, targets a single site to inhibit fungal respiration and so possesses a high risk of fungicide resistance development. Continued reliance on one to two applications of this product per season has prompted investigations of azoxystrobin sensitivity in Michigan *R. solani* populations. Isolates were collected from research and commercial fields in the Michigan sugar beet growing region. Two additional baseline isolates, collected prior to azoxystrobin use in sugar beet, were included for comparison. Isolates were screened in half-strength clarified V8 agar amended with salicylhydroxamic acid at 10 µg ml^{-1} and azoxystrobin at concentrations: 0, 0.01, 0.1, 1, 10, and 100 µg ml^{-1}. After 96 hr, mycelial mats were removed from media, air dried for 48 hr, and mass was measured. Percent inhibition was calculated and effective concentrations for 50% inhibition of colony mass were determined using three-parameter logistic regression. In a preliminary screen, azoxystrobin insensitivity was not observed in Michigan *R. solani* populations, however, additional testing is ongoing. Evaluation of azoxystrobin efficacy, and potential alternatives, for RRCR management will be conducted at the Saginaw Valley Research and Extension Center in Frankenmuth, MI.

Investigation of *in vitro* and *in planta* transfer of chromosomally-encoded copper resistance in *Xanthomonas perforans*
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Copper bactericides have been used since decades for the control of bacterial plant pathogens. Repeated use of copper bactericides against bacterial spot (BLS) xanthomonads has led to spread of the copper resistance trait in the pathogen population. Transfer of copper resistance genes via large conjugative plasmid has been a well characterized mechanism in spread of copper resistance in BLS pathogens. We recently identified copper-tolerant *Xanthomonas perforans* strains that contained chromosomally-encoded copper resistance genes on a genomic island. Interestingly, the structure of this island differs from the previously reported chromosomal island of *X. vesicatoria* strain XVP26. In this study, we investigated the transfer potential of chromosomally-encoded copper resistance genes from copper-tolerant *X. perforans* strains to the copper sensitive *X. perforans* strains under *in vitro* and *in planta* conditions. Co-infiltration of copper-tolerant and copper-sensitive strains into tomato leaves failed to obtain copper-tolerant transconjugants in the sensitive background. However, dip-inoculation of the mixture of copper-tolerant and copper-sensitive strains allowed us to obtain

copper-tolerant transconjugants of sensitive strains. This observation indicates that transfer of chromosomally-encoded copper resistance genes was successful during epiphytic colonization of pathogen.

Study of copper tolerance and resistance genes *copA* and *copB* in the plant pathogen *Xylella fastidiosa*
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Xylella fastidiosa (Xf), a Gram-negative bacterium, is a xylem-limited plant pathogen that causes diseases worldwide in many economically important crops such as grape. Copper (Cu)-containing antimicrobials are widely used in Xf hosts in vineyards and orchards. The accumulation of Cu in field soil and plants could be a challenge for Xf survival. Here we define Cu tolerance as the ability to growth under chronic elevated Cu levels while Cu resistance is the ability to survive acute Cu stress. Cu-genes *copA* and *copB* have been studied in model bacteria *E. coli* and plant pathogenic bacteria *Pseudomonas spp.* However, the functions of these genes differ among bacterial species, and the mechanisms of Cu tolerance/resistance is still unclear in Xf. Here we study *copA* and *copB* function in Xf through site-directed mutagenesis. Both *copA* and *copB* mutants were more sensitive to Cu than wild-type (WT) Xf strains. During acute exposure to high concentrations of Cu, the *copA* mutant showed significantly lower survival, while the *copB* mutant did not differ from WT. Under chronic Cu stress, the copA mutant shows sensitivity to Cu concentration at 0.125 mM while *copB* mutant is sensitive to 0.030 mM Cu. We hypothesize that *copA* and *copB* function differently towards Cu tolerance/resistance in Xf. CopA likely works as an oxidase to help Cu detoxification, while CopB is a putative Cu exporter. Future studies will address the Cu-binding capacity of CopA and CopB, and damage caused by Cu in the mutants. This will help to develop disease management strategies against this plant pathogen.

Resistance to thiophanate-methyl in *Botrytis* spp. from greenhouse crops in Kansas
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Gray mold, caused by *Botrytis cinerea* and other *Botrytis* species, is a cosmopolitan disease affecting diverse plants, including greenhouse crops. Greenhouse growers use routine fungicide applications as part of gray mold management, and resistance to several fungicide classes has been reported. Our objective was to determine sensitivity to methyl benzimidazole carbamate (MBC) fungicides in Kansas isolates. In 2018, 34 single-spore isolates were cultured from greenhouse crops at 14 sites in Kansas. Isolates were tested on 9 cm diameter 1% Malt Extract Agar plates amended with 100 µg/ml thiophanate-methyl. Each isolate was plated on three fungicide-amended and three unamended control plates. The plates were incubated for 5 days in the dark at 22° C. Two colony diameters were measured, excluding the 5mm starter plug, and relative mycelial growth (RMG) was calculated as the average diameter on fungicide-amended media divided by the average diameter on the control times 100. The entire experiment was conducted twice. Of the 34 isolates, 27 exhibited resistance, with RMG ≥75%. Two isolates were highly sensitive, with RMG ≤5%. Five isolates exhibited intermediate RMG values. Results of the study indicate that resistance to MBC fungicides is common in gray mold in Kansas greenhouses. Ongoing and future studies will include additional isolates and sequence-based identification of the *Botrytis* isolates.

Allele-specific detection methods for QoI and DMI fungicide resistant *Erysiphe necator* isolates in vineyards
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Powdery mildew, caused by the fungus *Erysiphe necator*, is a constant threat to worldwide grape production. Management requires the repeated use of target-specific fungicides. Resistance to commonly used quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs) fungicides is known to occur in subpopulations of *E. necator* and is associated with single nucleotide polymorphisms (SNPs) in the *cytochrome b* and *cyp51* genes, respectively. DMI resistance in *E. necator* is further complicated by *cyp51* copy number variation. To track this resistance, two TaqMan-based monitoring tools were developed for both QoI and DMI resistance loci. For *cyp51*, a marker was developed to monitor both SNP and copy number variation simultaneously. Specificity and sensitivity of these assays showed that these tools could discriminate SNPs and were effective on mixed samples. We implemented these diagnostic tools to survey *E. necator* isolates collected from leaf and air samples from major grape growing areas in Washington, Oregon and California and findings were corroborated with EC50 values from fungicide-amended agar germination bioassays in the case of the *cytochrome b* gene and QoI resistance. Our findings showed that results from these TaqMan probe-based allele-specific assays and bioassays are highly correlated. Overall, these diagnostic tools will help grape growers make informed decisions on fungicide selections and applications, which in turn, will facilitate grapevine powdery mildew disease management and improved grape production systems.

Monitoring Fungicide Resistance in *Botrytis cinerea* on grapes in Michigan
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Botrytis cinerea is an important pathogen on small fruits worldwide. On grapes, it causes bunch rot at both pre- and post-harvest stages, where losses can reach up to 100%. Chemical control primarily relies on the prophylactic use of synthetic site-specific fungicides. Repeated applications of these products raise the risk of fungicide resistance development in *B. cinerea* populations, resulting in crop management failures. To determine the extent of resistance, *B. cinerea* isolates were collected from grape clusters in the northwest and southwest grape growing regions of Michigan in 2014 (n = 115) and 2018 (n = 125). These isolates were phenotyped using discriminatory doses of 8 fungicides to determine the levels of resistance. Fungicide resistance increased from 2014 to 2018 with a higher increase in fenhexamid, fluopyram, and iprodione resistance. *B. cinerea* isolates resistant to multiple fungicides were detected in 2014 and 2018 with a higher frequency being observed in 2018. Our data provide essential information to growers about the efficacy for

B. cinerea control using the available botryticides. Future work will develop molecular markers for resistance that can be used for early fungicide resistance detection in the field and in fungicide stewardship programs. The developed markers will be transferred to diagnostic clinics to assist growers in the management of bunch rots before resistance related control failures occur.

Detection of *Sdh*B mutations in *Botrytis cinerea* isolates from strawberry using a High-Resolution Melting (HRM) assay
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Mutations in the succinate dehydrogenase (*sdh*) complex subunit B are associated with different levels of resistance to succinate dehydrogenase inhibitor (SDHI) fungicides in *Botrytis cinerea*, the causal agent of Botrytis fruit rot (BFR) of strawberry. Due to the emergence of *B. cinerea* populations resistant to the various SDHIs deployed in strawberry fields, it is important to elucidate the genotype of such populations to limit the use of at-risk SDHIs. Thus, the objective of this study was to develop and validate a High-Resolution Melting (HRM) assay for the rapid detection of *sdh*B mutations using conidia from infected strawberry tissue. Two sets of HRM markers were designed based on the *sdh*B gene of *B. cinerea* from strawberry to detect either the H272R/Y or the P225F mutations. *Sdh*B characterization and mutation detection of 25 *B. cinerea* isolates collected during the 2017–18 Florida strawberry season were performed using the HRM and Sanger sequencing assays. Both assays confirmed the presence of the H272R and H272Y mutations in four and two isolates, respectively, whereas the P225F was only detected in one isolate. Our study presents a fast and cost-effective method to detect frequent (H272R/Y) and powerful (P225F) *sdh*B mutations. Furthermore, HRM analysis could be implemented in plant diagnostic clinics to monitor predominant genotypes in strawberry fields and inform growers about SDHI fungicides with high risk for selection of resistance that would limit BFR control.

Assessing the impact of the G137S substitution in *Venturia effusa* on QoI efficacy in commercial pecan orchards
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Every year Georgia pecan growers lose millions of dollars to pecan scab, caused by *Venturia effusa*. Scab management relies heavily on fungicides, including the quinone outside inhbitors (QoI; FRAC group 11). Recently, reduced QoI efficacy has been observed in a few Georgia orchards and previous research revealed that an amino acid substitution from glycine to serine at position 137 (G137S) within the *V. effusa cytochrome b* gene leads to partial QoI-resistance. However, the effect of G137S on QoI efficacy in the orchard is unknown. Thus, field trials were conducted in 2018 across six locations in southwest Georgia to determine the impact of G137S. At each location, individual terminals were treated biweekly with Abound (azoxystrobin) at a rate of 0.88 L/ha, for 10 applications/year. Nut scab severity was evaluated in August and approximately 20 isolates were collected from unsprayed portions of trees at each location to screen for mechanisms of resistance. Percent control of nut scab ranged from 25.7 to 75.2% in 2018 and G137S was found in three of the six orchards, ranging in incidence from 0.0 to 30.8%; no other mechanisms were present. Percent control decreased linearly with increasing G137S frequency, indicating that the reduced QoI efficacy may have been a result of a greater number of G137S isolates in the population. Monitoring Georgia pecan orchards for isolates harboring G137S could allow for the detection of major shifts in efficacy before they occur.

A high throughput bioassay to rapidly characterize *Erysiphe necator* resistance to demethylation inhibitor fungicides
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Fungicide resistance of *Erysiphe necator* to demethylation inhibitor (DMI, FRAC 3) and Quinone outside oxidase inhibitor fungicides is widespread in the Western US *E. necator* populations, increasing resistance pressure on more modern fungicide chemistries. Currently, resistance to DMI fungicides can only be detected using time and resource expensive live leaf assays because resistance to DMIs is quantitative and the genetic control is not fully understood. We hypothesize that the conidium does not contain enough metabolic precursors to synthesize the sterol, ergosterol, before having to synthesize *de novo*; thus, fungicide resistance will be expressed as increased germination tube development on fungicide amended matrix. *Erysiphe necator* conidia were dispersed onto a 24-well plate with fungicide amended gellan gum medium and imaged immediately, and again after 24 hours to measure the development and elongation of germination tubes. The images were processed in ImageJ using the “HyphaTracker” macro. The percent change in the germination tube growth from the untreated control over 24 hours was measured for each concentration of the fungicide. At 3 µg/ml active ingredient, sensitive isolates germination tube development is reduced by at least 50%. This method can objectively assess 4 to 5 isolates with only 3–4 hours of labor and a 24-hour incubation period, as compared to leaf disks bioassays, which require 12 hours of labor, a 14-day incubation.

Characterizing the sensitivity of Midwestern *Phytophthora infestans* isolates to mefenoxam
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Phytophthora infestans, the causal agent of late blight, is one of the most devastating and widespread pathogens affecting potato and tomato crops across the globe. US-23 (A1 mating type) has been the dominant *P. infestans* clonal lineage in the Midwest during 2017 and 2018 and is currently controlled with the fungicide mefenoxam. With the re-emergence of clonal lineage US-8 (A2 mating type), shown to be highly tolerant to mefenoxam and of an opposing mating type to the common US-23, there is a need for continual monitoring and isolate characterization in order to optimize control. Twenty eight *P. infestans* isolates collected from production fields of the Midwestern U.S. during 2017 and 2018 were tested for sensitivity to mefenoxam (100ppm) using amended plate assays. Relative growth was calculated from pathogen growth on control and amended plates. Overall, there was greater variation in relative growth on mefenoxam-amended media among isolates of the US-8 clonal lineage than isolates of US-23. In 2017, relative growth responses of

US-8 isolates ranged from approximately 25 to 100%, while the US-23 isolates predominantly ranged from 10–25% (Tukey's HSD Test). Among the ten 2017, US-23 isolates, a single isolate demonstrated complete insensitivity and was significantly different in its growth response when compared to the other nine. In 2018, there were no significant differences in growth response among all of the US-23 isolates. The finding of a mefenoxam-insensitive US-23 isolate underscores the need for continual pathogen monitoring in support of grower decision-making.

Evolution and spread of SDHI-resistant alleles within field populations of Zymoseptoria tritici in the UK
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In vitro experimentation evolution studies have shown that Zymoseptoria tritici can easily develop resistance to different succinate dehydrogenase (Sdh) inhibitor fungicides upon UV exposure. A range of mutations (33) resulting in amino acid substitutions at 21 different positions within SdhB, C and D were identified. Different levels of SDHI sensitivity were linked with different mutations but high insensitivity levels were measured for B-H267L, C-S83G, C-N86K and C-H152R. The first SDHI-insensitive field strains were detected in the UK in 2015, four years after high usage of SDHIs to Septoria leaf blotch control. Strains with C-H152R showed the highest level of in vitro insensitivity to several SDHIs (e.g. boscalid, isopyrazam, bixafen, fluxapyroxad and benzovindiflupyr) and could not be controlled in planta. However, although C-H152R is accumulating after SDHI-based treatments in the field during the season, the frequency remains low at the start of the following season, indicating a fitness penalty that was also reported in experimentation evolution studies. A non-target site resistance mechanism, present as standing variation in field populations, was found to affect only fluopyram and isofetamid. Up to 2018, 22 different Sdh mutations have been reported in UK strains. Mutations resulting in B-H267L and C-N86K were for the first time detected in 2018, as well as strains carrying Sdh mutations in combination with efflux pump overexpression and further monitoring is required to follow the spread of these strains and impact on disease control.

Fungicide sensitivity, detection the G143A point mutation associated a QoI´s and competitive fitness of *Magnaporthe oryzae* populations from Brazil
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Magnaporthe oryzae, the cause of leaf and panicle blast, is the most damaging rice pathogen worldwide, causing yield severe losses. Blast management is based mainly on the application of fungicides; however, the sensitivity of the pathogen populations to the main fungicides is largely unknown. More or less sensitive phenotypes were identified when exposed to the fungicides most used for disease control in Brazil such as azoxystrobin, tricyclazole, trifloxystrobin + tebuconazole and tebuconazole. About 90% of the genotypes collected in Tocantins State (where disease is usually most severe) were resistant to azoxystrobin, based on the detection of G143A mutation (resistance marker for QoIs). The EC_{50} value for these isolates was >10µg. L^{-1}. Most of these resistant isolates were significantly less fit than the sensitive ones when inoculated *in planta* as measured by the respective latent periods, infection efficiencies, sporulation intensities and blast severities. The proportion of resistant and sensitive isolates in mixed inoculum of rice plants remained constant in the presence of azoxystrobin for four generations. Conversely, in the absence of the fungicide, the resistant populations were progressively reduced. While mutants with multiple resistances to diverse active ingredients in different fungicide groups may also arise, the fitness costs associated with resistance to more than one active ingredient may impair competitive abilities of such mutants even further.

Rapid sampling techniques to monitor quinone outside inhibitor fungicide resistant *Erysiphe necator*
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Quinone outside inhibitor fungicide resistant *Erysiphe necator* can be monitored using a previously developed competitive TaqMan qPCR assay. However, it is unclear how to sample vineyards to accurately assess resistance in a field population. A visual assessment with observed colonies collected by rubbing a cotton swab over the leaf surface (leaf swab) was compared to rubbing a swab over worker gloves (glove swab). Over 1500 samples from 326 row transects were collected throughout the 2018 season from 12 commercial Oregon vineyards via a linear stratified sampling pattern where each sample type was collected from the same row. For each row, the leaf swabs were aggregated. Comparing presence-absence of leaf vs glove swabs, there was a 65% agreement between techniques. In 31% of sampled rows, glove swabs detected the presence of *E. necator* without visual detection, indicating that glove swabs may be able detect disease at lower levels than traditional disease scouting. When *E. necator* was detected via both leaf and glove swab, the samples agreed 98% of the time for presence of the resistance genotype at the row level. Cooperating growers also collected glove swab samples and results agreed 77% of the time with the researcher glove samples. These results indicate that swabbing vineyard worker gloves may be a viable technique for *E. necator* sampling and fungicide resistance monitoring with minimal interruption to grower production practices or added collection costs.

Fungicide Resistance Profiles In *Botrytis cinerea* From Commercial Cut Flower Roses And Influence of Cultural Practices on Spore Movement
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Botrytis cinerea is a fungal pathogen that causes gray mold in cut roses. The infection affects rose quality and results in significant economical loses during production, and retail environments. Commercial growers rely on fungicide applications as one of the primary strategies for disease management. However, increasing reports of *B. cinerea* isolates resistant to different single-site fungicides in different crops around the world have become a concern. Crop sanitation and microclimate manipulation are also considered key pieces for disease management. Six commercial shipments containing rose cultivar Orange Crush were received from two greenhouses at a farm in Colombia. Additionally, four commercial shipments of cut roses cultivar Freedom were received from Africa, South and Central. A high proportion of isolates collected from Orange Crush showed resistance to thiophanatemethyl, iprodione, boscalid, and cyprodinil. Resistance to thiophanatemethyl and cyprodinil was frequently observed in Freedom isolates. The frequency of resistance to penthiopyrad, fenhexamid, fluopyram, isofetamid, and fludioxonil was variable. No resistance was observed to pydiflumetofen. Hierarchical clustering analysis was used to evaluate relationships between *Botrytis* spore count and production activities. A higher spore count was observed during the weekdays, compared with weekend days. High spore count was observed when dumping refreshing solutions, fungicide application, lime application, harvest, pinch, removal diseased tissue, supplemental watering, and sweeping of the central aisle occurred

Survey for quinone outside inhibitor fungicide-resistant *Cercospora sojina* in Kentucky
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Quinone outside inhibitor (QoI) fungicides can have a high level of efficacy in controlling diseases. However, this class of fungicide has a high risk of selecting for resistant individuals within a fungal population. QoI fungicides have been used to control frogeye leaf spot (FLS) in soybean, caused by the fungus *Cercospora sojina*. Resistance to QoI fungicides in *C. sojina* isolates was first reported in 2010. Since these initial findings, *C. sojina* isolates resistant to QoI have been detected in 15 states, including Kentucky. The objective of this survey was to estimate the frequency of QoI-resistant in *C. sojina* populations collected in different soybean growing regions in Kentucky in 2018. A total of 131 single-spore isolates were obtained from soybean leaves with FLS symptoms collected in 9 counties. A discriminatory dose of 0.1 µg/ml technical grade azoxystrobin, or a molecular assay was used to distinguish QoI-resistant and –sensitive isolates. The frequency of QoI-resistant *C. sojina* isolates was 70%. This survey reports the presence of *C. sojina* isolates resistant to QoI fungicides in all counties, where samples were collected. To avoid widespread occurrence of QoI-resistant *C. sojina* isolates and economic losses, farmers should adopt integrated pest management strategies such as planting resistant cultivars, rotating to non-host crops, and applying different or mixed modes of action fungicides.

A rapid method for detection of *Sclerotinia sclerotiorum* sensitivity to Fluazinam and Carbendazim fungicides
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Chemical control is the most used method for white mold (*Sclerotinia sclerotiorum*) management by farmers, but fungicide effectiveness can be hampered by the emergence of resistant *S. sclerotiorum* isolates. The objective of this work was to develop a method to screen *S. sclerotiorum* resistance to fungicides by employing 96-well microplates and Alamar Blue dye, used as a cell viability indicator. Experimental procedures involved 20 isolates of *S. sclerotiorum* from different Brazilian regions, tested at 1×10^2, 10^3 and 10^4 ascospores mL^{-1}, along with Carbendazim and Fluazinam fungicides at 10, 100 and 1000 ppm. Evaluations included incubation periods of 72, 96, 120 and 144 hours, and comparisons of isolate aggressiveness after inoculation of common bean plants. Resistance to Carbendazim was observed in 90% of isolates, compared to 55% of isolates resistant to Fluazinam. Isolates SS48 and SS67 showed multiple resistance to both fungicides (100ppm) and high aggressiveness. Resistance to fungicides was not associated to geographical origin of isolates, according to the hierarchical grouping test by level of resistance. Despite an average of 40 days necessary to obtain ascospores from carpogenic germination in the laboratory, the microtiter method consists in a fast procedure, with reduction of labor, bench area and toxic laboratory residues. This is a new method that allows laboratory routines for screening *S. sclerotiorum* resistance to fungicides from any host plant.

Mapping of a clubroot resistance gene from rutabaga using bulked-segregant RNA-Seq
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Clubroot, caused by the obligate pathogen *Plasmodiophora brassicae* Woronin, is an important disease of brassica crops. Use of genetic resistance is currently the most effective strategy for clubroot management. The aim of this research was to identify and map resistance to clubroot in rutabaga cultivar 'Wilhelmsburger' (*Brassica napus*). A BC_1 population was developed from a cross of 'Wilhelmsburger' with DH16516 (susceptible canola line). The BC_1 population and parental lines were tested for resistance to pathotype 3 (Williams' system) of *P. brassicae* collected from canola in Alberta, Canada. Resistance and susceptibility segregated in a 1:1 ratio in the BC_1, indicating that a single major gene controlled the resistance. A new mapping method based on the percentage of polymorphic variants (PPV) from bulk-segregant analysis-RNA Seq was used to map the resistance gene. In this analysis, the resistance gene is generally located on the chromosome with the highest PPV; chromosome A03 had by far the highest PPV. KASP assays are being conducted to confirm the location as chromosome A03 and to fine map the gene.

Mapping disease resistance in the presence of random meiotic pairing: A case study in rose
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Genetic mapping in polyploid organisms that undergo random pairing during meiosis is a difficult process. Early genetic maps were constructed using single dose allele markers. These markers cannot be used to identify homoeologous chromosomes. Software capable of identifying allelic dosage for SNP markers and mapping software that allows for the use of higher-dose markers has recently become available, allowing for the creation of high-density maps of segmental allopolyploids, like hybrid roses. The unmapped black spot resistance gene *Rdr3* was previously identified in the tetraploid rose 'George Vancouver'. A mapping population was developed by crossing 'George Vancouver' with the susceptible tetraploid 'Morden Blush'. Phenotyping was conducted via a detached leaf assay with *Diplocaron rosae* race 8 and the population was genotyped with the WagRhSNP 68K rose Axiom array. Allelic dosage assignment and genetic mapping were conducted using the R packages 'fitPoly' and 'polymapR', respectively. *Rdr3* was mapped to a chromosome 6 homoeolog. To date, three additional black spot resistance genes (*Rdr1, Rdr2,* and *Rdr4*) have been mapped. *Rdr1* and *Rdr2* map to a chromosome 1 homoeolog and *Rdr4* maps to a chromosome 5 homoeolog. *Rdr3* is a unique gene and not an allele of the other genes as it maps to a different region. Mapping *Rdr3* demonstrates how new tools can be used to create high-density maps to begin answering complex genetic questions surrounding polyploids.

Response of sorghum enhanced in monolignol biosynthesis to stalk rot pathogens

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To increase phenylpropanoid constituents and energy content in the C_4 grass *Sorghum bicolor* (L.) Moench, sorghum genes encoding for the following proteins related to monolignol biosynthesis were overexpressed: SbMyb60 (transcriptional activator), SbPAL (phenylalanine ammonia lyase), SbCCoAOMT (caffeoyl coenzyme A 3-*O*-methyl transferase), Bmr2 (4-coumarate:CoA ligase) and SbC3H (coumaroyl shikimate 3-hydroxylase). Overexpression lines were evaluated for responses to yield-reducing stalk pathogens under greenhouse and field conditions. Greenhouse-grown plants were inoculated with *Fusarium thapsinum* (Fusarium stalk rot) and *Macrophomina phaseolina* (charcoal rot). No significant differences in mean lesion lengths between wild-type and *F. thapsinum*-inoculated overexpression lines were observed, except for smaller lesions on two of three *SbMyb60* and one of two *SbCCoAOMT* lines. No significant differences in lesion sizes were observed in *M. phaseolina*-inoculated overexpression lines as compared with wild-type except one (of two) *SbPAL* line with larger lesions. Field-grown *SbMyb60* overexpression lines inoculated with *F. thapsinum* had mean lesions similar to wild-type but of two *SbCCoAOMT* lines, one had larger lesions compared to wild-type. These data suggest that lines with increased phenylpropanoid and energy content can be effectively deployed in the field without significant yield losses occurring from stalk pathogens.

The quest for fruit resistance in American cranberry

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The primary threat to cranberry production in the northeast is losses due to fruit rot in the field. The problem is increasing in other parts of the U.S., exacerbated by climate warming trends. Even with well-timed fungicide regimes, fruit rot incidence losses continue to reach 25% or more. Fruit rot is caused by a complex of pathogenic fungi from several genera. We have identified four distinct sources of broad-spectrum resistance from our germplasm collection. Crosses to pyramid putative resistance genes in horticulturally elite genetic backgrounds have resulted in populations segregating for fruit rot resistance and higher productivity. Utilizing our 3rd generation genome assembly and genotyping by sequencing (GBS), we have identified nine quantitative trait loci (QTL) associated with resistance. QTL for other key traits have also been mapped, e.g., yield. Research continues to validate the QTL in advanced breeding populations and marker development for marker-assisted selection. The mechanism(s) of fruit rot resistance is also being explored.

Evaluating Bacterial Wilt Resistant Tomato Lines for Resistance QTLs and Their Disease Reaction Against Phylotypes I and II Strain of *Ralstonia* spp.

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Bacterial wilt caused by *Ralstonia* spp. causes substantial yield losses on tomato. Disease management by chemicals are limited and host resistance is the cheapest and easiest means of control. However, the location and strain specific nature of resistance makes it difficult to breed varieties that are resistant across wide geography. We characterized a global set of bacterial wilt resistance tomato sources available at the World Vegetable Center, Taiwan, for presence of two major disease resistance QTLs, *Bwr*-6 and *Bwr-12*. We also correlated the genotypes of the resistant sources with their disease reactions against one strain each of phylotype I (*R. pseudosolanacearum*; Pss4) and phylotype II (*R. solanacearum*; Pss1632) by drench inoculation (10^8 CFU/ml) in the greenhouse. Out of sixty-seven entries tested, five had *Bwr*-6, nineteen had *Bwr-12* and six entries had both QTLs present. *Bwr-12* contributed to stable resistance against phylotype I but not against phylotype II strain. *Bwr-6* contributed to stable resistance against both phylotypes I and II. The entries with both QTLs performed the best overall. Interestingly, entry '94T765-24-79' that lacked both QTLs demonstrated high resistance against the phylotype II strain and may be a source of new disease resistance QTL/s. Understanding the interaction between distribution of resistance QTLs and corresponding disease reaction against phylotype I and II strains may help to unravel molecular genetics behind location specific and strain specific nature of bacterial wilt resistance in tomato for effective disease management.

Genome wide association studies of Fusarium wilt resistance in spinach (*Spinacia oleracea* L.)

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In the U.S., the Pacific Northwest (PNW) is the only area suitable for spinach (*Spinacia oleracea* L.) seed production. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *spinaciae* (*Fos*), is responsible for major losses in PNW spinach seed production. Most parental lines used in hybrid spinach seed crops are very susceptible to *Fos*. Genome wide association studies (GWAS) were initiated to identify sources of resistance to *Fos*. Spinach genotypes (n = 351) were evaluated for reactions to *Fos* using moderate and high inoculum levels from a mix of three isolates (Fus058, Fus254, and Fus322), with two replicates of a factorial design of genotypes and inoculum levels. A Fusarium wilt severity index (FWSI, 0 to 1) and area under the disease progress curve (AUDPC) calculated for wilt severity rated 21, 28, and 35 days after planting (DAP) in moderate risk soil ranged from 0 to 0.95 and 0 to 10.8, respectively. SNP markers (7,887) identified with genotyping by sequencing were used for marker-trait association with mixed linear model (MLM) in TASSEL 5.2.51. Population structure analysis showed two subpopulations ($Q1$ = 35.8%, $Q2$= 34.4%), with 29.6% of accessions showing admixture. Nine SNPs were associated with AUDPC in moderate risk soil (R^2 = 3.8 to 9.3%, -log P = 3.01 to 5.06). Five SNPs also were associated with FWSI 35 DAP (R^2 = 4.4 to 6.7%, -log P = 3.22 to 5.35). The SNPs may be used to develop high-throughput markers to breed for Fusarium wilt resistance in spinach.

Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2018
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Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), occurred in more than 16 states and stripe rust of barley, caused by *P. striiformis* f. sp. *hordei* (*Psh*), occurred mainly in the western U.S. 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. Twelve *Psh* races were detected, including 11 previously existing and one new race, of which PSH-33 (virulent on barley differentials Topper and Abed Binder 12) and PSH-97 (virulent on Topper, Abed Binder 12, and Bancroft) were the predominant races with frequencies of 20.0% and 17.1%, respectively. Of 26 *Pst* races identified, 24 were previously existing and two new races, PSTv-378 virulent on *Yr1*, *Yr6*, and *Yr8* and PSTv-379 virulent on *Yr1*, *Yr6*, *Yr9*, *Yr27*, *YrSP*, *YrTr1*, and *Yr76*. The five predominant races were PSTv-37 (41.4%), PSTv-52 (11.9%), PSTv-322 (6.6%), PSTv-39 (6.1%), and PSTv-201 (5.7%) with PSTv-37 detected in all sampled states, PSTv-52 in five eastern and western states, and PSTv-39, PSTv-201, and PSTv-322 almost restrictively in the western states. The frequencies of virulence to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2* were high (60.4-89.3%) while those to *Yr1*, *Yr10*, *Yr24*, *Yr32*, *YrSP*, and *Yr76* were low (2.2-27.6%). No virulence was detected to either *Yr5* or *Yr15*. The results are useful for breeding resistant cultivars and control of stripe rust.

ISF guidelines on the nomination of novel plant pathogen races and strains
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Disease resistance is a major goal in breeding new varieties and plays a key role in vegetable crop production and integrated pest management practices. It is also carefully described to differentiate new varieties from older ones on the market. The objective of the International Seed Federation Working Group on Disease Resistance Terminology is to promote the consistent use of terminology in relation to disease resistance. The Working Group codes pathogens for which companies claim resistance in their varieties, promotes harmonized terminology across the industry to avoid any liability due to miscommunication, develops host differentials and establishes procedures based on peer-reviewed scientific publication and industry practices to identify pathogen races/strains. To support the decision process associated with the nomination of novel races, the Working Group has formulated recommended guidelines. The recommendations constitute the minimum criteria to be fulfilled in order to nominate a novel race for a specific plant pest. An important selection criteria is the economic importance of the emerging resistance breaking event. The pest caused significant economic damage, the extent of this event is significant and the event is recurrent and observed over multiple growing seasons and/or years. The guidelines include basic rules for nomination and numbering of novel races. The nomination criteria and examples of numbering and differentials will be presented.

Characterizing the genetic basis of silver scurf resistance in the diploid potato clone 'C287'
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Silver scurf of potato, caused by the fungus *Helminthosporium solani,* is a tuber blemish disease of great and increasing concern to the United States potato industry. There are currently no commercially available silver scurf-resistant cultivars, nor are there consistently effective cultural management practices. Previous studies have identified potato breeding clone C287 with resistance to silver scurf. We aim to determine the genetic attributes responsible for silver scurf resistance in C287. This work is useful for breeders, as they can integrate this resistance into their breeding programs to further enhance management of this disease. C287 was crossed with M6, a diploid self-compatible inbred line of the potato wild relative *Solanum chacoense,* which is susceptible to silver scurf. From the progeny, F1-22 was selected (based on disease resistance and tuber type) and self-pollinated to create 135 F2 plants. Replicated inoculated field trials were conducted in 2017 and 2018 to screen for resistance. Preliminary results indicate that there are significant differences among F2 clones, including tuber weight and number, indicating that segregation occurred for these traits in this population. Disease symptoms were not visually apparent, so quantitative PCR was used to quantify pathogen presence. Results show variation in resistance between F2 clones. We will use single nucleotide polymorphisms to look for quantitative trait loci associated with silver scurf resistance. This will determine whether a marker for silver scurf resistance can be identified in potato.

Fusarium Root Rot Resistant Genotypes and Genomic Regions Identified in Two Major Common Bean Gene Pools
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Accelerating crop improvement in common bean (*Phaselous vulgaris* L.), a staple food across many parts of the world, is key to ensuring global nutritional security; however, root rot is a yield limiting constraint worldwide. Identification of resistant lines and genomic regions associated with key resistance genes can facilitate breeding for resistance. The Andean (ADP) and Middle American (MDP) diversity panels, representing common bean genetic diversity from two major gene pools, were screened in the greenhouse for root rot using a mixture of nine *Fusarium solani* isolates as a source of inoculum. The assay was highly repeatable across five control genotypes based on the relative effects. Lines with high resistance to *F. solani* were identified in ADP and MDP based on the reaction of the control lines. To identify loci underlying variation related to Fusarium root rot, genome-wide association study was performed on quantitative (disease score and disease index) and qualitative (binary and three class) phenotypic data. Common genomic regions were observed for disease score, disease index and the three-class system on Pv08 and Pv07 in the ADP and MDP, respectively. There were no common significant intervals between ADP and MDP, indicating separate genetic controls for resistance in the two gene pools. The information provides a basis for bean improvement through use of resistant genotypes and marker-assisted breeding for more durable root rot resistance.

Enabling gene pyramiding of rust resistance genes in common bean by untangling their epistatic interactions
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Genetic resistance to *Uromyces appendiculatus*, the rust pathogen of common bean, is conferred by single and dominant genes. Gene pyramiding is essential to manage the extensive and shifting virulence diversity of *U. appendiculatus*. However, the pyramiding of genes exhibiting epistasis is difficult. Alas, epistasis is frequent among rust resistance genes of common bean. In epistasis, one gene (epistatic) masks the presence of another gene (hypostatic) and prevents the identification of the masked genes. In this study, we wanted to investigate the presumed epistasis between *Ur-3* and *Ur-5* genes that have been used extensively in breeding programs. *Ur-3* confers resistance to 55 and *Ur-5* to 73 of 94, races of *U. appendiculatus*. The resistance reactions of *Ur-3* and *Ur-5* are hypersensitive spots and tiny pustules, respectively. We used an F_2 population from a cross between common bean cultivars Aurora (with *Ur-3*) and Mexico 309 (with *Ur-5*), four specific races of the rust pathogen, and the new SS68 and SS183 KASP markers developed using high-throughput genotyping and sequencing technologies. Inoculation of the F_2 plants with the rust pathogen races revealed a 12:3:1 phenotypic segregation ratio (hypersensitive spots: tiny pustules: large pustules), indicating that *Ur-3* was epistatic to *Ur-5*. The SS68 and SS183 markers that were tightly linked to *Ur-3* and *Ur-5* respectively, also established that *Ur-3* was epistatic to *Ur-5*. In addition, these markers will facilitate and speed up the development of dry and snap bean cultivars combining *Ur-3* and *Ur-5* with other rust resistance genes.

High-density SNP linkage mapping and single marker association analysis of soybean RILs segregating for resistance against Phytophthora root rot
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Phytophthora root and stem rot (PRR) is a major disease affecting soybean crops worldwide. PRR is caused by *Phytophthora sojae*, a soilborne pathogen that is managed by the deployment of major genes known as *Rps* (Resistance to *Phytophthora sojae*). To date, 32 *Rps* genes have been mapped but not all are effective, or their effectiveness has been evaluated in the US. The PI 424354 was identified as a potential source of novel *Rps* genes. A recombinant inbred mapping population, PI 424354 x Williams, was advanced to $F_{5:7}$ by single seed descent. Seedlings from 81 recombinant inbred lines (RILs) were phenotyped to different pathotypes of *P. sojae* corresponding to isolates OH1 (vir 7), OH4 (vir 1a, 1c, 7), OH7 (vir 1a, 3a, 3c, 4, 5, 6, 7), and OH25 (vir 1a, 1b, 1c, 1k, 7), and further genotyped using an Illumina Infinium SNP-6K BeadChip array. Single marker association analysis was first done using ANOVA, and significant markers were used to create linkage maps. High-density SNP mapping and single marker association analysis detected two new putative *Rps* loci on chromosomes 11 and 19, as well as previously reported resistance loci to *P. sojae* isolates on chromosomes 3, 13 and 18. Five independent *Rps* genes were mapped in this RIL population PI424354 x Williams, 2 towards OH 7, and 3 towards OH1, OH4, and OH25. This indicates that there are effectors within isolates of *P. sojae* for additional *Rps* genes and emphasizes the importance of screening new sources towards a wider range of isolates prior to breeding.

***Phytophthora sojae* pathotype variability across Ohio, Indiana and Kentucky**
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Phytophthora sojae, the causal agent of Phytophthora root and stem rot (PRR) of soybean, is an oomycete pathogen that can cause significant soybean yield reductions. A 2014 study estimated that annual yield losses attributed to PRR across the North Central region of the United States were 33 million bushels. This disease can be managed effectively through R-gene mediated resistance, but previous studies have shown an increasing number of *P. sojae* isolates that can adapt to the resistance conferred by several commonly deployed *Rps* (Resistance to *Phytophthora sojae*) genes. This study examined the population pathotype diversity among *P. sojae* isolates collected from Ohio, Indiana, and Kentucky to evaluate the effectiveness of a set of R genes. Each isolate of *P. sojae* was tested on a set of 16 differential lines containing the following *Rps* genes: *Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, *Rps1k*, *Rps2*, *Rps3a*, *Rps3b*, *Rps3c*, *Rps4*, *Rps5*, *Rps6*, *Rps7* and *Rps8*. Preliminary results show an average pathotype complexity of 6.74 on 15 differential lines based on 416

isolates from Ohio, Indiana, and Kentucky. Specifically, 206 *P. sojae* isolates from Ohio had an average pathotype complexity of 8.02, while 122 isolates from Indiana and 88 isolates from Kentucky had average pathotype complexities of 5.67and 5.22, respectively. The results of this study provide valuable and current information to soybean breeders and farmers about the effectiveness of different *Rps* genes across these three states, as well as showing the importance of combining multiple types of resistance to manage this disease.

Phenotyping the sorghum nested association mapping (NAM) parents for charcoal rot resistance
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Sorghum charcoal rot, caused by *Macrophomina phaseolina* (MP), is an economically significant disease in Kansas. An effective strategy to control this disease is to understand the genetic variation underlying charcoal rot resistance. The lack of accurate phenotyping and environmental variation has constrained genetic improvement of charcoal rot resistance. The sorghum NAM parents (ten diverse global lines crossed with an elite breeding line) have been used as a powerful population for trait dissection. The objective of this study was to phenotype the sorghum NAM parents for charcoal rot resistance. Sorghum NAM parental lines have been phenotyped against MP in the greenhouse using stalk injections with liquid cultures at 14 days after flowering (DAF). Mock-inoculated checks were injected with phosphate buffer (10 mM). Plant height, yield parameters, lesion length (measured from split stalks), and nodes crossed by the lesion were collected at 56 DAF. The results showed no significant difference between P898012, SC1345, SC971, Ajabsido, and Segaolane based on mean lesion length. However, comparison with the other six parents suggested the aforementioned lines are susceptible. SC1103 showed the lowest lesion length. Phenotyping will be repeated from greenhouse and field for confirmation. Based on phenotyping, transcriptome analysis will be performed to understand and dissect the molecular mechanisms of charcoal rot resistance using a NAM parental population.

Evaluation of resistance to *Ralstonia solanacearum* in blueberry and identification of functional resistance genes
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Florida blueberry production is expanding rapidly due to the development of low chill varieties. An emerging blueberry disease, bacterial wilt, caused by *Ralstonia solanacearum,* was identified throughout the state, threatening blueberry production. Discovery of resistance genes (R-genes) in blueberry species will assist the development of resistant varieties, which is the most effective method to control bacterial wilt. In this study, 18 blueberry genotypes including highbush (*Vaccinium corymbosum*) and rabbiteye (*Vaccinium ashei*) species were screened for resistance to three different populations of *R. solanacearum* isolated from diseased blueberry fields in Florida. *V. corymbosum* 'Arcadia' (susceptible) and *V. ashei* 15-506ASH (resistant) genomes, were sequenced using Illumina HiSeq X Ten platform, and were assembled and annotated predicting 118,772 and 87,512 ORFs, respectively. Using a known set of 106 R-gene sequences to build a profile and scan the genomes using a Hidden Markov Model software, we predicted 483 and 453 candidate R-genes respectively. A preliminary phylogenetic placement of candidate R-genes identified at least five families. Currently, we are testing protocols for RNA-seq with the long-read MinION™ sequencing platform with the ultimate goal of discovering functional R-genes against bacterial wilt. *In silico* predicted R-genes will be used to increase the catalog of R-genes in blueberry and to design R-gene oligos for enrichment sequencing (RenSeq).

Phenotyping soybean lines for resistance to multiple *Fusarium* species
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Fusarium root rot is among the top ten most destructive diseases of soybean in the United States. It is caused by a complex of *Fusarium* species which interact and increase symptoms of other soil-borne diseases. Management relies primarily on seed treatments which are limited to early growth stages and lack efficacy against all species of Fusaria. Breeding for genetic resistance in soybean cultivars is a viable alternative for managing diseases caused by these pathogens. In this study, virulent isolates of *F. graminearum*, *F. oxysporum*, and *F. solani* were used to identify resistant parental lines of soybean populations previously characterized for *F. virguliforme* resistance. Of the ten parental lines, only one showed resistant reactions to all three species of *Fusarium*, while four lines showed resistance to *F. oxysporum* and *F. solani*. This information can be used to guide investigations to identify markers for resistance to the Fusarium root rot complex in soybean breeding populations. The identification of soybean germplasm conferring resistance to this disease complex provides information to improve available cultivars and better manage seedling diseases.

Disease development in cotton when co-inoculated with the soilborne fungi *Fusarium oxysporum* f. sp. *vasinfectum* race 4 and *Rhizoctonia solani*
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Fusarium oxysporum f. sp. *vasinfectum* (FOV) race 4 is a virulent wilt pathogen of cotton, that can also cause seedling damping-off when high levels of inoculum are present in the field. *Rhizoctonia solani* can also cause seedling disease in cotton. Previous speculations have suggested there is an increase in wilt symptoms caused by FOV race 4 when *R. solani* is simultaneously present in the field. Therefore, the goal of this study was to evaluate the possible interactions between FOV race 4 and *R. solani* in a co-inoculation assay. Planting material included one FOV race 4 susceptible line and four FOV race 4 resistant lines. Oat-infested inoculum, was prepared using isolates of FOV race 4 and *R. solani*. The infested oats were mixed with potting soil (1-part fungal inoculum to 3-parts

potting soil) and cotton seeds were planted into one of the following treatments; FOV race 4 alone, *R. solani* alone, co-inoculation with both pathogens, and non-inoculated oats were used as a control. After six weeks, data for stand count, plant height, foliar and vascular symptoms were recorded. For all data collected the co-inoculation treatment was significantly different from the other fungal treatments ($P < 0.05$), producing the greatest amount of disease symptoms. There was no significant difference between varieties. Our results suggest infection by *R. solani* may compromise the plants ability to compensate for FOV race 4 stresses thereby increasing FOV race 4 symptoms in the field.

Genotypic and phenotypic evaluation of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 isolates collected from cotton in California

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Fusarium oxysporum f. sp. *vasinfectum* (FOV) race 4 is an aggressive wilt pathogen of cotton that is also capable of causing seedling mortality. FOV race 4, first identified in California in 2001, has been a recurring and expanding threat to California cotton. The goal of this research was to genotype and phenotype current field populations of FOV race 4 in California. A total of 138 isolates were collected from seven commercial or grower fields in the San Joaquin Valley during the 2017 and 2018 growing season. Isolates were genotyped using FOV race 4 specific primers, and DNA sequencing of the translation elongation factor gene. Previously only one haplotype of FOV race 4 was identified in California, however our results identified two unique haplotypes. Sixteen representative isolates were evaluated for wilt and seedling disease capabilities using a root dip inoculation and rolled towel assay, respectively. Plant material for both assays included the FOV race 4-moderately resistant Upland cultivar FM-2334 and susceptible Pima cultivar PHY-830. For the root dip assay, plants were evaluated at six weeks for foliar and vascular discoloration symptoms. For the rolled towel assay, seedlings were rated at ten days using a disease severity index and ordinal rating scale. There was a significant difference for isolate and variety ($P < 0.0001$) for all data collected in both assays. All isolates were able to produce wilt and seedling symptoms on cotton.

Pathogenicity diversity of korean race K3a of *Xanthomonas oryzae* pv. *oryzae*

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In Korea, *Xanthomonas oryzae* pv. *oryzae* is divided into K1, K2, K3, K3a, K4 and K5 race by using differential varieties of Milyang23, Cheongcheongbyeo, Pungsanbyeo, Hangangchalbyeo and Milyang42. There is a difference between theory and current situation. The reason is that this differential varieties are Tongil-type and discrimination system constructed from the 1970s to the 1980s. So this study was carried out for reclassification of race of *Xanthomonas oryzae* pv. *oryzae*. Isolates collected from 2001 to 2018 are divided using differential varieties. also, pathogenicity precisely checked up using the IRBB series (IR24, IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14, IRBB21), which is a Near-isogenic lines. As a result of comparing the pathogenicity with the classified race, it was confirmed that some of the pathogens classified as K3a race were different pathogenicity from the previously reported pathogenicity of K3a race. In other words, although K3a has been reported to show a resistance response to *Xa21*, a group of K3a race showing certain susceptibility to *Xa21* has been identified. In this way, although the classification system can be classified into the same race, it can be divided into different groups by confirming the pathogenicity by monogenic near-isogenic lines. Based on this, it is necessary to establish a detailed and efficient classification system of korean race classification.

Identification of a New Class of Transcription Activation-Like Effector, *pthXp1,* Among *Xanthomonas perforans* Strains Collected in Alabama

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Bacterial leaf spot of tomato, caused by *X. perforans,* is an endemic disease in the southeast United States and major limiting factor to the production of tomatoes worldwide. The emergence of novel *X. perforans* lineages with varying type 3 secreted effector repertoires has important implications in the sustainability of genetic resistance as an effective disease management tool. One such resistance, *Bs4*, is found in several tomato varieties and confers a hypersensitive response to *Xanthomonas* sp. with a cognate avirulence gene, either *avrBs3* or *avrBs4*. In a recent survey of the *X. perforans* population responsible for bacterial leaf spot of tomato in Alabama, we identified the presence of a transcription activation-like (TAL) effector with homology to *avrBs3*. As the strains carrying this gene failed to induce a *Bs4*-mediated resistance in tomato cv. Bonny Best, we obtained the completed TAL-effector sequence using a Phusion PCR approach. The sequence differed from the *avrBs3* and *avrBs4* effectors commonly found in related *Xanthomonas* sp. in that it was composed of 15.5 tandem repeats of 34 aa in length, rather than 17.5 and displayed 90 to 92% aa identity. Analysis of the repeat variable di-residues showed that the gene shared several blocks of homology with *avrBs4*; however, could not be assigned to same class as any TAL effector for which sequence data was available. As the predicted binding-specificity and host recognition of the gene was distinct from previously described TAL effectors in bacterial-spot xanthomonads, we propose the name *pthXp1*.

Application of *in vivo* imaging system (IVIS) and labeled *Xanthomonas* strains to quantify bacterial colonization as a measure of disease resistance

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Biological assessment of disease resistance in plants often relies on subjective visual assessment by experts, is costly and time consuming, and may be subject to high environmental and experimental variation resulting in low heritability. *In vivo* imaging technology is a non-invasive, rapid and unbiased tool that can be used to study the plant host-pathogen interaction and assess disease severity. We developed a strain of *X. gardneri* that expresses bioluminescence (lux operon) as a way to visualize and quantify infection. The objectives of this study were to determine the correlation between luminescence signals and bacterial populations and to make comparisons between resistant and susceptible tomato lines. Four week-old tomato seedlings were sprayed with a bacterial suspension (10^8 CFU/ml) and kept under high humidity. Leaflets were excised and luminescence signals were measured at 3, 4, 5, 8, and 9 days post-inoculation (dpi). Bacterial counts were estimated using serial dilution plating after each time point. Regression analysis displayed a positive relationship between bacterial counts and luminescence in infected plant tissue (P= <.0001, R=.71). Tomato lines with different resistance loci were evaluated at 9 dpi in a completely random design. Quantification of bacterial populations using IVIS could distinguish between susceptible and resistant lines and the heritability was 0.24. This study illustrates the use of IVIS for phenotypic evaluation of disease resistance in tomato. Future studies will focus on evaluating larger collections of germplasm for resistance.

Phenotypic and genotypic characterization of resistance to *Ustilago maydis* from teosinte and maize-teosinte introgression lines
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Ustilago maydis (*U. maydis*), the causal agent of corn smut, is an important agricultural pathogen that causes about $1 billion yield loss annually in the United States. The lack of resistant maize cultivars necessitates the identification of new sources of resistance. Teosinte, a wild progenitor of maize, has demonstrated resistance to various diseases and insects. We identified two maize-teosinte near isogenic lines (NILs) from a maize (B73)-teosinte (*Zea parviglumis*) introgression population that were resistant to *U. maydis*. Genotypic analysis of the two resistant NILs identified the same 3.6 Mbp and 3.9 Mbp teosinte introgression that were present only in the two resistant NILs. Teosinte has not been sequenced. Therefore, comparative analysis of the introgressed regions and the maize reference genome identified seven protein kinase genes that were collinear to the teosinte introgressed regions in two NILs. This study aims to 1) Characterize the seven-protein kinase R-genes (resistance genes) in teosinte and the two NILs, and 2) Perform whole genome sequencing of the teosinte parent. We designed gene-specific primers and used a PCR-based approach to clone and sequence the seven R-genes. Whole genome sequencing of the teosinte parent is ongoing. Data from this work will identify the R-genes and defense related genes in the two maize-teosinte NILs that are associated with the resistance. The selected R-genes can potentially be used to improve resistance to *U. maydis*.

Fighting a cereal killer: Novel sources of resistance to wheat head blast
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Wheat head blast (WHB), caused by the fungus *Magnaporthe oryzae Triticum* (MoT) is a major concern in South America and in South Asia. To date, the 2NS translocation is the only useful source of resistance to WHB effective against MoT prevalent races. Our objective was to identify non-2NS sources of resistance to WHB among elite cultivars, breeding lines, and wild-relative accessions. Over 780 accessions were evaluated under field and greenhouse conditions in Brazil and Bolivia and in biosafety level-3 laboratories in the U.S. The MoT isolates B-71 (2012), 008 (2015), and 16MoT001 (2016) were used for controlled experiments, while isolate 008 was used for field experiments. A wheat differential set was used to characterize the aggressiveness of the isolates. Resistant and susceptible checks were included at all experiments. Four spring wheat CIMMYT breeding lines (CM22, CM49, CM52, and CM61) and four wheat wild-relatives, *Ae. tauschii* (10142, 1624, 1667, and 10140) were identified as resistant (<5% of severity) or moderately resistant (5% severity <25%) to WHB. Experiments conducted at the seedling stage showed little correlation with disease severity at the head stage. MoT isolate 16MoT001 was significantly more aggressive against 2NS-based varieties. The low frequency of WHB resistance and the increase in aggressiveness of newer MoT isolates highlight the threat that the disease poses to wheat production worldwide and the urgent need to identify and deploy novel sources of resistance.

Do Resistance Genes Act Synergistically Against Grapevine Powdery Mildew (*Erysiphe necator*)?
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Stacking resistance genes has been suggested as a path to durable resistance. For the *Vitis / Erysiphe necator* pathosystem, we assessed the impact of two major (*RUN1* and *REN6*) and two moderate (*REN1* and *REN7*) resistance loci combined in 16 unique full-sib host genotypes (verified by AmpSeq markers). Each genotype was inoculated using 3 *E. necator* isolates with differential virulence on *RUN1* vines (avirulent NY90, fully virulent Musc4, and moderately virulent NY1-137). Disease progress was assessed non-destructively at 2, 4, 6 and 9 days post inoculation using a high-throughput robotic system and convolutional neural network, verified by manual microscopic examinations. Disease severity increased as a linear or exponential function over time in all genetic backgrounds. These preliminary data suggest that stacking a second gene with *REN1* or *REN7* provided more resistance than *REN1* or *REN7* alone, but in contrast *RUN1* or *REN6* resistance was not significantly improved when more genes were added. An exception was observed with Musc4, which coevolved with *V. rotundifolia* and is fully virulent on *RUN1* vines; it colonized more tissue than the other isolates on *RUN1*-containing germplasm. NY1-137, which was recently isolated from *RUN1* vines, was intermediate in virulence. The foregoing

results suggest that efficacy and durability of gene stacking depends on the resistance genes used and the virulence carrying capacity of the pathogen.

Races of *Puccinia graminis* f. sp. *tritici* with virulence to stem rust resistance genes effective against Ug99
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Wheat stem rust, caused by *Puccinia graminis*f. sp. *tritici* (*Pgt*), is a reemerging disease posing threat to wheat production worldwide, as exemplified by the occurrence and spread of *Sr31*-virulent races in the Ug99 race group. In an effort to mitigate the impact of Ug99, several resistance genes effective against races in the Ug99 group were identified and bred into commercial varieties. In the last six years, several stem rust outbreaks and local epidemics have occurred in East Africa, Europe and Central Asia. Surprisingly, all of these recent stem rust events were caused by *Pgt*races not related to Ug99. Some of these races, such as TKTTF and TTRTF, have been found with a wide geographic distribution, as they have been identified in the Caucasus, Europe, and East Africa. Besides causing severe damage on bread and durum wheat crops and significant losses to wheat farmers, many of these *Pgt*races have significant virulence combinations on genes that are effective against races in the Ug99 race group. Virulence to *Sr*genes alone or in combinations have been observed on *Sr13a*, *Sr22*, *Sr32*, *Sr35*, *Sr37*, *Sr40*, *Sr45*, *Sr53*, and $Sr1RS^{Amigo}$. These results highlight the need for continued efforts in identifying new stem rust resistance genes that are effective not only against races in the Ug99 group, but also effective against these newly emerged races with broad virulence combinations.

Mapping a Promising Susceptibility Gene for Fusarium Head Blight in Wheat
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Fusarium Head Blight (FHB), caused by *Fusarium graminearum,* is one of the most devastating diseases of wheat as it reduces both the yield and quality of the crop. Deploying genetic resistance is the most economical and sustainable strategy to manage FHB. However, only a few genes conferring partial quantitative resistance against FHB are known. The susceptibility genes pave a new way to the genetic control as compared to much commonly used resistance genes. The objective of this study is to map a susceptibility gene for FHB on chromosome 7A of wheat. Deletion lines and substitution lines for 7A along with Chinese Spring and Nulli7A as controls were used for the study. Genome specific markers were developed and each deletion line was molecularly characterized. Plants were screened in the greenhouse for their response to FHB by point inoculations with *F. graminearum* at pre-anthesis stage. The disease scoring was done at 14, 21, and 28 days after inoculation. Comparative analysis of the molecular and phenotypic data revealed the gene to be present in the pericentromeric region. Further work on reducing the genetic interval having the potential susceptibility gene is in progress and results will be presented.

Developing a holistic *in vitro* assay to screen *Prunus* species to Armillalria root rot
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Currently available approaches to screen resistance of various *Prunus* (stone fruit) rootstocks to *Armillaria* spp. may only provide partial evidence for resistance and are labor and time-intensive. Our objective was to overcome the limitations of the available methods and develop a holistic *in vitro* assay to screen tolerance/susceptibility of *Prunus* spp. to *Armillaria* spp. Sterile root segments of three *Prunus* spp. were placed next to or on top of 14-day-old cultures of *A. solidipes*. At 21-days after inoculation, the success of fungal penetration, the length of the fungal colonization, and host responses were evaluated. Also, an anti-*Armillaria* and antifungal compound detection assays were conducted by utilizing the root periderm, the first tissue that needs to be penetrated by the fungus. These methods revealed the variation in the relative tolerance/susceptibility of three *Prunus* spp. to *A. solidipes*. The overall success of fungal colonization in the wounded and intact root, host defense, and antifungal activity significantly differed among various *Prunus* spp. This holistic screening technique allowed associated active and passive host defenses to be assessed in three weeks. Results suggest that host tolerance/susceptibility to *Armillaria* spp. relies on the active and passive defenses of mature root periderm and this *in vitro* method can be used as a preliminary step in screening tree species to *Armillaria* spp.

Screening various *Prunus* species to find sources of resistance to *Armillaria* species
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Armillaria root rot (ARR) caused by *Armillaria solidipes*, *A. tabescens*, and *A. mellea* is one of the principal causes of premature mortality of *Prunus* species (stone fruit trees) in the United States. Presence of a high level of *Armillaria* spp. not only kills trees, but also passes a “death sentence” on *Prunus* spp. orchards. Unfortunately, there is no known control of ARR. The easiest strategy for growers to mitigate tree losses from *Armillaria* spp. would be to utilize *Armillaria* resistant rootstock. Our objective was to determine variation in tolerance of 24 different *Prunus* germplasms to *Armillaria* spp. by utilizing wounded and intact root/shoot periderm inoculation assays and anti-fungal screening assays. The success of fungal penetration through wounded and intact root/shoot periderm and the length of fungal colonization were significantly different among various *Prunus* germplasms. *Armillaria* spp. successfully invaded peach germplasms and were unable to penetrate through the intact bark of most of plum and plum derived roots and cherries, *P. avium*, and *P. maackii*. Histological examination of the inoculated roots revealed that the tolerant germplasms had the ability to form barrier zones to deter the invading fungus. Also, the concentration of anti-fungal compounds was relatively higher in germplasms that that were less prone to *Armillaria* spp. infection. Our results imply that intra-species variation in tolerance of *Prunus* spp. to *Armillaria* spp. exists.

Identification of orange fleshed sweet potato clone resistant to three different species of rootknot nematodes
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One of the main obstacles to food production is the damage caused by nematodes. On sweet potato symptoms are associated to reduction of fibrous roots, plant growth and yield loss. The objective of this work was to characterize the resistance level of four sweet potato clones to the *Meloidogyne javanica*, *M. incognita* and *M. enterolobii*. In addition to the clones, four sweet potato commercial cultivars, and one susceptible tomato control was used. The experiment was installed in 5L plastic pots and conducted under greenhouse conditions on a completely randomized design, with six replications. Soil was infested with 5000 eggs and eventual second stage juveniles (J2) from each species on separate pots. Plants were evaluated 47 days after soil infestation. The classification of resistance levels was performed according to the Reproduction Factor (RF), gall index (GI), egg mass index (IMO) and number of eggs per gram of root (NEGR). The four clones were classified as resistant according to the FR of *M. javanica* and *M. incognita.* The other parameters evaluated were also statistically different from the susceptible sweet potato commercial cultivars and the tomato cultivar. One of clones (BD 74) presented a FR of 0,14 and was considered also resistant to *M. enterolobii* since the threshold for resistance is FR lower than one. This finding is very relevant because despite being present in Brazil since 2001 few species and cultivars are resistant to *M. enterolobii.*

Screening commercial cucumber cultivars for resistance to anthracnose using image analysis
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Anthracnose was first reported on Guam in 1979. *Colletotrichum orbiculare* was identified as the causal agent in 2017. Four replications of 22 commercial cultivars were grown in pots, at the fourth leaf stage, they were spray inoculated to point of runoff with a 1×10^4 conidia/ml suspension, then afterwards placed under a plastic tent at 100% RH for 24 hrs. On the fifth day, the third leaf was removed and pressed between sheets of paper towel. This flattened the leaf and arrested further disease development. Leaves were scanned and images were evaluated using Adobe Photoshop and Imagej software. Statistical analyses consisted of one independent variable (cultivars) and four dependent variables: VR (Thompson and Jenkins 1985 Visual Rating scale), TL (Total Leaf area), PerDis (Percent Diseased area within a 5 cm diameter ascribed circle), and LesionsC (Lesions per cm^2, in the ascribed circle). Tukey's studentized range (HSD) Test was <0.001 for all dependent variables. Contrast testing of Resistance Vs Susceptible revealed the following significant pairs: 26 among 231 for VR, 7 among 231 for PerDis, and 19 among 231 for Lesions. Following visual inspection of cultivar groupings of t-test (LSD) results across VR, PerDis, and LesionsC, it was determined that Summer Top, Olympia (trd), Olympia, Blessing, Poinsett76, Soarer, and TI-09E were the most resistance cultivars, while those identified as the most susceptible included Early Triumph, Tasty King, and Turbo.

A sustainable approach to *Phytophthora*-infested landscape beds: The search for tolerant or resistant annuals and herbaceous perennials
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Many ornamental nursery crops are susceptible to species of *Phytophthora*, a genus of fungal-like organisms that can persist in soil for several years. Since chemical applications are not practical in the landscape, knowledge of *Phytophthora*-resistant or tolerant plant species would provide a sustainable management approach. In 2018, one or two cultivars of 15 annuals and 12 herbaceous perennials were evaluated based on desirability and anecdotal evidence of tolerance or resistance to *Phytophthora*. Six plant cultivars served as susceptible controls. Three landscape beds were established in North Carolina and infested with three species of *Phytophthora*: *P. nicotianae*, *P. tropicalis*, and *P. drechsleri*. Plants were regularly rated for disease incidence and appearance and symptomatic plants were assayed to determine the presence of *Phytophthora*. Ten cultivars of annuals and seven cultivars of herbaceous perennials appeared excellent throughout the season. *Phytophthora* spp. were recovered from six and five cultivars of the evaluated annuals and herbaceous perennials, respectively. *P. nicotianae, P. drechsleri*, or *P. cryptogea* were recovered from each susceptible host. *P. tropicalis* was recovered from one evaluated plant cultivar. *P. cryptogea* was recovered from three plant cultivars, although this species was not intentionally introduced in the landscape beds. This study identifies 17 cultivars that can be used as alternatives in landscape beds infested with *Phytophthora*.

The search for brown rot resistance in potato
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Brown rot of potato is a devastating disease caused by *Ralstonia solanacearum* (*RS*) (Smith, 1896; Yabuuchi et al.1995). Traditionally populations of *RS* were restricted to southern states since it is unable to infect and survive under temperate climate conditions. Increases in temperatures within potato growing regions and an influx of emerging temperate-tolerant *RS* populations are two major factors that could potentially affect potato production within the Northern US. Finding potato cultivars with resistance to this pathogen is important as disease is eminent and could be disastrous. The objective of this study is to evaluate resistance within potato species, cultivars, and accessions to populations of *RS* that are pathogenic at low temperatures. Eight *Solanum* species (*S. brevicaule, candolleanum, chacoense, gandarillasii, tuberosum subsp. andigenum*), nine *S. tuberosum* hybrids from the International Potato Center collection, seven common US cultivars, and the sequenced potato (RH89-039-16) were tested for this study. Pathogenicity screening was done in environmental chambers at 18°C and 30°C, and tests were repeated three times. Plants were inoculated with *RS* strain UW551, sequevar 1, phylotype IIB, a strain classified as a Select Agent due to its pathogenicity in potatoes at low temperatures. During the study, we

found a large variation to brown rot resistance. However, the resistance in most cases is broken at warm temperatures (30°C). Some cultivars showed resistance that can be used for breeding purposes and to mitigate disease in fields by cultivar rotation.

Screening citrus genotypes for *Phytophthora nicotianae* tolerance

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Seeds from four citrus genotypes including sour orange, Bitters-C22 citrandarin, Sarawak pummelo x Rio Red grapefruit, and Sarawak pummelo x Bower mandarin were exposed to high amounts of *Phytophthora nicotianae* to screen for tolerance. Inoculation of pre-germinated seeds (PGIS) and non-pre-germinated seeds (NPGIS) was carried out. The average *P. nicotianae* propagule counts from the soil samples where these seedlings were raised ranged from 424-1361 cfu/cm^3. The proportion of live/dead plants was recorded at eleven months post-inoculation. Sarawak x Bower performed significantly better compared to other genotyes. Evaluation of the rootstocks eighteen months post-inoculation resulted in only one surviving sour orange plant which suggests potential genotype resistance.

Evaluation of resistance in rootstock materials to manage Armillaria root disease in peach orchards of Mexico

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Armillaria root disease (caused by *Armillaria mexicana* and/or *A. mellea*) causes peach (*Prunus persica*) mortality in orchards of the Mexican sub-tropics. Three *Prunus* rootstocks were evaluated for resistance/susceptibility reactions to *A. mexicana* and *A. mellea* under greenhouse and field conditions. In the greenhouse, *A. mexicana* and *A. mellea* were independently inoculated on 21 trees for each of three rootstocks: *P. persica* × *P. davidiana* 'Nemaguard', *P. persica* landrace 'Criollos of La Goleta' genotypes, and *P. salicina* Japanese plum 'Mondragon', each with four non-inoculated trees as controls. After greenhouse inoculations with *A. mexicana*, 'Mondragon' showed the lowest (2.5%) incidence of infected roots, while 'Criollos of La Goleta' and 'Nemaguard' rootstocks showed the highest (21.6% and 24.6%, respectively). Root dry weights, root volumes, and plant dry weights were greater (2.2-4.8 X higher) in the 'Criollos of La Goleta'; however, abundant mycelial infections were observed in the root systems. Greenhouse inoculations with *A. mellea* resulted in lower (17.5%) incidence of root infection. In a commercial orchard infested with *A. mexicana*, 21 plants of 'Mondragon' (cutting-derived), 'Criollos of La Goleta', and *P. mume* 'Japanese apricot' were inoculated with *A. mexicana*. 'Mondragon' showed the lowest susceptibility to *A. mexicana*. Identifying *A. mexicana*-resistant 'Mondragon' rootstock is key for management of Armillaria root disease in peach orchards of Mexico.

Idaho spring wheat varies in susceptibility to Fusarium head blight

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Fusarium head blight (FHB) is an emerging disease of small grains in the Pacific Northwest (PNW) due to increasing corn acreage and prevalence of *Fusarium graminearum*. The use of resistant varieties reduces both FHB and deoxynivalenol (DON) contamination. However, varieties with specific end-use quality characteristics tend to be susceptible to FHB and have higher levels of DON. To facilitate wheat variety improvement, screening nurseries comprised of widely-grown cultivars and advanced breeding lines were established at Aberdeen, ID in early May of 2017 and 2018. Corn inoculum was applied at a rate of approximately 30 g/m^2 three weeks before anthesis of the earliest maturing variety. Plot severity and FHB incidence were evaluated at soft dough while DON were determined from harvested grain samples. FHB index (IND) values were calculated for the 37 entries that were evaluatedfor both years. Under high disease pressure in 2017, significant varietal responses ranged from 1.4 to 49.9% for IND and from 0.4 to 28.1 ppm for DON. Under moderate FHB pressure in 2018, IND values similarly ranged from 0.2 to 52.9% but the range of DON concentration of 0.3 to 19.5 ppm was significantly less. Low correlation (r) between arc-transformed IND (arcIND) and log-transformed DON (logDON) of 0.3 and 0.4 in 2017 and 2018, respectively, confirms that breeding efforts must target both FHB symptom development and DON accumulation. Disease variables will be analyzed with weather data to develop FHB prediction models specific to the arid, irrigated production areas in Idaho and other PNW states.

***Phlox* species have varied susceptibility to powdery mildew isolates from the Eastern United States**

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Growers, landscapers, and homeowners alike face challenges growing the native ornamental plant *Phlox* because of its susceptibility to powdery mildew (PM) caused by the fungi *Golovinomyces magnicellulatus* and *Podosphaera* sp. To fight off the disease, many economically and environmentally expensive fungicides are frequently used. In this study, we collected 15 powdery mildew isolates throughout the Eastern U.S. and used them to carry out resistance screening of perennial *Phlox* germplasm using a recently developed *in vitro* assay. Separate groups of micropropagated plantlets of *P. paniculata, P. amoena, P. subulata,* and *P. glaberrima* were inoculated with 18-day-old colonies of each PM isolate. Disease incidence and severity were assessed every four days for a month, and spore

production was quantified one month after inoculation. Additionally, we used a Multilocus Sequence Typing analysis to assess the genetic diversity of our PM isolates. Primers targeting five housekeeping genes (tef-1a, csI, ITS, H3, tub2) were designed from Whole Genome Sequencing of *G. magnicellulatus* strain FPH2017.1. To date, phylogenetic analysis by the Maximum Likelihood method using the H3 and ITS loci showed a certain degree of genetic diversity in the collected isolates. Moreover, we found that the tested *Phlox* species varied in susceptibility to PM isolates. *Phlox paniculata* 'Dunbar Creek' showed resistance across isolates, which is consistent with its observed performance in the landscape.

Evaluation of global collection of triticale to *Pyrenophora tritici-repentis* (tan spot) races 1, 5, and Ptr ToxA
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Triticale (× *Triticosecale*Wittm. ex A. Camus.) serves as an excellent choice for cover crop and builds soil health. It has also served as a bridge between wheat and rye to bring resistance genes to many fungal diseases into wheat. Triticale is generally considered less prone to biotic and abiotic stresses; however, it has shown susceptibility to leaf spot diseases including tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Ptr). The objective of this study was to characterize triticale germplasm for its reaction to Ptr race 1, race 5, and Ptr ToxA. A global collection of 366 triticale genotypes representing 21 countries was obtained from USDA- Small Grain Germplasm Bank, Aberdeen, ID. Two-week-old seedlings of all 366 genotypes were inoculated individually with a conidial suspension of Ptr race 1 and 5 under controlled greenhouse conditions. Also, three leaves of each genotype were infiltrated with Ptr ToxA. The evaluated genotypes exhibited a range of reaction from susceptible, moderately susceptible, moderately resistant, and resistant to both races. About 50% of the evaluated genotypes showed sensitivity to Ptr ToxA. Our results indicate that triticale varies in its reaction to tan spot, therefore when employing triticale genotype for wheat improvement caution should be taken to avoid accidental incorporation of tan spot susceptibility.

