2019 Northeastern Division Meeting Abstracts

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Auxotrophic *Erwinia amylovora* mutant pathogenicity patterns in apple

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The bacterium *Erwinia amylovora* causes fire blight disease of apples and pears. While the virulence systems used by *E. amylovora* to cause disease have been studied extensively, relatively little is known about the parasitic interactions of *E. amylovora* with the host. A series of auxotrophic *E. amylovora* mutants was isolated and characterized for metabolic defects and ability to cause disease in apple. The ability to perform *de novo* synthesis of arginine and leucine was essential for *E. amylovora* to grow and cause disease in apple fruitlets, while synthesis of cysteine, glutamine, methionine, or serine was dispensable. Defects in asparagine, isoleucine, lysine, proline, threonine, tryptophan, and purine and pyrimidine nucleotide biosynthesis, produced moderate to severe defects in virulence on apple fruitlets. This study has revealed the availability of a range of host metabolites to *E. amylovora* cells growing in apple fruitlet and tree shoot tissues, and whether these metabolites are available in sufficient quantities to render bacterial *de novo* synthesis of these metabolites partially or even completely dispensable. This provides information about potential trophic interactions between *E. amylovora* and the apple host, particularly as related to amino acid and nucleotide metabolites and the tricarboxylic acid cycle. This information could be useful for novel fire blight management tool development.

Verticillium wilt of potato caused by *Verticillium dahliae*: The story of a soilborne pathogen that hides as an endophyte

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Crop rotations are the backbone for sustainable management of recalcitrant soilborne diseases, but they are often not effective. Management of Verticillium wilt of potato caused by *Verticillium dahliae* (Vd) without soil fumigants focuses on rotating potatoes with crops considered non hosts. Past studies showed that Vd infects some crops as endophyte, which could partly explain the poor disease management provided by crop rotations. Our objective was to investigate the genetic diversity and pathogenicity of Vd populations infecting symptomatic and asymptomatic host plants from potato fields affected by Verticillium wilt. The hypothesis was that populations infecting as endophytes may constitute non-pathogenic lineages and be genetically distinct from those pathogenic. Population genetic structure and evolutionary relations were explored using vegetative compatibility groups (VCG), SNPs and microsatellites in a collection of Vd isolates. Asymptomatic hosts were infected by Vd lineage VCG4B, a known lineage with a broad susceptible host range, which also was found infecting some symptomatic hosts. Analyses indicated that lineage VCG4B isolates infecting symptomatic and asymptomatic hosts were not significantly genetically differentiated. Pathogenicity tests showed that a Vd

VCG4B isolate infected oat as an endophyte and potato as a pathogen in a rotational cropping system. We will discuss implications of these results in disease management and population biology of *Vd*.

Survey for grapevine leafroll viruses in Pennsylvania

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Grapevine leafroll disease, caused by grapevine leafroll associated viruses (GLRaVs), is a serious disease of cultivated *Vitis* species. Determining the incidence of these viruses across Pennsylvania and their impact on yield and fruit quality are essential for quantifying the potential economic impact of GLRaVs and best management practices. Therefore, a two-year statewide survey was conducted among commercial vineyards for GLRaV-1 and 3, the most important GLRaV species in grapevines. Sixty-three vineyard blocks of *Vitis vinifera* 'Cabernet franc' (18), 'Pinot noir' (10), 'Chardonnay' (16), and 'Riesling' (8) and *Vitis* interspecific hybrid 'Chambourcin' (11) were sampled from 24 sites. Leaf petiole samples from symptomatic and a-symptomatic vines (30 vines/block) were collected between veraison and harvest and analyzed via ELISA. GLRaV-1 and/or 3 was present in a third of the blocks examined; one of 11 Chambourcin blocks sampled (9%), and 20 of 52 *V. vinifera* blocks sampled (38.5%) contained vines that tested positive for GLRaV-1 and/or 3. In the second year of the study, two vineyards of Cabernet franc with a low incidence of the virus were selected to evaluate the effects of GLRaV-1 and/or 3 on yield and fruit chemistry at harvest, as well as bud freeze tolerance during the dormant season. Vines testing positive for the virus(es) had lower fruit soluble solids in one vineyard, and higher titratable acidity in the other. There were no significant effects on yield.

