2013 Northeastern Division Meeting Abstracts

Abstracts presented at the APS Northeastern Division meeting in Southbury, Connecticut, October 23–25, 2013. The abstracts are arranged alphabetically by the first author's name. Recommended format for citing division meeting abstracts, using the first abstract below as an example, is as follows: Abawi, G. S., Kikkert, J. R., Moktan, K., Lange, H. W., Smart, C. D. 2014. First report of resistance to *Quadraris* among populations of *Cercospora beticola* causing leaf spot of table beets in New York State. (Abstr.) *Phytopathology* 104(Suppl. 1):S1.1. <http://dx.doi.org/10.1094/PHYTO-104-3-S1.1>

First report of resistance to *Quadraris* among populations of *Cercospora beticola* causing leaf spot of table beets in New York State

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Leaf spot of table beets caused by *Cercospora beticola* is a major and widespread disease, impacting beet production in New York. In 2012, leaf spot severity was very high in an 80 acre beet field in western NY, as 100% of the plants were infected and there was an average of 28.3 lesions/leaf on August 24. Surprisingly, the beets in this field were already sprayed three times with *Quadraris* (azoxystrobin at 9.2 Oz/A). An isolate of *C. beticola* recovered from infected leaves was confirmed in laboratory tests to be highly resistant to *Quadraris*, as it grew on PDA amended with up to 100-ppm a.i. of *Quadraris*. However, this isolate and others were found to be sensitive to Tilt (propiconazole) in similar tests. Typical lesions of leaf spot developed on *Quadraris*-sprayed (9.2 Oz/A in 30 gal. water) and unsprayed beets growing in an experimental plot that were inoculated with infected leaves collected from this field. In a replicated field trial consisting of fungicides with different mode of actions, Tilt (4 Oz/A), Bravo (2 lbs./A) and Manzate (3 lbs./A) gave excellent control of *C. beticola*, whereas *Quadraris* (9.2 Oz./A) failed to control this pathogen and was not different from the untreated check plots. In 2013, 30 isolates of *C. beticola* were recovered from 10 commercial beet fields in central-western NY and all, but one grew on PDA amended with up to 1000-ppm a.i. of *Quadraris*. Characterization of the DNA sequences of resistant and sensitive isolates of *C. beticola* from NY is in progress.

Real-time PCR typing of *Colletotrichum cereale* from modern and historical collections

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Colletotrichum cereale is a pathogen and endophyte of cool-season grasses in the Poaceae family. The fungus has increased in frequency over the past decade as a destructive pathogen of golf course putting greens. DNA fingerprinting has revealed two distinct phylogenetic clades of the fungus, designated clade A and clade B. Little is known about the distribution of these

two clades in natural environments, or what role they may play in the trajectory of recent disease outbreaks. In this study, our objective was to evaluate modern and historical collections of *C. cereale* to determine the frequency of pathogenic and endophytic clade A and clade B isolates in the environment. A real-time PCR assay was developed using the *Apn2* marker to discriminate between the two *C. cereale* clades. A total of 701 *C. cereale* samples were screened from four sources: 1) 576 samples from axenic cultures, 2) 87 samples from preserved fungarium specimens, 3) 17 samples from *Poa annua* (annual bluegrass) plants with symptomatic infections, and 4) 21 samples from asymptomatic wheat plants that exhibited setae on sampled tissue. *C. cereale* was detected from 98.4% (n = 690) of all isolates screened. Of these samples, 87% were diagnosed belonged to clade A, 11.7% were part of clade B, and 1.3% were mixed A/B clades. From the two turfgrass hosts examined, *Agrostis stolonifera* (creeping bentgrass, n = 79) and *P. annua* (n = 179), 86% of all *P. annua* isolates were typed as clade A, whereas only 57% of *C. cereale* isolates from *A. stolonifera* were members of this clade. This distribution was also influenced by geography, with Clade A predominating in the southern U.S., and clade A and B being divided evenly in northern states, suggesting that temperature may play a role in the distribution of this pathogen.

Taxonomical status of *Lophophacidium dooksii* and *Canavirgella banfieldii*, causal agent of a white pine needle disease

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In 2009, unusual white pine (*Pinus strobus* L.) needle discoloration was observed in eastern Canada and USA. While the symptoms were similar in most locations, symptom severity varied depending on location and time of year. This slight variation in symptoms lead to the disease being diagnosed as *Canavirgella banfieldii* in several locations and Dooks needles blight caused by *Lophophacidium dooksii* in several other locations. Due to the similarity in symptom development and morphological features it is possible that *L. dooksii* and *C. banfieldii* are either the same species or represent closely related but phylogenetically distinct lineages. Therefore, the objective of this study was to clarify the taxonomical status of the relationship between *Lophophacidium dooksii* and *Canavirgella banfieldii*. For morphological measurements, DNA extraction and phylogenetic analysis, 20 fungal specimens representing *C. banfieldii* and *Lophophacidium dooksii* from pines, including the two type specimens were selected based on their geographical localization and their quality. Phylogenetic analysis confirmed morphological observation that the isolates of *C. banfieldii* and *L. dooksii* are indeed the same organisms. In addition, as this was the first time the ITS region from *C. banfieldii* and *L. dooksii* was sequenced, we were able to determine that the closest known relative is *Lophodermella arcuata*, which causes a very similar needle cast disease on Southwestern white pine (*Pinus flexilis*), as well as several species of *Lophodermium*. As *L. dooksii* was described first, this name should take priority. The results from this project will assist researchers and

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forest health professional accurately diagnose needle disease and provide consistency to diagnoses across locations. The study has also provided the first evidence of the evolutionary relationship of *L. dooksii* to other pine pathogens.

