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Antifungal potential of essential oils of *Cymbopogon citratus* and *Eucalyptus citriodora* against Brown Spot disease of Rice

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Rice brown spot produced by *Bipolaris oryzae* Breda de Hann (formerly, *Helminthosporium oryzae*) (teleomorph = *Cochliobolus miyabeanus*) is considered as an important production restraint of rice and occurred in all rice-growing areas of the world. Essential oils obtained from Lemongrass and Eucalyptus leaves were checked for their antifungal potentials against *Bipolaris oryzae*. Selected essential oils were checked in different concentrations of 1000 ppm, 500 ppm, 250 ppm and 150 ppm for their ability to inhibit the growth of the test fungi in-vitro and also in-vivo. The in-vitro and in-vivo studies revealed that the essential oils of lemongrass have the potential to inhibit the growth of test fungi completely at all concentrations. Essential oils of lemongrass were more effective than Eucalyptus. Formulating a Bio-Fungicide from these essential oils provides a more safer and Eco-friendly control of Brown Spot of Rice as it is an emerging disease in all rice growing areas of the world.

Survival of *Peronospora belbahrii* sporangia affected by temperature and relative humidity

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Basil downy mildew is a destructive disease of sweet basil (*Ocimum basilicum*) caused by *Peronospora belbahrii*, a biotrophic oomycete which requires extended periods of high relative humidity and leaf wetness to produce sporangia and initiate new infections. There is no known resting spore stage produced by the pathogen in North America, and sporangia cannot overwinter in Northern climates. Thus, destruction of the affected crop following a disease outbreak should be an effective means of preventing further disease spread if no new inoculum is introduced. Sporangia that do not come into contact with plant tissue may be released onto greenhouse benches, potting materials, and other surfaces. This study aims to improve cultural control methods for managing basil downy mildew by defining a timeframe of *P. belbahrii* sporangia survival. Two approaches were used to evaluate the long-term viability of *P. belbahrii* sporangia: an *in vitro* assay to assess germination, and a plant infection bioassay to determine infectivity. Sporangia were isolated from plants and stored for 24-hour intervals in 20 and 25°C temperature treatments and 84% and 96.5% relative humidity (RH) treatments. Significant reductions in germination *in vitro* were observed after 24 hours, and reduction of disease incidence was significant after 48 hours across all treatments. Relative humidity was found to have a more significant impact on the length of sporangia survival compared to temperature treatments; results showed that sporangia remained viable beyond 144 hours when stored at 96.5% RH, whereas viability was reduced to 96–120 hours in 84% RH.

Detection and identification of ‘*Candidatus Phytoplasma asteris*’-related phytoplasma associated with a witches’ broom disease of cassava in Thailand

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Cassava witches’ broom disease (CWBD) is of economic importance on *Manihot esculenta* Crantz a crop widely cultivated across South East Asia. In 2012, cassava growing provinces at Chachaengsao, and Rayong in Thailand were affected, with significant yield losses and reduced starch content. Observed symptoms were typical witches’ broom, reduction in leaf size, leaf proliferation, yellowing, shortened internodes, stem necrosis and stunting. Total DNA was extracted from leaf, root and shoot samples of symptomatic and asymptomatic plants and amplified using nested PCR with primers pairs P1A/P7A, R16F2N, R16R2, R16 (I)F1/R16(I)R1. A 950 bp amplicon was obtained from symptomatic samples. To confirm the presence of a phytoplasma restriction fragment length polymorphism analyses of nested PCR-amplified fragments from Thailand CWB phytoplasma isolates and representative phytoplasmas from other phytoplasma groups using *Mse*I restriction enzyme indicated that the cassava phytoplasma isolates are related to the aster yellows (16SrI-C) group. Sequence analyses of partial 16S rRNA fragments showed that Thailand CWB phytoplasmas have 99% to 100% similarity with ‘*Candidatus phytoplasma Asteris*’ (16SrI-C) group. To our knowledge, this is the first report of cassava infection with a phytoplasma related to the 16SrI-C subgroup in Thailand.

Discriminant haplotypes to identify common races of *Phytophthora sojae* found in Canada

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Phytophthora sojae, causing Phytophthora Root Rot (PRR), is responsible for 2 billion \$ in losses per year worldwide. In Canada, the rapid expansion of soybean gives this pathogen a new niche to establish its devastating presence. The best method to control this pathogen is through the use of varieties carrying resistance genes, following the gene-for-gene interaction where soybean resistance genes (*Rps*) provide immunity against *P. sojae* isolates carrying the corresponding *Avr* gene. Breeders and growers are thus confronted with the need to introgress *Rps* genes based on the *P. sojae* pathotypes found in the environment. This project aims to determine the presence and distribution of pathotypes of *P. sojae* in Canada, based on the seven most important *Rps/Avr* genes relationships. For this purpose, a collection of *P. sojae* isolates representing the most common pathotypes found in Canadian fields has been established, and 31 isolates from the collection were targeted for whole-genome-sequencing. Different gene mutations directly linked to those seven *Avr* genes from *P. sojae*, mostly gene suppression and SNPs, were discovered. These genomic data give discriminant haplotypes allowing the identification of common races found in Canada. The next step will be to develop molecular markers based on these genomic signatures to guide both breeders and growers for the choice of resistance genes that will provide protection against PRR.

Interaction between soybean cyst nematode (*Heterodera glycines*) and *Phytophthora* root rot (*Phytophthora sojae*) on soybean

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Over the last 10 years, areas cultivated with soybean in the province of Québec have increased by 67% to reach 325 000 ha in 2016. This large expansion, however, has contributed to the establishment of new pests. Currently, the most damaging disease of soybean is Phytophthora root rot, caused by *Phytophthora sojae*. Yet, the soybean cyst nematode (SCN), *Heterodera glycines*, is quickly making its way into Québec. To manage these diseases, the most efficient way is the use of resistant cultivars. However, the existence of several pathotypes is challenging. Moreover, it is known that the presence of SCN can repress the expression of resistance genes to certain diseases (e.g. brown stem rot) and make the infested plant more susceptible to other pathogens (e.g. SDS). It is therefore important to verify if the interaction between SCN and *P. sojae* could affect their virulence. A multiple pathotypes isolate of *P. sojae* and a virulent SCN population (Hg 2.6.7) were inoculated on four soybean cultivars, susceptible or not to one or both pathogens. Our results demonstrated that the number of cysts was significantly decreased when *P. sojae* was present on the susceptible cultivar. This suggests that *P. sojae* might influence SCN development through the stimulation of plant defenses. On the other hand, resistant plants were not affected by the presence of both pathogens indicating that these cultivars will remain a valid option for the management of SCN and *P. sojae*.