Nanoparticles of Cu for suppression of Fusarium root diseases

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The micronutrient Cu promotes defense mechanisms in plant roots. For example, phenols are enzymatically produced by polyphenol oxidase (PPO) which is activated by Cu. However, obtaining sufficient levels of Cu in susceptible roots can be compromised by their poor availability in slightly acid-neutral soils and by their poor mobility in plants. However, when Cu is applied as CuO or Cu₃(PO₄)₂ nanoparticles (NP) (1-100 nm), they provide more disease suppression than their larger bulk or ionic equivalents. We exposed young seedlings of begonia, chrysanthemum, cyclamen, soybeans, tomatoes, and watermelon to NP of Cu (500 µg/ml) and observed that Fusarium wilt and root rot were suppressed and growth were enhanced. Many times a single application resulted in season long suppression. In tomato and watermelon, analyses of gene expression (by RT-qPCR) in Fusarium infected tomatoes and watermelon revealed upregulation of PPO and pathogenicity-related genes (PR1) in plants treated with NP Cu. In tomato up regulation was within a week of treatment and before the onset of symptoms. These findings suggest that NP CuO may serve as a highly effective delivery agent for Cu and that when applied to seedlings at a young age can promote root health and impact yield. NP of Cu could help sustain food production as climate changes increase the threat of drought, stress, and disease.

Antifungal activity of new quinolone and acridone-derivatives

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Development of fungal resistance decreases fungicide effectiveness and leads to spoilage and potential contamination of food and feed products with mycotoxins. Chemical synthesis is the most common way to develop new antifungal agents. The goal of this study was to assess the antifungal activity of newly synthesized 3-hydroxy-quinolones and amides of acridone-carboxylic acid derivatives. Many triazole-based, synthetic fungicides initially interfere with ergosterol and melanin synthesis. The primary targets of quinolone and acridone derivatives are enzymes in the biosynthesis pathway of nucleic acids (polymerases and/or topoisomerases). In our experiments, both groups of the newly synthesized compounds demonstrated growth inhibition, affected the development of hyphae and conidia, and caused colony discoloration of the plant pathogens, *Fusarium* spp., suggesting activity towards ergosterol and melanin synthesis. The compounds studied also demonstrated effective inhibition of the *in vitro* model transcription assays, based on bacteriophage T7 DNA-dependent RNA polymerase. Molecular docking also revealed the formation of a nonproductive, enzyme-inhibitor-DNA complex that leads to a loss of catalytic activity of the polymerase. The results from this study demonstrate that the 3-hydroxy-quinolones and amides of acridone-carboxylic acid derivatives appear to have several cellular targets responsible for the antifungal properties.

Field efficacy of SDHI active ingredients on multiple SDHI mutations of *Sclerotinia homoeocarpa*

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Clariireedia spp. (formerly *Sclerotinia homoeocarpa*) causes dollar spot disease on turfgrass and is one of the most economically damaging pathogens on golf courses in North America. The pathogen has developed resistance to many systemic fungicide classes such as benzimidazoles, dicarboximides, demethylation inhibitors (DMI), and recently succinate dehydrogenase inhibitors (SDHI). Out of the four fungicide classes, SDHI resistance mechanisms in particular are rather complex due to multiple mutations that lead to differential in vitro sensitivities and field efficacy to five SDHIs labeled for turfgrass diseases: boscalid, fluxapyroxad, isofetamid, penthiopyrad and fluopyram. Recent studies have shown SDHI fungicides induced variable point mutations in SdhB, SdhC, and SdhD subunits of many plant pathogenic fungi including *Clariireedia*. Furthermore, in vitro bioassays using those five SDHIs revealed that each of seven different point mutations discovered so far in *Clariireedia* spp. confer partial and negative cross-resistance profiles. In 2018, field experiments were conducted on inoculated putting green plots at the Joseph Troll Turf Research Center at the University of Massachusetts Amherst and on natural fairway plots on a golf course with confirmed SDHI resistance. We will discuss the results of these field experiments in which we aimed to determine the relationship between in vitro resistance profiles and field efficacy of different SDHIs.

