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Roles of alternate alleles of the gene *cloA* in ergot alkaloid biosynthesis

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Ergot alkaloids are specialized metabolites produced by several fungi, including the sorghum pathogen *Claviceps africana*. Most ergot alkaloids are made from lysergic acid (LA) and are vasoconstrictive, but the ergot alkaloids of *C. africana* are made from dihydrolysergic acid (DHHLA) and are vasorelaxant. This difference affects their pharmaceutical activities related to cognitive function. To produce LA, the enzyme CloA fully oxidizes a methyl group on the substrate agroclavine. To better understand the pathway to DHHLA, we analyzed activities of CloA from different fungi by expressing alleles in a *Neosartorya fumigata* mutant that accumulates festuclavine, the precursor to DHHLA. Transformants expressing *cloA* from an *Epichloë* sp. did not oxidize festuclavine to DHHLA. When supplied agroclavine externally, these transformants oxidized it to LA, indicating CloA was functional. A native version of *cloA* from *C. africana* was expressed in *N. fumigata*, but introns were retained rendering the gene non-functional. We thus expressed a synthetic, intron-free version of *C. africana cloA* which resulted in DHHLA accumulation as assessed by HPLC and LC-MS. *Claviceps africana* CloA also oxidized agroclavine to LA, indicating a broader substrate specificity than observed with CloA of *Epichloë* sp. We conclude that the pharmaceutically important compound DHHLA can be produced in engineered *N. fumigata* and that CloA expressed from different alleles varies in substrate specificity.

Integrating biocontrols in the management of foliar diseases on turfgrass in the mid-Atlantic

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Turfgrass managers are hesitant to integrate biopesticides into their management programs, as synthetic products are generally regarded to be more efficacious. However, few studies have evaluated programmed rotations of either biopesticides or biological and conventional pesticides. In 2016, a 2×4 factorial study was initiated in Derwood, MD to evaluate fertilizers and biofungicides for control of grey leaf spot (*Pyricularia grisea*) on perennial ryegrass (*Lolium perenne* L.) maintained as a golf course fairway. The randomized complete block design treatments were comprised of fertilizer source factor [Fish meal based (Organic) vs Sulfur Coated Urea (Synthetic)] and disease control factor [conventional fungicides (CNV) vs biofungicides (ORG) vs alternated conv. and bio. fungicides (HYB), and none (NON)]. Fungicides were applied in 815 L of water per hectare using a CO₂ backpack sprayer. Treatments were applied along with measurements of turf quality and disease incidence every 14 d, from 27 April to 24 August. Differences were not observed with fertilizer source in 2016, whereas fungicide programs affected both disease and turf quality. Both CNV and HYB programs produced higher quality turf and reduced pest incidence compared to ORG and NON treatments, regardless of fertilizer source. The HYB program used 45% less synthetic pesticide and was as effective as CNV, making it a viable option for extending fungicide application intervals in turfgrass fairways.

Genetic analysis of the ergot alkaloid biosynthetic capacity of the maize ergot fungus *Claviceps gigantea*

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Ergot alkaloids are a family of secondary metabolites produced by several species of fungi. They are structurally similar to neurotransmitters, which allows them to treat several neurological and cognitive diseases. Dihydroergot alkaloids, such as dihydrolysergol and dihydrolysergic acid (DHLA), are suitable for treating dementia. Understanding the genes involved in dihydroergot alkaloid synthesis may facilitate more efficient semisynthesis of dihydroergot alkaloid-derived pharmaceuticals. The maize pathogen *Claviceps gigantea* was reported to produce dihydrolysergol, which is an oxidized product of the precursor festuclavine. Since the enzyme CloA oxidized a similar substrate, we hypothesized that expression of *C. gigantea cloA* in a festuclavine-accumulating mutant of the model organism *Neosartorya fumigata* would yield dihydrolysergol. We engineered such a strain, and HPLC and LC-MS analyses demonstrated that the modified strain produced DHLA, the fully oxidized product of dihydrolysergol. Since dihydrolysergol was expected to be the pathway end product of *C. gigantea*, we analyzed genomic sequence data and field-grown *C. gigantea*. Results supported our finding that DHLA rather than dihydrolysergol is the end product of the *C. gigantea* pathway. *C. gigantea* is the first known organism to produce DHLA as its pathway end product and expression of its *cloA* gene may serve as the basis of a more efficient approach to production of DHLA for pharmaceutical purposes.