The bioSAFE project: Developing tools for the genomic biosurveillance of forest invasive alien pests in Canada

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The capacity of forests to provide long-term fibre supply and ecosystem services is threatened by new introductions of Forest Invasive Alien Species (FIAS). The bioSAFE project is developing a biosurveillance pipeline to generate genomic tools that will facilitate biosurveillance to increase preparedness and facilitate early action against FIAS. Two insect pests (Asian Longhorned Beetle and Asian

Gipsy Moth) and two pathosystems (Sudden Oak Death and Dutch Elm Disease [DED]) are used as models for developing accurate identification means, identifying pathways of spread, and predicting fitness and outbreak-related epidemiological traits that impact the outbreak outcome. Ongoing research on DED includes comparing whole genomes of DED fungi and their relatives for population genomic studies; identifying genes contributing to parasitic fitness and invasiveness using transcriptomics, genome-wide association studies, and quantitative trait locus analysis of progeny from controlled sexual crosses; and developing efficient gene knockdown/knockout protocols for functional validation of candidate genes. Recent results include the assessment of genetic diversity in natural populations of DED fungi, confirmation of polygenic control of virulence, and production of knockdown mutants. The bioSAFE project will generate transformative changes to address the challenges of biosurveillance of FIAS by speeding up and improving decision-making to inform FIAS mitigation and management.

Assay tool development for emerging and re-emerging diseases

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Some plant diseases continue to be a problem even with modern agricultural practices, and new diseases continue to emerge and threaten agricultural and natural ecosystems. The spread of these plant pathogens is often facilitated by increases in global trade, leading to the need for governmental regulations to restrict pathogen movement on infected plant material at a local, regional, national, and international level. These regulations rely heavily on plant disease diagnostics, and it is well known that some fungi/oomycetes can be difficult or impossible to culture, differentiate from closely related species, or correctly identify. Diagnostics methods that are currently used involve classical isolation coupled with morphological identification, immunological approaches, and molecular diagnostics. The presence of ecological niches including soil, different plant parts, irrigation water, or in the air is making challenging the development of diagnostics tools that are effective in a variety of situations. Examples of development tools to emerging or re-emerging diseases will be presented following research and development work based on regulatory agency needs for detection and genotyping. Molecular biology tools development and how they are created and implemented for *Verticillium*, *Phytophthora* species and other fungi organisms targeted by our agency will be presented with qPCR assays, microsatellites, and use of the Next Generation Sequencing (NGS) with genomic and metagenomics case studies.

Strawberry powdery mildew (*Podosphaera aphanis*): Past, present, and future

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Powdery mildew is a disease which is raising concern in Eastern Canadian strawberry production. Considering the increasing complexity of disease management and cost of strawberry production and the large number of diseases affecting this crop, it is crucial to develop tools to help growers make optimal management decisions. Strawberry powdery mildew is difficult to predict because of the wide range of weather conditions favorable to its development. Weather, disease, inoculum, and host data collected at three, five and four sites in 2006, 2007 and 2008, respectively, for a total of 12 epidemics were used in this study. At all sites, data were collected in day-neutral strawberries (cv. Seascape) cultivars. First, temperature-based disease risk index was evaluated but found unreliable under the weather conditions of Eastern Canada. Hence, we explored other forecasting approaches including classification trees and dynamic simulations. A total of 36 variables were tested but only seven variables were used to construct the powdery mildew severity classification tree. These variables were related to airborne inoculum, amount of susceptible leaves, and during the previous six days. These variables were incorporated into the development of a quantitative dynamic simulation model, which is currently under validation. Using this tree model, 85% of the 342 days analyzed were correctly classified for powdery mildew severity. These tools could be combined with the previously develop action threshold to tune fungicide applications strategies and prevent fruit infections.

A programmable *Arabidopsis* resistance response conserved in barley?

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Plant immunity frequently relies on detection of effector molecules secreted by an invading pathogen into the host cell, which can trigger cell death through the hypersensitive reaction (HR). Deployment of diverse plant resistance genes that directly or indirectly detect effectors has been essential for combatting plant diseases in the field, but is often limited by the specificity of each resistance gene to one effector or pathogen. PBS1, a conserved protein in flowering plants, was recently shown to be a programmable part of an *Arabidopsis* innate defense response. A guard NLR protein, RPS5, detects cleavage of PBS1 by the *Pseudomonas* effector AvrPphB and triggers HR. Modification of the cleavage motif in PBS1 enables recognition by different proteases, such as those used by other plant pathogens, while still initiating HR when cleaved. The potential for increased disease resistance through expanded effector recognition led us to investigate if an analogous PBS1-mediated immune response is present in monocots, specifically barley (*Hordeum vulgare* ssp. *vulgare*). We confirmed the presence of AvrPphB-cleavable PBS1 homologues in barley and identified barley accessions that show an AvrPphB-induced HR. Using a genome wide association study, we mapped the AvrPphB response to a single locus on chromosome 3H, which we hypothesize contains an NLR that functions as a PBS1 guard. Silencing and gain-of-function assays for NLRs in this locus are underway. Determining conservation of disease resistance mechanisms among plant species will accelerate translation to many crop fields, especially through gene-editing for expanded effector recognition.

Understanding *Byssoschlamys nivea*: A new problem for apples?

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Byssoschlamys nivea as an important heat-resistant spoilage mold that produces the mycotoxin patulin. It is known as a soil-borne fungus, but we hypothesize that infection of apples may lead to contaminated food products post-processing. The ability of *B. nivea* to infect apples, both in the orchard and under post-harvest conditions, was assessed to study pathogenicity and complete Koch's postulates. We found that *B. nivea* can infect apples through wounds in the orchard and post-harvest. Infection produced rot symptoms similar to Bull's Eye Rot and Black Rot. Post-harvest infected 'Gala' and 'Golden Delicious' both displayed lesions with diameters greater than 40 mm after 3 weeks. Using *B. nivea* infected fruit to make apple juice concentrate, we determined that sexual spores of the fungus developed in apples survive thermal processing. Viable *B. nivea* propagules were significantly reduced but not eliminated during five stages of commercial processing ($p=0.017$). *Byssoschlamys nivea* may be an overlooked apple pathogen, perhaps mistaken for other fungal diseases, that can survive thermal processing and cause spoilage in apple products. The ability of *B. nivea* to infect apples pre- and post-harvest, may explain the episodic nature of food spoilage.