A proposed new model for beech bark disease development in aftermath forests

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Neonectria ditissima and *N. faginata* are causal agents of beech bark disease (BBD) in North America. Infection of American beech (*Fagus grandifolia*) by these fungi is believed to follow a single predisposing factor, infestation by the beech scale (*Cryptococcus fagisuga*). However, recent work does not support this model and suggests the influence of alternative or additional predisposing factors. Because BBD related research does not commonly operate at the species level, fundamental similarities and differences between the *N. ditissima* and *N. faginata* pathosystems have gone unexamined. An understanding of these pathosystems is integral to developing effective management strategies for BBD aftermath (long-affected) forests. Although several physiological and nutritional factors differ between healthy, infested, and infected beech, the possible role of these factors in predisposing infection has not been examined. A case-control study was conducted to investigate potential entomological, nutritional, and physiological factors predisposing beech to infection by *Neonectria* pathogens, individually and together. Infection by *N. ditissima* in 2012 was positively related to *Xyllococcus betulae* densities and the concentration of an unidentified phenol (P57) in 2011. Infection by *N. faginata* in 2012 was positively related to beech scale densities and the concentration of two unidentified phenols (P12 and P74) in 2011. Low bark phosphorus in 2011 was a predisposing factor important to the pathosystems of both fungi. These findings considerably expand the beech scale predisposition model of BBD development for aftermath forests and have important implications for managing BBD-affected forests.

Real-time PCR detection of *Puccinia chrysanthemi* causing brown rust of chrysanthemum

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Fungi responsible for rust diseases are among the most challenging organisms to identify. In the U.S., numerous rust fungi are quarantine-significant plant pathogens. As such, accurate identification is crucial to prevent the inadvertent introduction of destructive pathogens and to facilitate trade. Three species in the Pucciniales cause rust diseases of ornamental chrysanthemum, *Phakopsora artemisiae*, *Puccinia horiana*, and *Puccinia chrysanthemi*. *P. horiana* is a quarantine-significant pathogen causing white rust disease, which renders infected plants unmarketable and produces substantial economic losses. *P. chrysanthemi*, causing brown rust, is ubiquitous in the U.S., but losses due to this pathogen are trivial. *P. artemisiae* is known only from Japan and China. The objective of this study was to develop a real-time PCR assay for the detection of *P. chrysanthemi*, which can be difficult to distinguish from *P. horiana* in the early stages of infection. A fluorescence-labeled hydrolysis probe with four locked nucleic acids was designed from a region of the internal transcribed spacer region (ITS1) rDNA region. The probe region is unique to *P. chrysanthemi*, and falls within an 88-bp PCR amplicon. The assay was used to screen 206 specimens of rust fungi on chrysanthemum held at the U.S. National Fungus Collections (Beltsville, MD). *P. chrysanthemi* was detected in 181 of 187 specimens (97%) previously morphologically identified as *P. chrysanthemi*, including specimens >100 years old. Subsequent morphological examination of specimens that yielded negative results showed that three specimens appeared to have insufficient material for determination and three specimens were *P. horiana* misidentified as *P. chrysanthemi*. Negative results were obtained from ten specimens of *Phakopsora artemisiae* and nine specimens of *P. horiana*. The *P. chrysanthemi* diagnostic assay will confirm the presence of *P. chrysanthemi*, which may aid in correct diagnosis of *P. chrysanthemi* or *P. horiana* in symptomatic plants.

Epidemiological time scales: A self-similar approach

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The methods of dimensional analysis often times used in the physical sciences and engineering is applied to plant epidemic models. This leads to a time-

related epidemiological variable, X , that is dependent on the basic reproductive number, R_0 , the mean time, T , between spore generations, and the ratio of the initial amount of disease to the maximum amount of disease, f . The “ X ” variable is scaled with T and the natural logarithm of R_0 . The resulting curve is shifted in time by a value proportional to the natural logarithm of f . Plots of the time course of disease development versus “ X ” fall along a sigmoidal curve that is very similar for many plant disease epidemics, both theoretical and experimental. Deviations from this “ideal” epidemic are discussed in terms of the details of the temporal structure of the reproductive curve. The “ X ” variable is scaled with T and the natural logarithm of R_0 . The resulting curve is shifted in time by a value proportional to the natural logarithm of f .

The integrated use of host resistance, fungicides and disease forecasting for the management of late blight (US-23) on tomato in Pennsylvania

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Late blight of tomato (*Phytophthora infestans*) continues to require intense management annually. Common practices include the use of host resistance, fungicides, and disease forecasting. We evaluated the integrated use of these factors to manage tomato late blight in two locations in 2013. Treatments included two cultivars: Mountain Fresh Plus (susceptible) and Plum Regal (heterozygous *Ph-3*); two preventive fungicides: copper and chlorothalonil; and three fungicide application timings: 7-day schedule, based on Blitecast or the Cornell Decision Support System (DSS), which incorporates cultivar, Blitecast and Simcast. Disease severity was assessed every 7 days until the untreated susceptible cultivar reached 100%. Fungicide applications based on the forecasting models resulted in a reduction of sprays by 11–16% for Blitecast and 41–44% for the DSS, compared to a 7-day program. Disease severity significantly differed for cultivar ($P < 0.0001$), and fungicide ($P = 0.0010$, $P = 0.0372$) in Centre and Lancaster Co. trials, respectively. Interactions between cultivar x timing ($P = 0.0015$), and cultivar x fungicide ($P = 0.0121$) were found only in Centre Co. In this trial, significantly higher disease severity was observed in Plum Regal following the DSS compared to Blitecast and a 7-day program because no sprays were recommended due to the host resistance status of this cultivar. Treatment effects on fruit incidence were observed in Centre Co. for cultivar ($P < 0.0001$), fungicide ($P = 0.0001$) and interaction of cultivar x fungicide ($P = 0.0002$). Overall, our results suggest that cultivar selection has a major impact on disease severity and fruit incidence and the type of fungicide applied was more important for the susceptible cultivar. Both forecasting models would significantly reduce grower input and labor costs by reducing the number of fungicide applications needed for disease management compared to the 7-day program. For the DSS, differentiating cultivars containing known resistance genes and gene combinations into resistant and moderately resistant categories would further refine the forecasting model.

