



2015 Potomac Division Meeting Abstracts

Abstracts presented at the APS Potomac Division meeting in Rehoboth Beach, Delaware, March 11–13, 2015. 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: Atha, B. W., Sechler, A., King, J., Blanc, J., and Schneider, W. 2015. Development of a PCR assay for the tunicamycin gene cluster of *Rathayibacter toxicus*. (Abstr.) *Phytopathology* 105(Suppl. 3):S3.1. <http://dx.doi.org/10.1094/PHYTO-105-9-S3.1>

Development of a PCR assay for the Tunicamycin Gene Cluster of *Rathayibacter toxicus*

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Rathayibacter toxicus is a nematode-vectored bacterium responsible for Annual Rye Grass Toxicity (ARGT) in Western Australia. ARGT is a complicated interaction involving a forage grass, a nematode vector, *R. toxicus*, and an associated bacteriophage which possibly promotes the bacterium's production of a deadly toxin. The toxin is often consumed by grazing livestock causing neurological damage and eventually death. No robust molecular assay is currently available for identifying *R. toxicus*' presence and the goal of my project was to begin development of such an assay based on PCR. Using our recently sequenced and compiled *R. toxicus* genome, we have identified the genes believed to be responsible for toxin production in a putative tunicamycin gene cluster (TGC). Five primers and probe sets were developed targeting regions inside the TGC, along with one set directly outside the cluster in *R. toxicus*. Two primer and probe sets were also designed for potential genes of interest in the associated phage CS14Φ. All eight sets have been used in real-time PCR to rapidly identify *R. toxicus* and determine the presence of phage CS14Φ. After optimization and specificity testing of primer and probe sets are confirmed, they can be used in later qRT-PCR experiments analyzing the expression of toxin and phage genes under various situations. The resulting assay has potential to rapidly determine toxin production capabilities in related bacteria from around the world.

Developing thresholds and field specific risk maps for *Meloidogyne incognita*, southern root knot nematode, in lima beans

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Southern Root-Knot Nematode (RKN), *Meloidogyne incognita*, causes loss in a wide host range of crops. On the Delmarva Peninsula, RKN is a major yield limiting pest in lima beans (*Phaseolus lunatus*), a regionally important vegetable crop. Currently, there are no known RKN threshold levels in lima beans to determine when specific control actions should be taken in a field. To rectify this situation, we began by conducting a survey of eight lima bean fields with a history of RKN in 2014. Fields were divided into one hectare grid units and sampled before planting, during mid-crop growth, after harvest,

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and after overwintering. RKN were extracted from soils and counted. In addition, bioassays were conducted using collected soil from each grid with cucumber test plants in spring. RKN severity maps were created from these assays and population changes are reported. Grid units were also evaluated for edaphic factors including soil moisture, field capacity, electrical conductivity (EC), depth to hardpan, pH, P content, and soil texture. Preliminary results indicate that EC is well correlated with RKN populations. Microclimate and cultural data were collected from each field as well including overwintering soil temperatures and crop rotations. Edaphic, cultural, and microclimate data collected will be used to assess potential of different areas of fields to harbor RKN and to develop risk models.

Fungicide sensitivity of five commonly encountered *Phytophthora* species in Maryland nurseries

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Phytophthora is a commonly encountered pathogen in Maryland nurseries. In this study, a collection of isolates from five Maryland nurseries and forests in the Northeastern United States was screened against several fungicides commonly used to manage the pathogen. In total, 243 isolates (77 *P. cinnamomi*, 23 *P. citrophthora*, 21 *P. pini*, 15 *P. multivora*, and 107 *P. plurivora*) sampled from a variety of hosts and substrates were screened for sensitivity to mefenoxam and fosetyl-AI. Insensitive isolates [$\geq 50\%$ growth rate relative to controls (RG)] were further tested using higher concentrations of these fungicides along with dimethomorph, dimethomorph + ametoctradin and fluoxastrobin. Mefenoxam and fosetyl-AI-insensitive isolates were detected only for *P. cinnamomi*, *P. multivora* and *P. plurivora* and included 4%, 13% and 12% of screened isolates, respectively, and remained insensitive at the higher concentrations. These isolates were also insensitive to fluoxastrobin, but completely sensitive (0% RG) to dimethomorph and dimethomorph + ametoctradin. Based on our previous genotyping study using AFLP it was found that, while the most common genotypes found in Maryland nurseries appear to be sensitive or intermediately sensitive (<50% RG), the least common genotypes included the insensitive isolates. Our studies suggest discrepancies in fungicide sensitivity among these five common *Phytophthora* species, signifying that a genus-level fungicide application plan is insufficient for the management of all species in ornamental nursery production. Insensitive isolates are present within the population and thus the inclusion of chemicals such as dimethomorph and ametoctradin into management plans is recommended.