Recent investigations of biological control of Japanese hops, (*Humulus japonicus*) using endemic fungal pathogens

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Japanese hops, *Humulus japonicus* (family *Cannabaceae*), is now invasive along a riparian area of the Monocacy River in Frederick, MD and in many other similar habitats. It was imported into the U.S. in the late 1800s as an ornamental vine. Because infestations of Japanese hops dominate these riparian areas, it has become a target for biological control in the U.S.A. In 2016, diseased leaves were collected and sent to the Foreign Disease Weed Science Research Unit (FDWSRU) in Frederick, MD. Surface disinfested leaves were placed in moist chambers, and after several days at 20–25°C, acervuli formed on the irregular tan necrotic lesions. Pure cultures (FDWSRU 16-008) were obtained by transferring spore masses with sterile glass needles onto 20% V-8 juice agar. Five rooted cuttings of *H. japonicus* were inoculated by spraying an aqueous suspension of spores (10⁶ per mL to runoff) collected from two-week-old cultures. Inoculated plants were placed in a 20–24°C dew chamber for 18 hours and then placed in a 25°C greenhouse. Two weeks later, diseased leaves of each species were harvested, and the fungus was re-isolated. The causal agent is tentatively identified as a *Colletotrichum* species, based upon morphology of spore and appressoria and observation of setae on leaves and in culture. Molecular characterization of this isolate is planned, which will help with identification. The use of endemic fungal pathogens represents a new direction in weed biological control at FDWSRU.

Identification and characterization of virulence genes in *Erwinia amylovora*

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The bacterium *Erwinia amylovora* causes fire blight in apples and pears. This project seeks to identify novel *E. amylovora* genes that are necessary for fire blight disease development in apples. In order to study *ptxR* as well as other genes, *E. amylovora* was mutagenized with an engineered transposon and screened for loss of virulence. The transposon inserts itself randomly into the bacteria's genome, interrupting the spelling of DNA, creating gene mutations with a variety of effects. After three rounds of screening, mutants that were less or asymptomatic had their disrupted gene identified. The exact site of the insertion was determined by the use of the Basic Local Alignment Search Tool. This project also seeks to characterize the *ptxR* gene in *E. amylovora*, an uncharacterized gene found through this virulence screen. PtxR, a transcriptional regulator contains a helix-turn-helix (HTH) DNA-binding domain. Currently, we predict that PtxR plays a role regulating virulence factors in *E. amylovora*. Here, we observe changes in siderophore and amylovoran production by mutating *ptxR*. The decrease in virulence factors may explain why the *ptxR* mutant causes reduced disease in apples. Future directions include examining the region of DNA bound by PtxR's HTH domain using Chip-seq. This will allow us to better understand the role of PtxR in fire blight disease, and can allow scientists to focus their targets when creating methods to manage fire blight in agricultural settings.

Monitoring and managing white mold disease on lima bean in mid-Atlantic region, U.S.A.

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Lima bean is severely affected by white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. However, management options to control white mold in the region are limited to application of fungicides with active ingredients such as boscalid (Endura 70W) or a biocontrol agent, *Coniothyrium minitans* (Contans WG). There are currently no specific fungicide application guidelines and low adoption rates for Contans. Efficacy trials with Endura (2014–2016) in Lewes, DE and Contans (in 2016) in Salisbury, MD for white mold management were conducted. Endura (@0.76 kg ha⁻¹) was applied at 20% flowering; one week later; two weeks later; three weeks later; and at 20% flowering plus one week later (treated control); and non-sprayed (untreated control). Disease incidence (DI) was measured as number of infected plants/m sections of row. Disease severity (DS) was rated as percentage of infected tillers per plant/m sections of row. Pod weight was recorded as kg from a 6 m² subsection of row area. Proc mixed model in SAS was used to

determine statistical differences among DS, DI, and yield. Both DI and DS were significantly influenced by timing of Endura applications, the treated control being the better timing. DS was also significantly influenced by years. Yield was significantly different between locations. Contans spray timings did not significantly reduce DS, DI, or yield.