Boxwood blight in the Long Island, New York landscape

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Boxwood blight caused by *Calonectria pseudonaviculata* has brought about major financial losses in the American nursery and landscape industries since it was first seen in the U.S. in 2011. Infected plants develop leaf blight and distinctive non-girdling black shoot cankers; affected areas of the shrubs subsequently defoliate. On Long Island, the disease has been intercepted by New York State horticultural inspectors at retail, and found by home gardeners and commercial landscapers in established plantings. Based on samples received for diagnosis, environmental conditions have favored symptom development in both spring and fall from 2012–2017, with most outbreaks noted in fall. The pathogen is commonly introduced to landscapes along with newly-purchased boxwood. In a number of cases, however, symptoms in closely monitored landscapes did not appear until more than a year after new boxwood were introduced, even though environmental conditions were suitable for disease development every year. One implication of this observation is that the pathogen might possibly be introduced through some means other than through movement of contaminated planting material. Companies that specialize in boxwood shearing are a possible source of inoculum for boxwood blight, and are an important audience for extension education efforts.

A new phenotyping procedure to identify QTLs conditioning soybean resistance against *Phytophthora sojae*

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Although the deployment of resistance genes that confer complete immunity against *Phytophthora sojae* is currently the most attractive means to reduce soybean losses, the constant evolution of new pathotypes can eventually lead to a breakdown in resistance. A complementary approach is to rely on horizontal resistance, that is not dependent on a gene-for-gene interaction. The objective of this study is to identify QTLs in a soybean population that has been obtained from an early maturing line adapted to Canadian conditions, and a line showing high levels of horizontal resistance to *P. sojae* both in field and greenhouse observations. Over 150 F5 lines were selected on the basis of their field performance and brought to F6 stage. Each line was phenotyped exhaustively for horizontal resistance against *P. sojae* in a miniature hydroponic bioassay. In parallel, GBS was performed on all lines and the resulting reads (~1M/line) will be used for SNP calling and constructing a genetic map. Phenotypic data have revealed discriminant levels of resistance among the lines, which suggests the presence of strong QTLs separating the lines.

Evaluation of different salts for the control of lettuce varnish spot: When phytotoxicity rules

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Varnish spot (*Pseudomonas cichorii*) is a disease affecting lettuce (*Lactuca sativa* L.) plants worldwide. The disease leads to important economic losses in commercial lettuce production in Canada. No chemicals are registered in Canada to control the disease and cultural practices do not provide effective control. In this study, five GRAS (Generally Recognized as Safe) salts used in food industry that were previously shown to control different plant diseases were tested for their potential use as chemicals for the control of varnish spot. Phytotoxicity of salts was first estimated using greenhouse and in vitro assays. Greenhouse assays showed that potassium sorbate, sodium benzoate, and sodium carbonate at higher concentrations caused a marked decrease of growth along with foliar phytotoxicity symptoms while sodium metabisulfite and sodium bicarbonate did not cause such a decrease of growth. On the basis of the phytotoxic doses 5% (PD5s) determined in vitro, salts were ranked in ascending order of phytotoxicity as follows: sodium bicarbonate, potassium sorbate, sodium carbonate, sodium benzoate, sodium metabisulfite. For each salt, PD5 allowed a very good approximation of the phytotoxicity dose as determined on lettuce plants grown in greenhouse. When applied at concentrations causing mild to moderate foliar symptoms of phytotoxicity and no marked effect on growth, tested salts failed to reduce survival of *P. cichorii* on lettuce leaf tissue and varnish spot severity.

Host community as a predictor of *Fusarium graminearum* populations

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Fusarium graminearum, the incitant of head blight in cereals, is also associated with a wide range of gramineous hosts, including non-cultivated species that occur in proximity to agriculture. Debris of these grasses may act as pathogen reservoirs, contributing primary inoculum to crops and facilitating sexual recombination by providing ample substrate for growth, survival, and reproduction. We isolated *F. graminearum* from wild grasses to measure incidence in these hosts and the inoculum potential from their debris. Inflorescences and overwintered stem tissue were collected from farms and grasslands in two regions of New York with relatively high and low intensities of cereal production. Host community characteristics were recorded at each site and used to analyze data on *F. graminearum* presence. Intensity of cereal acreage best predicts the abundance of *F. graminearum* in a region. However, within individual fields, the ground coverage by grasses and their species diversity were good predictors of *F. graminearum* abundance and inoculum potential. The high frequency of *F. graminearum* in these wild grasses could inform the siting of cereal fields relative to natural grasslands and the management of fallow areas surrounding cereal fields.

Induced Viable But Nonculturable (VBNC) state in *Dickeya dianthicola*

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Dickeya dianthicola has been the primary causal agent of blackleg and soft rot of potato in the Northeastern U.S. since the 2015 outbreak. To understand the survival and spread of *D. dianthicola*, a viable but nonculturable (VBNC) state was investigated with the bacterium. *Dickeya dianthicola* cell suspensions were treated with copper sulfate and cold temperature. After various lengths of treating time, the bacterial cells were washed free of chemicals and placed in mixture of syto9, which binds DNAs of all types of bacterial cells, and propidium iodide, which only reacts with DNA of dead cells. The DNA combined with the dyes was run through a flow cytometer to count live and dead cells. *Dickeya dianthicola* was confirmed to have a VBNC state. Copper sulfate at concentrations of 50 and 500 μ M induced a VBNC of *D. dianthicola* in 2 hr. After copper treatment, at least 90% cells of *D. dianthicola* entered into a VBNC state. They remained viable for 2 months at 28°C, which was the length of the study. This may protect the bacterium from being eliminated by the application of copper products in seed treatment. It is possible that *D. dianthicola* survives the winter by entering a VBNC state.

Dollar spot control affected by bentgrass susceptibility and fungicide programming

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Creeping bentgrass (*Agrostis stolonifera* L.) susceptibility and fungicide timing affect dollar spot (caused by *Sclerotinia homoeocarpa* F.T. Bennett) control. This 3-yr field trial investigated fungicide timing effects on dollar spot on a susceptible ('Independence') and highly tolerant ('Declaration') cultivar. The trial was a $2 \times 8 \times 2$ factorial in a randomized complete block design with 5 blocks. Factors included bentgrass cultivar; initial fungicide timing [threshold-, calendar-, logistic regression model-, or growing degree day (GDD) model-based (20-30, 30-40, 40-50, 50-60, or 60-70 GDD; base 15°C and biofix 1 April)]; and subsequent fungicide timing [logistic regression model or disease threshold]. Response to treatments was limited in 2016 due to dollar spot suppression from fludioxonil applied to control anthracnose. Cultivar had the greatest impact on dollar spot incidence during 2015 and 2017 (to date). Cultivar and subsequent fungicide timing interacted to influence disease control and annual fungicide inputs during 2015. The logistic regression model- and disease threshold-based subsequent applications produced excellent control on 'Declaration'; whereas, only the logistic-regression model produced excellent control on 'Independence'. Three threshold-based applications were made to 'Declaration' plots during 2015 and 2017 (to date); whereas, six and five threshold-based applications were made to 'Independence' during 2015 and 2017 (to date), respectively.