Hybrid rice outperforms inbred rice in resistance against sheath blight and narrow brown leaf spot
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The introduction of hybrid rice in the United States provides producers an alternative to traditionally cultivated, conventional inbred rice. Because of its high yielding potential, hybrid rice acreage has continually increased over the past decade. The objective of this study was to compare hybrid rice with inbred rice for resistance to sheath blight (*Rhizoctonia solani* AG1-IA) and narrow brown leaf spot (NBLS, *Cercospora janseana*), two important diseases affecting rice production in Texas and other southern United States. Field evaluations were conducted on more than 40 hybrid cultivars and breeding lines and 160 inbred cultivars and breeding lines at two locations in Texas over 3 years. Sheath blight and NBLS severities were rated prior to harvest on a scale of 0 to 9 where 0 = no symptoms and 9 = most severe in symptoms and damage. Sheath blight developed on all hybrid and inbred entries evaluated. However, the average sheath blight severity over locations and years was 30% lower in the hybrid entries than in the inbred entries (4.7 vs 6.7). All hybrid entries had complete resistance to NBLS with no development of the disease whereas the inbred entries exhibited a range of NBLS severity from 0 to 8, with an average disease severity of 3.5. The results demonstrate that hybrid rice is less susceptible to sheath blight and has a higher level of resistance against the NBLS disease compared to inbred rice.

Resistance to anthracnose in 76 cultivars and elite breeding lines of strawberry
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Anthracnose, caused by *Colletotrichum acutatum* is an economically important disease of strawberry (*Fragaria* × *ananassa*). All parts of the plant are susceptible, resulting in fruit rot as well as plant mortality. Host plant resistance to anthracnose exists but could be better utilized if susceptibility of strawberry genotypes was widely known. In this experiment we evaluated the susceptibility of 76 genotypes to anthracnose during the 2018–19 season in San Luis Obispo, CA. Strawberry genotypes were selected from six breeding programs: University of California Davis, University of Florida, Driscoll's, Plant Sciences, Planasa and Lassen Canyon. Bare-root transplants of each genotype were placed in a 1-gal plastic bag, mixed with 100 ml of inoculum (1×10^6 spores/ml) and shaken for one minute immediately prior to planting (10 plants per rep; 4 reps per genotype) in the field. A non-inoculated control was included for each genotype. Weekly plant mortality assessments started three weeks after planting, when plants began to show symptoms and signs of infection. The causal agent was confirmed as *C. acutatum* using standard plating techniques. At 4.5 months after planting, susceptibility of all genotypes ranged from 0 to 100% mortality with the average across all genotypes at 45.0%; 23 genotypes had >70% mortality while 27 genotypes had <25% mortality.

Assessing susceptibility of University of Florida southern highbush blueberry cultivars to bacterial wilt
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Blueberries are a high value crop in Florida, where our climate allows for an early harvest during an advantageous market window. Bacterial wilt of blueberry is caused by *Ralstonia solanacearum* and was first reported in 2012 in New Jersey on the cultivar 'Bluetta.' In 2016, bacterial wilt was detected in Florida on the southern highbush blueberry (SHB) cultivar 'Arcadia.' Since 2016, the disease has spread to farms throughout central Florida, and 'Arcadia' continues to be the cultivar in which bacterial wilt is most frequently detected.

Developed by the University of Florida (UF), this cultivar is popular among growers for its large fruit size and good tolerance to leaf diseases. To help assess this new threat to Florida blueberry production, we screened cultivars that account for greater than 90% of planted acreage for susceptibility to bacterial wilt. Rooted cuttings of 13 SHB cultivars, 'Bluetta,' and one early ripening rabbiteye blueberry were wound inoculated with bacterial suspensions in the summer of 2017. Time from initial symptom development to death was recorded over a period of 8 months. The presence of *R. solanacearum* was confirmed when plants died, or at the close of the experiment. Frequency of disease detection varied significantly among cultivars, and Arcadia was in the most susceptible statistical grouping. The assay we developed may be useful for selecting less susceptible varieties to bacterial wilt in the UF blueberry breeding program.

Forty-one accessions have possible resistance to *Diaporthe gulyae* causing Phomopsis stem canker of sunflower (*Helianthus annuus*)

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Phomopsis stem canker of sunflower (*Helianthus annuus* L.) is a yield-limiting disease in the U.S. states of Minnesota, Nebraska, North Dakota, and South Dakota. Between the two causal fungi, *Diaporthe gulyae* and *D. helianthi*, *D. gulyae* is a new pathogen of sunflower. In this study, 205 accessions were selected from the cultivated sunflower germplasm and screened for resistance to a single isolate of *D. gulyae* using the mycelial-contact inoculation method in the greenhouse. The confection inbred 'HA 288' was used as the susceptible check. At V4 to V6 (four to six true leaves) growth stage, a 6-mm-diameter plug of potato dextrose agar infested with *D. gulyae* was placed in contact with the stem of the plants at the fourth node and covered with petroleum jelly. After inoculation, the sunflower plants were misted for 2 minutes every 2 h for 3 days. Fourteen days after inoculation, disease severity was evaluated on a 0 to 5 rating scale. Disease data were analyzed using non-parametric statistics and expressed as relative treatment effects (RTE). Of the 205 accessions, 41 accessions were determined to be significantly less susceptible to *D. gulyae* when compared to 'HA 288'. The findings in this study suggest that the 41 accessions could be useful resources to breed for resistance to *D. gulyae*. Future studies will focus on the identification of single nucleotide polymorphism markers associated with *D. gulyae* resistance using genetic mapping strategies.

Resistance of solanaceous rootstocks to *Ralstonia pseudosolanacearum* strains

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Grafting is a promising approach among the options available for the management of bacterial wilt of tomato. Bacterial wilt is caused predominantly in Africa by *Ralstonia pseudosolanacearum* phylotype III, while in Asia phylotype I predominates. However, due to genetic variability for virulence within *R. pseudosolanacearum*, rootstocks must be evaluated for resistance to multiple strains. Our study aimed to identify appropriate wilt-resistant solanaceous rootstock candidates for the management of bacterial wilt in multiple regions. We used a split plot completely randomized block design to evaluate responses of 11 Ohio tomato lines to three virulent *R. pseudosolanacearum* strains from East Africa and South Asia. We also evaluated the tomato line MT 56 (Makerere University Uganda) and the eggplant line EG 190 (World Vegetable Center) against fifteen additional strains, with tomato 'Moneymaker' as a susceptible control. Wilt incidence and severity were assessed, and latent infections identified by PCR (primers 759/760). Significant differences in wilting incidence were observed among cultivars ($P < 0.001$). EG 190 and OH-WG-120 exhibited no wilting symptoms to the strains tested, but latent infections were detected. Four Ohio lines (WG 220, 121, 211 and 215) were highly susceptible (up to 100% wilting in most of isolates), while the remaining lines had variable responses to the strains tested

Susceptibility of Geranium Cultivars to *Botrytis cinerea* in the Greenhouse

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Botrytis cinerea incites leaf, stem, and flower blight and occurs on greenhouse-grown crops including geranium. We evaluated the following geranium cultivars for *B. cinerea* susceptibility: 'Maverick', 'Multibloom', 'Ringo', 'Nano', 'Bullseye', 'Pinto', 'Quantum', 'Horizon', 'Pinto Premium', and 'Ivy'. Seed was planted, and plants inoculated 71 days later by spraying a *B. cinerea* (1×10^6 conidia/ml) solution obtained from a geranium isolate; plants were incubated in plastic bags to achieve high relative humidity. Ten single plants per cultivar were evaluated in research greenhouses. The number of blighted leaves and blighted leaves with sporulating *B. cinerea*, the number of foliar lesions, and overall disease severity ratings (0=no disease; 10=severe disease) were assessed three times beginning 7 days after inoculation. 'Ringo', 'Multibloom' and 'Maverick' were more susceptible based on blighted leaves (>24), blighted leaves with sporulating *B. cinerea* (>18), number of foliar lesions (>26) and disease severity (>6). 'Ivy' had the lowest number of blighted leaves (13), blighted leaves with sporulating *B. cinerea* (< 9), foliar lesions (<14) and the lowest disease severity (<5). Overall, 'Ringo', 'Multibloom' and 'Maverick' were highly susceptible, 'Nano', 'Bullseye', 'Pinto' and 'Quantum' were moderately susceptible, and 'Pinto Premium', 'Horizon' and 'Ivy' were less susceptible. When the experiment was repeated, differences among the cultivars were not observed.

Susceptibility of geranium and snapdragon cultivars to *Pythium irregulare*

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The U.S. floriculture industry was valued at $4.4 billion in 2015, with Michigan as a top producer. Pythium root rot affects many economically important plants, including popular annuals such as geranium and snapdragon. Disease symptoms include stunted growth, delayed flowering, and plant death. To reduce fungicide reliance, twelve cultivars each of geranium and snapdragon were evaluated for root rot susceptibility in a greenhouse study. Seedlings were transplanted into millet infested with *Pythium irregulare*. Plant health and height were compared to non-inoculated (control) plants. 'Maverick White' and 'Horizon White' geraniums were the most susceptible; height of inoculated plants was reduced by 81% and 80%, respectively, and received poor health ratings. In contrast, inoculated 'Tornado White' geraniums were 17% smaller than the control and were rated highly for plant health. Other inoculated geranium cultivars were 37% to 77% the size of the control plants. 'Bells White' snapdragons were reduced in size by 47% when inoculated with *P. irregulare* compared to the control and had the lowest health rating. 'Floral Showers White' snapdragon was slightly reduced in size (3%) when inoculated compared to the control. All other inoculated snapdragon cultivars ranged in size from 63%-88% of the control plants. Choosing cultivars that are less susceptible to *P. irregulare* can be used in an integrated management program that also includes sanitation and other strategies.

Strawberry cultivars have differing susceptibility to the two major *Colletotrichum* pathogens, *C. acutatum* and *C. gloeosporioides*
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Several species of *Colletotrichum* are known to infect strawberries worldwide. The two most important species in Florida are *C. acutatum* and *C. gloeosporioides.* The predominate symptom of *C. acutatum* is anthracnose fruit rot. *C. gloeosporioides* predominately infects the crown causing mortality. The diseases are managed with a combination of chemical control and cultivar resistance. Fourteen cultivars were evaluated in the field for incidence of anthracnose fruit rot (*C. acutatum*) and incidence of wilt and disease progress over time (*C. gloeosporioides*) for the last twelve or nine seasons, respectively. Each cultivar was evaluated for a minimum of two seasons, but seven cultivars were evaluated for at least seven seasons. 'Cabrillo', 'Monterey', 'Petaluma' and 'San Andreas' are susceptible to both species, whereas 'Winterstar' and 'Florida Elyana' are moderately resistant and resistant to both species, respectively. Sensation® 'Florida127' and 'Fronteras' are both moderately resistant to *C. gloeosporioides* but are resistant and susceptible, respectively, to *C. acutatum.* 'Strawberry Festival', 'Florida Beauty', 'Florida Brilliance', 'Florida Radiance' and 'Sweet Charlie' are all susceptible to *C. gloeosporioides* but moderately to highly resistant to *C. acutatum*. 'Treasure' is highly resistant to *C. gloeosporioides* but highly susceptible to *C. acutatum.* These results are consistent with the recent reports that the resistances are controlled by different genes.

Resistance of strawberry cultivars to crown rot caused by *Phytophthora cactorum* and *P. nicotianae*
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Phytophthora crown rot is an important disease of strawberry worldwide. *Phytophthora cactorum* is the most common causal agent, however *P. nicotianae* was also recently reported causing crown rot in the U.S. Therefore, the goal of this study was to evaluate whether the resistance of strawberry cultivars from Florida and California differs between the two species causing Phytophthora crown rot. Sixteen strawberry cultivars were evaluated during two Florida strawberry seasons. Prior to planting, roots of each cultivar were submerged in a zoospore suspension (1×10^4 ml^{-1}) for 5 min. The plants were then transplanted into previously fumigated beds. Plant wilting and mortality was assessed weekly, and AUDPC was calculated. Cultivars Sensation® Florida127, Winterstar, and Florida Radiance were highly susceptible, whereas Elyana, Camarosa, Fronteras, Sweet Charlie, and Strawberry Festival were highly resistant to both Phytophthora species. However, 'Florida Brilliance' and 'Cabrillo' were both resistant to *P. nicotianea* yet susceptible to *P. cactorum.* Contrarily, 'Florida Beauty', 'Ruby Gem' and 'Albion' were susceptible to *P. nicotianea* and resistant to *P. cactorum.* These results emphasize the importance of screening for disease resistance to guide management recommendations in commercial strawberry production as well as the need for proper pathogen identification since cultivar susceptibility might differ.

Resistance of commercial dry bean cultivars to soybean cyst nematode
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Soybean cyst nematode (SCN), *Heterodera glycines*, is an important nematode pest which can reduce dry bean yields. The nematode now occurs in major dry bean growing areas in North Dakota, a major producer of dry bean. However, information on how SCN impacts various dry bean cultivars is limited. A diverse collection of 69 commercially available cultivars in 8 bean classes was evaluated for resistance to SCN in a greenhouse. Seed was pre-germinated, planted in conetainers with 110 cm^3 of pasteurized Prairie loam soil then inoculated with HG 0 at VC growth stage (3,000 eggs/plant). Conetainers were placed in racks over a heating pad and the racks were enclosed in foam insulation on a greenhouse bench to maintain the root zone at 27° C. Plants were grown for 40 days with 16 h of natural and artificial light then SCN females were extracted and a female index (FI) calculated or each variety using Barnes soybean as the susceptible check. Large differences in FI were observed within and between bean classes with some varieties showing resistant to moderately resistant reactions to SCN. Bean varieties with high levels of resistance will be needed for management of this nematode as SCN spreads in bean production areas.

Zoospore production and inoculation techniques for screening Aphanomyces root rot of Alfalfa
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Root rot of alfalfa (*Medicago sativa* L.) caused by *Aphanomyces euteiches* Drechs. is controlled by breeding for resistance. Resistant plants are selected for breeding through disease screens, which use either zoospore or comminuted mycelial inoculum. In the present study, a comparison was made between these two sources of inoculum. Concentrations of 800-900 zoospores per ml and half-plates of mycelium grown on Potato Dextrose Agar blended in 1 liter of water were used. Seedlings were rated using a Disease Severity Index. No significant differences in disease severity occurred in seedlings when inoculated with zoospores versus comminuted mycelium. A large amount of variation between different isolates in regard to zoospore production was observed. Some isolates of the pathogen never produced more than a few zoospores while some individual isolates were variable, and some isolates reliably produced large numbers of zoospores. Production of zoospores increased in both number and reliability as the temperature of incubation was lowered from 24 to 16 C. Significant increase in time and material input for zoospore production versus mycelial inoculum was noted. A temperature effect on disease expression in the disease screen also occurred. It was concluded that comminuted mycelial inoculum was an acceptable inoculum source for the purpose of Aphanomyces root rot disease screens for the selection of resistant plants and that temperature has a profound impact on the system.

Evaluating breeder lines for adult plant resistance to wheat leaf rust

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Leaf rust (LR) of wheat, caused by *Puccinia triticina*, is a major foliar disease of wheat with incorporation of genetic resistance being a primary approach to help manage this disease. Ideally adult plant (all-stage) resistance (APR) is used to determine lines to advance, but in dry years APR field evaluations can be poor or lacking when natural infections are low or absent. To overcome this problem, over 589 wheat breeder lines were evaluated annually for seedling and APR in the greenhouse. Seedling resistance was evaluated by inoculating eight seedlings/line using long-established procedures. APR evaluation was conducted by planting wheat seeds into button cells (8 plants/line). Three to six days after planting, germinated seeds were vernalized for 6 weeks in a cold storage room at 7° C under LED grow lights. After vernalization, plants were transplanted into containers typically used to test for seedling reaction. After 7 weeks, adult plants were inoculated with *P. triticina*, and LR APR was evaluated on flag, F-1, and F-2 leaves 8–11 days post inoculation. This procedure is being used to evaluate LR APR in a greenhouse environment and provides APR data in years when APR reaction in field trials is absent.

Comprehensive Disease Survey on USDA-ARS Cucumber Germplasm Collections in Search for Downy Mildew Resistance at Charleston SC

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Downy mildew (DM) caused by *Pseudoperonospora cubensis* can affect a wide variety of cucurbit crops leading to significant yield losses. Cucumber cultivars with resistance to DM were available for over 30 years and widely grown across the U.S.A. After breakdown of resistance in 2004, intensive fungicide programs are being used to prevent DM from completely destroying the crop. We focused on identifying new sources of DM resistance in the USDA-ARS Cucumber Germplasm Collection against local *P. cubensis* isolates. In summer 2017, 180 plant introductions (PIs) were evaluated for DM resistance in field trials. The DM tolerant ‘Peacemaker’, the old resistant ‘Expedition’ (prior to 2004), and the susceptible ‘SMR-58’ were used as checks. Twenty-three PIs with varying levels of tolerance/resistance were identified and re-screened in fall 2017 and summer 2018. Six PIs were tolerant and two PIs had high levels of resistance compared to ‘Peacemaker’. In summer 2018, an additional 608 PIs were screened and of these 52 were tolerant and 3 were resistant. Severe DM was observed on ‘SMR-58’ and ‘Expedition’ in 2018. Differences in DM severity on ‘Expedition’ and ‘Peacemaker’ were observed indicating potential differences in the pathogen populations in 2017 and 2018. The pathogen population is being characterized using microsatellite markers. The five resistant cucumber PIs will be useful for breeding resistant commercial cultivars

Integrated use of brassica seed meal and cultivar resistance for management of seedling disease in organic rice production

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Managing stand loss caused by seedling disease (*Rhizoctonia solani* and other soilborne pathogens) is a challenge in organic rice production because no synthetic fungicides can be used for seed treatment. The use of brassica (mustard) seed meal as a biofumigant to control soilborne pathogens could offer an alternative to seed treatment fungicides. A 2-year study was conducted as a split plot design in a field under organic management to evaluate the performance of mustard seed meal in combination with rice cultivar resistance for reduction in seedling disease. Main plots were soil amendments (mustard seed meal at 1,700 and 3,400 kg/ha, ryegrass green manure, and fallow) and subplots were rice cultivars (Cocodrie, Presidio, and a hybrid). “Pescadero Gold” mustard seed meal and winter annual ryegrass cover crop were incorporated into the soil prior to rice planting. Both mustard seed meal treatments reduced % discolored root area and increased stand compared to fallow and green manure. The hybrid cultivar had a lower % discolored root area and a greater stand than did Cocodrie. Rice yield was greater with the low rate mustard seed meal amendment than with fallow over 2 years. The level of yield was higher in the hybrid than Presidio. Brassica seed meal soil amendment can reduce seedling disease and its combined use with a resistant cultivar such as a hybrid can be an effective approach to reducing stand loss and improving yield under organic production.

Evaluation of tomato cultivars and OMRI fungicides for late blight
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U.S. organic tomato production was valued up to $174 million in 2017. Late blight (LB) (*Phytophthora infestans*) is a destructive disease causing foliar blighting and fruit rot. Cultivar resistance and OMRI-approved fungicides were tested in growth chamber and field studies and evaluated for LB (0 to 100%); early blight (EB) (0 to 10 scale) and bacterial spot (BS) (0 to 100%) in the field were noted. Twelve untreated cultivars were inoculated with *P. infestans* isolate US-23. In the growth chamber and field, disease incidence was lower in 'Mountain Magic', 'Defiant', 'Matt's Wild Cherry', and 'Tomato Stellar' (<40% LB in growth chamber; 0% LB, < 2.5 EB, and < 30% BS in field) compared to 'Early Girl', 'Better Boy', 'Rugged Boy', 'Amish Paste', 'Mountain Merit', 'Damsel', 'Cherry Bomb', and 'Mr. Stripey' (<95% LB in growth chamber; <20% LB, <3.8 EB, < 70% BS in field). In a separate field study, 'Mountain Merit', 'Damsel', 'Mr. Stripey', and 'Better Boy' were inoculated as previously described and treated with Kocide 3000-O (copper hydroxide), Zonix (rhamnolipid biosurfactant), Ef-400 (herbal extracts) or not treated (control) every 7 days. Levels of LB, EB, and BS for 'Better Boy' sprayed with Zonix were similar to the control. LB, EB, and BS disease for all cultivars was reduced when Kocide 3000-O was applied compared to either Zonix or EF-400. 'Mountain Merit' or 'Mr. Stripey' treated with Kocide 3000-O had the lowest levels of disease.

Integrating resistance inducers, biopesticides and vermicompost for alternative management of tomato Fusarium wilt in Colombia
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In Colombia, tomato production is the second most important food crop after onion, representing 18% of production in the horticultural sector. Phytopathogenic fungi and bacteria affecting tomato and other crops are mainly controlled using pesticides. The average use of chemical pesticides for the year 2015 in Colombia was 14.7 kg/ha (global average; 3.9 kg/ha). The vascular wilt disease caused by the soil borne fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) is one of the major constraints for tomato production throughout the country. Colombian farmers use agrochemicals to control Fusarium wilt and, in most cases, without knowledge. Alternative approaches such as resistance inducers and biopesticides have proved to be effective against a broad range of pathogens and could be promising to control tomato crop diseases. In this study, we have evaluated the effectiveness to control *Fol* of silicon, phosphites, chitosan, *Trichoderma* spp. and organic amendments using both *in vitro* and *in planta* approaches. Individual application of silicon, phosphites and chitosan were effective to control *Fol in vitro* inhibiting mycelial growth until 100, 99 and 96%, respectively; and reduced disease severity in 25, 44 and 70%, respectively. Additionally, *Trichoderma* spp. and vermicompost were able to inhibit the growth of *Fol* in 56% and 95%, respectively. Particularly for vermicompost, experiments have been carried out on the filed including farmers participatory research methodologies. This study contributes to develop integrated management alternatives of the wilt disease in Colombia.

Variety genetics and oomicide seed treatments effect on stand, health, and yield of soybeans under *Phytophthora sojae* disease pressure
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The causal agent of Phytophthora stem and root rot, *Phytophthora sojae*, is a major pathogen of soybean. Management of *P. sojae* consists of cultivars with effective resistance genes, partial resistance (field tolerance) and the use of seed treatments containing oomicides such as ethaboxam, mefenoxam, and/or oxathiapiprolin. In this study, soybeans were inoculated with *P. sojae* to investigate the interaction of variety genetics and seed treatment on soybean health and yield. Nine cultivars with different Phytophthora resistance packages were treated with three different seed treatments: 1.) non-treated, 2.) ipconazole and clothianidin, and 3.) ipconazole, clothianidin, ethaboxam and mefenoxam. Soybean stand and dry weights were recorded at VC, V2 and R4. Quantitative polymerase chain reaction (qPCR) of soybean roots was performed at V2 and R4 to assess levels of colonization by *P. sojae* throughout the season. Soybean stand and plant dry weights were significantly increased with treatments containing oomicides in 9 and 5 of the cultivars tested, respectively. Significant increases in yield were observed in the oomicide containing seed treatment in 2 varieties. Significantly, this study finds that oomicide seed treatments are useful in protecting seed, allowing for increased emergence under disease pressure.

Effects of cover crops, rotation, and biological control products on soil properties and productivity in organic vegetable production in the Northeast
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The potential benefits of different cover crops, biological control amendments, and vegetable rotation on soil chemical and biological properties, crop development and yield, and disease development in organic vegetable production, as represented by legume (green snap bean), cucurbit (yellow summer squash), and solanaceous (sweet pepper) vegetable crops, were evaluated in a multi-year field trial in Maine. Cover crops evaluated included winter rye/hairy vetch, mustard green manure, a multi-species mixture (8 crops), and a fallow control. All cover crops improved biological properties compared to fallow soil, but had only marginal effects on soil chemical properties. The multi-species mixture and rye/vetch cover crops were associated with earlier emergence in beans and squash. All cover crops improved yield in beans and squash by 7–13%, but only the cover crop mixture increased yield for pepper (by 7–11%). Minimal crop diseases were observed throughout these studies, and biological control amendments, which included commercial formulations of

Streptomyces, *Trichoderma*, and *Bacillus* spp., in general, did not positively affect yield, but reduced powdery mildew on squash and leaf necrosis on beans by 10–28%. Vegetable rotation also affected yield, with beans yielding 8% higher following squash vs. pepper, squash yielding 15% higher following beans vs. pepper, and pepper yielding 11% higher following beans vs. squash. These results help define specific management practices to improve organic vegetable production and provide useful information and options for growers.

Evaluation of fungicides and resistant cultivars to reduce use of chlorothalonil for powdery mildew in pumpkin and winter squash

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Seven field experiments were conducted in three states to evaluate strategies to reduce use of chlorothalonil-based fungicides due to concern about potential impact on bees. A powdery mildew-resistant pumpkin cultivar (Bayhorse Gold) was less severely affected than the susceptible one (Gold Challenger) in 2016 but not in 2017 in NY and PA. Two alternative multi-site mode of action fungicides were compared to Bravo Ultrex or Weather Stik used alone and in a program with targeted fungicides (Luna Experience, Quintec, and Vivando). Efficacy similar to Bravo was achieved with Microthiol Disperss (sulfur) in most experiments and with Tritek (mineral oil) in some experiments. Based on AUDPC values, severity was suppressed on adaxial leaf surfaces by 89/95, 93/94, and 58/60%, respectively for these fungicides applied alone to the susceptible and resistant pumpkin cultivars, respectively, in NY in 2016; 92/99, 53/98, and 21/49% in PA in 2016; 97, 96, and 86% in NY in 2017; and 99, 98, and 36% in PA in 2017. Level of suppression on butternut squash cv. Atlas in 2018 was 98, 97, and 80% in NY; 100, 89, and 21% in PA, and 98, 98, and 26% in MA. Suppression on abaxial surfaces was limited, reflecting the contact activity of these fungicides. The targeted program tank-mixed with the alternatives was as effective as the one tank-mixed with Bravo in all experiments. Microthiol Disperss could be a viable alternative to Bravo for managing fungicide resistance and reducing potential negative impacts to bees while not compromising disease control especially when coupled with host resistance.

The RNA-dependent RNA polymerase coding region of turnip crinkle virus contains critical secondary structures required for efficient replication

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Virus replication is an essential step for viral infection and symptom development in crops. Both virus-encoded proteins, such as RNA-dependent RNA polymerase (RdRP), and RNA secondary structures formed through intra-molecular interactions of viral genomic RNA play crucial roles in RNA virus replication. However, it has been difficult to study some RNA structures because they are part of sequences that encode replication proteins. To study the predicted RNA secondary structures located within the RdRP coding sequence of Turnip crinkle virus (TCV), we developed a system that supplies TCV RdRP in *trans*, so that the impact of the RdRP coding capacity and the secondary structures become decoupled. The coding sequence of TCV RdRP was then subjected to systematic mutagenesis to identify and characterize *cis*-acting RNA elements therein. Using this system, a secondary structure designated Fa was found to be essential to the replication of TCV viral genome. Additionally, a tertiary pseudoknot structure within Fa was also found to be indispensable to the replication of TCV genome. More detailed findings will be reported at the meeting.

Generation of a Cowpea Severe Mosaic Virus Infectious Clone

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Infectious clones are important for functional studies of plant RNA viruses. Cowpea severe mosaic virus (CPSMV; Genus *Comovirus*, Family Comoviridae) is an RNA virus with a bipartite genome consisting of two single-stranded, plus-sense RNA segments. CPSMV is a well-known pathogen of cowpea, soybean, and other legume species. The RNA1 encodes proteins involved in replication, whereas the RNA2 encodes the movement protein and two coat proteins. In this study, we generated an infectious clone of a CPSMV variant able to infect tobacco (*Nicotiana benthamiana*) and soybean (*Glycine max*). The full-length cDNAs obtained from the CPSMV CB strain were cloned separately into the pAI101 binary vector downstream of a CaMV35S promoter with duplicated enhancers, and confirmed by restriction enzyme digestion and sequencing. Two amino acid changes were observed in the RNA1 polyprotein (S1098P and K1651R) when compared to the NCBI reference sequence (NC_003545.1), and none in the RNA2 polyprotein. The construct was introduced into lima bean cotyledons using particle bombardment. Inoculation with lima bean extracts resulted in systemic infection in both tobacco and soybean hosts. Infectivity was also retained throughout passages from soybean to tobacco. Surprisingly, *Agrobacterium* (C57C1) transformed with the construct harboring RNA1 cDNA grew into extremely small colonies on the selection plate, and did not grow at all in liquid culture without introducing mutations. Analysis of CPSMV mutants containing systematic deletions showed that the toxicity was imparted by a region in the N-terminal portion of the viral RNA-dependent RNA polymerase. This CPSMV infectious clone can be used as a plant virus vector to express foreign proteins or RNA, to study synergy between different comoviruses, and to study superinfection exclusion among bipartite viruses.

Repression of turnip crinkle virus replication by its replication protein p88

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Viruses with single stranded, positive sense RNA genomes are among the most economically important plant pathogens. Turnip crinkle virus (TCV) is a commonly used model for studying the genome replication of this group of viruses. In our previous study, we found

p28, one of the two TCV replication proteins, repressed the replication of a TCV replicon encoding wildtype p28. The other replication protein p88 contains the entire p28 at its N terminus. Here we further examined the effect of p88 on the replication of TCV replicons encoding either wildtype or mutated forms of p88. We found that transient, replication-independent expression of p88 permitted the complementation of a p88-defective replicon, but repressed the replication of another replicon encoding intact p88. Surprisingly, lowering p88 protein levels enhanced trans-complementation, but weakened repression. Testing a series of N-terminal deletion mutants of p88 demonstrated that repression by p88 can be attributed to multiple domains within p88, including those outside p28. Finally, both trans-complementation and repression by p88 were accompanied by preferential accumulation of subgenomic RNA2, and a novel class of small TCV RNAs. Our results suggest that repression of TCV replication by p88 may manifest a viral mechanism that regulates the ratio of genomic and subgenomic RNAs based on p88 abundance.

Transcriptome analysis of the interaction between *Lasiodiplodia theobromae* and *Theobroma cacao*
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Lasiodiplodia theobromae, a broad host pathogen, can attack *Theobroma cacao* (cacao) pods causing rots although the more common symptom is stem tip die back. The *L. theobromae*/cacao interaction was characterized using RNA-Seq to identify responsive genes in both species. A 43.75 Mb *de novo* assembled genome of *L. theobromae* was used to identify 13,061 protein-coding genes, 11,860 of which were transcriptionally active. In a 48-hour leaf disc infection assay, 1,255 of the predicted genes were induced compared to their expression in cultured mycelia. Many of the induced genes were involved in carbohydrate, pectin and lignin catabolism, cytochrome P450s, necrosis-inducing proteins and putative effectors. On the plant side of the reaction to infection, which included three isolates of *Lasiodiplodia*, 3520-4706 cacao genes were induced (1934-2900 with at least 2-fold induced) and 3000-4339 cacao genes were repressed (1807-2609 with at least 2-fold repressed). KEGG pathways with multiple genes showing repression were related to nucleic acid biosynthesis and metabolism while the pathways showing induction were associated with plant defense including cell signaling, biosynthesis of antimicrobial compounds, and production of and response to active oxygen. Most cacao genes showing induction in leaves have also been shown responsive to infection by other pathogens in distinctly different tissues and likely define the general response of cacao to infection.

Role of *pil* genes in movement and natural competence of the plant pathogen *Xylella fastidiosa*
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X. fastidiosa, a Gram-negative bacterium that causes disease in many economically important crops worldwide, is naturally competent. This feature allows it to take up DNA fragments from the environment and incorporate them into its own genome, which may lead to host shift and emergence of novel plant diseases. Type IV pili (TFP) play a central role in both natural competence and twitching motility of this bacterium. Many proteins, encoded mainly by *pil* genes, are responsible for TFP biogenesis and function. However, only few *pil* genes were evaluated for their role on twitching motility and natural competence in *X. fastidiosa*. Thus, in this study we aim to assess the role of different *pil* genes on movement and natural competence of *X. fastidiosa* by performing site-directed mutagenesis. Results showed that mutation of *pilB*, *pilP*, *pilR* and *pilT* resulted in loss of twitching motility. In addition, Δ*pilP* and Δ*pilT* mutants lost the natural competence ability, while Δ*pilB* and Δ*pilR* recombined DNA via natural competence at a lower rate than the WT. Overall, results showed that all analyzed genes are essential for twitching motility of *X. fastidiosa*, while only *pilP* and *pilT* are essential for the natural competence feature of this bacterium. Understanding the mechanisms by which this pathogen acquires exogenous DNA and lead to host shift may contribute to the development of new management approaches, mediating the transfer of knowledge to agricultural practices.

Understanding the compatible and incompatible host-pathogen interaction of *Setosphaeria turcica* races with maize leaves by histological studies
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Northern Corn Leaf Blight (NCLB) is an important leaf disease in maize worldwide. *Setosphaeria turcica,* causal agent of NCLB, infects and colonizes leaf tissue and induces elongated necrotic lesions. Disease control is based on fungicide application and resistant cultivars. Genetic resistance is mainly based on qualitative resistance. A race monitoring in Europe and South America indicated the presence of a distinct composition of races in different geographic regions. Moreover, histological studies were done to compare the pathogenesis of *S. turcica* races in maize exhibiting compatible and incompatible interactions. Maize plants from the reference line B37 with resistance genes *Ht*1, *Ht*2, *Ht*3 and *Htn*1 were inoculated with either incompatible or compatible races of *S. turcica*. Leaf segments from healthy and inoculated plants were collected one, three, six, twelve and eighteen days post inoculation (dpi) and stained with Chlorazol Black E. *S. turcica* formed appressoria and penetrated directly in both interactions. In a compatible situation, however, the pathogen was able to penetrate into xylem vessels at six dpi and to colonize the mesophyll at twelve dpi. In an incompatible interaction, the pathogen was not capable to colonize neither the xylem vessels nor the mesophyll. Further studies are underway in order to better understand the resistance mechanisms involved in incompatible interactions and the individual functions of the different known resistance genes.

The polyketide synthase VdPKS1 and the transcription factor VdCmr1 are required for pigment production and UV protection in *Verticillium dahliae*
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Verticillium dahliae is a soilborne fungus that causes vascular wilt diseases on numerous plant species worldwide. The production of darkly melanized microsclerotia is crucial in the disease cycle of *V. dahliae*, as these structures allow for long-term survival in soil. The biochemical steps of the dihydroxynaphthalene (DHN) melanin biosynthetic pathway, which are conserved among many fungi, were initially characterized in *V. dahliae* decades ago. In this study, we further characterized DHN melanin regulation genetically by focusing on functional characterization of genes in a pigment biosynthesis-related gene cluster encoded in the V. dahliae genome. Specifically, we explored roles of cluster-specific transcription factor VdCmr1, as well as two other genes within the cluster encoding a polyketide synthase (VdPKS1) and a laccase (VdLac1), enzymes at the initial and endpoint steps in DHN melanin production. *VdCmr1* and *VdPKS1* are required for melanin production, but neither is required for microsclerotia production. None of the three genes was required for pathogenesis on tobacco and lettuce. Exposure of the mutant *VdCmr1* and wild type strains to UV irradiation or high temperature (40°C), reduced survival in the *VdCmr1* mutant strain by more than 50%, relative to the wild type strain. Expression profiles revealed that some melanin biosynthetic genes are in part dependent on wild type *VdCmr1*. These data indicate VdCmr1 is a key regulator of melanin biosynthesis, and that via regulation of melanogenesis, VdCmr1 affects survival of *V. dahliae* in response to abiotic threats.

eQTL analysis of *Salix purpurea* shrub willow in response to *Melampsora americana* willow rust infection
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Melampsora spp. willow rust is the most threatening disease of *Salix* spp. shrub willow for short rotation coppice bioenergy feedstock production. Control of this pathogen is limited to host resistance via breeding and polyculture, as application of fungicides are economically limiting for this cropping system. Previous work has investigated the population of *Melampsora* rust in the northeast US, while simultaneously identifying QTL's for resistance to this rust in *Salix* spp. breeding populations. We have built upon our understanding of this pathosystem by using RNAseq methods to investigate host resistance though eQTL analysis in order to discover essential transcription factors associated with rust resistance. An F2 mapping population was surveyed for rust susceptibility and 60 *S. purpurea* genotypes (30 resistant and 30 susceptible to rust) were artificially inoculated in a greenhouse with *M. americana*. A preliminary experiment identified 2 and 3 days post inoculation as the time points with the greatest number of differentially expressed genes, therefore these timepoints were used in the eQTL experiment. RNA has been extracted and data analysis is ongoing to identify QTL's for variation in gene expression between the resistant and susceptible pools. This work will identify markers associated with transcription factors that regulate the resistance response, providing important information for future breeding work.

small RNA *rprA* modulates the pathogenesis of *Erwinia amylovora*
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Fire blight, caused by *Erwinia amylovora*, is a devastating disease infecting many rosaceous plants, such as apple and pear. *E. amylovora* initiates the infection through migration from nectarthodes of host floral cups to xylem vessels. Formation of biofilms within xylem vessels blocks water transport and cause wilting of a host. *E. amylovora* cells that detach from biofilms are translocated and cause infections in the new site. A previous study of the RNA chaperone protein Hfq in *E. amylovora* yielded the identification of a small RNA *rprA*, which contributes to the pathogenesis of *E. amylovora* on both immature pear fruits and apple shoots, respectively. In this study, we demonstrate that *rprA* positively regulates two critical virulence factors of *E. amylovora*: amylovoran, the main exopolysaccharide in *E. amylovora*, and HrpS, the global regulator of type III secretion system in *E. amylovora*. Interestingly, we also found that *rprA* acted as a negative modulator of levansucrase activity, cellulose production and motility of *E. amylovora*, which function synergically in biofilm formation of *E. amylovora* cells. In view of these results, we further showed the positive involvement of *rprA* in coordinating dispersal events of biofilm cells in an *in vitro* assay. Ongoing research related to the *in vivo* visualization of the *rprA*-mediated dispersal movement of biofilm in *E. amylovora* will be discussed. Taken together, our results shed light on a better understanding of the decision-making process of *E. amylovora* in transitions from biofilm to planktonic modes of growth within a host.

A sticky situation: Elucidating the role of putative adhesins in *Ralstonia solanacearum* virulence
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Attachment to host surfaces (adhesion) and to fellow cells (cohesion) are critical steps in bacterial pathogenesis. The bacterial wilt pathogen *Ralstonia solanacearum* (*Rs*) needs adhesion and cohesion at two distinct points in its life cycle: early in infection, when *Rs* must attach to root epidermal cells and form microcolonies, and later in infection, when *Rs* attaches to xylem walls and forms biofilm-like aggregates. Cell surface proteins called adhesins help bacteria stick to diverse surfaces, facilitating interactions with animal and plant hosts. An *in planta* transcriptomic analysis identified seven putative adhesins differentially expressed in a quorum sensing mutant (Δ*phcA*) that is locked in a low cell density (early infection) mode. Three genes were upregulated in the *phcA* mutant, while four were repressed. We hypothesized that the upregulated adhesins are important for virulence early in infection when *Rs* is at a low cell density, while the repressed adhesins are needed later in disease when *Rs* is at a high cell density. Targeted single and poly-mutants lacking the putative early adhesins did not attach to tomato roots as well as wild-type *Rs*. Consistent with the early-function hypothesis, they did not contribute to *Rs* virulence beyond this first critical host-pathogen interaction. These results suggest *Rs* strategically deploys adhesins at specific stages of disease in diverse host environments.

The role of Actin Network in the recruitment of host glycolytic proteins for tombusvirus replication
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Plus-stranded (+)RNA viruses are the largest and most widespread group among plant viruses, causing important economic losses in different crops. Positive-strand RNA viruses have a limited coding capacity, thus to replicate and infect the host cell they need to co-opt selected host components and subvert specific pathways. Tombusviruses, plant viruses with a small (+) RNA genome, use the actin network to modulate cell dynamics and promote viral replication. Additionally, to build the viral replication compartments and supply the energy required at the replication site Tombusviruses also co-opt the ATP generating enzymes: phosphoglycerate kinase (PGK1) and pyruvate kinase (PK). To study the importance of the actin network in the recruitment of PGK1 and PK enzymes, we used bacterial effectors that target the actin filaments and affect actin dynamics. We discovered that disruption of the actin network dynamics affects viral replication and the recruitment of the glycolytic enzymes to the replication site. Moreover, my results also showed that the efficient recruitment of the glycolytic enzymes through the actin network is necessary to productively generate ATP within the viral replication compartment. Overall, my research sustains the major role of the actin dynamics to facilitate or restrict tombusvirus replication, because subversion of the host cellular actin network is a key activity for many plant pathogens, our discoveries will lead to improving plant disease resistance against viruses and possibly other plant pathogens.

Stringent Response Regulators DksA and ppGpp Positively Regulate Virulence and Host Adaptation of *Xanthomonas citri*
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The stringent response is a response of bacteria to nutrition deprivation and other stress conditions. Regulation of this process involves small signal molecule guanoisine pentaphosphate pppGpp, guanosine tetraphosphate ppGpp (collectively referred to ppGpp) and the RNA polymerase-binding transcription factor DksA. Accumulation of ppGpp in bacteria is mediated by the ppGpp synthetase RelA and the bifunctional ppGpp synthase/hydrolase SpoT. Here, we investigated DksA and ppGpp of *Xanthomonas citri* subsp. *citri* (Xcc), the causal agent of citrus canker. Δ*dksA* and Δ*spoT*Δ*relA* caused reduced virulence and compromised bacterial growth in host plants, indicating that stringent response regulators are required for full virulence of Xcc. To characterize the effect of DksA and ppGpp on gene expression, transcriptome analyses were conducted on Δ*dksA*, Δ*spoT*Δ*relA* double mutant and the wild-type strains grown in XVM2 medium. Transcriptome analyses showed that DksA and ppGpp repress the expression of coding genes for tRNAs, ribosome proteins, iron acquisition, flagellar assembly and enhance the expression of coding genes for histidine metabolism, T3SS, T2SS and TonB-dependent transporters. Phenotypically, the Δ*dksA* and Δ*spoT*Δ*relA* strains display altered motility, enhanced siderophore production and are unable to cause hypersensitive response on non-host plant. In conclusion, DksA and ppGpp play an important role in virulence, nutrition uptake and host adaptation of Xcc.

Effects of pospiviroid infection on the hidden half of tomato plants – the roots - and evaluation of methods to reduce crop losses
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Tomatoes are one of the world's most consumed vegetable crops with production of fresh market and processing tomatoes increasing in greenhouse, high tunnels, and the field. Several viroids (small, unencapsidated, single-stranded, covalently closed circular, highly structured, noncoding RNAs) are seed transmitted and easily spread mechanically in tomato; control relies on limiting introduction of the viroid to prevent disease outbreaks. Disease symptoms-including stunting, reduced vigor, flower abortion, reduced size and number of fruits, and reduced root growth-can result in significant crop losses. These dramatic alterations in plant development are the result of differential gene expression patterns, including the transcriptional activation of genes encoding transcription factors and a protein kinase involved in phospholipid signaling. Silencing of the protein kinase gene in tobacco resulted in substantially increased vigor, including increases in plant and root biomass. Our studies involved a two-pronged approach to determine if symptoms of viroid infection can be mitigated to reduce crop losses in tomato: 1) silencing of key genes responsible for symptom production, and 2) grafting susceptible tomato cultivars onto either tolerant, engineered, or commercially employed rootstocks to evaluate changes in gene expression, water use efficiency, plant vigor, and the effects of viroid infection on fruit development.

To Stem Rot or Not: Gene regulation of *Sclerotinia sclerotiorum* in the soybean pathosystem
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Sclerotinia sclerotiorum is a broad-host range necrotrophic pathogen which is the causative agent of Sclerotinia stem rot (SSR), a major disease of soybean (*Glycine max*). A time course transcriptomic analysis was performed in both compatible and incompatible soybean lines to identify pathogenicity and developmental factors utilized by *S. sclerotiorum* to achieve pathogenic success. A comparison of genes expressed during early infection identified the potential importance of toxin efflux and nitrogen metabolism during the early stages of disease establishment. Analysis of genes highly upregulated in-planta revealed a temporal regulation of hydrolytic and detoxification enzymes, putative secreted effectors, and secondary metabolite synthesis genes. Redox regulation also appears to play a key role during the course of infection, as suggested by the high expression of genes involved in reactive oxygen species production and scavenging. Finally, distinct differences in early gene expression were noted based on the comparison of *S. sclerotiorum* infections of resistant and susceptible soybean lines. Although many potential virulence factors have been noted in the *S. sclerotiorum* pathosystem, this study serves to highlight soybean specific processes most likely to be critical in successful infection. Functional studies of genes

identified in this work are needed to confirm their importance to disease development, and may constitute valuable targets of RNAi approaches to improve soybean resistance to SSR.

Amino acids D(168)N, G(215)S, and I(217)V in PIPO of new TuMV isolates affect P3 subcellular localization and pathogenicity in *Nicotiana benthamiana*

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Two Korean isolates of turnip mosaic virus (TuMV), KIH1 and HJY1, show severe and mild symptoms, respectively, in *Nicotiana benthamiana*. Infectious clones under control of both 35S and T7 promoters allow infection by agroinfiltration or by *in vitro* transcripts. Analysis of chimeric constructs between KIH1 and HJY1 localized the major determinant of symptom severity in *N. benthamiana* to the N-terminal 3.9 kb of KIH1; amino acid sequence identities of P1, HC-Pro, P3, 6K1, and the CI N-terminal domain were 90.06%, 98.91%, 93.80%, 100%, and 100% respectively. Expression of either P1 or P3 from a potato virus X (PVX) vector yielded symptom differences only between P3 of KIH1 and HJY1, implicating P3 in symptom severity in *N. benthamiana*. However, over-expression of P3 from PVX failed to induce systemic necrosis. As an additional coding region, PIPO, lying within the sequence of the P3 gene is expressed by polymerase slippage resulting in a frameshift into the +2 reading-frame, we also examined the differences in the predicted P3N-PIPO sequences of isolates KIH1 and HJY1. Differences in the cell wall localization of GFP:P3, dependent on co-expression of either a P3N::PIPO fusion or PIPO of KIH1 and HJY1 were observed in agroinfiltrated *N benthamiana*. There were only three residue differences in the P3N portion, and three substitutions in the PIPO segment - (D(168)N, G(215)S, and I(217)V, that are possibly responsible for the differential symptoms in *N. benthamiana*.

Amino acids in P1 (A241) and CI (E1459) of a new TuMV isolate from China are associated with induction of stem necrosis in *Nicotiana benthamiana*

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Leaves were collected in 2017 from radish plants in fields in northern China with virus-like symptoms, and four infectious clones of turnip mosaic virus (TuMV) were generated from these samples (CN3:MH735111, CN7:MH735112, CN8:MH735113, and CN9:MH735114). The genomic sequences of these four isolates were determined and showed 90.53% - 99.96% nucleotide identity, and 97% - 99.94% amino acid identity. Among these four isolates, CN7, CN8, and CN9 were most closely related to Chinese isolate CCLB (KR153038), and CN3 was most closely related to Korean isolate HJY1 (KX674727). Phylogenetic analysis of TuMV genomic sequences showed that all four isolates clustered into the basal-BR group. Only four differential amino acids in the N-terminal half of the polyprotein (one in P1, two in HC-Pro, and one in CI) of three of these isolates were associated with varied symptom development in *Nicotiana benthamiana*. Although CN3 had the most divergent polyprotein sequence, the symptoms induced by CN3 and CN9 were the most similar among the four isolates. Residue V241 in P1, and HC-Pro residues G485 and S646 were unique to CN8, while CI residue E1459 was unique to CN7. In previous work we have found no association of HC-Pro differences between 20 Korean TuMV isolates and symptom severity. Chimeric constructs between stem necrosis-inducing isolate CN7 and mild isolate CN8 suggest that amino acids A241 in P1 and E1459 in CI are most likely required for induction of stem necrosis.

A core component FpDep1 in the Rpd3L histone deacetylase complex is essential for development and virulence in *Fusarium pseudograminearum*

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Fusarium crown rot of wheat caused by *Fusarium pseudograminearum* occurs in many arid and semi-arid cropping regions of the world and is an emerging disease in China. Lack of resistant cultivars makes this disease difficult to be controlled. To better understand the molecular mechanisms regulating pathogenesis, in this study, we identified and characterized the *FpDEP1* gene that is orthologous to *Saccharomyces cerevisiae DEP1* encoding a component of the Rpd3 histone deacetylase complex in *F. pseudograminearum*. The *Fpdep1* gene deletion mutant was significantly reduced in growth rate and aerial hyphae on PDA plates. It failed to cause typical lesions on the leaves and had limited expanding on coleoptiles in infection assays with wheat seedlings. Histological analysis indicated the *Fpdep1* mutant failed to overcome host defense response as visualized by detection of ROS and expand from the initially colonized cells to neighboring plant cells, which was consistent with its sensitivity to hydrogen peroxide in vitro. Expression of the FpDEP1-GFP fusion complemented the mutant phenotypes and the FgDep1-GFP fusion protein localized to the nucleus in conidia and hyphae**.** Affinity purification coupled with LC-MS/MS and yeast two hybrid assayed showed that the Rpd3L-like HDAC complex contains at least FpDep1, FpSds3, FpSin3, FpRpd3, FpRxt3, FpCti6, FpRho23, and FpUme6 in *F. pseudograminearum*. These results indicated that FpDep1 is involved in a HDAC complex that regulates fungal development and virulence in this important wheat pathogen.

The *rps10* gene as a new barcode locus for oomycetes and its utility in metagenomics studies of environmental samples

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The current barcode loci for identification of oomycetes are a portion of the mitochondrially encoded *cox1* gene and the nuclear encoded ITS region of the rDNA. Both loci have the advantage of being high copy number, thereby providing a greater sensitivity when generating templates from environmental samples. However, both loci also have the disadvantage that amplification primers are not specific, leading to background amplification. Comparative genomics of mitochondrial genomes from a wide range of oomycetes has identified a unique gene order of conserved tRNA genes flanking the mitochondrially encoded *rps10* gene that is not present in plants or the Eumycota. Highly specific *rps10* primers for oomycetes were developed that are useful for template amplification from environmental samples and obligate pathogens in host tissue. Studies to date have confirmed the ability of this locus to correctly identify organisms to a species level with evaluation of additional taxa in progress. Primers were modified to generate an amplicon suitable for MiSeq sequencing. Amplification of a mock library of DNA from known cultures and diverse environmental samples provided proof-of-concept that only oomycetes were amplified, improved identification over ITS and enabled the description of complex microbial communities. A draft reference database and website to query and curate information for metabarcoding projects are available for feedback from the oomycete community.

Understanding the molecular basis of *Phytophthora cinnamomi*-plant interactions using *Nicotiana benthamiana* as model system
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Phytophthora cinnamomi (*Pc*), the causal agent of phytophthora root rot, is the major constraint of avocado production worldwide. Despite its economic importance, the molecular basis of plant immunity and *Pc* pathogenicity are largely unknown. Functional genomic studies in avocado are challenging due to limitations associated with tree biology and lack of efficient transformation assays and a reference genome. A detached leaf *Pc* inoculation assay using *Nicotiana benthamiana* was used to conduct a dual RNAseq study at 6, 12, 24, 36, and 48 hours post inoculation (hpi). Genes involved in hormone signaling, oxidative stress, production of antimicrobial compounds, as well as receptor-like kinases, NLRs, transcription factors such as WRKY, and pathogenesis-related proteins were differentially expressed at different infection stages. Approximately, 53% of the *Pc* predicted RXLR effectors were expressed *in planta* and several effectors exhibited a dynamic expression during the infection. The peak for RXLR effector expression *in planta* occurs at the biotrophic stages of infection (24 hpi), which is consistent with the pathogen haustorium formation. Transient overexpression of host genes and pathogen effectors are being conducted in *N. benthamiana* followed by *Pc* inoculation to determine their effects in plant immunity and pathogenesis respectively. The information gained using this system can be translated to avocado using biotechnology or marker assisted selection.

Negative regulation of siderophore biosynthesis by a GATA transcription repressor in *Alternaria alternata*
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Iron acquisition and storage in all cells must be carefully regulated to avoid iron-induced toxicity. *Alternaria alternata* produces a dimethyl coprogen siderophore to acquire iron from surrounding environment. The ability to acquire iron is also required for detoxification of reactive oxygen species and virulence. Genetic analysis reveals that a *SreA* gene encoding a GATA type transcription factor negatively regulates siderophore production. *A. alternata* strains lacking *SreA* (ΔsreA) increase siderophore production by 3.7-fold compared to wild type. ΔsreA displays wild-type virulence in citrus, even though the mutant reduces 77% radial growth of wild type grown on PDA. An iron chelator and EDTA partially rescue mutant growth. ΔsreA reduces 43% growth on minimal medium and slightly increases sensitivity to iron, potassium superoxide, and H_2O_2. However, ΔsreA decreases sensitivity to diethyl maleate, rose Bengal, eosin Y, Congo red, and calcofluor white. The expression of *SreA* is induced by iron and repressed by H_2O_2, and negatively regulated by the oxidative stress-responsive transcription activator Yap1. Deletion of *SreA* leads to the increased expression of the genes involved in siderophore biosynthesis and transportation and reductive iron assimilation. EMSA reveals that the *E. coli*-expressed SreA protein could bind the promoter of the *Nps6* gene encoding a nonribosomal peptide synthetase required for siderophore biosynthesis. Our results demonstrate that SreA plays a profound role in iron homeostasis and cellular sensitivity to xenobiotics.

A maize line carrying an *O*-methyl transferase HIGS construct accumulates lower aflatoxin under field conditions
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Maize (*Zea mays*) is one of the most important staple crops and is highly susceptible to both *Aspergillus flavus* infection and aflatoxin contamination. Host Induced Gene Silencing (HIGS) has shown promise for plant pathogen management. For this reason, the *A. flavus O*-methyl transferase (*omtA*) gene, which encodes a key enzyme involved in aflatoxin biosynthesis, was selected as a target for suppression through HIGS. Positive transformation events containing a portion of the *omtA* gene in B104 maize zygotic background was first produced in 2013. In 2018, T4 lines from two selected transformation events were planted and self-pollinated. Ears were inoculated with *A. flavus* 14 days after pollination and harvested at maturity for analysis of aflatoxin production. One inoculated homozygous T4*omtA* event showed a 3-fold reduction in aflatoxin accumulation compared to the null and B104 inoculated controls ($P > 0.025$). Small RNA sequence analyses of immature maize kernels also revealed the significant difference in sRNA production with high levels of gene specific sRNA being detected in homozygous *omtA* lines and only background level of sRNA being detected in the null

and B104 kernels. The suitability of using the *omtA* gene in HIGS for managing aflatoxin contamination in maize is being evaluated with additional studies.

Interaction of bell pepper endornavirus with *Colletotrichum* sp. and *Meloidogyne incognita*
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Information on plant-pathogen and pathogen-pathogen interactions is useful to develop approaches to control plant diseases. Only a few studies have been published regarding the interaction of plant viruses with non-viral plant pathogens. We conducted a study using two near-isogenic lines of bell pepper (*Capsicum annuum*); one infected with bell pepper endornavirus (BPEV) and the other virus-free and two pepper pathogens; the fungus *Colletotrichum* sp., which causes anthracnose and the root- knot nematode *Meloidogyne incognita*. Detached leaves of the two bell pepper lines were inoculated using spore suspensions of *Colletotrichum* sp. Furthermore, in independent experiments the two lines were also inoculated with eggs of *M. incognita.* Leaves of the BPEV-infected line inoculated with *Colletrotrichum* sp. showed significantly greater lesion area compared to the BPEV-free line. The two bell pepper lines inoculated with *M. incognita* did not show differences in egg production, juvenile populations, plant dry, weight, or root weight. However, RT-qPCR analysis of BPEV showed a significant reduction of the virus titer in the nematode-inoculated plants. These results suggest that infections of bell pepper with BPEV might result in an increase of the severity of anthracnose. Nevertheless, infections of bell pepper with BPEV do not seem to affect the reproduction and damage potential of *M. incognita*.

***In-vitro* effect of two digestive enzymes and pH on the dsRNA of endornaviruses of bell pepper and melon**
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The terms acute and persistent have been used to describe symptomatic and asymptomatic host reactions to plant viruses respectively. Endornaviruses of pepper (*Capsicum annuum*) and melon (*Cucumis melo*) are persistent viruses that have been detected in many commercial bell pepper and melon cultivars. Therefore, when eating the fruits of these two plant species, we consume endornaviruses. We conducted experiments on the *in-vitro* effect of two common digestive enzymes and pH on the structural integrity of the viral dsRNA of bell pepper endornavirus (BPEV) and Cucumis melo endornavirus (CmEV), evaluated by gel electrophoresis and reverse-transcription polymerase chain reaction (RT-PCR). The effect of the amylase, pepsin, and pH treatments on the dsRNA of both viruses was similar. Amylase did not appear to affect the structural integrity of the viral dsRNAs. In contrast, gel electrophoresis analysis of pepsin-treated dsRNA samples showed an abnormal electrophoretic migration and evidence of partial dsRNA degradation. DsRNAs were partially degraded when exposed to a pH value of 2.0 and completely degraded at a pH value of 1.0. Our results suggest that under the simulated gastric conditions used in this investigation, the structural integrity of the dsRNA of BPEV and CmEV can be negatively affected. Nevertheless, it is likely that our lower digestive organs are exposed to both, fragmented and full viral replicative intermediate dsRNA of these two endornaviruses.

The role of mating-type genes in pathogenicity of *Fusarium graminearum* to wheat
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Fusarium graminearum, the primary cause of Fusarium Head Blight (FHB) of wheat and barley in North America, is a homothallic ascomycete capable of outcrossing. Ascospores produced in early spring from perithecia on infested crop debris serve as primary inoculum for the establishment of epidemics. The sexual behavior of this fungus is regulated by the mating type locus *MAT1*, which is comprised of two linked idiomorphs, (*MAT1-1-1* and *MAT1-2-1*). It is assumed that the products of these two genes interact to form heterodimers that regulate homothallic sexual development. We generated several independent knockout mutants (KOs) of *MAT1*, *MAT1-1-1*, and *MAT1-2-1* in the PH-1 strain of *F. graminearum.* As expected, all of the KOs lost their ability to produce ascospores. The *MAT1-1-1* KOs, when paired with the *MAT1-2-1* KOs, formed fertile perithecia. Markers segregated 1:1 among the progeny of these perithecia, confirming that the *MAT1-1-1* and *MAT1-2-1* KOs are heterothallic. The *MAT1* KOs were unaffected in aggressiveness to point-inoculated winter wheat (susceptible variety Pioneer 2555) in the greenhouse. In contrast, KOs of the individual specificity genes, especially of *MAT1-1-1*, resulted in significant reductions in aggressiveness and, in most cases, in DON production. These results suggest that the *F. graminearum MAT1-1-1* and *MAT1-2-1* specificity proteins individually regulate genes that negatively impact the aggressiveness and toxigenicity of the heterothallic strains.

Importance of *Xylella fastidiosa* RNA metabolism for cold survival and plant virulence
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Xylella fastidiosa (*Xf*) is a xylem-obligate plant pathogenic bacterium that causes diseases in diverse crop plants. In grapevines the infection known as Pierce's disease, is limited to regions with warmer climates. In previous research, it was observed that grapevines infected with *Xf* can lose the infection during the winter season ("cold curing"), but this phenomenon is not completely understood. Compared with other bacterial pathogens, *Xf* lacks certain aspects of cold response, including expression of cold-inducible cold shock proteins. Due to the importance of cold shock proteins in RNA stabilization, this study investigates the role of RNA metabolism in *Xf* in response to cold temperatures, specifically a polynucleotide phosphorylase homolog (PNPase). PNPase has been characterized in other

bacterial systems for RNA exonuclease and polymerization activity essential to survival at low temperatures. An *Xf* PNPase knockout mutant (*Δpnp*) is currently being evaluated for cold survival, *in planta* virulence, and cold-inducible gene expression. The results will provide important information regarding *Xf* cold response which is relevant to understanding the mechanism of cold curing of *Xf* in grapevine.

Characterizing the role *Ralstonia solanacearum*'s catabolic pathways play in pathogenesis in the xylem & root tomato environment

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The soilborne pathogen *Ralstonia solanacearum* (*Rs*) causes bacterial wilt disease by colonizing plant xylem vessels, triggering wilt and death. *Rs* affects over 200 species, including tomato. Although xylem sap has been considered nutrient poor, *Rs* populations in wilting plants surpass 10^9 CFU/g of stem and *ex vivo* sap from infected plants supports bacterial growth. Previous genomic, transcriptomic, and metabolomic analyses offered clues about the pathogen's carbon sources during infection. These datasets suggested *Rs* can use dozens of carbon sources at low cell densities, as in soil or very early disease. However, once the bacterium is established in host xylem, a major regulatory switch limits its available carbon sources to some present in xylem sap like sucrose, glucose, fructose, and myo-inositol. We tested the hypothesis that *Rs* has adapted to use a narrow set of carbon sources abundant in host xylem sap during disease. Using bacterial mutants lacking specific catabolic pathways, plant virulence assays, and quantitative metabolomics of xylem sap, we defined the link between xylem sap composition and virulence in the *Rs* tomato pathosystem. These studies showed that sucrose is the most important carbon source for the pathogen when it is growing in tomato stems. Overall, the sugars that were important in xylem colonization were not needed for successful root colonization and vice versa. The contribution of each carbon source to *Rs* fitness aligned with infection stage.

Very long chain fatty acid modification of *Liberibacter crescens* lipid A may be essential for culturability

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Lipopolysaccharide (LPS) is the major surface molecule of Gram-negative bacteria consisting of O-antigen polysaccharides, the core and the hydrophobic lipid A anchored in the outer membrane. *Ca.* Liberibacter spp. are uncultured, phloem-limited bacterial plant pathogens associated with plant diseases such as citrus greening (Huanglongbing) and potato zebra chip. *L. crescens* (Lcr) is a nonpathogenic, but cultured surrogate for functional genomic studies of pathogenic *Ca.* Liberibacters. The lipid A from Lcr has a traditional penta-acylated chitobiose backbone with two ester-linked C16:0 (3-OH) and two amide-linked C14:0 (3-OH) fatty acid residues. Lcr lipid A also contains a very long chain fatty acid (VLCFA) C28:0 (26-OH), a unique feature of bacteria such as *Sinorhizobium meliloti* and *Brucella abortus* that form chronic intracellular infections within eukaryotic hosts. Absence of VLCFA-LPS in *S. meliloti* increases sensitivity to salt, detergents and pH etc. *S. meliloti* genes *lpxXL* (SMc04268) and *acpXL* (SMc04278) encode a lipid A C28 acyltransferase and an acyl carrier protein involved in the biosynthesis of VLCFA-modified lipid A. Homologs for both these genes are present in Lcr (B488_RS04675 and B488_RS04700, respectively) but absent in all pathogenic Liberibacters. Knockout mutations of several Lcr genes were readily obtained, but not mutations of *lpxXL* or *acpXL*, suggesting that VLCFA-modified lipid A may be essential for axenic growth of pathogenic Liberibacters.

The Subcellular Localization of *Ralstonia solanacearum* K60 Type III Effectors

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Ralstonia solanacearum is a soil-borne bacterial pathogen that causes Bacterial Wilt disease in over 200 plant species in 53 botanical families (Genin 2010). This detrimental disease is found globally and is caused by various strains of *R. solanacearum*; one example is the K60 strain which is found in the United States. Bacterial pathogens like *R. solanacearum* use their type III secretion system (T3SS) to suppress host immune responses and cause disease. T3SS is composed of a needle-like structure that pierces the host cell wall and secretes type III effector (T3E) proteins into the host cell. The function of the T3Es in the K60 strain is not well understood. A small list of K60 T3E proteins was selected to be studied; the first step in this experiment is to determine the subcellular localization of these T3Es. A green fluorescent protein (GFP) was attached to the C-terminus of the individual T3E, and was transiently expressed in two systems: the hairy root system in tomato and the *Nicotiana benthamiana* leaf system. The subcellular localization of each T3E was observed with the Zeiss LSM 880 Upright Confocal Microscope. Preliminary results suggest that two T3Es, *Rs*K60-8 and *Rs*K60-17, localize at the plasma membrane, *Rs*K60-15 at the nucleus, and two, *Rs*K60-3 and *Rs*K60-6, localize at actin filaments. Future work will use secretion assays to confirm secretion of the T3Es, as well as yeast two-hybrid and mutant analyses to determine the interacting partners and function of the individual T3Es.

Characterization of *Sclerotinia sclerotiorum* isocitrate lyase gene in virulence on canola

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From differential gene expression data, genes involved in peroxisome associated pathways like fatty acid ß-oxidation and glyoxylate cycle were identified as novel potential pathogenicity candidates. The role of these pathways/genes has been shown to be essential for pathogenicity in several plant pathogens, including *Leptosphaeria maculans*, another important pathogen of canola. Based on these findings, isocitrate lyase (SS1G_04975 – *SsICL*), a key glyoxylate pathway gene was selected as candidate pathogenicity/virulence gene

for functional characterization. Gene disruption mutants for *SsICL*were developed by targeted gene replacement following split marker approach. PCR assays confirmed correct replacement of the gene. The effect of deletion of *SsICL*did not result in significant reduction in mycelial growth on PDA or production of oxalic acid. However, *SsICL* deletion mutant significantly affected the ability of *S. sclerotiorum* to utilize oleic acid as sole source of carbon on minimal media. Pathogenicity assays on leaf inoculation indicated that *SsICL* is required for full virulence on canola.

Disruption of the Rice Blast genome to identify genes involved in regulation of Reactive Oxygen Species
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Rice Blast disease, caused by the filamentous, ascomycete fungus *Magnaporthe oryzae,* can result in devastating losses to crops worldwide. It is a virulent pathogen, which can lead to complete crop loss when conditions are favorable. *Magnaporthe oryzae* is a major roadblock to increasing rice production and furthermore, infects other important crops such as barley, finger millet and more recently, wheat. We seek to address the plant-fungal interactions between two hosts, rice and barley, and *M. oryzae* that occur at the beginning of the infection cycle. Understanding these interactions could provide us the ability to better equip our hosts with a strengthened basal immunity and help protect the crop against the pathogen. While there is a substantial amount of research on rice as a host, our specific goal is to uncover the fungal genes that weaken the host's basal responses, specifically related to reactive oxygen species (ROS). Reactive oxygen species play an important role in the plant's basal defenses; during pathogen attack the plant produces ROS causing a toxic environment. Previous work in our lab generated a fungal strain containing an endogenous sensor (HyPer) of ROS, which was demonstrated to specifically detect hydrogen peroxide during these plant-fungal interactions. Our goal will be addressed through two objectives: first, through generation and screening of a random insertion mutational library in a HyPer *M. oryzae* line to identify ROS-related phenotypes, and second, through characterization of the mutant lines using live-cell imaging *in planta*.

Role of type VI secretion system in latent infection of plant pathogens
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Type VI secretion systems (T6SSs) are versatile macromolecular machines in about 25% of the Gram-negative bacteria. The genome of *Xanthomonas perforans* (*Xp*), a pathogen causing bacterial leaf spot disease on tomato and pepper, carries two gene clusters (~40kb), T6SS-I and T6SS-III, whose predicted products have homology with T6SS-associated gene products from other bacteria. Despite evolutionary conservation of large T6SS clusters, the contribution of these large clusters towards the infection strategies of *X. perforans* remains investigated. Here, we present evidence that T6SS-III plays an important role in the pathogenesis of *Xanthomonas perforans* on tomato plants. We constructed a knockout mutant of *X. perforans* carrying an in-frame deletion in the core T6SS-III gene, *icmF3*. *In planta* growth assays using *Xp*Δ*icmF3* mutant and the wild type strain, individually and in competition, indicated that the mutant had a significant advantage in terms of growth in tomato plants as well as degree of disease severity. *Xp*Δ*icmF3* mutant also exhibited higher disease index in seedlings, indicating possible role of T6SS-III in seed to seedling disease transmission. Delay in overall disease symptom development along with slower *in planta* growth might provide a significant fitness advantage to a hemi-biotrophic pathogen. Our results demonstrate possible role of T6SS in contributing towards latent infection period of a pathogen.

Identification of novel *Penicillium expansum* virulence factors
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Penicillium expansum is the causative agent of blue mold of pome fruits, a post-harvest disease. The presence and further infection of this fungus in apple and pears results in significant economic loss worldwide and significant health concerns due to the production of the mycotoxin patulin in apple tissues. To date, there is no substantial host resistance to blue mold in commercial apple varieties and thus disease management relies on fungicides. Previous work has showed that CreA, a carbon catabolite repressor (CCR), is required for *P. expansum* virulence although its downstream regulatory pathways are yet to be elucidated. The deletion of *creA* resulted in a strain of *P. expansum* which is nearly avirulent on apple and unable to produce patulin. When comparing *P. expansum* WT strain and Δ*creA* infections on apple, RNA-*seq* data analysis showed there are 399 differentially expressed genes in the Δ*creA* mutant. For this study we have prioritized 36 genes that were down regulated in the Δ*creA* mutant by selecting gene categories that seemed most likely to regulate virulence (e.g. transcription factors, transporters, and proteins with DNA binding domains). Currently, deletion strains are being generated for the selected genes. In order to assess if these genes are involved in virulence, we will screen the generated mutants by comparing their growth on apples, patulin production, and spore germination compared to a WT strain. Genetic analysis of these regulated genes may provide suitable targets for controlling blue mold of pome fruits.

Assessment of HIGS as a Control Strategy for Sclerotinia Stem Rot in Soybean
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Sclerotinia Stem Rot (SSR) caused by *Sclerotinia sclerotiorum* is one of the most devastating diseases on soybean and causes significant yield losses worlodwide. Current disease management strategies largely rely on fungicide applications. To develop additional tools to manage this disease, we considered targeting specific virulence factors in *S. sclerotiorum* using RNAi approaches. Oxalic acid is a known virulence factor for this fungus on soybean, and genes involved in its biosynthesis were identified. A gene (*Ssoah1*) encoding an oxaloacetate acetyl hydrolase is required for oxalic acid (OA) synthesis and was shown to be important for the pathogenic development of this fungus on soybean. *Ssoah1*-double-stranded/small interfering RNAs (dsRNA/siRNA) were synthesized and fluorescently labeled with Cy3 dye. Confocal imaging shows that *S. sclerotiorum* was able to uptake labeled ds/siRNAs, and this

uptake resulted in decreased *Ssoah1* transcript levels. *Ssoah1*-dsRNA/siRNA were also generated *in planta* using Virus-induced Gene Silencing (VIGS). Infections assays revealed that VIGS plants showed a decreased *Ssoah1* gene expression and lower disease levels compared to empty vector control plants. Results of both assays indicate that *Ssoah1*-ds/siRNAs were successfully taken up by *S. scleotiorum* both *in vitro* and *in planta*, and conferred enhanced resistance to this pathogen. Our results suggest that HIGS is a viable control strategy against SSR in soybean.