Genotypic diversity of common *Phytophthora* species in Maryland nurseries

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Phytophthora causes disease in many important agricultural, forest, and nursery crops. Plant trade has been instrumental in the transmission of *Phytophthora* in the ornamental nursery industry, potentially increasing the diversity of genotypes or facilitating new host-pathogen associations. In this study, the genetic diversity of a collection of *P. cinnamomi* (102 isolates) and

P. plurivora (186 isolates) from Maryland nurseries and forests in the Mid-Atlantic United States was characterized using amplified fragment length polymorphism (AFLP). Cluster analysis divided *P. cinnamomi* isolates into four clusters (1 = 15, 2 = 5, 3 = 43 and 4 = 39) and *P. plurivora* into six (1 = 24, 2 = 14, 3 = 18, 4 = 32, 5 = 26, and 6 = 72). Genetic diversity indices showed a high level of diversity among the *P. plurivora* isolates (Shannon's diversity index, $H = 6.01$), but relatively low for *P. cinnamomi* ($H = 4.72$). Analysis of molecular variance revealed that most of the variation for *P. cinnamomi* occurred within groups. Only 3% and 1% of the variation was associated with the isolate origin (nursery vs. forest) and isolate source (symptomatic/asymptomatic host), respectively. Little variation (4%) existed among the forest isolates from different States. In contrast to *P. cinnamomi*, greater amounts of variation existed among groups for *P. plurivora*: 24% of the variation was associated with nursery origin and 8% with the isolate source. Isolates with identical AFLP profiles were detected in both species, suggesting a clonal component to the pathogens' populations. Some of these clonal groups included isolates originating from both forest and nurseries, suggesting that both species may have been introduced from nurseries to forests or vice versa.

Delineating the oak strain of *Xylella fastidiosa*: A comparative genomics approach

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In 2000, *Xylella fastidiosa* had the distinction of becoming the first bacterial phytopathogen to have its genome sequenced. In the preceding decade and a half, no fewer than sixteen *X. fastidiosa* genomes now occupy the NCBI database. Despite the plethora of data, much of the comparative genomics work of major *Xylella* subspecies is housed in literature from the earlier parts of this fifteen year period. Working with the hypothesis that differences remain to be unearthed from continued comparative analyses, a simple database-driven pipeline is posited for the exploration of a recently sequenced *Quercus palustris* strain (RNBI) relative to three *X. fastidiosa* genomes isolated from *Vitis*, *Prunus*, and *Quercus rubra*. This approach expands assignment of putative functionality to numerous hypothetical ORFs, and in one case, identified a potentially important procytic acidic repetitive protein suggested to play a role in vector retention. The approach also rapidly segregates poor scoring reciprocal query sequences, underlying the discovery of unsuspected dissimilarities between both closely and distantly related subspecies strains. The added implications also extend to supplementation of both existing multilocus findings and novel phylogenetic topologies given possible new candidate loci. The expanded breadth of all *X. fastidiosa* genomic data may, therefore, lead to new insights in the areas of acquisition/infection, control, and refined taxonomic assignment.

Genome-assisted analysis of the fungarium phytobiome

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Fungal evolutionary relationships are traditionally explored by examining standard DNA markers generated using universal primer sets. Although these markers are easily amplified from a broad range of taxonomic groups, they have a number of drawbacks, including the undesirable amplification of DNA from non-target organisms. This is especially true when applying these markers to unculturable samples such as biotrophs or fungarium specimens. Such samples are typically in close association with plant substrates, and may be impossible to separate from host tissue and the associated phytobiome. However, these samples can be a tremendous resource for phytopathology research. In particular, fungarium samples are often the sole source of type specimens, and include fungi collected over many years. Additionally, fungaria are the primary source of exemplar samples for some groups. For instance, the largest collection of Rutstroemiaceae fungi is housed in fungaria. Using this family as an example, our objective was to generate genomic markers specific to the Rutstroemiaceae in order to generate sequence data to further studies in this family. Genome sequence assemblies were generated for eight representative isolates of the closely related grass pathogen *Sclerotinia homoeocarpa*, and the saprophytes *S. echinophila* and *Rutstroemia sydowiana*. Single copy genes were identified from whole genome alignments and were used to design 30 PCR primer sets with Illumina adapters. After preliminary PCR screening, 12 marker amplicons (average length = 117 bp) were sequenced using Illumina NGS. PCR success with the markers was 95% or greater, while PCR with standard ITS and LSU primers only yielded amplicons from 40% and 17% of fungarium specimens, respectively.