RNA-Seq and heterologous yeast expression provide evidence for novel fungicide resistance determinants with parallels to pesticide detoxification in plants and insects

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We recently reported findings from *de novo* RNA-Seq to investigate mechanisms of reduced sensitivity to fungicides in *Sclerotinia homoeocarpa* (Bennett), the causal agent of dollar spot on turf. This work identified genes overexpressed from metabolic pathways likely involved in reduced sensitivity to DMI fungicides, which mirror known genetic determinants of metabolic resistance to herbicides and insecticides. Further evidence for the involvement of these transcripts comes from quantitative PCR of isolates that display field resistance to demethylation inhibitor (DMI) fungicides and reduced sensitivity to additional fungicide classes. To further validate the involvement of putative fungicide detoxification genes, we are employing heterologous yeast expression using a galactose-inducible expression vector to express full-length cDNAs of genes representing multiple pathways mined from RNA-Seq data. Data from yeast expression assays confirm that at least one of the novel genes can reduce DMI fungicide sensitivity in yeast, and current work is also aimed at co-expressing multiple genes to look for additive or synergistic gene interactions with regard to reductions in fungicide sensitivity. The pipeline presented here holds potential for future discovery of gene functions and interactions underlying fungicide detoxification in other non-model fungal plant pathogens.

High-resolution DNA melting analysis as a tool to screen for genetic polymorphism in fungicide resistance genes for two important fungal plant pathogens, *Sclerotinia homoeocarpa* and *Colletotrichum cereale*

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Previously we reported on the development of tools for genetic screening of fungicide resistance alleles in the dollar spot pathogen, *Sclerotinia homoeocarpa*. By mining of transcriptome data and utilizing high-resolution melting (HRM) technology we have developed assays for detecting polymorphism in fungicide resistance determinants for two fungicides classes, dicarboximide and methyl benzimidazole carbamate (MBC). Using previously published DNA sequence data, we have also developed HRM assays for detecting genetic polymorphism linked to resistance to MBC and quinone outside inhibitor fungicides in another important turf pathogen, *Colletotrichum cereale*, causal agent of anthracnose of turf. Finally we demonstrate that this method can be adapted for field collected leaf blades of creeping bentgrass infected with dollar spot. Future work will focus on developing a similar protocol for screening anthracnose-infected turf. These assays hold promise for one day being offered as a diagnostic test, which could help to minimize costs and mitigate the environmental consequences of overuse and misapplication of fungicides on golf courses.

Potentials and mechanisms of *Bacillus amyloliquefaciens* BAC03 in plant disease control

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Bacillus amyloliquefaciens strain BAC03 is a potential biological control agent having all characteristics of plant growth promoting rhizobacteria. To determine the spectrum of pathogen and plant that are impacted, and elucidate the mechanisms of BAC03 in disease control, laboratory and greenhouse studies were conducted. Co-culturing with one of the test pathogens on agar media showed that BAC03 significantly inhibited the growth of *Streptomyces* spp., *Agrobacterium tumefaciens*, *Pseudomonas syringae*, *Clavibacter michiganensis*, *Cladosporium cucumerinum*, *Fusarium solani*, *Cryphonectria parasitica*, *Phytophthora* spp., *Penicillium citrinum*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. The inhibition zone of culture colonies ranged from 0.6 to 2.6 cm depending on the test organisms. The active compounds corresponding to antimicrobial activities were LCI protein and volatiles, confirmed by bioassays, DNA sequencing and mass spectrum analysis. Direct seed treatment with BAC03 in liquid culture (10^6 colony forming unit or CFU/ml) negatively affected most of the test plants for germination and emergence. However, if BAC03 was applied after seedling emergence, growth of all test plants were enhanced, including potato, radish, beet, turnip, carrot, tomato, pepper, cucumber, and squash. In a greenhouse trial, radish was grown in potting soil infested with *S. scabiei* (10^6 CFU/cm³), followed by applying BAC03 at 10^6 CFU/cm³. Quantitative polymerase chain reaction was used to detect and quantify the population of both BAC03 and *S. scabiei* in soil. BAC03 population decreased from their application and stayed at a stable level around 30 days after application. BAC03 significantly increased radish biomass ($P < 0.05$), higher (337% increase of fresh roots and 365% increase of fresh leaves) than the control (no BAC03 applied). The highest population reduction of *S. scabiei* occurred when BAC03 was applied 5 days after radish planting. Thus, BAC03 can be a candidate of biocontrol agent with a broad spectrum of target plant pathogens.