Phylogeography of *Ophiostoma novo-ulmi*, the fungal pathogen responsible of Dutch Elm Disease

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Considerable threats on forests are introduced by invasive species, such as *Ophiostoma novo-ulmi*, the fungal pathogen responsible for the Dutch Elm Disease (DED). DED was first described in the Netherlands in 1921 and has led to the mortality of millions of elm trees (Genus *Ulmus*) all over the world. DED has been the object of much attention during the 1980–1990s. Since then, no concrete control solution has been established. The disease is caused by a complex of species: *O. ulmi*, responsible for the first wave of infection, and *O. novo-ulmi*, responsible of the second. *O. novo-ulmi* is divided in two subspecies: the North american population (*O. novo-ulmi americana*) and the European population (*O. novo-ulmi novo-ulmi*). In the present work, we propose to address the question of the two species origin and phylogenetic relationship, by performing a comprehensive study of population genomics and using a phylogeographic approach to determine populations structure and origins. Based on the whole genome sequencing of 96 historical samples, we were able to show a complex genetical pattern in our species of interest, providing some insight on the evolutionary history of one of the most aggressive fungal pathogen in the world.

Multidrug resistance conferred by a xenobiotic detoxification pathway in *Sclerotinia homoeocarpa*, casual agent of dollar spot

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Sclerotinia homoeocarpa, the causal agent of dollar spot on turfgrasses, has developed resistance to multiple fungicide classes. In addition, multidrug resistance (MDR) has been recently reported. Model eukaryotes are known to utilize transcriptional regulation of genes encoding metabolizing enzymes and efflux transporters in a three-phase system, modification (Phase I), conjugation (Phase II) and secretion (Phase III) for MDR by detoxifying xenobiotics. To date, it is unknown how fungi transcriptionally regulate and coordinate different phases of this detoxification system. We have identified three Phase I cytochrome P450 enzymes (CYP450s) and two Phase III ATP-binding cassette (ABC) efflux transporters. Furthermore, we have also identified a fungal specific transcription factor (Shxdr1) regulating the Phase I and III genes for the detoxification in *S. homoeocarpa*. The genetic mechanisms of a fungal xenobiotic detoxification system in *S. homoeocarpa* will be presented.

Temporal shifts in genetic structure and differentiation of *Cercospora beticola* populations in table beet fields of New York

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Cercospora leaf spot (CLS), caused by *Cercospora beticola*, is an important fungal foliar disease of table beet (*Beta vulgaris* ssp. *vulgaris*). In broad-acre table beet production CLS may cause defoliation, which prevents mechanized harvesting and leads to substantial crop loss. Previous studies have characterized high allelic and genotypic diversity within *C. beticola* populations in table beet fields in New York, which may be suggestive of an active sexual morph. Rapid evolution of resistance to fungicides as a function of this diversity threatens the durability of the dominant control tactic for this disease. This study quantifies temporal changes in genetic structure and differentiation of *C. beticola* populations from three locations in New York, using 12 microsatellite markers. High allelic (Nei's index of gene diversity (H_e) = 0.34 to 0.61) and genotypic diversity (Simpson's complement index (λ) = 0.75 to 0.97) was present in each population. Indices of differentiation between years varied, with pairwise index of differentiation (ϕ_{PT}) values ranging from 0.02 to 0.25 for clone-corrected data, indicating significant genetic differentiation in some locations. No multilocus genotype was shared between years or locations. Hypotheses to explain significant temporal shifts in genetic diversity and population differentiation are being investigated. The diversity and variation in *C. beticola* populations reveals the potential for rapid genetic changes, including an increased risk of fungicide resistance development. This information highlights the importance of adhering to best management guidelines for fungicides in conventional table beet production.

Development of microsatellite markers for quantifying genetic diversity of *Pleospora betae*

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Pleospora betae (syn. *Phoma betae*) is an important fungal pathogen of table beet, causing foliar disease, damping-off, and root decay. New York is the second largest producer of table beets in the United States for the processing industry and fresh markets. Microsatellite markers were developed for population genetics studies to quantify genetic and genotypic diversity of *P. betae*, and investigate hypotheses concerning sources of inoculum and disease spread. A *de novo* genome assembly of *P. betae* was used to identify microsatellite loci with tri- to hexa-nucleotide repeat motifs in non-exonic regions. Forty potential markers selected for primer development were tested on eight *P. betae* isolates. Fifteen of the markers were labeled with fluorescent dyes and tested on 24 *P. betae* isolates for reproducibility and polymorphism. Fragment analysis identified 11 of the markers to be reproducible (error rate = 0) with only one peak per isolate. These markers were highly polymorphic with an allelic range of four to 18 and an average of 7.4 alleles per locus. The markers were used to develop two sets of multiplex assays containing five and six markers, respectively. The microsatellite markers will be used to genotype *P. betae* populations from New York and the Pacific Northwest, the primary table beet seed production region of the United States.

Calonectria pseudonaviculata dispersal mechanisms and implications for boxwood blight management

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The boxwood blight pathogen, *Calonectria pseudonaviculata* (*Cps*), has resulted in over \$5 million in boxwood losses in Connecticut since 2011. The pathogen produces sticky masses of conidia on leaf and stem lesions under warm, wet conditions. We conducted replicated experiments to investigate *Cps* conidial dispersal from both sporulating cultures on 1-cm³ half-strength PDA (½ PDA) and sporulating lesions on infected 'Green Mountain' boxwood leaves. *Botrytis cinerea* infected blossoms were used as a control. Dispersal of *Botrytis* conidia was confirmed by capture using an Allergenco air sampler at 15 liters/min and by microscopic observation and growth of conidia on 15-cm-d ½ PDA plates. *Cps* conidia were not dispersed by either dry or moist air currents directed at conidiophores from 2 mm away at air speeds up to 44 mph for 10 min. *Cps* spores were successfully dispersed by splash of water droplets and by a simulated wind-driven rain that moved water droplets at air speeds of 20 to 44 mph. Movement of the pathogen,

sometimes long distances, may also occur by movement of infected plants, dispersal of dropped infected leaves, and by conidia adhering to equipment such as pruning shears, humans and animals. Inspection and prevention of disease on planting stock, pruning boxwood under dry conditions, sanitation of equipment and management of the pathogen with fungicides prior to wet periods conducive to spread will all contribute to reduced *Cps* spread and subsequent disease.