Organism-specific markers generated reliable DNA sequence data from preserved Rutstroemiaceae fungi > 100 years old, allowing this fungaria collection to be included in ongoing phylogenetic analyses. Employing genomics-based methods circumvents the disadvantages of conventional methodologies, and facilitates analysis of complex phytobiome samples.

Utilizing microbial partnerships to enhance nitrogen fixation in legumes

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In recent years, biofertilizers have emerged as an important component for biological nitrogen fixation. It offers an economically attractive and ecologically sound route for augmenting nutrient supply for crop production. Soil microorganisms are very important in the cycling of nutrients from both inorganic and organic sources in the soil and also in the maintenance of soil health and quality, and microbial communities can be optimized to promote production. Soil-plant-microbe interactions are complex and the need of the hour is to enhance the efficiency of the meager amount of external inputs by employing the best combinations of beneficial microbes for sustainable agricultural production. The current knowledge pertaining to how different microbes work synergistically to improve nitrogen fixation is limited. Previously, we have shown a rhizospheric isolate *Bacillus subtilis* strain UD1022 facilitates plant growth and disease protection. The present study was conducted to evaluate the response of pea (*Pisum sativum* cv. Early Alaska) seeds and seedlings to a mixed inoculum of *Bacillus subtilis* spp. strain UD1022 and *Rhizobium leguminosarum* bv viciae strain RL2 at different time points. Our results indicate that the beneficial microbes work synergistically to promote growth response in plants. The results from our studies demonstrated that the co-inoculation of the pea seeds and seedlings with host-specific rhizobial species (RL2) and rhizobacteria UD1022 significantly increased the plants total biomass and resulted in formation of large functional nodules as compared to sole inoculation with RL2. Different treatments as well as the explant developmental stage exhibited significant variation in shoot dry weight, root dry weight, total biomass, number of nodules thus affecting the total Nitrogen content and nutrient uptake within different treatments. The promising results from this study will be decisive in our future experiments for harnessing the positive influence of different rhizobacteria interaction on the host plant. Our work will also advance the understanding of root-association by studying the interaction of beneficial rhizospheric bacteria with the plant host; this is a sophisticated, practical system for discovering the intricate communication and coordination mechanisms used by bacteria and their hosts.

Serological detection and diagnosis of '*Candidatus Liberibacter asiaticus*' in citrus

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'*Ca. Liberibacter asiaticus*' (CaLas), the pathogen associated with citrus Huanglongbing (HLB), is a non culturable member of the α -proteobacteria. Simple and cost effective confirmatory or presymptomatic diagnostic tests are lacking. In the present study, a simple *in situ* direct tissue blot immuno assay for the detection of CaLas was optimized. An anti OmpA polyclonal antibody was highly effective for the detection of CaLas from citrus petioles, stems, seeds and roots in a direct tissue blot immuno assay. When field samples of known CaLas-infected citrus were tested, about 80% of all samples analyzed tested positive with both the direct tissue blot immuno assay and qPCR; whereas 95% were positive with at least one of these two methods. When asymptomatic citrus tissues were tested, the direct tissue blot immuno assay method gave a higher rate of detection (83%) than the qPCR method (64%). This result is consistent with a lower concentration of CaLas DNA, but a higher proportion of viable cells, in the asymptomatic tissues. The direct tissue blot immuno assay also preserves the detail of the spatial distribution of CaLas in diseased citrus tissues. The antibody was also used for immuno capture of bacteria from both citrus and periwinkle extracts for successful detection by PCR without further purification of the DNA. The limit of detection for the immunocapture PCR assay was about 2.3×10^6 bacteria per g of tissue based on qPCR assay of the starting plant materials. The assays were equally effective when used to detect CaLas obtained from Florida, California, Japan and Thailand indicating wide applicability of the technology. The assays also offer the advantages of low cost, high throughput, ease of scaling for multiple samples and simplicity over current PCR-based methods for the detection of '*Ca. Liberibacter asiaticus*'.