Systemic infection of *Impatiens balsamina* through inoculation of roots with vegetative sporangia of the *Impatiens* downy mildew (*Plasmopara obducens*)

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Impatiens downy mildew (IDM) infects bedding *Impatiens*, threatening a crop valued annually at more than \$130 million. The life cycle of the pathogen is not clearly understood, but oospores of IDM are known to be seed borne in *I. balsamina*, and seed-borne oospores can lead to systemic plant infections. As a preliminary step to investigating the risk of systemic infection to seed-propagated *Impatiens* by seed-borne oospores, and to determine the risk of root inoculation by vegetative sporangia to transplanted bedding *Impatiens*, we attempted to infect *I. balsamina* systemically by inoculating roots with vegetative sporangia. In two trials, the roots of 20 seedlings were immersed in a suspension of sporangia collected from infected leaves (2×10^3 sporangium/ml); the roots of ten additional plants were immersed in sterile water as a control. After 48 hr, six plants from each treatment were removed from water and planted in sterile potting mix. The remaining plants were kept in water and examined at 10 and 14 days after inoculation. For plants transplanted to potting mix, we observed symptoms of systemic infection (dwarfing and chlorosis) in two of six root-inoculated plants in first trial and three of six in the second; for plants kept in water, we observed symptoms on 10 out of 14 root-inoculated plants in both trials. A subsample of symptomatic leaves placed in moist chambers at room temperature showed sporulation of the pathogen after 24 hr. Control plants showed no symptoms.

Both boxwood blight pathogens (*Calonectria pseudonaviculata* and *C. henricotiae*) can infect boxwood roots

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In North America, boxwood blight is caused by *Calonectria pseudonaviculata* (*Cps*) although a second species, *C. henricotiae* (*Che*) is also present in Europe. *Cps* is known to infect roots, but it was unclear whether *Che* could, therefore side-by-side comparisons were carried out. In two trials, the roots of 10–12 two-month-old boxwood cuttings (*Buxus sempervirens*) were dipped in a water suspension of conidia of either 4 pooled isolates of *Cps* or *Che* (at 4000 conidia/mL); 20 were dipped in water alone as controls. Three to four wk after inoculation, root segments were surface-sterilized and plated on semi-selective media for *Calonectria* and the number of positive root necrotic lesions (as determined by sporulation of the pathogens) was rated. Most of the inoculated cuttings developed symptoms of stem cankers and root necroses while the controls remained asymptomatic. Both pathogens formed microsclerotia in roots, and previous work has shown that microsclerotia survive for long periods of time in leaf and stem litter. It is possible that survival in root tissue may also be a significant method of overwintering.

Investigating the poison ivy drupe microbiome and avian phytosanitation

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We previously demonstrated that the endemic fungus *Colletotrichum fioriniae* on poison ivy drupes results in a lethal blighting of poison ivy seedlings. The purpose of this study was to characterize the fungal and bacterial microbiomes of poison ivy drupes collected in 2014 and 2015. Drupes were subjected to bead beating to extract microbes from exocarp and mesocarp tissues. The culturable fungal and bacterial CFUs were estimated on PDA and NA media, respectively. A small proportion of fungi and bacteria were associated with the external exocarp, whereas the majority of microbes were associated with internal mesocarp tissue. There were significant differences in total fungal and bacterial CFUs between drupes collected in 2014 and 2015. Fungal DNA and bacterial DNA were also isolated from mesocarp extracts and subjected to ITS and 16S rRNA sequencing, respectively. In both 2014 and 2015 phytopathogenic *C. fioriniae* were present on poison ivy drupes; however, in 2015 the fungal populations were less diverse (by Shannon indices) largely due to a high proportion of *Cladosporium* species. Poison ivy drupes that were fed to captive house finches and then defecated showed significantly lower fungal CFUs. Drupes showing greater degrees of avian digestion had significantly lower fungal CFUs. These findings indicate that avian digestion of poison ivy drupes has the potential to aid in both drupe dispersal and phytosanitation to reduce *C. fioriniae* seedling blight.

Monitoring *Impatiens* beds for overwintering of *Plasmopara obducens* (*Impatiens* downy mildew) and development of a baiting assay for soil

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Impatiens downy mildew (IDM) threatens a crop valued annually at more than \$130 million wholesale. This study investigated whether IDM is capable of overwintering in infested soil (and infecting new plants in the spring) and whether infested soil can be baited in order to detect IDM. Monitor plots emended with IDM-infested plant debris in fall were planted to *Impatiens balsamina* the following spring

and observed for disease as seedlings emerged and matured. In one of three infested monitor plots, seedlings emerged with IDM symptoms. In baiting experiments, infested soils from 14 locations were stored at 4°C, transferred to 0°C for one week, then warmed to room temperature for 3 days and screened to collect the organic fraction and fine silt. Millipore water was poured on over soil in Petri dishes and allowed to sit undisturbed for 4 and 6 days before subsamples were pipetted onto bait leaves in moist chambers. The chambers were placed in darkness for 24 hours then incubated under natural lighting at room temperature. One week later the leaves were checked for the IDM infection. The assay yielded positive results in two of the three trials. This research provides evidence that the pathogen can survive in soil for long periods of time and can be detected with baiting methods.