Optimizing white mold management in processing snap bean in New York

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White mold, caused by the fungus, *Sclerotinia sclerotiorum* continues to be a high priority for processing snap bean growers in New York. A major challenge in disease management is the uncertainty in identifying fields in which epidemics occur. Management therefore relies upon preventative fungicides. However, despite prophylactic application, suboptimal management is common. This research explored fungicide resistance and application timing as potential factors. *In vitro* screening of fungicide sensitivity found all *S. sclerotiorum* isolates tested were sensitivity to fluazinam and boscalid, but 30% had reduced sensitivity to thiophanate-methyl. In timing trials, application of thiophanate-methyl at early flowering was optimal and a second application at late flowering did not improve management. In contrast, if thiophanate-methyl was delayed until late flowering, white mold control was poor. Timing of boscalid and fluazinam was more flexible. To identify site-specific risk factors associated with white mold, a historical dataset (2006 to 2008) was analyzed using boosted regression trees. The most influential predictors (in decreasing order) were canopy openness during pod development, the number of days after planting, hydrological soil group, canopy openness during bloom, and elevation. Our continuing research into digital agriculture tools for improving profitability of processing snap bean production through improved white mold management will also be discussed.

Assessing the ability of bacteria isolated from soil to control early blight disease of tomatoes

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Early blight is an economically important disease of tomato caused by the fungal pathogen *Alternaria solani*. The disease can lead to significant yield losses. Despite the use of fungicides and breeding tomato plants for resistance, early blight remains difficult to control. Microbial agents with antifungal activity can offer a more durable, safer and sustainable alternative to control and eradicate the disease. These potential biocontrol agents can be found naturally in soils of organic crop fields. In this project we have isolated bacteria from the soil of organic fields at Rodale Institute. The bacteria were then tested for their ability to inhibit the growth of *A. solani*. Out of the 104 bacteria isolated, nine exhibited potent antifungal activity against the pathogen. Molecular characterization of six of these isolates showed that they belong to different species of the genus *Bacillus*. Plant infection assays are underway to confirm that these isolates are able to provide protection against early blight disease *in planta*. Data from this project will provide insight on how to develop effective biocontrol methods for early blight management.

Bacteriocin-mediated antagonism: A potential new method for biological control?

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Bacteriocins are antibacterial protein toxins that target closely related bacteria and due to their narrow-spectrum host range could act as biological control agents. *In vitro*, bacteriocin activity provides a fitness benefit for the producer in a structured environment (e.g. agar plate surface). A series of plant infiltration experiments tested the interactions of bacteriocin-producer *Pseudomonas syringae* pv. *syringae* (*Psy*), or the bacteriocin-deficient mutant of *Psy* (*Psy* Δ_{bac}), against the sensitive strain *P. syringae* pv. *phaseolicola* (*Pph*). The leaf bacterial populations were measured over an eight-day period. *Psy* and *Psy* Δ_{bac} populations were not significantly different in 1:1 co-infiltrations. At day 4 bacteriocin production increased the killing of sensitive cells with a 100- and 10-fold reduction of *Pph* by *Psy* and *Psy* Δ_{bac} , respectively. Altering the initial co-infiltration cell density to 1:9 (*Psy*:*Pph*), *Psy* achieved a significantly greater population at day 6 compared to *Psy* Δ_{bac} , showing a bacteriocin production benefit when in the minority. Furthermore, *Psy* reduced *Pph* to a greater extent than *Psy* Δ_{bac} for all sample days, indicating a combination of direct (e.g. bacteriocin-mediated antagonism) and indirect (e.g. resource competition) interactions between co-colonizing pathogens within plant leaves. Under certain antagonistic conditions, bacteriocin production can significantly promote *Psy* fitness and inhibit the growth of the competing population.