Evidence of physiological tolerance and heterogenous resistance to a phage-tail like bacteriocin in *Pseudomonas syringae*
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Bacteriocins are bacterially-produced, narrow-spectrum, antimicrobial proteins that are proposed as antibiotic alternatives. Long-term use of bacteriocins in pathogen control requires understanding of various survival mechanisms the target cells use against bacteriocins. We utilized the model plant pathogen, *Pseudomonas syringae*, where we exposed *P. syringae* pv. *phaseolicola* (*Pph*) cells to partially-purified bacteriocin of *P. syringae* pv. *syringae* B728a. *Pph* cells at two growth stages were exposed to bacteriocin and surviving population enumerated 1, 4, 8, and 24 hours post-treatment. Significantly higher survival was obtained with stationary than the log cells. Upon re-exposure, majority of the cells did not gain any detectable resistance, indicating survival by tolerance mechanism. We also recovered a high-tolerant mutant with increased bacteriocin survival. Bacteriocin treatment also selected complete and incomplete resistant mutants that differed in bacteriocin sensitivity. By genome sequencing, we identified various lipopolysaccharide related genes as the candidates for resistant and a hypothetical protein containing a signal peptide and ten trans-membrane domains for the high-tolerant mutant. Moreover, by lipopolysaccharide analysis, we showed that the complete resistant mutants lack the lipopolysaccharide O-antigen, while the incomplete resistant and high-tolerant mutants contain the O-antigen but likely in an altered form to survive bacteriocins.

The effect of XopQ and XopJ4 on host specificity varies with the *Xanthomonas perforans* genetic background
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Xanthomonas perforans (*Xp*), causal agent of bacterial spot of tomato, uses type III secreted effectors to infect hosts. Phylogenetic comparisons have identified at least 3 distinct groups (designated 1-3) of *Xp* strains that vary in effector profile. We compared the effector repertoires among *Xp* strains and identified three *xopQ* alleles in which one was associated with group 2 *Xp* strains that were pathogenic on pepper. We also identified *xopJ4* in all tomato strains, but not in an *Xp* strain, Xp2010, that was isolated from pepper and determined to be pathogenic on this host. Based on these observations we hypothesized that XopJ4 may be a negative factor for virulence in pepper, while the XopQ alleles, found in Xp2010 and other tomato strains that cause disease on pepper, is a positive factor in pepper pathogenicity. The *xopJ4* gene was conjugated in Xp2010, and disrupted in representative tomato strains. Likewise, *xopQ* was disrupted in *Xp* strains representing different phylogenetic groups, including Xp2010. Strains were compared for bacterial growth in pepper leaves. Expression of XopJ4 in Xp2010 suppressed bacterial growth in pepper, whereas disruption of *xopJ4* in tomato strains had no effect. Meanwhile, disrupting *xopQ* lowered bacterial growth only for group 2 *Xp* strains in pepper. Our results suggest that contribution of individual effectors towards overall pathogenicity depends on genetic background of strains.

Multi-disciplinary approaches to identify novel sources of resistance to minor-effect virulence effectors of *Xanthomonas oryzae* pv. *oryzae*
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An optimal approach to manage bacterial diseases of plants is the use ofresistant varieties. However, effective and durable disease resistance is a continuous challenge due to the pathogen's evolution and adaptation on cultivated varieties. *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the causative agent of bacterial blight (BB) or rice, produces Transcription Activator-Like (TAL) effectors, which activate expression of host susceptibility genes. TAL effectors differently contribute to a strain's virulence, and some are essential to pathogen fitness. We hypothesize that effective and durable disease resistance is attainable by targeting conserved virulence factors in the bacterium. To test this hypothesis, we used a set of *indica*Multi-Parent Advanced Generation Inter-Cross (MAGIC) inbred lines, screening them for resistance against *Xoo*strains PXO99A and PXO99A overexpressing the conserved minor-effect TAL effector Tal7b (+Tal7b). Genome-wide association and interval mapping analyses revealed 13 disease resistance QTL, six specific to PXO99A +Tal7b and seven to PXO99A. Our data suggest that Tal7b targets more than one gene and that resistance may result from polymorphisms in the candidate Tal7b gene targets, disabling activation of the susceptibility genes. Future research will focus on the mechanism underlying these resistance phenotypes.

Functional analysis and subcellular localization of EF-hand protein in *Xanthomonas*
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Bacterial spot of tomato and pepper is caused by *Xanthomonas euvesicatoria* (Xe), *X. vesicatoria, X. perforans* (Xp) and *X. gardneri*. A gene, putatively named as Efh-Xfa was identified in *X. fuscans* subsp. *aurantifolii* (Xfa) that has a possible role in elicitation of HR

when inoculated in tomato. Bioinformatic analysis predicts the presence of two EF-hand motifs and a single transmembrane domain in Efh-Xfa. Sequence analysis indicated that Efh-Xfa homologs are present in Xp and Xe. Efh-Xfa and its homolog in Xe strains from pepper shares a core region of 138 amino acids. Xe and Xp strains pathogenic to tomato are mutated in this gene such that the protein is truncated upstream of the EF-hand motifs. The Efh-Xfa gene contains additional amino acids at the N terminus. Xp expressing the Efh-Xfa was unable to grow to high populations in tomato while Xp expressing the Xe homolog, designated Efh-Xe grew to slightly higher populations but lower than Xp alone. Furthermore, Xp expressing Efh-Xe did not elicit an HR in tomato. We mutated the N terminus of the Efh-Xe by moving the translational start upstream to match that of Efh-Xfa gene. Infiltration of Xp expressing the mutated Efh-Xe resulted in rapid confluent necrosis, although bacterial growth in tomato leaves was similar to Xp expressing Efh-Xe gene. Our preliminary data indicate the importance of the N-terminus of the Efh-Xfa gene and its possible role in rapid confluent necrosis. Subcellular localization using an *Efh-Xfa-phoA* fusion based assay suggests that Efh-Xfa protein is localized to periplasm

Identification of putative FliA sigma factor motifs in methyl-accepting chemotaxis protein genes of *Dickeya dianthicola*
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Dickeya dianthicola, a member of the *Pectobacteriaceae* family, has caused significant losses in the US potato production since 2014. Genomic comparison of the *Pectobacteriaceae* with closely related animal pathogens in the order *Enterobacteriales* identified an elevated number of methyl-accepting chemotaxis protein (*mcp*) genes in this family. For instance, *Escherichia coli* has 5 *mcp*, while *D. dianthicola* has 42. The high number of chemotaxis genes in plant pathogenic bacteria compared to animal pathogens suggests that chemotaxis is important for *Pectobacteriaceae* in navigating their environments and host plants. The purpose of this study is to determine how *mcp* are regulated in *D. dianthicola*. The alternative sigma factor FliA (σ^{28}) is the only sigma factor required for transcription of chemotaxis and motility-related genes in *E. coli*. Based on the consensus motif of FliA (5'-TAAAG-N14-GCCGATA-3'), we analyzed the 500-bp upstream region of *mcp* in *D. dianthicola* to identify putative FliA binding sites. Out of the 42 *mcp*, 15 were identified to have a FliA consensus binding site upstream of the coding sequence. The remaining 27 *mcp* lack a putative FliA binding site. Our results suggest that other sigma factors besides FliA activate transcription of these 27 chemotaxis genes.

Net charges at the N-terminus of capsid protein interfere with *Potexvirus* systemic movement
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Pepino mosaic virus (PepMV) is a member of the *Potexvirus* genus. The virus has a 6.4 kb single stranded (+)-sense RNA genome, encoding for RNA-dependent RNA polymerase, triple gene block, and capsid protein (CP). PepMV CP is required for both viral cell-to-cell movement via plasmodesmata and systemic movement via phloem. Little is known about the mechanisms of CP's involvement in viral movement. We engineered a mutant PepMV with seven additional amino acids at the N-terminus of its CP, including three highly positive-charged arginine residues. This N-terminal modification prevented the systemic movement of the recombinant virus but did not affect its cell-to-cell movement. Progeny viruses with restored systemic movement were recovered and found to contain compensatory mutations in the modified N-terminal region of PepMV CP, leading to an overall lower positive charge. We hypothesize that the increased positive charge of the CP N-terminal region interferes with the systemic movement function of PepMV CP. Five additional mutants with various net charges at the CP N-terminus were designed and tested to determine if altered charges caused the impaired systemic movement of PepMV. Our results indicated that increased positive charges at the N-terminus of PepMV CP blocked the systemic movement of the virus, and that the roles of PepMV CP in the cell-to-cell movement and in systemic movement can be uncoupled.

A tale of two transporters: Cellobiose transporters as the key to trigger virulence in the plant pathogenic bacterium *Streptomyces scabies*
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Pathogenicity by the plant pathogenic *Streptomyces scabies*, the causative agent of common scab disease, on various economically important root and tuber crops is triggered by cellobiose, a subunit of plant cell wall polymer cellulose. Cellobiose induces production of thaxtomin A, the main virulence factor of this species. This phytotoxin affects cellulose synthase leading to stunted growth and tissue necrosis. The ABC transporter CebEFG-MsiK has been shown to be the primary importer of cellobiose, hence, a deletion of this transporter showed a significant decrease in thaxtomin production and a consequent attenuated virulence on plants. However, bacteria missing this transporter were still viable on minimal medium with cellobiose as the only carbon source (TDMc). Homology searches revealed there to be two other transporter candidates. Of these, the transporter with the highest homology seems to be absent in other pathogens but is found in a select group of non-pathogenic *Streptomyces* species, while a third transporter is located within the pathogenicity island of the pathogenic species. Mutants in the genes coding for these transporters showed, under the conditions tested, at least one other transporter is important during the pathogenic interaction with the plant host. Under more complex conditions, as would be in plant-microbe interactions, the loss of this transporter could not be compensated for by the first transporter as shown by the results of plant bioassays and the inability of this mutant to produce toxin on plant-based media known to induce thaxtomin production.

Interaction of laccase3 with small heat shock protein 24 of the chestnut blight fungus *Cryphonectria parasitica*
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Laccase3 is an important virulence factor and induced by the presence of tannic acid, a group of phenolic compounds function as a natural barrier against pathogen infection. Heat shock protein 24 in *Cryphonectria parasitica* contributes to the stress adaptation and virulence of a eukaryotic pathogen. In the previous study, the expression pattern of *CpHsp24*, induced by tannic acid and hypovirus, was similar to that of laccase3 and the accumulation of *lac3* transcript in response to tannic acid supplementation was hampered in the *CpHsp24*-null mutant. It is tempted to speculate that *CpHsp24* might function as a molecular chaperone for the protein product of the *lac3* gene. The focus of the current study was to examine whether the protein product of the *CpHsp24* gene plays a part as a molecular chaperone for the LAC3 protein. Two genes were cloned and co-expressed using *Saccharomyces cerevisiae* heterologous expression system and initial attempt of binding was performed using GST-fusion based assay. The specific functional interaction of LAC3 with heat shock protein 24 was validated by laccase assay using the tannic-acid supplemented medium and spectrometric assay using DMOP as a substrate. The co-expressed transformants showed darker brown coloring and higher enzyme activity, whereas less color reactions were observed in single gene expressed cells. These findings suggest that the molecular association of heat shock protein 24 with LAC3 may contribute to pathogenesis.

One plus one does not equal two: The interaction of *Ditylenchus dipsaci* and *Fusarium oxysporum* f. sp. *cepae*
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The combination of a nematode and a fungal plant pathogen often results in a synergistic interaction where the disease symptoms or crop losses are greater than expected from either pathogen alone. Anecdotal evidence suggests that basal plate rot in garlic can be caused by the interaction between the nematode, *Ditylenchus dipsaci* and the fungus *Fusarium oxysporum* f.sp. *cepae* (Foc). A pot study under controlled environment conditions was conducted to determine if there was an interaction between these two pathogens on garlic and if the interaction was additive, synergistic or antagonistic. Sprouted garlic cloves were planted into pasteurized soil. Each pot was then inoculated with either 100 fourth stage juveniles of *D. dipsaci*, 40 ml of 3×10^5 conidial suspension (60% marcoconidia and 40% microconidia) of Foc, or both. There was also treatments with *D. dipsaci* applied one week before Foc and Foc applied one week before *D. dipsaci*. Disease severity (DS) on the garlic bulbs was high (53- 61%) with *D. dipsaci* alone and the simultaneous inoculation. Hence, no effect of adding Foc. There was an antagonistic relationship when Foc was applied 7 days before *D. dipsaci* with only 8% DS. Inoculation with Foc alone resulted in 23% DS. Thus there was no synergism, but early infection by Foc reduced the development of basal plate rot, even compared to the fungus alone. All inoculated garlic had lower, but similar, bulb weight than the noninoculated check. Symptoms on the leaves were not closely related to disease severity on the bulbs.

Effects of secondary metabolites produced by PGPR amended with orange peel on the mortality of second-stage juveniles of *Meloidogyne incognita*
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The effects of *Bacillus velezensis* (Bv) PGPR strains amended with orange peel powder (OPP) on the mortality of second-stage juveniles (J2) of root-knot nematode (RKN) (*Meloidogyne incognita*) were investigated *in vitro*. We observed that Bv strains grown on OPP strongly expressed many secondary metabolites, and their metabolome was distinct from that of Bv strains grown on glucose; therefore, we hypothesized that Bv strains grown on OPP would have enhanced expression of metabolites that increase the mortality rate of J2 compared to Bv strains grown on glucose. The treatments included inoculation of J2 with cell-free supernatants (CFS) from Bv strains grown on OPP or glucose, J2 with OPP and non-inoculated controls. The results showed that CFS from Bv strains AP136 and AP203 grown on OPP significantly increased J2 mortality compared to other treatments ($P < 0.05$). The J2 mortality rate increased with OPP-grown strain AP203 (85.2%) or AP136 (75.2%) compared to these strains grown on glucose (52.3% or 49.1%, respectively) or with the presence of OPP (44.2%) or the non-inoculated control (4.5%). The unexpected increase in J2 mortality in the presence of OPP alone may be due to effects of OPP on nematode-associated microbiota. A draft genome of Bv AP136 was used to predict the presence of eight different biosynthetic gene clusters, some of which do not correspond to known metabolites. Ongoing LC-MS analyses are being conducted to determine the effects of growth substrate (glucose or OPP) on the expression of Bv secondary metabolite(s) responsible for death of the J2 RKN.

Occurrence of soybean cyst nematode and nematode communities in organically certified fields in central Illinois
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Soybean cyst nematode (SCN) is one of the most important pathogens of soybean. Our hypothesis is that organic production systems impact SCN and other nematode populations. Soil samples were collected from four organically certified soybean fields with long or short-term rotations in central Illinois. Cysts were detected in three of four fields. To determine if these soils were suppressive to SCN, an SCN susceptible soybean cultivar was planted into pots containing these four field soils and infested with SCN eggs. After 35 days, soils from the two fields with long term rotations (5-7 years) had 47 and 39% lower cyst counts, respectively, than the control. Soils from the fields with shorter rotations (3 years) had 0 and 38% higher cyst counts, respectively, than the control. To examine if the nematode community impacts SCN, vermiform nematodes were extracted from the original soil samples. The most abundant plant parasitic nematode was *Hoplolaimus* sp. followed by *Pratylenchus* sp., *Meloidogyne* sp., and *Xiphinema* sp. Indices for soil health indicated the soil communities were enriched but less structured suggesting that nematode communities in these systems are characterized by families of opportunistic life history characteristics. Less suppressive soil was shown to be more structured than more suppressive soil indicating that the natural suppressiveness might be associated with other members of the soil food web.

Identifying cover crops as non-hosts for management of soybean cyst nematode, *Heterodera glycines*
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Soybean cyst nematode (*Heterodera glycines*) reproduces on a wide range of plants, including some cover crops. With the increasing acreage of cover crops planted in the Northern Great Plains for improving soil health, quality, and ecosystem, it is important to investigate whether these cover crops have an additional benefit for suppression and control of soybean cyst nematode (SCN), a major pest in soybean production. Forty-one cover crop species/cultivars from four plant families (Brassicaceae, Fabaceae, Linaceae, and Poaceae) were evaluated for their host status to SCN under controlled greenhouse conditions. Evaluation experiments were conducted in a walk-in growth chamber with two SCN populations, HG type 0 and HG type 7, from two soybean fields of North Dakota, using naturally infested soils and artificially inoculated sandy soils. After 35 days of growth, white SCN females were extracted from individual plants, and then identified and counted to determine a female index (FI) for each crop. Out of the 41 cover crops tested, SCN white females developed only in 10 crop species/cultivars, suggesting as either hosts (FI ≥ 10) or poor hosts (0 < FI < 10). The other 31 crop species/cultivars did not support any reproduction of both SCN populations across all the experiments and were classified as non-hosts (FI = 0). The non-host crops identified in this study can be planted as cover crops or rotational crops in SCN-infested soybean fields to reduce SCN numbers to manage this damaging disease.

Characterization, pathogenicity, and fungicide sensitivity of *Pestalotiopsis* spp. on strawberry in the U.S.
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Pestalotiopsis spp. have been known to cause strawberry fruit rot. In recent reports from Florida and other strawberry producing areas, the fungus was associated with root and crown rots. The taxonomic status is confusing as two novel genera, *Neopestalotiopsis* and *Pseudopestalotiopsis*, have been described. During the 2018–19 season, a severe outbreak was observed in two Florida fields where leaf, fruit, petiole, crown, and root symptoms were present. Currently, there are no fungicides labeled to control this disease in the U.S. Therefore, morphological and molecular characteristics, pathogenicity, and fungicide sensitivity of isolates from Florida were evaluated. Colonies were white, circular, and cottony on the upper side. On the bottom, root, crown, and petiole isolates were pale luteous to orange; and leaf and fruit isolates were white to pale yellow. Optimum temperatures for mycelial growth and sporulation were 25 and 30°C, respectively. In pathogenicity tests, all isolates were able to produce root and crown rot symptoms. However, on fruit, disease incidence and severity were higher with leaf and fruit isolates. Thirty-eight isolates were screened *in vitro* for their sensitivity to azoxystrobin, captan, fluopyram, penthiopyrad, flutriafol, tetraconazole, fludioxonil, and thiophanate methyl. Only captan and fluodioxonil significantly inhibited mycelial growth. Moreover, resistance to QoI fungicides was confirmed by the presence of the G143A mutation in the *cytb* gene. Molecular studies are currently being performed to determine the taxonomic status of Florida isolates.

***In silico* approach to reveal viral populations in two different pear cultivars using transcriptome data**
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The co-infection of diverse viruses in a host plant is common; however, little is known about viral populations and their quasispecies in the host. Pear is a relatively popular pome fruit belonging to the genus *Pyrus*. To examine viruses and viroids infecting pear in Korea, we collected 110 pear leaf samples from 35 orchards in five major pear producing area from June 2017 to October 2018. Nine viruses including six viruses in the family *Betaflexiviridae* and one viroid were identified in two different pear libraries. Most virus-associated contigs were matched to sequences of known viruses in pears, including *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), and *Apple stem grooving virus* (ASGV). However, some contigs were matched to sequences of *Apple green crinkle associated virus* (AGCaV) or *Grapevine yellow speckle viroid* (GYSVd), which is the first detection of the virus and viroid in pear. Phylogenetic analyses indicated that AGCaV was separated from ASPV isolates and GYSVd was grouped with 10 type 1 GYSVd isolates. Furthermore, we analyzed the amount of viral RNA and the copy number for the identified viruses and viroid. Based on the number of virus-associated reads, ASPV and ASGV were dominant viruses infecting two pear cultivars in Korea. Taken together, we describe the viral population and provide a comprehensive overview of the diversity of viral communities in two different pear cultivars.

The role of *Pythium* spp. in root rot of clary sage in North Carolina
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Clary sage (*Salvia sclarea* L.) is a winter specialty crop grown in northeastern North Carolina for the production of sclareolide, which is a fixative used in the fragrance industry. Poor stand establishment, plant stunting, and reduced yields are common problems that are exacerbated under wet conditions. In October 2018, 15 isolates of *Pythium* spp. were recovered from the roots of severely stunted clary sage plants from Bertie County, NC. Seven species were identified based on morphological features and by sequencing the ITS region of the rDNA. The most frequently recovered species were *P. spinosum* (5), *P. sylvaticum* (3), *and P. irregulare* (3). Seedling pathogenicity assays were performed on clary sage cultivars 'English' and 'Early Selection' by inoculating the embryonic plant root with a pathogen-colonized rice grain, and all isolates were pathogenic. Selected isolates also were pathogenic on seedlings grown in potting mix. All isolates were sensitive to mefenoxam at 100 µg/ml. To our knowledge, this is the first report of root rot of clary sage caused by *Pythium* spp. in North Carolina.

First detection of a phytoplasma associated with witches' broom of industrial hemp in the United States
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In 2017–2018, industrial hemp (*Cannabis sativa*) crops in Nye and Humboldt counties exhibited severe stunting and abnormal growth. The percentage of affected plants was approximately 25–30%. The disease was primarily found on 'Cherry Wine' and 'Berry Blossom' varieties and symptomatic plants were useless to harvest. Typical symptoms of the disease were excessive apical branching and crowded growth of underdeveloped leaves, giving a shoot proliferation or clusters of witches' broom appearance. Leaves in a cluster were yellowish, often distorted, and small. These symptoms suggest a phytoplasma infection. DNAs were extracted from 200 mg of leaf midrib and petiole tissue of symptomatic plants. A multiplex real-time PCR assay was performed using phytoplasma-specific primers (JH-F1/JH-F all/JH-R) and probe (JH-P uni). Phytoplasma DNA was detected in 9 of 61 tested samples with Ct values ranging from 14 to 33. Conventional and/or nested PCR was then performed using primer pairs P1/P7 and R16F2n/R2, and PCR amplicons of approximately 1.8 kb (PI/P7) or 1.4kb (nested) were obtained. The PCR products were purified, subcloned into pGEM®-T vector, and sequenced. DNA sequences of 5'- and 3'- ends of PCR amplicons were obtained. BLASTn search suggests that this phytoplasma is closely related to the clover proliferation group, and its identity is currently under study. To our knowledge, this is the first detection of a phytoplasma from industrial hemp crops in the United States.

Identification and pathogenicity of species of Botryosphaeriaceae causing canker and dieback in apple trees in Chile
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Chile is one of the major exporters of fresh apples worldwide with a production area of 37,000 ha. The fungal diseases are one of the major problems for fresh apple production, and in recent years, symptoms of Botryosphaeria canker and dieback of arms and twigs have become an important threat for apple growers in Chile. However, a systematic study of the importance of Botryosphaeria canker and dieback in the field has not yet been performed. Therefore, field surveys were conducted throughout the main apple-growing regions to determine the prevalence and to identify the Botryosphaeriaceae species associated with cankers and dieback in apple trees in Chile. A total of 115 samples of symptomatic branches or twigs were plated on PDA plus antibiotics and Igepal. Cultures were identified morphologically and molecularly using the ITS region of rDNA, a portion of the βtubulin and a part of EF1α genes. The results allowed to determine a prevalence between 8 and 40% of canker and dieback in apple orchards. Based on morphologic and phylogenetic analyses, we identified three species belonging to Botryosphaeriaceae family: *Diplodia seriata*, *Neofusicoccum arbuti* and *Lasiodiplodia theobromae*. All the isolates were pathogenic on 1-years old dormant cuttings and on twigs in the orchard. Additionally, these isolates caused rot lesions on inoculated apple fruits and were sensitive *in vitro* to several fungicides. The fungus *D. seriata* was the most frequent Botryosphaeriaceae specie obtained from apple trees with canker and dieback in Chile.

Genetic analyses of the laurel wilt pathogen, *Raffaelea lauricola*, in Asia provide clues to the source of the clone causing the current USA epidemic
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Laurel wilt is caused by the fungus *Raffaelea lauricola* T.C. Harr., Fraedrich and Aghayeva, a nutritional symbiont of its vector, the redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff. Both are native to Asia but were found in Georgia in the early 2000s and since spread to much of the southeastern United States killing >300 million trees in the Lauraceae plant family. Our goals were to elucidate the genetic structure of populations of *R. lauricola*, examine its reproductive strategy, and investigate the number of times the pathogen was introduced to the USA. A panel of 12 simple sequence repeat (SSR) markers identified 15 multilocus genotypes (MLGs) among 59 isolates from the USA (34 isolates), Myanmar (18), Taiwan (6) and Japan (1). Limited diversity in the USA isolates and the presence of one MAT idiotype (mating type locus) indicated that *R. lauricola* was probably introduced into the USA a single time. Only three closely related MLGs were detected in the USA, the most prevalent of which (30 of 34 isolates) was also found in Taiwan. MLG diversity was far greater in Asia than the USA with isolates from Myanmar being distinct from those from Japan, Taiwan and the USA. Although both MAT idiotypes were present in Myanmar and Taiwan, only the population from Taiwan had the genetic structure of a sexually reproducing population. The present results suggest that a Taiwanese origin is possible for the population of *R. lauricola* in the USA, although more work is needed.

Rapid `ōhi`a death pathogens cause two distinct diseases on *Metrosideros polymorpha* in Hawai`i
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Rapid `Ōhi`a Death (ROD) is an emerging disease on two islands of Hawai`i that has led to extensive mortality of the keystone forest species *Metrosideros polymopha* on Hawai`i Island since 2011. Artificial inoculation trials were conducted with isolates of the ROD pathogens, *Ceratocystis lukuohia* (Cl) and *C. huliohia* (Ch), on forest trees in 2017. Mature trees naturally-infected with either pathogen were also identified for comparative study. Visual results from whole tree dissections of all study trees and results from numerous isolations of the pathogens revealed two distinct diseases that differ in their rate and pattern of colonization within the host and in crown symptom progression. Inoculation of tree main stems with Cl led to 100% crown wilt in five and partial wilt in three of the nine study trees within one or two months of inoculation in mid-June or late August, respectively. Extensive, vertical xylem staining of stems was observed on all nine trees. In contrast, long elliptical bark cankers underlain by reddish-brown sapwood resulted on stems inoculated

with Ch, but no crown symptoms were observed within 54 or 91 days after late May and early August treatments, respectively. No disease symptoms were observed on control trees for Ch or Cl trials. After comparing results of artificially-inoculated and naturally-infected trees, we conclude that ROD is actually two diseases, i.e. a systemic wilt disease caused by Cl and a canker disease caused by Ch.

Occurrence of new phytoplasma disease on date palm in Jordan
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Date palm (*Phoenix dactylifera* L.) is one of the most important cash crops in Jordan, occupying 33000 donum, where 450000 trees produce 12000 ton of dates annually. A symptoms of mild leaf stunting, yellow streaking and a reduction in fruit and stalk size had been observed in date plam orchards. A total of 262 healthy and symptomatic samples were collected from different date palm cultivars, herbs growing under trees and different possible hosts growing in the orchards in addition to leafhoppers. Total DNA was extracted from all samples. Nested PCR was performed using phytoplasma P1/P7 primer pair at the first PCR round while R16F2n/R2 primer pair was used in the second round. Sequencing of representative PCR products was done in both directions. BLAST analysis of the obtained sequences revealed over than 98% similarity with different reference phytoplasma strains deposited in the Genebank and it shows 98–100% similarities with Candidatus Phytoplasma Ulmi strain.
To our knowledge, it is the first report about detection and identification of phytoplasma infecting date palm in Jordan.
Key words: *Phoenix dactylifera,* 'Candidatus Phytoplasma Ulmi', date palm, phytoplasma, 16SrI DNA, Jordan

Identification of *Cycas necrotic stunt virus* (CNSV) in alfalfa (*Medicago sativa*)
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Cycas necrotic stunt virus (CNSV), genus *Nepovirus*, family *Secoviridae*, was first detected in the gymnosperm *Cycas revoluta* in Japan. Prior to this report, it has not been identified in alfalfa (*Medicago sativa* L.). While analyzing alfalfa transcriptome datasets generated by others (SRR7751381, SRR7751384, SRR7751386), we found a total of 4,209 reads of CNSV that were subsequently assembled into an essentially complete viral genome. The virus has a bipartite single-stranded positive sense RNA genome; RNA1 and RNA2 are of 6,891 and 4,196 nucleotides in length, respectively. RNA1 encodes polyprotein 1 (P1) with predicted motifs for a protease cofactor, an NTP-binding domain, viral genome-linked protein, a cysteine protease, and a RNA-dependent RNA polymerase; RNA 2 encodes polyprotein 2 (P2) with predicted domains for a movement protein and a coat protein. P1 consists of 2020 amino acids (aa) and shows 96.3% overall identity to the P1 of CNSV (AEN25475) while the P2 is 1260 aa-long and has 93.4% overall identity to the P2 of CNSV (AEN25476). The Pfam-predicted coat protein (CP) region of P2 shared ~ 97.8% identity with the CP of CNSV (NP620620) and pfam-predicted RNA dependent RNA polymerase (RdRP) region showed 93.9% identity to the RdRP of CNSV (NP620619). Based on these observations, the virus represents a new strain of CNSV adapted to *Medicago sativa*, for which we propose the name CNSV-A.

***In silico* and experimental identification of an amalgavirus in U.S. alfalfa (*Medicago sativa* L.) germplasm**
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Amalgaviruses are a new group of monopartite double-stranded RNA viruses identified in several plant species. Recently, genome sequences of an amalgavirus were reported from alfalfa (*Medicago sativa* L.), (PMID:27596539), and tentatively named Medicago sativa amalgavirus 1 (MsAV1). The MsAV1 genome was identified from alfalfa transcriptomic data released by a Chinese group (PMID:25799491). Here we present a first identification of MsAV1 by Illumina RNA-sequencing in seven out of 18 tested samples of U.S. alfalfa cultivar ZG 9830. A total of 7,910 virus reads were found among all positive samples, averaging 1,130 per sample. A complete viral genome was obtained by *de novo* assembly of paired-end reads (2 × 150 bp) using SPAdes or Trinity assemblers and then mapped to the available MsAV1 genome (NC_040591). Presence of the virus was experimentally confirmed by reverse-transcription polymerase chain reaction using primers derived from the *de novo*-assembled genome, following by cloning and sequencing of the resulting amplicons. Additionally, 5′ and 3′ rapid amplifications of cDNA ends were performed using a SMARTer RACE 5'/3' kit. The U.S. isolate of MsAV1 was 100% identical to the isolate NC040591 from China at both the nucleotide and amino acid levels, indicating the same origin. This is the first experimental confirmation of MsAV1 infection in alfalfa in the United States, significant because amalgaviruses are known to be vertically transmitted through seeds.

An emerging pathogen (*Diaporthe* sp.) of hop (*Humulus lupulus*) associated with foliar necrosis in commercial production yards in Michigan
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Hop (*Humulus lupulus*) production in the U.S. is expanding with 304 ha of hops harvested in Michigan (2018). In August 2018, two Michigan commercial hopyards reported a high incidence of necrotic leaf lesions with concentric circles on 'Chinook', 'Centennial', and 'Crystal'. Preliminary identification of the isolates recovered from lesion margins was based on pycnidia morphology and the ITS region. A BLASTn search of the ITS region indicated partial homology (96.6% pairwise identity) to *Diaporthe nomurai* (AB302238 or KC343154.1) for 15 out of 19 isolates recovered. Neighbor-joining trees were created for the ITS and TEF1 loci using a NCBI Popset and eight representative *Diaporthe* sp. isolates recovered from hop leaf tissue. All eight *Diaporthe*sp. isolates from hop clustered together on the same branch for both loci. The closest relatives were *D. nomurai* (KC343154.1), *D. scobina* (KC343195.1), and *D. paranesis* (KC343171.1) but based on these two markers the specific species could not be resolved. To test pathogenicity a detached leaf assay was performed. Ten-days post inoculation, irregular necrotic lesions with light brown pycnidia developed on all inoculated

abaxial leaf surfaces. Multi-locus sequence typing is underway to determine the species identity. This work serves as a basis for future studies of the management of this emerging hop pathogen.

Fitness, virulence and fungicide sensitivity of a newly reported pome fruit pathogen *Phacidium lacerum*
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Phacidium lacerum (*Ceuthospora pinastri*) is a recently reported quarantine fungal pathogen responsible for postharvest rot in apples and pears. Very little is known about its epidemiology and best management practices. We tested a representative sample of *P. lacerum* isolates for their ability to grow and sporulate under various conditions and their ability to cause decay on several apple cultivars. While *Phacidium* in vitro growth was established on all six media tested, sporulation was only observed on oatmeal agar. Among nine cultivars tested, Honeycrisp and Gala were the most susceptible, whereas WA 38 (Cosmic Crisp™) was the least susceptible ($P < 0.01$). Detached fruit assays were used to assess the sensitivity of *P. lacerum* to six pre and postharvest fungicides. After 6 months of storage at 1°C, fludioxonil (FDL) and difenoconazole (DIF) provided full control followed by pyrimethanil (PYR) and boscalid (BOSC) + pyraclostrobin (PYRA) with 70% control on non-wounded apples. On wounded fruits, FDL, DIF and PYR were as effective as on non-wounded fruits, whereas the efficacy BOSC+PYRA decreased to 7%. Thiabendazole was the least effective fungicide regardless of the inoculation method. Findings of this project will provide basis for development of efficient control strategies against *Phacidium* rot.

Pectolytic bacteria associated with bacterial stem canker disease of hop (*Humulus lupulus* L.)
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In late summer fall of 2014, nearly all hop (*Humulus lupulus* L.) plants in a newly established yard of cultivar 'Cashmere' in Idaho suddenly wilted just prior to harvest. Affected plants had dark brown, occasionally water-soaked, cracked cankers often exuding a cream-colored ooze near the base of the plant. Exudate from the bleeding cankers had a distinctive odor that attracted numerous insects. Since then, the problem has been observed regularly on cultivar Cashmere in multiple regions of the U.S. Isolations from the margins of symptomatic material have consistently yielded an *Enterobacter* and *Brenneria* sp., but have also yielded a *Corynebacterium* sp., several species of yeast fungi, and a *Pseudomonas* sp. Attempts to reproduce symptoms with the recovered organisms have largely been unsuccessful unless plants are incubated at elevated temperatures (37–40°C). A multi-locus sequence analysis placed the most virulent isolates in a monophyletic clade with the known endophyte and plant pathogen, *Enterobacter cloacae,* and in a unique clade within the genus *Brenneria*, causal agent of drippy pod of lupine and bark canker diseases of several woody hosts. Research is underway to conclusively identify the causal organism, determine if these bacteria have additive detrimental effects as a disease complex, and elucidate the role of abiotic stress in disease expression on hop.

Biological, pathological and molecular characteristics of a new potyvirus, Dendrobium chlorotic mosaic virus, infecting *Dendrobium* orchid
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Dendrobium smillieae is one of the popular orchids in Taiwan. This report described a new potyvirus tentatively named Dendrobium chlorotic mosaic virus (DeCMV) causing chlorotic and mosaic symptoms in *D. smillieae*. ELISA tests using six antisera against orchid-infecting viruses revealed that only a monoclonal antiserum against the potyvirus group reacted positively with crude saps prepared from a symptomatic dendrobium orchid. Potyvirus-like, flexuous filamentous particles were observed under an electron microscope, measuring approximately 700-800 nm in length and 11-12 nm in diameter. Sequence analyses revealed that DeCMV coat protein gene shared 59.6-66.0% nucleotide sequence identity and 57.6-66.0% amino acid sequence identity, whereas the DeCMV complete genome shared 54.1-57.3% nucleotide sequence identity and 43.7-49.5% amino acid sequence identity with those other known potyviruses. Thus, DeCMV can be considered as a new potyvirus. The whole DeCMV genome contains 10,041 nucleotides (MK241979) and encodes a polyprotein which is predicted to produce ten proteins by proteolytic cleavage. In pathogenicity test, results of inoculation assays demonstrated that DeCMV can be transmitted to dendrobium orchids by grafting and mechanical inoculation, as verified by ELISA using the DeCMV polyclonal antiserum and by RT-PCR using the CP gene-specific primers. The inoculated orchids developed the similar chlorotic and mosaic symptoms. In conclusion, DeCMV is a novel orchid-infecting potyvirus and this is the first report of a new potyvirus that infects dendrobium orchids in Taiwan.

Pathogenicity, phylogenetics, and cucurbit susceptibility to a soilborne disease complex of winter squash (*Cucurbita maxima*) in Oregon
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Winter squash (*Cucurbita maxima* cv. 'Golden Delicious') grown in Oregon for edible seed has experienced yield losses due to an undiagnosed soilborne disease. *Fusarium solani, F. culmorum, F. oxysporum, Plectosphaerella cucumerina,* and *Setophoma terrestris* were associated with symptomatic tissues in field surveys. Phylogenetic analyses of ITS and EF1α gene regions of 241 isolates of these species confirmed their identity. *Fusarium solani* isolates clustered with *F. solani f. sp. cucurbitae* race 1. In pathogenicity trials, *F. solani* produced the most severe symptoms, followed by *F. culmorum, F. oxysporum, P. cucumerina,* and *S. terrestris*. Mixed fungal species inoculum produced symptoms greater than *F. solani* alone. While these trials indicate the disease is primarily caused by *F.*

solani, the complex of *F. solani, F. culmorum, F. oxysporum,* and *P. cucumerina* enhances symptoms. To understand the host range, 35 cultivars representing seven cucurbit species were grown at four commercial farms in 2015 and 2017. Farms with a history of cucurbit production had higher disease severity than farms with no cucurbit history. Winter squash and cantaloupe (*Cucumis melo*) had the highest disease severity, and cucumbers (*C. sativus*) and a squash hybrid (*C. maxima x C. moschata*) had the lowest severity. Though winter squash had high severity overall, cultivars 'Sweet Mama' and 'Winter Sweet' exhibited resistance to the disease and could be grown as alternatives to industry standards.

Tar Spot of Corn: Predicting Local Epidemics

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Tar spot of corn, caused by the obligate fungal pathogen *Phyllachora maydis*, was first detected in the United States in 2015 in Illinois and Indiana. Since then, tar spot has been detected in Florida, Iowa, Michigan, Ohio, and Wisconsin. The epidemiology of the tar spot fungus has been studied in Mexico. A limited amount of information is available on the weather conditions that favor the development of tar spot and if these conditions can predict future epidemics in the Midwest. Tar spot is favored by cool conditions (monthly average of 15-21°C) and high relative humidity (monthly averages above 75%). Based on this information and data from epidemics in Wisconsin and Michigan in 2018, a preliminary disease prediction model was developed for testing in 2019. The underlying framework of the prediction model uses logistic regression with monthly mean air temperature and relative humidity inputs. The output results in a prediction of risk of tar spot development on a daily basis and suggests if a fungicide application is necessary to reduce disease. The model has been programmed into a smartphone application called Tarspotter, which is being beta-tested in 2019. Accurately predicting local tar spot epidemics in corn can help farmers make accurate, and environmentally and economically responsible fungicide applications.

Tar Spot of Corn: Hybrid Reaction and Susceptibility

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An unforeseen epidemic of tar spot of corn, caused by *Phyllachora maydis*, had a significant yield impact on corn production in the upper Midwest during 2018. Tar spot was first observed in 2015 in both Illinois and Indiana, and has since been confirmed in Florida, Iowa, Michigan, Ohio, and Wisconsin. The 2018 tar spot epidemic was the first time yield losses were documented in the U.S. Prior to this epidemic, no field research had been done in North American to screen corn germplasm for resistance to tar spot. Preliminary data from state hybrid trials in Indiana, Illinois, Michigan and Wisconsin demonstrated a range in hybrid susceptibility and reaction to tar spot and it is estimated that a 0.4 – 0.8 bu/A loss occurred per 1% increase in tar spot. Tar spot was observed on all hybrids assessed, although some hybrids showed lower tar spot severity and the crop canopy remained greener for a longer duration. All hybrids evaluated across the upper Midwest are considered susceptible based on data collected from this epidemic, consequently farmers in areas with a history of tar spot are urged to scout their fields during the growing season to ascertain if the disease is present. Although fungicides are available to manage the disease, hybrid and germplasm screening for susceptibility will be an important component of tar spot IPM in the future.

Tar Spot of Corn: Using Extension and Outreach Tools for Rapid Communication

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University Field Crops Pathology Extension programs in the Midwest annually monitor for field crop diseases to provide timely and effective information in the event of a disease threat to crop production. These programs were uniquely situated to provide a rapid response to the tar spot epidemic of corn in 2018. The team used a combination of social media, blogs, websites, and newsletters to engage and interact with farmers, industry, and crop consultants. This interaction led to a large number of sample submissions to diagnostic clinics and the development of distribution maps to chart epidemic progress. This interaction continued into the off-season with the development of a tar spot fact sheet released through the Crop Protection Network (CPN). Furthermore, Extension specialists have given over 100 presentations to over 10,000 people about tar spot that included information on symptomology, epidemiology and potential management strategies. These included presentations to crop management workshops, state Certified Crop Advisor conferences, winter county Extension meetings, and agribusiness gatherings. Videos and webinars on tar spot and how to manage it in future seasons have also been outputs of this successful Extension group.

Cotton leafroll dwarf virus: An emerging threat to cotton production in GA

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During fall 2018, cotton plants in several fields in Georgia were observed with symptoms resembling “Cotton blue” disease like leaf curling, reddening and drooping of leaves, subsequent distortion of leaf growth and shortening of upper internodes and their discoloration to deep green. The disease is caused by phloem-limited Cotton leafroll dwarf virus (CLRDV), transmitted by aphids. Symptomatic and asymptomatic leaves tissues were collected from different cotton growing regions in Georgia. Using the three different sets of specific primers, products of predicted sizes were amplified from the symptomatic tissues, but not from asymptomatic tissues collected from the fields. The complete genome of the virus is elucidated by primer walking and small RNA sequencing. Based on the sequences available, the virus detected from the symptomatic tissues is closely related to the South American CLRDV isolates. In 2018, the virus was detected from fourteen counties across the coastal plains of Georgia. In January 2019 commonly growing winter weeds, cotton stalks surviving through the winter and cotton regrowth from the previous crop were collected to understand the disease epidemiology. Preliminary results suggest that the virus could overwinter in the winter weeds and cotton stalks that survived the winter, potentially act as “green bridge” and virus reservoir. Understanding this could help us disrupt the disease cycle and develop management recommendation for this emerging virus. The yield loss due to the virus disease and the role of overwintering hosts need to be investigated.

Outbreak of *Xanthomonas* in Ohio – a real *Paeonia* in the neck
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The bacterial genus *Xanthomonas,* comprises roughly 30 species that infect hundreds of different plants. This host range includes ornamental plants in the floriculture industry, valued at $223 million in Ohio and $4.37 billion in the United States. The spread of these pathogenic bacteria and damage from severe outbreaks or misidentification is a cause of concern for many producers. In 2013, potted peony plants at an Ohio nursery showed dark purple, necrotic lesions on the leaves. Diagnostic procedures at The Ohio State University identified *Xanthomonas hortorum* as the causal agent based on colony morphology, partial genome sequencing and fulfillment of Koch’s postulates. Sequence data is nearly identical to a recent *Xanthomonas* outbreak in Virginia, suggesting that the pathogen is crossing state lines. Currently, there are no damage estimates or rapid screens available. In this study, we generated genome-enabled diagnostic primers specific to *X. hortorum*. This set of primers is designed to identify *X. hortorum* from other closely related *Xanthomonas* species to enable rapid identification and monitoring during future occurrences.

Marssonina Blotch of Apple Caused by *Marssonina coronaria* in New York
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Marssonina blotch (MB) is a fungal disease of apple leaves and fruit caused by *Marssonina coronaria* (Ellis & Davis) Davis (teleomorph *Diplocarpon mali* Harada & Sawamura). Favored by excessive number of rain events in 2017 and 2018, MB is a rising problem in apple production of New York and eastern United States leading to premature tree defoliation and fruit infections at the end of summer. To identify the causal pathogen, we isolated fungi from symptomatic apple fruit of cultivar (cv.) ‘Rome’ on peptone potato dextrose agar at 25°C in darkness. The five recovered isolates uniformly showed slow growing dark grey colonies after three weeks of incubation. The phylogenetic analysis by sequencing of the conserved regions of 5.8S rRNA and internal transcribed spacer (ITS) revealed that our isolates were 99% similar to *Marssonina coronaria* reference strains in NCBI GenBank. High sequence similarity (98~100%) among our isolates confirmed these results. Pathogenicity test on 4-month-old potted apple seedlings (cv. Rome) inoculated with suspension of 1×10^6 conidia ml $^{-1}$ and placed in humid conditions showed small dark brown to black spots developed on leaves 10 days after inoculation. Our results for the first time confirm the presence of MB caused by *M. coronaria* on apple in New York.

Two species of *Diaporthe* cause needle-loss, branch dieback and cankers on Colorado blue spruce (*Picea pungens*) in Michigan
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Since 2006 there has been a decline in Colorado blue spruce (CBS; *Picea pungens*) planted as landscape trees and for Christmas tree production throughout the Lower Peninsula of Michigan, United States. This decline is characterized by a gradual loss of needles in the lower portion of the tree starting at branch tips, followed by entire branch dieback which gradually progresses upwards over several years. This dieback is linked to shallow branch cankers visible in the phloem when the bark layer is removed. Isolates in the fungal genus *Diaporthe* have been consistently isolated from lesion margins on symptomatic branches. To determine the species of *Diaporthe* linked to the decline of CBS in Michigan, seven gene regions were sequenced from a collection of *Diaporthe* isolates collected in 2011–2018 from CBS and other woody hosts. Subsequent phylogenetic analyses indicated that *Diaporthe eres* and a novel *Diaporthe* clade were present on symptomatic CBS in Michigan. The new species *Diaporthe vadumcancri* is described. Koch’s postulates were confirmed for *D. vadumcancri* and *D. eres* and *D. vadumcancri* is significantly more virulent than *D. eres* on CBS. Due to sampling only of the terminal ends of branches and the inconspicuous nature of cankers, it is likely that the etiology of spruce decline has commonly been attributed to a range of needle cast diseases, instead of the canker causing *Diaporthe* spp. Further research focused on better understanding the epidemiology of *Diaporthe* spp. is essential to adequately manage for this disease issue.

Infection by *Neopestalotiopsis* spp. occurs on unwounded eucalyptus leaves and is favored by long periods of leaf wetness
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Brazil is the global leader of forest productivity; however, one of the major challenges to fiber crops is the existence of fungal diseases. In this specific instance, *Pestalotiopsis*-like fungi were continuously observed in a privately-owned forest nursery in Brazil, reaching epidemic levels with incidence levels greater than 70% in the eucalyptus micropropagation stages. The objectives of the present study were to identify the causal agent associated with leaf spot and dieback on eucalyptus, assess its pathogenicity to different commercial clones, and evaluate which conditions favor infection by the pathogens. Results showed that *Neopestalotiopsis protearum* and *N. rosae* caused symptoms on unwounded eucalyptus leaves and disease development was strongly correlated to long leaf wetness periods (≥ 72 h). All of the commercial clones tested were susceptible to both pathogens and the set of data disputes the assumption that pestalotioid fungi are weak and opportunistic. The data produced in the study implies that single-gene phylogenetic trees constructed from ITS and TEF regions showed unresolved polytomies, while TUB and multi-locus phylogenies provided sufficient resolution tree topologies. This is the first report of different phylogenetic species of *Neopestalotiopsis* causing dieback, leaf and stem lesions on eucalyptus seedlings. Hence, this study can provide a basis for developing novel management strategies in forest nurseries.

Is an invasive forest pathogen moving into a new environment? *Phytophthora ramorum* found on chaparral plants in Coastal California

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Phytophthora ramorum, causal pathogen of Sudden Oak Death and ramorum blight, was discovered in California in the mid 1990ies, and spread quickly along a narrow band close to the coast characterized by mild temperatures and abundant year-long moisture. The foliar host California bay laurel (*Umbellularia californica*) is an essential driver for the spread of the disease to 'dead-end hosts', such as coast live oak (*Quercus agrifolia*). Recently, *P. ramorum* was detected on several chaparral plants (manzanita, *Arctostaphylos spp.;* chaparral pea, *Pickeringia montana*) on a high, sun-exposed ridge in Marin County. During 2018, a severe outbreak of disease was observed on Mt. Tamalpais, with symptoms including wilting, branch dieback and occasionally plant death. Several plants showed a positive reaction for *Phytophthora spp*. using immuno-strips; and *P. ramorum* was detected using PCR from a manzanita stem. In addition, *Neofusicoccum australe* (Botryosphaeriaceae) was isolated from a symptomatic plant. Potted rhododendron plants were placed near symptomatic plants on Mt. Tamalpais to monitor the possible spread of airborne inoculum and the effect of environmental parameters such as rainfall on the timing and appearance of disease symptoms. While it is still unclear whether the observed symptoms are caused by a disease complex, and which role *P. ramorum* has in it, mounting evidence indicates that *P. ramorum* is expanding its host range and moving into new environments.

Characterization pectolytic bacteria associated with blackleg and soft rot in Pennsylvania potatoes

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Recent outbreaks of potato blackleg and soft rot have resulted in yield loss across the mid-Atlantic due to stem and tuber rot and poor emergence. Surveys were conducted to identify the *Pectobacterium* spp. and more recently *Dickeya* spp. associated with these outbreaks. The objective of this study was to characterize select pectolytic isolates from Pennsylvania potatoes exhibiting potato blackleg and soft rot symptoms. Symptomatic plants were collected from Pennsylvania fields in 2016, 2017 and 2018. Pectolytic bacteria were isolated using the selective media, SL-CVP. Isolates were characterized by rep-PCR and 16S rRNA sequence analysis and a few isolates from each year were selected for whole genome sequencing. The phylogenetic relationships between isolates and *Dickeya* and *Pectobacterium* spp. type strains were compared using neighbor-joining analyses. Additional analyses using whole genome sequences and multilocus sequences on four housekeeping genes (*dnaA*, *dnaX*, *dnaJ* and *gyrB*) has been initiated. To date, most isolates have been identified as *Pectobacterium carotovorum* spp. though other *Dickeya* spp., namely *D. dianthicola*, have also been identified. An accurate evaluation of species diversity present in Pennsylvania is crucial for developing effective management methods. This knowledge can be used to elucidate how factors such as cultivar, location, season influence the bacterial species causing blackleg and soft rot.

A novel *Pyricularia*-like species on *Microstegium vimineum* in Indiana

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The family *Pyriculariaceae* includes several agronomically important pathogens of primarily grass hosts. The best studied example, *Pyricularia oryzae*, includes several divergent lineages including the causal agents of rice blast, wheat blast, and gray leaf spot of several turfgrass species. While other members of this genus are host specific, artificial inoculation and other studies have demonstrated cross-infectivity on a wider variety of grass species than typically found in naturally occurring epidemics, creating the potential for pathogen co-occurrence, genetic exchange, and adaptation to novel hosts. *Microstegium vimineum* is an invasive annual C4 grass that has colonized many forests in the Eastern USA. In the summer of 2018, a *Pyricularia*-like species was isolated from foliar lesions on *M. vimineum* in Indiana, where we are studying *M. vimineum* as a model for the emergence and accumulation of pathogens on non-native species. Symptoms included diamond to elongated, foliar, water-soaked, gray to olivaceous lesions with necrotic borders; some lesions underwent further secondary development and resembled blast-like symptoms on the leaf. Fungal isolates had pyriform, two to three-celled conidia born sympodially on erect conidiophores. A blast search of Genbank of ITS sequences revealed no results with greater than 95% nucleotide identity. Our preliminary results indicate these are isolates of a novel species within the *Pyriculariaceae* causing a new disease of *M. vimineum* in Indiana. We hypothesize that this represents adaptation to a new host by a pathogen of a native grass.

High-throughput sequencing identifies a new robigovirus in sweet cherry

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During the past decade the application of high-throughput sequencing (HTS) has facilitated the identification of several new fruit tree viruses. In 2009, during a screening of sweet cherry orchards in Northern Greece, a divergent viral sequence was identified sharing 75% nt identity with a conserved portion of the betaflexiviruses polymerase gene. Further HTS analysis of total RNA from this sample revealed the presence of several known sweet cherry viruses as well as a potential new species showing highest similarity to members of the genus *Robigovirus*. The genome of the new virus has a size of approx. 8100 nt and encodes for five open reading frames (ORFs). ORF1 encodes for an RNA polymerase, ORF2, ORF3 and ORF4 encode for movement proteins and ORF5 encodes for the capsid protein. Phylogenetic analyses clustered the new viral agent along with *Cherry green ring mottle virus* (CGRMV), *Cherry rusty mottle associated virus* (CRMaV), *Cherry necrotic rusty mottle virus* (CNRMV) and *Cherry twisted leaf associated virus* (CTLaV). These pathogens are associated with various disorders in sweet and sour cherries and for that reason are included in phytosanitary indexing and sweet cherry certification schemes. Since the new virus was identified from a sweet cherry cv Ferovia tree, the tentative name Robigo virus F (RVF) is proposed. The distribution of the new virus in Greek sweet cherry orchards and its pathogenicity in the infected trees are under study.

Pennsylvania Orchards for Rapid Apple Decline

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In 2018, the Pennsylvania Department of Agriculture and Pennsylvania State University conducted a survey of PA orchards for Rapid Apple Decline (RAD). Forty-four apple orchard blocks in 16 farms located in seven counties were inspected for symptoms of RAD, including presence of many declining trees, unseasonal tree discoloration and defoliation, brown cankers around graft union, and presence of rootstock suckers. A total of 516 samples were collected from both symptomatic and asymptomatic trees and tested. The main target was a newly described apple luteovirus 1 (ALV1), which may play a role in RAD. A one-step RT-PCR assay was developed and used for the ALV1 detection in the samples. One hundred and twenty samples (23%) including scion leaves, bark, blossoms, fruits, and rootstock sucker leaves were found positive for ALV1. The virus was detected from several apple cultivars including Crimson Crisp, Fuji, Gala, Honeycrisp and Golden Delicious. Most ALV1 positive trees (67%) were grafted onto M9 rootstocks. Our results also show the virus does not distribute equally within the tree, and the number of positive tree scaffolds increases during the infection process. ALV1 was detected in some trees in combination with 1-3 apple latent viruses (apple mosaic virus, apple stem grooving virus, apple stem pitting virus, apple chlorotic leaf spot virus); however, there was no consistency in virus combinations. Additional studies are necessary to further understand any connection of ALV1 to RAD.

Study of cotton leafroll dwarf virus in the Mississippi: Year zero

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In the fall of 2018, cotton fields in Mississippi (MS) exhibiting a range of virus-like symptoms were scouted and cotton plant material tested in the laboratory for possible virus infections. Symptomatic plants exhibited crinkled leaves, shortened internodes, reduced boll set, and abnormal plant growth extending out the top of the cotton canopy. Disease incidence in the fields tested was on the order of < 1% to ≥ 20%, depending on geographic location. Lab analyses revealed infections by a distinct strain of cotton leafroll dwarf virus (CLRDV), a virus previously reported to affect cotton production in South America and Asia, and most recently reported from Alabama, Georgia and MS. The virus was detected in a pool of 60 samples collected in 13 out of 17 surveyed counties, with an overall incidence exceeding 60%. Analyses of partial sequencing of the viral coat protein and RNA-dependent RNA polymerase genes of several MS isolates suggest high level of conservation. In addition to the initial observation of the virus in infected cotton material during the fall of 2018, winter weeds were screened during the winter of 2019 for CLRDV infections in order to better understand the ecology and epidemiology of this virus. Results of our study, along with data on the same virus from the southern United States, call for an urgent and coordinated multi-state effort to evaluate potential impact of this virus on the U.S. cotton industry.

Genetic diversity of tomato brown rugose fruit virus in North America and development of RT-qPCR for species-specific detection

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Tomato is one of the most important vegetable crops grown in many countries. With an increasing trend in greenhouse tomato production, growers are facing serious challenges from seed-borne and mechanically-transmitted viruses. A new tobamovirus, tomato brown rugose fruit virus (ToBRFV) first identified in 2014 in Jordan and Israel, has recently been reported on disease outbreaks of greenhouse tomatoes in southern California, USA and in Baja California, Mexico. Initial efforts to eradicate this emerging virus through total removal and hygiene practices have achieved some success. Unfortunately, new disease outbreaks were reported in other states. Several questions remain unanswered, including: 1) Was the virus introduced to North America through seeds or seedlings? 2) Were the outbreaks in Mexico and the U.S. resulted from the same or different introductions? In characterizing ToBRFV isolates in Mexico and the U.S., we identified two distinct types of genetic variants of ToBRFV. Understanding the genetic diversity of ToBRFV will improve the design of sequence-based detection tools. Due to cross serological reactions among tomato-infecting tobamoviruses, the potential presence of three additional tobamoviruses (i.e., tobacco mosaic virus, tomato mosaic virus and tomato mottle mosaic virus), and their varying levels of response to the resistance $Tm2^2$ gene, sensitive species-specific detection of ToBRFV is needed. In this study, we developed a RT-qPCR protocol, which will be useful for seed and plant health tests to assist in monitoring and controlling disease outbreaks.

Diseases of hydroponic microgreens

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Microgreens have become increasingly popular since their introduction in the early 1990s. Their use has expanded from that of a simple garnish to an important source of phytonutrients. Microgreens are produced for fresh-cut distribution or adhered to their growing media for harvest by the end-user. Species may be selected for aesthetics or for distinctive flavor characteristics. In a recent series of studies performed to evaluate the horticultural characteristics and volatile compounds associated with flavor, microgreens were produced using a hydroponic channel mat system under greenhouse conditions. Of the twenty-one microgreen varieties evaluated, three developed fungal diseases resulting in a loss of production. Red orach (*Atriplex hortensis*) seed rot was caused by *Penicillium* spp. and *Rhizopus stolonifera*. Loss of cilantro (*Coriandrum sativum*) was associated with seed infected by *R. stolonifera* and damping-off of basil (*Ocimum basilicum*) resulted from *Rhizoctonia solani*. In a pilot study using arugula (*Eruca sativa*), mustard (*Brassica juncea*), and anise (*Pimpinella anisum*), potential seed disinfestation treatments, acetic acid, hydrogen peroxide, peracetic acid, clove oil, and thymol were evaluated for their impact on microgreen germination and productivity. Acetic acid had no detrimental impact on biomass accumulation for arugula and mustard. All other treatments had a negative impact on arugula biomass production, while thymol and clove oil reduced mustard microgreen harvest weights. Anise germination was poor regardless of treatment.

Discovery of a new *Vitivirus* in an American hybrid bunch grape cultivar Blanc du Bois in Texas

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During 2018 season, we explored the virome of a declining 32-yr. old Blanc du Bois (*Vitis* spp.: 'Florida D 6-148' × 'Cardinal') vineyard in Texas. Total RNA extracts from 51 samples were pooled and subjected to Illumina high throughput sequencing (HTS). Analyses of the obtained ~23.5 million raw reads (76 nt each) revealed contigs specific to viruses and viroids, including 10 contigs (206 to 4,405 nt) with similarities to vitiviruses. RT-PCR tests of individual vines with two primer pairs designed from the HTS sequences revealed 11.8% (6/51) incidence of the *Vitivirus*. The complete ssRNA (+) genome of the virus, provisionally named as grapevine virus M (GVM), was determined via its RT-PCR amplification as two pieces of overlapping DNA fragments, and verification of its 5′ and 3′ extremities by RACE assays. The assembled Sanger-sequenced genome was 99.7% nt identical to the HTS-derived sequence. The complete genome of GVM is 7,387 nt, excluding the polyA tail and consists of five ORFs that are homologous and phylogenetically related to ORFs of grapevine-infecting vitiviruses. The replicase and coat protein genes of GVM were most identical to those of GVH at 66% nt & 75% aa and 79% nt & 89% aa levels, respectively. Based on current ICTV species demarcation criteria, GVM is proposed as a new species in the genus *Vitivirus*. The discovery of GVM is shedding more light on the evolution of vitiviruses across grapevine-producing regions of the world.

Identification, prevalence and host specificity of edamame pod diseases in Virginia

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Demand in the United States for edamame *Glycine max* (L.) Merr. has increased over the past two decades and is the second largest soyfood consumed in the US (25,000-30,000 tons annually). While domestic edamame production is on the increase, at least 70% of edamame consumed in the US is imported from China and Taiwan. A research trial in 2018 wasperformed at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter to evaluate new edamame breeding lines for the purpose of potentially increasing edamame production in the US. The objective of this preliminary study was to evaluate common diseases found on edamame in Virginia and assess potential differences in cultivar susceptibility. Despite minor levels of foliar diseases, significant pod disease was noted in certain cultivars. Incidence of damaged pods ranged from 7% to 62% across the 24 entries in this trial. Fungi were isolated from pods showing circular, discolored, and sometimes sunken lesions. No bacteria were recovered. Fungi were morphologically and molecularly identified, using the ITS4/5 primer set and sequencing the DNA. *Colletotrichum truncatum*, a number of *Fusarium* spp., *Phomopsis* spp., and *Cercospora kikuchii* were recovered. This trial will be repeated in 2019 with preliminary results being presented.

Characterization of *Botrytis* spp. associated with calyx-end rot in apple fruits in Chile

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Calyx-end rot (CER) is an important disease on apple in Chile, affecting the quantity and quality of fruits. Recently the incidence of CER has been reported between 0.1 to 0.2% to harvest reaching an 2% during storage at 0°C. However, a deep study of the characterization of species of *Botrytis* obtained from apples with CER has not yet been studied. Therefore, twenty isolates representatives obtained from symptomatic apples were characterized culturally, morphologically, molecularly and pathogenically. Botrytis isolates were classified according to sporulation degree in culture medium potato dextrose agar acidulated (APDA) and pea agar (PA). Shape and size of conidia and conidiophore were evaluated in PA media. Sclerotial production was evaluated on APDA. All isolates were molecularly characterized by amplification of glyceraldehyde-3-phosphate dehydrogenase (G3PDH), heat-shock protein 60 (HSP60), DNA-dependent RNA polymerase subunit II (RPB2) and necrosis and ethylene-inducing proteins (NEP1) genes. Pathogenicity tests were carried out on cultivars Cripps Pink, Fuji, Granny Smith, Modi, Premium Gala, Braeburn, Scarlette and Red Chief. Based in cultural, morphological and phylogenetic analysis the isolates were identified as *B. cinerea* and *B. caroliniana*. All

isolates were pathogenic in the apple cultivars tested, showed difference of virulence between isolates of both Botrytis species. Additionally, this isolates were sensitive *in vitro* to several fungicides. B. cinerea was the most frequent species of Botrytis causing CER in apple fruits in Maule Region, Chile.

***Phytophthora palmivora*: Another threat to English lavender in the United States**
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English lavender (*Lavandula angustifolia*) is one of the most widely grown lavender species worldwide. Phytophthora root rot (PRR) was reported on *L. angustifolia* at a nursery in the U.S. in 1991, and *Phytophthora nicotianae* was identified as the pathogen. PRR has now been reported around the world on multiple species of lavender and is caused by several species of *Phytophthora*. Since 2015, we have collected samples of lavender plants with PRR symptoms from 25 states in the U.S., and *P. nicotianae* has been isolated most frequently. However, *P. palmivora* was also isolated from *L. angustifolia* plants from multiple states. Previously, this pathogen has been reported on *L. angustifolia* only in Italy and Turkey. Two isolates of *P. palmivora* and one isolate of *P. nicotianae* isolated from roots of *L. angustifolia* plants were used to test pathogenicity. Four-month-old plants of *L. angustifolia* 'Hidcote' were inoculated with individual isolates or were used as non-inoculated control plants. Plants were grown in a greenhouse for 9 weeks and evaluated weekly for foliage symptoms. At the end of the trial, multiple disease parameters were measured. The plants inoculated with either *P. palmivora* or *P. nicotianae* developed typical PRR symptoms and had some mortality, and both pathogens were isolated consistently from diseased roots. Control plants remained pathogen- and symptom-free. Therefore, *P. palmivora* is capable of causing PRR on *L. angustifolia* and poses another threat to lavender production in the U.S.

Potato tuber necrosis induced by alfalfa mosaic virus depends on potato cultivars rather than on virus strains
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Alfalfa mosaic virus (AMV) was previously identified as the casual agent of internal tuber necrosis in a potato cultivar. To further characterize the isolate (CaM), it was inoculated to seven potato cultivars. Sporadic needle-sized necrotic spots occurred on the inoculated leaves of four cultivars. Thereafter, yellowish spots started to appear on the newly emerged leaves; which may be coupled with the needle-sized necrotic spots, depending on cultivars. As time progressed, the calico symptoms become more profound whereas the necrotic spots remained unchanged. All tubers of CaM-infected Innovator and Shepody plants developed sporadic necrotic spots in tuber flesh. So did ~23% and 8% tubers of CaM-infected Yukon Gold and Rochdale Gold-Dorée, respectively. Sequence analysis of the CP gene of CaM with that of other potato isolates indicated that CaM shared >97.1% sequence identity with all but four Egyptian isolates. Phylogenetic analysis of all available sequences demonstrated that RNA1 and RNA3 can be grouped into three major clades each whereas RNA2 can be clustered into two clades. Interestingly, CaM and Ca175-1, an isolate that was deemed non-necrotic in a previous study, had different phylogenetic clade patterns, indicating different RNA1-RNA2-RNA3 haplotypes: IA-I-IB (CaM) vs. Ca175-1 (IB-II-IA). Despite the differences, CaM and Ca175-1 induced similar levels of internal necrosis in tubers of Innovator and Shepody. The results suggest that the internal necrosis in AMV-infected tubers depends on potato cultivars rather than on AMV strains/haplotypes.

Bermudagrass Decline: A Complex Disease
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Gaeumannomyces graminis (Gg) is understood to be the causal agent of bermudagrass decline (BD), however a complex of ectotrophic root-infecting (ERI) fungi were identified by Vines (2015) at Mississippi State University. To investigate the natural occurrence of an ERI complex in bermudagrass putting greens, two greens at Mississippi State University Golf Institute were selected. One green was symptomatic for BD and the other asymptomatic. A fishnet grid was designed using ArcGIS to overlay each green to establish areas of interest (AOI). A summer core aerification provided root material for each AOI to detect ERI fungi using genomic DNA and multiplex qPCR. Fungi of interest included Gg, an undescribed *Gaeumannomyces* sp. (Gsp), *Magnaporthiopsis cynodontis* (Mc), and the novel *Candidacolonium cynodontis* (Cc). A four-fungal complex was present in 8% of AOIs within the BD green. Gg had the greatest number of DNA copies (# DNA) within 80% of AOIs while Cc had the greatest # DNA in the remaining 20% AOIs. This complex was also present within 7% of AOIs on the asymptomatic green. However, Gsp had the greatest # DNA within 80% AOIs and again Cc had greatest # DNA in the remaining 20%. Various combinations of the four ERI fungi were widespread within AOIs for both greens confirming the natural occurrence of an ERI fungal complex associated with bermudagrass roots. This may lead to the decline of affected turf. The impact on plant health will be evaluated in future analyses.

Tar Spot of Corn: Distinguishing the fungal communities of tar spot and fish-eye symptoms through amplicon sequencing
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Tar spot is a fungal disease complex of corn that has been destructive and yield limiting in Central and South America for nearly 50 years. *Phyllachora maydis*, the causal agent of tar spot, is an emerging corn pathogen in the United States. The tar spot disease complex putatively includes *Monographella maydis* (syn. *Microdochium maydis*), which increases disease damage through the development of

necrotic halos surrounding tar spot lesions. These necrotic halos, termed “fish-eye” symptoms, have been identified in the United States, though *Monographella maydis* has not yet been confirmed. In this study, next-generation sequencing of the internal transcribed spacer-1 (ITS1) ribosomal DNA was used to identify fungal taxa that distinguish tar spot infections with or without fish-eye symptoms. Fungal communities within tar spot only lesions were significantly different from communities within fish-eye lesions. Interestingly, a single OTU was found to be significantly more abundant in fish-eye lesions compared to tar spot lesions. This OTU was positively associated with fish-eye symptom networks, but not in tar spot symptom networks. Many OTUs identified as *Phyllachora maydis*, suggesting that different isolate genotypes may be capable of causing both tar spot and fish-eye symptoms, independent of other fungi. We conclude that *Monographella maydis* is not required for fish-eye symptoms in tar spot of corn in Michigan.

Viability of CGMMV recovered from naturally-contaminated cucurbit seeds
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Cucumber green mottle mosaic virus (CGMMV) is a devastating cucurbit disease, first reported in the U.S. in 2013. The damage to host plants and fruit can be extensive, resulting in substantial yield and quality loss. Although contaminated seeds are considered an important way to introduce the virus into new production fields, the potential of CGMMV particles from naturally-contaminated seeds to start a new epidemic is not fully understood. The objective of this study was to investigate the viability of the virus recovered from naturally-contaminated cucurbit seed samples by bioassay. Two CGMMV-positive cucurbit seed samples were used, one squash and one melon. Twenty subsamples, containing 100 seeds each, were tested by ELISA to confirm the presence of the virus. The squash and melon seed samples presented medium (12/20) and high (17/20) CGMMV contamination levels, respectively. *Nicotiana benthamiana* was initially used to assess the pathogenicity potential of extracted CGMMV sap from contaminated seeds. Plants inoculated with extraction buffer (PBS) and viable CGMMV particles were used as mock- and positive-controls, respectively. Typical leaf distortion and blistering were observed only on the indicator plants inoculated with extracts from melon seeds and positive control. Vertical transmission of the virus was only detected from the positive control. Additional pathogenicity tests on cucumber (‘Straight 8’), melon (‘Expedition’), and watermelon (‘Sugar Baby’) are underway.

First detection of *Cadophora sp.* involved in cranberry fruit rot in Eastern Canada
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Vaccinium macrocarpon, the most cultivated species of cranberry, hosts a large spectrum of pathogenic fungi responsible for 25–33% of production losses through cranberry fruit rot (CFR). Given that many fungal species can be involved in CFR, the diagnosis of the disease has always been challenging. To overcome this problem, a new molecular tool was recently developed to diagnose the 12 most important fungal pathogens causing CFR. However, during the validation phase of the molecular tool, an unidentified fungus was frequently isolated from infected berries. Based on ITS sequences, compared with publicly available sequences, the fungus was identified as belonging to the genus *Cadophora.* Koch’s postulates confirmed the pathogenicity of the fungus on cranberry fruit. Previously, a *Cadophora* sp. has been isolated from cranberry fruit in Washington and Oregon but its impact as a factor of CFR appears to be limited. Based on our observations, it was the first most frequently observed fungus on cranberry fruits, which would indicate that it has become an important component of the CFR complex in the Northeast. Specific primers targeting the genus have been developed and the precise specie identification is currently under study. Furthermore, the specific markers are effective when used on samples collected through spore traps. These strategies will be used to monitor the presence and progression of the fungus in cranberry beds to determine if it represents a serious threat for cranberry growers so they can be alerted of the need for preventive treatments.