***In planta* detection of *Puccinia horiana* infection by *in situ* hybridization**

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Puccinia horiana, known to infect *Chrysanthemum x morifolium*, causes the disease Chrysanthemum White Rust (CWR). To monitor the progress of infection by this obligate pathogen, we developed an *in situ* hybridization assay using biotinylated DNA probes. Chrysanthemum leaf tissue inoculated with *P. horiana* isolate PA11-1 was fixed, paraffin embedded, and mounted on positively charged glass microscope slides for use with this protocol. Probes targeting 18S ribosomal RNA were designed by aligning a portion of the 18S sequence of *P. horiana* to representative fungi from many genera and selecting a common variable region. Experiments were conducted to optimize proteinase K digestion, formamide concentration, and final wash temperature. Results show that a 20-minute treatment with 10 µg/mL proteinase K was necessary for probe penetration, with increased concentrations resulting in lower signal and reduced tissue morphology. Conditions providing optimal signal production included 10% formamide in the hybridization mixture, a 52°C post-hybridization wash in 1X SSPE, and treatment with 0.4 ng/µL streptavidin-horseradish peroxidase conjugate. This study describes the optimization of a general *in situ* hybridization protocol for use in the detection and visualization of *P. horiana* in paraffin embedded plant tissue.

Soft rot of onion caused by the yeast *Wickerhamomyces onychis*

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An unknown yeast was isolated from sweet onions grown in Northumberland County, Virginia, and submitted to the Virginia Tech Plant Disease Clinic in 2010. The bulbs exhibited symptoms of soft rot of inner scales similar to those caused by the yeast *Kluyveromyces marxianus* var. *marxianus*. To perform Koch's postulates, 1 mL of yeast suspension with concentrations ranging from 1.9×10^5 to 8.5×10^7 CFU/ml was injected by hypodermic syringe into whole sweet onion bulbs. All inoculated bulbs developed water-soaked lesions and tissue disintegration, and yeast was recovered from symptomatic tissue. Bulbs injected with sterile water did not develop any symptoms of soft rot. The yeast recovered from the original sweet onions and the yeast recovered from inoculated sweet onions were both identified as *Wickerhamomyces onychis* by sequencing the internal transcribed spacer region of ribosomal DNA. This cosmopolitan species was first isolated from an infected fingernail and has been found in both terrestrial and marine environments. To our knowledge, this is the first report of *W. onychis* as a plant pathogen and the first report of a yeast causing soft rot of sweet onions grown in Virginia.

Incorporation of the standard scientific name for fungi into national regulatory and extension databases and other nomenclature support for safeguarding

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In order to provide accurate information for pest screening efforts, the National Plant Diagnostic Network (NPDN), Federal identifiers and quarantine inspectors must consistently identify and reference scientific names of microorganisms. The International Code of Nomenclature (ICN) for algae, fungi, and plants (Melbourne Code) no longer accepts two names representing sexual and asexual stages for a single fungus, requiring use of a single name, causing confusion in naming for a large number of plant pathogens. The USDA ARS Systematic Mycology and Microbiology Lab (SMML), U.S. National Fungus Collection, and Mycobank are used for reference, and also utilized by USDA APHIS PPQ National Identification Services and CPHST. The Center for Environmental and Regulatory Information Systems (CERIS), Purdue University, manages databases for the NPDN and USDA APHIS Cooperative Agricultural Pest Surveys (CAPS). Approximately 3,000 names of fungi and oomycetes, including 200 key plant pathogens were reviewed for accuracy under the new ICN and updated in the NPDN database, and at SMML. A total of 7,322 names were updated in the SMML database, including the records directly related to the NPDN repository and names from

recent works on plant pathogenic fungi. Approximately 1,500 entries were added or verified for newly described type specimens of fungal pathogens, voucher specimens from published first reports and specimens intercepted at ports of entry. Updates are available to the public through the U.S. National Fungus Collections website. Database design at CERIS ensures usability and commonality for all upload software systems for NPDN. NPDN lab management systems are able to change lab specific software to use the new naming convention for uploads or searches in the repository, and modifications allow for pest groupings and a larger number of synonyms per pest. The work continues and educational efforts are ongoing through NPDN Newsletters and trainings.