Bloat nematode in Maine garlic -- A new pathogen in Maine

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Commercial garlic (*Allium sativum* var. *ophioscorodon*) is grown in all 16 Maine counties. Many garlic producers in Maine are market gardeners producing many crops with the contribution to farm income from garlic disproportionately large when compared to the area planted. Garlic grown in Maine is predominately distributed within Maine but some does go to other states. In July of 2011, garlic plants were observed with symptoms consistent with garlic bloat. Affected plants were stunted with leaf yellowing and were dying prematurely. Bulb symptoms included distinct swellings with underdeveloped and deformed bulbs. A general absence of roots or the absence of roots on one side of the basal plate was observed. Infected plants were collected from the field. *Ditylenchus dipsaci* Berk was extracted from the affected bulbs. The nematode is a recent introduction to Maine. Nematode buildup in seed stocks and spread to uninfested areas is a concern in Maine. The current practice of importation of seed stock and exchange of live plant

material may contribute to new appearances and further spread of the nematode. Garlic samples showed localized garlic bloat distribution in 2011 and 2012 and very limited distribution in 2013. The reduction in nematode distribution is attributed to increased awareness of the nematode and purging of affected seed lots.

A simple *in vivo* bioassay for estimating fungicide residues on peach fruit

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Protectant fungicides are routinely applied to peach trees during the period from shuck-split through early pre-harvest. These cover sprays are primarily applied to control peach scab, caused by *Fusicladium carpophilum*, and latent and early-ripening brown rot, caused by *Monilinia fructicola*. In the case of preharvest brown rot development, residual activity from late-cover protectant fungicides could help reduce selection for resistance among site-specific fungicides typically applied at this time. To determine the extent of this residue, a simple *in vivo* bioassay was developed by assaying *M. fructicola* spore germination directly on sampled fruit. A 1.5 cm section of polyvinyl tubing (12.7 mm I.D.) was placed upright on harvested fruit to create a miniature well. The tubing-fruit interface was sealed using silicon vacuum grease. A suspension of *M. fructicola* spores at 50,000 conidia/ml, harvested from seven-day-old PDA cultures, was pipetted into the well. The spores were suspended in a buffer-substrate medium consisting of 0.025M potassium phosphate, 0.1% sucrose, and 0.1% yeast extract. A rubber stopper with 2 mm aeration hole was inserted into the well's top and the fruit placed in an incubator at 25°C. To determine the optimum incubation period, a time-course study was conducted by removing non-treated incubating fruit at 1.5 hour intervals and assessing germination with a hemacytometer. Results showed that approximately 85% of spores had germinated after six hours incubation. To determine sensitivity of the bioassay, peach fruit were first sprayed with varying concentrations of captan fungicide ranging from field rate (1X) to one-ten thousandth field rate (0.0001X), then subjected to the bioassay procedure. Fitting of the logistic decline model to the data indicated that captan residue levels as low as 0.01X to 0.001X could be detected using spore germination as the predictor. The development of standard curves and other uses for the bioassay will be discussed.

Genome of *Pseudozyma flocculosa* reveals singular path towards bio-control activity

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Most fungi species from order Ustilaginales are renowned plant pathogens. The black and powdery masses they cause on plants are characteristic of what are called smut diseases. However, an intriguing species belonging to this group is more likely to be found in association with another fungus than with a plant. *Pseudozyma flocculosa* was first isolated from powdery mildew-infected leaves and was then shown to antagonize the plant pathogen. In an effort to identify the genetic traits that explain *P. flocculosa* lack of plant pathogenicity and strong antagonism against powdery mildews, we compared its genome to that of the closely related plant pathogenic species *Ustilago maydis*, *U. hordei* and *Sporisorium reilianum*. The results were quite revealing. On the one hand, the genome of *P. flocculosa* was found to be similar in size and number of genes to the genomes of other sequenced Ustilaginales. They also possess canonical mating genes, a similar number of plant and fungal cell wall degrading enzymes and gene clusters for synthesis of secondary metabolites. On the other hand, *P. flocculosa* has fewer genes encoding candidate secreted effector proteins and those clearly diverged from what is found in plant pathogenic species. Most interestingly, all secreted proteins proven to have an effect on *U. maydis* pathogenicity were absent from *P. flocculosa*. Also, we found a number of species-specific genes that could explain its biocontrol activity. In sum, the evidence suggests that *P. flocculosa* has diverged from a plant pathogenic lifestyle to become an efficient fungal antagonist. More importantly, this work constitutes an important step towards a better understanding of plant pathogenicity and biocontrol activity using the tools of genomics.

Improved stakeholder input – The IR-4 project nomination process

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The mission of IR-4 is to support the registration of pest management tools for specialty crops such as fruits, vegetables and ornamental horticulture. Pest

management tools include conventional pesticides as well as biological control agents (biopesticides). The IR-4 research process is dependent on the active participation of growers, researchers and extension personnel. A Project Clearance Request Form (PCR) is the first step in the process to get the pest management use on the IR-4 agenda for consideration. This can be done either through the IR-4 Northeast Region Field Coordinator (RFC), Edith Lurvey, the IR-4 website (<http://ir4.rutgers.edu/>) or IR-4 State Liaison Representative. PCR's are reviewed by the registrants to insure that they are willing to proceed with the use. Each year's priorities are chosen at the IR-4 priority setting workshops. Food use priorities are as follows: A priorities will have research started in the following growing season; B priorities may be researched as funds allow; C priorities are kept on the researchable project list for future consideration. Ornamental Horticulture priorities are established focusing on a specific pest or production needs. These priorities are arrived at by consensus among the participants from the four IR-4 regions, with additional options for projects of regional importance. Please note that the Northeast region solicits input via email on regional priorities prior to the workshop. Final selection of regional A priorities is made at a regional meeting in August where stakeholders get together to discuss the most important needs. For more information, please contact the RFC, Edith Lurvey (ell10@cornell.edu). Biopesticide projects continue to be selected as competitive grants for proposals.