Coffee quarantine pathogens: A high risk to the Colombian economy within unrestrained markets and immigration dynamics
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Coffee is one of the most valued commodities produced for nearly 25 million families in more than 60 countries. Colombia is the third largest producer with 14 million 60 kg bags annually, being the most important crop planted by 540,000 families in 887,000 hectares. Currently, there is a high risk for entry of very aggressive quarantine pathogens due to legal or illegal movement of plant and bean materials, and human immigration from countries where those pathogens exist. Based on biology, epidemiology and economics, the most threatening quarantine pathogens to Colombia´s coffee are *Colletotrichum kahawae* subsp. *kahawae*, *Fusarium xylarioides*, *Pseudomonas syringae* pv. *garcae*, *Xylella fastidiosa*, and *Coffee ringspot virus*, among others. They can increase production costs, yield and quality losses, and restrictions on international trade, with severe economic, social and environmental impacts. Consequently, FNC - Cenicafé works on a framework to exclude, prevent entrance, mitigate risks, and manage these pathogens based on International Standards for Phytosanitary Measures. The strategy suggests technical and legal measures to the National Plant Protection Organization, working with government, farmers, academics, industry, and research institutions, based on Pest Risk Analysis for entrance and establishment, and Contingency Plans for opportune and effective response when an outbreak is detected. Development and standardization of diagnosis methods, surveillance on ports of entry and fields are required for early detection, to support eradication actions of possible outbreaks.

Identification of new ‘*Candidatus* Liberibacter solanacearum’ haplotypes in *Solanum tuberosum* and psyllid family Aphalaridae in Klamath Basin, Oregon
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In the United States, two haplotypes of '*Candidatus* Liberibacter solanacearum,' A and B, have been shown to cause zebra chip disease symptoms in potato, *Solanum tuberosum*. In 2017, a single potato tuber from the Klamath Basin of Oregon showed classic zebra chip symptoms of darkened, striped vascular tissue and tested positive for '*Ca.* L. solanacearum' by conventional polymerase chain reaction. Analysis of the 16S ribosomal RNA gene, 50S ribosomal proteins L10/L12 genes, and the outer membrane protein gene identified a new haplotype of '*Ca.* L. solanacearum,' designated as haplotype F. Since potato psyllid is the known vector of '*Ca.* L. solanacearum' haplotypes A and B, psyllids were collected from sticky cards placed in or near potato fields in the Klamath Basin to identify the insect vector of '*Ca.* L. solanacearum' haplotype F. No potato psyllids were found, but psyllids of the Aphalaridae family (which likely do not develop on potato) were numerous, and a subset tested positive for '*Ca.* L. solanacearum.' Further analysis of the 16S ribosomal RNA gene, 50S ribosomal proteins L10/L12 genes, and the outer membrane protein gene identified another new haplotype of '*Ca.* L. solanacearum' in the Aphalaridae psyllids, designated here as haplotype G. These findings are the first reported identification of '*Ca.* L. solanacearum' in the Klamath Basin of Oregon. Further research to explore the impact of these haplotypes on potato in this region of the Pacific Northwest is needed.

Full-length genome sequence of *Cotton leafroll dwarf virus* infecting cotton in Alabama

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During 2017, previously unreported virus-like symptoms were observed in cotton fields in Alabama (AL). High-throughput Illumina RNA sequencing yielded a ~1,300 bp poleroviral fragment that had its closest match with *Cotton leafroll dwarf virus* (CLRDV), associated with cotton blue disease (CBD) in Africa, Asia, and most recently in Argentina, and Brazil. CLRDV-specific primers confirmed virus presence in symptomatic cotton plants collected from multiple AL locations during 2017–2018. The objective of this study was to validate an apparently full-length CLRDV-like genome sequence determined by Illumina sequencing of a 2018 AL isolate (5,865 bp), by cloning and sequencing a full-length genome for two AL CLRDV isolates representing Barbour and Macon counties in AL. The CLRDV-like genomes share ~95.0% pairwise nucleotide identity with an isolate of CLRDV from Brazil (HQ827780). The Illumina- and Sanger- DNA derived sequences encode six open reading frames (ORFs). The ORFs 1-5 share 92–100% amino acid (AA) similarity with isolates from South America. The most divergent coding region, ORF0, encodes for a silencing suppressor, sharing 82.4–88.5% AA similarity with CLRDV isolates from South America, indicating that AL isolates represent a previously unreported CLRDV strain, phylogenetically distinct from typical and atypical CBD-associated CLRDV strains.

A complex of badnavirus species associated with cacao swollen shoot disease in West Africa: High genetic variability in virus populations

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Since ~2000, the productivity of cacao (*Theobroma cacao* L.) has declined in part due to cacao swollen shoot disease (CSSD), which is attributed to infection by a group of badnavirus species. To better-understand CSSD-badnavirus species diversity and genomic variability, symptomatic cacao leaf samples were collected in Cote d´Ivoire and Ghana, and the total DNA was subjected to high-throughput sequencing. The RT-RNaseH and complete genome sequences were evaluated with respect to phylogenetic informativeness and species demarcation. Nucleotide diversity and potential for recombination were determined for the badnaviral genomes. Three badnaviruses were identified among the 30 new genomes, *Cacao swollen shoot virus* (CSSV), *Cacao swollen shoot CD virus* (CSSCDV), and Cacao red vein virus (CRVV). The RDP4 analysis revealed eight independent recombination events involving West African (WA) isolates. This result was supported by the network analysis (SplitsTree4), which also evidenced recombination affecting the diversification of WA badnaviruses. High values of nucleotide diversity (π) were observed for CSSV (π=0.06691) and CSSCDV (π=0.07111), with CRVV (π=0.21911) being the most variable among the three species. Purifying selection was the main selective force identified among the CSSV isolates. Phylogenetic trees reconstructed for the viral ORFs 1-2, RT-RNaseH, and MP, separately, were incongruent with one another and with the complete CSSD-badnaviral genome tree.

Hemp leaf spot, caused by a *Bipolaris* sp., is a major disease threat for industrial hemp

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Hemp leaf spot has become a major disease threat for field-grown hemp. Between 2014 and 2018, disease was confirmed in nine counties among at least 16 different fields in Kentucky; similar cases have been reported in at least five other states. Disease affected fiber, grain, and cannabidiol (CBD) cultivars similarly. Disease symptoms began in July or August and became progressively more severe until harvest in September. During rainy weather or in cases of early infection, growers deemed fields too severely affected for harvest. Leaf spots typically expanded to 1-2 mm, and spots frequently coalesced. Affected plants became blighted and sometimes stunted; floral materials such as calyces and sugar leaves became necrotic. Morphological characteristics of the causal fungus matched published descriptions of *Drechslera gigantea*. Large conidia and conidiophores, each documented at over 400 µm long, deemed this pathogen distinct among other leaf-spotting fungi. Microconidia developed in culture, but none were identified from field samples. Sclerotia-like structures developed in culture, as well. Phylogenetic analysis placed this pathogen within the genus *Bipolaris*, with sequence matches to *D. gigantea*. Thus, we propose that the causal agent of hemp leaf spot is *D. gigantea*, with support for reclassification to *Bipolaris*. Implications of this finding are mostly unknown, as all previous reports of *D. gigantea* are from monocot hosts, in contrast to *Cannabis sativa*, a dicot. Expanding acreage of industrial hemp in the US may experience severe losses as a result of this new disease.

Distribution and population of viruses in maize (*Zea mays*) in the United Republic of Tanzania
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Maize chlorotic mottle virus (MCMV) coinfection with any of several viruses in the family *Potyviridae*, causes maize lethal necrosis (MLN), characterized by severe necrosis and yield loss. The *Potyviridae* viruses are endemic to Tanzania, while MCMV was first reported in 2012 in northern Tanzania. Little information is available regarding the distribution of MCMV in Tanzania. Therefore, a survey was conducted in 2016/2017 to test the prevalence and distribution of viruses in maize across the country. Sampling for symptomatic maize and a second unbiased sampling at regular intervals across field transects was used to collect 1100 samples from 110 fields representing 11 regions. Next generation sequencing (NGS) and serological assays were used for virus discovery and diagnosis. Sequences mapped to important maize viruses, including sugarcane mosaic virus, MCMV and maize streak virus. MCMV distribution varied widely by region. Of all the reads that mapped to MCMV, 70.3% mapped to Northern region samples but only 0.22% mapped to Southern regions samples. Sequences with top hits to maize yellow dwarf virus (MYDV-RMV2), Johnsongrass mosaic virus, maize associated totiviruses, maize associated pteridoviruses and taro vein chlorosis virus were found. Analysis of transect samples showed that the incidences of potyviruses were similar across regions, whereas; MCMV was found across the country but incidences were highest in the Northern regions

Extent and significance of Botryosphaeria and Anthracnose diseases in avocado orchards of California
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Severe Botryosphaeria (Bot) canker and dieback and anthracnose blight have been observed in California avocados in the past few years. These diseases, caused by a complex of species in the Botryosphaeriaceae and Glomerellaceae family members, can easily infect woody trees directly or through injuries. To determine the extent of Bot canker and anthracnose problems in avocado-producing counties in California, 18 experimental and commercial groves were surveyed and sampled in the fall of 2018. Isolations from symptomless and infected plant organs (branch, twig, leaf…) were performed on acidified potato dextrose agar. Although, *Botryosphaeria* and *Colletotrichum* spp. were recovered from all sampled tissues of the Hass and Lamb Hass cvs., they were most frequently isolated from symptomatic branches and leaves, respectively. The predominance of Bot species in infected branch and twig tissues confirmed their role in avocado branch canker formation, either acting solely or synergistically with *Colletotrichum* spp. However, the later pathogens were less common within the xylem elements, but along with *Alternaria,* they dominated the fungal population within the extra-cambial tissues. These pathogens were also detected in symptomless tissues revealing the presence of latent infections, while their respective fruiting structures (pycnidia and acervuli) were observed on infected twig and leaves. The highest incidence of *Colletotrichum* spp. was found in a high-density Hass orchard in San Luis Obispo, suggesting that the local conditions are likely conducive for anthracnose development.

Virus survey in California reveals the presence of novel vitiviruses infecting grapevine
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Virus species in the genus *Vitivirus* (family *Betaflexiviridae*) are associated with the etiology of rugose wood disease in grapevine. This genus was named after *Vitis* spp., the host of *Grapevine virus A*, the type species. Recently, through the application of high-throughput sequencing (HTS), five novel *Vitivirus*-like viruses were discovered and named grapevine virus G, H, I, J and L (GVG, H, I, J and L); GVJ and GVL were identified in the USA. Given the history and importance of the well-known vitiviruses, grapevine viruses A, B and D, we targeted novel vitiviruses in an extensive survey of ca. 2,500 samples to evaluate their prevalence in different California grapevine populations. As a result, all five novel vitiviruses were detected in several grapevine selections via reverse transcription PCR (RT-PCR) and later confirmed by Sanger sequencing; specifically, 4, 36, 16, 1 and 15 samples tested positive for GVG, H, I, J and L, respectively. This study represents the first report of GVG, GVH and GVI in the USA. The variability between GVG, GVH, GVI and GVL isolates was investigated using distance matrices and phylogenetics. Finally, co-infection by novel and previously known vitiviruses was also evidenced by RT-PCR.

Chaetomium globosum*, a pathogen of hemp, *Cannabis sativa
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In the United States, there is an unprecedented demand for *Cannabis sativa* with low levels of the psychoactive drug, tetrahydrocannabinol (THC), because of other medicinal compounds and fibers produced by the plant. Cultivation of hemp has been limited in the United States because of its close relationship to THC-containing varieties (i.e., marijuana), thus, little is known about its pathogens. Putative pathogens were isolated from symptomatic leaves of two greenhouse grown fiber varieties, Futura and Fedora. Four isolates were identified as *Chaetomium globosum* based on colony characteristics, and ascospore and perithecium morphology. Pathogen identification was confirmed by amplifying and sequencing the ITS region, which had a 100% nucleotide sequence identity with *C. globosum* (GenBank:HG423571.1). In detached leaf assays, original symptoms were duplicated on the two fiber varieties from which the original isolates were collected; similar symptoms were produced on another fiber variety, Delores. The pathogen was re-isolated from leaves and grown in axenic culture. Greenhouse grown plants were inoculated on leaves with mycelial plugs, and original symptoms were duplicated on ‘Fedora’ and ‘Futura’, but not on ‘Wife’, a high CBD variety. The relationship between secondary metabolite concentrations and the lack of symptom development on ‘Wife’ in plants, and in subsequent detached leaf assays is unknown. Therefore, additional high CBD varieties will be tested.

Characterization of *Cytospora* species from South Carolina Peach Trees
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The humid climate in the southeastern United States is conducive for bacterial and fungal diseases of peach. A survey of prematurely declining peach trees in South Carolina orchards revealed the presence of *Cytospora spp* isolates in many production fields. On potato dextrose agar the isolates revealed highly variable colony morphologies. To further investigate the isolates on the molecular level, we sequenced the ribosomal Internal Transcribed Spacer regions 1 and 2. Six genotypes were identified that formed distinct clusters within published *Cytospora leucostoma* Genbank entries. The isolates were sensitive to thiophanate-methyl and pyraclostrobin but resistant to boscalid. Preliminary results show two more isolates that most closely match *Cystospora donestzica* in Genbank, but more information is needed to confirm identity. Two peach cultivars were inoculated with one representative each of the eight genotypes and canker formation was monitored over four months. Koch's postulates were confirmed for all isolates, but we found highly variable virulence among single tree replicates of the same cultivar. The variability could not be explained with deficiencies in soil nutrient content. Although there were a variety of different plant pathogenic nematodes in the soil, there were no statistical differences in the nematode count in soil of experimental tree replicates. By evaluating these isolates we gain a better understanding of the genetics and diversity of *Cytospora* species affecting peach trees in South Carolina.

Identification and characterization of *Fusarium* spp. associated with root rot of dry pea in Montana
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Fusarium spp. are a major components of a root rot disease complex, which threaten pulse crop production systems. Information is limited on the diversity of *Fusarium* spp. in dry pea growing regions of Montana. The objectives of the study were to identify the *Fusarium* spp. isolated from dry pea roots and seeds from Montana, and determine their pathogenicity on barley, chickpea, dry peas, lentil, and wheat. Of the 301 *Fusarium* isolates obtained, 228 and 73 were from roots and seeds. Internal transcribed spacer region of the rDNA and the translation elongation factor genes were used to confirm the identity of the isolates. The *Fusarium* isolates were 31% *Fusarium avenaceum*, 19% *F. tricinctum*, 14% *F. acuminatum*, 8% *F. sporotrichioides*, 6.3% *F. poae*, 6% *F. oxysporum,* 3% *F. culmorum*, 2.3% *F. redolens*, 2% *F. graminearum*, 2% *F. solani*, 0.9% *F. equisitii*, and 0.3% *F. torulosum*. Pathogenicity of 43 isolates representatives of all the *Fusarium* spp. were evaluated. Significant pathogenicity was caused by isolates (from roots and seeds) of *F. avenaceum* on pea, chickpea, lentil, barley, and wheat; *F. solani* on pea, chickpea, and barley; *F. culmorum* on barley, lentil, and wheat; *F. acuminatum* and *F. redolens* on barley. Due to the cross-pathogenicity of the *Fusarium* spp. on the pulse and cereal crops, their rotation could propagate the root rot pathogens. Our results suggest the pathogens are seedborne and seed could be a source of inoculum. Effective seed treatments are necessary along with a need for alternative non-host crops for rotation with pulses to break the disease cycle.

Through the lens of social science: How the IPM Innovation Lab is helping smallholder farmers achieve food security in the developing world
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The Feed the Future Innovation Lab for Integrated Pest Management (IPM IL), a USAID funded collaborative research program managed by Virginia Tech, continues to make transformative impacts on the livelihoods of smallholder farmers around the world through improving crop production, nutrition, health, income, and food security. The IPM IL develops and implements ecologically based IPM technologies, practices, and strategies in developing countries as well as transfers technologies and scales them up. The program examines the gendered components of pest management by addressing two broad questions: 1) How do gender relations, norms, and attitudes affect the impact and outcomes of IPM IL research activities? 2) How do IPM IL research activities affect gender relations, norms, and attitudes at the household and community level? The IPM IL places special emphasis on gender analysis at the onset of and throughout IPM research and dissemination activities, so that women's and men's priorities, problems, and concerns are at the forefront. The IPM IL works to understand the gendered pathways of IPM knowledge and technologies and is making strides in dissemination and application of IPM technologies, promoting the well-being of both men and women, and increasing women's ability to make pest management decisions associated with knowledge acquired through the IPM IL program.

Secret relationships with serious consequences: Understanding the endophyte-tall fescue symbiosis
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Outreach from the research community to the broader population can impact people of various age groups and inform decisions. We developed an outreach unit tailored to reach diverse learners from school students and teachers to agricultural producers introducing them to the inconspicuous symbiotic relationship of endophytes in grasses. Endophytes are beneficial symbionts that can improve forage grasses by providing protection to their host from biological and environmental stresses. However, these benefits can have serious consequences because chemicals produced by the endophyte may harm grazing livestock. We established a Noble Education Fellowship with the focus of providing science teachers with research experience and resources they can take back to the classroom. We feature an exercise revealing endophytes in common grasses using microscopes and cell phones to record findings. Technical aspects of

this outreach unit can be adjusted depending on the materials and time frame available for a given group. For example, an activity from this unit has been implemented to demonstrate to agricultural producers the presence of endophytes inside tall fescue. This heightens their awareness of factors to consider when planting and maintaining fields intended for grazing. The learning activity can stand alone for outreach events or may be used as a component of a broader unit covering symbiosis with particular emphasis on mycology, microbiology or molecular biology.

Opportunities through the Agricultural Microbiomes Research Coordination Network
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Recent advances in sequencing technologies have resulted in a wealth of microbiome studies to catalog and describe plant-associated microbial communities. However, a lack of coordinated efforts, standard protocols, and communication among microbiome researchers has limited our capacity to understand the factors that influence the diversity, composition, and benefits of plant and soil microbiomes across diverse cropping systems. The Agricultural Microbiomes Research Coordination Network (RCN) seeks to advance understanding of agricultural microbiomes and their relationships with sustainable crop production and disease management. To accomplish this, we are building a coordinated global network of scientists from public and private entities working on developing and applying concepts, technologies, and analytical approaches for agricultural microbiome research. Network participants have the opportunity to engage in training and collaborative activities, and contribute to identifying research priorities and community needs to move agricultural microbiome science forward. The RCN facilitates the development of collaborative synthetic and cross-disciplinary research opportunities, taking advantage of existing research platforms and available resources. Diverse opportunities for plant pathologists to participate in RCN-sponsored collaborative research and publication projects, as well as in workshop attendance and organization, are currently available.

Engaging Florida's small fruit and vegetable industry with hands-on plant disease identification workshops
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The Florida vegetable and small fruit industry consists of over 40 crops that commercially generate over $1 billion in annual sales. With such diversity, accurate and timely disease identification is key to economic success. Our objective was to create and apply a 90-min hands-on training during the Florida Ag Expo hosted at the University of Florida, Gulf Coast Research and Education Center, to train industry participants in the identification of vegetable and small fruit diseases. The annual training focused mostly on tomato and strawberry diseases, with 30 to 45 participants per year over the last 3 years. Topics covered during the training included the plant disease clinic, disease symptoms and signs, traditional and molecular diagnostics, plant disease management, and available web resources. Symptomatic plant materials were prepared prior to the event, including slides for microscopic viewing of specimens. Results of a survey given at the end of the last workshop indicated that all participants were interested in attending future workshops, highlighting the importance of offering additional training. For the next workshop, we plan to include additional crops, as well as incorporate recent research findings.

Future scientists and farmers: Youth outreach to promote concepts of Integrated Pest Management
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Youth education (ages 5-18) is an important component of university extension's mission to share research-based information with the public, including providing current and future growers with the best tools for disease, insect and weed management. Youth are early adopters and an example for others in their community to follow. The Integrated Pest Management (IPM) Program at Iowa State University has offered youth programs for ten years, with increased programming in recent years. The programs include crop scouting competitions, crop scouting workshops, online lessons, field days, and two National 4-H curricula on pollinators. The Iowa and Regional Crop Scouting Competitions let youth learn about pest management from experts. Survey results from the competitions show that youth increase their knowledge about IPM and are better prepared for careers in agriculture after participating. The pollinator curricula emphasize land management decisions to increase profitably and biodiversity. Survey results from these shows increased knowledge about the importance of pollinators and increased interest in science and agriculture. A field day on crop management with a focus on career opportunities including research is in development. Exposing youth to IPM concepts and the importance of using research-based information generates interest in careers in plant pathology and other crop sciences and can impact adoption of best practices in disease, insect and weed management.

Planting Diversity: Novel Paths for Increasing Diversity and Inclusion in the Plant Sciences and Beyond
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Despite significant investments made over the past several decades to recruit individuals and increase participation of underrepresented minorities in sciences, these groups are still highly underrepresented in many plant science disciplines, including plant pathology. The

Plant Science Research Network (PSRN), an NSF-funded RCN, organized a workshop to explore innovative ways of effectively broadening participation in the plant sciences and beyond. Through facilitated training and discussion, workshop participants began the 3-day retreat by developing a common language through lessons focused on cultural fluency, intersectionality, and equity. Participants engaged in dynamic conversations and facilitated activities aimed at envisioning new approaches to promote more inclusive and equitable plant science programs. Participants proposed significant, measurable, and potentially amplifying actions for advancing equity, diversity, and inclusion in plant sciences. Opportunity gaps and systemic barriers that contribute to disparate levels of academic preparedness and research training skills were identified. Enrichment of equitable mentoring relationships beyond academic advising was recognized as critical to providing more inclusive plant science education at all levels. Pilot projects were proposed to take action in implementing innovative strategies for professional networking, increasing early exposure to plant science, and improved mentoring. Join us at plantae.org/PSRN to contribute to the discussion and learn more about the strategic recommendations and pilot projects.

Crop Protection Network: A platform for regional, national and international extension outputs

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The Crop Protection Network (CPN) was established in 2014 as a multi-state and international collaboration of university/provincial Extension specialists and public/private professionals that provides unbiased, research-based information to farmers and agricultural personnel. The goal of CPN is to streamline the collaborative process of creating regional Extension outputs while retaining the unbiased perspective of the land grant universities. Currently, CPN has created several publication series focusing on identification and management of corn, small grains and soybean diseases, and general crop management issues. Over 270,000 publications have been printed and distributed through CPN or downloaded from the CPN website: www.cropprotectionnetwork.org. In addition to crop-specific publications, CPN publishes annual crop yield-loss estimates due to diseases, fungicide efficacy guides, feature articles, videos, interactive web-based tools and annual impact statements. All CPN outputs are as inclusive as possible, with Extension specialists in each state having the opportunity to review outputs related to their specialty. To date, authors from 28 institutions have contributed to CPN publications. These collaborative outputs help CPN achieve the goal of delivering high-quality research-based information to stakeholders.

Evaluation of isothermal recombinase polymerase amplification (RPA) to detect the four major soilborne diseases of strawberry in California nurseries

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The soilborne pathogens *Verticillium dahliae*, *Macrophomina phaseolina*, *Fusarium oxysporum* f. sp. *fragariae* and *Phytophthora* sp., causal agents of Verticillium wilt, Macrophomina crown rot, Fusarium wilt, and Phytophthora crown rot, respectively, have emerged as prevalent pathogens in California strawberry production after the phase-out of methyl bromide. There is a need to detect these pathogens in nurseries to minimize their spread on transplants. To evaluate the sensitivity and specificity of isothermal recombinase polymerase amplification (RPA) assay, 125 plant samples from 13 locations of high elevation strawberry nurseries were collected in August 2018. All crowns were plated on APDA, ACMA, NP10, and PARP media. Extracted DNA (1µl) was added to the master mix and each pathogen's presence was determined using a TwistDx T16-ISO instrument (RPA) and High-Resolution Melting (HRM) methods. The results showed that 31(25%), 32(26%) and 43(34%) samples were positive for *Phytophthora* sp. using plating, RPA and HRM, respectively. The remaining pathogens were not detected by either method. The RPA diagnostic sensitivity was 74% while its specificity was 100% compared to HRM. Since the RPA instrument is easily transportable, highly sensitive with the same specificity as PCR, but faster, RPA could be used as a rapid method for nurseries to detect important soilborne pathogens of strawberry in the field before shipping plants to fruit production fields around the world.

A probe panel assay for detection and quantification of soil-borne pathogens in soybean fields

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The fungal species *Fusarium oxysporum, F. graminearum, F. acuminatum, F. verticilliodes, F. equiseti, F. proliferatum, F. solani, F. sporotrichioides* and *Rhizoctonia solani* are soil-borne pathogens responsible for yield loss of multiple crops and are known causal agents of important diseases in soybean, including root rots and seedling diseases. The early and accurate identification of these pathogens represents a decisive step in managing these diseases. Therefore, we have designed highly species-specific primer-probe assays for the identification of these soil-borne pathogens. The primers and probes were designed based on the intergenic spacer ribosomal RNA (IGS) and translation elongation factor alpha gene (EF1α). The specificity of the designed panel was tested against 21 non-target pathogenic fungal species. All the designed sets showed early amplification using genomic DNA of the target species and were specific enough to not amplify all non-target species. The designed primer-probe sets were also successfully used in identifying and quantifying target pathogens in roots and soil samples collected from in soybean fields across different states in the US. We have developed a precise set of primer and probes to detect and quantify major soil-borne pathogens in fields, which could guide decision-making in disease management by providing early detection of these pathogens in the soil.

Comparative study of methods for detecting *Xylella fastidiosa* causing bacterial leaf scorch in blueberry
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Bacterial leaf scorch, caused by *Xylella fastidiosa,* is a major threat to blueberry production in the southeastern United States. Management of this devastating disease is challenging and often requires early detection of the pathogen to reduce major loss. There are several different molecular and serological detection methods available to identify the pathogen. Knowing the efficiency and suitability of these detection techniques for application in both field and laboratory conditions is important when selecting the appropriate detection tool. Here, we compared the efficiency and the functionality of four different molecular detection techniques (PCR, real-time PCR, LAMP and AmplifyRP® Acceler8™) and one serological detection technique (DAS-ELISA). The most sensitive method was found to be real-time PCR with the detection limit of 25 fg of DNA molecules per reaction (≈9 genome copies), followed by LAMP at 250 fg per reaction (≈90 copies), AmplifyRP® Acceler8™ at 1 pg per reaction (≈350 copies), conventional PCR with nearly 1.25 pg per reaction (≈ 440 copies) and DAS-ELISA with 1×10^5 cfu/mL of *Xylella fastidiosa*. Considering robustness, portability, and cost, LAMP and AmplifyRP® Acceler8™ were not only the fastest methods, but also portable to the field and didn't require any skilled labor to carry out. Among those two, AmplifyRP® Acceler8™ was faster but more expensive and less sensitive than LAMP. These findings should enable growers, agents, and diagnosticians to make informed decisions regarding selection of an appropriate diagnostic tool for *X. fastidiosa* on blueberry.

Rapid species differentiation of *Calonectria henricotiae* and *C. pseudonaviculata* by high-resolution melting analysis of PCR products
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Two *Calonectria* species*, C. henricotiae* and *C. pseudonaviculata,* are recognized as the causal agents of boxwood blight, a recently emerged threat to the annual $1.3 million U.S. boxwood industry. The two species are difficult to distinguish based on morphology and pathogenicity, although some studies indicate that *C. henricotiae,* which is distributed only in Europe*,* exhibits greater fungicide tolerance. Fast, accurate, and inexpensive methods to detect and differentiate species allows accurate diagnosis of boxwood blight on plants as well as monitoring and detecting fungicide resistance. We designed primer pairs based on available sequences of three genes - calmodulin, histone H3, and β-tubulin – and tested their ability to differentiate the two *Calonectria* species. Genomic DNA was extracted from 15 isolates of *C. henricotiae* and *C. pseudonaviculata* and was subjected to a real-time PCR amplification in the presence of fluorescent dye, followed by high-resolution melting analysis (HRMA). Primer pairs were identified that can differentiate the two species, and the 15 isolates were clustered into two distinct groups representing the two species, *C. henricotiae* (3) and *C. pseudonaviculata* (12). This HRMA is a fast high-throughput method to differentiate the boxwood blight pathogenic fungal species that doesn't require gel or capillary analysis.

Development and application of a real-time RT-PCR assay for the direct detection of *potato mop top virus* in soil
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Potato mop top virus (PMTV) is a continuing threat to potato production throughout the world. It has the potential to persist in the soil for long periods in the sporosori of its vector *Spongospora subterranea* f. sp. *subterranea* (Sss) which serve as an important source for PMTV infection and dissemination. In this study, we used real-time reverse transcription PCR of the total RNA directly extracted from the soil to develop a simple, fast and sensitive method to detect PMTV. The effort was made to identify a sensitive and specific primer with high efficiency despite a minimal amount of viral RNA or the possible presence of various PCR inhibitors in soil extracted RNA. The assay detected PMTV from all soil types used and it supported the detection of less than 10 PMTV copies μl^{-1} in the RNA sample. Detection was linear with amplification efficiencies ranging from 93% to 105% for silt loam, loamy sand, sand and sandy loam in various experiments with R^2 values >0.99. Additionally, the assay developed successfully detected PMTV from different types of naturally infested soil with PMTV carrying Sss sporosori ranging from 6.2×10^2 g^{-1} to 1.2×10^6 g^{-1} in soils with pH ranging from 4.9 to 7.5, and organic matter ranging from 0.9% to 5.1% indicating this assay has the potential to detect PMTV from a wide variety of soil.

A loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Fusarium oxysporum* causing fusarium wilt of watermelon
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Fusarium oxysporum f. sp. *niveum* (FON), the causal agent of Fusarium wilt of watermelon, is a devastating pathogen that is responsible for significant losses of watermelon in many states in the US and in the world. Differentiation of this pathogen from other formae speciales of *F. oxysporum* based on morphological characteristics is difficult and time-consuming. A few molecular methods have been developed to specifically detect FON, primarily based on conventional PCR. Loop-mediated isothermal amplification (LAMP) provides a cheaper and quicker molecular detection tool compared to traditional PCR, which can also be used in the field for rapid on-site detection. In this study, we developed a highly sensitive LAMP assay to detect *F. oxysporum* pathogens of watermelon based on the *SIX6* avirulence protein gene that can detect a quantity as low as 0.1 pg/µl of genomic DNA. Findings from this study are helpful for designing sound strategies to detect and manage this disease efficiently.

Development of AmplifyRP assays for three important grapevine viruses through recombinase polymerase amplification
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Focusing on a higher level of sensitivity, the specificity, and the simplicity of detection application, Agdia has developed three virus-specific AmplifyRP® XRT assays for three important grapevine viruses: Grapevine leafroll associated virus 3 (GLRaV-3), Grapevine pinot gris virus (GPGV), and Grapevine red blotch virus (GRBV). For each assay, all reaction reagents are lyophilized into a single pellet in a PCR tube and rehydrated in a buffer prior to adding testing sample. Crude extracts are prepared by grinding leaf petiole or leaf base in 1:10 extraction buffer ratio (wt/vol) in extraction bags and directly used for testing. Assays can be completed within 20 minutes in a portable reader, AmpliFire®. AmplifyRP® XRT for GLRaV-3 can detect the GLRaV-3 variant representatives from eight known groups (I, II, III, V, VI, VII, IX and X) while excluding other grapevine leafroll viruses. AmplifyRP® XRT for GPGV can detect 27 GPGV isolates collected from Italy, Canada, and USA without cross-reacting to other grapevine viruses. AmplifyRP® for GRBV was previously developed as the Acceler8® format and recently the XRT format was generated. AmplifyRP® XRT has demonstrated to be as sensitive as RT-PCR or PCR. Both GLRaV-3 and GPGV can be detected from crude infected plant extracts diluted to $1:10^4$ while GRBV can be detected in crude extracts diluted to $1:10^6$. These new assays provide convenient and fast tools for growers to monitor key viruses in their vineyards.

Detection of viable *Phytophthora* spp. associated with Christmas trees in Michigan using qPCR combined with propidium monoazide (PMA)

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Phytophthora root rot can cause widespread mortality in numerous species of plants. Conventional PCR and qPCR assays used to detect and quantify pathogen presence may overestimate the number of active pathogens due to the lack of discrimination between viable and non-viable propagules of the pathogens. Detection of a pathogen and determination of live or dead status is critical to evaluate the efficacy and success of pathogen mitigation treatments. A quantitative PCR (qPCR) assay using pre-treatment of propidium monoazide (PMA), a DNA-binding dye penetrating broken membrane of dead cells and inhibiting PCR amplification, was evaluated to test the viability of *Phytophthora* spp. in artificially inoculated and field samples. The PMA-qPCR (*atp9-nad9* region) protocol developed in this study, was specific and multiplexed with genus and species-specific for the detection of *Phytophthora* spp. in the samples tested. Detection sensitivities for the genus and species-specific assays down to 10 and 100 fg/µl of genomic DNA extracted from pure culture, respectively. Artificially inoculated soil samples treated with 40 µM of PMA showed a signal reduction (3.03 cycles) in qPCR between live and heat-killed zoospores of *Phytophthora* spp. We demonstrated that our PMA-qPCR treatment is a suitable approach to distinguish between viable and non-viable populations of *Phytophthora* spp. Further studies will focus on the validation of the PMA-qPCR assay using environmental samples.

A diagnostic *taqman* real-time PCR assay for detection and quantification of *Colletotrichum theobromicola*, causal agent of Boxwood dieback

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Boxwood dieback, caused by *Colletotrichum theobromicola*, is spreading at an alarming rate in the boxwood industry in the United States. Although *C. theobromicola* has been accepted as a distinct species within the *C. gloeosporioides* species complex, it is difficult or impossible to distinguish from other closely related species on the basis of morphology. Moreover, molecular identification requires amplification and sequencing of multiple loci, which can be expensive and time consuming. Because boxwood dieback produces above-ground symptoms similar to those produced by Phytophthora root rot, visual disease diagnosis may be inaccurate and result in costly and ineffective management decisions. Furthermore, previous pathogenicity reports have shown that the symptoms of boxwood dieback appear 10–12 weeks after inoculation under greenhouse conditions. This delay in the onset of disease symptoms may lead to infected boxwood liners being traded in the horticulture industry and unwitting dissemination of the causal agent. Therefore, an accurate and a rapid diagnostic tool is required to detect *C. theobromicola* in suspected liners at early growth stages. This will allow boxwood producers to implement effective disease management strategies, and prevent the spread of the disease. The main objective of this study is to develop a diagnostic *taqman* real-time PCR assay for early detection and quantification of *C. theobromicola* from boxwood.

Near complete genome sequence of Tulip virus X obtained by high-throughput sequencing from naturally-infected lemon balm (*Melissa officinalis*)

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Three plants of *Melissa officinalis* with mosaic symptoms were obtained from a local nursery. Transmission electron microscopy (TEM) of extracts revealed flexuous particles typical of potexviruses; TEM of thin sections showed loose cytoplasmic arrays of similar particles. *M. officinalis* is known to be susceptible to Alternanthera mosaic virus (AltMV), and to tulip virus X (TVX). ELISA with AltMV-specific antibodies yielded negative results, while ELISA with antibodies to Plantago asiatica mosaic virus (PlAMV; closely related to TVX) was positive. Inoculation to bioassay plants susceptible to AltMV and/or PlAMV yielded no symptoms or positive ELISA results. A cDNA library for high-throughput sequencing was generated from total RNA from pooled leaves of the three lemon balm plants, and sequenced by paired-end protocols (Illumina MiSeq); raw reads (3,337,739; of 80-251 nt) were trimmed to remove adapter linkers, and *de novo* assembled into contigs using Geneious Pro R9. Contigs were analyzed by BLASTX against viral sequences from GenBank; those with high identity to potexviruses were further edited and assembled into a 6034 nt nearly full-length sequence, lacking 3-17 nt at the 5', and 2 nt at the 3', by comparison to the reference (TVX-J) and other TVX isolates. The predicted RdRp,

TGB1, TGB2, TGB3, and CP share 89%, 91%, 86%, 75%, and 94% amino acid identity to those of TVX-J. RT-PCR and sequencing of cloned PCR products to validate the genome sequence is in progress.

Development of a real-time duplex isothermal assay for the detection of *Tobacco rattle virus* and an endogenous internal RNA control

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Tobacco rattle virus (TRV), the causal agent of corky ringspot in potato, is capable of infecting upwards of 400 plant species. Reliable detection of all infectious TRV isolates is often limited to molecular methods due to the expansive host range, genomic arrangement, and absence of the RNA2 segment in some isolates. Agdia presents a novel isothermal diagnostic tool for the detection of *Tobacco rattle virus*. The isothermal assay utilizes recombinase polymerase amplification to detect over 25 diverse TRV isolates in a 20-minute real-time detection scheme and matches the sensitivity of existing real-time and conventional PCR methods. The assay incorporates an endogenous RNA control to assess if inhibition from fragrant ornamentals, succulents, and starchy potato tubers occurs in molecular diagnostics of TRV. The assay demonstrates competency of target detection in over 70 plant species utilizing a crude extraction comparable to common serological assays. Paired with a portable fluorometer the assay will allow for a broader screen of TRV across different hosts in diverse environments.

Prevalence and early detection of bermudagrass root-infecting pathogens accessed by novel real-time PCR assays

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Bermudagrass Decline (BD) is a destructive disease of ultradwarf bermudagrass (UDB) putting greens in the southeastern United States. Previous studies reported a single ectotrophic root-infecting (ERI) fungus associated BD. Recently, three novel ERI fungi have been associated with BD of UDB. Current diagnostic methods for identification of ERI fungi can be inconclusive and inaccurate for distinguishing ERI fungal species. Here we present the development and application of novel real-time PCR assays designed to detect four ERI fungi associated with BD. The distribution of those fungi in UDB samples collected from putting greens across the southeastern United States was also accessed. Assay validation was carried out using DNA and plasmids and the Limit of Detection (LOD) was calculated for each assay. Results revealed novel real-time PCR assays effectively distinguished all four fungi associated with infected UDB root samples. The LOD was established at 1pg/µL of DNA proving the sensitivity of the method to detect very low amounts of fungal DNA. Fungal presence was detected in twenty-nine of thirty UDB root samples, with one fungus presenting a wider distribution and two fungi having higher DNA quantities when compared to UDB root material. The novel assays provide a reliable method for accurate detection of the four ERI fungi investigated. Due to the assay's high sensitivity, this method can be used as a tool for early detection of BD in roots of UDB putting greens.

Protecting the U.S. pome fruit industry from foreign pathogens at the USDA APHIS using high-throughput sequencing technologies

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The pome fruit industry is a multibillion dollar business with apples as the most consumed fruit in the United States. Improved varietal development depends on access to valuable imported pome germplasm, mainly introduced into the U.S. through the National Clean Plant Network in Washington and the USDA-APHIS Plant Germplasm Quarantine Program (PGQP) in Maryland. The PGQP Pomes Program screens imported germplasm for a wide variety of well-characterized high-risk pathogens including viruses, viroids and phytoplasmas using PCR techniques, bioassays, and field indexing. If pathogen(s) are detected, germplasm undergoes therapy to generate pathogen-free trees before their released. Heightened demand for imported germplasm and limitations with conventional pathogen detection increases the risk of undetected pathogens during the screening process. To reduce this risk, the PGQP Pomes Program is combining high-throughput sequencing (HTS) technologies and improved bioinformatic pipelines to rapidly detect quarantine pathogens and uncharacterized disease agents. Ten unique apple and pear fruit trees routinely used as positive controls were subjected to HTS as a proof-of-concept. Illumina TruSeq cDNA libraries were constructed from ribosomal RNA-depleted total RNA extracts. Approximately 50 million reads (1x75 bp) were obtained per sample. Bioinformatic analyses identified most expected plant pathogens as well as unreported agents. A summary of our findings will be presented.

Connecting diagnostic labs with a system for sharing protocols and maintaining version control

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Diagnostic labs are the foundation for protecting agriculture from new pathogens and providing reliable information to direct effective pest management decisions. To date, there has been no formal system for sharing protocols between labs within the National Plant Diagnostic Network. While many labs are willing to share diagnostic procedures and resources like the online Diagnostician's Cookbook exist, the updating of diagnostic procedures and notifications of updates requires significant time and may not happen efficiently. This can lead to improvements not being relayed in a timely fashion and makes it difficult to compare what protocols are used by different diagnostic labs.

A new system has been launched to facilitate communication between diagnostic labs as well as other groups providing testing protocols. All protocols published in the system are available for adoption by any participating labs. As updates are published by the originating lab, all adopting groups are notified of the change and given the opportunity to accept the revisions or start maintaining their own version of the protocol. The system provides a forum for commenting on protocols between and within labs, public listing of protocols that a lab offers within a geographic area, and services to integrate with a lab's data management system.

Detection of RNA viruses using RT-qPCR and HTS in potato seed-tubers in Antioquia (Colombia)
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Virus diseases are one of the most important factors affecting potato production worldwide, especially in developing countries, where only a small minority of potato farmers has access to certified seeds. The continued use of uncertified tuber-seeds can result in poor crop harvests as a consequence of seed degeneration, which is caused by the accumulation of pathogens and pests after successive cycles of vegetative propagation. In this study we evaluated the incidence of four RNA viruses (PVY, PLRV, PYVV and PVS) in both informal and commercial tuber-seeds marketed in the province of Antioquia (Colombia) using RT-qPCR. Viruses were detected in tuber-sprouts and their corresponding four-week-old plantlets. The presence of these viruses in commercial potato fields was confirmed by Sanger and High-throughput (HTS). Results revealed that commercial tuber-seed lots have a high incidence of PVY (81.7%), PYVV (70.0%), PVS (30.0%) and PLRV (11.7%). No statistically significant differences were found in the levels of viral infection between informal and commercial tuber-seeds. HTS analyses of bulked leaf-samples revealed a high abundance of PVY and PVS, which ranked as the 2nd (10,047 TPM) and 24th (2,761 TPM) most abundant transcripts. This work reveal urgent need to update current potato seed-certification programs in Colombia.

Evaluation of factors associated with real-time PCR quantification of the stubby root nematode *Paratrichodorus allius* from field soil DNA
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The stubby root nematode *Paratrichodorus allius* is a prevalent vector that transmits *Tobacco rattle virus* causing corky ringspot disease of potato in the United States. Factors relating to SYBR Green quantitative real-time PCR (qPCR) quantification of *P. allius* using soil DNA were assessed. Soil pre-treatments showed that autoclaved field soil significantly reduced the DNA amount and quality, and air or oven dried soil yielded a lower amount of DNA with a similar purity compared with natural field soil. Loamy sand soils from potato fields were used. PCR inhibitors were detected in soil DNA without additional purification targeting pBluescript II SK(+)-plasmid DNA, and $Al(NH_4)(SO_4)_2$ treatment significantly reduced the inhibitors compared with polyvinylpolypyrrolidone column. PCR inhibitors on qPCR were suppressed by bovine serum albumin used as a PCR enhancer. The largest nematode individuals produced significantly lower Cq values than the smallest individuals. Quantification results did not significantly change when increasing the number of DNA extractions from three to six per soil sample when a grid sampling strategy was used. Two standard curves, generated from serial dilutions of plasmid DNA containing *P. allius* ITS1 rDNA and soil DNA with known nematode numbers, produced similar quantification correlation and amplification efficiency. This study will be useful for the setup or optimization of qPCR-based quantification of plant-parasitic nematodes from soil DNA to improve detection efficiency and accuracy.

Development and validation of a TaqMan PCR method for rapid detection of *Phytophthora fragariae* in high volume root samples of strawberry
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Phytophthora fragariae is the causal agent of red stele disease in strawberries. In the Netherlands, every year ~600 samples are collected from strawberry propagation companies to test for presence of *P. fragariae*. Samples consist of up to 200 gram of strawberry root tips. Until now samples are tested using the Duncan bait test. This test requires greenhouse space with controlled climate conditions and takes over five weeks to finish. In this project, we developed a root extract (RE) TaqMan PCR assay for detection of *P. fragariae* in large root samples as an alternative screening method for the bait test. DNA is purified using a magnetic bead based method and analyzed using TaqMan PCR. Quality of test results is monitored with a process control. The method is high throughput and results can be obtained within few days. In our validation study, we demonstrated that the RE TaqMan PCR was at least as sensitive as the bait test. The analytical specificity of the method, tested both in silico and in vivo, was high. The TaqMan PCR detected only *P. fragariae* from a panel of selected relevant *Phytophthora* spp. Finally, based on repeatability and reproducibility, the method appears very robust. The root extract TaqMan PCR allows more cost-effective and rapid testing for *P. fragari*ae in comparison with the traditional bait test. A method description and summary of the validation study of the *P. fragariae* RE TaqMan PCR assay will be presented.

A viability-qPCR test for quantifying living pathogens of bacterial spot in tomato seed
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Bacterial spot is one of the most serious diseases of tomato. It is caused by four different species of *Xanthomonas*: *X. euvesicatoria*, *X. gardneri*, *X. perforans,* and *X. vesicatoria*. Contaminated and/or infected seed serve as a major inoculum source for this disease. The use of certified pathogen-free seed is one of the primary management practices to reduce the inoculum load in commercial production.

However, current seed certification protocols rely mainly on culturing and PCR, and both methods have limited capability to accurately quantify viable pathogen cells. To improve the seed certification process, a viability-qPCR assay in conjunction with a crude DNA extraction procedure was developed to selectively quantify viable xanthomonads in tomato seed. A DNA-binding dye, propidium monoazide (PMA), was used to treat seed extract where it selectively binds to the DNA of dead cells, thus preventing the DNA from dead cells from being amplified in downstream qPCR. The procedure was tested with tomato seed samples spiked with mixtures of viable and dead cells in different concentrations and proportions. The assay accurately quantified viable cells within mixtures of 1:10,000 (live:dead), and use of the crude DNA extract provided results similar to those obtained using purified DNA prepared from commercial kits. This assay will provide a sensitive, specific, and cost-effective way to certify tomato seed free of the bacteria spot pathogens.

Tools for early detection and monitoring of the koa wilt pathogen (*Fusarium oxysporum* f. sp. *koae*) in Hawai'i

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Koa (*Acacia koa*), Hawaii's second most common endemic tree that is ecologically, economically and culturally important to Hawai'i, is under threat from koa wilt disease, particularly in the low- to mid-elevation forests caused by *Fusarium oxysporum* f. sp. *koae* (Fo koae). *Fusarium oxysporum* is a cosmopolitan vascular wilt pathogen of diverse plant hosts. Morphological identification methods have little utility for pathogenic and non-pathogenic *F. oxysporum* strains because differences are unreliable. After screening for pathogenicity, genetic analyses of 24 isolates showed a clear and well-supported clade of pathogenic Fo koae isolates. Because highly virulent strains of Fo koae are genetically but not morphologically distinct for non-pathogenic isolates, our objectives were to develop a DNA-based probe to detect pathogenic Fo koae and examine population genetics among isolates from different Hawaiian Islands. Using whole-genome sequencing of one highly virulent isolate and one non-pathogenic isolate of Fo koae, a SNP-based DNA probe was developed to identify pathogenic strains of Fo koae. This probe was verified on 21 characterized isolates (16 pathogenic and 5 non-pathogenic). To confirm probe specificity, greenhouse assays were conducted on newly collected Fo koae isolates from across four Hawaiian Islands. The results will provide guidance to koa restoration efforts and resistance-breeding and screening programs for koa.

Targeted early detection of citrus Huanglongbing causal agent '*Candidatus* Liberibacter asiaticus' before the symptoms expression

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Citrus Huanglongbing (HLB) is the most severe disease of citrus plants caused by *Candidatus* Liberibacter asiaticus (Las) and transmitted by the insect vector Asian citrus psyllid (ACP). To date, no effective curative measure is available against HLB. For citrus production areas without HLB or with low HLB disease incidence, removal of Las inoculum is critical to prevent HLB spread. Such strategy requires robust early diagnosis of HLB for inoculum removal to prevent ACP acquisition and transmission of Las. However, early diagnosis of HLB is challenging because the citrus trees remain asymptomatic for several months to years after Las transmission by ACP. In this study, we report a new method for a targeted early detection of Las in Valencia sweet orange before HLB symptom expression. We take advantage of the fact that Las remains around the ACP feeding site immediately following transmission into the young flush and before flush maturation. The ACP secretes salivary sheaths at their feeding sites, which can be visualized using Coomassie brilliant blue staining due to the presence of salivary sheaths secreted by ACP. Epifluorescence and confocal microscopy indicate the presence of salivary sheaths beneath the blue spots on ACP-fed-leaves. Quantitative real-time PCR and conventional PCR assays are able to detect Las in the ACP feeding surrounding areas as early as 2–20 days after ACP feeding. This finding lays a foundation to develop much-needed tools for early diagnosis of HLB before symptom expression, thus assisting Las inoculum removal and preventing HLB from spreading.

Identification of a novel endornavirus in *Geranium carolinianum* and occurrence in plants within three agroecosystems

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Plant endornaviruses do not cause visible symptoms and are transmitted only vertically. In the spring of 2015, a putative endornavirus was detected in Carolina geranium (*Geranium carolinianum*) in Louisiana, USA. The virus was provisionally named Geranium carolinianum endornavirus 1 (GcEV1). With the objective of characterizing the virus, we sequenced the viral RNA using Illumina MySeq and reads were assembled with Spades 3.8.0. The viral genome consisted of 14,625 nt containing a single ORF coding for a putative polyprotein of 4,815 aa with conserved domains for a helicase_1, peptidase_C97, UDP-glucoronosyl and UDP-glucosyl transferase, and RNA-dependent RNA polymerase (RdRP)_2. The 5'end consisted of 130 nt while the 3'end of 54 nt ending in nine cytosine residues. The closest relative to GcEV1 was Phaseolus vulgaris endornavirus 3, a putative endornavirus from common bean. In phylogenetic analyses using the RdRP, both endornaviruses formed a distinct cluster. GcEV1 was detected in 181 of 184 *G. carolinianum* plants collected from three distinct agroecosystems. The virus was not detected in seven other species of the genus *Geranium*. There was no correlation of a particular phenotypic trait of the host with the presence of the virus. GcEV1 was transmitted at a rate of 100% in seeds of a self-pollinated *G. carolinianum* plant. At the present time, the type of symbiotic interaction between GcEV1 and *G. carolinianum* is not known.

PCR assay for rapidly differentiating aflatoxin-producing taxa in *Aspergillus* section *Flavi*
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Humans and animals are exposed to aflatoxins, toxic, carcinogenic fungal metabolites, through consumption of contaminated food and feed. The most important species associated with aflatoxin contaminated crops are members of *Aspergilllus* section *Flavi*. Most aflatoxin producers within section *Flavi* display similar aflatoxin profiles and morphological characteristics resulting in frequent misidentification. DNA based identification of taxa has improved our understanding of the cryptic diversity within *Aspergillus* section *Flavi*, but this can require several PCR amplifications, a sequencing facility, and expertise for data analysis. To develop a rapid, accurate and inexpensive assay for identifying aflatoxin producing taxa within *Aspergillus* section *Flavi*, primers were designed using ITS, nitrate reductase, β-tubulin, calmodulin, aflatoxin transcription factor (*aflR*), and cytochrome P450 monooxygenase (*cypA*) genes. A multiplex PCR assay was performed on 45 isolates. The PCR assay was capable of differentiating aflatoxin producing species with similar morphology and aflatoxin profiles. The β-tubulin, nitrate reductase and calmodulin genes were suitable for differentiating among *Aspergillus* clades while the *aflR* and *cypA* genes were suitable for differentiating within the *A. flavus* clade.

Development of race-specific diagnostic assays for detection of *Podosphaera macularis*
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Hop powdery mildew (*Podosphaera macularis*) was confirmed in the Pacific Northwest in 1996. Prior to 2012, the most common race of *P. macularis* was able to infect plants that possessed powdery mildew resistance based on the R-genes Rb, R3, and R5. Post 2012, two additional races of *P. macularis* were discovered that can overcome the resistance gene R6 and the partial resistance found in the cultivar Cascade. Current methods for race determination for *P. macularis* involve challenge assays onto differential cultivars and are slow, costly, and labor intensive. We sought to develop a molecular assay to determine race in *P. macularis.* We sequenced the transcriptomes of 46 isolates of *P. macularis* to identify loci that differentiate R6-virulent isolates from the other races. Sixteen loci that differentiated R6-virulent isolates from the others were identified. Primers were designed to flank single nucleotide polymorphisms present in the highly differentiated loci and were sequence verified. Locked nucleic acid probes capable of differentiating the races based on the conserved mutations in these loci are in design. Progress towards development, validation, and application of the assays will be presented and discussed.

Development of a qPCR Assay for Hop Powdery Mildew Field Specific Differentiation and Late-Season Dispersal Patterns
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Podosphaera macularis, the causal agent of hop (*Humulus lupulus*) powdery mildew (HPM), causes severe economic damage annually across the Pacific Northwest through expensive fungicide applications, hop cone degradation, and quality defects. The fungus emerges as flag shoots, focally located, within hop yards in the spring and spreads throughout the season. Determining the late season dispersal patterns of powdery mildew between yards may improve management practices, potentially allowing for detection of the pathogen before disease onset and enabling more strategic use of fungicides. However, conclusive detection of *P. macularis* within a field through spore trapping has proven difficult because both *P. macularis* and *P. clandestina,* powdery mildew of cherry, share a high degree of sequence similarity in the internal transcribed spacer region. The single copy genes β -tubulin and minichromosome maintenance 7 (MCM7) were investigated for their suitability for developing a quantitative PCR assay (qPCR) capable of detecting and differentiating *P. macularis* from other fungi. Several single nucleotide polymorphisms have been identified in β -tubulin and MCM7 which are unique to HPM isolates. Impaction spore traps were deployed near commercial hop yards in western Oregon during 2018 and 2019 to associate airborne inoculum density to disease levels on leaves. Development of the assay and its use as a component of a disease forecasting system for powdery mildew will be discussed.

Fungal pathogens associated with rice seedling disease in the Southern United States
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Seedling disease, also known as seedling blight or damping off, is a disease complex caused by several seedborne and soilborne pathogens. The distribution and importance of causal pathogens in the rice-producing regions in the U.S. remain largely unknown. Survey was conducted in 2018 from 35 rice fields of Arkansas (6), Louisiana (6), Mississippi (4) and Texas (19), and 10 symptomatic rice seedlings were collected from each field. Fungi were isolated from the roots and mesocotyls, and their pathogenicity was determined in a plant growth chamber. Pathogenic fungi were identified based on morphology and internal transcribed spacer rRNA gene sequences. *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium* spp. were confirmed to cause seedling disease in 42, 10 and 8% of plant samples collected, respectively. Results of this study provide better understanding of the distribution and frequency of fungal pathogens associated with rice seedling disease in the Southern U.S., which will serve as the foundation to develop and implement effective management options for control of this important disease in rice.

Wheat Head Blast Detection Using Deep Convolutional Neural Networks
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Wheat blast caused by *Magnaphorthe oryzae* pathotype *Triticum* (MoT) is a threat to food security. High-throughput phenotyping methods are needed for the detection of symptoms and accurate identification of wheat head blast (WHB). Current methods for wheat phenotyping include visual assessment for disease severity performed by human experts. However, significant intra and interrater variability often occur. Automated, sensor-based methods with higher reliability and accuracy have the potential to maximize phenotyping. The development of a new framework is essential to assess WHB intensity. The objective of this study is to develop an accurate deep learning framework to detect WHB symptoms. To this end, spike severity was visually estimated from experiments that included eight wheat genotypes with different levels of susceptibility to WHB. Treatments included MoT inoculated and non-inoculated wheat heads. 3155 RGB images were captured from plants grown under a controlled environment room in Bolivia. Different convolutional neural networks (CNNs) were evaluated for autonomous classification of infected heads from healthy ones in the captured images. Several experiments were conducted to illustrate the capabilities as well as the limitations of the proposed approach. Experimental results showed that the classification accuracy of our methods was 89.7% which was a significant step towards autonomous high-throughput WHB phenotyping under controlled environments.

Improved detection method for the spinach downy mildew pathogen, *Peronospora effusa*
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Peronospora effusa is an obligate oomycete and the causal agent of spinach downy mildew. *P. effusa* results in losses of up to 30% or higher in organic spinach production. Control options for this disease in organic production are limited to the development of disease resistant cultivars since fungicides are not available to use in organic production. Different pathotypes of *P. effusa* have been identified using a list of differential cultivars. There is a continuous need to update the differentials nearly every year due to the quick evolution of the pathogen. Early detection of the pathogen, especially in the field, can contribute to improved management strategies in both conventional and organic spinach production. We currently are testing a recombinase polymerase amplification assay (RPA) to detect the pathogen in the field. The RPA assay process works at low constant temperature due to a stabilizing enzyme and the use of specific primers. To confirm the specificity of the targeted locus, primers and a qPCR probe based on a mitochondrial locus for specific detection of *P. effusa* was developed. The assay was tested on 15 different isolates of *P. effusa* and eight closely-related species. The qPCR assay was specific for *P. effusa.* However, using only the primers without the qPCR probe, nonspecific detection of DNA from the related species *P. obovata* and *P. lepigoni* was observed. The next step is to adapt the *P. effusa* specific probe to in-field RPA-based detection.

Towards the development of a multiplexed qPCR assay to distinguish Cauliflower mosaic virus (CaMV) infection from GM plants
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To facilitate transgene expression, many genetically modified (GM) plants contain a promoter from the plant virus, *Cauliflower mosaic virus* (CaMV). The relative ubiquity of this CaMV 35S promoter (P35S) in GM constructs means that assays designed to detect plant GMOs often target the P35S DNA sequence. However, because this promoter is derived from CaMV, these detection assays can yield false-positives from non-GM plants infected by these naturally-occurring viruses or their relatives within the Caulimoviridae. Here we report the development of an assay designed to distinguish CaMV-infected plants from GM plants in a single multiplexed qPCR reaction. With literature-derived PCR primers targeting P35S, TNOS (a terminator often used in GM constructs), and P3 (a CaMV-specific gene), we have successfully amplified target sequences in plasmids cloned to contain these genes, both in PCR and qPCR reactions. We have also successfully tested these PCR primers on plant DNA extracts from organic watercress and both organic and GM canola, all with and without CaMV infection. Four qPCR probes with different fluorescence wavelengths have been designed to target P35S, TNOS, P3, and actin (a positive-control plant gene). Results from multiplexing these probes with the aforementioned primers in single qPCR reactions containing template DNA from organic and GM plants with and without CaMV infection will be presented as a demonstration of the utility of this new assay.

Improved detection of *Clavibacter michiganensis* subsp. *sepedonicus* using droplet digital PCR
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The Gram-positive bacterium *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) is the causal agent of bacterial ring rot (BRR) in potato. In the United States, Cms is a zero-tolerance pathogen in seed potato production, and the presence of Cms in a single plant or tuber on a farm can result in rejection of whole seed lot. Assay results near threshold levels for common Cms detection assays, including enzyme-linked immunosorbent assay (ELISA) and quantitative real-time PCR (qPCR) assay, are common and this makes it difficult to decide whether the pathogen is present. We developed a droplet digital PCR (ddPCR) assay for Cms and compared its accuracy and sensitivity to currently used assays. We found that ddPCR is more sensitive than qPCR and that unlike qPCR, it could reliably detect one infected potato tuber core mixed with 800 uninfected tuber cores. We also tested field samples that gave ambiguous results with ELISA and qPCR, presumably due to low levels of Cms and found that ddPCR provided unambiguous test results for these field samples. A limited sampling of tubers from a local grocery store showed that Cms is still present in the potato system. Our results provide evidence that ddPCR offers a robust alternative to PCR and ELISA for CMS testing.

An RNA Extraction Method for Improved Molecular Diagnostics of *Plum Pox Virus* in PPQ-CPHST Beltsville Laboratory
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Plum pox virus (PPV) is a potyvirus that infects all known commercial, ornamental, wild *Prunus* spp. and some weeds. PPV was first reported in the USA in 1999 and has been under federal quarantine and eradication ever since. For federal confirmatory diagnostics, any PPV-suspect sample identified by screening laboratories is forwarded to the CPHST Beltsville Lab. As a part of the confirmatory protocol, Immunocapture (IC) of PPV particles was performed prior to RT-PCR analysis. IC allows for using the same ground sample prepared for DAS ELISA, thus overcoming the challenge of PPV's uneven distribution in the sample. Improvements were identified for the current system: a need of a plant internal control for improved reliability of RT-PCR and a need to overcome defined specificity of antibodies that may not capture all variants of the virus. We have developed a total RNA extraction method starting from the same DAS ELISA sample suspension. A real-time RT-PCR assay was used to validate the RNA extraction method. We found that the total RNA extraction method is more sensitive than IC, as it produced lower Ct values. When using the same amount of starting suspension (100 µL) as IC, total RNA extraction produced lower Cts by an average of 1.92. When the suspension volume was increased to 300 µL, the RNA extraction method produced an average of 3.94 Cts lower than IC. Additionally, the total RNA extraction method was found to be faster and easier to perform than IC.

Reliable detection and identification of *Tomato brown rugose fruit virus* (ToBRFV) and other tobamoviruses in seeds of solanaceae

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First occurrence of ToBRFV on commercial scale was in Jordan in 2014. The virus, which overcame Tm-2^2 resistance that has effectively controlled tobamoviruses in tomato for decades, is a threat to solanaceae seed and produce industries. Spread of tobamoviruses by solanaceae seed is prevented by testing representative seed samples and disinfecting infected seedlots. Tobacco plants are used as an indicator for presence of viable virus in solanaceae seed and if seed is infected with a tobamovirus, leaves inoculated with the seed extract develop small necrotic lesions. ELISA can be used as prescreen and if no virus is detected by ELISA the seedlot is considered free from tobamoviruses. However, as ELISA detects both infectious and non-infectious virus particles, the indicator plant test should follow after a positive ELISA to determine the presence of infectious tobamovirus. Some countries require specific testing for ToBRFV and a PCR test on symptomatic leaves in the indicator plant test can identify the virus, if present. PCR tests on seed extract after a positive ELISA prescreen can also identify the virus. For the vegetable seed industry it is crucial that all tobamoviruses associated with solanaceae seed are detected in seed health tests. The seed industry is developing and adopting new methods. These methods must be as robust and reliable as the indicator plant test and ELISA prescreen have been in preventing tobamovirus outbreaks for many decades.

Seed extract TaqMan RT-PCR for high throughput prescreening of ToBRFV in seeds of tomato

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Tomato brown rugose fruit virus (ToBRFV) is a new Tobamovirus of tomato. Spread of ToBRFV by mechanical transmission is common, but potentially seed transmission is a pathway. ELISA and an indicator plant test are used to detect ToBRFV in seed extract, but do not discriminate between ToBRFV and more common tobamoviruses like ToMV and TMV. Two complementary ToBRFV-specific TaqMan RT-PCRs in a multiplex format were developed and implemented for seed health testing. One ToBRFV-infested tomato seed was diluted in healthy tomato seeds with variable subsample sizes. After grinding of seeds *Bacopa chlorosis virus* was spiked in the extract as a process control to monitor inhibition. RNA was purified on a Kingfisher platform. Finally, TaqMan RT-PCR was performed to detect presence of ToBRFV. Validation results of the ToBRFV seed extract (SE) TaqMan RT-PCR method will be presented. The SE TaqMan RT-PCR prescreening is high throughput, cost-effective and a specific method for ToBRFV in comparison with ELISA. A disadvantage of SE TaqMan RT-PCR and ELISA is that both methods cannot discriminate between infectious and non-infectious virus like the indicator plant test does. This SE TaqMan RT-PCR is a reliable prescreening method for ToBRFV and is complementary to the reference indicator plant test that distinguishes between infectious and non-infectious ToBRFV among other relevant tobamoviruses.

From Branches to Bands: Detecting TCD with Conventional Gel Electrophoresis

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Thousand canker disease is a disease complex formed between susceptible walnut, butternut, and wingnut host trees, the fungal pathogen *Geosmitha morbida*, and the primary bark beetle vector *Pityophthorus juglandis*. Together, the plant pathogen and its principle vector induce cankers and excavate galleries beneath tree bark. TCD infestation and resulting damage cause tree decline, which when severe, can kill a tree in three to four years. Disease identification using traditional fungal isolation on agar cultures can take six weeks or more. A molecular protocol has been developed that can help identify *G. morbida* and *P. juglandis* within a day or two; however, the protocol requires an automated gel system that may be too expensive for most diagnostic laboratories. This research study is focused on enabling sample visualization using a conventional gel system rather than the more expensive equipment and better understanding the lower identification limits of an optimized DNA detection protocol. Optimized detection was assayed using both an automated system and conventional gel methodologies. Both systems provided a positive test result with as few as seven *G. morbida* hyphal cells or conidia. These findings enable an alternative detection approach that can replace the need for more expensive

equipment. The resulting procedural improvements can be readily adopted by regulatory agents and diagnosticians charged with reducing the spread of thousand canker disease.

A spore trap network for the early detection of plant pathogens in Idaho
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Many important crop pathogens are dispersed via air-borne spores. Typically, these pathogens are managed on calendar-based schedules, often without knowledge of the local optimum conditions for disease development or if spores are present in the area at the required threshold for disease development. Recent advances in spore sampling technology and molecular diagnostics have enabled airborne spores of these pathogens to be detected and the results disseminated in a timely manner. Spore traps placed in a network can potentially provide a powerful tool to warn growers of disease risk, thereby allowing early treatments or conversely alleviating the need for unnecessary treatments in the absence of disease pressure. Recently the University of Idaho and industry partners have invested in a network of 15 Burkard multi-vial cyclone spore samplers. These samplers can sample continuously for 7 days, automatically changing the sampling vial daily. Spore samplers were deployed throughout southern Idaho from May to September 2018 and real-time PCR was used to determine the relative levels of spores from potato pathogens (*Phytophthora infestans, Alternaria solani*, *Alternaria alternata, Botrytis cinerea* and *Sclerotinia sclerotium*). In addition, the network was used to monitor spores of sugar beet pathogens (*Erysiphe betae* and *Cercospora beticola*) and cereal pathogens (*Puccinia striiformis* f.sp. *tritici* and *Blumeria graminis*). Seasonal trends of airborne spore levels will be presented as well as the potential use of such a spore trap network to provide early detection of plant diseases.

In silico detection of multiple oomycetes in metagenomic data by using E-probe Detection of Nucleic acid Analysis (EDNA)
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Plant diseases can be better managed if pathogens are detected early and identified accurately. Metagenomic diagnostic methods allow identification of multiple pathogens from a single sample without pure culture isolation or specific gene-targeted sequencing. This projects utilized E-probe Diagnostic Nucleic acid Analysis (EDNA), a computational pipeline that couples metagenomic sequencing and bioinformatics, to detect multiple oomycete plant pathogens in metagenomic data. EDNA-MiFi, a new bioinformatic tool based on the EDNA concept, consist of of two parts: MiProbe, for selection of pathogen-specific sequences (E-probes); and MiDetect, for the rapid identification of target organisms from metasamples. E-probes 20-120 nucleotides length were obtained for nine pathogenic oomycete species. To avoid false positives, e-probes with similarity to non-target sequences in NCBI's Nucleotide database were removed. Curated e-probe databases were validated in silico on mock-sequencing databases (MSDs) created using MetaSim, simulating Illumina average read length and average error rates. MSDs contained the genomes of a known host and the target oomycete at different percentages (10 to 10^{-4}). A metasample was positive for the presence of a pathogen when a significant number of e-probes were found in a MSD. Accurate pathogen detection in silico was achieved even at very low pathogen concentrations. EDNA-MiFi is a highly sensitive diagnostic tool for oomycetes from metasamples.

Whole genome analysis for the development of a conventional multiplex PCR for *Peronosclerospora* species of regulatory concern
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Graminicolous downy mildews are economically important pathogens of grasses. These pathogens are most commonly found in tropical regions of Asia where crop losses can be devastating. *Peronosclerospora philippinensis* and *P. sacchari* cause destructive diseases of maize, while *P. sorghi,* the only *Peronosclerospora* species reported in the US, is a pathogen of sorghum. In this study we performed comparative genomics analyses on a selection of *Peronosclerospora* genomes for the development of a conventional multiplex PCR assay for the specific detection of the species of regulatory concern, *P. philippinensis* and *P. sacchari*. In addition, we designed internal control primers for *Peronosclerospora* at the genus-level and a plant internal control. The assay was optimized and evaluated against other graminicolous downy mildew pathogens. Sensitivity tests using environmental samples resulted in a limit of detection of 0.05 ng/µl to all the tested species. We observed no cross-reaction against closely related pathogens and other *Peronosclerospora* species. The conventional multiplex PCR assay we developed works effectively for the target species *P. philippinensis/P. sacchari*, *P. sorghi* and the internal *Peronosclerospora* genus and plant controls. This species-specific conventional multiplex PCR assay requires basic molecular laboratory equipment and techniques, making it ideal for the quick detection of many samples.

Ultrasensitive and in-situ detection of a plant virus by a nanotube-filtering device and isothermal amplification
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Plant virus detection at the site of initial infection could prove useful for identification prior to transmission by vectoring insects. This would allow for earlier implementation of a management system and could prevent epidemics. However, the low viral titer during early infection renders detection and identification difficult and requires the use of highly sensitive laboratory techniques that are often incompatible with field application. To obviate this challenge, we designed a Recombinase Polymerase Amplification, a molecular

technique that makes millions of copies of a predefined region in the genome, in this case of the tomato spotted wilt orthotospovirus (TSWV) genomic segment M. The reaction occurs at 39°C and can be used directly from crude plant extracts without nucleic acid extraction. A positive result can be seen with the naked eye as a flocculus made of newly-synthesized DNA and metallic beads. This technique can be coupled with the use of our nanotube-based microfluidics devices to trap viruses by size and to concentrate them at detectable levels for any downstream technique. The objective of the combined procedure is to create a portable and affordable system that can isolate and identify viruses in the field, from infected plants and suspected insect vectors, and can be used by scientists and extension managers for making informed decisions for viral management.

Initial Evaluation of a CANARY™ Biosensor-based Assay for Detection of Potyviruses

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Potyviruses, members of the genus *Potyvirus*, collectively infect almost every major crop throughout the world, and in many crops a potyvirus may be the most economically important viral pathogen. Efficient management of viral diseases requires detection and identification tools and protocols for quick, reliable and accurate identification of the pathogen. Several years ago, the USDA-ARS-USNA Beltsville Lab developed a hybridoma cell line that secretes a broad-spectrum reacting PTY-1 monoclonal antibody (McAb), which detects almost all known aphid-transmitted potyviruses, was patented and licensed to Agdia, Inc., and forms the basis of Agdia's "POTY Group Test" reagents, ELISA kits, and ImmunoStrip Test. CANARY™ (Cellular Analysis and Notification of Antigen Risks and Yields) is a fast, sensitive, and easy-to-use biosensor-based technology that has been successfully utilized for the detection of bacteria, viruses, and toxins. Several PTY-1 hybridoma-derived CANARY biosensor clones were created at MIT-LL, initially tested in the APHIS-CPHST Beltsville Lab, and later evaluated collaboratively in the PathSensors and USDA-ARS labs, for assay feasibility, bead capture efficacy, specificity and sensitivity of the reaction to detect target potyviruses. The optimized POTY biosensor can detect diverse purified potyviruses down to less than 5 ng/ml and can detect potyviruses in naturally infected sources, such as various isolates of potato virus Y in potato leaves and tubers.