Evaluation of nematocides and alternative control measures for root-knot nematodes in lima beans

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Root-knot nematodes (*Meloidogyne incognita*) are among the most destructive plant pathogens and are infectious on a range of crops, including lima bean (*Phaseolus lunatus*). Control of root-knot nematode (RKN) cannot be limited to crop rotation or the fallowing of fields due to an extensive host range and survival characteristics that enable the pathogen to persist in the soil, even in non-ideal conditions. In the past, the most effective chemical controls such as methyl bromide and organophosphate nematocides were found to be harmful to the environment and other, non-target soil biota, spurring a revival in research to control RKN. In this study, the impact of new chemicals, biologicals, and cultural practices on RKN populations were examined. Two nematocides currently being labelled for vegetable crops, fluensulfone and fluopyram, were studied for RKN control in lima bean. Both chemicals reduced RKN populations and lima bean root galling; however, phytotoxicity was observed with fluensulfone, when placed near the seed. Cultural approaches were also found to reduce RKN populations. Organic matter additions via composts and chicken manure were tested; only chicken manure reduced RKN populations significantly. In addition, the use of biofumigant mustard and sorghum cover crops, which can reduce RKN levels by releasing compounds that react in the soil with an end-product resulting in natural fumigation, were investigated. Results from 2014 were inconclusive; biofumigant treatments will be repeated in 2015. Biological controls, including antagonistic soil bacteria and fungi, are currently being evaluated as well. The goal of this study is to estimate the efficacy of chemical, biological, and cultural control mechanisms for the development of an Integrated Pest Management (IPM) approach for reducing RKN population levels in lima bean fields.

Potential for resistance to isofetamid in fungal pathogen populations

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Succinate dehydrogenase inhibitor (SDHI) fungicides (FRAC group 7) are an important control mechanism for fungal diseases in a wide variety of crops. This control is compromised by the accumulation of fungicide resistance in pathogen populations. Several mutations in the succinate dehydrogenase (*Sdh*) gene across the fungi have been shown to confer resistance to the SDHI fungicides, although each member of the fungicide class are differentially affected by each known mutation. Individual isolates of *Botrytis cinerea* that carry these resistance mutations in *Sdh* have been shown to possess a fitness penalty, suggesting that buildup of resistance in a population can be easily mitigated by chemistry rotation or by removal of SDHIs from use. Isofetamid, a new SDHI fungicide from Ishihara Sangyo Kaisha, is the perfect foundation fungicide on high value crops. It has been shown to be effective in controlling several pathogens carrying most of the *Sdh* mutations currently known to confer resistance to the SDHIs. This places isofetamid as an effective disease control mechanism with a limited potential for buildup of field resistance.

Using fluorescent labeling to gain insight into fire blight disease development

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Fire blight is an economically damaging disease of apples and pears caused by the bacterium *Erwinia amylovora*. We have developed a versatile tool to help understand the roles of bacterial virulence genes in fire blight disease development: a strain of *E. amylovora* that carries an indispensable plasmid marking both the location of the bacteria and the expression of target

virulence gene transcriptional promoters. This plasmid reporter system allows us to visualize the spatiotemporal expression of genes necessary for disease development within plant tissues. We are currently using this system to observe the *in planta* expression of *hrpN*, a gene necessary for fire blight disease development, with epifluorescent and confocal microscopy. The knowledge gained from these experiments will give us a better understanding of exactly when and where *E. amylovora* virulence genes are expressed *in planta*, and this may define novel targets for intervention in disease prevention.

Evaluation of fungicides for efficacy against pod rot of lima bean caused by *Phytophthora capsici*

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Pod rot caused by *Phytophthora capsici* is a devastating disease of lima bean (*Phaseolus lunatus*) on the Delmarva Peninsula. Management of pod rot is often limited to fungicides. However, only two fungicides, mefenoxam (Ridomil Gold), registered since 1992, and more recently, cyazofamid (Ranman) are registered for the management of pod rot. Because growers have relied on mefenoxam for management, resistant populations of *P. capsici* have developed. Ten fungicides, in addition to Ridomil and Ranman, were tested for their efficacy in controlling pod rot of lima bean. Two separate field trials were conducted at the University of Delaware's Carvel Research and Education Center in Georgetown, Delaware in 2014. The first and second field locations had moderate and heavy disease pressure, respectively. Results indicate that all tested fungicides significantly reduced disease incidence when compared to the water control treatment in both trials. Applications of Ridomil, Ranman, Omega, and Zorvec consistently achieved in the greatest reduction of pod rot. There was no significant difference in yield among treatments in the first field location (moderate disease pressure). However, the control plots in the second field location (heavy disease pressure) yielded significantly less, with potential yield loss over 86%. These trials indicate that there are additional fungicides with efficacy against *P. capsici* on lima bean which merits further research to support their labelling.