Cross resistance among some SDHI fungicides documented in *Podosphaera xanthii*, the cucurbit powdery mildew pathogen

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Succinate dehydrogenase inhibitor (SDHI) fungicides (FRAC code 7) are important for managing cucurbit powdery mildew because their mobility in leaves enables control on the lower leaf surface where the pathogen, *Podosphaera xanthii*, develops best, and due to high potential for resistance development in the pathogen, applying an SDHI fungicide in alternation with other mobile fungicides also at risk for resistance development is recommended for managing resistance. Boscalid, the first SDHI fungicide developed, was registered in 2003. Strains of *P. xanthii* resistant to 500 ppm boscalid were first detected in the US in 2008. These strains were associated with control failure with the fungicide Pristine, which has boscalid as an active ingredient. The goal of this study was to determine if sensitivity to new SDHI fungicides was correlated with sensitivity to boscalid (phenomenon called cross resistance). Sensitivity was determined with a leaf disk bioassay for 14 *P. xanthii* isolates collected in September 2012 from research and commercial cucurbit crops that had been treated with fungicides to manage powdery mildew. Boscalid-resistant isolates were able to grow on disks cut from cotyledon leaves sprayed with 500 ppm penthiopyrad (formulated as Fontelis) and on disks with 500 ppm fluxapyroxad (Merivon). These isolates were more sensitive to fluopyram (Luna Privilege), being able to tolerate 10 ppm but not 50 ppm fluopyram. Only one of the seven boscalid-sensitive isolates tested was able to grow on leaf disks treated with 50 - 500 ppm penthiopyrad and 50 - 500 ppm fluxapyroxad. Luna and Fontelis were registered in the US for cucurbit powdery mildew in February and March 2012. Merivon is not registered yet. Based on these results, fluopyram appears to differ in activity from other SDHI fungicides and is recommended for managing cucurbit powdery mildew.

Molecular mechanisms of thifluzamide resistance in *Rhizoctonia solani*

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Thifluzamide is a succinate dehydrogenase inhibitor (SDHI) fungicide that effectively controls diseases caused by multi-nucleic *Rhizoctonia solani*. The fungicide takes effect when combined into succinate dehydrogenase (SDH) pocket of the fungi, which contains iron-sulfur protein (SDHB) and two membrane-anchor subunits. Mutants of *R. solani* have been obtained from a field and under laboratory conditions, but no further information of fungicide resistance on the molecular basis is available. In this study, four succinate dehydrogenase genes (*sdhA*, *sdhB*, *sdhC* and *sdhD*) in both mutants and wild-type isolates of *R. solani* were cloned and their sequences were analyzed. Mutation conferring resistance to thifluzamide was only found in genes related to SDHB subunit. Two types of thifluzamide-resistant mutants were observed based on the genotype and thifluzamide susceptibility. Type I mutants were heterokaryons with both histidine (H249) and tyrosine (Y249) showing at position 249 in SDHB subunit, which had thifluzamide-resistance level (EC₅₀ values) less than 100 times compared to their parents. Type II mutants were homokaryons with one allele of Y249 showing in SDHB

subunit, which had EC₅₀ values 200 times greater than their parents. Docking studies indicated the point mutation at position 249 reduced the affinity of SDH pocket to thifluzamide. One out of seven heterokaryotic mutants evolved into homokaryons via multi-subcultures on fungicide-free or -amended media, or in planta; however, the sensitive isolates and homokaryotic mutants had no changes in their genotypes both in vivo and in vitro. Some mutants had even higher fitness than sensitive isolates confirmed in greenhouse studies, which implied the possibility of enhanced risk of thifluzamide resistance in *R. solani* population. The data suggested that point mutation of *R. solani* resulted in thifluzamide resistance; mutants in heterokaryotes could evolve into homokaryotic mutants; and this mutation did not sacrifice the fitness of *R. solani*.

White pine blister rust occurrence on cultivated resistant and immune *Ribes* varieties and the impact to local white pine resources

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White pine blister rust (WPBR) has been a threat to both forest resources and agricultural commodities since its introduction to North America in the early 1900s. Eastern white pine (*Pinus strobus*) is the most economically important timber species in New Hampshire. From 1917 to 1970 millions of man hours were spent eradicating *Ribes* throughout the state to protect the timber industry. Prior to eradication efforts, it was not uncommon to find stands of pine with WPBR incidence greater than 50%. Since the enactment of the Federal Plant Quarantine Act in the early 1900s, the interstate movement of *Ribes* was controlled. After the lifting of the Federal quarantine and the more recent availability of immune and resistant *Ribes* varieties, States in the Northeast have amended their restrictions to allow for commercial plantings and homeowner requests. In 2011, WPBR was reported for the first time on immune black currant *Ribes nigrum* cv. *Titania* in Preston, Connecticut. Results from a 2012 preliminary survey in New Hampshire indicated that immune black currant were infected with WPBR and a complete survey of these plantings and the impact on local white pine forests was needed. During 2013, 42 sites with cultivated *Ribes* were surveyed. Incidence of WPBR ranged from 20% to 50% and 100% for immune and resistant *Ribes* varieties, respectively. As expected, WPBR severity on resistant *Ribes* was limited to <5% of leaf area. In contrast, mean WPBR severity on immune *Ribes* was 20% leaf area. Mean incidence of WPBR on white pines planted within 300 meters in western or eastern directions of cultivated *Ribes* was 16% and exceeded previously reported Statewide incidence of 3-7%. The breakdown in of WPBR immunity in cultivated *Ribes* could have potential deleterious effects on the white pine resource.