Early detection of Bull's-eye rot (*Neofabraea vagabunda*) from apple fruits using qPCR in Chile

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Bull's-eye rot caused by *Neofabraea vagabunda* (= *N. alba*) is one of the most important postharvest disease of apple, being the only described specie associated with this rot in Chile. The symptoms develop in slow progress, requiring at least three months in cold storage to be visible during postharvest, starting from a preharvest latent infection at the fruit lenticel. Therefore, the aim of this study was to early detect latent infections of *N. vagabunda* in apple lenticels, using TaqMan probe based qPCR method. The samples analyzed were apple fruits cv. Cripps Pink apparently healthy (n=100); inoculated with *N. vagabunda* (isolate Nv-UTalca 6.1) (n=50), and with a natural infection developed during cold storage (n=50). The negative controls used were DNA of *Diplodia seriata* (Ds) and *Phacidiopycnis washingtonensis* (Pw) isolates obtained from natural infected Chilean apples and DNA of *N. kienholzii* (Nk) and *N. malicorticis* (Nm) from pure cultures provided by Wageningen Plant Research. Our results indicated that a qPCR assay was developed and validated to detect DNA sequences specific to *N. vagabunda*. Consequently, DNA of Ds; Pw; Nk and Nm was not detected using the developed TaqMan probe. Therefore, the early detection of *N. vagabunda* was positive in fruits apparently healthy (76.2%), natural infected (100%) and *N. vagabunda* inoculated apples (100%). This method would be also useful for epidemiological studies of Bull's-eye rot in Chile.

***Rose rosette virus* detection methodology: Is asymptomatic detection possible?**

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As Rose Rosette Disease (RRD) continues to cause issues for rose producers, growers, and enthusiasts, the question has been posed whether early/asymptomatic detection is possible. RRD is a viral disease caused by the *Rose rosette virus* (RRV), which is vectored by a species of eriophyid mite. Plants have been documented to take upwards of two years to display symptoms after being exposed to RRD, potentially allowing for the plant to serve as an asymptomatic disease reservoir for continued spread by the mite vector. Therefore, effective and sensitive detection tests are needed to enable early removal of infected plants. Our experiment focused on approximately 500 samples of RRV from symptomatic, asymptomatic, and clean plants. It was found that the use of primer set RRV (Di Bello et al. 2018), allowed for effective asymptomatic detection compared to all other RRV primers. This RT-PCR primer set targets the third segment of RRV's seven segmented genome. While primer set RRV was comparable to other primers (RT-PCR and qPCR) for symptomatic plants, it was more effective at detecting asymptomatic, PCR positive plants than other published primers. Results were verified through Sanger sequencing, with 96-99% sequence identity to RRV. Identification of asymptomatic, RRV infected plants will enable the early removal of diseased plants and reduce potential spread. Asymptomatic detection testing can also allow producers to screen plants from any RRV infected fields, determine the infection status, and reduce the amount of asymptomatic RRV positive plants being sold to consumers.

Development and Validation of a TaqMan qPCR Assay for the Detection of Citrus chlorotic dwarf associated virus
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Citrus chlorotic dwarf disease (CCDD) is an emerging graft-transmissible disease first observed in the mid- 1980s in Turkey and in 2009 in China. CCDD is also transmitted by vector bayberry whitefly (*Parabemisia myricae*). CCDD symptoms include chlorotic flecking on young leaves, and crinkling, inverted cupping, variegation, chlorosis and dwarfing on mature leaves. A novel *Geminivirus* was discovered in association with CCDD in 2012 using high throughput sequencing, named Citrus chlorotic dwarf associated virus (CCDaV), and is listed as a quarantine pathogen in the United States. To prevent the introduction of the disease and to improve the existing CCDaV diagnostic methods, a triplex TaqMan qPCR assay was developed that targets the Coat Protein and RepA genes of the CCDaV genome along with the plant internal control COX gene. To develop a specific and sensitive molecular assay, CCDaV isolates from China, Italy and from the Exotic Pathogens Citrus Collection (EPCC) in BARC Beltsville MD, USA were obtained. The triplex TaqMan qPCR assay was optimized and validated by determining the selectivity, specificity, efficiency, repeatability and intermediate precision of the assay. No adverse effects were detected in the triplex assay when compared to the duplex assay for detecting one of the CCDaV targets and a COX plant DNA internal control gene. The described TaqMan qPCR assay can be used for quick and reliable detection of CCDaV from infected plant material.

Comparative genomics approach to develop a highly reliable duplex TaqMan qPCR assay for sensitive detection of genus *Dickeya* and *Dickeya dianthicola*
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Dickeya, a genus of important and high consequence bacterial plant pathogens, is listed among the quarantine pathogens of the European Union and is associated with potato disease outbreaks and economic losses worldwide. Early, accurate, sensitive and reliable detection of the pathogen is needed to prevent further dissemination and establishment of disease. A genome-informed multiplex TaqMan qPCR labeled with different fluorophores was developed for accurate and sensitive detection of *Dickeya* pathogens. A whole genome comparative genomics approach was used to identify unique and conserved regions: alcohol dehydrogenase in *D. dianthicola* strains and *mglA*/*mglC* for the genus *Dickeya*. The developed multiplex TaqMan qPCR was validated using an inclusivity panel to confirm broad range detection capability and an exclusivity panel to exclude cross-reactivity with strains of closely related genera and species. The developed assay was validated with plant samples naturally and artificially infected with *Dickeya* and/or *D. dianthicola*. Both sensitivity and spiked sensitivity assays showed the limit of detection down to 10 fg/reaction. No false positives or false negatives were detected. This assay will serve as a practical tool for screening propagative material, monitoring the presence and distribution of pathogens, and quantification of target pathogens in a breeding program. It also has applications in routine diagnostics, biosecurity, and microbial forensics.

Application of Classical and Molecular Techniques for Screening Sweetpotato Germplasm Imported into the United States
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Certain plant genera are known to carry pathogens not readily detected at inspection stations, such as viruses and viroids, and are restricted from entering the United States. The USDA-APHIS-PPQ-FO-Plant Germplasm Quarantine Program (PGQP) imports crops such as potatoes, sweetpotatoes, apples and sugarcane that belong to these prohibited genera. The Sweetpotato Program uses diagnostic tools, including PCR-based molecular testing and indicator plants, to identify such pathogens. Infected accessions must undergo tissue culture therapy prior to release. In 2018, 27 sweetpotato accessions were tested for viruses, including Geminiviruses, Potyviruses and Carlaviruses, using reverse transcription PCR (RT-PCR). Quantitative reverse transcription PCR (RT-qPCR) was used to screen for the West African and East African strains of *Sweet potato chlorotic stunt virus*. Seven accessions tested positive for a Potyvirus, although only two tested positive at the seedling stage. All seven accessions, when grafted onto the susceptible indicator plant *Ipomoea setosa* displayed symptoms, and the respective *I. setosa* tested positive for a Potyvirus by RT-PCR. The use of indicator plants complemented with molecular testing has proven critical in germplasm testing, and we are now beginning to implement high-throughput sequencing (HTS) technologies for diagnostics. Several positive controls for viruses of economic significance have now been sequenced, allowing for improved germplasm screening.

Robust and highly reliable loop-mediated isothermal amplification (LAMP) assay for specific and sensitive detection of *Dickeya solani*
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Dickeya species cause serious bacterial soft rot diseases of potato, an economically important crop worldwide. *D. solani* is the most aggressive species within soft rot causing bacterial pathogens, but fortunately is not yet reported in the United States. Symptoms produced by soft rot causing bacterial species are nearly indistinguishable. A rapid, field-deployable tool is required to monitor potato

farms and to inspect potato tubers at ports-of entry for *D. solani*. Comparative genomics approach was used to identify signature target regions within the *D. solani* genome to develop a robust and field-deployable loop-mediated isothermal amplification (LAMP) assay. The specificity of the developed assay was tested with members of inclusivity (*D. solani* strains from distinct geographical locations) and exclusivity (strains from near-neighbor species and genus including all species from *Dickeya* and *Pectobacterium*) panels. No false-positives or -negatives were detected. A detection limit of 100 fg was obtained using DNA from pure cultures. However, a detection limit of 1000 fg was observed when target DNA was mixed with host genomic DNA. The developed LAMP assay has numerous applications from routine diagnostics at point-of-care, biosecurity, surveillance to disease management.

Multiplex TaqMan qPCR targeting unique genomic regions for specific, sensitive and robust detection of *Pectobacterium* species and *P. parmentieri*

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Pectobacterium, a pectinolytic phytobacterium, is listed among the top 10 most destructive plant pathogens. The newly defined species *P. parmentieri*, which lacks a type III secretion system (T3SS), has emerged as a particularly aggressive pathogen by eliciting soft rot and blackleg diseases that have impacted the potato productivity and jeopardized food security. The purpose of this research was to develop a robust and highly accurate multiplex TaqMan qPCR to specifically detect all known *Pectobacterium* species and the recently characterized *P. parmentieri*. Comparative genomic approaches were used to determine conserved signature genomic regions for the genus and species. A triplex TaqMan qPCR was developed to target unique gene regions of *Pectobacterium* and *P. parmentieri*, and a universal internal control (UIC) was incorporated to enhance the reliability of the assay. The assay was validated using diverse strains, meticulously selected for exclusivity and inclusivity panels. No false positives or false negatives were detected during the assay validation. The detection limit of qPCR is down to 10- and 100-fg per reaction for *Pectobacterium* and *P. parmentieri*, respectively. The assay developed in this study will contribute to tracking the geographic distribution and dissemination of *Pectobacterium,* and will be an excellent tool for detecting and quantifying the target species especially in seed certification programs, microbial forensics and routine diagnostics.

Microbe Finder (MiFi): Pathogen detection in metagenomic sequence data

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Bioinformatic analysis of metagenomic sequence data has been a bottleneck for diagnostic analysis. Most available tools require that the user have computer coding skills and access to high throughput computing. Taxa assignments using bioinformatic tools often lack enough resolution to detect microbes at the species/strain level. Microbe Finder (MiFi) is a web application that allows microbial community detection at species and strain level resolution without the need of coding skills. Obtaining metagenomic sequence data, from both host tissue and resident pathogens, is cheaper and easier. Ability to simultaneously screen for all possible pathogens in a single sample will enable more timely response, mitigation and management of potential plant disease introductions and outbreaks. MiFi relies on the successful development and validation of unique signature sequences (e-probes) for targeted query of metagenomic sequence databases for pathogen detection. Whole genome sequencing (WGS) coupled with targeted detection such as with MiFi e-probes has promise to overcome some of the technical difficulties in detection of and/or declaring freedom from a set of pathogens in a sample. Until recently, WGS has been constrained by cost, data volume, and limited bioinformatic tools for analysis, creating a bottleneck to adoption by most diagnostic clinics. With decreasing sequencing costs and bioinformatic tools like MiFi, the constraints are resolved. Here we introduce MiFi as a tool for plant pathologists that want to develop and use it for their research and daily diagnostics.

Evaluation of the disease severity of *Phyllachora maydis*, *Mycosphaerella fijiensis* and *Moniliophtora roreri* in three important crops in Los Ríos, EC

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Accurate assessments of disease severity are critical for several research areas in plant pathology. Several methods have been described to assess disease severity but visual evaluation remains as the main option despite any bias the researcher might have. Recently, a portable application called LeafDoctor was developed using six different foliar diseases, however this software has not been not tested in other crops and with different tissue organs. Black spot of maize (*Phyllachora maydis*), black sigatoka of banana (*Mycosphaerella fijiensis*) and frosty pod rot of cacao (*Moniliophtora roreri*) are the most devastating diseases that affect the main crops at Los Ríos, Ecuador. After sampling corn and banana leaves and cacao fruits using visual rating of symptoms and the LeafDoctor evaluation, linear regression data shows that the software can be used in black spot of maize but not in black sigatoka or frosty pod rot. Future refinements could be used in banana to increase its correlation values, however the time spent to increase their correlation values might be time restrictive for the researcher.

Quantification of an atoxigenic strain of *Aspergillus flavus* (AF36) using real-time PCR

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Aspergillus flavus and *A. parasiticus* are commonly found in nut-growing areas in California. Some isolates of these species produce aflatoxins that very occasionally contaminate nut kernels. The *A. flavus* strain AF36, which does not produce aflatoxins, is registered as a biocontrol for use in tree nuts in California. AF36 success in aflatoxin reduction relies on the capacity to displace the native toxigenic isolates. Currently, the biocontrol AF36 is quantified by vegetative compatibility group analysis, which is expensive and time consuming. We developed a quantitative real-time PCR (*q*PCR) procedure to quantify the proportions of both AF36 and the toxigenic isolates at reduced time and assay costs. Specific primers to target the AF36 strain, and *A. flavus* and *A. parasiticus* were designed. The standard curves were generated to quantify the amounts of DNA based on the threshold values (Ct) for each strain of interest. Verification tests showed significant correlations between known concentration of spore mixtures and the amounts quantified by the *q*PCR assay. The *q*PCR assay was used to quantify the proportions of AF36 from leaves, nuts, and soil samples, demonstrating its usefulness to accurately quantify AF36 and the natural strains of the pathogen. This study demonstrates the potential of *q*PCR assay to increase the number of samples that can be quantified based on available resources and on time and improve the study of the epidemiology of aflatoxin producing fungi.

Comparison of newly developed ELISA and RT-PCR assays for the detection of all known genetically diverse variants of GLRaV-3

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Grapevine leafroll-associated virus 3 (GLRaV-3) is the main etiological agent of grapevine leafroll disease, one of the most important virus diseases of grapevine worldwide. The long-distance spread of GLRaV-3, caused by the movement of infected vines, can be controlled effectively if GLRaV-3 is accurately identified and clean stock is made available to growers. However, the development of reliable detection assays is complicated by the high genetic diversity of GLRaV-3. Phylogenetic studies based on whole genome sequences have placed GLRaV-3 isolates into ten different genetic groups. Additionally, new divergent variants have been discovered recently around the world. To cover this genetic diversity, we developed new enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase quantitative PCR (RT-qPCR) assays for GLRaV-3 detection. These assays were tested using a large number of geographically diverse grapevine samples that included plants infected with different GLRaV-3 variants. Both assays detected all known GLRaV-3 variants characterized to date and represent two new alternatives for high throughput sample testing that are both sensitive and specific for GLRaV-3. In the case of the ELISA, this assay brings the advantage that reagents are relatively cheap and no special equipment is needed. These new detection tools will benefit growers, researchers, and diagnostic labs involved in the grapevine industry in the US and around the world.

Improved detection of fruit tree viruses and viroids by real-time quantitative PCR

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Viruses and viroids can cause economic losses in fruit trees by reducing growth, vigor and yield. Efficient and reliable diagnostic methods are critical in identifying viral infection in fruit trees. This study evaluated the broad-range detection capacity of currently available PCR-based assays for pome and *Prunus* viruses and viroids (16 different pathogens) and developed new reverse transcription quantitative PCR (RT-qPCR) assays when current tests were inadequate. Thus, published PCR assays for targeted pathogens were evaluated *in silico*, using GenBank nucleotide sequence data, to determine their ability to detect specific virus and viroid isolates. Assays with nucleotide mismatches in the primer sequences were selected for modification. Modifications consisted of either the addition of primers/probes to the existing assay or a new assay design targeting a more conserved region of the virus/viroid genome. To validate proposed assay designs, select fruit tree populations were screened for targeted viruses and viroids via high throughput sequencing (HTS) compiling a representative set of isolates. Consequently, all the updated or new RT-qPCR assays successfully detected the different pathogens, including the diverse isolates. The development of robust, sensitive and reliable detection methods is needed for large scale pathogen testing, which will contribute to maintain the highest quality nursery stock.

Epidemiological studies of a complex viral infection in red ginger in Hawai‘i

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Red ginger (*Alpinia purpurata*) is among the most popular landscape plants in Hawai‘i and can be subject to serious diseases. Members of the potyvirus and badnavirus genera have been previously reported in red ginger in Hawai‘i displaying mosaic, leaf streaking and severe dieback symptoms. Symptomatic plants can be infected by single and/or mixed infectious of canna yellow mottle virus (CaYMV) and banana bract mosaic virus (BBrMV), but only mosaic symptoms caused by BBrMV are easy to identify by sight. Also, in some symptomatic plants, the range of symptoms could not be attributed to either virus, whether alone or in a mixed infection, suggesting the presence of additional pathogens. The aims of this project are to characterize viral populations in red ginger, to associate virus presence with expressed symptoms, and to provide insight into the epidemiology of these diseases. Samples displaying the dieback and viral-like symptoms, as described above, were collected from several locations on the island of O‘ahu. Multiple samples tested positive using degenerate potyvirus and badnavirus detection primer sets, while testing negative for BBrMV and CaYMV, respectively. Sequencing of amplicons revealed the presence of two additional viruses infecting red ginger, banana streak virus and bean common mosaic virus. The latter virus has been found infecting additional hosts in Hawai‘i. Additional work is currently being done to examine

the etiology of the disease using PCR-based assays to discern the involvement of the above-mentioned viruses and to develop management strategies.

E-probe: A new diagnostic tool for detection of Dichorhaviruses associated with Citrus leprosis syndrome
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Diagnosing of causal agents associated with Citrus Leprosis (CiL) syndrome has been difficult because of the large number of potential viruses. There are five cytoplasmic viruses that includes Cilevirus; Citrus leprosis virus (CiLV) -C, CiLV-C2, SJP strain of CiLV-C (CiLV-C-SJP), Hibiscus strain of CiLV-C2 (CiLV-C2H), Hibiscus green spot higrevirus -2 (HGSV-2), and five nuclear viruses including Dichorhavirus; CiLV-N, CiLV-N0, Citrus strain 1 and 2 of Orchid fleck virus (OFV-Cit1 and OFV-Cit2) and Citrus chlorotic spot virus (CiCSV). More viruses causing CiL are being discovered every year, making the development of a single RT-PCR based detection protocol a nearly impossible. Therefore, we created and validated a suite of diagnostic E-probes for Dichoraviruses (generic), Dichoraviruses infecting citrus, and near neighboring viruses on Orchid, Hibiscus, Clerodendrum and Coffee. There are 19 unique e-probe sets, each with *in silico* sensitivity analysis, analytical limit of detection and specificity curation. E-probes for all CiLV strains and near neighbors were selected to be 30nt long. Identifying (discriminatory) unique sequences per virus ranged from 2 – 20 e-probe sequences. Sensitivity was validated using simulated metagenomic databases containing the complete genome of navel orange and the generic Dichoraviruses for nuclear type species. The limit of detection *in silico* was 0.0034% relative abundance when using one million reads. Specificity of e-probes to discriminate different strains within a mixture of Dichoraviruses remains to be established.

***Ficus carica* plants displaying severe viral-like symptoms harbor a complex virome**
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In 2009, edible fig plants (*Ficus carica*) displaying severe mosaic, mottling, and leaf distortion symptoms were reported from a nursery on Kauai, Hawaii. To determine if viral-like agents were present in these plants, double-stranded (ds)RNAs were extracted from root tissue. The extracted dsRNAs ranged from 1 to 20 kb and were used to generate a randomly-primed cDNA library that underwent high throughput sequencing (HTS) using a 454 GS FLX Titanium platform. About 263k reads (134 Mb) mostly between 400 and 600 nt in length were generated. After quality control, trimming and de novo assembly, 1183 contiguous sequences (contigs) were assembled. BLASTX/BLASTN comparisons to existing databases revealed these contigs were most similar to fig mild mottle-associated virus, fig leaf mottle-associated viruses 1-3, citrus yellow vein-associated virus, fig latent virus 1, fig badnavirus 1, grapevine badnavirus 1, fig mosaic emaravirus, and members of the genera *Endornavirus*, *Partitivirus* and *Totivirus*. Additionally, some contigs shared sequence homology to citrus exocortis viroid and a putatively novel viroid most similar to avsunviroids. Testing is currently underway to determine whether the viruses and viroids identified by HTS can be detected using PCR-based assays in edible and ornamental ficus plants growing in Hawaii.

Metagenome sequence calculator for effective pathogen detection
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Nucleic acid based analysis is a prevalent technology for plant disease diagnoses, particularly using amplification and primers designed on a unique sequence, primarily from the ITS region. Future diagnostic technology will turn to whole metagenome sequencing, without isolation or amplification, using *in silico* searches for any number of pathogen-specific unique nucleic acid signatures. A host-pathogen metagenome, comprised of full sequence data of the host and resident pathogen(s), then can be described in probabilistic terms, based upon relative abundance and size of the pathogen genome within the metagenome. Abundance of pathogen within a host tissue can be a gradient from high titer (highly symptomatic tissue) to low titer (pre-symptomatic infection). Generalizing pathogen genome size: viroids range from 0.2-0.5Knt, viruses from 10-100Knt, bacteria from 3Mnt-5Mnt, and fungi from 30 to 100Mnt. The ratio of (high to low abundance) pathogen sequence to host sequence can be calculated to inform the diagnostician how deep to sequence in order to positively detect the pathogen sequence within the metagenome. In this poster, we demonstrate how many sequenced reads are required in a metagenome in order to positively detect pathogen sequence with high probability, based on host genome size, pathogen genome size, and expected abundance of the pathogen within the host tissue.

Detecting viruses and bacteria of grapevine with Microbe Finder (MiFi) and an Oxford Nanopore sequencer
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Grapevines can be infected by numerous viruses and bacteria. Infections may be mixed complicating detection and diagnosis. Simultaneous detection of multiple viruses has been performed using High Throughput Sequencing (HTS) and MiFi virus e-probes. Portable sequencers like the minION from Oxford Nanopore Technologies (ONT) allow fast, inexpensive access to HTS. We have developed a graphical-user interphase (GUI), MiFi, that contains e-probe libraries that will allow unequivocal detection of 12 pathogens of grapevine in metagenomic sequences. E-probes are unique signatures of each pathogen of interest used for a targeted query if the pathogen sequence is found in HTS data. Currently there are specific e-probes for bacteria *Xylella fastidiosa subs.* fastidiosa (14,236) and for *Agrobacterium vitis* (4,034), and 19 viruses including strains of Grapevine Leafroll associated virus (GLRaV). Sensitivity was assessed *in silico* where relative gradient of pathogen abundance was simulated using host and pathogen genomes from NCBI. Bacterial pathogens had higher sensitivity (< 0.001%) than viruses (average 0.02%). Positive control samples were sequenced with the minION

and sensitivity was assessed using different viral concentrations. Validation with real sequence data (*in vitro*) has been done for GLRaV-2, GLRaV-3 and TRSV. Preliminary results have shown that MiFi is able to detect the pathogens in minION metagenomic sequence data.

Detection of *Clavibacter michiganensis* and *C. michiganensis* ssp. *nebraskensis* using multiplex recombinant polymerase amplification coupled with LFD
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Clavibacter michiganensis (*Cm*) is an agriculturally important species comprising nine host-specific subspecies including *C. m*. subsp. *nebraskensis* (*Cmn*), which causes Goss's wilt and blight of maize. A robust, reliable, simple and field deployable method is required to specifically detect *Cm* and *Cmn* in infected plant tissue for timely disease management. A multiplex Recombinase Polymerase Amplification (RPA) coupled with a Lateral Flow Device (LFD) was developed for sensitive and rapid detection of *Cm* and *Cmn* directly from an infected host. Comparative genomic analyses were performed to identify unique and conserved genomic regions for long primer and probe design. The multiplex RPA assay was evaluated using 54 strains representing all nine subspecies of *Cm* and other closely related bacterial species. No false positives or false negatives were detected. The multiplex RPA reactions were also incubated in a closed hand at body temperature; results were again specific. The assay does not require DNA isolation and can be directly performed using host sap. The detection limit of 10 pg and 100 fg was determined for *Cm*- and *Cmn*-specific primers/probe, respectively. The developed multiplex assay is accurate and has applications at point-of-care diagnostics without the need for DNA isolation. This is the first multiplex RPA for any plant pathogen.

Enhanced Detection of Bacterial Blights on Common Bean
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The common bean growing region in North Dakota and Minnesota accounts for over 35% of acres harvested nationwide. A bacterial blight complex affects nearly all common beans in the region to some extent annually. The three most common bacterial diseases in this region include: common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) *and X. fuscans* subsp. *fuscans* (*Xff*), bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* (*Pss*), and halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*). Simplex PCR assays exist for each of these pathogens; however, the development of a multiplex qPCR assay will enable the quantification of each pathogen in the complex simultaneously from seed, pod, and leaf tissue. Primer sets of *Xap, Xff,* and *Psp* optimized only the target bacteria among 27 target and non-target bacterial species tested for primer specificity. Standard curves for the multiplex assay established for *Xap, Xff, Psp,* and *Pss* produced efficiency values of 90.9%, 93.1%, 94.4% and 91.5%, respectively. R^2 values were 0.99 for all assays. Pathogen detection of the four target species was successful on 30 diseased leaf tissue samples. Preliminary results indicate a significant positive correlation between traditional plating methods and qPCR detection from seed in field trials conducted in Oakes, ND in 2016 ($P < 0.0001$; $r = 0.85$) and Fargo, ND in 2017 ($P < 0.0001$; $r = 0.56$).

Aerobiology of *Xanthomonas hortorum* pv. *carotae* in carrot seed crops of Central Oregon
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Bacterial blight of carrot, caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is a common seed-borne disease of carrot. The biennial nature of seed-to-seed carrot seed production requires 13 months from planting to harvesting, resulting in a possible "green bridge" that facilitates *Xhc* survival from year to year. Consequently, the crop is potentially exposed to bacterial blight inoculum from the seedling stage until harvest. The objective of this research was to quantify airborne *Xhc* during the potential green bridge period in the biennial carrot seed cropping cycle. Burkard 7-day volumetric spore samplers were used to sample air continuously in three fields harvested in 2018 and two fields intended for harvest in 2019. DNA was extracted from Burkard samples and pooled into weekly samples for a quantitative polymerase chain reaction assay specific to *Xhc*. *Xhc* was detected in 52 out of 54 weekly air samples at levels ranging from 10^2 to 10^6 bacteria/week. *Xhc* levels in air samples increased from 10^2 bacteria/week in July to between 10^4 and 10^6 bacteria/week at harvest. In contrast, *Xhc* levels declined from 10^5 bacteria/week in August to 10^3 bacteria/week by November in air samples from one field and varied from 0 to 10^5 bacteria in air samples from another field. Leaves taken in November showed *Xhc* populations were between 10^3 and 10^4 bacteria/g leaf tissue, demonstrating that sizeable *Xhc* populations can be established on seed-to-seed carrot fields prior to winter.

Does the proximity of corn planted to terminated winter rye cover crop influence seedling disease caused by *Pythium*?
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Yield loss of corn following a winter rye cover crop discourages farmers in Iowa from adopting cover crops to manage soil and water quality. The addition of cover crops into the cropping system alters microbial community within the rhizosphere of both winter rye and corn by increasing *Pythium* species that may cause seedling disease. *Pythium* is more prevalent in corn seedlings following winter rye. Growth chamber experiments were done to evaluate the susceptibility of corn to seedling disease as a function of corn seedling proximity to winter rye plants. Corn seedlings were planted at three distances, 0 cm, 2-3 cm, and 8-10 cm, from terminated winter rye plants. Root rot severity was assessed at crop development stage V2. Quantitative PCR was used to estimate the abundance of *Pythium* Clade B and Clade F members present in corn roots. Radicle and seminal root rot severity was greater the closer the seedling was to the

winter rye plants ($P = 0.01$). Moreover, a greater abundance of *Pythium* Clade B was detected in corn grown within the terminated winter rye compared to corn planted further away ($P = 0.01$). No effect of distance between corn and winter rye was detected for *Pythium* clade F. This study will inform management practices for farmers to reduce occasional yield loss of corn following a winter rye cover crop.

Dead twigs: A reservoir for *Phyllosticta citricarpa* inoculum leading to citrus black spot (CBS)
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Phyllosticta citricarpa, the causal agent of CBS, is an emerging fungal pathogen of citrus causing heavy yield loss and a reduction in fruit marketability. New research has determined the heterothallic nature of the fungus, mating gene structure, and population structure in Florida. Currently, only one mating type is present in Florida leading to new questions about the mechanism for disease progression. Our objective was to assess dead twigs as a potential inoculum source contributing to new secondary infections. Field collection of twigs was conducted in Immokalee, FL from 2016 to 2018, to determine the presence of *P. citricarpa*. Citrus trees were sampled biweekly and a total of 10,176 twigs were evaluated. DNA obtained from conidia and bark samples had a positive correlation with CBS fruit severity ratings ($P < 0.0001$). Conidia production was negatively affected by growing degree-day (base 10°C) accumulation over a 7-day period (gdu7) and number of days with measurable precipitation (>0.254 mm) over a 28-day period (rainday28). However, the interaction between gdu7 and rainday28 increased conidia production. Similarly, *P. citricarpa* DNA titers obtained from bark samples were negatively affected by average temperature and rainday28 but the interaction between them increased fungal growth. To conclude, *P. citricarpa* DNA was correlated with CBS development and fungal inoculum production occurred with increasing temperature in the presence of precipitation.

Cover crop rotation effects on growth and development, seedling disease and yield of corn and soybean in no-till production system
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A two-year research trial was established in Fall 2016 in a corn-soybean rotation field. The cover crops, winter cereal rye (*Secale cereal* L.) and winter camelina [*Camelina sativa* (L.) Crantz] were planted soon after each main crop harvest to study the effect of two cover crops used either in both years or in rotation with each other, on corn (*Zea mays* L.) and soybean (*Glycine max.* L.) growth and development, root disease and yield in 2017 and 2018. Corn growth and development was better following a cover crop of camelina and had reduced root disease, a lower *Pythium* population in seedling roots and greater yields compared to corn planted after a cover crop of rye. Cover crop treatments had a positive or no effect on soybean growth and development, root disease and yield. Clade B *Pythium* species were predominantly detected in corn seedlings, while *Pythium* Clade F were predominantly detected in soybean seedlings. Moreover, *Pythium* Clade B populations were greater in corn seedlings planted after a rye cover crop compared to those planted after a cover crop of camelina, while Clade F populations were greater on soybean seedlings planted after a cover crop of camelina compared to those planted after a cover crop of rye. This study demonstrated a cover crop of winter camelina before corn, with or without winter rye in a cover crop rotation system, could be a potential option to mitigate the negative effect of winter cereal rye on corn growth and development.

Epidemiology of Cotton leaf roll dwarf virus in Georgia, USA
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During 2018–19, Cotton leafroll dwarf virus (CLRDV) has been reported from the southern United States in Alabama, Florida, Georgia, Mississippi, and South Carolina. This phloem restricted virus, transmitted by aphids in a persistent, circulative and non-propagative manner. Since January 2019, in coordination with UGA-Extension, an extensive survey is initiated in GA to investigate the alternate hosts that could potentially act as a green bridge. Different weeds commonly found in GA including HenBit deadnettle (*Lamium amplexicaule*), Mouseear chickweed (*Cerastium vulgatum*), Purple cudweed (*Gnaphalium purpureum*), Curly dock (*Rumex crispus*), Carolina Geranium (*Geranium corolinianum*) and Wild radish (*Rhaphanus raphanistrum*) were collected from commercial cotton fields. In addition, cotton stalks and leaves regrowth from the remains of the previous year crop were also sampled. The samples were analyzed using RT-PCR to detect the presence of the virus. The virus is detected from the cotton stalk, suggesting its overwintering host as well. Cotton being a perennial crop and could survive the winter as a reservoir. Proper sanitation measures, like roughing, or plowing needs to be included for the management of the virus to break the disease cycle. The economic impact of the virus and sanitation practice is to be investigated. Processing of the weeds and other hosts is underway. These results could help us develop an appropriate management strategy for this emerging virus disease.

Modeling the temporal dynamics of *Leptosphaeria maculans* ascospore dispersal in North Dakota
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Blackleg, caused by *Leptosphaeria maculans,* inflicts greatest canola yield losses when plants are infected before reaching the six-leaf growth stage. During this period, fungicide applications made more than one week after initial infection provide significantly less protection. The objectives of this study were to characterize the dynamics of *L. maculans* ascospore dispersal and to identify environmental factors associated with this phenomenon. Airborne ascospores concentrations were monitored using Burkard 7-day volumetric samplers between mid-May and mid-July in Langdon, ND in 2017 and 2018. Ascospores were identified and counted under compound microscope. Concentrations > 5 ascospores/m^3 of air were considered peaks. Ascospores concentrations peaked during mid to late June in both years when canola plants were at early bud to flowering stage. Logistic regression analysis indicated there was a significant association between ascospore peaks and seven-day moving averages of daily mean relative humidity and soil temperature. In this way, there is a 50% probability that ascospores peaks would occur when the daily mean relative humidity is at 50% and the soil temperature is at 15°C. These results suggest that a warning-system could be developed; however, additional locations-years are needed to strengthen it before it is deployed.

Tracking the distribution and spread of the invasive pathogen *Fusarium virguliforme* in Minnesota
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Fusarium virguliforme is the soilborne fungal pathogen that causes sudden death syndrome (SDS) of soybean. SDS causes root rot and interveinal necrosis and chlorosis, often leading to significant economic losses. This disease was initially identified in the southern USA, and risk assessment studies suggested the pathogen would not survive in Minnesota due to cold stress. However, in 2002 SDS was confirmed in southern Minnesota and has since spread northward. The pathogen was documented in 38 counties in southern Minnesota by 2012. Due to increasing soybean production in northern Minnesota and warming winters, we are conducting work to clarify the distribution and risk factors for this disease in central and northern Minnesota. Plant samples were collected in 2018 from multiple fields and counties based on symptomology, and roots were analyzed via fungal isolations and a species-specific qPCR assay. Based on this work, the pathogen was confirmed in four additional counties on the northern and western edge of its known range in Minnesota. This study also seeks to understand traits that contribute to survival strategies of this invasive pathogen in different environments. Results from this study will help to clarify areas at risk for SDS for forward-thinking disease management planning.

Movement of '*Candidatus* Liberibacter asiaticus' and progression of disease in split-root 'Swingle' trees
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'*Candidatus* Liberibacter asiaticus' (CLas), an alpha-proteobacteria associated with huanglongbing (HLB) and transmitted by *Diaphorina citri* is the most devastating pathogen of citrus. After initial infection, CLas quickly colonizes the root system before visible canopy symptoms develop. Understanding how CLas moves within the plant and local and systemic effects of CLas on different tissues is fundamental to improve HLB management. Using split root rhizotrons and one-side graft inoculation below the trunk split in late summer was used to study the role of root infection on disease development. Root dieback increased on both halves of the root system in as little as 6 weeks, even though CLas infection remained isolated in the inoculated half of the root system through late summer and fall root flush. More root growth occurred in the non-infected half of the root system of HLB-affected trees during late summer and fall root flush compared to healthy trees and live root length after each seasonal flush. Increased root dieback removed this gain by the next root flush. The first detection of CLas in canopies occurred in the spring flush, with 6 canopies CLas-infected at the end of the experiment (11 months). The results showed that movement of CLas from infected root systems is linked to season, where it can move up and around the trunk after the spring flush; however, systemic effects on the root system are not dependent on CLas movement.

Primary inoculum, etiology, and oospore production of the spinach downy mildew pathogen, *Peronospora effusa*
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Downy mildew of spinach, caused by the obligate pathogen *Peronospora effusa*, continues to be the most important constraint in the major spinach production areas in the US. Spinach downy mildew can potentially be initiated by asexual sporangia, sexually derived oospores, or dormant mycelium. However, the relative importance of the various types of primary inoculum is not well known. The ability of *P. effusa* sporangia to withstand abiotic stress, such as desiccation, and remain viable during short- and long-distance dispersal, and the ability of oospores to germinate and infect seedlings have not been established. Thus, the primary objectives of this research were to evaluate the impact of desiccation on sporangia survival and infection efficiency and evaluate the occurrence, production, and germination of oospores. The initial results indicate that desiccation significantly reduces sporangia viability and infection potential. Mating tests using isolate pairs on detached leaves, as well as intact plants, demonstrated that oospores can be produced in certain controlled crosses. Oospores were also observed in numerous commercial cultivars in both CA and AZ, although the frequency of occurrence varied between the two locations. Understanding the variables that effect sporangia viability and oospore production will help in improving the management of the spinach downy mildew pathogen.

Bayesian Evolution Analyses Suggests the Global Spread of *Xanthomonas cynarae* pv. *gardneri Occurred During the Global Adoption of Hybrid Tomato Seed*
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Xanthomonas cynarae pv. *gardneri* (formerly, *X. gardneri*, *Xg*) is one of the four species causing bacterial spot of tomato and pepper. For more than four decades, only the reference strain isolated from Yugoslavia in 1957 was known. While two strains were isolated from Costa Rica in the early 1990s, recently, especially in the late 1990s to the 2000s, strains were recovered from several countries around the world where they cause significant yield loss. In this study, we sequenced genomes of strains from Brazil, Canada, Costa Rica, Ethiopia, Reunion, Uruguay, USA and South Africa to study global diversification and spread. Pangenome analysis was carried out using Roary while recombination was inferred using ClonalFrameML. Aligned core genes were used for inferring maximum likelihood phylogeny. For calling Single nucleotide polymorphisms, the Snippy pipeline was used. After correcting the dataset for molecular clock signal using TempEst, we used the program BEAST to infer time-calibrated Bayesian analyses under various evolutionary models. Our results show a near clonal population for *Xg,* with few strains having unique recombination hotspots. Time-calibrated Bayesian analyses suggests the reference strain and the current global population diverged more than two hundred years ago. The major diversification of the current global population took place in 1975–1985, which coincides with the period of expansion in the global use of hybrid tomato seeds. Our results are important in understanding the evolution and global spread of plant pathogens as trade in seeds become globalized.

Development of a controlled-environment assay for studying tar spot in corn

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Tar spot is an emerging threat to U.S. corn production. In the United States, *Phyllachora maydis* has been the only pathogen associated with the tar spot outbreaks. No reliable, high throughput phenotyping protocol is available to study aspects of the ecology, epidemiology, and management of tar spot under controlled conditions. This study focused on developing a controlled-enviroment assay to facilitate *P. maydis* research. Dried corn leaves with tar spot symptoms were surface sterilized and blended to release asci and ascospores from stromata. Spores were then suspended in water and Tween 20 and concentration adjusted to 30,000 spores ml^{-1}. Corn plants at the V5 growth stage were inoculated in a greenhouse by spraying the spore suspension directly onto the upper and lower surfaces of leaves until runoff. Plants were left inside a humidity chamber at 95% RH for five days at 14 h/10 h day/light. Plants were taken from humid chamber and put under greenhouse conditions at an average of 20°C. Initial chlorotic symptoms began to develop four days after inoculation, and 12 days later, severity reached 5% on individual leaves. Stromata developed between 25–30 days post inoculation. The pycnidial anamorph (*Linochora*) of *P. maydis*, was microscopically confirmed in black stromata and no ascospores were observed in microscopic examinations of these lesions. Experiments will continue to refine and develop a reliable method for inoculation in controlled environments.

***Spongospora subterranea* detected in commercial peat-based potting mix in the United States**

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Spongospora subterranea (Ss) is a soil-borne pathogen that infects multiple plant species and causes powdery scab disease on potato. Recently, growers in multiple states reported powdery scab symptoms on tubers grown in peat-based potting mix considered to be free of potato pathogens. Peat-based potting mix is commonly used to grow ornamental plants and in the production of seed potatoes in greenhouses. We obtained two unused commercial potting mix samples from growers who observed powdery scab on their tubers and tested them to determine if potting mix was the source of Ss. The number of Ss sporosori (resting spore aggregates) was determined with a validated qPCR assay. The number of sporosori per gram of potting mix (sp/g) ranged from 0 to 140 sp/g or 0 to 78 sp/g, making it difficult to determine whether the samples contained Ss. For confirmation, two bioassays were performed, using tomato plants as bait. For each assay, six tomato plants were grown in potting mix samples, in potting mix inoculated with 9 sp/g of Ss (positive control), and in autoclaved potting mix (negative control). Plasmodia were observed in the roots of plants grown in the potting mix samples and the positive control, but not in the negative control. The presence of Ss in tomato bait roots was confirmed using qPCR. These data provide evidence that Ss can be found in commercial potting mix. Our findings suggest that potting mix could contribute to the spread of Ss in the U.S. and could impact research and production of potatoes and other plants.

***Watermelon green mottle mosaic virus* seed transmission and incidence in California**

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Watermelon green mottle mosaic virus (WGMMV) is a recently described virus that was discovered in 2007 infecting commercial watermelon fields in Taiwan. It is a cucurbit-infecting tobamovirus with a genome size and organization similar to other viruses in that subgroup. In 2017 and 2018 WGMMV was discovered infecting several species of cucurbits in three counties in California. Each of the detections occurred on small farms (>20 acres) for in-state markets. To determine if there was a single introduction followed by intrastate movement, or multiple introductions, we completed high-throughput sequencing (HTS) on RNA derived from crude virion

purifications of each isolate and compared the sequences. We also performed greenhouse host range studies and found that WGMMV has a wide cucurbit host range, can systemically infect *Nicotiana benthamiana*, and form local lesions on *Chenopodium amaranticolor*. Additionally, an epidemiolocially important feature of many tobamoviruses is seed transmissibility. To examine the seed transmissibility of WGMMV we produced seeds from infected cucumber in the greenhouse, then tested individual seeds for the presence of the virus with and without surface disinfestation treatment. Untreated and treated seeds used as inoculum caused systemic infections in healthy host plants. This indicates infectious virus is present on and within the seed coat, as previously described for *Cucumber green mottle mosaic virus*. Infected seeds were also grown and tested for systemic infection. These results indicate that WGMMV is seed transmissible.

Infection of blueberry cultivar Emerald with a California grapevine strain of *Xylella fastidiosa* and acquisition by glassy-winged sharpshooter

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Bacterial leaf scorch disease caused by *Xylella fastidiosa* (*Xf*) occurs in southern highbush blueberry varieties in the southeastern United States. However, blueberry cultivar susceptibility to *Xf* is variable and these interactions are often specific to the pathogen strain. In the San Joaquin Valley of California where Pierce's disease in grapevines caused by *Xf* subsp. *fastidiosa* has been problematic over the last twenty years, blueberry acreage of southern highbush cultivars is expanding, but little is known concerning the potential for spread of *Xf* from grape to blueberry in this area. Glassy-winged sharpshooter (*Homalodiscus vitripennis*), a major vector of *Xf* in the San Joaquin Valley is known to feed on blueberry suggesting potential for emergence of bacterial leaf scorch in California if susceptible blueberry cultivars are planted near *Xf* infected grapevines. Experimental inoculations showed that a California Pierce's disease strain of *Xf* subsp. *fastidiosa* (Bakersfield-1) causes disease in blueberry cultivar Emerald, and glassy-winged sharpshooter was able to acquire *Xf* Bakersfield-1 from artificially inoculated blueberry plants under laboratory conditions. The possibility for spread of *Xf* between blueberries and nearby vineyards has implications for area-wide disease and vector control in the San Joaquin Valley and other regions where these two crops are grown.

Diversity of *Ralstonia solanacearum* infecting multiple hosts in Southeast Asia

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The soilborne plant pathogen *Ralstonia solanacearum* causes bacterial wilt of over 200 different plant species, including tomato, potato, eggplant, and peanut. This bacterium is particularly important because of its lethality, wide host range, and global distribution. Additionally, the pathogen forms a heterogenous species complex, meaning resistance breeding and management strategies must be tailored to local strains. Although bacterial wilt is present in Cambodia and Vietnam, little is known about the distribution and diversity of *R. solanacearum* in these countries. We therefore isolated Cambodian and Vietnamese *R. solanacearum* strains from various hosts, including tomato and potato, and performed Koch's postulates to confirm host range. Notably, we isolated *R. solanacearum* from tomato (*Solanum lycopersicum*), long bean (*Vigna unguiculata* subsp. *sesquipedalis*), and bitter gourd (*Momordica charantia*) in a single field plot from a mixed cropping system in Cambodia. Long bean and bitter gourd were not previously known hosts for *R. solanacearum* in these countries. Our results identify the diversity of *R. solanacearum* within a geographic region and the continually expanding host range of this pathogen. Not only do these results fill a knowledge gap on pathogen diversity, they also provide a foundation for development of varieties specifically resistant to bacterial wilt for Southeast Asian farmers.

Differential fruit colonization patterns of group I and II *Acidovorax citrulli* strains

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Acidovorax citrulli causes bacterial fruit blotch of cucurbits (BFB), and can be assigned to two genetically distinct groups: I and II. The natural association of group I and II *A. citrulli* strains with different cucurbit hosts suggests host preference. Additionally, in field experiments we observed significant differences in BFB incidence between representative group I and II *A. citrulli* strains on watermelon and melon fruits, but not on foliar tissues. Thus, we hypothesized that *A. citrulli* host specificity occurs in cucurbit fruit. The objective of this study was to further characterize differences in the abilities of group I and II *A. citrulli* strains to colonize melon fruit tissues. Group I and II *A. citrulli* strains were inoculated on detached melon fruits by syringe-inoculation and attached melon fruits by swab-inoculation. Both group I and II *A. citrulli* strains colonized melon fruit mesocarp tissues after syringe inoculation and the type 3 secretion system was important for bacterial colonization of internal fruit tissues. However, only group I strains penetrated into pericarps of attached melon fruits after swab-inoculation. These data support the hypothesis that *A. citrulli* groups display a preference for certain cucurbit fruits and indicate that the mechanisms of host preferential association involve the initial bacterial penetration into the fruit pericarp.

'Candidatus Liberibacter solanacearum' haplotypes differentially affect seed-potato sprouting

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'*Candidatus* Liberibacter solanacearum' (Lso), putatively causes potato zebra chip and there are currently two widely recognized haplotypes (A&B) of Lso infecting potatoes in the US. However, the relative impact of the haplotypes on tuber sprouting is unknown. To address the issue, two separate studies were conducted in which sixteen-to-twenty tubers, verified to be infected with individual haplotypes (A, B, or A+B), were planted in the greenhouse in 2018–2019 in a randomized complete-block design. Emergence was monitored daily over a six-week period. At the end, plant height, stem width, leaf area (LA), and plant biomass were determined. In both studies, plant emergence from tubers infected with Lso A (80%) was not significantly different from those of uninfected controls (97%). However, plant emergence from tubers infected with Lso B was significantly lower (60%) than that of the controls (Chi Square, $\propto$=0.05). Emergence of plants from tubers infected with A&B was not consistently different from those of uninfected tubers although it was numerically greater than those from tubers infected with Lso B. Plants from tubers infected with Lso B took a longer time to emerge than those from tubers infected with Lso A or uninfected controls, which had similar length of time for emergence. All plant measurements (biomass, height, LA, and stem width) were significantly higher for plants that emerged from the controls than those from infected tubers regardless of the haplotype ($\propto$=0.05).

Norton grapevine resists grapevine vein clearing virus
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Norton is a North American hybrid grape with a major genetic background of *Vitis aestivalis*, which is native to the Midwest region of the United States. Norton has been known to be resistant to the fungal pathogens causing powdery mildew and downy mildew. Grapevine vein clearing virus (GVCV) is endemic to the same region and associated with a disease that causes vine decline and death of cultivated grapevines. In a project assessing grape cultivars' resistance to GVCV by graft-transmission, we did not detect GVCV in grafted Norton either as a rootstock or a scion by PCR assays or Next Generation Sequencing. In a survey of a foundation vineyard over five years, we did not find GVCV infection in all 74 Norton vines, although susceptible grape cultivars in adjacent rows were infected. Additionally, Norton was not infected with GVCV by aphid-transmission in greenhouse experiments. These results demonstrate that Norton is resistant to GVCV, revealing a new trait. Genetic mechanisms of Norton's resistance to a virus and assessment of its resistance to other grapevine viruses merit further investigation.

Spread and host-specificity of tomato ringspot virus on sweet cherry (*Prunus avium*) in Oregon
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Oregon ranks second among U.S. states for sweet cherry (*Prunus avium*) production. Recent statewide surveys identified the presence of the dagger nematode (*Xiphinema* spp.) transmitted tomato ringspot virus (ToRSV) in Hood River, The Dalles, and the Grande Ronde Valley. Little is known about this virus on sweet cherry. A ToRSV-infected orchard in The Dalles was assessed for foliar enations, rosetting, dieback and rapid decline symptoms. Visual assessments of symptoms on 1500 trees were made by ranking severity on a 0-4 scale (0 = no symptoms, 4 = most severe). Leaves were collected from a sub-region of 400 trees for diagnostic testing using a ToRSV-specific double-antibody sandwich ELISA (Agdia, Inc., Elkhart, IN). A section of 50 trees was selected for nematode sampling within the sub-region. A composite of four soil cores from around each tree base were collected in October 2018. Nematodes were extracted by sugar flotation and identified with microscopy. The visual assessment of symptoms matched 92.9% (n = 352) of the ELISA results. A total of 28 mismatches occurred, 26 (6.9%) were positive when no symptoms were observed and 2 (0.5%) tested negative that had symptoms. Dagger nematodes were present in soil near trees with and without ELISA-positive ToRSV diagnoses. Host-specificity of the ToRSV sweet cherry isolate is being evaluated in a field study by interplanting apple, peach, cherry, blueberry, raspberry, and grape around ToRSV-infected sweet cherry trees.

Pathotypes of *Plasmodiophora brassicae*, the causal agent of clubroot, in Manitoba and Ontario, Canada, and in North Dakota
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Plasmodiophora brassicae causes the disease, clubroot, on plants in the family Brassicace, including canola and many Brassica vegetable crops. The pathogen exists as several pathotypes that are determined by the reaction on differential hosts. *Plasmodiophora brassicae* has been present in many regions of North America for several decades, mostly on Brassica vegetables. The disease was identified on canola in Alberta, Canada, in 2003, and has been recently reported on this crop in other Canadian provinces including Ontario and Manitoba. Clubroot has also recently been reported in North Dakota on Brassica vegetables. Clubroot samples were obtained from field surveys and from researchers in these three regions and the pathotype was assessed using the Williams (1966) differential set. New pathotypes have recently been identified that can overcome the clubroot resistance of previously resistant canola. The resistant canola hybrid 45H29 was inoculated to determine if any of the collections were new virulent pathotypes. The pathotypes found in Ontario in surveys in 2016 and 2017 were 2 and 6, similar to what was found 50 years ago, and also 5 and 8. Two samples of pathotype 2 were able to overcome the resistance in 45H29, so have been temporarily named 2x. Pathotype 9 was found in Manitoba. The pathotypes from North Dakota were 2 and 8. The differences in pathotypes in these regions suggests independent introduction events, and not transport from region to region. This was especially important for Manitoba and North Dakota, as some of the agricultural areas share a border.

Investigating environmental impacts on *Phytophthora infestans* gene expression and virulence
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Continued sub-culturing of *Phytophthora infestans* on artificial media results in virulence reduction. Typically, inoculation of the pathogen back on to host plant tissue restores virulence. This response suggests that the change is not due to genetic mutation but may be the result of a more sophisticated and fluid method of regulation. We investigated the virulence of *P. infestans* after continued sub-culturing on artificial media and analyzed changes in effector expression within the pathogen at key stages. The isolate was sub-cultured (6X) and maintained at 18°C on Rye-A medium over 4 months. Sporangial suspensions of the 0, 3rd, and 6th sub-cultured isolates were inoculated onto susceptible ('Katahdin' potato) and resistant ('Katahdin' potato transformed with RB). Disease severity was determined and lesion size was measured. The mixed model generated for lesion development showed that sub-culturing 3 times resulted in a non-significant 1% decrease in the likelihood of lesion development while sub-culturing 6 times resulted in a significant 22% decrease in the probability of lesion development (p-value 8.36e-11). RNA was extracted from inoculated plants and RT-qPCR analyses were conducted to assess changes in effector expression. At 72 hours post inoculation, the relative expression of the Avr3 effector was significantly reduced by an average of roughly 5-fold from the 0 sub-culture event to the 3rd, with no significant change in expression to the 6th. Further work is underway to continue elucidating this host-pathogen interaction.

Investigations on host specificity within the *Alternaria alternata* species complex

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Members of the *Alternaria alternata* species complex (AASC) can be found as both saprophytes and pathogens. Although a wide host range has been reported for this species complex, including hosts in different plant families such as Amaranthaceae, Solanacea, Fabaceae and Rosaceae, there still is some uncertainty about whether the same species and strains affect all these potential hosts. Therefore, this study focuses on examining the host specificity of AASC strains by inoculating AASC isolated from different hosts on a set of test plants representing various plant families. Spore suspensions of AASC isolates originally collected from sugar beet and potato were inoculated to potential host tissue, such as tomato and apple fruits, as well as leaves of potato and sugar beets to test the variation in symptom production among isolates. AASC spore suspensions were inoculated into wounds of tomato and kept in a moist chamber until lesions appeared. A similar inoculation method was performed on sugar beet and potato leaves, on both wounded and non-wounded sites. The results showed a difference in symptom production by lesion diameter for the same isolate among hosts, as well as for varieties of a particular host. Additional testing is ongoing by inoculating more isolates from different crops to various hosts. Potential host specificity in the AASC could be important for crop rotation programs in agriculture.

Cross pathogenicity of *Xanthomonas arboricola* pv. *pruni* in stone fruits in Korea

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Bacterial shot hole disease caused by *Xanthomonas arboricola* pv. *pruni* (XAP) is a major plant disease in stone fruits as peach, plum, apricot, Japanese apricot and plumcot in South Korea. Aim of this study was tested cross pathogenicity of XAP in stone fruits. Bacterial isolates of XAP were obtained from diseased leaves of stone fruits. Cross pathogenicity of the isolates was studied by inoculating each isolate into three stone fruits as peach, apricot, plumcot. Three Inoculums were cultured 2 days on LB broth and adjusted to 1×10^7 cell/mL. Each Inoculum was sprayed on healthy leaves of peach, apricotand plumcot. After being kept in 100% humidity for 24 hours, the inoculated plants were incubated at 25 degrees. The development of symptom as water socked was observed visually 2~3 days after inoculation (DAI). Three isolates were infected three different stone fruit leaves. These findings suggest that XAP was possible to cross infection and occurrence disease for stone fruits. This results will be helpful to know biological characteristics of XAP in stone fruits and set up management strategies.

Cross infectivity of powdery mildew isolates originating from hop (*Humulus lupulus*) and industrial hemp (*Cannabis sativa*) in New York

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Production regions of hop (*Humulus lupulus*) and industrial hemp (*Cannabis sativa*) significantly overlap in North America. Demand for hops are driven by craft brewers seeking locally-sourced ingredients. The 2014 Farm Bill created hemp pilot programs for fiber, seed, and cannabidiol production. Both are hosts of powdery mildew pathogens, but prior reports do not indicate cross infectivity of the pathogen species most commonly reported on each host: *Podosphaera macularis* for hop, and *Golovinomyces spadiceus* for hemp. We transferred two isolates of powdery mildew pathogen originating on hop to hemp, and two isolates from hemp to hop. The hemp isolates were derived from two NY greenhouse hemp operations. Based on morphology and sequencing of 28S and ITS regions, both matched reports classifying the pathogen as *G. spadiceus*. The two hop isolates were obtained from feral hops in NY and were similarly confirmed as *P. macularis*. The highly mildew-susceptible hop variety 'Symphony' was sparsely colonized by *G. spadiceus,* which produced hemp-infective conidia on hop, albeit at a lower density than on hemp. *P. macularis* did not grow on the CBD hemp variety 'TJ's CBD', inducing a non-host incompatibility response. However, on the dual-purpose hemp variety 'Anka', *P. macularis* isolates representing both MAT1-1 and MAT1-2 mating types grew rapidly and sporulated profusely. The foregoing cross infectivity may significantly affect disease risks and regulatory concerns as production regions continue to expand.

Morphology and pathogenic variation of 15 *Puccinia melanocephala* single pustule isolates causing Sugarcane Brown Rust at the Cauca valley in Colombia
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Brown Rust (BR) is one the most important diseases of sugarcane worldwide, causing yield losses between 10 to 40% in susceptible varieties, requiring breeding towards resistance. Considering pathogen´s coevolution, planted area and environmental factors, BR overcame resistance in several varieties at the Cauca valley in Colombia, indicating progressive adaptation. To address this issue, this work standardized BR inoculations on 2–3 months old plants at semi-controlled conditions, and determined morphology and pathogenicity of 15 single pustule BR pure isolates from four varieties with economic and breeding importance to Colombia´s industry. The 15 isolates were cross- inoculated on 11 sugarcane varieties, contrasting in origin, genetic background and BR response. Morphometric characterization of urediniospores and paraphyses showed significant differences between isolates and found relationships with BR severity. Cross inoculations showed statistical differences between isolates and within isolates obtained from the same variety, with high variation in severity, incubation and latent periods, and reaction or disease phenotype. There was not relationship between isolate origin and severity on the variety source. Based on severity and reaction, varieties Pindar, Hinds Special and Q-117 showed differential responses. Results supported BR adaptation through the historical transition of commercial varieties MZC 74-275, CP 57-603, CC 84-75 and CC 85-92 in the Cauca valley, providing also elements to study pathogen diversity and incomplete resistance to BR in sugarcane varieties.

Fitness cost associated with increased aggressiveness of *Phytophthora nicotianae* on a tobacco genotype with *WZ* resistance
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Phytophthora nicotianae causes tobacco black shank worldwide. The ability of *P. nicotianae* to rapidly overcome single-gene resistance was documented following deployment of the *Php* gene in the 1990s. Increased aggressiveness in *P. nicotianae* has been observed following exposure to tobacco varieties with varying levels of partial resistance. The *WZ* genomic region from *Nicotiana rustica* has been demonstrated to confer a very high level of resistance to *P. nicotianae*. However, a greenhouse study showed *P. nicotianae* was able to adapt to WZ resistance within several host generations. Our objective in this study was to investigate the fitness cost of this increased aggressiveness of *P. nicotianae* on the tobacco genotype K326WZ that has the *WZ* resistance. We selected an isolate of *P. nicotianae* adapted on K326WZ for 6 generations and then maintained it on either K326WZ or Hicks (a susceptible cultivar) for an additional six host generations. Hicks-exposed isolates exhibited a decreased aggressiveness on K326WZ comparing to K326WZ-exposed isolates, indicating a fitness cost associated with aggressiveness on tobacco genotype K326WZ. This observation could aid researchers in optimizing resistance deployment strategies, thus increasing the durability of different sources of partial resistance to tobacco black shank.

Pathogenesis study of *Rhizoctonia solani,* sclerotia in Sugar beet
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Rhizoctonia solani Kühn is a genetically complex soil borne pathogen that causes pre and post-emergence damping off to a wide range of crops. However, its life cycle and pathogenesis is not thoroughly well characterized. The primary form of the pathogen in nature is mycelia. This pathogen can survive in soil for many years through forming sclerotia, which is a resting spore produced under unfavorable condition. *R. solani* can be cultured in different artificial media but the development of sclerotia in the artificial media in the laboratory is not always easy to study the morphology of the pathogen. Currently, *R. solani* is grown on barley and other grains and used as inoculum for research purposes. One of our aims is to develop a system for producing sclerotia in vitro for large scale greenhouse and field trials for studying the natural infection process in sugar beet. In order to develop sclerotia; we cultured a number of Rhizoctonia isolates on different media, such as amended CV8 media, 50% potato dextrose agar, water agar, Metalaxyl-Benomyl-Vancomycin, and cornmeal agar media. We observed variations in the colony morphology and growth pattern of different isolates. Profuse production of sclerotia observed in the amended CV8 media compared to other media. Further study will be followed by using this sclerotia to understand pathogenesis at different growth stages for different germplasm lines and varieties of sugar beet. Gene expression study between the infected tissue and mock will improve our understanding in the soil-borne pathogen and host interaction.

Pathogenicity tests of fungal isolates from Loblolly pine (*Pinus taeda*) trees growing in southern Mississippi
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Characterization of fungal isolates obtained from five (5) year old loblolly pine trees was conducted in this study to better understand the pathogenic fungi that cause pine forest decline in southern Mississippi. Fungal isolates with different morphological variations were tested for extra-cellular enzyme production. Comparative pathogenicity tests using juvenile *P. taeda* seedlings as host were conducted by root inoculation with two of the isolates (ASP1 and ASD18). The control seedlings were mock inoculated with sterile water. To study the fungi infection process, the first (1st) and second (2nd) 10mm root tips were cut from inoculated and un-inoculated (control) seedlings, 3, 6, 10 15 days post inoculation. Of the 19 fungal strains isolated from symptomatic *P. taeda*, 21% showed a positive Bavendamn reaction, indicating the ability to produce phenol oxidase, and depletion polysaccharide and lignin components in wood. ASP1 and ASD18 fungal strains showed strong enzyme reaction, 36%, and 38% respectively. Both tested fungal isolates caused browning tissues in the roots from 3 days post inoculation. Observations on seedlings, day 15-post-inoculation showed severe browning

of the root tissues in both root regions (1st and 2nd mm regions) challenged with ASP1 when compared to similar regions of ASD18 and the controls, indicating that the necrotic cells could not prevent the fungal colonization in loblolly pine seedlings. More studies are proposed to establish the pathogenic capability of ASP1 and ASD18 fungal strains on field-grown loblolly pine seedlings.

Infection by '*Candidatus* Liberibacter solanacearum' haplotypes and psyllid survival in different solanaceous hosts
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'*Candidatus* Liberibacter solanacearum' (Lso), transmitted by the potato psyllid (*Bactericera Cockerelli*), putatively causes potato zebra chip (ZC). The bacterial organism has a wide host range, primarily in the Solanaceae family, including peppers, potatoes, and tomatoes. Currently there are two commonly detected haplotypes of Lso (A & B) in the US, which can be detected individually or as an A & B mix in psyllids or plant samples. Experiments were conducted in 2017 and 2018 in the greenhouse to determine whether there are interactions between Lso haplotypes and different solanaceous hosts. Peppers, potatoes, and tomatoes were infested with six psyllids per plant carrying either Lso A, B, A & B mix, or Lso-negative psyllids. Infested plants were maintained in replicated cages of each haplotype. Seven weeks after infestation, plants were sampled and tested with qPCR for Lso detection and adult psyllids in each cage were counted. Individual psyllids carrying haplotypes B, A & B mix, and those free of Lso reproduced well on all three hosts, and plant samples, except those infested with Lso-negative psyllids, tested positive for the respective Lso haplotype. However, psyllids carrying Lso A did not survive on peppers but did survive and reproduce on potatoes and tomatoes. In addition, samples from peppers infested with psyllids carrying Lso A tested negative for Lso. However, peppers infested with psyllids carrying Lso A & B mix tested positive for Lso A, indicating that the presence of Lso B may be required for pepper infection by Lso A and psyllid survival on peppers.

AgriVectors: A systems biology portal for plant pathosystems and arthropod vectors of plant diseases
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Arthropod vectors of pathogens cause enormous economic losses and are a fundamental challenge for sustainable increases in food production. To more effectively fight plant diseases, data pertaining to a disease system needs to be consolidated, made searchable and amenable to data mining. The proposed AgriVectors platform is an open access and comprehensive resource for growers, researchers and industry working on plant pathogens and pathosystems spread by arthropod vectors. The portal connects established public repositories with pathosystem-specific data repositories. The AgriVectors system will provide tools to enable technologies such as RNAi, CRISPR, screening bioassays, etc. to leverage current and emerging knowledge across disciplines. The portal will be based on the Citrusgreening.org (https://citrusgreening.org/) community resource for the Huanglongbing pathosystem that was developed as a model for systems biology of tritrophic disease complexes. It includes a biochemical pathway database for each organism in this disease complex, and an expression atlas for the psyllid vector and citrus host. The AgriVectors portal will extend this model beyond gene-centric omics data to the broader Pathosystem-wide information, with integrated pest management, behavioral, plant health, soil health and climate data to incorporate rapid phenotyping information from research trials, building a foundation for more effectively identifying solutions to combat plant diseases.

Why vectors like mixed infected plants? The more, the better?
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Plants can be naturally infected with more than one plant virus. Here we report how mixed-infection affects vector's behavior and plant defenses in a pathosystem with two related orthotospoviruses, *Tomato spotted wilt orthotospovirus* (TSWV) and *Impatiens necrotic spot orthotospovirus* (INSV). TSWV and INSV can be vectored by the same thrips species and in plants have an antagonistic interaction. First and second instar thrips play an essential role during virus acquisition, therefore where eggs hatch could impact Orthotospoviruses' transmission. Our results show that female thrips prefer mixed-infected plants to lay eggs, even if they show symptoms similar to singly infected ones. Surprisingly, mixed-infection did not change the profile of plant volatiles that could be used as chemical cue to attract vector thrips. We chose then to characterize the small RNA profile of plants infected with single or double TSWV/ INSV infection, since they play a key role in determining the efficacy of RNA interference, the main antiviral defense in plants. Our data show an under-representation of viral small RNAs(vsRNAs) mapping to the intergenic region of the S and M segments in both viruses, and an increase in the total amount of 21nt and 22nt vsRNA in mixed-infected plants by different Dicers. Our study enhances the understanding of the response of plants and vectors to dual virus infection and can explain the outcome of antagonistic virus interactions.

Evaluation of "*Candidatus* Liberibacter solanacearum" induced effects on the feeding behavior of *Bactericera cockerelli* by two distinct haplotypes
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The potato psyllid, *Bactericera cockerelli* (Hemiptera: Triozidae), is a significant pest of potatoes throughout Central and North America, as well as New Zealand. Crucially, this psyllid is the vector of the fastidious bacterium "*Candidatus* Liberibacter solanacearum" (Lso), which is the causative agent of diseases in several solanaceous crops, including the economically important zebra chip disease of potatoes. Currently, five Lso haplotypes have been identified, two of which (LsoA and LsoB) are exclusively transmitted by potato psyllids in their shared geographic range within the Americas. However, relatively little is known about the interactions of either Lso haplotype with its plant and insect hosts, due to its fastidious nature. In these studies, we performed 8-hour recordings using

the electrical penetration graph (EPG) technique to elucidated the feeding behavior of Lso-infected (LsoA or LsoB) and non-infected psyllids. Critically, these data improve our understanding of the pathogenesis of Lso in its insect vector and provide new information concerning Lso-potato psyllid interactions that ultimately benefit pathogen transmission of only one Lso haplotype.

Host response of loblolly pine (*Pinus taeda*) to fungal associates of the southern pine beetle (*Dendroctonus frontalis*)
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The southern pine beetle (*Dendroctonus frontalis*) (SPB) is the most damaging beetle pest of pine trees in the southern United States, causing large losses in loblolly pine plantations and natural ecosystems. SPB is associated with three fungal species, two mutualistic and one antagonistic. *Ceratocystiopsis ranaculosus* and *Entomocorticium* sp. A, the mutualistic species, serve as a nutritional source for developing larvae, whereas the antagonistic one, *Ophiostoma minus,* competes for the substrate with SPB larvae, and is also antagonistic to the other two mutualistic fungi. While direct competition for space might be one of the reasons why *O. minus* hinders these two fungal species, other mechanisms might also be involved. We hypothesize that *O. minus* induces a defense response in the host plant that is more detrimental to the mutualistic fungi than to itself. In fact, the mutualistic species, unlike *O. minus*, are not pathogens and hence have not evolved to withstand tree defense responses. Objectives of this study are the following: (i) to determine which compounds are induced in loblolly pine after the inoculation of the three fungal species, and (ii) *in vitro* testing of the previously identified compounds to determine their toxicity against each of the fungal species. Results of this work will assist in elucidating the interactions within the complex SPB system, as well as clarifying what defensive chemical traits of loblolly pine are important in fungal arrest.

Grapevine vein clearing virus is prevalent in grape aphids
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Grapevine vein clearing virus (GVCV) is known to be associated with a disease that causes a variety of severe symptoms in grapevines in the Midwest region of the United States. Recent surveys showed that GVCV is prevalent in wild Vitaceae that are native and distributed abundantly in this region. In a recent study, we found that grape aphids (*Aphis illinoisensis*) are able to transmit GVCV from plant to plant, and that a variant of GVCV infecting a wild *Ampelopsis cordata* plant caused severe disease on the Chardonel grape cultivar. To investigate the incidence of GVCV in grape aphids feeding on wild vines, we collected aphids and colonizing leaf tissues randomly from native Vitaceae and used PCR assay to determine the presence of the virus in plant tissues, groups of 10 aphids, and single aphids. We found that 81% of the groups of aphids and 52% of single aphids contained GVCV while wild *Vitis* spp. and *A. cordata* vines were infected with GVCV at 10% and 34% incidence, respectively. Sequencing of the open read frame (ORF) II, the most diverse region in the GVCV genome, showed that same variants of the virus in single grape aphids were also found in wild Vitaceae and cultivated grapevines. These results provide timely knowledge into the epidemics of GVCV in native habitats and for implementing strategies of preventing GVCV spread in vineyards.

Influence of flow dynamics on transmission of *Xylella fastidiosa*
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Xylella fastidiosa is an important bacterial plant pathogen, causing expensive crop losses each year. The disease is transmitted from one plant to another by insect vectors, including the blue-green sharpshooter (BGSS). *X. fastidiosa* forms biofilms in the foregut of insects. These biofilms experience harsh flow environments due to the high flowrates associated with sharpshooter xylem feeding, which have yet to be quantified. During this study, a novel estimate for liquid flowrates in the foregut of the BGSS was simulated using published electrical penetration graphs along with a newly created 3D model of the BGSS foregut. The 3D model was used in COMSOL Multiphysics® simulations to compute fluid velocity profiles in the foregut. MATLAB® was used to probe the 3D solutions for velocities relevant to the forces that biofilms experience. These results, coupled with published bacterial detachment force data, enabled us to generate a testable model for insect acquisition and transmission of *X. fastidiosa*.

Population establishment potential and virus transmission efficiency of Montana wheat curl mites and the cereal rust mite
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Wheat streak mosaic (WSM) is the most important viral disease of cereals in the Great Plains region of the U.S. *Wheat streak mosaic virus* (WSMV; family *Potyviridae*) is transmitted by the wheat curl mite (WCM; *Aceria tosichella*). This eriophyid mite is also a known vector of *Triticum mosaic virus* (TriMV; family *Potyviridae*). In recent years, WCMs have been identified as a cryptic species complex composed of genetically distinct biotypes with variable host ranges and vector capacities. During the WSM epidemic in Montana in 2016, two WCM populations were collected from different locations in the state and maintained in the greenhouse in addition to a population of cereal rust mites (CRM; *Abacarus hystrix*). While CRMs infest wheat, they're not a known vector for any of the WCM-transmitted viruses. The three mite populations were assessed for their ability to establish on wheat plants and to transmit WSMV and TriMV. Non-viruliferous mite colonies were reared on wheat plants inoculated with WSMV, TriMV, or a mixture of viruses. Single viruliferous mites were then transferred to individual healthy wheat plants grown in mite-proof containers. Initial results suggest consistently high establishment success rates for CRMs and substantiate that CRM is a not a vector of WSMV or TriMV. In contrast,

mite establishment success was more variable for the different WCM/virus combinations, indicating potential differential interactions between the WCMs, WSMV and TriMV. This study contributes to the understanding of eriophyid mite diversity and vector capacity.