MelCast fungicide applications scheduling for mid-Atlantic watermelons: A re-evaluation

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Anthraxnose and gummy stem blight (GSB) are major diseases of watermelons in the U.S. MelCast is a weather-based spray advisory program for anthracnose and GSB that uses hours of leaf wetness and temperature during the leaf wetness period to calculate an environmental favorability index (EFI). Historically, mid-Atlantic watermelon growers who implemented MelCast using the threshold of 30 EFI reduced fungicide use and maintained disease control as compared to a weekly schedule. However, fungicides used on watermelons have changed in recent years. An experiment to evaluate the anthracnose and GSB severity in plots sprayed according MelCast vs. weekly with "newer" fungicide products was conducted as a randomized complete block design in 2012 and 2014, with five fungicide treatments and four replications. Plots received one of two fungicide programs applied on a MelCast and weekly schedule (tebuconazole alternated with chlorothalonil or difenconazole + cyprodinil alternated with chlorothalonil) or were untreated. In both years, six sprays were applied under the Melcast schedule and nine sprays were applied weekly. In 2012, the weekly schedule with either fungicide program reduced defoliation more than the MelCast program using the same respective products ($p < 0.0001$). Likewise in 2014, the weekly schedule with either fungicide program reduced GSB more than the MelCast schedule ($p < 0.0001$). Due to these results, we are re-evaluating the use of MelCast for fungicide scheduling.

Transmission of *Candidatus Liberibacter Asiaticus* strain JPB430b and development of a strain specific PCR assay

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Huanglangbing (HLB, citrus greening) is considered the most destructive citrus disease that threatens US agriculture. JP430b is a strain of *Liberibacter Asiaticus* from Japan that causes no visible symptoms and low titer infections. HLB transmissions are typically more successful in cases of high titer symptomatic infection. The objective was to determine if JP430b could be acquired and transmitted by the citrus psyllid vector, and to develop an assay which specifically detects only the JP430b strain. By comparing the genome of the Japanese isolate to a Florida isolate, sequences that were specific to the Japanese isolate were identified and used to make PCR primers. Healthy psyllids were fed on a JP430b infected plant. Some psyllids were removed for DNA extraction and testing while the remaining psyllids were moved to healthy citrus plants to test for transmission. Our results showed that JP430b could be picked up by the psyllids as well as transmitted to healthy plants. These results indicate that even though JP430b is a low titer infection, it is still capable of being picked up and transmitted by psyllids. This raises the possibility that there are strains of HLB that are low titer and symptomless but still highly transmissible.

***Salmonella*-induced systemic acquired resistance in tomato and its impact on *Salmonella* colonization of tomato leaves**

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Tomatoes are vectors for the human pathogen *Salmonella enterica*. This pathogen-commodity pair ranks second in foodborne illness risk among produce commodities. The *Salmonella* host environment is the gastrointestinal tract, but it is adapted to colonize plants. *Salmonella* induces pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI) in plants, however systemic acquired resistance (SAR) has not been explored. This research investigated SAR activation in tomato and whether SAR restricts epiphytic *Salmonella* populations, using standard bacterial enumeration and expression analysis of SAR marker genes. Reactive oxygen species production was assessed using 3, 3'-diaminobenzidine (DAB) staining in cv. 'Primo Red' leaves inoculated with *S. Newport*, Typhimurium and Senftenberg. All serotypes triggered staining relative to the control ($X^2(8) = 23.4, p = 0.003$). *S. Newport* induced expression of pathogenesis-related gene PR5 and salicylic acid carboxyl methyltransferase (SAMT) ($p < 0.05$), in distal leaves of 'Primo Red'. Treatment with the SAR activator acibenzolar-*s*-methyl also increased expression of PR1 ($p < 0.05$). *NahG* mutants deficient in SAR supported higher *Salmonella* populations ($p < 0.05$). However, *S. Newport* inoculation did not impact levels of *Pseudomonas syringae* pv. tomato or *Salmonella* on distal leaves in 'Primo Red' and 'Heinz'. It appears that SAR is insufficient to restrict *Salmonella* growth on tomato, despite being triggered by this human pathogen.