Observation of drippy nut disease on oak in southeastern NY in 2017–2018

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Drippy nut of oak, first described in California in 1967, causes premature drop and death of acorns and cankering, but not oak mortality. The causal agent, *Lonsdalea quercina* (also known as *Erwinia quercina* and *Brenneria quercina*) is one of four species in the genus *Lonsdalea* that have been observed globally, causing diseases on various hosts. In Nassau and Suffolk Counties, NY beginning in late July 2017, numerous calls were received by diagnostic labs and arboricultural firms, reporting blobs of clear, sticky exudate on decks, outdoor furniture, and driveways over a two-week period. Acorns of *Quercus rubra*, *Q. alba* and *Q. velutina* were determined to be the source of the exudate. A bacterial isolate (BP7103) was obtained from a discolored acorn of *Q. velutina* by directly streaking exudate from beneath the acorn cap onto potato-dextrose agar. The 16S rRNA gene of BP7103 was amplified, sequenced, and compared to sequences from the type strains of the *Lonsdalea* species and species from related genera. The isolate was tentatively identified as a member of *Lonsdalea* because the sequence of the 16S rRNA gene of BP7103 was 97 percent similar to that of *L. quercina* and 96.7 percent similar to that of *L. iberica*. Koch's postulates are yet to be carried out and further testing is needed to definitively identify the species, but a bacterium likely to be *L. quercina* has been associated with symptoms of drippy nut in east coast oaks for the first time.

Evaluation of biopesticides used alone and with conventional fungicides for managing Phytophthora blight in pepper and pumpkin crops

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Four replicated experiments were conducted in 2017 and 2018 in a field with a history of Phytophthora blight (caused by *P. capsici*) to evaluate a single program of organic biopesticide products applied to soil early and then to foliage in two crop types. Biopesticides were selected to reflect the range of available active ingredients labeled for this disease: Actinovate (*Streptomyces lydicus*), Bio-Tam 2 (*Trichoderma asperellum* and *T. gamsii*), Double Nickel and Stargus (both *Bacillus amyloliquefaciens*), Regalia (extract of *Reynoutria sachalinensis*), SoilGard (*Gliocladium virens*), and Taegro 2 (*B. subtilis*). A conventional grower standard fungicide program was applied on the same dates as the foliar biopesticides: Revus plus K-Phite alternated with Presidio plus K-Phite. A fourth treatment in the pumpkin experiments consisted of the soil-directed biopesticide applications followed by the conventional fungicide foliar program. Phytophthora blight became severe in the untreated control plots in all experiments (57–88% plants affected at last rating). The biopesticide program was not effective in any experiment; however, the conventional fungicide program was also ineffective in the pepper experiment in 2017 and the pumpkin experiment in 2018 reflecting high disease pressure. Disease severity was not further reduced by applying biopesticides to soil before starting the conventional fungicide program; although in 2018 this treatment had numerically fewest affected plants.

First report of Magnaporthe oryzae causing gray leaf spot on tall fescue in the Mid-Atlantic

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Gray leaf spot (GLS) is an important turfgrass disease caused by *Magnaporthe oryzae*. *M. oryzae* has been documented to cause GLS on tall fescue, perennial ryegrass and St. Augustinegrass in the southeastern U.S. However, outbreaks of GLS in the Mid-Atlantic region of the U.S. have been confined to perennial ryegrass and not reported on tall fescue, despite wide adaption. In 2018, GLS symptoms were observed on tall fescue at sod farms in Maryland and Delaware. Disease symptoms included tan, distinct leaf spots with brown margins. Hyaline, pyriform, septate conidia were observed on diseased tissue. Fungal morphotypes were isolated and grown in pure culture. To demonstrate pathogenicity, tall fescue seedlings were inoculated with a conidia suspension of both fungal isolates. Seedlings were incubated in a growth chamber at 26°C and 12-hr day/night cycle. Disease symptoms appeared 48 to 72 hr after inoculation and morphotypes were reisolated from infected tissue. To confirm pathogen identity, the ITS region of DNA from both isolates was amplified as well as the Pot2 transposon, known only to the *M. oryzae* genome. Based on symptoms, morphological characteristics, pathogenicity tests, and DNA fingerprinting, the pathogen was identified as *M. oryzae*. To our knowledge, this is the first report of *M. oryzae* causing GLS on tall fescue in the Mid-Atlantic and documents the growing potential for GLS to be a devastating disease of tall fescue in Mid-Atlantic and Northeast regions.