Lonsdalea quercina occurs on insects visiting drippy blight diseased oak trees

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Drippy blight is a recently recognized disease of red oak trees caused by the bacterial pathogen *Lonsdalea quercina* in conjunction with the kermes scale insect *Allokermes galliformis*. The name drippy blight is descriptive of the disease, as copious amounts of bacterial exudates occur at kermes scale feeing sites throughout the canopy. Their combined damage causes branch abscissions and limb dieback, which over time results in progressive canopy thinning and tree death. Although the majority of damage occurs at kermes scale feeding sites, these insects are relatively immobile and not likely involved in long-distance dispersal of the bacterium. However, the associated bacterial exudates are visited by a variety of arthropods that may become contaminated with the bacterium. In order to test for natural dissemination of *L. quercina* on the arthropod community, trapped specimens were tested for bacterial contamination using DNA extraction and PCR protocols with species specific primers. Furthermore, collections were made of arthropods actively visiting trees. These arthropods were assayed on nutrient agar, and the resulting cream colored bacterial colonies were identified using colony PCR. Overall, 158 arthropod samples were tested over two field seasons, with eight percent carrying the bacterium. Contaminated specimens exhibited differing life histories and were from three insect orders and eight families. Insects in the order Hymenoptera (including ants, bees, and wasps) were the most frequently contaminated, but a variety of insects likely play a role in the spread of disease.

Strain specificity of _Potato virus Y_ encoded helper components provides evidences for transmission bias of recombinant strains over ordinary strains

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Recombinant *Potato virus Y* (PVY) strains ($PVY^{N:O}$, PVY^{NTN}) have replaced the long dominant ordinary strain (PVY^{O}) in the US potato crop. Transmission bias by aphid vectors is contributing; aphids preferentially transmit the recombinant strains when acquired sequentially with the ordinary strain. To understand the mechanisms behind this, we separated purified virus and active helper component proteins (HC), mixed them in homologous and heterologous combinations, and then fed them to aphids. HCs showed strain-specific properties. Mixtures of PVY^{O} HC with either recombinant virus, $PVY^{N:O}$ or PVY^{NTN}, resulted in efficient transmission. $PVY^{N:O}$ HC also facilitated the transmission of PVY^{O} and PVY^{NTN}, albeit at reduced efficiency. PVY^{NTN} HC failed to facilitate transmission of either PVY^{O} or $PVY^{N:O}$. To better understand transmission bias towards specific HCs, mixtures of two purified viruses were combined with a single HC and fed to aphids. In these experiments both viruses were transmitted at higher efficiencies than in the single virus treatments when mixed with PVY^{O} HC or $PVY^{N:O}$ HC. Treatment combinations mixed with PVY^{NTN} HC resulted in zero transmission. In this case, non-specificity could be due to factors of virus or HC concentration affecting virus binding and release efficiency. Ongoing research is evaluating transmission using serial dilutions of purified virus mixed with similar amounts of HCs.

Interactions between _Fusarium virguliforme_ and soybean aphid on shared soybean hosts

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Fusarium virguliforme (*FV*), the casual-agent of soybean sudden death syndrome (SDS), and *Aphis glycines*, the soybean aphid, are widespread and can occur simultaneously in North America. It was unknown whether they interact on shared host plants. Field studies were conducted to determine whether *FV* influences soybean aphid population growth rates and if soybean aphids influence SDS development. Caged soybean plots were established in Rosemount, MN and Nashua, IA in 2017 and 2018 with four treatments: plots with no aphids or *FV*, plots infested only with aphids, plots infested only with *FV*, and plots infested with aphids and *FV*. Aphid counts and foliar SDS incidence and severity were recorded weekly. At crop maturity, 16 plants per plot were sampled and visually assessed for root rot severity. A logarithmic model of aphid population growth was fit to each plot and the model coefficients were analyzed using a linear mixed effect model to determine whether SDS influenced aphid population growth. Results from preliminary analysis suggest that SDS may have a negative effect on aphid population growth. Other potential interactions between soybean aphid and *FV* are being explored.

What happens in storage: Mold-moth interactions on conventional and transgenic maize hybrids

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Grain quality of maize after harvest is reduced primarily by mold and insect infestations. When stored under high temperature and moisture conditions, *Aspergillus flavus* contamination, associated with aflatoxin production, has been known to considerably increase in levels. Indianmeal moth (IMM) or *Plodia interpunctella*, a Lepidopteran stored-grain pest, directly feeds on kernels thereby potentially predisposes grain to infection and mycotoxin contamination. Insect-mold interactions have been studied thoroughly in the field but not in storage, particularly the effect of Lepidopteran events on IMM. In this study, Bt and non-Bt maize hybrids at 16–17% moisture content, were artificially inoculated with *A. flavus* and kept at 32°C and 80–85% relative humidity in continuous dark to simulate

storage conditions. IMM was allowed to feed and complete one-generation cycle for 28 days. Survival of IMM on transgenic grain expressing Bt proteins Cry1Ab, vip3Aa20, and Cry1Fa2 solely or in combination were none or lower compared to the non-Bt grain. Feeding damage incurred by IMM was contingent on the suitability of grain for IMM development. Deterred feeding of IMM resulted in the reduction of *A. flavus* and aflatoxin levels. The use of transgenic hybrids with genes for Lepidopteran resistance hinders IMM feeding and mitigates the risk of mold and mycotoxin contamination. This is particularly valuable especially in areas where keeping maize at low moisture levels during storage remains a challenge.

Investigating Virus Acquisition and Transmission Characteristics Resulting in Mixed Infections of Tomato Infecting Begomoviruses (Hemiptera: *B. tabaci*)
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Tomato yellow leaf curl virus (TYLCV) and *Tomato mottle virus* (ToMoV) (*Geminiviridae, Begomovirus*) are two tomato-infecting viruses. TYLCV is one of the most damaging pathogens of tomato, and it limits production of tomato worldwide. Though viruses are usually studied as a single infection, mixed viral infections have become a major problem in agriculture. Mixed infections of plant viruses can be important factors in influencing disease severity, genetic diversity of the co-infecting viruses, transmission rates, and virus evolution. The role of the vector in propagating mixed infections has been largely ignored. In this study, we investigated the role of vector transmission on the propagation of single and mixed infections of TYLCV and ToMoV by its whitefly vector, *Bemisia tabaci*, in tomato. A transmission experiment was designed to investigate how acquisition of these viruses together in mixed infections or sequentially in single infections by *B. tabaci* influences the probability of transmission. After whitefly transmission, plant heights and disease severity scores were recorded 7, 14, 21, and 28 Days Post Inoculation. At 28 DPI plant samples were collected from all plants and virus infection confirmed using PCR. Results from these experiments will be presented.

Soil microbiomes from two levels of management intensity differ in their capacity to protect a high-value hardwood from an above-ground pathogen
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The capacity of the microbiome to protect agricultural crops from disease depends on management practices, but whether this applies to forest trees is unknown. We investigated the extent to which microbiomes from forest and plantation soil modify the susceptibility of *Juglans nigra* to *Geosmithia morbida*, the phytopathogen responsible for Thousand Cankers Disease. This fungal pathogen causes necrotic lesions in the phloem of *J. nigra* when introduced by colonizing walnut twig beetles. Seedlings were grown in potting mix amended with bulk soil from a forest, walnut plantation, or sterilized soil. Stems were inoculated with *G. morbida*, and necrotic area and callus formation were measured and compared among treatments. Fungal endophytes were isolated from roots and community composition was compared among treatments. There was a 15% reduction in necrotic area and significant increase in callus formation in seedlings that received the forest soil amendment compared to the control group. Plantation soil had an intermediate effect on necrosis. *Rhizoctonia* and *Fusarium* spp. were isolated more frequently from roots of seedlings that received control soil, suggesting plantation and forest microbiomes suppress secondary pathogens. Our results support the hypothesis that the microbiome of forest soil has a greater capacity to enhance defense against *G. morbida* compared to plantation soil.

Microbiomes of *Xylella fastidiosa* infected grapevine in California
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Xylella fastidiosa (Xf) causing Pierce's Disease (PD) of grapevine is known to associate with endophytes (microbiome) during infection and pathogenicity development. The increase of Xf genetic resources in online databases, along with improved sequencing and genetic analysis technology, has provided a framework to study the PD microbiome. In this study, DNA was extracted from a PD symptomatic grapevine inoculated with Xf strain Stag's Leap in greenhouse (Greenhouse Grape - GG), and a PD symptomatic grapevine from a vineyard in Bakersfield, CA (Field Grape - FG). Illumina HiSeq was used on GG and generated 190 M 100-bp short sequence reads. Illumina MiSeq was used on FG and generated 39 M 250-bp short sequence reads. Percentages of Xf reads were 1.7 for GG, and 0.5 for FG. Genomic variations of Xf under different conditions (field, greenhouse, sampling time) were studied. The microbiomes of GG and FG were analyzed to determine the major taxonomic groups of bacteria and fungi through metagenomic approach with the help of Kaiju software. The most abundant bacterial genera present in GG was Xf. The most abundant bacterial genera present in FG was *Paenarthrobacter*, with Xf ranking-the third. Many endophytic bacteria such as *Enterococcus*, *Staphylococcus*, and *Paraburkholderia* were observed. These results provide new information on grape microbiome associated with Xf infection under greenhouse and field environment.

Carbon amendments alter composition and pathogen-inhibitory capacities of indigenous soil bacterial communities
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Organic amendments can enhance microbial activity and improve soil health. However, the impacts of specific carbon amendments on indigenous microbiomes and their functions in agricultural soils remain underexplored. We investigated the effects of carbon amendments on antibiotic inhibitory capacities of indigenous soil *Streptomyces*, and composition of bacterial communities. Mesocosms were established in sterile jars with soil from two field sites varying in nutrient status, and were amended at intervals over nine months

with glucose, fructose, malic acid, a nutrient mixture, or water control. In initially low-nutrient soils, carbon amendments increased frequencies of *Streptomyces* inhibitory against both bacterial (*Streptomyces scabies*) and fungal (*Fusarium oxyxporum*) pathogens. Sequencing of 16S rRNA genes showed that community composition differed significantly among carbon-amended and non-amended soils. Carbon type and dose had significant effects on bacterial community composition in low-nutrient soils, while communities were only responsive to differences in carbon substrates among initially high-nutrient soils. In total, these results suggest that nutrient additions to soil may play an important role in disease management by enhancing pathogen-inhibitory microbial communities in agricultural soils, but the effects vary with nutrient type, dose, and initial soil nutrient status.

The Impact of Soil Diversity on the Tomato Seedling Microbiome
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Bacterial endophytes colonize the interior of plants without causing disease and can produce beneficial effects to the host. These are transmitted vertically via seed or from soil by colonizing cracks in plant roots. Research is needed to understand what drives endophyte communities and management strategies which promote colonization of beneficial microbes. The benefits of chicken crop-livestock rotations on bacterial microbiomes is unknown. We aim to characterize the endophytic bacteria of tomato seedlings grown in chicken-grazed (CG) soils and determine the impact of soil diversity gradient in endophyte microbiome. Two tomato varieties were grown in the greenhouse in five treatments: 100%, 75%, 50% and 25% CG soil and 100% sterile soil substrate. Five-week-old tomato seedlings were measured for fresh weight and height and endophytes were isolated. Seedlings grown in 100% and 75% treatments had significant lower biomass. 88 bacterial endophytes from 14 genera, were recovered. 50% of the isolates obtained from higher diversity soils were *Bacillus* sp. and 20% of the isolates obtained from lower diversity soils were *Microbacterium* sp. Our data suggests that soil diversity gradient affects endophyte richness and plant biomass. Microbiome characterization of stem, root and leaves will be done using next generation sequencing. This project will lead to greater understanding of the impact of sustainable management strategies on the microbiome of this important commodity.

Bacterial Communities Associated with Soil Health in Wheat
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The microbial components of soil heath are poorly understood, despite the increasing popularity of this concept. Widely used soil health tests, such as Haney and Solvita, rely on a measure of short term microbial activity, but relationships between these tests and specific bacterial populations have not been explored. We sampled 118 locations at two depths (0-10 and 10-20 cm) in two fields; one long-term no-till and the other conventionally-tilled. Depth had a larger effect on soil communities than tillage. Samples from the shallow depths were enriched in Proteobacteria and Bacteroides, while samples from deeper depths were enriched in Actinobacteria and Acidobacteria. Significant correlations between the abundances of bacterial families and soil variables were found in the shallow soil layer. We found significant positive correlations between grain yield and Caulobacteraceae and negative correlations with Micromonosporaceae. Oxalobacteraceae, Cytophagaceae, Comamonadaceae, Verrucomicrobiaceae and Pseudomonadaceae were positively correlated with the Haney and Solvita tests. Further, random forest regression identified key taxa related to soil health measures, soil characteristics, and grain yield. In conclusion, there are strong associations between specific bacterial families and components of soil health measurements. Although it remains unclear whether relationships are causal, the bacteria tended to represent root-associated taxa and reside in the upper soil depths where root density is highest.

Dynamics of endophyte communities associated with carrot
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Despite the importance of endophytes to plant health, their dynamics in carrot, one of the most important vegetable crop worldwide, are not fully understood. Thus, we investigated carrot endophytes dynamics in greenhouse, field and laboratory trials using a set of carrot genotypes with broad genetic backgrounds and physiological characters. Endophytes communities of seed, seedling and roots of examined carrot were studied using both traditional culture based techniques and low and high throughput sequencing technologies. Results showed that carrot are colonized by divers assort of endophytic microbial taxa. Many of them could fix nitrogen, solubilize phosphorus, produce auxin and siderophores and reduce *Alternaria dauci* infection, confirming that endophytes play a role in carrot health and productivity. Some of detected endophytes were vertically transmitted to progeny via seeds and could be a part of carrot core microbiome that is needed for early seedling establishment. We also detected that some of carrot endophytes could be acquired through horizontal transmission from soil and with the greater soil health in an organic relative to a conventionally managed system a higher endophytes population with greater antagonistic activity against *A. dauci* were detected in organic trials. Finally, different carrot genotypes showed different endophytes communities within their tissue and some genotypes were more responsive to the beneficial endophytes inoculation, which indicate that it could be possible one day to start breeding for those beneficial plant microbial relationship.

Comparing approaches for capturing bacterial assemblages associated with symptomatic and asymptomatic mushroom caps
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Bacterial blotch, caused by *Pseudomonas tolaassi*and related species, may be influenced by the microbial community of the mushroom cap. The objectives of this work were to compare methods for bacterial assemblage extraction and to assess the bacterial microbiome in mushroom caps with and without blotch symptoms. Mushrooms with blotch symptoms (n=50) and asymptomatic mushrooms (n=50) were collected at an organic farm in Pennsylvania and processed using sonication (resulting in washate and post-washate samples) or a whole mushroom processing strategy, prior to DNA extractions. The V4 hypervariable region of the 16S rRNA gene was sequenced using Illumina MiSeq. The washate and whole mushroom methods showed differences in the microbiome depending on the mushroom health status. However, regardless of treatments, Proteobacteria and Bacteroidetes were the most predominant phyla. An overrepresentation in the relative abundance for the classes Gammaproteobacteria and Aphaproteobacteria was found for the washate and post-washate methods, respectively. Forty-nine exact sequence variants (ESV) were related to *Pseudomonas*spp., from which thirteen, three, and one ESV grouped, respectively, with blotch pathogens, *P. tolaasii*, *P. agarici*and *P. constantinii*. Bacterial populations differed depending on the isolation method and the mushroom health status.

Changes of microbial community in the soybean plots treated with biochar and poultry litter
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Soil microbial communities play a significant role in nutrient cycling and soil conditioning that promotes plant growth and health. Four different soil amendments, which include untreated control, biochar at 11.2 Mg ha-1, poultry manure at 0.8 11.2 Mg ha-1 dry weight, and a combination of biochar and poultry manure, were applied annually for three consecutive years (2016, 2017, and 2018) in soybean plots in Red River Research Station, Bossier City, Louisiana. DNA were directly extracted from the soil samples in each treatment and were analyzed using QIIME2. Results showed that the composition and structure of bacterial communities varies from phylum to genus. In the phylum level, the top five dominant phyla were *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia*, and *Gemmatimonadetes*. The ten most abundant culturable genera throughout all the treatments were *Blastocatella, Sphingomonas, Nitrospira, Desulfurellales, Nitrososphaera, Gemmatomonas, Bryobacter, Ramlibacter, Bacillus*, and *Paenibacillus*. Interestingly, nitrifying bacteria of the phylum *Nitrospirae* known to promote plant growth and antimicrobial peptide-producing bacteria of the phylum *Firmicutes* were among the dominant phyla in the biochar and the poultry treated soils, respectively. Noteworthy, the microbial distribution in soil tends to become more even by repeated treatments of biochar for three consecutive years. This research provides the information about the significant changes of microbial community from the treatment of biochar, which could have an impact on soil-borne diseases of soybean.

Association between soil and root microbiomes and within-farm-spatial-variation of soybean yields I: The bacterial perspective
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The occurrence of high and low yielding sites within a farm has been a common yield limiting issue in soybean growing states. The causes behind such yield variations are currently unknown. We hypothesize that the potential differences of soil/root associated bacterial communities contribute to within-farm-spatial-variation of soybean yields (WFSVSY). Therefore, the objective of this study was to explore the bacterial communities of samples collected from high and low yielding sites at V1 and R8 soybean growth stages (SGS). Bulk soil (BS), rhizosphere soil (RS), and soybean root (R) samples were collected from eight farms in Pennsylvania (five high and low sites per farm). DNA extracted from samples were subjected to PCR targeting bacterial 16S rRNA genes (515F/806R primers) and amplicons were sequenced using MiSeq platform. The β-diversity analysis did not distinctly cluster data points based on yield (high/low), farm, or SGS for BS and RS samples. For R samples, a clear clustering was observed based on SGS. In BS, α-diversity was similar among high and low yield sites, however higher α-diversity was observed in high yield sites for both RS and R. While RS showed higher α-diversity at V1 compared to R8, the opposite was observed for R. There were no noticeable differences in relative abundances at the phyla level across high and low sites at V1 and R8. In-depth analysis will be performed to see whether specific bacterial taxa drive WFSVSY in Pennsylvania.

Tare soil microbiome of seed Potato (*Solanum tuberosum*) varies by geographic location and seed growing operation
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Tare soil refers to soil that adheres to the potato tuber surface after harvest. This soil is part of the geocaulosphere, the zone of soil in proximity to and associated with the stolon and tuber system. Seed potatoes are transported along with their tare soil from seed farms to the fields of potato growers around the country. Tare soil has the potential to harbor both pathogenic and beneficial microbes, thus understanding the factors influencing tare soil microbiome composition may help develop management strategies that promote plant health. To determine if the tare soil microbiome varies among cultivars and/or origin of seed potato production (i.e. state, growing operation), we characterized bacterial and fungal communities of tare soil from 38 seed lots in 2018. Our preliminary analysis indicates that geographic location and individual seed growing operation combined explain a significant proportion of variation in both fungal (PERMANOVA $F_{6,16}$= 3.14, $p < 0.001$) and bacterial ($F_{6,16}$=1.91, p=0.002) community composition. Our ongoing analyses seek to determine the relative importance of geographic distance, soil type, and potato cultivar as factors influencing the composition of the tare soil microbiome.

Bacterial community response to bacterial spot disease and resistance in tomatoes
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Bacterial spot of tomato and pepper is a disease complex caused by *Xanthomonas* spp. Because bacterial spot resistance does not exist in tomato, accelerated genetics have been used to transfer broad spectrum bacterial resistance by incorporating the pathogen recognition receptor EFR from *Arabidopsis thaliana* to enhance pattern triggered immunity (PTI) as well as Bs2 from pepper for effector triggered immunity (ETI) specific to *Xanthomonas* spp. The aim of this project is to determine how disease as well as broad and narrow spectrum resistance influence phyllosphere bacterial and fungal community structure. In order to investigate this, 16s and ITS amplicon sequencing were performed on leaf wash extracts collected from three separate field trials inoculated with *X. perforans* in Florida. Across all plants in each of the three seasons, *Xanthomonas* was the most abundant taxon. In concordance with bacterial spot disease rating data, *Xanthomonas* represented a significantly smaller proportion of the community in plants expressing Bs2. The effects of EFR expression were less pronounced than Bs2 and varied by season. While not as dramatic as the effect of Bs2 expression, plants expressing EFR had slightly lower disease ratings, but no difference in the relative abundance of *Xanthomonas*. Fungal phyllosphere communities did not consistently differ by plant genotype. Further analysis seeks to delimit whether expression of ETI or *Xanthomonas* reshape bacterial community structure.

Dynamics of soil microbial communities associated with cover crops in soybean-corn rotation systems in Nebraska
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Cover crops can play an important role in improving the environmental sustainability of agricultural production systems. Nevertheless, adoption of the practice in the Corn Belt remains relatively low. Factors identified as major barriers to the use of cover crops by farmers in Nebraska are concerns about possible negative impacts to the commodity crop. To better understand of possible impacts and management of cover crops, we aimed to characterize how cover crops impact soil microbial communities in three locations across Nebraska. We used soybeans as a model grain crop and planted different maturity groups (MG) with a range of harvest dates from late summer to late fall. A 50:50 mixture of oats and rye were planted at harvest or remained fallow in the control treatment. Soil samples were collected during cover crop planting in fall 2017, and before cover crop termination in spring 2018. Bulk DNA was purified and 16S (V4V6) and ITS2 regions were sequenced using the Illumina Mi-Seq platform. Soil chemical properties, environmental, and agronomic variables were measured at each site. Sequence variants were obtained using dada2 plugin in QIIME2 (version 2018.8). Filtering, visualization, and comparison of microbiome data will be analyzed using phyloseq. Results will be used to determine how soybean MGs impact microbial diversity and whether this correlates with increased plant biomass and yield. Our aim is to identify microbial species that can be beneficial/antagonistic or are indicators healthy soils, high yield, or disease incidence in the commodity crops.

Interkingdom interactions in the soybean root microbiome with different soybean seed treatments
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Soybean seeds and seedlings are vulnerable to many soil-borne pests and pathogens and protection of emerging soybeans is essential. Seed treatments are widely used in soybeans but, little is known about the ecological significance of chemical seed treatments on microbial communities. The objective of this research was to investigate interactions between oomycetes (RPS10), fungi (ITS1-2), and bacteria (16s) within soybean roots and to understand if soybean seed treatments impacted these interactions through direct and indirect means. Roots were collected from two locations in Michigan from non-treated soybean, or soybeans treated with ethaboxam, mefenoxam, ethaboxam plus mefenoxam or fluopyram. DNA was extracted from soybean root tissue and amplicon libraries were constructed then sequenced using Illumina MiSeq. Co-occurrence networks were constructed and investigations into the interactions among soil microbes revealed that the majority of interkingdom interactions was negative (indicating possible competition) but that intrakingdom interactions were positive. Negative interkingdom interactions could reveal potential biocontrol candidates. Investigation into the effect of seed treatment chemistry onto microbial network structure in soybean roots is underway and will reveal how seed treatment chemistry may alter network structure for improved plant health.

Genome wide identification and characterization of small signaling peptides (SSPs) involved in plant defense against pathogens
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Small signaling peptides (SSPs) are small molecules generally less than 200 amino acids with a diverse role in many plant physiological processes such as development and stress responses. SSPs belong to two distinct groups; small post-translationally modified peptides or cysteine-rich, based on structural characteristics arising from protein processing and post-translational modifications. To date, there are no studies available in the literature on genome-wide transcriptomic analysis of SSPs involved in pathogen responses in Arabidopsis. To address this, we generated a time-course RNA-seq experiment using host and nonhost bacterial pathogens for infection in Arabidopsis using the flood inoculation method. From the initial analysis, we found 214 differentially expressed SSPs, after inoculation with the host pathogen *Pseudomonas syringae* pv. tomato DC3000, from a total of 1,998 SSPs identified in Arabidopsis using PlantSSPdb. From this list of 214 transcripts, 36 belong to known gene ontology categories associated with defense. We selected the top induced SSPs in response to pathogen from our list of 214 SSPs for experimental validation. We obtained Arabidopsis T-DNA

knockout mutants for some of these pathogen-induced SSPs and tested the disease phenotype of mutants in response to pathogen infection. RNA-seq analyses of Arabidopsis leaves infected with several other host and nonhost pathogens is underway and the results will be presented.

Virulence of *Magnaporthe oryzae* isolates and resistance to blast disease

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The fungus *Magnaporthe oryzae* presents high variability in virulence which can vary depending on the wheat genotypes. There is also a intense demand for sources of resistance to wheat blast. Since 2016, the *Aegilops ventricosa* 2NS chromosomal segment in wheat genotypes has been used as the sole source of blast resistance. The objectives of this study were to: a) characterize and compare the virulence of *M. oryzae* isolates in Brazil based on the reaction of a set of wheat genotypes; b) compare the wheat genotypes in relation to the reaction to *M. oryzae* infection on seedlings and detached heads; and c) relate the presence of the chromosomal segment 2NS of *A. ventricosa* in wheat genotypes and resistance to blast. Seedlings and detached heads of wheat genotypes were used in experiments conducted under controlled conditions in which individualized suspensions of conidia of 91 wheat *M. oryzae* isolates were used in inoculation procedures. Presence of the 2NS chromosomal segment in wheat genotypes was determined via PCR. It was verified that *M. oryzae* virulence groups are widely distributed in the country and that, due to the high resistance to blast demonstrated, Santa Fe wheat cultivar, carrying 2NS chromosomal segment, plays a prominent role as a source of resistance to blast. However, the presence of the 2NS chromosomal segment in the genome does not necessarily associated with resistance to blast. The evaluation of wheat *M. o*ryzae isolates for virulence generates a very distinct classification patterns depending on whether the object of infection analysis is leaf or head.

Dual Transcriptome Analysis Reveals Insights into Innate and Phosphite-Induced Resistance of Tanoak to *Phytophthora ramorum*

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Phosphites have been used in the control of sudden oak death, however, the precise mode of action of these compounds is not fully understood. We designed an inoculation experiment on four open-pollinated tanoak families, previously defined as partially resistant. Stems of treatment-individuals were sprayed with phosphite, and 7 days later distal leaves were inoculated with the sudden oak death pathogen *Phytophthora ramorum*. Leaves from treated and untreated control plants were harvested for RNA extraction before and seven days after inoculation, and transcriptomes of both host and pathogen were analyzed. We found that sets of genes associated with innate resistance and with phosphite-induced resistance largely overlapped within a more susceptible but phosphite-treatment responsive tanoak family, supporting the hypothesis that phosphite treatment increases the resistance of susceptible host plants to *Phytophthora* infection. In addition, we found that genes of the pathogen involved in detoxification, such as ATP-binding cassette (ABC) transporters and vitamin B_6 biosynthesis genes, were upregulated in phosphite-treated plants, but not in untreated plants. In summary, our dual RNA-Seq supports a dual mode of action of phosphite compounds, including a direct toxic effect on *P. ramorum* and an indirect enhancement of resistance in the tanoak host.

Genetic resistance to fire blight in three pear populations maps to chromosome 2

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Fire blight, caused by *Erwinia amylovora*, is a constant problem for pear (*Pyrus* spp.) growers in the U.S. Resistant cultivars are the best option for managing fire blight. Fire blight resistance has been observed in the cultivars Potomac and Old Home and the selection NJA2R59T69. Mapping populations ('El Dorado' × 'Potomac', 'Old Home' × 'Bartlett', and NJA2R59T69 × 'Bartlett') were developed to identify the genomic regions associated with fire blight resistance in these sources. Progeny were genotyped with the recently developed Axiom Pear 70K Genotyping Array. Phenotyping was conducted in 2017 and 2018 by inoculating multiple actively growing shoots of field grown trees with *E. amylovora* isolate E153n via the cut leaf method. Severity was calculated by determining the percentage of blight on the current season's shoot. Chromosomal linkage groups were created for each population and an integrated two-way pseudo-testcross approach was used to map QTLs. In each population, a single resistance QTL was identified at a similar region on chromosome 2. Fire blight resistance QTLs have been identified in this region for 'Harrow Sweet' and 'Moonglow' (parent of 'Potomac'). The resistance in NJA2R59T69 originated from the *P. ussuriensis* selection 'Illinois 76' and not a *P. communis* source like 'Potomac' and 'Old Home'. Additional work is needed to further characterize this resistance region.

Does tomato domestication process alters the plant interaction with above-ground pathogens and below-ground beneficial microbes?

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Crop domestication events followed by targeted breeding practices across plant taxa was a pivotal achievement for human civilization to introduce superior agronomic traits needed to meet human needs, and adapt varieties to local agronomic environments. However, domestication also caused a strong reduction in the genetic diversity of modern cultivars compared to their wild relatives. It remains undetermined how plant domestication may have influenced plant interaction with aboveground pathogens and belowground beneficial

microbes. We examined the levels of induced resistance and changes in hormonal signaling pathways by *Trichoderma harzianum*, a beneficial soil microbe, against two foliar pathogens (*Botrytis cinerea* and *Phytophthora infestans*) in 30 diverse tomato genotypes across a domestication gradient, including wild relatives, landraces and modern commercial cultivars (conventionally or organically bred). Our results have demonstrated that *Trichoderma* enhanced both aboveground canopy and belowground root biomass in all tested genotypes. Wild relatives and landraces tomato were more responsive to *Trichoderma* against foliar pathogens in comparison to early modern cultivars. In addition to these finding, transcriptional profiling of responsive and non-responsive genotypes will be discussed. These findings have important implications for understanding the mechanisms behind differences in the induction of resistance among genotypes, and may someday be used to identify molecular markers for efficient selection of responsive cultivars.

***OsSPL9* regulates rice antiviral response through the miR528-L-ascorbate oxidase (AO) pathway**
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MicroRNAs (miRNAs) are critical regulators of plant antiviral defense. However, little is known as to how miRNAs respond to virus invasion on transcriptional level. We established previously that defense to *Rice stripe virus* (RSV) invasion entailed a reduction of miR528 accumulation in rice due to the down-regulation on transcriptional level, thus alleviating miR528-mediated degradation of *L-Ascorbate Oxidase* (*AO*) mRNA, bolstering the antiviral activity of AO. Here we show that a large number of GTAC motifs existed on the promoter of miR528, which was considered as the binding site of SQUAMOSA Promoter Binding Protein–Like (SPL) transcription factor family protein. To investigate which SPL transcriptional factor regulates the transcription of miR528, we used Yeast one hybrid and other technique, and show that miR528 is regulated by the SPL9 specifically among 19 SPLs in rice. SPL9 displayed high affinity binding to specific motifs within the promoter region of miR528 and activated the transcription of miR528 promoter *in vivo* and *in vitro***.** To confirm our hypothesis, we obtained *SPL9* overexpression and knock out mutant (Crispr/Cas9) rice lines. Knock-out *spl9* significantly reduces the expression of miR528, but increasing the expression of the target of miR528, *AO* expression and rice resistance to RSV, while overexpression of *SPL9* increases the expression of miR528, but reduces the expression of *AO* and host antiviral defense. In conclusion, SPL9-mediated transcriptional activation of miR528 expression adds a novel layer of regulation of *miR528-AO* antiviral defense.

Age Related Resistance in Arabidopsis in Response to Tobacco Mosaic Virus Infection
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Age related resistance (ARR) is a phenomenon in which older plants are able to better cope with infections compared to younger plants and is often associated with a particular stage in the life cycle of the plants like flowering or leaf maturity. Arabidopsis- *Tobacco mosaic virus* (TMV) is an ideal system to study if adult plants deploy ARR towards viral infections. Previously, it was shown TMV interacts with the phloem specific auxin/indole acetic acid responsive protein 26 (IAA26) and disrupts its localization, thus altering the phloem transcriptome. In this study, wild type Arabidopsis shahdara, IAA26-knockout and -overexpression lines were used to investigate the effect of the TMV-IAA26 interaction on ARR. To test if Arabidopsis plants show higher levels of resistance towards TMV infection, 10 plants one week before flowering and one week after flowering were inoculated with TMV. Samples were collected one week after inoculation and quantitative polymerase chain reaction (qPCR) analysis was performed. Results showed that transcript levels of TMV were 12 fold higher in younger plants before flowering compared to older plants after flowering. However, none of the host resistance genes tested i.e. pathogenesis related protein 1 (PR1), Jasmonate ZIM domain 1 (JAZ1) and Pectinesterase 5 (PME 5) showed significant difference before and after flowering, which indicate that there are other defense response pathways involved in ARR in Arabidopsis challenged with TMV.

QTL-seq of young fruit resistance to *Phytophthora capsici* in cucumber
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Phytophthora fruit rot caused by *P. capsici* is a devastating disease for cucumber. As young fruit are especially susceptible, we sought to identify quantitative trait loci (QTL) associated with young fruit resistance using QTL-seq analysis. A cucumber accession, PI 109483, was identified as a source of young fruit resistance, and a resistant breeding line, MSU109483-53, was developed. Crosses were made between the susceptible breeding line, Gy14, and two resistant MSU109483-53-derived lines: an S_6 line, B5, and a doubled haploid line, DH A4-3. *P. capsici* isolate Barley's 1 was used in all experiments. In the summer of 2017, F_2 progeny of Gy14 x B5 (n=397), and in 2018 Gy14 x A4-3 (n=362), along with parental lines and F_1 were grown in the field. For accurate phenotyping, plants were trellised to reduce wounding due to soil removal during cleaning and reduce possibility of contamination from other pathogens. Replicate harvests providing 10-50 fruits per plant allowed replicated scoring for each individual. Normally distributed disease scores suggested that young fruit resistance is a quantitative trait. Individuals with extreme resistant and susceptible phenotypes were selected for QTL-seq analysis, performed using QTLseqR. QTL were identified on chromosomes 1, 5, and 6 from both experiments. To narrow the genomic regions and verify QTL, kompetitive allele specific PCR (KASP) markers will be used to genotype an F_2 population of Gy14 x A4-3 in summer 2019.

Generation of *Xanthomonas oryzae* PthXo1 mutants to gain insight into TALE-dependent *Xo1*-mediated resistance in rice
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Xanthomonas oryzae pv. *oryzae*, the causal agent of bacterial blight of rice, produces Transcription Activator-Like Effectors (TALEs) that activate target gene expression in the plant by binding to gene promoter regions. To circumvent pathogen infection, plants have evolved TALE-associated resistance, including loss of susceptibility, dominant TALE-dependent, and dominant transcription-independent resistance. The recently described *Xo1* gene from rice is included the latter category. Little is known about this type of resistance. To understand the mechanism of *Xo1*, which is independent of gene activation, we created mutations in the surface-exposed amino acids of PthXo1, a TALE which induces resistance in a rice variety carrying *Xo1*, Carolina Gold. The wild-type alleles triggered resistance when inoculated to Carolina Gold, while the variants lost resistance triggering ability. Our results suggest that the surface exposed amino acids of PthXo1 are critical for triggering *Xo1*-mediated resistance and give a deeper insight into the mechanism governing non-transcriptional resistance in rice.

Bradyrhizobium japonicum IRAT FA3 differentially modulates _Arabidopsis_ thaliana root phenotype in response to abiotic stresses

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Plant growth-promoting bacteria (PGPR) improve plant growth and biotic or/and abiotic stress tolerance. In nature plants are challenged by multiple stressors simultaneously and plants under abiotic stress are more susceptible to disease. Therefore, it is imperative to understand how PGPR modulate plant stress response to achieve overall plant health. However, the underlying mechanisms are not fully understood. Our objective was to investigate how the PGPR *Bradyrhizobium japonicum* IRAT FA3 modulates plant responses to individual and multiple stresses. Using the model plant *Arabidopsis thaliana* (Col-0) and an auxin receptor mutant, tir1afb2, the effects of heat stress, salt stress, or a combination of both in the presence of *B. japonicum* were investigated. After 8 d of cocultivation with *B. japonicum*, shoot and root fresh weight increased. *B. japonicum* drove developmental plasticity in roots by inhibiting primary root elongation and promoting lateral root and root hair formation. Compared to Col-0, tir1afb2 displayed fewer lateral roots, indicating that functional auxin perception is required for *B. japonicum*-stimulated root architectural changes in *Arabidopsis*. Stress induced a reduction in photosynthetic pigments and an increase in lipid peroxidation. *B. japonicum* rescued plants exposed to moderate stresses. Transcription of key antioxidant enzymes increased significantly in treated plants. These results suggest that B. *japonicum* improves plant performance under moderate salt and heat stress through increasing root phenotypic plasticity and priming of antioxidant responses.

Resequencing of bottle gourd germplasm and using QTL-Seq to map PRSV-W resistance in bottle gourd (_Lagenaria sinceraria_)

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Bottle Gourd, native to Africa, is used as an important rootstock to improve cold tolerance and disease resistance in cucurbits. Viral diseases are considered a major threat to cucurbit crop production. The most prevalent viruses infecting bottle gourd are aphid-transmitted papaya ringspot virus watermelon strain (PRSV-W) and zucchini yellow mosaic virus (ZYMV). The genetic source of resistance to PRSV-W and ZYMV has been identified, but incorporating the disease-resistant gene(s) into a commercial cultivar through traditional breeding would take years or decades. With the recent advancement in the sequencing technologies, we aim to accelerate the molecular breeding for trait improvement. We recently assembled the bottle gourd genome and used genotyping-by-sequencing (GBS) to map the PRSV-W resistance locus in an F2 population. A dominant monogenic locus *Prs* was mapped in a 317.8-Kb region on chromosome 1 still containing 39 annotated genes. In the current study, through QTL-Seq analysis on pooled libraries with 10 most PRSV resistant or susceptible individuals from six F3 populations, we aim to map the *Prs* locus and to identify the candidate resistance gene for PRSV-W. In addition, through screening the USDA collections of bottle gourd germplasm, significant variations in the level of resistance to PRSV-W were identified in 154 Plant Introductions (PI) evaluated. Through genome re-sequencing of these PI lines, we intend to use genome-wide association studies to confirm genes or SNPs associated with the PRSV-W resistance in bottle gourd.

Exogenously applied ROS/RNS scavengers reduce _Macrophomina phaseolina_-induced lesion development in soybean and sorghum

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Charcoal rot, caused by the soilborne necrotrophic fungus *Macrophomina phaseolina* (MP), is a prevalent and economically significant plant disease. MP infection in sorghum or soybean generates excessive amounts of reactive oxygen (ROS) and nitrogen species (RNS) as a basal defense mechanism that causes cellular damage/senescence. Necrotrophs, such as MP, take advantage of this built-in cell senescence mechanism as they depend on dead tissue for nutrition. ROS/RNS generation has been validated from both hosts by transcriptomics, physiologcial assays, and exogenous application of ROS/RNS scavengers. In vitro sensitivity tests showed that MP was sensitive to sodium pyruvate, DMTU and ascorbic acid at high concentrations (50-100 mM) but not at in-planta working concentrations (10-20 mM, 0.28 M, or 100 µM). For in-planta assays, the apical portion of soybean seedlings (V2 stage) were clipped and pretreated with scavengers followed by MP inoculation. With the exception of sodium pyruvate, the scavengers showed reduced lesion development in soybean. Based on ROS scavenging by ascorbic acid in soybean, sorghum adult plants stalks were pre-treated with ascorbic acid followed by MP inoculation. Ascorbic acid reduced lesion length in sorghum adult plant. Detection and quantification of ROS/RNS and transcriptome analysis from both hosts during scavenger quenching will be performed. These experiments will be useful in understanding host resistance mechanisms against MP.

Functional characterization of a member of Arabidopsis Acyl Carrier Protein in plant defense
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Plant fatty acids and lipids have essential functions in storing energy and acting as structural components for cell membranes. They are critical for plant growth and defense. Acyl Carrier Protein (ACP) is a small acidic protein, which can carry the acyl group to elongate fatty acid chains. The *Arabidopsis thaliana* ACP family has 8 ACP members. By applying integrated genetic, molecular, and biochemical approaches, we have discovered that one member of ACPs functions as a broad negative regulator in plant immunity, since the mutant plants can enhance plant resistance against the bacterial pathogen *Pseudomonas syringae* and fungal pathogen *Botrytis cinerea*. The comparative analysis of fatty acid levels and related gene expression in diverse defense-related pathways indicated that the *acp* mutant may enhance plant disease resistance through up-regulating the Salicylic Acid (SA) pathway and repressing the Jasmonic Acid (JA) pathway. The increased callose deposition of mutant may also contribute to the enhanced disease resistance. Hormone contents and reactive oxygen species levels of the mutant plants will be further investigated. It is found that this ACP interacts with an uncharacterized TIR-NBS (TN) protein that functions in plant defense based on our result. However, the functional mechanism of their interaction in plant disease resistance are not known. Our further investigation of the related mechanisms and networks will strengthen our understanding of the function of the ACP and TN proteins in plant immunity and their applications for enhancing plant health and yield.

Genetic dissection of rice resistance to sheath blight and bacterial panicle blight using recombinant inbred lines of Jupiter and Trenasse
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Varietal development is one of the key components of managing sheath blight (SB) and bacterial panicle blight (BPB) in rice. Identifying host resistance loci through mapping is an important tool for breeding resistance. In this study, 255 recombinant inbred lines (RILs) derived from the susceptible variety Trenasse and the moderately resistant variety Jupiter were evaluated based on disease reactions for SB and BPB. Disease index score and other agronomic traits were recorded for individual RILs. Genetic linkage analysis was carried out using 170 simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers. Composite interval mapping identified six additive quantitative trait loci (QTLs) for SB, conferring 3.1-23.5% phenotypic variations (PVE), and twelve additive QTLs for BPB, conferring 2.6-17.7% PVE. Trenasse allele had an increasing effect on most of the QTLs found for SB and BPB. The SB QTLs were located on chromosomes 2, 3, 4 and 6; while BPB QTLs were on 1, 3, 4, 5, 6, 9 and 11. One common major QTL was detected on chromosome 3 for both SB and BPB. The same QTL was also associated with heading, corresponding to 17-20% PVE. Genes found in this QTL were predicted to be involved in the signaling pathway, heading and disease resistance. Because most of the QTLs identified have small effects, marker-assisted recurrent selection should be used to accumulate favorable alleles for improving the resistance using the resistant RILs. The RILs will also provide more accurate information about the resistance mechanisms of rice for the diseases.

Visualization of TAL effector activity in barley host-cells during *Xanthomonas tranlsucens* leaf infection
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Many Gram negative bacteria require a Type III secretion system (T3SS) to cause disease. Translocation of Type III-secreted effectors into host cells interferes with host immunity and modifies host physiology. For most pathosystems, researchers have focused on effector function, but we lack a spatial understanding of host cells targeted by Type III effectors. The pathogenic bacterium *Xanthomonas translucens* depends on a T3SS to cause bacterial leaf streak on barley. Type III secreted transcriptional activator-like (TAL) effectors found in *X. translucens* bind promoters of host genes and induce plant gene expression. We developed a tool to visualize cells specifically targeted by *X. translucens* TAL effectors. We engineered transgenic barley that only express green fluorescent protein (GFP) in the presence of TAL effectors translocated by *X. translucens*. We created artificial, designer TAL effectors (dTALEs) that specifically bind the the promoter of the *GFP* gene. We confirmed through quantitative PCR that specifically *GFP* was only induced by *X. translucens* in a TAL effector-dependent manner. With fluorescence laser confocal microscopy we found that the mesophyll and stomatal cells were targeted by *X. translucens* during leaf infection. We expect this tool to bring new insights in TAL effector-targeting of host cells during barley infection by *X. translucens*.

Identification of QTL associated with resistance to root-lesion nematode (*Pratylenchus neglectus*) in wheat using genotyping-by-sequencing
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The root-lesion nematode, *Pratylenchus neglectus* is one of the major soil-borne pathogens that reduces wheat productivity throughout the world. As very few management practices are available, utilization of host resistance is considered to be the most effective and preferred method to mitigate effects of root-lesion nematode in wheat. Phenotyping of resistance to *P. neglectus* in wheat is labor-intensive and time-consuming, which is the major constraint in breeding programs to develop resistant cultivars. Molecular marker assisted selection may enhance the process of developing resistant cultivars. An $F_{2:5}$ recombinant inbred line population was developed from a cross between a soft white spring wheat "Louise" (susceptible parent) and an Iranian landrace "Persia 20" (resistant parent). The recombinant inbred lines were evaluated for *P. neglectus* resistance reactions under controlled greenhouse conditions, and then were genotyped using genotyping-by-sequencing (GBS). Using Tassel5 GBSv2 Pipeline, a total of 2,427 high-quality SNPs were identified and used to construct a linkage map. We detected one major QTL (LOD = 5.3) showing association with *P. neglectus* resistance, located on chromosome 4A and accounted for 35.4% of the total phenotypic variation. The SNP markers closely linked with this QTL will be converted to PCR-based markers that may provide an efficient tool to breeders for developing *P. neglectus*-resistant cultivars using marker-assisted selection.

Photosynthetic cost associated with the inducible defences to *Plasmopara viticola* in grapevine

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Downy mildew, caused by *Plasmopara viticola*, is the most destructive disease of *Vitis vinifera* worldwide. Grapevine breeding programs had introgressed *P. viticola* resistant traits into *V. vinifera* genotypes and yielded downy mildew resistant hybrids. The objective of this work was to estimate the cost, at leaf level, of inducible responses to the infection of *P. viticola* on resistant cultivars (Solaris, Cabernet Blanc, and Phoenix) as well as to determine and to compare the limitations to photosynthesis imposed to *P. viticola* in resistant and susceptible cultivars. The net photosynthetic rates (An) were reduced (–15%) in inoculated 'Solaris' plants, even before the appearance of hypersensitive response-like cell death. A similar pattern was noticed in 'Cabernet Blanc' and 'Phoenix' plants. The susceptible cv. Riesling did not show any variation in leaf gas exchange before the appearance of visual symptoms. However, drastic reductions in An and stomatal conductance were found in 'Riesling' plants after 12 days of inoculation. Decreases in the maximum Rubisco carboxylation rate were noticed in 'Riesling' plants after pathogen inoculation, which was not found in 'Solaris'. Damage to the photochemical reactions of photosynthesis was likely associated with the oxidative burst found in resistant cultivars within the first 24 hours after the inoculation. Taken together, our data revealed that the defense response against *P. viticola* causes a photosynthetic cost to grapevines, which is not reversible even 12 days after the pathogen infection. (Funding - FAPESP 2017-02432-6)

Engineering recognition of SCN effector proteases using soybean PBS1-based decoys

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Soybean cyst nematode (SCN) is the most economically significant pathogen of soybean in North America. During the infection of host roots, it secretes a diverse array of proteins in its saliva to facilitate a productive infection. Included among these proteins are several different proteases. The goal of my project is to engineer decoy substrates for these proteases that will activate defense responses upon cleavage. Our laboratory has recently shown that the soybean PBS1-1 protein can be engineered to act as such a decoy substrate. Specifically, we have shown that insertion of a cleavage site for the NIa protease from Soybean mosaic virus into *Gm*PBS1-1 enables it to be cleaved by this viral protease, which then activates a programmed cell death response. Our goal is to extend this decoy technology to recognize proteases secreted by SCN during infection. To test whether our decoy approach can be expanded upon, we are developing a soybean root transformation system to determine whether *Gm*PBS1-1 cleavage activates a cell death response in roots. To compliment this approach, we are using protoplast transfection to test candidate decoy proteins for activation of cell death in response to cleavage by SCN proteases. Since other agriculturally significant bacterial, viral, fungal, oomycete, and nematode pathogens are known to secrete proteases, evidence that this technology can function in soybean roots will provide new alternatives to developing resistance to a variety of crop plant pathogens.

Pea aphid resistance in the *Pisum* Core Collection as a potential means to reduce spread of virus in pea production areas

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Pea aphid (*Acyrthosiphon pisum*) is the primary aphid vector of viruses in pea production areas of the U.S. Pacific Northwest. Aphid reproduction rates on different pea genotypes impact population levels, which can affect the movement of aphids and spread of the viruses they vector. Pea (*Pisum sativum*) accessions (302) from the USDA-NPGS *Pisum* Core Collection were screened for their ability to inhibit reproduction rates when a single aphid was placed on a leaf at the third node under greenhouse conditions and the number of nymphs and adults present on each plant were determined after 10 days in replicated, repeated trials. No accession completely inhibited aphid reproduction. Mean reproduction rates of aphids on accessions ranged from 2.2 to 60.3. Of the accessions screened, four limited aphid reproduction to less than five aphids and represent resistant germplasm that can be used in breeding programs. A genome-wide association study was used to determine quantitative trait loci (QTL) associated with the resistance and a highly significant QTL associated with the number of adult aphids present on the plants was located on Chromosome 4, explaining 7.7% of the total phenotypic variation observed. This information combined with screening of pea accessions for resistance to the major pea viruses impacting pea production will be utilized in an integrated pest management approach to limit the impact of virus and aphids on pea production.

Hormone signaling pathways elicited by *Lysobacter enzymogenes* C3 induced systemic resistance in soybean
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The biological control bacterium *Lysobacter enzymogenes* strain C3 can suppress fungal diseases by producing a suite of lytic enzymes and antimicrobial secondary metabolites. Previous studies have also shown that C3, when applied to grass and cereal plants, is capable of inducing systemic resistance against fungal pathogens. It is unknown, however, whether the bacterium has the ability to induce resistance in dicots and what signaling pathways are involved. In this study, the ability of C3 to trigger an induced resistance response in a dicot species was examined by applying the bacterium to leaves of soybean (*Glycine max* 'Williams82') and then measuring relative expression of salicylic acid (SA), jasmonic acid (JA), and ethylene pathway marker genes using qPCR. Allene oxide synthase (AOS) and aminocyclopropane-1-carboxylic acid synthase (ACS), upstream marker genes for jasmonate and ethylene pathways, respectively, were upregulated by C3 treatment, indicating activation of these pathways. A downstream marker gene for the SA pathway, PR-1, also was upregulated by C3, suggesting crosstalk between the pathways. The stage in the signaling pathways involved in the crosstalk is still unknown. Understanding the signaling events induced by C3 will provide a better understanding of the molecular mechanisms involved in disease suppression by the bacterium.
Key Words: Biocontrol, Induced Resistance, *Lysobacter enzymogenes*, defense signaling

Using network analysis to uncover interactions between host defense signaling pathways and virulence effectors of *Pseudomonas syringae* pv. *tomato*
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Interactions between plants and phytopathogenic bacteria depend on the interactions of host defense pathways and virulence effectors. Among the arsenal of effectors of *Pseudomonas syringae* pv. *tomato* (*Pto*), the phytotoxin coronatine (COR) and the functionally-redundant type-III effectors AvrE1 and HopM1 make key contributions to virulence on host plants. While these effectors perform partially-overlapping functions by interacting with distinct host targets, our understanding of how their activities are coordinated through downstream pathways is unclear. *Arabidopsis thaliana* employs a hormone-dependent signaling network to coordinate host defense, including against *Pto*. COR, AvrE1 and HopM1 directly and indirectly target the salicylic acid, jasmonate, ethylene, and indole glucosinolate-dependent sectors of this network. We have constructed polymutant sets of both *Pto* and *Arabidopsis* for all combinations of these effectors and pathways and will measure the interaction of these strains and plants, at both the level of bacterial growth and plant immune responses. This approach will enable network analysis to reveal contributions of interacting effectors and pathways during plant-bacterial interactions.

Transcriptional analysis of spinach cultivars during resistant and susceptible interactions with the downy mildew pathogen, *Peronospora effusa*
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Spinach is an important crop, cultivated worldwide for its dark green leaves that are often consumed in salad mixes. Nearly 350,000 tons of spinach are produced in the U.S. every year and nearly 40% of which are organically produced. Downy mildew of spinach, caused by the oomycete pathogen *Peronospora effusa*, remains a serious threat to production since synthetic fungicides cannot be applied for disease control in organic production. The use of resistant cultivars is an effective strategy to manage downy mildew, but much of the underlying genetic basis of susceptibility and resistance is not established in the spinach-downy mildew interaction. In this study, transcriptional profiles of *P. effusa*-inoculated resistant and susceptible spinach cultivars Solomon and Viroflay, respectively, were monitored at an early stage of pathogen establishment (24 hours post inoculation, hpi) to a late stage of symptom expression and sporulation (168 hpi). Approximately 10,000 (24 hpi mock) to 12,000 (24 and 168 hpi) genes were differentially expressed. Major up-regulated genes linked to the host defense included those encoding a zinc finger CCCH protein, a 1-aminocyclopropane-1-carboxylate oxidase homolog, an LRR receptor-like serine/threonine-protein kinase, WRKY transcription factor, nucleoside diphosphate kinase, pectin methyltransferase QUA2, and pollen receptor-like kinase. This study provides preliminary information for elucidating molecular mechanisms of spinach- *P. effusa* interactions and candidate genes for resistance against infection.

Investigation of a salicylic acid elicitor formulation in the bacterial leaf blight disease of rice using Synchrotron-based FTIR microspectroscopy
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We investigated the resistance of rice to bacterial leaf blight (*Xanthomonas oryzae* pv. *Oryzae*) induced by a formulation including salicylic acid and inert ingredients (Ricemate®). A Synchrotron Radiation-based Fourier-Transform Infra-Red (SR-FTIR) microspectroscopy technique was used to detect biochemical changes at the sub-cellular level. Rice plants were sprayed with SA-Ricemate® or with water as a control, the plants were inoculated, and disease severity was recorded. Rice leaf supernatant was used to determine salicylic acid (SA) content. Control and treated rice leaf samples were subsequently cut and subjected to SR-FTIR and spectra were collected. SA-Ricemate® reduced disease severity by approximately 41%, 54% and 60% at 7, 14 and 21 days after inoculation, respectively. The accumulation of SA showed an increase of approximately 50% at 24 hr after inoculation. In addition, the variation of biomolecular structure and intensity from average spectra showed higher intensity of CH_2, CH_3 from lipid groups (3000-

2800 cm^{-1}), C=O from pectin (1740 cm^{-1}) as well as C=O, N-H from protein amide I and amide II on treated rice leaf. These results suggested that an elicitor formulation could increase disease resistant by inducing defense compounds such as SA and possibly phenolic, lipid and protein compounds. SR-FTIR may be a useful tool to study the plant defense mechanisms at sub-cellular level.

Role of micronutrients on systemic acquired resistance in watermelon against *Fusarium oxysporum* f. sp. *niveum* and *Meloidogyne incognita*
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Fusarium oxysporum f. sp. *niveum* (FON) and southern root-knot nematode (RKN, *Meloidogyne incognita*) are devastating soil pathogens of cultivated watermelon in the southeastern United States. The role of controlled micronutrient feeding [iron (Fe), manganese (Mn) and zinc (Zn)] via hydroponics on genes related to systemic acquired resistance [SAR, salicylic acid (SA) pathway genes (PR1, PR5, NPR1) and jasmonic acid (JA) pathway genes (VSP, PDF and LOX)] in watermelon seedling was investigated. Micronutrients were fed to the plants at higher (3X) and lower levels (0.5X) of recommended dose (X= recommended dose) for 7-days and the expression levels of the above-mentioned SAR genes were evaluated. A sub-set of micronutrient-fed plants were later inoculated with either FON or RKN or both and were re-evaluated at 3 day post inoculation for above mentioned genes. Two independent trials were conducted with three replications per treatment. Our results showed that JA-pathway genes (VSP and LOX) were upregulated in plants that were fed with micronutrients for 7 days. However, expressions of other SAR-related genes were downregulated and significant differences among treatments was only observed in PR1 expression. Upon pathogen inoculation, expression levels of JA and SA genes varied considerably for different micronutrient treatments. These observations indicate that micronutrient feeding in watermelon for 7-days can induce SAR genes and expression of JA or SA pathway genes can potentially be affected when either FON or RKN or both are present, irrespective of nutrient feeding.

A high throughput method for determining protease target sequences
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Feeding the human population while reducing environmental costs requires crops resistant to a variety of pathogens. The PBS1/RPS5 decoy system can be used to this end. The Innes lab has shown that *Arabidopsis* detects the presence of the effector AvrPphB via its cleavage of PBS1 and resulting activation of the disease resistance protein RPS5, triggering a hypersensitive response. The AvrPphB cleavage site can be altered to be cleaved by other pathogen proteases that can then activate RPS5, as shown with the turnip mosaic virus protease NIa. For this to work, a protease's target amino acid (AA) sequence must be determined. On this poster, I will describe a high throughput system for determining a protease's target AA sequence. Specifically, we integrated into the *E. coli* chromosome an antibiotic resistance cassette repressible by a synthetic repressor. A randomized AA linker joins the repressor's two halves. If a protease cleaves this sequence, then antibiotic resistance will be derepressed and selectable. This system enables rapid screening of random 7mer AA sequences for cleavage by proteases of interest. Components of this system are currently being assembled, and results are forthcoming. Because PBS1 cleavage activates resistance in many crop species, this assay will be instrumental in creating an array of PBS1-based decoy systems, allowing plants to resist a wider variety of pathogens. This would reduce pesticide use, lessening agriculture's environmental impact.

***In planta* expression profiling of *Erwinia tracheiphila* effector genes to identify pathogenicity and host-specific virulence candidates**
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Erwinia tracheiphila (*Et*) causes bacterial wilt of cucurbits in the *Cucumis* and *Cucurbita* genera. *Et* strains fall into groups that induce rapid wilt on muskmelon versus squash; here we call these groups *Et subsp. melon* and *Et subsp. squash,* respectively. We characterized the *in planta* expression of *Et* effectors following recovery of *Et subsp. melon* from a primary host and a non-host, and of *Et subsp. squash* from primary and secondary hosts. Using qRT-PCR, we profiled the expression of 14 shared and two strain-specific effectors. An *Et subsp. melon* strain expressed several shared effector genes in both muskmelon and a non-host, squash. We found, however, that *hrpA,* which encodes the pilin protein of the type III secretion system (T3SS), was highly expressed in muskmelon but not in squash; this lack of expression of a T3SS may explain its lack of pathogenicity on squash. In fact, the *Et subsp. melon* effector gene expression in squash was higher than in melon, potentially due to feedback from a nonfunctional T3SS. In contrast, an *Et subsp. squash* strain expressed *hrpA* in both squash and melon, consistent with its induction of rapid wilt on squash and slow wilt on muskmelon. It also exhibited a very high level of expression of the effector gene *hopO1,* which is present in *Et subsp. squash* but not *Et subsp. melon* strains. Interestingly, it exhibited a 21-fold higher level of *hopO1* expression in squash than in melon, consistent with HopO1 contributing to faster wilt in squash than melon.

The role of polyacetylenic lipids during the interaction between *Daucus carota* and the necrotrophic fungus *Sclerotinia sclerotiorum*
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Carrot (*Daucus carota*) is an important US vegetable crop that has an annual value of $820 million (USDA NASS 2016). *Sclerotinia sclerotiorum* is a devastating necrotrophic pathogen that affects >400 plant species in the field and in storage. *S. sclerotiorum* alone accounts for up to 50% of carrot post-harvest losses. Although multigenic (QTL)-mediated partial resistance has been discovered in

specific cultivars of some crop species, no complete resistance to *S. sclerotiorum* has yet been identified. The capacity of plants to produce antibiotic compounds is one important pathogen defense mechanism. Carrots and other Apiaceous species produce polyacetylenic lipids, often in the form of falcarins such as falcarinol and falcarindiol, C_{17} aliphatic compounds with multiple triple bonds thought to be derived from linoleic acid ($C_{18:2}$). These falcarins are known to have potent antibiotic activities against human pathogens as well as anticancer activities. Although they have been described in primary literature for decades, their role in plant disease resistance and the genetic basis of their biosynthesis remain poorly understood. Results on the biosynthesis and functional role of falcarins in defense against *S. sclerotiorum* and other necrotrophic pathogens will be presented.

Elucidating the molecular mechanism of the Ptr-mediated rice blast resistance
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Rice blast caused by *Magnaporthe oryzae* is one of the most devastating plant diseases in the world, which frequently leads to an enormous yield loss. Cultivation of rice cultivars carrying resistance (R) genes is an effective, economical and ecologically sound approach to prevent the rice blast disease. Recently, we have isolated and functionally validated the broad-spectrum blast resistance gene *Ptr*, which encodes an atypical R protein without nucleotide binding site-leucine rich repeat (NLR) domains. In this study, a combination of molecular, biochemical and genetic approaches is being taken to elucidate the underlying mechanism of the Ptr-mediated rice blast resistance. A number of the Ptr-interacting proteins have been identified by the yeast two-hybrid screening and predicted protein interactions. These putative interacting proteins are being validated using *in vitro* protein pull-down and *in vivo* co-immunoprecipitation and bimolecular fluorescence complementation. Further molecular and genetic characterization of these interacting proteins is expected to help elucidate the molecular and biochemical basis of the Ptr-mediated blast resistance and facilitate the precision breeding of new rice cultivars with durable and broad-spectrum blast resistance.

Towards Understanding the Molecular Mechanisms Underlying *Fhb1*-mediated Resistance Against *Fusarium graminearum*
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Fusarium head Blight (FHB), caused by *Fusarium graminearum*, is one of the most devastating diseases of wheat. *Fhb1* is the most stable and effective quantitative trait locus (QTL) that confers resistance against FHB. A pore forming toxin-like (*PFT*) gene has been identified as the underlying gene for *Fhb1* mediated resistance. PFT is predicted to be a lectin protein with two agglutinin domains and one bacterial pore forming domain. The objective of this study is to understand the molecular mechanism by which PFT confers resistance to *F. graminearum*. The Arabidopsis - *F. graminearum* interaction has been demonstrated to be an effective pathosystem for studying basal resistance against *F. graminearum*. PFT has been ectopically expressed in wild-type and various Arabidopsis mutant backgrounds to determine the defense pathway(s) PFT may function in. Meanwhile, PFT was fused with GFP and RFP at the N or C terminus and transiently expressed in *Nicotiana benthamiana* for determining its sub-cellular localization. Our preliminary results showed that G(R)FP-PFT signal was found in the tonoplast, multi-vesicular bodies and the apoplast, suggesting that PFT is membrane associated protein and secreted out of the cell via exocytosis. Stable transgenic Arabidopsis plants expressing G(R)FP-PFT will soon be used to test their response against *F. graminearum* and confirm PFT's subcellular localization.

Redox regulation plays an important role in nitric oxide-mediated regulation of systemic acquired resistance
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Systemic acquired resistance (SAR) is a highly desirable form of resistance that protects against a broad-spectrum of pathogens. SAR involves the generation of a mobile signal at the site of primary infection, which arms distal portions of a plant against subsequent secondary infections. The last decade has witnessed considerable progress and a number of diverse chemical signals contributing to SAR have been isolated and characterized. Among these, salicylic acid (SA) functions in parallel to nitric oxide (NO)- and reactive oxygen species (ROS)-derived signaling leading to SAR. The plant galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) are also required for SAR and of these DGDG contributes to plant NO as well as SA biosynthesis. Interestingly, both NO deficient or overexpressing plants show compromised SAR, suggesting that NO levels play a critical role in SAR. Epistatic analysis shows that mutations leading to NO deficiency has an compensatory effect on compromised SAR phenotype observed in NO overexpressing plants. SAR in NO overproducer *nox1* mutant can also be partially compensated by overexpression of thioredoxin reductase (TRX), which facilitates formation of reduced disulfide bonds. Consequently, a mutation in TRX also compromises SAR. Detailed characterization of NO overproducing plants shows that they are able to make the mobile signal but remain non-responsive to chemical signals that operate in the NO-ROS branch of the SAR pathway. Together, these results suggest an important role of free radicals in systemic signaling.

Comparative transcriptome analysis between a resistant and a susceptible wild tomato accession in response to *Phytophthora parasitica*
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Phytophthora parasitica is one of the most widespread *Phytophthora* species, which is known to cause multiple diseases in tomato. Our current understanding of tomato-*Phytophthora parasitica* interaction is very limited. In this study, we have analyzed and compared the transcriptome of a resistant and a susceptible wild tomato accession in response to *P. parasitica* infection using the RNA-seq technology. We have identified 2657 and 3079 differentially expressed genes (DEGs) in treatment vs control comparison of Sp-R and

Sp-S samples respectively. Functional annotation of DEGs revealed substantial transcriptional reprogramming of diverse physiological and cellular processes particularly the biotic stress responses in both Sp-R and Sp-S upon *P. parasitica* treatment. However, subtle expression differences among some core plant defense related genes were identified and their possible role in resistance development against *P. parasitica* is discussed. Our results revealed 1173 genes that were differentially expressed only in Sp-R accession upon *P. parasitica* inoculation. These exclusively found DEGs in Sp-R accession included some core plant defense genes for example, several protease inhibitors, chitinases, defensin, PR-1 etc. were all highly induced. Whereas, several *R* genes, WRKY transcriptions factors and a powdery mildew susceptibility gene (*Mlo*) were highly repressed during the resistance outcome. Analysis reported here lays out a strong foundation for future studies aimed at improving genetic resistance of tomato cultivars against to *Phytopphthora* species.

Molecular Phylogeny of *Fusarium Oxysporum* forma specialis elaeidis from Oil Palm Plantations in Cameroon
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Oil palm, *Elaeis guineensis* Jacq., is an important economic crop in Cameroon. Fusarium is responsible for the most serious oil palm diseases in Cameroon, including a vascular wilt presumed to be caused by *Fusarium oxysporum* f. sp. elaeidis. Contemporary species identification relies strongly on molecular identification, yet resources for this identification are rare in developing countries. Traditional taxonomy of Fusarium is based on morphological criteria. However, the limited number of morphological characters available has resulted in cryptic Fusarium taxa. The aim of the study was to molecularly identify Fusarium species obtained from diseased palms and compare the isolates in order to devise appropriate control mechanisms and better pathogen detection methods. Samples of diseased palms were collected from the canopies and soil in five oil palm estates of the Cameroon Development Cooperation. Isolates were cultured, single spored and a portion of the translation elongation factor 1a gene was amplified from genomic DNA. The result revealed the presence of *Fusarium oxysporum* f. sp. elaeidis (FOE) from approximately 80% of the isolations. Cameroonian isolate within FOE clade 1 exhibited the greatest variability. Some grouped with FOE from Suriname and Brazil while others with previously characterized African isolates from Zaire and other isolates form a unique Clade 2 comprising solely of isolates originating from Cameroon. This study reveals that FOE in Africa is more diverse than previously reported and contains lineages not previously observed outside of Cameroon.

Assessing the occurrence of bacterial spot of tomatoes in Illinois and identifying species causing the disease
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Bacterial spot, caused by *Xanthomonas* spp., is one of the most important diseases of tomato in Illinois. Field surveys were conducted in 2017 and 2018 to assess occurrence of bacterial spot in commercial tomato fields in Illinois. Diseased samples were collected from three to five cultivars of tomatoes and from two to four different fields in each of southern, central, and northern regions of Illinois. Tomato cultivars included in this study were Biltmore, Brandywine, Carolina Gold, Chefs Choice, Dixie Red, Heirloom, Phoenix, Primo Red, Pony Express, Red Duce, Red Morning, and Rocky Top. Disease severity on leaves in 2017 ranges from 3% ('Rocky Top', central region) to 38% ('Heirloom', southern region). In 2018, disease severity on leaves ranged from 9% ('Red Duce', central region) to 91% ('Red Duce', northern region). Overall, severity of bacterial spot in 2018 was higher than that in 2017. During 2017–2018, 159 Xanthomonas isolates from 146 symptomatic leaf and fruit samples were collected and 102, 56, and 1 isolates were identified as *X. gardneri*, *X. perforans*, and *X. euvesicatoria*, respectively. The findings of this study are expected to help to develop effective management of bacterial spot of tomatoes.

Molecular identification and comparison of *Sclerotium cepivorum* isolates causing *Allium* white rot in Nevada
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White rot caused by *Sclerotium cepivorum* has been the biggest threat to *Allium* crop production in Nevada. Over the years, infections of white rot have been found in Washoe, Douglas and Lyon counties. Diagnosis of *Allium* white rot is generally based on symptoms and microscopic examination of fungal mycelium and sclerotia, and the molecular identity of the pathogen and its potential variants among those isolates have not been investigated. In this study, we used rDNA and ITS regions to compare 7 isolates collected from 3 counties during 2014–2017. An individual sclerotium was placed onto potato dextrose agar and incubated at 22°C in the dark. Genomic DNA was extracted from single sclerotium isolates (SSIs), regions of rDNA were amplified using ITS1/4 primers, and PCR products were subcloned into pGEM®-T vector and sequenced. A consensus DNA sequence of a 540-bp PCR fragment was obtained for each isolate by aligning multiple cloned sequences. Alignment analysis revealed that 5 out of 7 SSIs had the same amplicon sequence (Accession No. MK577439) that is 100% identical to the corresponding regions of *S. cepivorum* voucher NKI 286 (Accession No. FJ460433). Two SSIs had only one nucleotide substitution. Our data confirmed that these 7 isolates were *S. cepivorum* and there were no significant variations in the rDNA or ITS regions. Lack of molecular variation among these isolates suggests that white rot infections in Nevada may have originated from a common source.

Shifts in the blueberry stem blight pathogen complex and the appearance of fungicide resistant isolates
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Losses due to stem blight are increasing in many blueberry growing regions. Symptoms of the disease include drying of the leaves on one or more canes of an otherwise healthy bush. The disease can devastate young plantings and the mature bushes will die if the disease

reaches the crown. Bushes are infected at wound sites and fungicides are largely ineffective in controlling the disease. The disease was thought to be caused by the fungus *Botryosphaeria dothidiea*, but recent data show that *Neofusicoccum* spp. and *Lasiodiplodia* spp. are also casual agents in some regions. Understanding the pathogen complex is the first step in developing effective control recommendations. Canes with typical stem blight symptoms were collected throughout the blueberry growing region in New Jersey. Fungi were isolated from the growing edge of the lesions and further characterized. Our data show that stem blight in New Jersey is caused primarily by *Phomopsis vaccinii*, a pathogen typically associated with twig blight in New Jersey, and *Neofusicoccum* spp. Virulence assays show that isolates from these genera are more virulent than isolates of *B. dothidea*. Other isolation and testing suggest that *Pestalotiopsis* spp. and *Neopestalotiopsis* spp. can also cause stem blight. Selected isolates were tested for fungicide resistance. Fungal growth was not inhibited by some of the fungicides tested; this may partly explain why incidence of stem blight is increasing.