Screening *Phaseolus lunatus* for resistance to *Sclerotinia sclerotiorum*

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Delaware is the number one producer of lima beans (*Phaseolus lunatus*) for commercial freezing and canning in the United States. Unfortunately, most commercial cultivars of lima beans are susceptible to white mold (caused by the fungus *Sclerotinia sclerotiorum*), an economically devastating disease that causes substantial crop yield loss, annually. White mold is difficult to manage because overwintering sclerotia of the pathogen may remain in the soil for five years or more, infecting lima bean and more than four hundred other crops and many broadleaf weed species. Sclerotia of *S. sclerotiorum*, along with infected pods and stems were collected from a lima bean field in Lewes, Delaware. Single-hyphal isolations were made from the samples after surface sterilization and were used to evaluate thirty-eight *P. lunatus* cultivars and landraces. Plants were grown in the greenhouse and inoculated using the cut stem, or straw test method, and evaluated for resistance. Plants were rated on length of lesion, formation of sclerotia, and side shoot survival. One lima cultivar, and four Plant Introduction (PI) lines showed no infection in all or most plants. Seven PI lines exhibited partial resistance to white mold by maintaining overall vigor, strong side shoots, and pod set. The research is ongoing, as the trial is being repeated in the greenhouse, and all thirty-eight lines will be rated for disease avoidance in the field in the summer of 2015.

Temperature effects on the germination of sporangia and zoospores and colony establishment of *Phytophthora kernoviae*

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Phytophthora kernoviae, first reported in 2003 has the ability to infect and cause foliar necrosis on *Rhododendron ponticum* and other woody ornamentals. However, little is known about the specific conditions necessary for this species to infect host plants. Temperature plays a significant role in the life cycle of all *Phytophthora* spp. and preliminary studies showed it is important to the infection process of *P. kernoviae*. This study was designed to understand what factors in the infection process are controlled by temperature. Sporangia and zoospores from six *P. kernoviae* isolates were exposed to different temperatures ranging from 20 to 25°C. A study on infection of plant tissue showed that after 1 wk, foliar necrosis of detached *R. ponticum* and *Magnolia stellata* leaves only appeared between 20 and 23°C. Colony development studies originating from sporangia and zoospores show that germination is about equal across all temperatures, but the establishment of new colonies is significantly diminished above 23°C. However, a less noticeable difference in colony establishment originating from mycelia plugs is observed at these temperatures. In summary, germination of sporangia and zoospores for *P. kernoviae* occurs at all temperatures, but the establishment of new colonies from asexual propagules appears to depend on temperature conditions not exceeding 23°C. This also appears to impact disease development on host tissue at these higher temperatures.

The effect of temperature on *Phytophthora kernoviae* infection of *Rhododendron ponticum*

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Phytophthora kernoviae is a pathogen known only to exist in the U.K. and New Zealand. In the U.K., it is known to infect *Rhododendron ponticum* and other woody species. In New Zealand, it is known only from soil samples and *Annona cherimola*, even though *R. ponticum* exists where *P. kernoviae* is found. This study was conducted to determine if temperature may be a limiting factor in infection of *R. ponticum* by *P. kernoviae*. Detached *R. ponticum* leaves were inoculated with 500 sporangia from three isolates originating each from the U.K. and New Zealand. After 1 week at 10, 15, 20, 25, and 28°C, the necrotic area was measured. There was no difference in necrotic area between the non-inoculated controls and any of the isolates at 25 and 28°C and the pathogen could not be isolated afterwards from the inoculated leaves. At 10, 15, and 20°C, the necrotic area was higher after inoculation with most of the isolates than the non-inoculated control and the pathogen was isolated from the leaf tissue. The largest necrotic area occurred at 20°C. There was no difference in necrotic area between the U.K. and New Zealand isolates. Inoculation of whole plants of *R. ponticum* with *P. kernoviae* sporangia confirmed the results of the detached leaves. Necrosis and recovery of *P. kernoviae* occurred at plants kept at 20°C, while no necrosis or recovery of *P. kernoviae* occurred at plants kept at 25°C. More detailed studies showed necrosis between 20 and 22°C, but not between 23 and 25°C.