Effect of substrate on biopesticide efficacy to suppress Pythium in hydroponic systems

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Oomycete pathogens cause significant losses in greenhouse production due to their swimming zoospores and wide host range. Many greenhouse producers have utilized commercial biopesticides in addition to chemical treatments to protect plants from these pathogens. Few studies have evaluated the effect of newer substrate types on biopesticide colonization and disease suppression efficacy in horticulture crops. Cucumber, calibrachoa, and tomato cropping systems were utilized to evaluate the implications of propagation substrate on biopesticide efficacy. We predicted that there would be a significant difference in efficacy of biopesticides between substrate. Substrates currently used in commercial production (peat, rockwool, coco coir, and Oasis®), as well as newer alternative substrate blends of peat and wood fiber were evaluated. Biopesticide drenches of Rootshield® WP, Cease®, and Regalia® were applied twice in propagation at 7 and 14 days post seeding. At transplant, plants were challenged with *Pythium* spp. or a water control. Root rot

and root growth were evaluated at 21 days post inoculation. This experiment will provide initial data for determining the mechanisms driving variation in biopesticide performance. Results from this research will be shared with growers and biopesticide producers in an effort to improve on-farm performance and increase adoption of biopesticides as plant disease management tools.

Detection of internal decay in American elms: Is decay incidence higher for elms under regular fungicide injection to control Dutch elm disease?

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Fungicide injection is regularly performed to prevent and treat Dutch elm disease (DED) of American elm (*Ulmus americana*). In an effort to better understand the effects of long-term fungicide injection, sonic tomography (SoT) and electrical-resistance tomography (ERT) were used to determine the incidence and severity of internal decay in landscape elms. Overall, 253 sonic and electrical resistance tomograms were generated from 210 American elms. Trees were partitioned into two fungicide injection groups: (i) regular injection; and (ii) irregular or no known history of injection. Among all American elms, the incidence of internal decay in the lower trunk was 30% (63/210) with a mean percent decay, as determined by SoT, of 39%. Based on Chi-square analysis, there were no significant differences in the frequency of elms with decay by injection history ($p = 0.799$). Mean percent decay was significantly different by DBH class ($p = 0.005$) and while linear regression demonstrated a positive correlation between percent decay and DBH, most of the variability went unexplained ($R^2 = 0.182$). For elms with decay, there was a significantly higher frequency of trees in the lowest decay class (<25% of the cross section) compared to the highest decay class (>75% of the cross section). The results suggest that the wounding associated with regular fungicide injection does not increase the likelihood of internal decay and that American elms exhibit a low frequency and severity of decay.

***P. syringae* target cells survive bacteriocin exposure both by physiological tolerance and genetic resistance**

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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 resistance 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.

Evaluating genetic resistance to downy mildew in *Chenopodium* Species for use in breeding programs

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Quinoa is a potential new crop for New England; however, its susceptibility to downy mildew, caused by *Peronospora variabilis* is a key obstacle. The objective of this project was to evaluate differential resistance within the *Chenopodium* genus and identify novel genetic sources of resistance for use in future breeding programs. The long-term goal of this research is to develop a resistant variety of quinoa to be grown in North America. Field trials conducted at UNH evaluated downy mildew disease severity on ten *Chenopodium* accessions representing four species. Accessions were planted in a 2-factor randomized split plot design with three replicate plots per treatment. Disease severity for each treatment was compared and significant differences in disease severity was observed among treatments. *C. berlandieri* var. *macrocalycium* ecotypes collected from Rye Beach, NH and Appledore Island, ME exhibited the lowest mean disease severity of the season. *P. variabilis* was isolated from each of the 10 accessions and ITS and COX2 sequences were compared; phylogenetic analyses suggest no effect of host species; however, New Hampshire isolates formed a clear cluster when compared with Pennsylvania isolates. These results provide the first step in identifying potential New England native sources of resistance to downy mildew within the genus, and provide preliminary information needed to further investigate resistance at the genomic level in *Chenopodium* spp.