***Pseudozyma flocculosa*, a new paradigm in the mode of action of biological control agents**

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Powdery mildews are obligate biotrophic parasites that divert nutrients from their host through specialized feeding structures known as haustoria. The infection results in significantly lower productivity for the plant. In the case of tritrophic interactions where a biocontrol agent (BCA) parasitizes powdery mildews, the delicate biotrophic balance is thought to be deregulated either by competition, parasitism, antibiosis or induced resistance. However, none of these modes of action seems to explain the strong antagonistic activity of the BCA *Pseudozyma flocculosa* against powdery mildews. To uncover the intricate properties of *P. flocculosa*, we conducted the first comprehensive physiological and transcriptomic analyses of all three interacting organisms, *P. flocculosa*-powdery mildew-plant. Based on our observations, the BCA uses a unique strategy, whereby, for a short period of time, *P. flocculosa* modulates powdery mildew virulence, through the release of unique effectors, to divert nutrients extracted from the plant by the powdery mildew fungus for its own development. This phenomenon, hereby called hyperbiotrophy, causes a rapid collapse of powdery mildew haustoria, bringing an end to the interaction.

DNA sequence analysis of microbiomes on the surface of sour rot-infected grapes

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Sour rot is a grape disease about which research into its cause and management is limited. The disease negatively impacts fruit quality as ethanol oxidizes into acetic acid within the infected grape berries. The goal of this project was to determine which bacteria and fungi are more prevalent in the surface microbiomes of sour rot-infected versus asymptomatic grapes. Microbiome samples of 22 asymptomatic clusters and 29 sour rot-infected clusters were collected in a vineyard of *Vitis* interspecific hybrid cv. *Vignoles* in Geneva, NY in 2015. The total DNA was extracted from rinsate of 3-berry samples, and the 16S rRNA and ITS regions were amplified and sequenced. Qiime (Quantitative Insights Into Microbial Ecology) software was used to quality filter the sequences and identify the OTUs (operational taxonomic units) present in each sample. The taxonomies of these OTUs were assigned using the Greengenes and Unite databases. Statistical analysis was done in STAMP (Statistical Analysis of Metagenomic (and other) Profiles). Among 31 sequenced bacterial orders, the order *Rhodospirillales* had a significantly increased incidence in sour rot-infected grapes ($p < 0.001$) while *Burkholderiales* had a decreased incidence ($p = 0.024$). Among the 21 sequenced fungal species, *Talaromyces marneffeii* was the only species with an increased rate of detection in the sour rot-infected group ($p = 0.004$). An understanding of which microbes are present on the surface of symptomatic and asymptomatic grapes may lead to a better understanding of the sour rot disease complex and aid in developing targeted treatments.

PCR detection of *Erwinia amylovora* in apple rootstocks at fire blight infection foci edges after 2016 epidemic in Northeastern New York

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Unusually hot and humid conditions with rains and hail at the end of apple bloom in New York Champlain Valley, favored fire blight epidemic in 2016. Asymptomatic rootstock infections with *E. amylovora* (*Ea*), in contrast to the ones with visible cankers, presented a challenge in selecting the trees for removal in young, high-density orchards. Due to asymptomatic rootstock infections, seemingly healthy trees died at different times during the summer and fall 2016, and spring 2017, after first blight symptoms in tree crowns or whole dead trees were removed in 2016. We collected 360 rootstock samples from 7 apple farms on 1 Nov 2016. After PCR detection, the incidences of *Ea*-positive rootstocks in all selected infection foci edges per farm, each consisting of 5-8 surrounding rootstocks nearest to the central cankered rootstock, were 85, 79, 76, 94, 84, 82, 93% on 7 apple farms ($n=54, 52, 54, 54, 63, 55, 28$, respectively). Mean incidences of infected rootstocks for all foci edges, for each of 7 farms, were 87.5% (M.9 Nic29), 84.4% (M.26), 75% (M.9-337), 93.8% (M.26), 83.9% (M.9 Nic29), 83.3% (M.26) and 91.7% (M.26), respectively. For the same infection foci edges, these high incidence results contradicted lower incidences of (1) dead vs. alive trees: 2.1, 4.4, 35.4, 6.3, 25.4, 20.8, 0%, and of (2) cankered vs. non-cankered rootstocks: 56.3, 26.3, 41.7, 39.6, 35.4, 22.9, 20.8%, respectively. The results imply on need to remove the nearest trees around a cankered rootstock.

Update on successful strategies for the management of potato pink rot

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Pink rot of potato, caused by *Phytophthora erythroseptica*, is endemic in many potato-producing regions of the world. Typical management of pink rot has relied upon application of metalaxyl-m (mefenoxam) or more recently phosphites, either at planting or as a foliar spray during the growing season. However, reports of pathogen isolates with resistance to metalaxyl-m appeared as early as 2004 in Canada (and earlier in some parts of the U.S.A.). In Canada, national surveys have yielded isolates of *P. erythroseptica* with resistance to metalaxyl-m from across the country, with the highest frequency of resistant isolates recovered from eastern Canada. Replicated field trials have confirmed the capacity of metalaxyl-m to suppress disease caused by metalaxyl-m-sensitive strains, but not by metalaxyl-m-resistant strains. Phosphites have been shown to significantly suppress pink rot caused by all pathogen strains, when applied as multiple foliar sprays during the growing season, but not when applied in-furrow at planting. Novel chemistries including fluopicolide and oxathiapiprolin have been successful at suppressing pink rot in daughter tubers when applied in-furrow at planting. In addition, post-harvest applications of phosphites, fluopicolide or oxathiapiprolin were able to prevent disease in stored tubers inoculated with pathogen spores. Options are available for the successful management of pink rot, even when dealing with fungicide-resistant pathogen strains.

Estimate: A new mobile app to facilitate disease severity evaluation

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A new iPad application (app) called *Estimate* is introduced to facilitate rapid and mobile assessment of plant disease severity. The app may be downloaded from iTunes (<https://itunes.apple.com/WebObjects/MZStore.woa/wa/viewSoftware?id=1193605571&mt=8>) and is compatible with an iPad Air 2 or equivalent using iOS 9.0 or greater. The app is intended for use by researchers and crop scouts who would benefit from the ability to evaluate disease severity on a mobile device in the field with reference to standard area diagrams (SADs) prepared for specific diseases. *Estimate* allows for selection among photographic SADs representing logarithmic or linear intervals of disease severity. Data may be collected as a categorical variable representing the mid-point of these intervals, or as a continuous variable for severity in 1% increments. Data may be entered according to a user-specified framework involving varying numbers of plots, sub-plots, and sampling units at the plant and leaf levels. Data are recorded by touching the SAD image that best represents the disease severity of each sampling unit. The data may be interactively edited by the user within the app and sent as a comma-separated-value (csv) file for further manipulation. *Estimate* offers substantial savings in time during data collection, entry and verification, and disease severity categories are represented by realistic, color photographs to facilitate estimation. The app was released with separate SADs for assessing *Cercospora* leaf spot severity of red and yellow table beet cultivars. SADs for five additional diseases will be included in *Estimate* within a year. We invite collaborators to develop and/or contribute new SADs for inclusion in new versions of the app.