US23 was the dominant genotype of *Phytophthora infestans* in north-eastern U.S.A. in 2013

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During the summer of 2013, late blight was reported from Connecticut, Massachusetts, Maine, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island and Vermont. Many potato and tomato samples with symptoms of late blight from these states were analyzed with microsatellite markers to determine the specific clonal lineage of *Phytophthora infestans* that caused the disease. Samples were received from early July through September. More than 140 samples were processed through September. Of these, all except two from the Northeast were US23. One potato sample with the US8 clonal lineage was detected from Pennsylvania and one potato sample with US24 was detected from Maine. US23 was first detected in 2010. During the summer of 2013, the mefenoxam sensitivity of US23 isolates collected in 2012 was assessed in an "in vitro" assay. All of these isolates were identified as sensitive to mefenoxam. This finding is important for growers because fungicides containing this active ingredient are highly effective against sensitive strains of *P. infestans*. The US8 lineage, which dominated before 2009, is resistant to mefenoxam. Three potato samples with apparent late blight lesions on leaves and one tomato sample with apparent late blight lesions were diagnosed with *Phytophthora nicotianae*. Information about samples submitted in 2013 and results from their analysis were posted at <http://usablight.org/>.

Evaluation of the efficacy of natural resistance in 'Honeycrisp' to reduce fungicide applications for *Venturia inaequalis* (Cooke) Wint.

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Most fungicide applications on apples in Vermont are targeted at managing apple scab. Due to the susceptibility of cultivars like 'McIntosh' and wet weather, it can be difficult to manage the disease. 'Honeycrisp' is regarded as being "slightly susceptible" to apple scab. The objective of this study was to evaluate the efficacy of natural resistance inherent in 'Honeycrisp' apples to determine if reducing fungicide applications is possible without significantly increasing disease incidence or severity. The study was conducted at the University of Vermont Horticultural Research Center in South Burlington, VT, in an orchard planted in 2011, which contained rows of 'Honeycrisp' and 'McIntosh' trees. The experiment was conducted on 'Honeycrisp' trees in 2012, and separately on 'Honeycrisp' and 'McIntosh' trees in 2013, using a completely randomized design of six replications of three-tree plots per fungicide treatment. There were six treatments: a non-treated control, a treatment that covered all primary infection periods, and treatments where applications were delayed and only applied after one, two, or three primary infection periods. Dilute applications of mancozeb and/or captan at standard label rates were applied by a handgun. No fungicides were applied during secondary scab infection periods. Two foliar assessments (June and August) were performed each year. For each assessment, five apical, vegetative terminals were selected from the canopy of the middle tree of each three-tree plot and each leaf was evaluated for scab incidence in June, and scab incidence and severity in August. There were no significant differences in foliar scab incidence and severity among 'Honeycrisp' treatments in both years; scab levels were low and were in contrast with the foliar scab levels that developed on 'McIntosh'. This research documents that fungicide applications can be reduced on 'Honeycrisp' with no significant increase in foliar scab incidence or severity.

Influence of nitrogen fertility on bacterial rot incidence and overall yield of onion in Pennsylvania

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Bacterial rots, including center rot (caused by *Pantoea agglomerans* and *P. ananatis*), are the most significant diseases of onion in Pennsylvania, resulting in over 50% loss on some commercial farms. From a 28-farm study in 2011, bacterial rot incidence was negatively associated with harvest levels of soil ammonium and leaf tissue nitrogen at midseason, suggesting an impact of nitrogen on bacterial disease incidence. To examine the roles of different nitrogen forms [nitrate (NO₃) or ammonium (NH₄)] in the development of center rot, growth chamber and field studies were undertaken in 2012 and 2013 in which CaNO₃ and (NH₄)₂SO₄ were compared as primary nitrogen sources. In the growth chamber, disease severity was initially lower in inoculated plants fertilized with NH₄, as opposed to NO₃-fertilized plants, while there was no effect on plant size. In a replicated field trial comparing nitrogen forms in combination with fertilizer application timing (pre-solstice or full-season), no differences in disease incidence or marketable yield were suggested from plots fertilized with different nitrogen forms. However, onions fertilized with (NH₄)₂SO₄ had significantly higher sulfur content, a key component in onion pungency (P < 0.0001). Collectively, plots inoculated with *Pantoea* spp. had significantly higher disease incidence than uninoculated plots (P = 0.009), and center rot incidence and marketable yield trended based on interactive effects between inoculation status and fertilization timing (P < 0.16). Effects of different fertilizer regimes on plant tissue micronutrients will also be discussed. While preliminary, these results suggest the importance of nitrogen fertilization, timing, and their effects on micronutrients in the management of center rot of onion.