Characterization of the small RNA profiles of *Impatiens necrotic spot orthotospovirus* from infected *Nicotiana benthamiana*

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RNA interference (RNAi) is the main pathway used by plants to fight against viruses, and viral small RNAs (vsRNA) play a key role in the outcome of this process. *Impatiens necrotic spot orthotospovirus* (INSV) can infect economically important ornamental plants and vegetables worldwide, but characterization studies on INSV are limited and not much is known on how plants infected with INSV respond to viral infection. Here, we report the first analysis of the small RNA profile of *Nicotiana benthamiana* plants infected by an INSV isolate from the US. Our data show an under-representation of vsRNAs mapping to the intergenic region of the S and M segments, and an increase of the total amount of 21nt and 22nt vsRNA in infected plants, compared to uninfected. We also observed changes in the abundance of 21nt endogenous small RNAs in *N. benthamiana*, suggesting the accumulation of virus-activated small-interfering RNAs (vasiRNA). Our study enhances our understanding of the biogenesis of vsRNA in INSV infected plants and will contribute to the development of better RNAi-mediated disease control tools against INSV and plant viruses in general.

The feasibility of MinION sequencing for fast identification of regulated bacterial plant pathogens

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The MinION sequencing device (Oxford Nanopore Technologies) is part of the third generation of sequencing technologies and promises to provide rapid, real-time, long-read sequencing. However, external work validating and benchmarking the performance of this new technology is relatively scarce, and little has been done in the context of regulatory diagnostics. The goal of this study was to evaluate MinION library preparation protocols and sequencing for use in the diagnostics of bacterial plant pathogens of regulatory importance. Using pure cultures of *Ralstonia solanacearum*, we evaluated three DNA library protocols: 1D, 1D², and Rapid. All three protocols produced reads of comparable quality and length, although varied in the quantity of reads generated. Using data generated during just the first hour of sequencing we were able to assemble 99.11% of the genome with 99.71% accuracy and a mean depth of 56.25. We also examined how MinION could assist with genome sequencing in conjunction with next generation sequencing data. *De novo* assembly of Illumina MiSeq sequences from *Erwinia aphidicola* resulted in 27 contigs, with a maximum length of 1,621,424 bp. Combination with MinION data consolidated this into 4 contigs: one chromosome corresponding to *Erwinia aphidicola* and three potential plasmid sequences. Having established the feasibility of using this technology, this report also discusses the challenges of implementing this technology in the regulatory sphere.

Survey of *Fusarium* species associated with Michigan asparagus

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Fusarium crown and root rot of asparagus is an important contributor to the reduction of yield and crop longevity. Historically, *F. proliferatum*, *F. oxysporum* f. sp. *asparagi* and occasionally *F. moniliforme* have been associated with Michigan asparagus plantings. Our objective was to conduct a survey of the *Fusarium* spp. associated with asparagus cultivars that currently dominate Michigan's industry. Asparagus crowns (491 total) were sampled from three Michigan growers and included 'Jersey Supreme' (100), 'Guelph Millennium' (258), and 'Guelph Eclipse' (133). Samples from 'Guelph Millennium' included those grown in soils fumigated pre-plant with metam sodium (131) or not fumigated (127). Tissue from the crown and roots was plated onto a *Fusarium*-selective medium. Isolates were subcultured, then hyphal tipped onto potato dextrose agar. *Fusarium* spp. were isolated from 'Jersey Supreme' (39%), 'Guelph Eclipse' (47%) and 'Guelph Millennium' (30%). Crowns grown in soils that were non-fumigated or fumigated yielded *Fusarium* spp. from 25% and 36% of the tissue samples, respectively. Overall, a higher percentage of *Fusarium* spp. was isolated from roots (45%) than the crown (28%). Species identification was achieved via DNA extraction, PCR using EF-1 and EF-2 primers, and Sanger sequencing. Results of this study will be used to develop approaches for growers to improve management strategies for *Fusarium* crown and root rot in their asparagus fields.