Host range and genetic characterization of the stem and bulb nematode, *Ditylenchus dipsaci*, from Eastern Canada

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The stem and bulb nematode, *Ditylenchus dipsaci*, is a plant-parasitic nematode affecting over 500 plant species worldwide. Since 2012, garlic producers from Ontario and Quebec have been particularly affected with economic losses caused by this pest. Reproduction of *D. dipsaci* on a particular host depends on its biological race which is unknown for these populations. Other crops such as onion could be at risk. In this study, the host range of four populations of *D. dipsaci* from Quebec and Ontario was determined in a greenhouse test on 12 crops. Garlic, onion and scallion showed great susceptibility to the nematode while reproduction on potato was poor. No development was observed on maize, soybean, barley, alfalfa, mustard, carrot, leek and lettuce. These plants could therefore be used in rotation crops in a control program. The susceptibility of five garlic cultivars (Arnaud, Cledor, Flavor, Messidor and Music) was also tested in field microplots. No significant difference was observed. Nineteen populations of *D. dipsaci* were also genetically characterized using genotyping-by-sequencing. The comparison of allele frequencies at 502 SNPs showed that most of the populations had a genotype similar to a reference population from northern Ontario. However, a sample from eastern Quebec exhibited a very distinct genotype and will require further phenotyping in greenhouse to preclude the possibility of a different race.

Partial cross-resistance to SDHI fungicides in *Sclerotinia homoeocarpa* field isolates from U.S.A. and Japan

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Succinate dehydrogenase inhibitor (SDHI) fungicides have been applied to control dollar spot (caused by *Sclerotinia homoeocarpa*) on turfgrass for the last 14 years without documented resistance. Two *S. homoeocarpa* field isolates sampled (2016) from a golf course in Japan exhibited resistance to boscalid in a monitoring survey. Six SDHI active ingredients (boscalid, fluopyram, flutolanil, fluxapyroxad, isofetamid, and penthiopyrad) were examined to determine cross-resistance among the two suspected SDHI resistant and

six SDHI sensitive reference isolates. The two resistant isolates displayed cross-resistance to boscalid, penthiopyrad, and fluxapyroxad. The SDHI target gene, iron-sulfur protein subunit of succinate dehydrogenase (SdhB) was sequenced. The two resistant isolates harbor an amino acid substitution (H267Y) in SdhB gene, but the six sensitive isolates did not harbor the mutation. SdhB^{H267Y} mutants were generated from a SDHI sensitive isolate through a genetic transformation system and confirmed the mutation is the direct determinant of SDHI resistance. Additional isolates were collected in 2017 from the same location in Japan (9) and suspected SDHI resistant isolates were collected from a Rhode Island golf course (8). *In vitro* assays confirmed the additional Japan isolates share the same cross-resistance properties, however, the Rhode Island isolates are cross-resistant to boscalid, fluxapyroxad, penthiopyrad, and isofetamid. The Rhode Island isolates do not harbor the same amino acid substitution and additional sequencing of SdhC and SdhD subunits is being conducted. This is the first report of SDHI field resistance and the molecular mechanism responsible for SDHI resistance in *S. homoeocarpa*.

Comparative transcriptomic analysis of soybean root response to *Phytophthora sojae* reveals new insights into silicon-mediated disease resistance

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Silicon (Si) is reputed to offer protection to plants against biotrophic and hemibiotrophic pathogens. Considering that *Phytophthora sojae* is a hemibiotroph that relies heavily on effectors for its virulence against soybean, the pathosystem *P. sojae*-soybean is perfectly suited to both validate the prophylactic role of Si and offer insights into the elusive role of Si. In this context, the present study had two main objectives: 1) to assess the protective role of Si against *P. sojae* in soybean, and; 2) to investigate how Si fertilization affects both soybean and *P. sojae* through dual RNA-seq analysis of the interaction. Based on the phenotypic response, Si fertilization prevented disease development and mortality in soybean plants inoculated with *P. sojae*. Surprisingly, comparative transcriptomic analysis of the interaction with and without Si treatment revealed a much higher expression of *P. sojae* effectors and plant receptors (NB-LRR), together with plant reactions, under the control treatment. These data suggest that Si plays a role in preventing *P. sojae* effectors from reaching plant receptors, which results in a form of non-host recognition and concomitant lesser infection.

Recovery of butternut and protection against the canker in Quebec: What have we learned after 6 years?

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In Canada, butternut is listed as an endangered species since 2005 and this precarious status is primarily due to a canker caused by *Ophiognomonia clavignenti-juglandacearum*. In 2010, we started a project in Quebec aimed at identifying, propagating and testing trees putatively resistant to butternut canker (BC). As butternut is shade intolerant, another goal was to release some trees to improve their vigour and their resistance to BC. As we had difficulty propagating butternut vegetatively, seedlings were obtained from nuts collected from putative resistant trees. These seedlings were inoculated and some resistance was noted. Defense mechanisms were then examined in bright-field, confocal, and transmission electron microscopy and the most obvious ones were linked to compartmentalization, where suberin and phenols seem to play a major role in limiting pathogen development. After dormancy, most of these defensive tissues were breached and BC symptoms then were evident. Release treatments did not improve the growth and the health status of butternut in the short term. Moreover, a qPCR assay failed to disclose any differences between the number of spores collected under control and released trees. Finally, some details gathered during this project when surveying putative resistant butternuts were used with other data in a recent study revealing that multiple factors, notably weather, influence the development of the disease.