Screening the local population of *Pseudoperonospora cubensis* for fungicide resistance in the mid-Atlantic region

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Cucurbit downy mildew (CDM) caused by *Pseudoperonospora cubensis*, is a devastating disease of cucurbit crops worldwide. Management of the rapidly evolving oomycete pathogen often relies on intensive fungicide spray programs. A bioassay was conducted to determine the efficacy of fungicides commonly used for control of CDM and the underlying resistance to the fungicides in the 2016, mid-Atlantic, *P. cubensis* field population. Ten fungicides were applied at full and half-label rates on cucumber (*Cucumis sativus*) seedlings before 48 hours of exposure to the natural *P. cubensis* population in an infested field. Plants were then placed in a growth chamber for five days at 21°C/18°C day/night, before rating disease severity. The fungicides cyazofamid (Ranman), cymoxanil (Curzate), zoxamide + chlorothalonil (Zing!), and fluopicolide (Presidio) all provided excellent control (96–100% disease reduction compared to the untreated control) at both full and half-label rates. While propamocarb (Previcur Flex) controlled CDM at the full-label rate, almost no reduction of disease was observed at the half-label rate (100% and 1.5% disease reduction, respectively, compared to the untreated control), suggesting a high percentage of the 2016, local, *P. cubensis* population was partially-resistant to the fungicide. Penthiopyrad (Fontelis), triflumizole (Procure), and azoxystrobin (Quadris) ranged from 32–65% disease reduction compared to the untreated control at the full-label rate, suggesting limited utility of these chemicals in CDM management. The bioassay results will be used to develop a fungicide spray program, balancing the efficacy of the products with the likelihood for development of resistance to the fungicides.

Discovering an even stickier situation with *Erwinia amylovora*

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Erwinia amylovora causes fire blight, an economically important bacterial disease of Rosaceous plants. *E. amylovora* produces copious quantities of a sticky extracellular polysaccharide (EPS) called amylovoran, which is essential for *E. amylovora* pathogenicity. The large, 14-gene *ams* operon directs amylovoran synthesis. Here, we describe the identification of a previously uncharacterized operon that may also be involved in amylovoran production. A genetic screen for loss of virulence mutants revealed that the *rfbX* gene is required for *E. amylovora* virulence and amylovoran production. *rfbX* encodes a predicted polysaccharide transporter involved in EPS moiety movement across the cytoplasmic cell membrane. *rfbX* is the second gene in a two gene operon along with *yibD*, a predicted glycosyltransferase. Surprisingly, deletion of the entire *yibDrfbX* operon or mutation of *yibD* alone did not disrupt virulence or affect amylovoran production. Furthermore, overexpression of *YibD* interfered with amylovoran production, further supporting a link between the *yibDrfbX* operon and this important EPS. Possible redundancy is now being explored between *yibDrfbX* and the tandem glycosyltransferase and polysaccharide transporter genes *amsK* and *amsL* at the distal end of the *ams* operon. A more complete mechanistic understanding of amylovoran production, including vulnerabilities, may facilitate development of novel fire blight management approaches.

Common *Fusarium* spp. causing fruit rots on *Cucumis melo* in the mid-Atlantic region of the U.S.

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Melons (*Cucumis melo*) are hosts to many fruit rots including those caused by several *Fusarium* spp. However, which *Fusarium* spp. cause cucurbit fruit rots in the mid-Atlantic region of the U.S., especially in the states of Maryland and Delaware is not known. Our objective was to identify *Fusarium* spp. causing fruit rot on melon cultivars ('Arava', 'Athena', 'Dulce', 'Eden's Gem', 'Escorial', 'Juane', 'Sivan', 'Snow Mass', and 'Spanish Sun'). A survey was conducted in melon production fields and two experimental farms. Fields were located in Maryland (four locations) and in Delaware (two fields) during the months of July and August 2016. Twenty five fruit with visible symptoms of *Fusarium* fruit rot (white, tan or brown sunken areas on fruit) were collected from each location and transported to the plant pathology lab for *Fusarium* isolation and identification. Seventy two putative *Fusarium* isolates were obtained from one hundred twenty five fruit collected. Virulence of mono-conidial cultures was tested by inoculating spores on wounded and non-wounded muskmelon and honeydew, which were purchased from local retail stores. Fungi were re-isolated from infected fruits for pathogenicity confirmation. All of the isolates were pathogenic on melon fruit. Isolates were morphologically identified to specie on Carnation Leaf Agar (CLA) medium. Morphological identification was based on the presence of microspores, macrospores, conidial shape, and other informative traits. DNA was extracted from mono-conidial isolates, amplified with PCR, and sequenced for final identification.