Bitter rot of apple causal species distributions and climatic correlations in Pennsylvania

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Bitter rot of apple has become a major problem in Pennsylvania in recent years, leading to grower requests for up-to-date management recommendations. Management is complicated by the various *Colletotrichum* species that can cause bitter rot, which differ in host preference, fungicide sensitivity, and optimal growth conditions. Historical climate data and grower surveys of bitter rot losses and cultural practices were compared to species identified in a state-wide sampling of apples with bitter rot. Multilocus sequence analysis of the ~500 *Colletotrichum* isolates collected revealed *C. fioriniae* of the *C. acutatum* species complex was the most abundant species, followed by four species in the *C. gloeosporioides* species complex; *C. fructicola*, *C. siamense*, *C. henanense* (first report) and *C. gloeosporioides* sensu stricto. 'Honeycrisp' was reported as one of the most susceptible apple cultivars. *C. fioriniae* relative abundance was higher in northern areas, and in farms with aggressive fungicide programs. Many growers reported bitter rot was concentrated in random "hot spots" in the orchard. The recent increase of bitter rot is weakly correlated to increases in growing season daily mean temperatures. These findings suggest that a combination of climate change, cultivar susceptibility, fungicide usage and cultural practices are likely responsible for the increase of bitter rot in Pennsylvania, of which the latter two are prime candidates for improved management practices.

Genetic diversity and movement of *Verticillium dahliae* lineage 4A: A lineage highly aggressive to potatoes

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Characterizing the diversity of pathogen populations is important for designing durable disease resistance and effective management, as well as for understanding the evolution of pathogenicity and virulence. *Verticillium dahliae* (*Vd*) is a soilborne fungal plant pathogen with a broad host range. *Vd* populations affecting crops have been genetically characterized into a limited number of clonal lineages. Interestingly, *Vd* lineage 4A is highly aggressive to potato crops and has been solely recovered from US potato agroecosystems. However, genetic variation and evolutionary relationships among 4A isolates remain unknown. Here, we aim to (1) investigate the genetic diversity within *Vd* lineage 4A and (2) elucidate the evolutionary and genetic relationships of 4A isolates. We hypothesize that the 4A lineage is highly clonal, has little diversity and has moved within the U.S. To test this, we performed Genotyping-by-Sequencing on a collection of 100 *Vd* 4A isolates collected during 1980s-2010s across different U.S. states from potato seed, plants, and field soil. Preliminary phylogenetic and population structure analyses using 23,754 SNPs across the genome supported our hypothesis. We will discuss results in depth, indicate the importance of this work for potato seed and disease management, and suggest directions for further research.

Identification of *Colletotrichum* species causing bitter rot on apple in New York and their sensitivity to fungicides

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Bitter rot is one of the most common apple rot diseases caused by *Colletotrichum* spp. and has highly destructive impact on fresh-market apple fruit yields each year. The goal of this study was to determine which *Colletotrichum* spp. cause bitter rot on apple in New York and their sensitivity to different fungicides. In 2017 and 2018, 12 isolates collected from different locations and apple cultivars, identified based on multi-locus sequencing analysis of ApMat, CAL, GAPDH, GS, ITS, and TUB2. Phylogenetic analyses indicated that the most dominant species was *C. fiorinae* from the *C. acutatum* species complex, one isolate identified as *C. fructicola* from *C. gloeosporioedes* species complex and one other species from *C. gloeosporioedes* species complex found for the first time on apple which needs to be further confirmed by morphological data. The sensitivity of all isolates to difenoconazole, thiabendazole, pyraclostrobin was determined based on mycelial growth inhibition. *C. acutatum* isolates were highly sensitive to pyraclostrobin and difenoconazole based on mean effective concentrations (EC50) values <0.1 µg/ml and sensitive to thiabendazole (mean EC50 values of 0.1 to 1 µg/ml). However, all *C. gloeosporioedes* isolates were very sensitive to all the tested fungicides (EC50 values of 0.1 to 1 µg/ml). Pathogenicity test confirmed the potential of all isolates to produce typical bitter rot symptoms on Honeycrisp apple fruit inoculated with mycelial plugs of each isolate.