Spatio-temporal severity patterns and association of certain key factors with butternut canker severity in Quebec

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Butternut is an indigenous tree species important from a conservation and biodiversity perspective in the mixed hardwood forests of eastern North America. An exotic fungal pathogen, *Ophiognomonia clavignenti-juglandacearum* (*Oc-j*), causes butternut canker that is characterized by dieback and trunk cankers that eventually kill the tree. This disease currently endangers the survival of butternut throughout its natural habitat in Canada and the United States. We studied the distribution patterns of butternut canker in Quebec using data collected from 24 locations and assessed the relationships of tree location and topography with trunk canker and dieback. We also assessed the temporal changes in severity of trunk canker and dieback on a small sample of trees from three locations. Butternut canker was found in all study locations in Quebec. Trunk canker showed a southwest to northeast declining trend in severity while dieback was more related to the altitude. Generalized mixed models showed a negative association of trunk canker with increasing latitude. Dieback showed a positive relationship with elevation. Trees on flat lands showed a greater probability of trunk canker than those on higher slopes, whereas dieback was unrelated to slope. Trunk canker and dieback worsened over time on individual trees. Given the spread of the disease and the strong future likelihood of extirpation of butternut by *Oc-j*, strategies to restore this tree species should be a high priority.

Functional analysis of *Erwinia amylovora* nucleobase cation symporter 2 transporters

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E. amylovora affects apples and pears deriving nutrients during disease progression. Nucleobase biosynthetic pathway mutants of *E. amylovora* differ in ability to cause disease, suggesting varied capacity for nucleobase uptake. We investigate nucleobase cation symporter 2 (NCS2) members in *E. amylovora*. Biochemical properties of the uracil transporter EaUraA, guanine/hypoxanthine transporter EaG/HXP, adenine transporter EaAdeP and xanthine transporter EaXanP are determined through heterologous expression of *E. amylovora* NCS2 in select *E. coli* Keio strains deficient in nucleobase transporter loci coupled with radiolabeled nucleobase uptake studies and toxic analog growth studies. EaUraA transports uracil with a high affinity (Km 0.56 mM) and a toxic derivative 5-fluorouracil. EaG/HX moves guanine with high affinity (Km 0.067 mM), hypoxanthine (Km 3.39 mM), 6-thioguanine (6TG) (Ki 3.71 mM) and transports 6TG in growth studies. EaAdeP transports adenine with high affinity (Km 0.43 mM), and is able to move guanine (Km 1.83 mM), but does not take up 6TG in growth studies. EaXanP transports xanthine with high affinity (Km 0.13 mM), while adenine, guanine, uracil and hypoxanthine - but not 6TG - act as weak competitive inhibitors. *E. amylovora* deletion mutants for EaUraA, EaG/HXP and EaAdeP, created using a Cam^R cassette, can cause disease in immature pears. Future research will investigate nutrient requirements fire blight disease development.

Investigating fungicide resistance in cucurbit downy mildew pathogen on Long Island, NY

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Cucurbit downy mildew caused by the oomycete *Pseudoperonospora cubensis* affects all cucurbit crop types and has become increasingly more impactful on cucurbit production since its re-emergence in 2004. Typically, effective control of this pathogen has been achieved through the use of multiple foliar fungicides. Recently, some of these fungicides have begun to lose effectiveness. Seedling bioassays were conducted on Long Island, NY, in 2016 and 2017 to attempt to identify potential resistance to labeled fungicides. Cucurbit seedlings were sprayed with fungicides, put for 1 to 2 days next to field-grown cucumber with downy mildew that had not been treated with resistance-prone fungicides, then kept in a greenhouse until symptoms developed. In 2016 fungicides Quadris, Forum, and Revus were ineffective based on comparing symptom severity to that of untreated control plants. Similar results were reported for bioassays conducted in other states. Previcur Flex, Zampro, and Presidio were effective. These fungicides ranged from effective to poor or ineffective in the other bioassays. In 2017, similar results were observed with Revus, Quadris, and Forum on Long Island. Additionally Presidio was shown to be ineffective. Information about potential existence of fungicide resistance based on efficacy in the bioassays will be used to develop fungicide recommendations for managing downy mildew in cucurbit crops.

A TaqMan real-time PCR for specific detection of *Pantoea allii*, a bacterial pathogen of onion

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Bacterial diseases of onion caused by *Pantoea ananatis* and *Pantoea agglomerans* have been reported in the U.S.A. and South Africa. In 2004, a new pathogen, *Pantoea allii*, was isolated from onions in South Africa and the U.S.A. *P. allii* has not been reported in Canada and as such precautionary measures are required to safeguard Canadian onion production. This work describes the development and validation of a TaqMan real-time PCR for specific detection of *P. allii* in pure cultures and infected plants. Comparative genomics of *P. allii* and closely related species followed by primer/probe design identified allii_leuS181F/allii_leuS235R (primers) and allii_leuS205pb (probe) as the best combination. The assay was evaluated for specificity using 45 *Pantoea* (28) and non-*Pantoea* (17) strains. Only *P. allii* strains consistently exhibited detectable fluorescence with a mean threshold cycle (C_T) of 20.64. All the other *Pantoea* and non-*Pantoea* strains did not show any detectable fluorescence. The assay detection limit of 1 pg compares favorably to that of a previously published conventional PCR technique. The generated standard curve showed an almost perfect regression line with a coefficient of determination of R²=0.98. The assay consistently detected the pathogen in all infected plants 21 days after inoculation. Viability of *P. allii* cells in infected plants after 21 days was confirmed by plating on culture medium followed by colony PCR amplifications and sequencing of the 16S rRNA and *leuS* genes. This assay should fill the current gap for a reliable diagnostic technique for screening onion imports to Canada.

Molecular evaluation of butternut canker sporulation under trees submitted to different release treatments

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We developed and thoroughly validated a specific and sensitive molecular assay for the butternut canker (BC) fungal pathogen, *Ophiognomonia clavignenti-juglandacearum* (*Oc-j*). The TaqMan real-time PCR assay discriminated *Oc-j* amongst the 35 other known *Ophiognomonia* species. Using an effective and consistent DNA extraction protocol, coupled with our molecular assay, we demonstrated a linear relationship between fluorescent signals and DNA extracted from serial dilutions of spores. The molecular *Oc-j* was then used to evaluate *Oc-j* sporulation under BC-infected trees that were released to promote their vigor either by pruning or cutting adjacent trees. *Oc-j* conidia were quantified in 977 samples collected from 49 trees, at two locations, and from two successive growing seasons. No significant differences were observed between control and silviculturally treated trees. The highly specific and sensitive assay developed in this study will become an important tool for evaluating various treatments against BC.