Evaluating the effect of increased salinity on the growth of root pathogens of dry beans

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Legumes such as dry beans and pulse crops produced worldwide are known as good sources of dietary protein. They are included in crop rotations for their ability to fix nitrogen. However, they are subjective to abiotic stresses such as drought and salinity, and are commonly affected by root diseases. As these abiotic stresses are on the rise particularly in the semi-arid and arid regions, there is a need to develop a better understanding of the effect of abiotic stresses on plant pathogens and disease development. Independent studies have been conducted on the effect of abiotic and biotic stressors on plant growth, however, studies on the combined effect of these stresses in systems involving root pathogens and disease development are limited. Two of the most important root pathogens of dry beans in the U.S. are *Rhizoctonia solani* and *Fusarium solani* f.sp. *phaseoli*. The goal of this study is to assess the effect of increased salinity on growth of these two pathogens and assess their ability to infect dry beans. The effect of increased salinity on fungal growth is being assessed by recording radial growth and biomass *in-vitro* on solid and liquid potato dextrose media amended with different concentrations of NaCl. Similar studies on disease development involve treating inoculated roots with saline water. Initial results suggest that increased salinity affects pathogen growth, however the extent of the effect varies between isolates and species. Among the isolates evaluated, generally a decrease in radial growth is observed. Preliminary results also suggest that salinity has an effect on disease development.

Evaluation of rose germplasm for resistance to Rose rosette disease

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Rose rosette disease (RRD) is a lethal disease of roses (*Rosa spp.*) caused by *Rose rosette emaravirus* and vectored by the eriophyid mite *Phyllocoptes fructiphilus*. It is one of the most devastating diseases of landscape roses, producing aesthetically disfiguring symptoms within months and eventually killing the plant. Unfortunately, there is no known resistance among commercial rose cultivars and the few rose species that have demonstrated resistance are not ideal candidates for introgression of resistance genes into commercially viable cultivars. This study hopes to identify rose genotypes that merit consideration as candidates in a breeding scheme for resistance to RRD. A total of 151 genotypes are being screened in an ongoing field trial at the University of Delaware in Newark, DE. In addition to high natural disease pressure, the field has received multiple augmentations since its establishment in May 2015. Augmentation consisted of using twist ties to affix symptomatic shoots of RRD-infected *Rosa multiflora* to actively growing shoot tips of target roses. The vector preferentially feeds on symptomatic tissue, so augmentation artificially enhanced vector populations. Any roses that developed symptoms were subsequently tested by RT-PCR to confirm the presence of RRV. Thus far 85 genotypes have been verified as susceptible to RRD. The study will continue through 2018 and any roses remaining symptom-free will be assayed to confirm the absence of viral replication.

Interaction of brown marmorated stink bug and bacterial soft rot in bell pepper fruits

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The brown marmorated stink bug (BMSB, *Halyomorpha halys*) is an introduced pest causing widespread damage to many crops in Mid-Atlantic states. We evaluated the interaction between BMSB and soft rot bacteria (Pc, *Pectobacterium carotovora*) on bell pepper. Adult BMSB were either fasted for 2 days in cages with distilled water or exposed in cages with Pc-infected pepper fruits and Pc-infested drinking water. Miniature bell pepper fruits (cv. Sweet Cute Gold Stuff) were surface disinfested in 1% NaOCl, and rinsed in sterile water. Single peppers were placed in Solo® ramekins, and ten each were treated with: 1) two adult BMSB exposed to Pc-infected fruits; 2) two unexposed BMSB; 3) Pc spray inoculated onto fruits; 4) Pc spray and two unexposed BMSB; or, 5) control pepper fruits sprayed with distilled water. Lids were attached, and fruit rot was assessed after incubating at 22 C for 4, 7, 11 or 14 days. Fruit rot was visually rated as 0 = no visible decay; 0.5 = 1–9% of fruit area with decay; 1 = 10–24% decayed, 2 = 25–49% decay; or 3 = 50% or more decayed. No bacterial soft rot decay was observed through 14 days after inoculation (DAI) on controls or fruits with unexposed BMSB only. One of ten fruits inoculated with Pc spray showed slight decay. Fruits with two exposed BMSB or fruits spray-inoculated with Pc plus BMSB had significantly more bacterial soft rot at 11 and 14 DAI. The two-way interaction between BMSB and Pc was statistically significant.