Grapevine leafroll disease is widespread in Connecticut

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Grapevine leafroll disease (GLD) is the most widespread viral disease to grapes worldwide and it is caused by at least eight different +ssRNA viruses - grapevine leafroll associated virus (GLRaV) -1, -2, -3, -4, -5, -6, -7, and -9. To investigate the potential presence of GLRaVs in Connecticut (CT), 56 leaf petioles were collected from symptomatic plants (one sample per plant) from six different vineyards in CT in the fall of 2018. All samples were assayed by DAS-ELISA using antibodies specific for four major GLRaVs (GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-4). ELISA positive samples were further tested by RT-PCR using primers specific for each of the four GLRaVs to confirm the ELISA results. Four samples representing three vineyards tested ELISA positive for GLRaV-1 and GLRaV-3, one sample was infected with GLRaV-2 and GLRaV-3, three samples from a single vineyard were tested positive for just GLRaV-1, and 10 samples from four vineyards were singly infected by GLRaV-3. GLRaV-3 was the most common virus species detected (12 samples from five vineyards). GLRaV-4 was not detected in any sample. All ELISA positive samples also tested positive by RT-PCR for the specific virus. This is the first study reporting the presence of GLRaVs in grapevines growing in CT, extensive surveys need to be conducted to evaluate the prevalence and economic impact of GLRaVs to Connecticut's vineyards.

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Preliminary phylogenetic and population structure analyses using 23,754 SNPs across the genome supported our hypothesis. We will discuss results in depth, indicate the importance of this work for potato seed and disease management, and suggest directions for further research.

Effect of plant cultivar on efficacy of biochemical inducers for suppression of plant diseases

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A major challenge in sustainable agriculture is development of non-toxic alternatives to traditional pesticides. Biochemical pesticides are naturally occurring substances which control pests through induction of natural plant defense mechanisms. Several studies have reported on the ability of biochemical pesticides to reduce disease on multiple crops. It is documented however, that plant genotypes respond differently to biochemical pesticides. The objective of this research was to evaluate the effect of plant cultivar on biochemical pesticide efficacy to suppress disease using apple and cucumber. Three experiments were conducted to evaluate Regalia®, Lifegard™, Actigard®, Pipecolonic Acid, and Hexanoic Acid. A foliar spray of these inducers was tested on four cucumber cultivars for suppression of Powdery mildew in a RCBD with 5 replicates. Foliar spray and drench applications were tested in suppression of *Erwinia amylovora* on four apple scion/rootstock combinations with 5 replicate trees per treatment. A significant effect of cultivar on suppression of powdery mildew was observed on cucumber. However, the efficacy of inducer to suppress fire blight symptoms was not affected by apple cultivar. Actigard® significantly suppressed powdery mildew on three of the four cucumber cultivars. More replications are needed to determine the effect of cultivar in both systems on biochemical pesticide efficacy.

Impact of signal molecules on *Phytophthora erythroseptica* for potato infection

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Pink rot (*Phytophthora erythroseptica*) of potato is a major concern in many potato production regions. We have found the correlation between the density of *P. erythroseptica* zoospores and level of potato infection was mediated by specific signal molecules found in zoospore exudates *P. erythroseptica*. By using liquid chromatography mass spectroscopy (LC-MS), the signal molecules have been identified as amino acids leucine and isoleucine. Exudates of select soil microorganisms (fungi, bacteria, and oomycetes) and potato roots contained homologous of the signal molecules that function to stimulate zoospore germination and infection of *P. erythroseptica*. Microorganisms closely related to *P. erythroseptica*, such as other *Phytophthora* spp. were more likely to have this type of signal molecules. Root exudates from the pink-rot-sensitive varieties induced zoospore germination while that from tolerant varieties had less effect, although there were exceptions. These results indicated that the biological activity of *P. erythroseptica* was not only self-regulated, but also affected by other environmental organisms and host plants.