Fungicide rotation strategies to manage *Sclerotinia homoeocarpa* populations resistant to DMI and benzimidazole fungicide classes

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Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) is the most economically important turfgrass disease in the Northeast. Dollar spot is primarily controlled by fungicide applications on golf courses and resistance to the sterol demethylation inhibitor (DMI) and benzimidazole fungicide classes has become increasingly widespread in the Northeastern region. Currently, there is limited information on the effectiveness of different fungicide rotation options for controlling *S. homoeocarpa* populations with confirmed fungicide

resistance. Furthermore, some golf courses are located in environmentally sensitive areas where usage of the multi-site, contact fungicide chlorothalonil is restricted. The objectives of this study were to determine if DMI fungicides could be used in rotation strategies and to determine the effect of DMIs with and without chlorothalonil for dollar spot control. A two-year field trial was conducted on two golf courses located in Amherst and Southwick, MA with prior confirmation of benzimidazole and DMI resistance to test different rotation strategies on dollar spot control. Five applications per rotation strategy were made each year and dollar spot infection centers or percent dollar spot was evaluated on a weekly basis. Results from both years indicate that rotation strategies utilizing DMI fungicides early in the season (May/June) generally provided similar control to non-DMI fungicides. Increased dollar spot was observed in rotation strategies that made DMI applications later in the season (July/August); however, season-long disease control was similar to strategies that used DMI's early in the season. Results also indicate that rotation strategies that did not utilize chlorothalonil can provide similar control to strategies in which chlorothalonil was used. Overall, our data indicate DMI fungicides can be used under lower disease pressure and rotation strategies without chlorothalonil can provide adequate dollar spot control. These results offer superintendents practical dollar spot control solutions, however, the implications on long-term fungicide resistance management are uncertain.

The *rpoN* gene is essential for *Erwinia amylovora* bacterium to cause fire blight disease in apples trees

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Fire blight is caused by the bacterium *Erwinia amylovora* and even though scientists have been studying it since 1794, to this day there is still much we do not understand about the disease. Since the 1980's, scientists have been looking for the genes in *E. amylovora* that cause fire blight in apples. Some of these genes have been identified, but scientists cannot fully understand the process of disease yet. In my lab we have found a new gene that is essential for the bacteria to cause disease, and its name is *rpoN*. In this study, we found that a mutation of the *rpoN* gene in the fire blight pathogen *E. amylovora* caused a nonpathogenic phenotype. The *E. amylovora rpoN* mutant was named *rpoN1250::Tn5*. The *rpoN1250::Tn5* mutant did not cause fire blight disease symptoms on shoots of mature apple trees. In detached immature apple fruits, the *rpoN1250::Tn5* mutant failed to cause fire blight disease symptoms and failed to grow in apple tissue. In addition, the *rpoN1250::Tn5* mutant failed to elicit a hypersensitive response when infiltrated into non-host tobacco plant leaves, and *rpoN1250::Tn5* cells failed to express HrpN protein when grown in hrp (hypersensitive response and pathogenicity)-inducing liquid medium. A plasmid-borne copy of the wild-type *rpoN* gene complemented all the *rpoN1250::Tn5* mutant phenotypes tested. The *rpoN1250::Tn5* mutant was prototrophic on minimal solid and liquid media, indicating that the *rpoN1250::Tn5* nonpathogenic phenotype was not caused by a defect in basic metabolism or growth. This study provides clear genetic evidence that *rpoN* is an essential virulence gene of *E. amylovora*. A better understanding of *E. amylovora* can help us to one day stop the bacterium from causing disease.

Impatiens under attack: Genetic variability of the oomycete pathogen *Plasmopara obducens* in the U.S.

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Impatiens downy mildew (IDM), caused by the fungus-like oomycete *Plasmopara obducens*, is an aggressive, newly emergent disease affecting cultivated impatiens (*Impatiens walleriana*) in the U.S. The first U.S. IDM outbreak was reported in 2004. By 2012, IDM was reported from 33 states, resulting in millions of dollars in losses. The factors driving these destructive outbreaks are unknown. It is unclear whether IDM is caused by the same organism recorded on wild impatiens throughout the 20th century, or if disease outbreaks are due to a new pathotype or species. In this work, we set out to determine the pathogen population(s) responsible for IDM in cultivated impatiens, and compare these genotypes with pre-epidemic populations on wild impatiens. Single nucleotide polymorphism (SNP) analysis was performed using real-time PCR, droplet digital PCR and Sanger sequencing to genotype 87 pre-epidemic *P. obducens* herbarium specimens (c. 1881-2005) and 830 IDM samples from modern disease outbreaks. The sample was diverse, with six major genotypes (n > 10) and 24 minor genotypes observed. Phylogenetic network analysis showed reticulations reflective of hybridization

or recombination between genotypic groups. Four major genotypes were exclusive to modern IDM samples ($n = 82$). Two major genotypes were observed from both modern IDM samples and herbarium specimens. Droplet digital PCR revealed allelic ratios departing from 1:1 for multiple heterozygote samples, with a substantial number of samples exhibiting a 3:1 allelic skew. Because *P. obducens* cannot be cultured and DNA was extracted directly from environmental samples, the observed skew could be a result of mixed genotypes in individual samples, the presence of heterokaryotic isolates, or both. Together, our present dataset shows that although some *P. obducens* genotypes have been residing in the US since 1880's, other genotypes are unknown from pre-epidemic populations. Allele frequencies and network clusters suggest heterokaryosis and recombination as possible contributors to the observed population structure.

Selecting and timing fungicides to control sooty blotch and flyspeck on apples in southeastern New York