Combining host resistance and hypovirulence to combat chestnut blight

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Chestnut blight, caused by the fungus *Cryphonectria parasitica*, is a canker disease of *Castanea* species. A backcross-breeding program is being employed by The American Chestnut Foundation (TACF) to generate blight-resistant hybrids. Biological control has been attempted by exploiting hypovirulence, a phenomenon that results from infections of *C. parasitica* by virulence debilitating mycoviruses. This study assessed the interactions between host resistance and hypovirulence. Plots containing American (*Castanea dentata*), Chinese (*C. mollissima*), European (*C. sativa*), and three generations of TACF hybrid (B2F2, B2F3, B3F2) chestnuts were used. Host resistance was screened for a subset of the chestnut species and hybrids using direct stem inoculations and subsequent canker measurements for one year. A repeated measures ANOVA for average canker growth revealed Chinese chestnut to be most resistant

with American, B2F3, and B3F2 chestnuts significantly more susceptible. Natural and artificial infections on half of the trees were treated with hypovirulent isolates, while the remaining trees were left untreated. Tree health (Dead (0) – Healthy (5)) and survival within all plots have been evaluated yearly since 2014. Survival of hypovirulence treated trees was up to 31% better, and average tree health ratings were 0.56 higher. These findings begin to describe the effects of combining increased host resistance and hypovirulence for the restoration of the American chestnut.

Potential use of biosurveillance to detect oak wilt

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Oak wilt, caused by the fungus *Ceratocystis fagacearum*, is a vascular disease that causes wilting and rapid mortality of members of the red oak group. Early detection and diagnosis of oak wilt is difficult, and the development of effective and efficient techniques is needed. Oak wilt spreads via root grafts and insect vectors. The primary insect vectors of oak wilt are Nitidulid beetles, which are attracted to oak wilt mats produced in the spring and fall. Beetles pick up spores while visiting these fungal mats and can disseminate the fungus to fresh wounds on adjacent trees. We provide an early assessment of the potential use of a Nitidulid-based biosurveillance detection method using baited insect traps. Beetles were trapped for 8 months at three oak wilt centers in Pennsylvania. Two of the 26 species identified, *Carpophilus sayi* and *Glischrochilus sanguinolentus*, accounted for 16 and 14%, respectively, of the total (2373) beetles trapped. A nested PCR protocol utilizing *C. fagacearum* selective primers was performed for genomic DNA samples extracted from washes of beetles. Gel electrophoresis was utilized to visualize any expected 280-bp products. A sample of these positive reactions were sequence confirmed. Despite being originally developed for diseased plant tissues, limited success has been observed using beetle washes. Further testing is currently being conducted to further develop this biosurveillance detection method for oak wilt.

Examining the population structure of the cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*, by host, location and time

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Cucurbit downy mildew, caused by the oomycete organism *Pseudoperonospora cubensis*, is a foliar disease that affects cucumber, cantaloupe, squash, pumpkin, and watermelon in the United States (U.S.) and worldwide. It has been previously demonstrated that populations of *P. cubensis* change in genetic structure from field to field in Michigan (MI) and Ontario (ON), and genetic structure can also change over the course of a growing season. The authors sought to determine the population structure of *P. cubensis* in the state of North Carolina (NC) where there is known to be high genetic diversity. Microsatellite markers were used to genotype samples of *P. cubensis* collected from 10 host species from eastern, central, and western NC. Samples were collected during the summer and fall cucurbit growing seasons and sampling was repeated over two years. Alleles were visualized through fragment analysis and were called and binned through Geneious. STRUCTURE analysis revealed that a vast majority of the *P. cubensis* isolates had high identity to either one of two distinct genetic clusters. A majority of *P. cubensis* isolates that grouped in Cluster 1 were from *Cucumis* hosts and a majority of the isolates that grouped in Cluster 2 were from *Cucurbita* hosts. *P. cubensis* isolates from cantaloupe tended to have more genetic diversity in regions with more diverse cucurbit production. These results demonstrate the role of host and location in pathogen diversity within a localized region.