Pythium Pathogens of Common Bean in North Dakota and Minnesota

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North Dakota and Minnesota rank number one and four in US common bean (*Phaseolus vulgaris* L.) production, respectively. Root rot is a destructive disease to common bean production worldwide. To effectively manage root rot, it is vital to know what pathogens are most damaging. *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani* Kühn are known to cause devastating root rot in common beans. A root rot survey conducted a decade ago established *Fusarium* and *Rhizoctonia* pathogen populations in the region. However, *Pythium* spp. were not included in the survey. Metalaxyl (mefenoxam) is commonly used as seed treatments to manage *Pythium* spp. in many crops. Metalaxyl resistance has been observed in some *Pythium* populations in the US in numerous crops. To date, metalaxyl resistant *Pythium* spp. have not been observed associated with common beans. The objectives of this research are to establish which *Pythium* species are important pathogens in common beans in this region by determining the pathogenicity and aggressiveness of isolates collected and to evaluate metalaxyl sensitivity in the *Pythium* population. To date, 16 *Pythium* spp. have been morphologically identified from 13 of 33 fields sampled in 2018. Preliminary results indicate that varying levels of aggressiveness exist among the *Pythium* spp. isolated. Metalaxyl sensitivity levels for the *Pythium* spp. identified also will be reported.

Molecular characterization of recombinant *Potato virus Y* (PVY) in potato from South Korea, PVY^{NTN} strain

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Information about viruses affecting potato in South Korea is limited. To fill in this knowledge gap, we conducted a small survey of potato viruses in seed potato grown in South Korea. In 2017, potato plants of five cultivars exhibiting foliar mosaic, crinkling and mottle were sampled in two seed potato production areas, in Gangwon-do and Jeollabuk-do Provinces, and subjected to virus testing and characterization. *Potato virus Y* (PVY) was found associated with mosaic symptoms, and samples were molecularly characterized. All analyzed PVY-positive samples were found to represent the same recombinant PVY strain: PVY^{NTN}. Three South Korean PVY isolates were subjected to whole genome sequencing using overlapping RT-PCR fragments, and all three were confirmed to represent strain PVY^{NTNa} after a recombination analysis of the complete genomes. In phylogenetic analysis, the three South Korean isolates were placed most closely to several PVY^{NTNa} isolates reported from Japan and Vietnam, suggesting a common source of infection. This is the first report and complete molecular characterization of a PVY^{NTN} strain present in South Korea, and since this strain induces tuber necrotic ringspot disease in susceptible cultivars of potato, appropriate management tools need to be implemented to mitigate potential tuber quality losses.

Molecular and morphological identification of Fusarium species associated with black pepper (*Piper nigrum* L.) in the main areas of Costa Rica

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Black pepper (Piper nigrum L.) has recently increased in Costa Rica, mainly as a minor crop. It has been named as one of the best qualities in the world due to the high piperin concentration. The crop is affected by aggressive soil diseases. Yellowing, leaf wilt, root rot and death of black pepper plants are symptoms associated with different species of Fusarium. Twenty nine samples from diseased plants were collected from the main areas of black pepper production, twenty one from Sarapiqui and eight from San Carlos. Diseased tissues were placed on selected medium, resulting in twenty one isolates of Fusarium spp. The characterization was performed based on morphological and molecular analysis. For the morphological identification, mycelium segments of each isolate were placed on PDA to observe the pigmentation of the colonies, and CLA to classify the macroconidia, microconidia, conidiogenous cells and chlamydospores. For the molecular identification, genomic DNA was extracted from mycelium of the twenty one isolates using the CTAB method. DNA amplification was performed using the elongation factor α (EF-1α) region. All PCR products were sequenced and compared with the Fusarium species available in the GenBank database. Species of Fusarium solani, F. oxysporum, F. concentricum and F. proliferatum were identified and the two latter pathogen species were specific to a geographical region. The identification of these species facilitates the design of an integrated management program, in order to increase the national production of high quality black pepper.

Genetic Diversity of *Grapevine leafroll-associated virus 3* in Washington State vineyards
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Grapevine leafroll disease (GLD) continues to be a significant constraint to sustainability of the Washington State wine industry. Among the four grapevine leafroll-associated viruses (GLRaVs) documented in Washington vineyards, GLRaV-3 (genus *Ampelovirus*, family *Closteroviridae*) is the most insidious and widespread. A project was initiated to examine GLRaV-3 genetic diversity in vineyards to understand its epidemiology for implementing control strategies. A total of 1,260 samples were collected from red and white wine grape (*Vitis vinifera*) cultivars showing and/or suspected for GLD symptoms from thirteen vineyards. Samples were also collected from juice grape (*V. labrusca*) cultivars. Samples were tested for GLRaV-3 by RT-PCR using primers specific to a portion of the virus-encoded heat shock protein 70 homologue (Hsp70h) gene. The approximately 580 base pair amplicons generated from the Hsp70h gene were cloned and sequenced, and the derived virus sequences were aligned with corresponding sequences of GLRaV-3 isolates reported from different grapevine-growing regions worldwide. A global phylogenetic analysis of the Hsp70h sequences revealed the presence of five distinct variant groups differing from one another by 10–20% at the nucleotide level. GLRaV-3 isolates belonging to variant group I were found to be the most predominant. These results provide insights into the genetic makeup of GLRaV-3 for improved diagnosis of the virus and GLD management.

Survey of viruses found in grapevine cultivars grown in Missouri
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In 2017 a survey was initiated of vineyards throughout Missouri for the presence of 26 different grapevine viruses. A total of 400 samples were collected with a sample consisting of 16 petioles (4 petioles from 4 different vines). A total of 26 grape cultivars were sampled. Vineyard blocks were sampled randomly without regard to the presence or absence of symptoms. After nucleic acids were extracted from petiole samples, nucleic acid samples were analyzed using reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) to detect viral RNA/DNA. At least one virus was detected in 90% of the samples. The most common virus detected in the survey was Grapevine stem pitting-associated virus, which was found in 59% of the samples, followed by Grapevine leafroll-associated virus 3 (in 53%), Grapevine red blotch (in 35%), Grapevine virus E (in 31%), Grapevine leafroll-associated virus 2 (in 19%) and its closely related variant Grapevine leafroll-associated virus 2RG (in 9%), Grapevine virus B (in 17%), Grapevine fleck virus (in 13.5%), Grapevine vein clearing virus (in 8%), Grapevine virus A (in 0.5%), and Grapevine leafroll-associated virus 4 strain 5 (in 0.2%). Two or more viruses were detected in approximately 65% of the samples, with an upper limit of seven viruses detected in one sample. An analysis of the survey results indicated that each grapevine cultivar appears to be infected by specific virus combinations that are for the most part, unique to that cultivar.

Survey of soilborne diseases in Ohio tomato high tunnels
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High tunnel tomato production continues to increase every year in Ohio, however the protected environment within high tunnels creates conditions conducive for the development of soilborne disease complexes. A soilborne disease complex consisting of corky root rot (*Pyrenochaeta lycopersici*), black dot root rot (*Colletotrichum coccodes*), Verticillium wilt (*Verticillium dahliae*), and root knot nematode (RKN; *Meloidogyne incognita and M. hapla*) has become an emerging problem in tomato high tunnel production in Ohio. A survey was conducted to determine the presence and distribution of this soilborne disease complex, with the purpose of providing growers with effective management strategies. Farmers were provided with a soil sampling guide and samples were submitted to regional produce auctions or to the OARDC. A bioassay was conducted to assess the presence and severity of root rot, taproot rot and RKN galling. DNA was extracted from soil samples and species-specific primers were used to identify each pathogen. *P. lycopersici* was detected in 32 of 68 high tunnels (47%) and 17 of 34 farms (50%). *C. coccodes* was detected in 61 of 68 high tunnels (90%) and 33 of 34 farms (97%). *V. dahliae* was detected in 31 of 68 high tunnels (46%) and 25 of 34 farms (74%). RKN was detected in 32 of 68 high tunnels (45%) and 19 of 34 farms (56%). Growers were informed of the presence of soilborne pathogens and personalized management recommendations were given to each grower.

Identification and pathogenicity of *Lasiodiplodia* species from grapefruit in Florida
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Lasiodiplodia, a genus in the Botryosphaeriaceae, consists of important fungal pathogens that cause leaf spots, cankers, dieback, or fruit and root rot on a wide range of hosts from tropical and subtropical regions. Species of *Lasiodiplodia* were isolated from asymptomatic fruit during a field survey for citrus black spot in Marion, St. Lucie, and Collier Counties, FL. Morphological characterization and single- and multi-locus phylogenetic analyses of the ribosomal DNA internal transcribed spacer region (ITS), translation elongation factor 1-alpha gene (TEF-1), and β-tubulin gene sequences were used to identify two species, *L. iraniensis* and *L. theobromae*. Their pathogenicity was determined by inoculation of detached Valencia sweet orange or grapefruit twigs. Significant variation ($P < 0.0001$) in length of necrotic lesions and/or cortex discoloration was detected among isolates of *L. iraniensis* and an interaction in terms of

pathogenicity to Valencia and grapefruit among the isolates ($P < 0.0001$). In addition, *L. theobromae* showed much slower development in host tissue compared to *L. iraniensis*. Two-year old Valencia were inoculated with mycelial plugs on pruned branches in the greenhouse. Both *Lasiodiplodia* spp. caused plant death (up to 2 of 5 inoculated/isolate) within 12 weeks post inoculation. Further collection from fruit and wood is underway to confirm results and the greenhouse trial will be repeated. This is the first report of *L. iraniensis* in the USA.

Phenotypic and genotypic characterization of recent isolates of *Phytophthora infestans* on *Solanum lycopersicum* crops in Colombia

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Late blight disease caused by the plant pathogen *Phytophthora infestans*, is one of the major threats for tomato crops in Colombia. All tomatoes grown in Colombia are susceptible to this disease and control requires a high input of fungicides. Knowledge on the population structure of *P. infestans* is important to determine if there are changes in the sensitivity to fungicides and aggressiveness on the host. Here, we characterized the genotypic and phenotypic diversity of 22 isolates of *P. infestans*. Isolates were collected in 2017 and 2018 on tomato crops in the departments of Cundinamarca, Boyacá, Antioquia, and Caldas. The genotypic diversity for this isolates was assessed using microsatellite markers and mitochondrial haplotyping. Furthermore, sensitivity to three systemic fungicides (mefenoxam, cymoxanil, and fluopicolide) and pathogen aggressiveness on three tomato cultivars was evaluated. Mitochondrial haplotyping showed that in Colombia, isolates of *P. infestans* collected on tomato are Ia and Ib, while isolates collected on potatoes are IIa. Furthermore, differences in sensitivity to fungicides were observed. Ninety percent of the *P. infestans* isolates were resistant to mefenoxam with an EC_{50} greater than 10 µg ml^{-1}, response to cymoxanil were intermediate with 54% of the isolates with an EC_{50} between 1 to 10 µg ml^{-1}, while 91% of the isolates were sensitive to fluopicolide with an EC_{50} less than 1 µg ml^{-1}. The information obtained in this study will help establish the actual state of *P. infestans* populations associated to tomato crops in Colombia.

Characterization of *Pseudomonas syringae* pv. *papulans* strains isolated from 'NY-1' apple fruit in New York

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Pseudomonas syringae pv. *papulans* (*Psp*) is the causal agent of apple blister spot, a disease typically affecting the cultivar 'Mutsu' and causing economical losses due to symptoms reducing the apple fruit marketability. Disease symptoms on other cultivars usually occur when infected 'Mutsu' trees are in the vicinity. However, in 2018 immature 'NY-1' apple fruit showing blister spot characteristic symptoms were sampled from an orchard in New York with no 'Mutsu' trees in the surroundings. In this work, for the first time, we isolated, identified and characterized *Psp* isolates from 'NY-1' fruit by classical and molecular methods. Characteristic levan-negative colonies on SNA were identified as *Psp* by specific PCR and 16S rDNA partial sequencing. The isolates were negative for cytochrome oxidase, arginine dihydrolase and pectin degradation, but triggered a strong hypersensitive response in *Pelargonium* sp. After inoculation, symptoms of blister spot and necrosis were reproduced on immature 'NY-1' fruit and leaves, respectively. The *Psp* isolates were streptomycin sensitive, but showed a high copper tolerance compared to other phytopathogenic bacteria. Studies on apple 'NY-1' as a possible new overwintering host for *Psp*, or on the characterization of copper resistance mechanisms in this bacterial species are currently ongoing, and will provide useful data for the management of blister spot disease.

Population Diversity of the Hop Powdery Mildew Pathogen (*Podosphaera macularis*) Suggests Continental Spread of the Pathogen

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The powdery mildew pathogen *Podosphaera macularis* can devastate commercial hops. The fungus was unknown in the Pacific Northwestern US, the primary hop production region in this country, until the mid-1990s. The disease has occasionally been reported in other regions of the eastern US as hop production has reestablished. We sequenced transcriptomes of 104 isolates collected throughout the US and Europe to better understand genetic diversity of the fungus and the possible origin of the population in the western US. Discriminant analysis of principle components grouped isolates into 3 to 5 geographic populations, dependent on stringency of the grouping criteria. Western US isolates were clonal, irrespective of pathogenic race, and consistently grouped with isolates from Europe. Eastern US isolates from wild hops were genetically unique, however isolates from cultivated hops in the eastern US were most often genetically similar to western US isolates. Mating types of isolates originating from cultivated western and eastern US hop were invariably MAT1-1. In contrast, a 1:1 ratio of MAT1-1 and MAT1-2 was observed with isolates from wild plants or Europe. The foregoing genetic and phenotypic patterns suggests a European origin of *P. macularis* populations in the western US, followed by spread of the pathogen from the western US to re-emergent production regions in the eastern US, perhaps via distribution on planting material.

Assessment of genetic diversity of Aspergillus spp. Section Nigri populations in Georgia peanuts by DNA fingerprinting techniques
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A population of 288 isolates of *Aspergillus* spp. Section Nigri were obtained from peanut (*Arachis hypogaea*) seeds and peanut plants from the state of Georgia. DNA of these isolates was subjected to fingerprinting analysis using 19 microsatellites (SSR) and 5 Indel (insertion/deletion) markers. These markers were developed by mining sequences from the National Center for Biotechnology Information (NCBI) and then using Clone Manager and Primer3 to align sequences and design primers. Fungal isolates of the section Nigri from the collection at NPRL were included in the analysis for comparison. Cluster analysis by Neighbor Joining and 3D-Principal Coordinate Analysis (3D-PCoA) of the fingerprinting results were performed to identify potential groups of isolates. In 3D-PCoA, the first three dimensions explained 78.9% of the genetic variation. The fingerprinting was used to separate genotypes into groups. Results of cluster analysis will be used to identify representative isolates for further study of whole genome sequencing. This work is part of a larger project that includes the potential fungicide resistance and ochratoxin production of the isolates, characteristics previously reported in some isolates of *Aspergillus* spp. Section Nigri.

Spread of the famine lineage of *Phytophthora infestans* into the African and Asian continents
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The FAM-1 clonal lineage of *Phytophthora infestans* caused late blight in the 1840s in the US and Europe. FAM-1 (HERB-1 mtDNA haplotype) was the only lineage detected in herbarium samples from the US and Europe collected from the 1840s through 1930. Spread of the lineage to other continents has been reported, but had not been extensively researched, and it was assumed that the US-1 lineage caused the first outbreaks on other continents. We examined 48 herbarium samples from the African and Asian continents collected between 1910 and 1991 and used 12 microsatellite (SSR) loci to identify FAM-1 and mtDNA sequencing to identify HERB-1. The famine lineage was detected in approximately 35% of the samples and was found in samples collected between 1910 and the 1950s. In Africa, the famine lineage was found in Uganda, Tanzania, Kenya, Zimbabwe, Cameroon, and Ethiopia, all of which were subject to European colonialism and/or occupation. In Asia, FAM-1 was found in the Philippines, India, and Malaysia, also countries subjected to European and/or US colonialism, and in Nepal. The US-1 lineage was not detected in any samples collected from Africa or Asia before the 1950s. The data from historic samples suggest that the famine lineage was spread during global colonization, most likely via potato tubers from Europe and/or the US, and was subsequently displaced by US-1 in the 1950s.

A preliminary investigation into the genetic diversity of *Fusarium* species causing Fusarium head blight (FHB) of wheat in Georgia and Alabama
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Fusarium head blight (FHB) has become a limiting factor to soft red winter wheat production in Georgia and Alabama in recent years partly due to an increase in acreage of corn, another host for some FHB fungi. FHB is caused by 16 biogeographically diverse species within the *Fusarium graminearum* species complex (FGSC) although *F. graminearum sensu stricto* (*s.s.*) with its 15ADON (type B trichothecene) chemotype is predominant in the US and Canada. To investigate the genetic diversity of this pathogen in GA and AL, we collected nearly 150 isolates from symptomatic wheat heads and corn debris from 23 counties of the two states in 2018. PCR-based identification with translocation elongation factor 1 alpha (*EF-1α*) primers indicated most isolates were *F. graminearum s.s.* with a few of the recovered isolates being *F. poae.* Chemotyping using multiplex PCR targeting the *Tri3* and *Tri12* genes revealed the isolates so far are 15ADON type. Phylogenetic analysis of representative isolates and reference strains of all known FGSC species based on the *EF-1α* gene showed that most of the isolates were clustered within the *F. graminearum* clade. To our best knowledge, this is the first investigation of FGSC along with chemotype identification in the region. However, additional sampling and further studies on the population structure, chemotype, and virulence of the isolates are needed to better understand the pathogen to assist in developing adequate disease management strategies.

Global Population structure of *Fusarium virguliforme* based on microsatellite marker analysis
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Fusarium virguliforme is a soilborne pathogen and the causal agent of soybean sudden death syndrome (SDS) in the USA. Since the first observation in Arkansas in the 1971, SDS has been reported in 22 states. SDS has also been reported in Canada, South America and South Africa and *F. virguliforme* was found in the soil in Malaysia. In South America, SDS can also be caused by several other *Fusarium* species. In an effort to examine global population structure of *F. virguliforme*, a collection of isolates, 34 from Argentina and 56 from the USA, were genotyped using microsatellite markers. Bioinformatics mining of four *F. virguliforme* genomes and experimental validation identified 16 polymorphic microsatellite markers and these markers were used to fingerprint these isolates. In total, 37 multi-locus genotypes (MLGs) were identified. USA isolates were distributed among 27 MLGs while Argentina isolates were distributed among 11 MLGs. Majority of our Argentina isolates were clustered with USA isolates collected from Indiana, Iowa, Arkansas and Illinois. Several diversity indexes, including the average number of different alleles, the number of effective alleles, and

Shannon's diversity index, showed that the USA isolates were more diverse than Argentina isolates. We are currently collecting more isolates from geographic regions not represented or under-represented in our collection.

Investigating the genetic structure of *Pseudoperonospora cubensis* populations after single-product fungicide application events
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Pseudoperonospora cubensis causes cucurbit downy mildew (CDM), the most economically devastating disease in the *Cucurbitaceae*. Frequent fungicide applications are required to control the disease in cucumber since the pathogen overcame previously effective host resistance. In this study, we aimed to determine changes in the genetic structure of *P. cubensis* populations following single-product fungicide applications. Ten previously described microsatellites were used to genotype 880 *P. cubensis* isolates sampled immediately following spray events during field experiments in 2016 and 2017. Previously described *P. cubensis* clade and fungicide resistance markers for Carboxylic Acid Amides and Quinone Outside Inhibitors were also used to genotype isolates. Bayesian clustering analysis revealed several genetic clusters in the population. When genetic cluster occurrence was evaluated by treatment, similarities in structure for tolerant varieties with similar genetic background were observed. Likewise, genetic structure was also similar between some fungicides even though treatments belonged to different modes of action. Our results demonstrate the role fungicide applications have in pathogen populations and give insight into the evolution of fungicide resistance in oomycetes. Our findings will ultimately allow for population-informed fungicide recommendations for sustainable disease management of CDM in the United States.

Diversity of *Colletotrichum* species on tea (*Camellia sinensis*) from three counties in Florida
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Anthracnose is a devastating disease on tea [*Camellia sinensis* (L.) O. Kuntze]. In the US, this disease has been associated with the pathogen *Colletotrichum camelliae*. No data currently exists on the species diversity of *Colletotrichum* on tea cultivated in Florida. We collected symptomatic leaves exhibiting typical anthracnose lesions from both field and nursery plants in three counties in Florida. Ten isolates were identified as *Colletotrichum* sp. based on microscopy and sequencing using the GS, GADPH, TUB2, and ApMat loci. A multi-gene phylogeny was constructed using sequences generated in this study and previously published sequences from *Colletotrichum* spp. isolated from tea and ornamental camellia. The Florida tea isolates cluster in two distinct clades. Our phylogeny also identified a second *Colletotrichum* species in the *C. siamense* complex as a potential causal agent of anthracnose on tea. The isolates collected from nursery and field grown plants in North Central Florida grouped with *C. camelliae*, but isolates from field grown tea plants in Central Florida clustered within the *C. siamense* complex. Our isolates in the *C. camelliae* clade were genetically similar to Chinese isolates collected from tea. Future research will require Koch's postulates to be performed to confirm the pathogenicity of *C. siamense* on tea.

Characterization of bacterial leaf streak of corn in Minnesota
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Bacterial leaf streak (BLS) is an invasive disease of corn caused by *Xanthomonas vasicola* (*XV*). This disease was first confirmed in the USA, including two counties in Minnesota, in 2016. The goal of this project is to understand the distribution and characteristics of this pathogen in Minnesota field corn and sweet corn production fields. Corn leaves with typical symptoms of BLS, i.e., brown to yellow narrow leaf lesions, were collected from multiple fields and counties across southern Minnesota in 2017 and 2018. Leaf samples were tested for bacterial streaming and for the presence of *XV* directly with a specific PCR assay and via isolations on media. BLS and *XV* have been confirmed in eight counties, and BLS has been reported based on symptoms in at least 10 additional counties in southern and central Minnesota. Three isolates of *XV* obtained from symptomatic leaves were confirmed via sequencing of the 16S rRNA gene, and virulence was tested on field corn and sweet corn in greenhouse experiments with four inoculation methods. Disease severity was assessed weekly for 3 weeks after inoculation. Isolates of XV differed significantly in virulence on field and sweet corn and the method of inoculation significantly influenced development of BLS.

Characterization of *Stemphylium* spp. from spinach based on molecular data, host response, and azoxystrobin sensitivity
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Stemphylium leaf spot of spinach causes losses under warm, humid conditions. The causal agent was described originally as *Stemphylium botryosum* based on anamorph and teleomorph morphology. As a result of increasing losses to this disease and evidence of diversity among *Stemphylium* isolates from spinach, molecular characterization, pathogenicity tests, and azoxystrobin resistance screening were performed on isolates sampled from symptomatic plants in Arizona, California, Florida, Oregon, Texas, and Washington as well as isolates from seeds grown in six countries, including the USA. The internal transcribed spacer 1 and 2 regions of ribosomal DNA, and regions of *glyceraldehyde-3-phosphate dehydrogenase*and *calmodulin* were sequenced. Two spinach cvs., Mandolin and Viroflay, were inoculated with each isolate. Isolates clustered into two phylogenetic lineages: 1) isolates most similar to *S. vesicarium*, and 2) isolates previously identified as *S. botryosum*. The former caused small (≤ 5 mm diameter) lesions on Mandolin 2 days after inoculation, but no symptoms on Viroflay, even after 21 days. The latter produced larger (5 to 15 mm diameter) lesions on both cultivars within 10 to 14 days. Isolates resistant to azoxystrobin (EC_{50}> 10 mg/liter) were detected, and mutations conferring resistance

are being characterized. Differences in the biology, epidemiology, and management of these two causal agents of Stemphylium leaf spot of spinach are being investigated.

Sweet corn production in the Columbia Basin of Washington: Causal agents of seedling blights and prevalence of mefenoxam resistance
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Washington State is a major producer of sweet corn in the USA with roughly 36,400 ha planted in the semi-arid Columbia Basin annually. However, cold spring soil conditions and pathogenic species of *Fusarium*, *Pythium*, and *Rhizoctonia* can cause significant yield losses. In 2018, conventional (n = 32) and organic (n = 16) sweet corn fields were surveyed in the Columbia Basin to assess the prevalence of damping-off and seedling blights. Isolations from stunted seedlings primarily yielded *Fusarium*, *Pythium*, and *Rhizoctonia*. *Pythium ultimum* comprised 95.2% of 63 *Pythium* isolates based on sequencing the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA), with only one isolate of each of *P. irregulare*, *P. rostratifingens*, and *P. sulcatum*. Of 66 *Rhizoctonia* isolates, 69.7% were *R. solani* AG-4 based on ITS rDNA sequences. Of 370 *Fusarium* isolates identified by sequencing the *translation elongation factor 1-alpha* gene, *F. equiseti*, *F. oxysporum*, *F. solani*, and *F. verticillioides* were most prevalent. Of the 63 collected *Pythium* isolates, 62 *Pythium* isolates screened for mefenoxam resistance, 38 and 35 were resistant to mefenoxam at 10 and 100 ppm, respectively. The high incidence of mefenoxam resistance may account for some stand losses in fields planted with mefenoxam-treated seed. Isolates from each genus are being tested for pathogenicity to sweet corn, and the most virulent isolates used to screen sweet corn germplasm for resistance to seedling blights.

Morphological and Genomic Diversities of *Rhizoctonia solani* on Sugar beet in the USA
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Rhizoctonia solani Kühn is a devastating soil-borne pathogen that can infect a wide range of crops, including sugar beet. It was estimated that 24% of sugar beet planted in US and 5–10% in Europe can be affected by this fungus. The fungal pathogen has been classified into thirteen main anastomosis groups (AGs). In Minnesota and North Dakota, AG 2-2 (IIIB and IV), which cause root and crown rot, is the most important for sugar beet production. In addition, AG 4 has been found to cause the seedling damping-off in sugar beet. Other AGs and sub-groups are also present at low frequency in US. Overall, the signs, symptoms, morphology and infection process of different AGs on sugar beet are not very clearly defined. Therefore, this study is to examine morphological, genetic and virulence diversity of *R. solani* in sugar beet. A total of 400 isolates of *R. solani* has been collected from sugar beet fields across US. Initial microscopic observations indicated conspicuous variation in colony morphology. The future work includes characterization of those isolate using microsatellite and genotyping-by-sequencing approaches (GBS). Primers have developed based on the draft genome of AG 2-2 IIIB available in the gene bank. GBS data would reveal the genomic complexity among the AGs. Furthermore, this research would provide knowledge and tools for a sustainable management of this important pathogen in sugar beet.

Genetic diversity of cucurbit-infecting begomoviruses in Malaysia
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Cucurbit crop production was seriously damaged by begomovirus diseases in Southeast Asia. However, the related information on this virus is shortage in Malaysia. In 2016 and 2017, the viral diseases in cucurbit production fields were observed in Eastern and Western Malaysia, and the disease incidence up to100% with symptoms of mosaic and yellow leaf curling was investigated. Seventy symptomatic samples were collected in 7 cucurbit crops from 15 locations, and 51 symptomatic cucurbit samples were tested for presence of begomovirus by PCR using primer pair- PAL1v1978B/PAR1c715H. Fifty-two PCR products were sequenced from selected 45 samples. Based on the phylogenetic analysis, those 52 sequences were grouped into five clusters. Considering the demarcation criteria 91% nucleotide sequence identity for virus species and 94% for virus strain, the majority *Tomato leaf curl New Delhi virus* (ToLCNDV) isolates were distributed in four clusters and could be considered respectively belonging to distinct strains. The *Squash leaf curl China virus* (SLCCNV) isolates from Eastern Malaysia formed the other cluster. Both SLCCNV and ToLCNDV are first detected in Malaysia, and the whole viral genomic DNA sequencing is ongoing for further characterization. However, our results reveal the explanting of cucurbit-infecting begomoviruses in Southeast Asia, and should be considered seriously as an important factor for cucurbit virus control in the area.

Fungicide sensitivity and population structure of *Rhizoctonia zeae* isolated from soybean and corn in the North Central U.S.
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Soybean and corn are the major crops in the North Central U.S. *Rhizoctonia zeae* was recently identified as a causal agent of seedling diseases of these crops in Nebraska. Fungicide seed treatments are commonly used to manage seedling diseases and include several modes of action, such as DMI, Phenylpyrroles, SDHI, and QoI. To establish the current control level and devise better control strategies, we examined the fungicide sensitivity and population structure of *R. zeae*. Effective concentration for 50% inhibition (EC_{50}) was estimated for 65 *R. zeae* isolates to four fungicides using serial dilution. Average EC_{50} for prothioconazole (DMI) was 0.173 ppm, fludioxonil (Phenylpyrroles) was 0.096 ppm, sedaxane (SDHI) was 0.066 ppm, and azoxystrobin (QoI) was > 100 ppm. To validate

insensitivity to azoxystrobin, *in planta* assays will be performed. EC_{50} did not differ significantly among isolates collected from different years (2015-2017) or crops for all fungicides ($P > 0.05$). A discriminatory concentration will be selected for each fungicide and EC_{50} of additional isolates will be estimated. Results will establish fungicide sensitivity of *R. zeae* and can be used to monitor sensitivity shifts in future. We sequenced five *R. zeae* isolates at >100X coverage and identified 1,593 candidate microsatellite markers to characterize its population structure. This will allow inference of the evolutionary potential, mating system, and dispersal mechanism in *R. zeae* populations. Collectively, this information will help to devise better control strategies for *R. zeae* in the North Central U.S.

Stone fruit commodity survey in Texas monitoring for pests and pathogens of high concern: 2018–2019
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The Texas Plant Disease Diagnostic Lab continued participation in the National Stone Fruit Survey in 2018, monitoring for pest and pathogens of concern for the stone fruit industry in Texas. Orchards in nine different counties in Texas were tested for the presence of plum pox virus (PPV). A total of 472 foliar peach samples were collected. For virus detection, each sample was tested by ELISA using Agdia ELISA kits following USDA APHIS PPQ and National Plant Diagnostic Network protocols. All 472 samples tested negative for all strains of PPV. Additionally, 868 visual observations were made for European stone fruit yellows (ESFY, causal agent '*Candidatus* Phytoplasma prunorum') and phony peach disease (PPD, causal agent *Xylella fastidiosa*). Disease symptoms were not observed in the surveyed orchards. Jackson traps were also set in each orchard and the Texas A&M stone fruit breeding plots to screen for the light brown apple moth (LBAM, *Epiphyas postvittana*). A total of 45 traps were collected and LBAM was not observed. These continued survey efforts ensure that stone fruit orchards in Texas remain free of these invasive threats and allow for their early detection if they are introduced. Surveying will continue in 2019, focusing on almond witches' broom (AlmWB), PPV, ESFY, PPD and LBAM.

Effect of cucurbit host, production region, and season on the population structure of *Pseudoperonospora cubensis* in Florida
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In Florida, cucurbit downy mildew (CDM), caused by *Pseudoperonospora cubensis*, is one of the most devastating diseases of cucurbit production. *P. cubensis* has been shown to overwinter in areas of central and south Florida, which serves as a source of inoculum for cucurbit production in Northern states. Thus, understanding the current population structure of *P. cubensis* in Florida is vital in properly managing CDM. The objective of the study was to identify differences in seasonal and regional genetic diversity and population structure of *P. cubensis*, in major cucurbit production areas of Florida. A total of 274 *P. cubensis* isolates from two cucurbit hosts (cucumber and butternut squash) and three production regions over a two-year period were evaluated. Ten simple sequence repeat (SSR) loci were utilized to establish population genetic statistics and potential structure. High levels of diversity were detected among all isolates based off various diversity estimates. Analysis of molecular variance (AMOVA) analyses revealed significant genetic differences (< 0.001) among host species, production region, and season. STRUCTURE analysis identified two clusters (k=2), which are best explained by cucurbit host species. Results from this study will further our knowledge of genetic variation among *P. cubensis* populations.

Genomic microsatellites evidence species diversity and origin of *Peronospora tabacina*, an important pathogen of tobacco
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Peronospora tabacina Skalický [D.B. Adam], an obligate oomycete causing blue mold of tobacco, is an important pathogen causing significant economic losses. The pathogen rarely reproduces sexually, and the windblown asexual sporangia from infected plants are the source of yearly natural epidemics. A collection of 110 isolates of *P. tabacina* with subgroups from across Europe, the Middle East, and the Americas was analyzed with nine genomic microsatellites to investigate population structure and pathogen diversity. Clone-corrected data revealed the presence of 34 alleles or 3 to 5 alleles per locus. Genetic variation among the 10 subgroups accounted for 7% of total variation indicating low levels of genetic differentiation and high gene flow among them. The Mantel test showed significant correlation between geographic and genetic distances ($r = 0.25$; $P < 0.001$). Evidence of linkage disequilibrium ($P < 0.001$) showed that several tested subgroups were partially clonal in character, whereas the markers used were distributed across the whole genome, without significant linkage disequilibrium among them ($P < 0.001$). Low genetic variation and lack of population structure among the analyzed isolates was attributed to continuous gene flow across continents via infected plant exchange and/or long distance dispersal of sporangia. Bayesian inference provided insights into pathogen history and origin based on several possible scenarios when linked to the host plant.

Genetic diversity of the spinach downy mildew pathogen based on hierarchical sampling
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Downy mildew disease, caused by the obligate pathogen *Peronospora effusa*, is the most economically important disease of spinach. Fourteen of the 17 named races and 11 novel strains of *P. effusa* have been identified in the past 30 years. However, the mechanism of the rapid evolution of virulence remains unknown. A total of 69 downy mildew isolates (an isolate is defined as a collection of nearby

symptomatic leaves within a given field) were collected from 14 states from 2010 to 2018. The genotypes of between 1 and 39 individual lesions per isolate were examined using a targeted sequencing technique to sequence 33 SNP loci. Genetic diversity was evaluated between individual lesions within a given isolate, between fields, host cultivars, geographic locations, and years. A total of 387 multilocus SNP genotypes (MLG) were identified among 731 sequence samples examined. Among the 387 genotypes, 315 (81%) were unique. The most common genotype (MLG374) was identified in 110 sequence samples from 16 isolates collected from CA and AZ in 2016. The variation for a given isolate ranged from no variation (a single genotype among lesions from that isolate) to many genotypes (highest 37) from lesions of that isolate. An index of association analysis indicated evidence for both asexual (clonal) and sexual reproduction. Four subpopulations were found by discriminate analysis of principal components. Host cultivar, origin and time of collection had effects on population differentiation, and genotypes specific to certain location or time period were identified.

Characterizing Pythium associated with Illinois soybean fields
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Root rots can significantly impact yields of field crops. There have been no recent, detailed surveys of Pythium in Illinois field crops for at least a decade. The objectives of this project were to isolate and identify different Pythium associated with soybeans in Illinois and assess their aggressiveness level and sensitivity to select fungicides commonly used to control Pythium in field crops. *Pythium* spp. 2016 and 2018 through soil baiting and symptomatic plants. Isolates were identified by sequencing of the ITS region and morphology. Five species of *Pythium* were identified from 61 isolates recovered from 12 counties. These included *P. ultimum* (68.9%), *P. dissotocum* (8.2%), *P. aphanirdermatum* (6.6%) *P. vexans* (4.9%) and *P. irregulare* (1.6%). An additional 9.8% were only identified to genus. A petri plate assay was used to assess the aggressiveness of 61 isolates on soybean seed. All Pythium species reduced germination (76-100%) relative to non-treated controls. *Phythium ultimum* and *P. aphanirdamatum* caused significantly greater reduction in germination than all other species except *P. irregulare*. All species except *P. vexans* increased seed colonization relative to controls by 14-33%. Significant variation in aggressiveness existed within species. Poison plate assays were used to assess the sensitivity of 11 Pythium isolates to azoxystrobin and using a discriminative dose. Results indicated that 27% of isolates were insensitive to azoxystrobin. Additional studies to assess isolate sensitivity to other fungicides as well as aggressiveness to corn are ongoing.

Competition between *R. solanacearum* sequevars causing Moko disease on banana (*Musa acuminata*) in Suriname
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Ralstonia solanacearum is the causal agent of Moko/Bugtok disease. *Musa acuminata*, Cavendish cultivar, accounts for 47 percent of global banana production and is susceptible to *R. solanacearum*. Our objective was to characterize isolates of *R. solanacearum* from Cavendish bananas collected in Suriname, August 2018. Sequencing of the endoglucanase gene identified sequevars 4 (P856) and 38 (P853). Previously, sequevar 4 had been associated with banana but sequevar 38 had not. Competition assays were performed *in vitro* and on banana with strains P853 and P856, using Rifampicin mutants of each strain. For *in vitro* assays, P853, P856, P853R, and P856R were grown in liquid CPG/ CPGR for 24 h and diluted to 10^6 cells/ml. Mixtures (1:1) were created with P853R/P856, and P856R/P853. Suspensions were dilution plated on CPG/ CPGR, incubated for 24 hours, and colonies were counted. Six plants were inoculated with each isolate or mixture by stem injection with 1 µl of a 1×10^6 cell/ml bacterial suspension. Plants were maintained in a greenhouse with controlled temperature and lighting. After three weeks, 100 µl of sap was extracted from each plant, suspended in 1.9 ml of liquid CPG/ CPGR, dilution plated onto CPG/ CPGR agar, and counted after 24h. In two repetitions *in vitro*, P856 outcompeted P853; however the reverse was observed in an initial repetition of the *in planta* assay. Additional repetitions are in progress, as are host range assays with both strains on *Solanaceae* species and squash. Preliminary tests reveal susceptibility to strain P853 on Solanaceae and Cucurbitaceae species.

Utilization of microsatellites for *Rhizoctonia solani* AG2-2 population genetics
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Rhizoctonia solani AG2-2 causes Rhizoctonia root and crown rot of sugarbeet (*Beta vulgaris*) as well as root rots of several other economically important crops. We have developed a set of microsatellite markers to examine diversity and population structure in *R. solani* AG2-2. We are utilizing these markers to investigate *R. solani* AG2-2 diversity at varying geographic levels and among isolates recovered from sugarbeet and several rotation crops. To examine the distribution of AG2-2 genotypes in Michigan fields, isolates collected by baiting with toothpicks are being genotyped using eight microsatellite markers. Furthermore, in order to understand how diversity is generated in AG2-2, we need to identify the extent to which recombination occurs. Since AG2-2 rarely produces a sexual stage, genetic material is expected to be exchanged through hyphal fusion. However, anastomosis reactions typically result in death of the cells adjacent to the fusion site. It is therefore unclear how much genetic exchange occurs between isolates. Preliminary evidence from microsatellite loci sequencing shows that isolates from different genetic groups have microsatellite alleles that are identical by sequence, which is suggestive of an exchange of genetic material. To test the extent of asexual hybridization, isolates from different genetic groups were paired on water agar and potential hybrids isolated. Several resultant isolates showed variation in cultural characteristics from the parent cultures indicating possible hybridization occurring. The microsatellite analysis is ongoing.

Diversity and fungicide sensitivity of *Phytophthora* pathogens of soybean in Illinois
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Several species of *Phytophthora* cause damping-off and root rot on soybeans. However, response to resistance genes and fungicide seed treatments can vary between species and isolates. The objectives of this study were to determine pathotype diversity of *Phytophthora* in Illinois and determine their sensitivity to fungicide active ingredients found in soybean seed treatments. *Pythium* spp., *P. sojae*, and *P. sansomeana* were isolated between 2016 and 2018 through soil baiting and from symptomatic plants. Isolates were identified by sequencing of the ITS region and morphology. Twenty-one isolates of *P. sojae* and six of *P. sansomeana* were recovered from 12 counties. The pathotype of each *Phytophthora* isolate was determined by inoculating 14 differentials with known *Rps* genes. Twenty and sixty percent of the isolates were able to cause disease on plants with the *Rps*1c or *Rps*1k, respectively. The sensitivity of *Phytophthora* spp. to the fungicides metalaxyl, mefenoxam, azoxystrobin, and ethaboxam were determined using poison plate assays and calculating EC_{50}. All *Phytophthora* spp. isolates were sensitive to metalaxyl, mefenoxam, azoxystrobin, and ethaboxam at concentrations lower than 1 µg a.i. ml^{-1}. The characterization of the population of oomycete pathogens causing disease on soybeans will enable better use of resistance and fungicide seed treatments.

Time-point field sampling of *Pythium* spp. pathogenic on cucurbits reveals a seasonal change in species recovery
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Pythium spp. cause root and stem rot in cucurbits, but no formal surveys have been conducted to identify which species are responsible. A panel consisting of bottlegourd, cucumber, Hubbard squash, and watermelon was transplanted in May, July, September, and November into four and five different fields in 2017 and 2018, respectively, in South Carolina to identify associated *Pythium* spp. Isolates (524) were collected and identified by sequencing the mitochondrial cytochrome oxidase I region. Of the 11 species recovered, the 4 most common were *P. spinosum* (38.9% of isolates), *P. myriotylum* (23.1%), *P. irregulare* (16.4%) and *P. aphanidermatum* (15.1%). *P. myriotylum* and *P. aphanidermatum* were predominantly isolated in May through September, whereas *P. spinosum and P. irregulare* were predominantly isolated in November. Representative isolates (63) from each species were screened in vitro against oxathiapiprolin. All isolates were insensitive to oxathiapiprolin, except those of *P. ultimum* var. *ultimum* and *P. sylvaticum*, both members of *Pythium* Clade I. Additional isolates of remaining *Pythium* Clade I species were obtained. EC_{50} values for Clade I species ranged from 0.50 to 1.05 µg/ml. Four *Pythium* spp. made up 93.5% of all isolates recovered from diseased cucurbits, and the community of pathogenic *Pythium* spp. changed throughout the year. Oxathiapiprolin is effective for managing many oomycetes but appears to selectively target only those *Pythium* spp. in Clade I.

Sugarcane cultivars susceptible to orange rust can host sub-populations *of Puccinia kuehnii* capable of infecting resistant cultivars
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Since the first outbreak of orange rust in Brazil in 2019, resistant sugarcane cultivars were used to control this disease caused by *Puccinia kuehnii.* Nevertheless, new rust epidemics occurred on cultivars previously considered resistant, suggesting variation in virulence of the pathogen. Variability in virulence of *P. kuehnii* was investigated herein using three different inoculum sources of *P. kuehnii* from Brazil. In an initial screening, 1,068 single-uredinium rust isolates from three susceptible cultivars were each inoculated in the laboratory on one of five sugarcane cultivars resistant to orange rust. A five-cm leaf section was brush-inoculated with all spores of each uredinium and plants were maintained in a humid chamber for 24 hours before transfer to a shed. The size of leaf areas with disease lesions was measured 21 days after inoculation with image analysis software Assess 2.0. Fourteen isolates of the pathogen produced uredinia on a leaf of resistant cultivars. Capacity of infecting a resistant cultivar was confirmed for one of these variants in replicated experiments with spores from previously inoculated leaves. Uredinia of *P. kuehnii* from sugarcane cultivar SP89-1115 that developed on leaves of three resistant cultivars were differentiated from uredinia infecting only a susceptible cultivar by microsatellite marker NPRL_PK_221_spka. Populations of *P. kuehnii* infecting susceptible cultivars therefore include sub-populations that could be the initial inoculum for development of new epidemics on cultivars resistant to orange rust.
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Genotypic diversity among *Botrytis cinerea* isolates from California
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Botrytis cinerea, the causal agent of grey mold, has high genetic diversity and a broad host range. In grape and *Prunus spp.*, *Botrytis* causes a fruit rot, and fungicide spray are routinely used to prevent loss pre- and post harvest. Five hundred thirty-five isolates of *B. cinerea* collected from grape and *Prunus spp.* in 2012, 2016, and 2017 were genotyped using 18 microsatellite markers and the transposable elements (TE) Boty and Flipper. Only nine markers, were considered informative and retained for the final analyses. Four hundred ninety-eight of the isolates were tested for resistance to six or seven different fungicides, representing 5 classes. After clone correction, 323 multi-locus genotypes (MLG) groups were retained across the three years, and four genetic sub populations were detected. High levels of clonality were observed across the dataset. Significant pairwise differentiation was detected among years, locations, TE composition, and host. However, most of the differentiation observed was within a subpopulation, and not between subpopulations. No differentiation was detected among fungicide resistance isolates when compared against individual products. When resistance to different numbers of fungicides were compared regardless of the chemistry, significant little differentiation was detected among resistance to two fungicides and three or four fungicide chemistries.

Epidemic dynamics of multiple introductions of the sudden oak death pathogen into Oregon forests
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Sudden oak death (SOD) has been actively managed in Oregon since the early 2000's. There are currently four recognized clonal lineages, with the NA1 lineage being primarily responsible for the epidemic in southwest Oregon forests. We compared population dynamics of the NA1 *Phytophthora ramorum* outbreak first reported in 2001 to an outbreak of an emerging EU1 *P. ramorum* clonal lineage first detected in 2015. We also tested if NA1 was reintroduced since the initial documentation of two introductions. Infested regions of the forest were sampled between 2013–2018 (n=903) and screened at 15 microsatellite loci. Most genotypes were transient, with 272 of 358 unique genotypes emerging one year and disappearing the next. Diversity of EU1 was very low and isolates were geospatially clustered (<8 km apart), suggesting a single EU1 introduction. Some forest isolates are genetically similar to isolates collected from a local nursery in 2012, suggesting introduction of EU1 from this nursery or simultaneous introduction to both the nursery and latently into the forest. In contrast, the older NA1 populations were more polymorphic and spread over 30 km. Principal component analysis supported two to four independent NA1 introductions. The NA1 and EU1 epidemics infest the same area but show disparate demographies owing to initial introductions spaced 10 years apart. Comparing these epidemics provides novel insights into patterns of emergence of clonal pathogens in forest ecosystems.

Road Trips for Redbuds: An Assessment of the Genetic Diversity and Spatial Distribution of *Cercis canadensis* in the US
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Cercis canadensis L. (Eastern Redbud) is a small, understory tree native to 24 states in the eastern United States (US) and Mexico. It is among the earliest deciduous trees that flower in the spring, has lush-green heart-shaped leaves and a wide range of fall foliage colors. Eastern Redbud has become a very popular landscape tree, contributing to more than $27M annually in sales of native trees and cultivars. Because of the wide range of morphological variation, we hypothesized that high genetic diversity among the wild collections of *C. canadensis* would be evident across its native geographical distribution. We utilized 12 previously developed microsatellite loci in genotyping 691 wild individuals from 74 collection sites across 23 states to determine the population structure and genetic diversity across the Eastern US. Our results indicated a presence of population structure, high genetic diversity (H_E=0.67), and high genetic differentiation (F_{ST}=0.19) among the collection sites. Two major genetic clusters were inferred by STRUCTURE in our collection. The majority of variation was retained within individuals (70.2%, $P < 0.0001$) revealed by AMOVA. Only 16.2% ($P < 0.0001$) of variation was retained among the samples within collection sites, and 13.5% of the variation ($P < 0.0001$) among the two major population clusters identified. The data suggested that high levels of genetic diversity and spatial structure of our *C. canadensis* collection could have important implications for habitat management efforts, and future breeding programs to improve desirable horticultural traits.

Population structure of *Globisporangium cryptoirregulare* from Rhododendron Nurseries in Oregon
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Globisporangium cryptoirregulare (syn. *Pythium cryptoirregulare*), is the causal agent of root rot and damping-off of a broad range of ornamental crops. Although, root rot disease in *Rhododendron* is often caused by *Phytophthora* species, studies performed in Oregon nurseries have found that *G. cryptoirregulare* is a prevalent pathogen in symptomatic rhododendron roots, causing mild levels of disease. The objective of this study was to assess the diversity and population structure of *G. cryptoirregulare* isolated from rhododendron in five Oregon nurseries over 4 years, to better understand the population dynamics of this pathogen. A total of 137 samples of *G. cryptoirregulare* were isolated from rhododendron roots from different nurseries between 2013 and 2016. Samples were analyzed using eight microsatellite loci. Forty-one multilocus (MLG) genotypes were observed. Two clonal genotypes were prevalent in all the nurseries studied. MLG 17 was prevalent from 2013 to 2016 (57 isolates), while MLG 9 was the most common in 2016 only (22 isolates). Low to moderate genetic differentiation was observed between nurseries. Our results suggest low diversity and a high likelihood of gene flow between the five nurseries, with a high likelihood of clonal lineages getting established locally once introduced. Nonetheless, new genotypes were introduced every year, and a new clonal population displaced the previously predominant clone in 2016 in three of the nurseries.

***Fusarium graminearum* population-specific differences during wheat infection**
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Fusarium graminearum (*Fg*) is the primary fungal pathogen responsible for Fusarium head blight (FHB), a devastating disease of wheat and barley worldwide. FHB reduces crop yield and contaminates grain with trichothecene mycotoxins that are harmful to plant, human and animal health. Three genetically distinct populations of *Fg*, each associated with a different trichothecene chemotype, have been identified in North America (NA1, NA2, and NA3). To determine how this population-level diversity influences pathogenesis and mycotoxin contamination, we inoculated moderately resistant hard red spring wheat variety Alsen with 15 representative strains from each NA population and evaluated disease progression, mycotoxin accumulation, and fungal biomass. Additionally, we evaluated *Fg*

population-specific differences in induced host defense responses. Population-specific differences in aggressiveness were observed and appear to be only partially attributable to differences in trichothecene chemotype. Differences in host defense response and in the relationship between trichothecene accumulation and fungal biomass were also observed between pathogen populations.

Fumigation-induced shifts in *Streptomyces* populations in a potato cropping system

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Fumigation is frequently used in potato production, yet the long-term effects on soil microbial communities remain underexplored. The potential for fumigation to impose selective shifts on the competitive abilities of soil pathogens may exacerbate disease potential in fumigated soils. We studied the phenotypes of *Streptomyces scabies*, including pathogen competitive and antagonistic capacities and aggressiveness, following vapam or chloropicrin fumigation and in non-fumigated soils. Scab-susceptible potatoes were grown in fumigated and non-fumigated soil for two seasons, and *S. scabies* were randomly isolated from tuber lesions in year two. Nutrient use, inhibitory phenotypes, antibiotic resistance, thaxtomin production, and 16S sequence were determined for every isolate. Pathogen populations from different treatments varied in competitive and antagonistic phenotypes. Isolates from chloropicrin-treated soil were significantly less growth efficient than isolates from vapam ($p=0.0109$). In contrast, vapam isolates were significantly more resistant to antibiotics than isolates from non-fumigated or chloropicrin-treated soils ($p=0.0002$). These data suggest that pathogen populations in vapam-treated soils have significantly greater saprophytic competitive abilities than those in chloropicrin-treated soils. Variation in aggressiveness and fitness tradeoffs between saprophytic and pathogenic phenotypes are under investigation. By inducing shifts in saprophytic competitive abilities and pathogen aggressiveness, fumigation may contribute to the intensification of disease severities.

The Incidence of Grapevine Viruses in Four Texas Blanc du Bois Vineyards

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Winegrape production in Texas continues to increase to support a ten-fold increase in wine production over the past two decades. Viruses are one of the most serious threats to grape quality throughout the world's major winegrape regions. Blanc du Bois (BdB), a white grape American hybrid bred for Pierce's Disease resistance, comprises the 5th most planted winegrape variety in Texas. Because of an unexplained curling and deformation of BdB foliage, a systematic sampling of four Blanc du Bois vineyard blocks in the Texas gulf coast region was conducted in May 2018. An average of 50 vines per block were sampled, pooled and subsequently processed for high-throughput sequencing (HTS) to determine the virome and potential role in symptom development, vine health and grape quality. *Grapevine leafroll-associated virus 3* was the most prevalent virus ranging from 0% to 63% incidence in the four vineyards. The next most prevalent virus was *Grapevine red blotch virus*, found only in one vineyard at 71% incidence. Other notable viruses of lesser incidence were *Grapevine fleck virus*, *Grapevine virus B*, *Grapevine virus E*, and grapevine virus M (GVM). GVM has only been recently described as a new *Vitivirus* species. These results have confirmed the presence of a complex virome in Texas vineyards. Further studies are underway to gain a better understanding of their impact.

Characterization of pathogenic *Streptomyces* spp. causing scab in Michigan potato, turnip and beet production

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Pathogenic *Streptomyces* spp. causes scab disease in a number of plants including potato, turnip and beet. Michigan is a national leader for growing potatoes for chip production and for root crop production including ranks first in turnip acreage in the US, and sixth for beet acreage. Common scab of potato and scab in turnip and beet is caused by several *Streptomyces* species. The objective of this study was to characterized plant-pathogenic *Streptomyces* spp. associated with common scab infected potato, turnip and beet roots harvested in Michigan. A total of 151 putative isolates, 39 from turnip roots, 13 beet roots, and 99 from potato tubers were successfully isolated. The isolates were identified by morphological and molecular methods and phylogenetic relationship was determined between isolates. Preliminary results showed out of 151 isolated 48 isolates that caused pitted and surface lesions have genes encoding for thaxtomin synthase (*txtA* and *txtAB*), tomatinase (*tomA*) and a necrosis protein (*nec1*). Maximum-parsimony analysis of 3 loci revealed three similarly clustered groups, with some exceptions. Preliminary results from isolates recovered from turnip and potato from scab lesions shared similar molecular characteristics and grouped together in phylogenetic analyses. Whereas, potato isolates recovered from pitted lesions shared similar molecular and morphological characteristics and grouped together based on phylogeny.

Genetic diversity of *Erysiphe necator* populations in a center of diversity: A case study of select vineyards in New York State

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The grape powdery mildew pathogen (*Erysiphe necator*), has a center of diversity in eastern North America. Genetic divergence of *E. necator* populations on native or introduced *Vitis* species in this region has not been reported in previous studies, except on muscadine grapes (*V. rotundifolia*). In this study, we used AmpSeq to genotype 6 polymorphic microsatellite markers to quantify the genetic diversity of *E. necator* populations in 3 commercial *V. vinifera* vineyards, a commercial vineyard of the interspecific hybrid

'Chancellor', and a vineyard hosting fungicide efficacy trials on *V. vinifera* 'Chardonnay.' Across 300 samples, AmpSeq returned an average of 7.7 alleles per marker compared to 6.2 for the same markers assayed by capillary electrophoresis in a previous study of 129 samples spanning the Eastern United States and Europe. Multidimensional scaling plots along with pairwise F_{ST} values ranging from 0.00 to 0.02 suggested that isolates collected from various fungicide treatments in the efficacy trial vineyard had the lowest within-site diversity. Across all markers, no private alleles were identified in isolates from fungicide trial treatments, compared to 3 across *V. vinifera* commercial sites and 12 in the Chancellor site. These preliminary data suggest that isolates from the interspecific hybrid vineyard were the most genetically diverged. Further studies are needed to determine how geography, fungicide use, and host impact the pathogen population.

Genetic diversity of *Sorghum mosaic virus* isolates from sugarcane in Louisiana, USA
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Sorghum mosaic virus (SrMV) and *Sugarcane mosaic virus* (SCMV) cause mosaic disease (MD) in sugarcane; however, SrMV has been the primary cause of MD in Louisiana sugarcane for over 70 years. Prior to the 2000s, strains of SCMV and SrMV were identified by symptoms expressed in a set of host plant differentials. Since 2002 a reverse transcriptase-polymerase chain reaction (RT-PCR) protocol has been used to distinguish between SCMV and SrMV and a restriction fragment length polymorphism (RFLP) analysis of the RT-PCR product to identify the strains of the SCMV or SrMV characterized by host differentials. However, the RFLP banding patterns of an increasing number of SrMV isolates collected annually in Louisiana do not match the patterns of recognized strains of SrMV. Phylogenetic analysis was performed using the RT-PCR sequences from 54 SrMV isolates collected between 2012 and 2018 from 31 released and near-release sugarcane cultivars. Those with similar RFLP banding patterns were grouped together. Five groups were identified and a phylogenetic analysis revealed similar genetic diversity among the SrMV isolates from Louisiana. An inoculated field experiment has been established to determine pathogenicity of isolates representing the genetic diversity among the SrMV isolates across sugarcane cultivars with different levels of mosaic resistance.

***Chondrostereum purpureum* diversity in symptomatic and reversed plants**
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Chondrostereum purpureum is a fungus that causes the silverleaf disease, producing wood necrosis and foliar silvering through an endoPG enzyme. The reversion of foliar symptoms has been observed and postulated that is driven by a non-genetic mechanism. Eventually, *C. purpureum* populations inhabiting these plants have lost their ability to produce disease. Thus, the objective was to characterize *C. purpureum* isolates from symptomatic and reversed plants based on morphologic, genetic and pathogenic traits. The pathogen was isolated from symptomatic and reversed plants. Isolates morphology was described and radial growth at 5, 10, 15, 20, 25, 30 and 35°C was measured. DNA was extracted, amplified and the banding patterns analyzed. Mycelial discs were inoculated on apple cuttings and after 20 days internal necrosis was measured. Moreover, expression levels of the gene that codifies for endoPG production was studied using qPCR. From all the *C. purpureum* isolates, 80% were obtained from diseased plants and 20% from reversed ones. Morphology was similar among them, however, there was a wide variation on mycelial growth and pathogenicity, from avirulent to the complete necrosis of the cuttings, as well as in the endoPG gene expression. Different genetic groups were found in diseased plants, but reversed ones weren't diverse. These results contributes to the reversion understanding, indicating that the C. *purpureum* features may participate on silverleaf symptom expression.

Development of a reference panel to standardize genotype data of *Sclerotinia sclerotiorum*
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Several population studies of *Sclerotinia sclerotiorum* have been conducted and resulted in important publications furthering our knowledge of this pathogen. Microsatellite genotype data from those studies has been published with the notion that this data could be re-used by other scientists. However, amplicon sizes are known to vary across labs due to primer modifications for fluorophore labeling and the instrument used for fragment analysis. Thus, it is necessary to create a reference panel of DNA that represents allele sizes known to be present within the population. Given recent completion of genotyping of more than 366 *S. sclerotiorum* isolates from across the U.S., development of a DNA reference panel that represents most is now possible. Here, we identified 22 *S. sclerotiorum* isolates that were collected and genotyped previously and represented all known possible alleles. These 22 isolates were genotyped at 11 loci using two different methods. Results showed that seven of the 11 loci were reliable in generation of amplicon sizes, which allowed standardization of the genotypes generated in each method. The next step will be to identify additional published microsatellite loci used for *S. sclerotiorum*, to create a comprehensive reference panel that provides standards for all microsatellite loci genotyped to date. Taken together, the present results suggest that this method of creating a DNA reference panel will be an effective method to allow integration of previously published population genetic data of this plant pathogen.

Incidence of bacterial and viral diseases in blueberry in Maryland
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Blueberry growers in Maryland have expressed concern with increasing incidence of diseases, especially those caused by viruses and some bacteria, and their impact of sustainable production. Due to confusing visual symptoms in plants and lack of information on their

occurrence, a survey was initiated in 2018, we collected 109 blueberry leaf samples from June to September at seven farms located throughout Maryland. These samples were tested for six viruses of concern in blueberry including: blueberry leaf mottle virus (BLMoV), blueberry scorch virus (BlScV), blueberry shock virus (BlShV), blueberry shoestring virus (BSSV), tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV), and *Xylella fastidiosa* (XF) using ELISA kits developed by different companies. Twenty-two additional samples were collected from two out of original six sites, in late October, 2018. These samples were tested for the above viruses as well as for blueberry fruit drop associated virus, blueberry necrotic ring blotch associated virus, blueberry red ringspot virus, blueberry virus A and blueberry mosaic associated virus, by RT-PCR and PCR. Three viruses and XF were recorded in a few blueberry samples during the survey by ELISA: BLMoV, BSSV, and ToRSV. None of the samples collected in October and tested by PCRs were positive for any of the viruses or XF. It is possible that sampling late in October leads to false negatives, thus comparisons will be done multiple times during the 2019 season. Further study of the incidence of plant viruses in berry crops is underway.

Sexual characterization of *Magnaporthe oryzae* populations associated with winter crops

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Brazil is a host spot of blast disease in winter crops, where reports of severe epidemics in wheat, barley, oat, and triticale are documented. Genotype replacement is the most employed method to their control, but has had limited success due to high pathogen diversity. Sexual recombination is suggested for the diversity of wheat blast pathogen due to high mating ability of isolates, presence of both mating type with even distribution; there is little information on sexual traits of *M. oryzae* from other hosts. This work employed biological and molecular tools to examined sexual characteristics of *M. oryzae* associated with blast disease of wheat, barley, oat and triticale. Mating type distribution of 600 isolates of *M. oryzae* (150 from each of the four hosts) was examined 'in vitro' assay in oat meal agar medium with two fertile parental strains collected from wheat; PCR approach employed primers L1/L2 (*MAT1-1*) and T1/T2 (*MAT1-2*). Our data showed existence of only one mating type among 150 *M. oryzae* isolates from barley (*MAT1-1*), black oat (*MAT1-2*), and wheat (*MAT1-1*, but one). Only *M. oryzae* from triticale showed a 1:1 rate (72:78) supporting hypothesis of sexual recombination in this field. Nevertheless, lab assay showed low mating ability of *M. oryzae* from all four hosts as no ascospore was formed. Only 16 isolates from barley and 3 from wheat crossed with fertile wheat isolates. Therefore, our study suggest that recombination does not play any role in *M. oryzae* from these fields, even when a 1:1 mating type distribution was found. Grant: FAPESP (2017/18587-9)

Diversity and Pathogenicity of *Rhizoctonia* spp. from the Sandhills Grassland of Nebraska

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The Sandhills of Nebraska is a complex grassland ecosystem, covering 50,000 km^2 in central and western Nebraska, predominantly virgin grassland. To date, little information is available on the diversity of *Rhizoctonia* spp. from the Sandhills. The objective of this study was to evaluate the diversity of *Rhizoctonia* spp. on major grasses in the Sandhills and determine pathogenicity of isolated strains, including their potential to cause disease in row crops. In 2016 and 2017, four dominant native grass species, namely, sand bluestem, little bluestem, prairie sandreed, and needle-and-thread, and soil samples were collected from 11 virgin soil locations in seven counties in the Sandhills. Fungi were isolated from discolored roots and baited from soil samples. A total of 17 isolates were collected: 5 isolates of *Rhizoctonia solani* AG-F; 2 isolates each from *R. solani* AG-B, AG-C, AG-3, and AG-4; 1 isolate of AG-L; 1 isolate of *Rhizoctonia zeae*. Pathogenicity was assessed using a rolled towel assay on soybean variety 'CR2603' and in the greenhouse on soybean, sand bluestem (*Andropogon hallii*), and needle-and-thread (*Hesperostipa comata*) grasses. Isolates of binucleate *Rhizoctonia* spp. were pathogenic on all grasses but did not cause significant necrosis on soybean. Isolates of AG-C and AG-3 caused severe necrosis on both grasses and soybean. These results suggest grasses in the Sandhills harbor unique AGs, some with cross-pathogenicity to cultivated crops.

Virulence diversity of *Puccinia graminis* f. sp. *tritici* in Ethiopia

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Stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a major production constraint in most wheat growing areas of Ethiopia. In 2013 a severe local epidemic caused by race TKTTF resulted in devastating losses to wheat growers in the main wheat growing regions of Bale and Arsi. The present study was conducted to investigate the virulence composition and diversity of *Pgt* in Ethiopia after the TKTTF epidemic. Stem rust samples collected from the major wheat growing areas in Ethiopia were race-typed at both the Ambo Plant Protection Center in Ethiopia and the USDA Cereal Disease Laboratory in Minnesota. More than 24 *Pgt* races were identified since the stem rust epidemic in 2013. *Pgt* races TKTTF, TTKSK, TTTTF, JRCQC, and RRTTF were widely distributed and have been identified almost at each cropping season. Although race TKTTF has been the predominant race in all survey years, its frequency has been declining as newly emerged races, such as TTRTF and TKKTF, are becoming more prevalent in the last two years. Two races with *Sr24* virulence were identified in 2016 (TKFTP) and 2017 (TTKST). This virulence is significant because several major cultivars rely on *Sr24* for stem rust resistance. The majority of the identified races are virulent to most of the wheat cultivars currently grown in the country. The continuous emergence of new virulent races in Ethiopia underlines the need for monitoring the pathogen population and identifying effective resistance genes that can be used in the national wheat-breeding program to control this devastating disease.

The effector diversity and population structure of a United States population of *Puccinia striiformis* f. sp. *tritici*
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One of the most devastating wheat diseases is stripe rust of wheat caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*). In the United States (U.S.), where *Pst* asexually reproduces, new pathotypes emerge due to events such as mutations within the resident population or through the introduction of exotic populations. The goal of our study was to use a field-transcriptomic approach to evaluate the population structure, evolutionary history, and effector diversity of *Pst* within the U.S. by determining the genetic diversity of *Pst* and comparing these results to older U.S. isolates and European isolates. During the 2017 growing season, 25 *Pst*-infected wheat leaves were collected from fields and compared to 20 *Pst* transcriptomes from northern Europe (identified in 2013 to 2017) and three additional older U.S. isolates. Our results show the isolates fell into three clusters, with the U.S. samples clustering together in one group regardless of year, and the European samples clustering into two separate groups. These results suggest that recently emergent European races have not been introduced to the U.S. to date, but rather the U.S. diversity may be caused by mutation and adaption within the resident population. The gene expression variability seen within the U.S. population occurs mainly in candidate effectors, which indicates the presence of potentially important genes for distinguishing between U.S. subpopulations.

Evaluation of discriminatory primers for *Fusarium oxysporum* f. sp. *niveum* on Australian and United States Fusarium isolates
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Fusarium wilt of watermelon is difficult to diagnose because *F. oxysporum* f. sp. *niveum* (FON) is morphologically indistinguishable from other formae speciales (f. spp.). Additionally, current molecular identification methods cannot consistently or reliably discriminate all FON isolates from non-pathogens or other f. spp. We assayed FON and other Fusarium isolates: 25 from the Northern Territory (NT) and nine from Queensland, AU, and 31 from the Mid-Atlantic region of the USA, with primers fp7315/7332 and fp7314/7331 that were developed in the Netherlands using comparative genomics and reported to differentiate FON from closely related f. spp. All isolates from the NT (12 of 12) that had tested as FON with primers Fon-1/Fon-2 developed in Taiwan also tested positive as FON with primers fp7315/7332 and fp7314/7331. In addition, one isolate out of four that had not tested as FON using the Fon-1/Fon-2 primers tested positive with these primer pairs. Interestingly, all isolates that had previously been designated as race 0 in pathogenicity testing (6 of 29 USA FON isolates) tested negative with fp7315/7332 and fp7314/7331. In addition, three USA FON isolates that had been designated as either race 1 or 2, also tested negative. These isolates are being retested for pathogenicity. The primers fp7315/7332 and fp7314/7331 may be effective in identifying FON from a wide geographic region, although they may not detect weakly pathogenic race 0 isolates.

***Xanthomonas euvesicatoria* populations are notably clonal irrespective of distant geographical distribution**
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Bacterial leaf spot of tomato and pepper (BLS), an economically important bacterial disease caused by four species of *Xanthomonas* [(*X. euvesicatoria* (*Xe*), *X. vesicatoria* (*Xv*), *X. gardneri* (*Xg*) and *X. perforans* (*Xp*)], is a global problem and can cause over 50% crop loss under unfavorable conditions; among the four species, *Xe* and *Xv* are prevalent worldwide. Characterization of the pathogens is crucial for management and regulatory purposes. In this study, we performed MLSA with 6 genes (*dnaA, hrcN*, *gyrB, gapA, hmbs* and *pdg*) to determine phylogenetic relationships within and among the four species using 77 strains from different serological groups and diverse geographical locations and also determined their phylogenetic positions relative to other xanthomonads. Both antibody and MLSA data showed that *Xv* was clearly separated from *Xe* and that the latter strains were remarkably clonal even though they originated from distant geographical locations. The *Xe* strains formed two separate phylogenetic groups; *Xe* group A consisted only of tomato strains whereas *Xe* Group B included strains from tomato and pepper. In contrast, the *Xv* group showed greater heterogeneity. Some *Xv* strains from South America were closely related to strains from California while others grouped closer to a strain from Indiana and more distantly to a strain from Hawaii. Using this information molecular tests can now be devised to track distribution of clonal populations that may be introduced into new geographic areas through seeds and other infected plant materials.

Phylogeny of *Dickeya zeae* isolated from different hosts and irrigation water using multilocus sequence analysis
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Soft rot *Dickeya* species (formerly known as pectinolytic *Erwinia* species) in the *Enterobacteriaceae* cause economically important diseases on vegetable and other crops. Both bacterial heart rot of pineapple and bacterial stalk rot of corn are caused by strains of *Dickeya zeae.* Recently, we found *D. zeae* associated with diseases of kale and taro, which are economically important crops in Hawaii and other areas. In this research, we used Multi-Locus Sequence Typing (MLST) to define the relationships among the strains isolated from different sources including pineapple, corn, taro and irrigation water. The housekeeping genes, *dnaA* (chromosomal replication initiator) and *gapA* (glyceraldehyde-3-phosphate dehydrogenase A), were selected based on their higher resolution capabilities for *Dickeya* species compared to the other housekeeping genes. In addition to 40 strains of *D. zeae,* 15 and 20 strains other *Dickeya* species and *Pectobacterium* species, respectively, were included in the study. The phylogenetic tree revealed a high nucleotide discrepancy among the *D. zeae* strains. The *D. zeae* strains found associated with kale and taro showed close relationships with the strains isolated from corn and irrigation water. However, strains from both kale and taro were in the same clade with strains isolated in 2006-07 from irrigation water.

Amylose-free ("*waxy*") wheat colonization by *Fusarium* spp. and response to Fusarium head blight
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Hexaploid *waxy* wheat (*Triticum aestivum* L.) has null mutations in *Wx* genes, which results in grain lacking amylose with increased digestibility, usability for specialty foods and improved self-life. The *waxy* cultivar Mattern is susceptible to Fusarium head blight (FHB) caused by *Fusarium graminearum* species complex that produces the mycotoxin deoxynivalenol (DON). In Experiment 1, conducted during low natural FHB, grain from *waxy* breeding lines, Mattern, and wild-type breeding lines and cultivars, were assessed for *Fusarium* infection and DON concentration. Nine *Fusarium* species and species complexes were detected from internally-infected (disinfested) grain; *F. graminearum*-infections were not different between *waxy* and wild-type. Surface- and internally-infected grain (non-disinfested) had more *Fusarium* isolates in *waxy versus* wild-type, but *F. graminearum*-like infections were similar; however, DON levels were higher ($P = 0.01$) in *waxy*. In Experiment 2, disease severity, *Fusarium*-damaged kernels (FDK), and DON were assessed for *waxy* breeding lines, Mattern, and wild-type cultivars during a severe epidemic. Disease severities and FDK were not significantly different from wild-type but DON was higher ($P < 0.01$) in *waxy* than wild-type lines. Across both experiments, two *waxy* breeding lines, Plant Introductions 677876 and 677877, responded similarly to FHB as moderately-resistant wild-type cultivar Overland, showing promise for breeding *waxy* cultivars with FHB resistance.