Susceptibility of fine fescue cultivars to *Colletotrichum cereale*, an aggressive pathogen causing Anthracnose Disease

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Anthracnose, caused by *Colletotrichum cereale*, is a serious disease in many cool-season turfgrasses but the fungus has not been reported as an aggressive pathogen on fine fescues (*Festuca* spp.). The objective of this study was to determine if an isolate of *C. cereale* obtained from symptomatic fine fescues was pathogenic to this host and if fine fescue cultivars and accessions varied in susceptibility. Pathogenicity of *C. cereale* isolate (FF1A) obtained from symptomatic fine fescues in Adelphia, NJ was evaluated on four hard fescue (*F. brevipila*) and four Chewings fescue (*F. rubra* ssp. *commutata*.) cultivars and accessions using a 5×10^{-4} conidial suspension in a growth chamber. Additionally an isolate of *C. cereale* (HF217C) from annual bluegrass was included for comparison. Disease symptoms were observed at five to 11 days post-inoculation with both isolates on all cultivars and accessions. Infection was confirmed by morphological evaluations, re-isolation from symptomatic tissue, and real-time PCR. Overall, the *C. cereale* isolate obtained from fine fescue was more pathogenic than the annual bluegrass isolate. Hard fescue cultivars 'Sword' and 'Beacon' exhibited mild susceptibility, and three non-commercial accessions (two Chewings fescues and one hard fescue) were very susceptible to the fine fescue isolate. This study confirmed that *C. cereale* can be a major pathogen of the fine fescues and should be considered by breeders when evaluating new germplasm.

Development of a NGS-based detection method to identify exotic forest pathogens from fungal spores suspended in air and occurring on insect vectors

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A significant threat to Canadian natural resources is the introduction and establishment of invasive and exotic fungal phytopathogens. Wood boring insects, which are recognized as vectors for fungal spores, and anthropogenic practices, such as agriculture, horticulture and international trade can facilitate the introduction and dispersal of fungal pathogen's spores. As detecting and identifying non-indigenous fungi are key initial steps in managing invasive species, we developed a new method based on next-generation sequencing (NGS), metagenomics and bioinformatics to allow for rapid and higher throughput species identification. We tested field samples collected over the summers of 2013–2015 from surveys conducted near high-risk areas across Canada to monitor several regulated fungal invasive species. Our amplicon-based technique used customized barcoded primers to target the ribosomal Internal Transcribed Spacer 1 (ITS1) in fungi and oomycetes and the mitochondrial ATP9-NAD9 region for specific screening of *Phytophthora* spp. Fifteen sequencing runs were performed on the Ion Torrent platform which yielded over 45 million sequences from our 398 samples. Using Real-time polymerase chain reaction (qPCR) assays for NGS results' validation, most species were detected by both techniques, though NGS was more sensitive to detect some species. In addition to the detection of *Heterobasidion* species including *H. annosum* s.s., we also found several species closely related to those from our target list, notably some *Phytophthora* species. We highlighted the major asset of this new methodology to increase surveying capacity and its detection potential for species of concern.

Application of CRISPR-Cas9 technology to study the *Epichloë festucae* antifungal protein gene

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Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is a major turfgrass disease. Strong creeping red fescue (*Festuca rubra* subsp. *rubra*) is often naturally infected with the fungal endophyte *Epichloë festucae*. This mutualistic symbiosis has been associated with enhanced resistance to dollar spot disease in the field. In a previous quantitative transcriptome study of the *E. festucae*–strong creeping red fescue interaction, the second most abundant fungal transcript was found to encode a protein similar to antifungal proteins found in *Penicillium* and *Aspergillus* species. The *E. festucae* antifungal protein was purified and did inhibit the growth of *S. homoeocarpa* in plate assays. We performed gene knockout to further study the role of this protein in the endophyte-mediated disease resistance. The conventional fungal gene knockout approach that relied on homologous recombination was unsuccessful, likely because this gene is flanked by repeated sequences. However, we successfully applied CRISPR-Cas9 technology to the non-model fungal endophyte *E. festucae*. The Cas9 endonuclease generated a double-strand break at our target site of the antifungal protein gene, and a mutation was introduced when repairing the break. This was confirmed by PCR and sequencing. Next, the knockout isolate will be reintroduced into endophyte-free strong creeping red fescue to evaluate susceptibility of the host plant to dollar spot disease using an established greenhouse assay.

Potato leafroll virus alters small RNA production in its aphid vector during plant viral infection to promote virus dispersal

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Potato leafroll virus (PLRV) is transmitted by the aphid *Myzus persicae* in a circulative, non-propagative manner. We performed small RNA (sRNA) sequencing of *M. persicae* to test the hypothesis that the vector suppresses PLRV replication using an RNA interference mechanism. While no sRNAs against PLRV were detected, the aphids were found to be infected with *Myzus persicae* *Densovirus* (MpDNV) and mounted a sRNA antiviral immune response to this insect-infecting virus. Feeding on PLRV-infected plants altered this immune response, as aphids exposed to PLRV-infected plants, but not purified PLRV or plants infected with the non-persistent *Potato virus Y*, generated an abundance of unusually long sRNA against MpDNV, a reduced number of 22-mers, and had higher titer of MpDNV when compared to aphids fed on noninfected plants. Feeding on plants expressing the PLRV silencing suppressor protein P0 recapitulated the increase in MpDNV titer in aphids, showing that P0 is the viral protein responsible for this effect. Winged aphids showed higher and more variable MpDNV titer compared to wingless individuals. We hypothesize that PLRV increases MpDNV titer in its vector to increase the production of winged morphs and hence, virus dispersal. If so, the sRNA interplay observed among the aphid, MpDNV, and PLRV-infection in the plant is some of the first molecular evidence supporting the vector manipulation hypothesis, that is, strategies of pathogens that enhance their spread to new hosts.

Development of molecular assays to detect the presence and viability of *Phytophthora ramorum* and *Grosmannia clavigera*

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In order to determine if living fungi of phytosanitary concern are present in wood or to evaluate the efficacy of treatments, the method of choice is to grow microbes in petri dishes for subsequent identification. However, some fungi are difficult or impossible to grow in cultures, and thus, to validate the effectiveness of existing and emerging wood treatments, a molecular methodology that can detect living fungi and fungus-like organisms is required. RNA-based molecular diagnostic assays were developed to detect the presence of living fungi and fungus-like organisms of phytosanitary concern. Since RNA represents the transcription of genes and can therefore only be produced by living organisms, it provides a marker to determine if an organism is alive. The assays were designed to target genes that are essential to vital processes, then used to assess their presence and abundance through real-time reverse transcription polymerase chain reaction (PCR). A stability analysis was conducted by comparing the RNA to DNA ratio over treatment time. The results illustrated that for treated samples, DNA remained stable over a period of 10 days post treatment, whereas RNA could not be detected after 24 hours for *Phytophthora ramorum* or 96 hours for *Grosmannia clavigera*. Therefore, this method provides a reliable way to evaluate viability of organisms following treatments and can have profound impacts on assessing both timber and non-timber forest products of commercial value.