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Sooty blotch and flyspeck (SBFS) are caused by a complex of more than 60 fungi that discolor the surfaces of apple fruit. The first infections occur soon after petal fall in spring and additional infections accrue throughout summer unless fruit are protected with fungicides. SBFS on fruit has an incubation period of 270 hr of accumulated wetting (HrAW) as measured with string sensors. Electronic sensors are less sensitive to wetting. In 2013, SBFS on Cameo apples was monitored weekly in replicated plots at Highland, NY. Rainfall totaling 4.7 cm on 6 to 8 June, in addition to 0.15 cm rainfall earlier in June, ensured that all fungicide residues from a 27 May scab spray were depleted by 8 June. No SBFS was visible on 26 July (188 HrAW from 8 June), but 69% of unsprayed fruit showed SBFS on 2 August (231 HrAW from 8 June). Thus, a conservative incubation threshold for SBFS would be 190 HrAW as measured with the electronic sensors in stations of the Northeast Weather Network (NEWA). Twelve fungicide treatments were evaluated for postinfection activity via applications on 22 July (162 HrAW from 8 June). Treatments were repeated on 6, 15, and 30 August. Captan (1.0 g a.i./liter) and Topsin-M were relatively ineffective when each was applied alone. Merivon, Pristine, Inspire Super, and Flint provided 72 to 90% control of flyspeck and 61 to 88% control of sooty blotch on Cameo fruit assessed on 17 September. In the same plots, 99% of Royal Court apples harvested on 18 September were unsuitable for USDA Extra Fancy grade due to SBFS whereas treatments involving Flint, Flint-plus-Captan, Pristine, and Topsin-plus-Captan had only 8, 14, 19, and 23% fruit out of grade, respectively. None of the fungicides completely eradicated SBFS. Thus, prophylactic treatments are required for commercial control of SBFS.

Climatic suitabilities of two forest pests of importance to Canada's forests

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Bark beetles are major disturbance agents in forest ecosystems worldwide. These insects are often associated with ophiostomatoid fungi, which play an important role in helping the bark beetles in overcoming host tree defenses. The mountain pine beetle, *Dendroctonus ponderosae*, and the European spruce bark beetle, *Ips typographus*, are the most important bark beetle species infesting conifer forests of western North America and Eurasia, respectively. The blue-stain fungus *Grosmannia clavigera* is a common associate of the mountain pine beetle, whereas *Ceratocystis polonica*, another blue-stain fungus, is the most pathogenic associate of the European spruce bark beetle. While not much is known about how the beetle-fungal interactions will vary under a changing climate, potential insect responses to climate change could offer an insight into where the associated fungi can be expected to occur in the future. Results of our investigations concerning potential changes in climatic suitability of the mountain pine beetle in western Canada under simulated climate change conditions will be presented. Current investigations into developing a bioclimatic envelope map for the European spruce bark beetle-associated *C. polonica* for Canada will also be discussed.

Investigation of the role of an ABC-G transporter from *Sclerotinia homoeocarpa* in resistance to multiple fungicide classes by a heterologous yeast expression system

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Sclerotinia homoeocarpa is an ascomycete fungus and causes dollar spot disease on turfgrasses. Resistance of *S. homoeocarpa* to fungicides is a common problem on North American golf courses. In a previous study, we showed that overexpression of ABC-G transporter, *ShMR4*, was associated with reduced sensitivity to multiple fungicides in *S. homoeocarpa* field isolates. Briefly, the field isolates exhibited significantly decreased sensitivity to a sterol demethylation inhibitor (DMI) fungicide, propiconazole, as well as reduced sensitivities to a succinate dehydrogenase inhibitor fungicide (SDHI), boscalid, and a dicarboximide fungicide, iprodione. These field isolates also showed constitutive and induced overexpression of *ShMR4* in the absence and presence of the three fungicides. In order to further validate the role of *ShMR4* in the multi-drug resistance, the substrate specificity of this efflux transporter will be examined using a heterologous yeast expression system.

Distribution in Canada of a new *Cronartium ribicola* race virulent on previously immune blackcurrant cultivars

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White pine blister rust (WPBR), caused by the fungus *Cronartium ribicola*, is an introduced species found throughout North America. The disease is causing extensive damage to natural and managed native five-needle pines. The fungal pathogen has to alternate between two hosts, currants and white pine, to complete its life cycle. As a mean to control the disease, resistance breeding programs were undertaken for both hosts. So far, two WPBR dominant resistance genes were deployed in white pine species and one in black currant. Breakdown of *Ribes Cr* resistance gene was reported in 2011. In order to characterize the distribution of this new *C. ribicola* race in Canada, we surveyed blackcurrant producers in Ontario, Quebec, New Brunswick, Nova Scotia and Prince Edward Island in summer 2013. Previously immune *Ribes nigrum* cultivars Titania, Consort, Tisel and Blackcomb were found rust infected. DNA barcoding was used to confirm the identity of the *C. ribicola* on immune *Ribes* cultivars. The new *C. ribicola* race was recorded in every surveyed Canadian provinces except Ontario.

Blueberry floral extract stimulation of *Colletotrichum acutatum*

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Anthraxnose of highbush blueberry (*Vaccinium corymbosum* L.), caused by *C. acutatum*, is one of the most significant diseases affecting fruit quality. There are several lines of evidence that indicate fungal sporulation and fruit infection are initiated during the bloom period. The purpose of this study was to test the hypothesis that plant signals produced during bloom play a critical role in infection and sporulation. Specifically, we examined the role of water-soluble flower extracts (FE) as possible signals for initiating fungal development and the onset of disease. Treatment of conidial suspensions with FE collected from the susceptible cultivar Bluecrop caused a significant increase in germination, microcyclic conidiation and appressorium formation compared to the water only control. Time course studies showed that appressoria develop in the presence of FE at a significantly faster rate and magnitude beginning at 6-hrs post inoculation. Comparison of floral extracts derived from susceptible and resistant cultivars also revealed a significant difference in the level of appressorium formation. Infectivity trials were conducted using healthy, ripe, detached blueberry fruit with and without FE. Results at 7-days post inoculation showed that floral extract treatments induced higher levels of disease incidence as compared to the water only and conidia plus water controls. These results provide strong evidence that flower extracts contribute a critical role in the disease cycle of *C. acutatum* on blueberry, and may be related to the expression of host resistance.