Development of a Plasma Sterilization System for Improved Storability of Paprika and Validation of its Effects
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Paprika are a important agricultural exports in Korea. Unfortunately, paprika are not suitable for long-distance exports as they do not store well. One major cause of damage to fresh agricultural produce including paprika is infection with pathogenic microorganisms that cause post-harvest diseases during storage and distribution. To address this problem, research is underway to develop a range of surface sterilization techniques. The recent confirmation of the sterilization effects of reactive oxygen species (ROS) generated from plasma discharge has attracted substantial research interest in applying the plasma technology to agriculture. As such, this study develops a plasma sterilization system with the aim of improving storability of paprika. This system consists of an atmospheric-pressure plasma generator, a plasma controller, and a chamber for plasma treatment. The sterilization effect of the present system was examined using *Erwinia carotovora* that causes bacterial soft rot in paprika. The *E. carotovora* samples were treated with plasma for 30 and 60 seconds, respectively, at the plasma power 500W, 700W, and 1,000W. It was found that the samples treated for 60 seconds showed a 100% sterilization effect. Then, to validate the effectiveness of the system in maintaining the freshness of paprika, paprika were treated at the plasma power 1,000W for 30 and 60 seconds, respectively. As a result, browning was observed in the stem end of untreated paprika due to withering, whereas this phenomenon was delayed for 26 days in the stem end of plasma-treated paprika.

Changes in the composition of wheat grain at elevated CO2 can influence Fusarium graminearum disease and deoxynivalenol contamination
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Increased photosynthetic carbon assimilation rate in C3 crops, such as wheat, at elevated atmospheric CO2 concentrations can dramatically alter grain nutritional quality. Typically, growth at elevated CO2 leads to grain with higher carbohydrate content which in turn also significantly alters the protein, mineral and lipid compositions. The production of the mycotoxin, deoxynivalenol (DON), by F. graminearum is highly dependent on the nutrient composition of its growing medium. Therefore, alterations in the nutritional composition of wheat when grown under conditions of elevated CO2 likely have a significant impact on F. graminearum infection. This investigation explores how changes in wheat primary metabolism due to elevated CO2 affect F. graminearum growth and DON production. Additionally, the impacts of growth at elevated CO2 on grain nutritional quality of moderately resistant and susceptible wheat varieties were compared and found to be significantly different.

Effects of sulfur dioxide-emitting polyethylene packaging on postharvest decay and fruit quality of blueberries
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Blueberry production has been rapidly growing in California during the last two decades. The vast majority of blueberries grown in California are destined for the fresh market, thus prolonging the storage and shelf life of blueberries is important to extending marketing opportunities and increasing exports. However, blueberries are highly perishable. Gray mold caused by *Botrytis cinerea* is one of the major limiting factors of storage and shelf life of blueberries. In addition to postharvest decay, water loss, leading to a decline in both textural and visual quality, is also a significant quality issue that arises during extended storage of blueberries. To search for methods to control decay and maintain fruit quality during storage, the effects of a newly-developed packaging that consists of SO_2-emitting low density polyethylene liners with different vent area (0.1, 0.3 and 0.9%) or in combination with modified atmosphere packaging (MAP) were evaluated on five southern highbush blueberry varieties. The use of MAP significantly reduced water loss and consequently reduced shriveled fruit compared to the liners with vent, while the use of SO_2-emitting liners effectively controlled fruit rots. The SO_2-emitting liner with MAP was the best combination to control postharvest decay while maintaining fruit quality during storage.

Genetic diversity of *Aspergillus flavus* and identification of atoxigenic isolates to reduce aflatoxin contamination of chilies in Nigeria
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The filamentous fungus *Aspergillus flavus* is the primary cause of aflatoxin (AF) contamination of several crops in warm regions of the globe. Aflatoxins are of severe health and economic concern due to their toxicity and carcinogenicity. Red chili (*Capsicum s*pp.), a widely produced and consumed spice in Nigeria is frequently contaminated by AFs. Aflatoxin contamination of chilies in Nigeria was previously attributed to *A. aflatoxiformans,* but knowledge of *A. flavus* genetic diversity associated with chilies and occurrence of atoxigenic isolates with potential for biological control of AF contamination remains unexplored. The current study examined the diversity and aflatoxigenicity of 325 *A. flavus* isolates recovered from Nigerian chilies (n=55) sampled in 2015-16. Isolates were diverse at 17 microsatellite loci, with 5 to 36 alleles per locus and 152 haplotypes. Among 120 haplotypes analyzed for aflatoxigenicity, 68 produced AFs (mean=75,365 ng/g) and 52 did not produce detectable concentrations of AFs (< 2 ng/g) on sterile maize. Isolates from 11 atoxigenic haplotypes had deletions in the AF cluster, which confirmed their inability to produce aflatoxins. Atoxigenic *A. flavus* isolates from chilies will be co-inoculated with toxigenic isolates to evaluate their potential to reduce AF concentrations in chilies. The atoxigenic isolates are a genetic resource for the development of biological control products for aflatoxin mitigation in chilies in Nigeria.

Fungal Symbionts Hold Potential for Reducing Mycotoxins and Increasing Resistance to Herbivores in Maize

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Aspergillus flavus and *Fusarium verticillioides* are well known maize ear rot pathogens and produce mycotoxins that are carcinogenic to humans and livestock. Insect damage to kernels increases the occurrence of these mycotoxins in maize. Many studies focus on the inhibition of aflatoxin production by direct competition with biocontrol agents *in vitro*. The effects of application of the endophytes to roots via seed treatment on reduction of mycotoxins are not well studied. Here, we present a study that tests the efficacy of symbiotic rhizosphere fungi on improving maize resistance to insects and to contamination with aflatoxins and fumonisins under the field conditions. Two fungal symbionts were applied as seed treatment to B73 inbred and four hybrids and the seed was planted in two locations. Aflatoxin and fumonisin accumulation and the damage by insect herbivory were quantified. Results showed a genotype- and location- dependent effect on mycotoxins and herbivory by these biocontrol candidates with potential for significant reduction of both toxins. We discuss here the potential for this novel approach to mitigate both mycotoxin contamination and yield losses by insect herbivores.

Evaluation of new germplasm associated with reducing losses associated with poor quality grain and organisms present post-harvest

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Poor grain quality resulting from delayed harvest can cause major economic losses in the U.S. soybean production system. Phomopsis seed decay, caused by Phomopis longicolla (PL), can contribute to reduced quality in harvested grain. The objective of this research was to determine differences in grain damage between soybean entries under environmental conditions conducive to effecting harvested grain quality and identify contributing organisms. Two rainout shelters were used for experiments. Each shelter contained 21 entries planted as single row plots on 76.2 cm centers and 3.6 m in length, replicated three times. Both shelters were inoculated with a PL spore suspension at R5.5. An application of 118.3 ml/ha of trifloxystrobin + prothioconazole was applied at R7 to plots in shelter 2, but not shelter 1. Shelters were overhead irrigated for 200 h. Plots were hand-harvested at R8. Symptoms associated with Phomopsis seed decay were observed on grain. Hundred kernel weights and germination percentages were determined from grain post-harvest. Significant differences were observed in the non-fungicide shelter with a 78% difference in weight between Progeny 4211 and 11030-541-210 and an 81% difference in damage between 11030-541-210 and DB06X06-093. No significant differences in plot weight, hundred kernel weight or grain damage were observed in the fungicide-treated shelter. The internal spacer region (ITS) was used to determine the fungi present on harvested grain.

Interactions among aflatoxigenic species of *Aspergillus* section *Flavi* during maize infection

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Fungi within *Aspergillus* section *Flavi* are the most important causal agents of crop aflatoxin contamination. Aflatoxins are natural carcinogens that contaminate food and feed impacting human and animal health and trade. The extent of crop contamination is partly determined by the infecting species. *A. flavus* and *A. parasiticus* are often implicated as causal agents of aflatoxin contamination. Recently, species with S morphology capable of producing high levels of aflatoxins have been identified. Several aflatoxin-producers frequently co-occur in agricultural regions and may coinfect crops. The current study assessed interaction among fungi with S morphology, *A. flavus* L strain and *A. parasiticus* on maize at 25C and 30C. Proportions of infecting mycelia and spores of each species were quantified with quantitative pyrosequencing during co-infection. *A. flavus* dominated sporulation at both temperatures. Fungi with S morphology outcompeted *A. flavus* during infection and *A. parasiticus,* both during infection and sporulation at 30C. Sporulation was reduced during coinfection, but crop contamination with aflatoxins was not significantly reduced or enhanced. Results suggest that the nature of coinfecting fungi and temperature can influence the fungal population structure, with temperatures around 30C selecting for

increases in S morphotype fungi. Efforts to develop aflatoxin management should consider influences of interacting fungi and shifting environmental temperature.

Insights on brown rot of chestnuts caused by *Gnomoniopsis* spp. in Michigan
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Post-harvest spoilage of chestnuts (*Castanea* spp.) severely impacts the chestnut industry by lowering the quality and subsequent marketability of the nuts. A 2017 survey of Michigan (MI) chestnuts identified *Gnomoniopsis smithogilvyi* in association with rot symptoms. *Gnomoniopsis smithogilvyi*, the causal agent of chestnut brown rot (CBR) is characterized by light to severe brown lesions on the endosperm and the embryo and affects the quality and storage capacity of the nuts. Prior surveys in MI have only confirmed the presence of nut rotting saprophytes such as *Penicillium* spp. and *Botrytis* spp. DNA barcoding of the ITSrDNA region of the *Gnomoniopsis*-like cultures isolated from symptomatic nuts resulted in the identification of several genotypes of *Gnomoniopsis* spp., distinct from *G. smithogilvyi*. To complete Koch's postulates we inoculated nuts from 3 cultivars of *Castanea* spp. (cv. Benton Harbor, Labor Day and Colossal) collected from trees grown in MI and Washington state with 2 strains of *G. smithogilvyi*. All nuts inoculated with *G. smithogilvyi* demonstrated CBR symptoms, however the percentage of the nut affected by rot was variable among the cultivars tested. A higher than expected level of rot occurred in control nuts, confounding informal identification of CBR versus other rots. Identification of other fungi from rotting nut tissue is currently under investigation. Understanding CBR caused by *G. smithogilvyi* and the various genotypes present in MI chestnuts will aide in the evaluation of potential pre- and post-harvest management strategies by the chestnut industry.

Comparative phenotypic analysis of *Fusarium graminearum* and *Fusarium meridionale* affecting maize and wheat in Brazil
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Fusarium graminearum with the 15-acetyl-deoxynivalenol (15-ADON) chemotype is the predominant cause of Fusarium head blight (FHB) of wheat in the U.S. and Brazil. In the U.S., the same pathogen population causes Gibberella ear rot (GER) and stalk rot (GSR) of maize, and infected maize serves as the major source of inoculum for wheat. In contrast, in Brazil *F. meridionale* with the nivalenol (NIV) chemotype is the main cause of GER and GSR. We hypothesized that this shift in dominance on maize is explained by greater fitness of *F. meridionale* (which doesn't occur in the U.S. on that host). A collection of Brazilian isolates of *F. graminearum* (10 from wheat and 10 from maize) and *F. meridionale* (10 from wheat and 10 from maize) were compared for several traits that are potentially relevant to fitness including aggressiveness to maize ears and stalks and to wheat heads; mycelial growth rate; macroconidial, perithecial, and ascospore production; and fungicide sensitivity. The two species could not be differentiated for aggressiveness to maize: there was more variation within species than between species for this trait. A few traits were more closely associated with species: most strains of *F. meridionale* were less aggressive on wheat, and produced fewer macroconidia, perithecia, and ascospores in culture, than most strains of *F. graminearum*. The goal of our studies is to improve management of FHB, GER, and GSR in Brazil, accounting for differences from U.S. conditions.

Incidence of potentially toxigenic *Fusarium* spp. on landraces of corn on Mexican highlands pathosystems
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Corn is a main crop in Mexico, where 69 landraces are cultured by peasants in a non-synthetic crop improvement, obtaining seed-grain for autoconsumption. Corn agriculture has developed pathosystems in which phytopathogenic fungi, including *Fusarium* spp. could damage seeds on their physiological and food qualities due to mycotoxins contamination. Therefore, this study estimated the association between their incidence and 21 corn native populations from two states of Mexico: Mexico and Tlaxcala, in which five races were previously identified by morphometric characteristics. They were microbiologically analyzed, using the freeze blotter test with subsamples of 50 grains and four replications, from 10 ears, identifying and quantifying incidence of: Number of Phytopathogenic Fungi Genera (NPFG), *Fusarium graminearum* (FG) and *Fusarium verticillioides* (FV). Additionally, germination tests and ELISA (AgraQuant®, Romer-Labs.) tests were applied to detect contamination of Deoxynivalenol (DON), Zearalenones (ZON) and Fumonisins (FUM). Main Components (MCA) and Cluster Analysis (CA) estimated the association among those variables (Minitab, 16.0). CA generated seven groups of landraces revealing a differential incidence between FG (0- 31%) and FV (21.5-62.5%) (r = - 0.225*). The NPFG were 2-8 genera biodiverse. MCA associated FV (32.17%), FG (22.33%), germination 74.3%, DON (1.640 ppm), ZON (0.330 ppm) and Cacahuacintle landrace in Mexico State; FV (50.13%), FG (9.5%), germination 93.5%, DON (0.400 ppm), ZON (0.000 ppm) and Cónico landrace in Tlaxcala. FUM was not detected.

Effect of 1,4-dimethylnaphthalene on fungal and oomycete storage pathogens of potato tubers
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Potato tubers to be used for the fresh market or processing are treated with inhibitors in storage to prevent sprouting and protect tuber quality. One of the currently used sprout inhibitors, the natural product 1,4-dimethylnaphthalene (1,4-DMN), is thought to also enhance

skin set and have antimicrobial activity, both of which may help protect tubers from storage diseases. 1,4-DMN was tested for antimicrobial activity against several post-harvest tuber pathogens. These included *Fusarium sambucinum*, *F. oxysporum*, *F. avenaceum*, *Helminthosporium solani*, *Phytophthora infestans* and *P. eythroseptica*. Cultures of each pathogen was exposed for 24 hours to 20 ppm 1,4-DMN (the concentration used in commercial storages) in sealed jars, and growth rates were subsequently measured over time starting at 24 hours. Growth of all pathogens, except *P. infestans*, was reduced, but none were completely inhibited. Pre- or simultaneous treatment of potato tissue with 1,4-DMN had no effect on infection by *F. sambucinum*, *F. oxysporum*, *F. avenaceum* or *P. erythroseptica* indicating no direct effects on the pathogen or any resistance inducing effects. Tubers treated with 1,4-DMN in a simulated storage experiment showed no reduction in the amount of silver scurf, caused by *H. solani.*

Pre-harvest monitoring of postharvest rot pathogens in European pear

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Several pathogens are involved in postharvest rot diseases in pears that affect both consumption and marketability of fruits. While some pathogens infect through wounds at harvest, some pathogens establish earlier in the season and remain latent until storage. A study was conducted to understand the seasonal prevalence of these pathogens in two pear orchards in southern Oregon. The samples were collected at pink bud, full bloom, petal fall, fruitlets, and field bins. Four pathogens previously reported as postharvest rot pathogens, *Botrytis cinerea, Penicillium expansum, Cladosporium herbarum,* and *Alternaria alternata* were identified. At pink bud stage, all the pathogens were isolated in equal frequencies. At full bloom until fruitlets stage *A. alternata* was most frequently isolated, followed by *B. cinerea* and *C. herbarum.* From the field bin samples, *C. herbarum* were frequently isolated. Several other fungal species were isolated along with the pathogenic species, the identity of which were established based on sequencing of the ITS and elongation factor 1-alpha genes. They were grouped into thirteen unique species, pathogenicity of which were tested in wound inoculated fruits. Out of thirteen, three new species, *Dothiorella iberica, Diaporthe rudis, and Fusarium avenaceum* were identified as pathogenic on wound inoculated fruits. The three new pathogenic species were present at full white, full bloom, and petal fall stages. This study suggests the possibility of developing postharvest rot disease management programs earlier in the fruit developmental stages.

Determining a threshold for use of deoxynivalenol (DON) contaminated durum wheat as seed

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North Dakota is the top producer of durum wheat in the United States, harvesting just over 1 million acres in 2018. Epidemics of Fusarium Head Blight (FHB) in recent years have been a severe constraint to production, resulting in mycotoxin contaminated grain unsuitable for use as food or feed. Use of contaminated grain as a seed source is not recommended, due to poor germination and plant vigor. However, data outlining tolerable levels of mycotoxins, functionally corresponding to levels of fungal contamination, in grain used for seed is lacking. Field evaluations of durum seed contaminated with varying levels (< 0.3 – 19.9 ppm) of the mycotoxin deoxynivalenol (DON) were conducted over two years. Treatments were split to assess the effect of the seed applied fungicide, tebuconazole, on plant population and yield. Seeding rates were adjusted to account for poor germination. Seed with greater than 10 ppm DON resulted in stand reductions in one out of two years. Treatments seeded with grain containing 19.9 ppm DON resulted in a 5.3 bu/ac yield decrease in one year of the study. Tebuconazole seed treatment had no effect on plant population or yield. Our data suggest that grain containing less than 10 ppm DON may be used as seed without yield loss. The effect of such a practice within a crop cropping system is still unclear.

Efficacy of pesticides for mitigating stink bug feeding injury and associated mycotoxin contamination in field corn

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Previous studies demonstrated associations between stink bug feeding and mycotoxins in corn and potential vectoring of mycotoxigenic *Fusarium* spp. by stink bugs. The objective of this study was to assess efficacy of pesticides for mitigation of stink bug-associated mycotoxin contamination. Fungicide (prothioconazole) and insecticide (bifenthrin) were applied to corn plots either individually or in combination at silking. A second experiment included prothioconazole treatment as the main plot, and subplot treatments (untreated, stink bug, *Fusarium*, and *Fusarium* + stink bug) were applied to bagged ears. Following harvest, stink bug feeding injury was visually assessed, and mycotoxins were quantified using Neogen Reveal Q+ kits. In the first experiment, prothioconazole and bifenthrin reduced kernel injury but not mycotoxins. However, there was a positive correlation between levels of stink bug feeding injury and concentrations of fumonisin and aflatoxin. In non-fungicide treated plots of the second experiment, kernels from stink bug + *Fusarium* treatment had higher fumonisin compared to kernels from stink bug only, *Fusarium* only, or non-treated corn ears (P= 0.004). However, in prothioconazole treated plots, application of stink bugs and *Fusarium* did not increase fumonisin (P=0.103). Results indicate both fungicide and insecticide may reduce stink bug associated mycotoxins in corn, but additional tactics are needed when contamination risk is high.

Effect of soil moisture on sporulation of two aflatoxin biocontrol products at different temperature regimes under controlled environments

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Aflatoxins, toxic metabolites produced by *Aspergillus* section *Flavi*, occasionally contaminate tree nuts in California, but due to strict regulations, they pose a high risk to international trade. The use of atoxigenic strains of *A. flavus* is a proven and registered method for aflatoxin control, but its efficacy depends on its timely sporulation and dispersal for toxigenic strains displacement. *A. flavus* AF36 Prevail sporulates abundantly in Californian orchards at temperatures over 25°C. Earlier applications could be more effective, but it is unknown if the product will sporulate sufficiently. The effect of soil moisture and low temperatures on sporulation of *A. flavus* AF36 Prevail and Afla-Guard were evaluated in pots with soil in a growth chamber. Soil moistures were based on field capacity (FC) and temperatures were set according to maximum and minimum temperatures from May to July in the Central Valley, CA. Sporulation was determined by the percent of sporulating grains and a sporulation index (0-4). Results indicate that sporulation of AF36 Prevail is delayed under low soil moisture and low temperatures, while Afla-Guard has good sporulation even at minimum temperatures of 15°C. AF36 Prevail has sporulation >80% and an index >1 at 16% to FC (21%) soil moistures and minimum temperature ≥25°C, while Afla-Guard reached these levels at the lowest temperatures. Good sporulation at low temperatures in earlier applications could be more effective on aflatoxin reduction.

Preliminary study on exogenous NAD^+-induced transcriptome changes in vegetable crops by RNA-seq

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Beta-nicotinamide adenine dinucleotide (NAD^+) is a ubiquitous electron carrier involved in many metabolic pathways. In the greenhouse, NAD^+ was confirmed for its potential to reduce disease severity of powdery mildew (*Podosphaera xanthii*) on squash and halo blight (*Pseudomonas syringae* pv. *phaseolicola*) on snapbean. However, the underlying molecular mechanisms in these crops following NAD^+ treatment remain unknown. RNA-seq has been developed to investigate a variety of biological responses by measuring the abundance of different RNAs. In this study, we monitored the change of genes in plants after NAD^+ treatment by RNA-seq. True leaves of both crops were collected 4 h after NAD^+ or water (mock) treatment. Total RNA was extracted and subjected to RNA-seq assays. Preliminary data analysis revealed that 15344 and 7552 genes were upregulated, and 8479 and 12502 genes were downregulated in squash and snap bean, respectively. These results indicate that exogenous NAD^+ induces profound transcriptome changes in both crops. Meanwhile, 25 and 13 lectin receptor kinase (*LecRK*) genes, which are homologous to the *Arabidopsis* extracellular NAD^+ ($eNAD^+$) receptor gene *LecRK-I.8*, were upregulated in squash and snap bean, respectively. These results suggest that squash and snap bean may also use LecRKs to sense $eNAD^+$. Further studies will focus on RNA-seq analysis of the kinetics of NAD^+-induced transcriptome changes and identification of the $eNAD^+$ receptors in squash and snap bean.

Pathogenic protein networks in *Phomopsis longicolla* underlying seed decay of soybean

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Phomopsis seed decay (PSD) is an economically important seed disease in soybean, *Glycine max* (L.) Merrill. This disease is primarily caused by a seed-borne fungus, *Phomopsis longicolla* T. W. Hobbs (syn. *Diaporthe longicolla*). Information about the pathogenic mechanism of the fungus is lacking. The objectives of this study were to analyze protein-protein interactions (PPI) and to identify conserved global networks and pathogenicity subnetworks in *P. longicolla*, which include orthologous pathways for cell signaling and pathogenesis. Genome-wide analysis of the predicted protein interactome with the interlog method revealed 215,255 unique PPIs among 3,868 proteins. A total of 1,414 pathogenicity related genes in *P. longicolla* were also identified using the pathogen host interaction database. In addition, 149 plant cell wall degrading enzymes (PCWDE) were detected. The most predominant class of PCWDE was a group of 60 glycoside hydrolase proteins. The glycoside hydrolase subnetwork was found to be interacting with 1,442 proteins. The orthologous proteins FUS3, HOG, CYP1, SGE1, and the g5566t.1 gene identified in this study could play an important role in pathogenicity. The *P. longicolla* protein interactome generated in this study can enhance our knowledge about PPIs in soybean pathogens and aid in developing new strategies for disease control.

***Maize stripe tenuivirus*, full genome sequence completed after nearly half a century of research**

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Since the early 1970s, *Maize stripe tenuivirus* (MSpV) has been reported to infect maize and other monocots around the world. MSpV was formally accepted as a species by the ICTV under the name Maize stripe virus in 1991 and finalized in 2018 as *Maize stripe tenuivirus*. MSpV has a multipartite genome comprised of four or five negative and ambisense RNAs. Nucleotide sequences of RNAs 2-5 were completed in the 1990s, but no complete genomic sequence of any MSpV isolate had been determined. We completed the full genomic sequences, including RNA1, of five MSpV isolates using high throughput sequencing, and partially verified them by Sanger sequencing. One isolate was from maize in Florida. Two maize and two *Rottboellia cochinchinensis* (itchgrass) isolates were from Papua New Guinea (PNG). Interestingly, only the isolate from FL had five RNAs. All four isolates from PNG lacked RNA5. All PNG isolates grouped together and distinct from the FL isolate in phylogenetic comparisons of RNAs 1-4. On the whole genome level nucleotide identities among the PNG isolates ranged from 97-98% for RNA 4 to 99-100% for RNA1. The nucleotide identity comparisons between each of the PNG isolates and the FL isolate was 89% for RNAs 1-2, 78% for RNA3, and 82-84% for RNA4. This research provides evidence of MSpV genomic diversity. The complete genomic sequences of multiple isolates are useful to better understand MSpV evolution and diversity and for developing diagnostic protocols.

Elucidating the barley metabolome during tissue-specific *Xanthomonas* infection
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The plant pathogenic bacterial species *Xanthomonas translucens* comprises diverse strains that cause vascular and non-vascular symptoms of barley. *Xanthomonas translucens* pv. undulosa (*Xtu*) infects the non-vascular, mesophyll tissue, while *X. translucens* pv. translucens (*Xtt*) colonizes, the water-transporting xylem vasculature. It remains unknown how these pathogens modify the plant environment during infection to get host nutrients. The aim of this study was to define the barley metabolites associated with the tissue-specific host colonization. Through barley metabolic profiling, we compared the healthy apoplastic and xylem fluids against fluids from apoplast infected with *Xtu* and xylem infected with *Xtt*. We described the metabolic changes in barley tissues caused by both pathogens during colonization. This work provides a model and intellectual framework to determine the basis tissue-specific behavior of vascular and non-vascular pathogens of important crop hosts.

Genomic characterization of beet oak-leaf virus: A putative member of the family *Rhabdoviridae*
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Beet oak-leaf virus (BOLV) is a soil-borne virus that induces oak-leaf shaped patterns on sugarbeet leaves and has been identified serologically from most US production regions. Symptomatic leaves collected from a sugarbeet plant at the USDA research station in Salinas, CA in 2016 were confirmed to be infected with BOLV by ELISA using antiserum previously developed against the original isolate of BOLV. To determine the sequence of BOLV, total RNA was extracted from leaves of this plant and examined by high throughput sequencing (HTS). HTS results revealed the presence of a varicosa-like virus, as well as *Beet cryptic virus 1*. Primers were designed to the varicosa-like virus sequences and used in RT-PCR, followed by direct sequencing, to obtain further genome sequence information. RT-PCR also confirmed the presence of the varicosa-like virus in the original extract used for HTS, and in archived, purified RNA samples of BOLV collected between 2008 and 2012. All amplicons shared 99-100% sequence identity, confirming the sequenced virus as BOLV. The L protein encoded by RNA1 shares 39% amino acid identity with that of *Lettuce big vein associated virus* (LBVaV; genus *Varicosavirus*, family *Rhabdoviridae*), its closest known relative, whereas the coat protein encoded on RNA2 shows only 30% identity with the LBVaV coat protein. These results demonstrate that BOLV is a new member of the family *Rhabdoviridae*, and a putative divergent member of the genus *Varicosavirus*.

Mitochondrial genome of *Phytophthora sansomeana*, a soybean root rot pathogen
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Phytophthora sansomeana is one of two pathogens that cause Phytophthora root rot of soybean. While much molecular and genomic information is available about the other pathogen, *P. sojae*, only sparse information in these areas about *P. sansomeana* is known. We initiated an effort to sequence and annotate the genome of *P. sansomeana*. Here we report the assembly and annotation of its mitochondrial genome. Sequenced using PacBio technology and error-corrected using Illumina sequences, the complete mitochondrial genome was assembled into a circular molecule of 39,618 bp, with 22.03% GC content. It encodes 39 protein coding genes, 25 tRNA genes, and two ribosomal RNA genes (rnl and rns). The protein coding genes include 14 genes in the respiratory complexes (cob, cox1, cox2, cox3, nad1, nad2, nad3, nad4, nad4L, nad5, nad6, nad7, nad9, and nad11), four ATP synthase genes (atp1, atp6, atp8 and atp9), 16 ribosomal protein genes (rpl2, rpl5, rpl6, rpl14, rpl16, rps2, rps3, rps4, rps7, rps8, rps10, rps11, rps12, rps13, rps14 and rps19) and five other genes. The tRNA genes encode transfer RNAs for 19 essential amino acids except threonine. Further analysis of this mitochondria genome will be presented.

Comparative Analysis of High-Throughput Sequencing Data Analysis Methodologies and Conventional Testing for the Identification of Quarantine Pathogens
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The USDA-APHIS Plant Germplasm Quarantine Program (PGQP) uses conventional testing (i.e. molecular, serological, biological indexing) and contemporary high-throughput sequencing (HTS)-based methods for the identification of viruses and virus-like pathogens in imported plant germplasm. Implementing HTS-based technologies, such as total RNA metagenomic sequencing, for pathogen diagnosis enables rapid detection and identification of quarantine pathogens. To test the efficacy of these diagnostic methods, we compared the diagnostic capabilities of conventional testing versus HTS data analysis pipelines using Poaceae quarantine germplasm. The HTS-based bioinformatics analyses included two approaches: 1) read assembly and subsequent BLAST-based pathogen detection pipeline; and 2) read mapping-based pathogen detection pipeline. By comparing the results for 142 quarantine Poaceae accessions, we demonstrated that HTS-based methods were capable of detecting all expected viruses in addition to novel viruses undetected by conventional testing. Comparable results were obtained for the detection of 20 of the 24 viral pathogens when tested with conventional testing and the BLAST-based HTS approach. The remaining four viruses were only identified by HTS. The read mapping-based HTS pipeline outperformed the BLAST-based HTS pipeline in detecting novel viruses and quantifying viral titer. These results demonstrate the effectiveness of HTS data analysis pipelines as a robust method for detecting plant viruses that can enhance our ability to diagnose viral pathogens of quarantine significance.

Genome and transcriptome analyses of the brown rot fungus, *Pyrrhoderma noxium*, reveal potential enzymes that degrade wood from diverse host trees
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Pyrrhoderma noxium (Corner) L.W. Zhou & Y.C. Dai, comb. nov. (formerly known as *Phellinus noxius*) is a destructive root decay pathogen that affects a wide variety of host trees in pan-tropical areas, including Asia, Oceania, and Africa. The ability of *P. noxium* to infect a broad host range implies a dynamic pathogenicity strategy that warrants investigation of gene expression related to pathogenic lifestyle, such as genes associated with plant cell wall degradation. The genome of *P. noxium* (isolate P919-02W.7 from Pohnpei State, Federated States of Micronesia) was sequenced and the gene expression profile was analyzed using different host wood substrates [ponderosa pine (*Pinus ponderosa*), mahaleb cherry (*Prunus mahaleb*), black willow (*Salix nigra*), and sugar maple (*Acer saccharum*)]. The assembled genome was 33.9Mbp and 2,954 contigs and contained 9,389 predicted genes. Only minor differences were observed in size and gene content in comparison with other *P. noxium* genome assemblies. Transcriptome analysis revealed 488 enzymes related to carbohydrate metabolism, many of which degrade plant cell wall components. This study provides the second annotated genome of *P. noxium* and highlights the vast suite of decomposing enzymes of *P. noxium*, which suggest a potential for degrading wood from diverse host trees. This information contributes to our understanding of pathogen ecology and lifestyle.

Deciphering the pangenome of *Macrophomina phaseolina* populations from Ohio and Paraguay
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Macrophomina phaseolina is responsible for significant yield loss in soybean worldwide and is capable of infecting more than 500 plant species including maize. Despite its broad host range, *M. phaseolina* reproduces exclusively through asexual means. The objectives of this study were to phenotype and genotype populations of *M. phaseolina* associated with soybean in order to understand the genetic basis of adaptation in this asexual pathogen. A total of 200 *M. phaseolina* isolates were obtained from soil collected from soybean and corn fields in Ohio and 300 isolates from soybean fields in Paraguay. We assessed pathogenicity on soybean and *in vitro* growth at various temperatures, and then sequenced the genomes of 12 selected isolates using a combination of short and long read technologies to obtain near-chromosome level assemblies. By building SNP-based phylogenies derived from whole genome alignments, we identified two major populations: one containing only isolates from Ohio and another containing isolates from both Ohio and Paraguay. Although all 12 isolates were nearly identical (>97%) at the nucleotide level, we found that genome size differed by as much as 25%, and predicted gene number varied by 30%. We also found that *M. phaseolina*'s pangenome is more open than the pangenomes of other obligate asexual fungi, suggesting that this pathogen has retained mechanisms for generating genotypic diversity despite its obligate asexuality.

Proteomic Analysis of the *Acidovorax citrulli* during the Viable but Nonculturable State and Resuscitating Process
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Bacterial fruit blotch (BFB) is a serious threat to cucurbits production worldwide. Its causal agent, *Acidovorax citrulli*, infects many cucurbits crops including watermelon and melon. A previous study showed that *A. citrulli* can enter into a viable but nonculturable (VBNC) state following exposure to copper sulfate and those VBNC cells can be resuscitated by addition of EDTA solution. VBNC *A. citrulli* cells challenged the traditional culture-based detection method and the resuscitated cells could be a great risk to the cucurbit production. Since copper-based bactericides are widely used for BFB management, a good knowledge of mechanism in *A. citrulli* VBNC state is quite important.
In this study, protein profiling in log-phase, copper-induced VBNC state and four stages of resuscitating *A. citrulli* cells was analysis by isobaric tags for relative and absolute quantification (iTRAQ) approach. A total of 60 proteins were differentially expressed in VBNC cells compared with log-phase cells, and 469 proteins were differentially expressed in resuscitated cells compared with VBNC cells. Expression levels of 15 in 18 selected genes tested by qPCR were consistent with the protein levels in the iTRAQ experiment.
Many proteins were associated with induction and resuscitation VBNC state in *A. citrulli*. In the VBNC state, energy generation associated proteins and a copper translocating P-type ATPase was remarkably enriched. While in the resuscitating process, the energy production proteins played an important role, as well as oxidative stress response and toxin-antitoxin system proteins.

Phylogenomic and pangenomic analyses to understand the evolutionary history of Claviceps purpurea and the Claviceps genus
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The genus Claviceps includes strict ovarian parasites of Poaceae. Some species, such as C. purpurea are defensive mutualists or weak pathogens which can infect 400+ species while others, like C. africana are aggressive pathogens and have a restrictive host range. Recent taxonomic advances in this genus have delimited cryptic species. The genus Claviceps represents an excellent system to study the evolution of fungal species that share identical infection patterns, but vary in aggressiveness and host range. The goal was to annotate the genomes of 21 Claviceps species and 25 isolates of C. purpurea to better understand the recent divergence in host adaptation and to identify genes under selective pressure to understand the evolutionary forces shaping the genus. A phylogenomic

analysis of 2,722 orthologs showed strong support for evidence of cryptic species within the genus and divergence of clade Claviceps that have overlapping host range. This suggests that host specialization might not be a factor for the divergence of cryptic species within C. purpurea. A pangenome construction revealed core (6652,481,118,384), accessory (2304,203,126,56), and singleton (817,27,17,5) gene, secreted gene, putative effector, and 2° metabolite ortholog clusters, respectively. In addition, we found clusters of redundant secreted metabolites and effectors that are present in clade Claviceps, but absent in clades with reduced host range. These results, suggests that creativity of secondary metabolite production may be responsible for the success of C. purpurea and its cosmopolitan distribution.

RNASeq: Virus infection alters terpenoid biosynthesis in *Chenopodium quinoa*
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Saponins of *Chenopodium quinoa* (quinoa) are triterpene glycosides synthesized primarily via the mevalonate pathway. Cultivars with high saponin content are bitter and must be processed to render seeds of the pseudograin palatable. There is no information on the modulation of terpenoid biosynthesis in quinoa in response to pathogens. The objective of this study was to determine if *Cucumber mosaic virus* (CMV) infection impacts regulation of saponin biosynthesis in quinoa. Seven-week-old seedlings of cultivars with different saponin content in seeds (sweet 'Jesse', medium 'QQ74' and bitter 'Red Head') were mock inoculated or inoculated with CMV. Leaves were harvested at 1 or 4 days post inoculation (dpi) and used for RNASeq library preparation. Raw data were trimmed and mapped to quinoa reference genome. Differentially expressed genes were identified by DESeq2 and RT-qPCR. At 1 dpi, triterpene saponin biosynthesis activating regulator 1 (TSARL1) was increased in all cultivars, but at 4 dpi, both TSARL1 regulation and virus symptoms in the high saponin cultivar differed from those with lower saponin. In 'Red Head', CMV infection was systemic and TSARL1 gene was downregulated at 4 dpi, whereas in the lower saponin cultivars, 'Jesse' and 'QQ74', there was a local lesion response and the TSARL1 gene was upregulated. The role of saponin in tolerance to CMV is uncertain, and more research is needed to ascertain relationships among these compounds and virus infection.

Copy number variation occurs in all four clonal lineages of the sudden oak death pathogen *Phytophthora ramorum*
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Phytophthora ramorum causes sudden oak death throughout the American west coast, where it infects tan oak, live oak, and bay laurel, as well as the UK, where it infects Japanese larch. Copy number variation (CNV) has been implicated as contributing to pathogenic phenotypes in clonal pathogens including *P. ramorum*, but it has not been determined how extensive CNV may be in *P. ramorum* populations. In order to explore this topic we first updated the genomic reference by sequencing Pr-102 using Pacific Biosciences technology. We then downloaded 189 read libraries from the SRA and sequenced 11 genomes from southern Oregon to explore copy number variation within three of the clonal lineages (NA1, NA2, EU1) as well as variation in southern Oregon. The updated reference resulted in a 75,183,546 bp assembly with no gaps, over 20 Mbp longer than the reference produced by the JGI using Sanger sequencing which was shorter and contained gaps. The assembly had a N50 of 1,287,631 indicating the fewer contigs were longer than the JGI reference that had a N50 of 308,042. While most isolates were observed to be predominantly diploid, copy numbers of three, four, and possibly five were observed for large contiguous regions in many but not all strains. CNV may thus be the predominant form of genetic diversity generation in clonal lineages. Our results suggest that CNV occurs in almost all lineages of *P. ramorum* and may play an important role in *P. ramorum* epidemics worldwide.

Virome of *Macrophomina phaseolina* collected from soybean fields in Mississippi
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In this study we investigated the "virome" of *Macrophomina phaseolina* (MP), an important and polyphagous phytopathogenic fungus causing "charcoal rot" disease in soybeans. In order to characterize community of viruses with RNA genomes in this host, total of 35 MP isolates collected from soybean fields in Mississippi were isolated in pure culture on PDA plates, submitted to dsRNA extraction, library preparation and subsequent custom-based pair-end Illumina sequencing. The majority of MP isolates examined in this study revealed presence of complex patterns of high molecular weight dsRNA molecules, suggestive of multiple infections. Presence of viruses was confirmed by transmission electron microscope observations of putative viral particles in partially purified and negatively-stained preparations from several dsRNA-containing fungal isolates. Computer-based assembly of Illumina-generated raw sequence data resulted in thousands of virus-specific contigs of different size. *In silico* analyses revealed presence of several viruses recently reported from this hosts, along with dozens of new ssRNA and dsRNA viruses belonging to currently recognized genera and/or families. In addition to characterization of new viruses, we completed genome sequences of several known viruses and investigated their population structure and distribution in studied MP isolates. Several viruses characterized in this work differ from known viruses in their genomic properties and may represent founder members of new taxa. These results further the knowledge about general mycovirus diversity.

Assessing the bias in gene content from resequenced Phytophthora infestans strains where reads do not map to the reference genome
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Next generation sequencing reads that fail to map against a reference genome are often neglected in the study and no further analysis is done. There is growing evidence that the reads which fail to map to a reference genome might provide insight into the biological functions. Thus, we hypothesized that unmapped reads represented the uniqueness of the strain and could be assembled to predict strain specific genes. We first mapped the short reads from 22 *Phytophthora infestans* isolates to the *P. infestans* reference strain T30-4 using bowtie2 and filtered the unmapped reads. The number of reads that did not map to the reference strain ranged from 3-58%. We then performed *de novo*assembly with the velvet assembler using those unmapped reads for each isolate and predicted the gene content using AUGUSTUS. Assembly size ranged from 1.43 - 16 Mbp and the gene content ranged from 394-3938. Orthofinder was used to identify orthologous gene clusters along with strain specific genes. We were able to identify 62 clusters with genes present from all 22*P. infestans* isolates anda total of 1554 singletons. These newly identified genes had orthologs in other *Phytophthora* species which suggests the unmapped reads are not just contaminations and can be used to improve the reference genome.

Comparative genome analyses of four rice-infecting isolates of *Rhizoctonia solani* belonging to anastomosis group 1-intraspecific group IA (RsAG1-IA)

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Rhizoctonia solani is a species complex composed of numerous isolates capable of infecting a wide range of economically important crops worldwide. Isolates belonging to *R. solani* anastomosis group 1-intraspecies group IA (AG1-IA) cause rice sheath blight (ShB), which significantly reduce the quantity and quality of rice production, threatening global food security. In this study, we sequenced the genomes of four *Rhizoctonia solani* AG1-IA isolates, namely B2 (USA), ADB (India), RNR (India) and YN-7 (China), using PacBio de novo sequencing and Illumina resequencing approaches. We obtained four high-quality genome sequences, comprised of 96 to 413 scaffolds with N_{50} values ranging from 1.21 Mb to 1.56 Mb. Genome synteny of approximately 66.4% to 70.9% was observed upon pairwise genome alignments, utilizing the B2 genome as the reference. Moreover, the genomes were predicted to harbor 9,722 to 11,505 putative protein coding genes, wherein 5,777 genes were identified to be shared by all four rice-infecting *R. solani* isolates. In addition, enrichment of polysaccharide lyase families (PLs) classified under carbohydrate-active enzymes (CAZymes) and pectin-degrading enzymes were observed, as well as the presence of monocot cell wall-degrading enzymes, which may have allowed the adaptation of rice-infecting *R. solani* isolates to their monocot host, rice. The outcomes of our study will enrich the *R. solani* genomic resource and allow better understanding of the pathogenicity of the rice sheath blight pathogen.

Host range and molecular characterization of two novel Ralstonia phages - RsoP1EGY and RsoP1IDN

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Bacterial wilt caused by *Ralstonia solanacearum* is one of the most devastating bacterial diseases in tropical, subtropical, and temperate regions of the world. Two lytic *R. solanacearum*-infecting phages isolated from soil in Egypt and Indonesia, respectively, were characterized in this study. They were designated Ralstonia phage RsoP1EGY and RsoP1IDN based on our phage identifier naming approach to reflect the phages' bacterial host species, characteristics and origins. Both phages have typical podovirus morphology and are similar in genome size. They are different, however, in host specificity and share no sequence homology. Genomic analysis revealed that phage RsoP1EGY has a dsDNA genome of 41,297 bp, contains 50 annotated open reading frames and has no significant sequence identity to any other reported phages. Phage RsoP1IDN has a linear dsDNA genome of 41,135 bp with 413-bp terminal repeats, and 41 open reading frames. The genome of RsoP1IDN was most closely related to that of Ralstonia phage RSB1, but different in that it contains a putative HNH homing endonuclease. When tested for host specificity, phage RsoP1EGY specifically infected only race 3 biovar 2 phylotype IIB sequevar 1, not non-race 3 biovar 2 strains of *R. solanacearum*, while phage RsoP1IDN had a narrow host range infecting only 3 out 16 tested strains. RsoP1EGY and RsoP1IDN are the first sequenced and characterized *R. solanacearum* phages isolated from Egypt and Indonesia respectively.

Comparative genomic and phylogenomic analyses of the sweetpotato soil rot pathogen, *Streptomyces ipomoeae*

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Previous phylogenomic analysis suggested that the common scab pathogen *Streptomyces scabiei* and the soil rot pathogen *Streptomyces ipomoeae* (*Si*) are derived from a shared pathogenic ancestor. Despite this relationship, these pathogens differ substantially in host range, virulence factor production and associated disease symptoms, and it is our hypothesis that such differences are related to niche development by *Si*. *S. scabiei* contains pathogenicity islands at conserved loci, including a mobile island at *aviXI*, which contains genes (*txt*) for production of the phytotoxin thaxtomin. To gain insights into the *Si* genome, here we have performed comparative analyses involving both virulent and avirulent strains of *Si*, as well as other plant pathogenic and saprophytic *Streptomyces* spp. We found that the *txt* cluster has apparently become fixed within the *Si* genome separately from *aviXI*, where a small excisable island still exists. Using ortholog gene searches, we have defined a *Si* pathogen-specific genome (*Si*PSG), which comprises 153 ortholog groups. It is anticipated that genes within the *Si*PSG contribute to the unique aspects of soil rot disease. Comparative *txt* gene analysis has provided evidence

that thaxtomin production in *Si* is under unusually strong regulation by *bldA*, a major regulator of secondary metabolism in the genus. Overall, the results here will facilitate elucidation of the mechanisms governing virulence in this novel *Streptomyces* plant pathogen.

Monilinia comparative genomics for pathogen diagnosis using gene based markers

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Monilinia species causing disease on stone and pome fruits have overlapping and similar host-ranges, disease symptoms and morphology. It can be difficult to reliably differentiate among native and exotic (regulated) species. HTS sequencing (Third Generation Sequencing; TGS) uses single molecule templates that have a higher error rate than alternatives, but that provide longer read lengths. TGS long reads can be used to create draft genomes with better assembly and increased potential for the discovery of structural variations and diagnostic markers. To better characterize a *Monilinia* genome, we used TGS to sequence an isolate of *M. fructigena* (Mfg5-SP-A). This is an exotic pathogen under regulatory control for the United States. We generated a PacBio and Mi-Seq hybrid genome assembly, then annotated the genome to identify unique targets between *Monilinia* species. We used comparative genomics with a custom *Monilinia* Genomics Database (MGDb) to predict more than 9600 genes in *M. fructigena*. We identified approximately 350 genes that represent potential diagnostics targets to distinguish among the especially closely related *M. fructigena* and *M. polystroma*. A more exhaustive comparison suggests that the number of truly discriminating targets may be considerably lower than those ~350. Our analysis for unique and common predicted genes shows all possible logical relations between 4 *Monilinia* species (two native and two exotic) that could be used to identify DNA markers and functional genes with potential to be used for diagnostic purposes.

***S.* Typhimurium strain and tomato genotypes affect the persistence of *Salmonella* in tomato plants**

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Little is known about the genes essential for the persistence of *Salmonella* in plant tissues. Our preliminary studies showed that *S. enterica* subsp. *enterica* serotype Typhimurium (MDD14) displayed a lower persistence in contaminated tomato leaf tissues compared to the six other *S.* Typhimurium strains tested (JSG626, JSG210, JSG3444, JSG637, JSG634, and EV030415). The reduced persistence of MDD14 in tomato plants was more attenuated in 'Tiny Tim' plants compared to the other four varieties ('Saladette', 'mountain Spring', 'OH4292', and 'Celebrity' varieties) tested. *In vitro* experiments showed that MDD14 had an equivalent growth rate and motility, and significantly higher abundance of biofilm compared to the other strains when grown in Luria-Bertani or M9 minimal medium at 28°C (P value less than 0.05). The whole genome of the seven *S.* Typhimurium strains was sequenced using Illumina paired-end MiSeq sequencing. The raw reads were quality checked, assembled, and annotated using FastQC, SPAdes/QUAST, and RAST, respectively. Preliminary results of the annotated genomes showed that only 1.84% of protein-encoding genes were inconsistent between strains. Interestingly, only 5 genes encoding hypothetical proteins were missing in MDD14 compared to other strains. Studies are underway to delineate the role of these unique proteins in S. Typhimurium persistence. In summary, our study suggested that the genotypes of *Salmonella* and tomato verities affect the persistence of *Salmonella* in tomato plants.

High-throughput sequencing of *Phytophthora ramorum* Ex-type using long-read MinION nanopore sequencing

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Sequence information of type specimens is critical in order to make correct molecular identification and develop robust diagnostic systems. *Phytophthora ramorum* is one of the species of biosecurity concern in the United States, United Kingdom and other areas of the world. Due to the significance of the species there are currently 22 genome sequences for isolates of *P. ramorum* deposited at the NCBI, however a whole genome sequence for the Ex-type of *P. ramorum* is not available. Here we report the whole-genome sequence for the Ex-type of *Phytophthora ramorum* generated using High Throughput Sequencing on the MinION platform from Oxford Nanopore Technology. The quality filtered 625,764 reads were assembled in to 1322 contigs indicated extended genome size of 52.4 Mb. We analyzed our Nanopore assembly of the *P. ramorum* (CPHST-BL 55G which is a duplicate of specimen P10103) with another isolate CC1011 of EU1 lineage available at the NCBI, and found 251,859 quality Single Nucleotide Polymorphisms (SNPs) genome-wide. When compared to the genome of isolate SOD69/12 of EU2 lineage 441,859 SNPs were found. Completion of Ex-type draft genome of *Phytophthora ramorum* is highly important since this is the representative specimen for the species of biosecurity concern at different regions of the world.

Full-length infectious clones of *Banana bunchy top virus*

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Banana bunchy top virus (BBTV) is an important pathogen responsible for a serious disease of banana worldwide. In this study, longer than unit-length DNA of the eight components of the BBTV genome was amplified and cloned into the plant expression vector pCAMBIA3300. The eight recombinant plasmids were subsequently transferred into *Agrobacterium tumefaciens* GV1301 individually, and co-inoculated into banana leaves by agroinfiltration. At 90 days post inoculation (dpi), typical BBTV symptoms were visible on

inoculated banana plants, and individual components of the BBTV genome were readily detectable in systemic leaves. However, the infection rate was relatively low. One possible reason is the high frequency of homologous recombination between repeated sequences in the longer than unit-length clones, resulting in either concatmers or deletions of BBTV sequences while being propagated in bacterial hosts. The simultaneous delivery of eight different BBTV components to the same cell also posed a great challenge in agroinfiltration. Despite the low infection rate, this is a first report of successful BBTV infectious clones. Further improvement and the use of a *rec*$^-$ bacterium host for cloning should substantially improve the infection rate of the BBTV infectious clones. The availability of the BBTV infectious clones is expected to accelerate studies on the functional genomics of BBTV and molecular interactions between BBTV and the banana host.

Identification of host factors interacting with the movement protein of *Ourmia melon virus* in Arabidopsis
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Ourmia melon virus (OuMV) movement protein (MP) forms tubules across the cell wall of epidermal cells of OuMV-infected *N. benthamiana*, and these tubules contain virions; however, at this point, it is not clear if the OuMV MP can be strictly categorized as belonging to the tubule-forming group, or if it follows a novel mixed-movement model. OuMV MP has an unusual intracellular distribution, including endoplasmic reticulum (ER), trans-Golgi network (TGN), plasma membrane, and plasmodesmata, suggesting that it possibly interacts with numerous plant host factors. It is also still unknown how OuMV MP reaches the cell periphery to enable cell-to-cell movement of OuMV, and how OuMV MP is regulated by host proteins. In this study, a total of 55 unique candidate OuMV MP-interacting proteins in Arabidopsis (*A. thaliana*) were identified by a maltose-binding protein (MBP) pull-down assay coupled with liquid chromatography-mass spectrometry (LC-MS/MS) analysis. Among these candidates, 12 would be novel proteins of great interest. Identified candidate OuMV MP-interacting proteins are involved in membrane trafficking, calcium signaling, transport across the plasma membrane, protein folding and homeostasis, proteolysis, and metabolism. This study provides insight to reveal missing molecular components of OuMV movement, and targets for studying virus-host interactions. A better understanding of interactions between viral and host proteins will help develop tools to better control viral diseases.

Genotyping of the downy mildew species *Pseudoperonospora cubensis* and *P. humuli* using target genome sequencing
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Technological advances in genome sequencing have rapidly improved our ability to catalog genomic variation, expanding the scope and scale of genetic studies over the past decade. Yet, for many agronomically important plant pathogens such as the downy mildews, these advances remain limited. This is, in part, due to the difficulties associated with maintaining obligate biotroph pathogens, and the logistical constraints involved in the genotyping of these species ie. obtaining DNA of sufficient quantity and quality from environmental samples. Adaptable methods such as whole genome sequencing (WGS) or reduced representation approaches for the genotyping of the downy mildews are required for the understanding of the pathogen populations from an evolutionary and ecological perspective. Here, we describe a targeted enrichment platform (TEP) able to effectively genotype isolates from two *Pseudoperonospora* species using DNA concentrations of less than 50 ng of mixed pathogen and plant DNA samples. We were successfully able to enrich 830 target genes across 84 samples and identified SNPs that discriminated along host preference. Future application of TEP should focus on more even distribution of targeted polymorphic regions across the pathogen genome. Our results support the ability of TEP to detect informative SNPs able to describe the population structure of *Pseudoperonospora* species over time and space. This platform provides an additional tool for genotyping of an obligate biotrophic plant pathogen and the execution of new genetic studies.

Small RNA profile in the *Chenopodium quinoa – Cucumber mosaic virus* interaction
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Seeds of the pseudograin, quinoa (*Chenopodium quinoa*), must be washed extensively prior to consumption to remove saponins that taste bitter. To reduce costs associated with saponin removal, low saponin (sweet) cultivars have been developed. Quinoa is usually tolerant of viral pathogens and is often a local lesion host. However, there is a lack of knowledge on the role of small RNAs in this tolerance. To fill this gap, an experiment was conducted on quinoa with different seed saponin content: sweet ‘Jesse’, medium ‘QQ74’, and bitter ‘Red Head’. Leaves were mock-inoculated or inoculated with *Cucumber mosaic virus* (CMV). Leaves, harvested 1 and 4 days post inoculation, were used in small RNASeq library preparation. Raw reads were trimmed and excluded from tRNA, rRNA, and snoRNA. They were then mapped to the quinoa reference genome. Novel and known miRNA were identified by miRDeep2. Differentially expressed miRNA (DE-miR) were detected by DESeq2. Target genes of DE-miR were determined by Target Finder. One mature miRNA, miR168, was significantly different and upregulated. The only significant upregulated target gene was “putative disease resistance gene At3g15700.” Higher expression of miR168 induced elevated regulation of this disease resistant gene during CMV infection. Using this conserved miRNA in breeding program may facilitate enhanced resistance to CMV infection in quinoa cultivars. Additional research on small RNAs mapping to CMV genome is ongoing.

Secretome annotation of the citrus stem end rot fungal pathogen *Lasiodiplodia theobromae*
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Fungal plant pathogens secrete an array of effector proteins in order to achieve colonization of the host plant. *Lasiodiplodia* theobromae is a fungal pathogen that causes soft rot of fruits shortly before or after harvest, and the disease in citrus is known as Diplodia stem end rot (SER). Our aim is to investigate the repertoire of secreted proteins (secretome) from *Lasiodiplodia theobromae* Lt_CITRA15 isolated from grapefruit. In this study, we sequenced and assembled the genome for the strain Lt_CITRA15 using PacBio which resulted in 475 contigs with a N50 of 178kb and with the sum of contigs equivalent to 43.3Mb. We have identified using Signalp software a set of 1,203 secreted proteins out of 10,456 predicted proteins in the genome. We will perform comparative analyses using blast with secreted proteins from *L. theobromoae* and the secretomes from *Diplodia corticola* and from the grapevine pathogen *Diplodia seriata*. Our long term goal is to characterize unique families of secreted proteins from *L. theobromae* and those shared with related species. These studies will contribute to the annotation efforts on pathogen effector proteins for this genus.

Insights into pathogenicity determinants of *Clavibacter michiganensis* subsp. *michiganensis* and their effects on disease expression

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Tomato canker is a geographically widespread and lethal disease of tomato. This seed-borne disease is caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) which produces copious amounts of extracellular polysaccharide (EPS). The aim of this study was to better understand the genomic determinants involved in pathogenicity. We have sequenced three strains using PacBio: virulent & EPS-producing, virulent & non-EPS-producing, and non-virulent & EPS-producing. Comparative genomic analyses revealed no truncated clusters associated with EPS production; however, large-scale rearrangement events were observed. Plasmid organization was different for each strain and the plasmid sequences showed significant differences in their genomic constituents. Plasmids pCM1 and pCM2 were present in virulent EPS-producing and virulent non-EPS-producing strains, respectively, but showed high discrepancy when aligned with reference genome NCPPB 382. Non-pathogenic strains showed disrupted and/or truncated pathogenicity islands (*tomA* and *chp* clusters). Scanning electron microscopy and qPCR results confirmed that the EPS-producing virulent strain colonized the host more rapidly, whereas, non-virulent strain showed no colonization of the stem vascular tissue (lacked the colonization facilitator gene: *chpG*), although it was detected in the root samples.

Blackbird: A novel high-throughput laboratory phenotyping system to quantify incidence and severity of powdery mildews

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Phenotyping of responses in the earliest stages of pathogen growth has required microscopy and observation by human experts, an expensive and slow process that severely restricted throughput. We developed a high-throughput phenotyping system that combined high-resolution, low magnification (4X) optics, a 47 MP 24X36 mm CMOS sensor with a pixel width of 4.2µm, long working distance, consequent maximum depth of field; and paired this with an X-Y-Z robotic stage, image-stacking to create fully-focused composite images, and a pre-trained convolutional neural network. Projecting a 4X image of the 4 µm-wide hyphae of the grape powdery mildew pathogen (*Erysiphe necator*) onto the camera sensor provided 4-pixel width coverage of the hyphal diameter. The system synoptically captures a fully-focused high-resolution image covering a 1-cm leaf disk in as little as 13 seconds. We named the system *Blackbird,* after the SR-71 reconnaissance plane. The current throughput rate ranges between 1200 to 2400 samples imaged per 8-hour workday. The assessments are also non-destructive; allowing repeated assessments of samples through multiple time points. Our present convolutional neural network estimates the percentage of infected area per sample at an agreement ratio of 91% compared to human expert assessments. Further QTL analysis showed that this automated phenotyping methodology has higher QTL association than the traditional manual method.

Impact of zebra chip on crop water-use and efficiency of the Irrigation Scheduling Supervisor Control and Data Acquisition (ISSCADA) System

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Potatoes in Texas High Plains are irrigated to maximize yield. However, the Ogallala Aquifer has declined significantly, causing an increase in pumping costs. Farmers are trying to improve crop water use efficiency (WUE) and conserve ground water with automated irrigation scheduling systems, such as the ISSCADA system, that are based on remote detection of crop canopy stress. The question was raised how a plant disease, such as zebra chip (ZC), might affect such a system. A study was conducted in which three irrigation treatments were established, 100, 80, and 60% full irrigation, and ZC subplots were established in each main plot. Remote imaging was taken weekly to monitor crop stress and neutron probe readings were taken in all irrigation treatments and ZC subplots to monitor soil water. In all irrigation treatments, soil water content started to accumulate three weeks post-infestation within diseased subplots, suggesting decreased root function of infected plants. Remote sensing at this time did not detect ZC. Soil water continued to accumulate in infested subplots for the remainder of the study. ZC subplots could be remotely detected 50 days after infestation, but not clearly differentiated from drought stress. ZC negatively impacted crop WUE and the efficiency of the ISSCADA system, and soil water measurements in combination with remote sensing of canopy temperature will be required to optimize irrigation scheduling in the presence of plant disease.

Early detection of soybean sudden death syndrome using high-resolution satellite imagery
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Sudden death syndrome (SDS) is a major yield-limiting disease of soybean in the Midwest. Effective management for SDS requires accurate detection in soybean fields. Since traditional scouting methods are time-consuming, labor-intensive and often destructive, alternative methods to monitor SDS in large soybean fields are needed. This study explores the potential of high-resolution (3 m) PlanetScope satellite imagery for early and accurate detection of SDS using random forest classification algorithm. We used four spectral bands including red, blue, green, and near-infrared (NIR) and calculated normalized difference vegetation index (NDVI) to detect healthy and SDS-infected quadrats (3 m wide × 1.5 m in length) in a soybean field experiment located in Boone, Iowa. Data collected during the 2016, 2017 and 2018 soybean growing seasons were analyzed in this study. The results indicate that spectral bands of PlanetScope imagery, along with calculated NDVI, can accurately predict SDS in soybean plots, even before foliar symptoms onset. Healthy and diseased soybean quadrats were detected with more than 85% accuracy and with kappa statistics (a measure of inter-rater agreement) of more than 68%, in all growing seasons. These promising results suggest that high-resolution satellite imagery has a high potential for early detection of SDS in soybean fields. Our findings highlight that this technology can facilitate large-scale monitoring of SDS and possibly other economically important soybean diseases to guide recommendations for site-specific management in current and future seasons.

Assessment of plant diseases using an unmanned aerial system with high resolution color and multispectral imagery
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Sudden death syndrome (SDS) is a soil borne disease, caused by the pathogen *Fusarium virguliforme* (*Aoki and O'Donnell*). This disease can be extremely detrimental to the soybean crop by decreasing yields and causing premature plant death. The most evident symptomology is the interveinal chlorosis and subsequent necrosis in the trifoliate leaves. This study is will determine the most efficient way to capture multispectral and color imagery by utilizing an Unmanned Aerial Vehicle (UAV). The objectives of this research are to quantify the changes in color and pattern associated with the symptoms of plant disease, to determine the correlation between vegetation reflectance and disease severity, and evaluate the advantages and disadvantages of using high resolution and multispectral imagery to assess disease. During the growing season, SDS disease ratings were collected to determine the incidence and severity. Sensors were used to capture imagery throughout the growing season. The multispectral imagery was used to create indices to determine which had the highest correlation to the ground disease ratings. ArcGIS indices were created to determine which index correlates best with the disease ratings in the field.

Differentiating between two clonal lineages of *Phytophthora infestans* with hyperspectral sensors
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Late blight of tomato and potato caused by the oomycete pathogen *Phytophthora infestans* continues to be one of the most challenging diseases to sustainably and proactively manage. As a heterothallic organism, *P. infestans* has two mating types capable of sexual recombination when in co-occurrence. Our previous work established a non-destructive method of early late blight detection based on hyperspectral reflectance that can identify infected plants with >85% accuracy before visual symptoms appear. The objectives of this work were to explore differences between spectral signatures and remotely sensed biochemical and physiological metrics of plant health between potatoes infected with two clonal lineages, US-23 and US-08 throughout disease progression. We conducted three controlled experiments in growth chambers using inoculated and control plants and measured continuous visible to shortwave infrared reflectance (400-2500 nm) on leaves with a portable spectrometer at 12–24 hr intervals for 5–7 days. We could consistently discriminate between lineages with accuracy of >80%. Infected leaves showed significant differences in total phenolics, sugar, and nitrogen content. The interaction of disease progression and clonal lineage was significantly different for leaf mass/area, normalized differential water index, and starch content. Shortwave infrared wavelengths (>1300 nm) were important for differentiation and biochemical trait model accuracy. Our results support the future use of hyperspectral sensors as precision disease management tools in potato and other cropping systems.

Spectral and thermal signatures of early infection of peanut with *Sclerotium rolfsii*
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Southern stem rot (SSR), caused by *Sclerotium rolfsii*, is a soilborne disease of peanut (*Arachis hypogaea* L.). Fungicides control SSR but are most effective when applied prior to or during early stages of infection. Previous studies demonstrated early detection of foliar disease using spectral reflectance and thermal imaging, but few studies have used these technologies to detect soilborne disease. Our objective was to identify spectral and thermal signatures of peanut infected with *S. rolfsii*. In greenhouse experiments, *S. rolfsii* inoculated and mock-inoculated lateral stems of peanut were inspected daily for symptoms, and measurements were taken with a forward-looking infrared (FLIR) camera and a Jaz spectrometer to detect plant surface temperature and spectral reflectance, respectively. Symptoms (wilting of terminal leaflets) were first observed approximately 1 week after inoculation. Reflectance curves for leaflets on inoculated and mock-inoculated stems differed in both visible and near-infrared ranges. Reflectance at 550 nm and 790 nm was used to calculate a SSR spectral disease index (SSRI). Three days after first observation of symptoms, inoculated stems had lower SSRI and higher temperature compared to mock-inoculated stems. Results indicate it is possible to detect signatures of SSR in peanut during early stages of symptom expression using spectral reflectance and thermal imaging, and this may have applications for early disease detection in the field.

***Phakopsora pachyrizi* monitoring by spectral reflectance**
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Soybean rust (SBR) is caused by the biotrophic pathogen *Phakopsora pachyrizi*. SBR is a polycyclic disease where urediniospores dispersed by the wind lead to different spatiotemporal epidemics. Threshold for disease management considers SBR severity and infection timeframe. Remote sensing techniques mesure spectral response of the canopy: characterized by the wave reflectance process. This technique can be successfully employed in disease management studies, due to the differences in the spectral responses of a healthy leaf and a diseased leaf. Based on this, the present study sought, through the FieldSpec 3 sensor, to determine the potential use of reflectance measurements to quantify the damages caused by the SBR. Plant leaves with different SBR severities (0, 0.1, 2, 3, 5%) were submitted to spectral evaluation on spectrophotometer FieldSpec 3. resulting in differences among diseased and healthy leaves ($p > 0.05$) to spectral bands 531, 720, and 810 nm. Correlations were establish, and as severity increase plant reflectance decrease 0.018, 0.0198, 0.013 nm to respectively bands 531, 720, and 810 nm (r^2= 0.76, 0.89, 0.93), respectively. Use of remote sensing is a tool able to quantify and identify SBR even with lower severity in the begin of the epidemy. This results contribute to SBR integrate disease management.

Known viruses infecting wheat in Oklahoma
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Wheat is one of the important crops in Oklahoma. Before the initiation of this work, no detailed information existed about viruses of wheat in the state. Therefore, a state-wide survey was conducted to determine which known viruses infect wheat crops in Oklahoma during 2015 and 2016 growing seasons. Approximately 100 growers wheat fields located in 33 counties of the eight main wheat growing districts of the state were surveyed for virus infection. More than 1,000 leaf samples were collected from wheat and weed plants and were tested serologically using dot-immuno-binding assay (DIBA) against the antisera of 15 viruses. The results showed that 10 out of 15 viruses were detected in wheat fields. The highest average incidence was recorded for BaMMV (55.9%) followed by WSMV (44.06%), BYDV-PAV (12.6%), BYDV-RPV (7.9%) and HPV (2.09%). The remaining five viruses (BaYMV, BYDV-MAV, SBWMV, JGMV and MCMV) were detected in less than 2% of the samples tested. Mixed infection of two or more than two viruses was also observed in some counties. Two of the 10 detected viruses were new in Oklahoma and were never reported previously. In addition, virus infection in weeds species naturally grown in wheat fields and heavy aphid's infestation will also be discussed.

A collection of curated rose viruses; an important part of the National Clean Plant Network
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Roses are one of the most economically important horticultural crops, used for cut flowers, landscape plants, culinary products, essential oils, and religious rituals. Roses are susceptible to about 20 viruses of which at least half affect their economic and/or aesthetic values. With the advancement of virus diagnostic technologies more plant viruses are being routinely characterized including rose viruses. Detection and control of viruses is very important to both rose research and industry programs necessitating proper diagnostic controls. With funding from the National Clean Plant Network (NCPN) the USDA National Germplasm Resources Laboratory partners with the Texas Plant Disease Diagnostic Laboratory, Texas A&M and Foundation Plant Services, University of California, Davis to screen rose varieties for viruses, and establish a curated rose virus collection. These infections will be verified by rigorous testing using transmission electron microscopy, conventional molecular methods and high throughput sequencing. Samples from the collection will be freely available to NCPN Centers, National Plant Diagnostic Network and commercial diagnostic laboratories as well as industry representatives. The outcome will be an established virus collection used as standardized positive controls in rose virus testing and indexing. This will reduce the spread of deleterious rose viruses and increase reliability in virus testing among stakeholders and partners of the rose industry.

Survey of virus diseases affecting cucurbits in Taiwan
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Cucurbits, including cucumber, chayote and bottle gourd, are some of the important vegetables in Taiwan. However, virus disease is a major constraint to the production. To identify predominant and emerging virus diseases of cucurbits in Taiwan, a field survey was conducted in 2016 and 2017. Samples from different cucurbit plants with virus-like symptom were collected from different areas of Taiwan and the virus(es) present in each were diagnosed by ELISA, RT-PCR and PCR. In cucumber samples, CCYV was predominant in southern Taiwan with approximately 76% of samples testing positive. About two thirds of CCYV positive samples also tested positive for presence of one or more other viruses. CCYV was not detected in the samples from eastern and central Taiwan. The major virus in eastern Taiwan was SLCuPV while MYSV was prevalent in central and northern Taiwan. It suggests that cucumber production in central and northern Taiwan is greatly affected by thrips-transmitted Tospovirus while whitefly-transmitted viruses are the major threat in eastern and southern Taiwan. Similarly, whitefly-transmitted SLCuPV was predominant in the samples of bottle gourd and chayote. Approximately 88% of chayote samples and 38% of bottle gourd samples tested positive for SLCuPV by PCR. The second important virus in bottle gourd was from the genus *Potyvirus*, including PRSV-W and ZYMV; approximately 32% of samples were infected with potyvirus. These results suggest that whitefly- transmitted viruses are the most important factor to limit production of cucumber, bottle gourd and chayote in Taiwan.

Survey for Virus Diseases in Vegetable Crops in Nepal

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The Feed the Future Innovation Lab for Integrated Pest Management (IPM-IL) funded by USAID is conducting adaptive research to mitigate crop losses due to viral diseases in subsistence agriculture in Nepal. Surveys were conducted in farmers' fields in different regions of Nepal to document viruses affecting the production of vegetable crops. Symptomatic samples suspected for virus infections were collected and tested for specific viruses by serological and molecular diagnostic assays. Symptomatic samples from representative crops were imprinted onto FTA™ PlantSaver Cards in the field, air dried and processed in the lab for virus testing. In addition, total RNA extracted from symptomatic samples was subjected to high-throughput sequencing for identification of viral sequences. The results indicated the presence of viral sequences highly similar to *Bean common mosaic virus* in beans, *Zucchini yellow mosaic virus* and *Zucchini tigré mosaic virus* in squash, pumpkin, bitter gourd and snake gourd, *Potato virus Y* (PVY) and *Potato virus M* in potato, PVY, *Cucumber mosaic virus*, *Peanut bud necrosis virus*, *Tomato chlorosis virus* and *Tomato leaf curl New Delhi virus* in tomato, *Chilli veinal mottle virus* in chillies, *Bhendi yellow vein mosaic viru*s in okra and *Turnip mosaic virus* in broadleaf mustard. This knowledge is currently being used for in-country capacity building to implement integrated pest management strategy for controlling viral diseases in vegetable crops.

Is Grapevine rupestris stem pitting-associated virus Involved in Graft incompatibility of Pinot noir Grapevines on 110 Richter Rootstock?

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Several Pinot noir (PN) (*Vitis vinifera*) clones grafted on 110 Richter (110R) (*V. berlandieri* x *V. rupestris*), a drought tolerant and phylloxera resistant rootstock, exhibit a necrotic graft union (NGU) that girdles the trunk leading to red canopy. To determine the causal agent of NGU, a bioassay was conducted using budwood from FPS 76, a PN clone released after microshoot tip culture of French clone PN 927, and from nine self-rooted PN 927 grapevines with history of NGU. Grafts of FPS 76 and PN 927 grapevines on 110R were established in a vineyard, in 2011 and 2012, respectively. In 2016, cambial scrapings were obtained from lignified canes of two composites from PN 927 budwood source, and from a PN grapevine with NGU in the same vineyard. Total RNA was extracted, barcoded cDNA libraries constructed after rRNA depletion, and subjected to Illumina sequencing. Sequence analysis revealed presence of Grapevine red globe virus (GRGV) and Grapevine rupestris stem pitting-associated virus (GRSPaV) in all three libraries. In 2018, observations on grafted grapevines revealed that 12 out of 23 grapevines of PN 927 had red canopy and the FPS 76 grapevines did not. RT-PCR tests conducted on the vines for GRBV and GRSPaV indicated that GRSPaV was the only virus present in all PN927 grapevines with red canopy but not in the asymptomatic grapevines. These results indicate that GRSPaV infection of PN 927 causes an incompatibility reaction leading to NGU when grafted on 110R.