Plant Health 2021 Abstracts of Special Session Presentations

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Aflatoxin impacts on insect and *Aspergillus flavus* fungal fitness M. DROTT, University of Wisconsin, Madison, WI, USA

Dr. Milton Drott is currently a post-doctoral researcher working with Dr. Nancy Keller at the University of Wisconsin–Madison. His research explores the evolution and ecology of fungal secondary metabolites, with a focus on agriculturally and medically relevant pathogens. Dr. Drott's doctoral work with Dr. Michael Milgroom at Cornell University specifically focused on the ecological factors that drive the persistence of both aflatoxigenic and non-aflatoxigenic isolates of *Aspergillus flavus*. Dr. Drott will discuss the advantages that aflatoxin can confer to aflatoxin-producing fungi in the presence of some insects. Intriguingly, different species of insect vary considerably in their resistance to this notorious mycotoxin, with several agricultural pests like the corn ear worm and the maize weevil showing high tolerance. Dr. Drott will explore how advantages conferred to the fungus in the presence of different insects and how costs of toxin production in the absence of insects can provide insights that allow us to better understand aflatoxin contamination of crops.

Impacts of bacteriophages on plants: From environmental microbiomes to pathogen biocontrol C. HERNANDEZ, University of California, Berkeley, CA, USA

Bacteriophages (phages; viruses that infect bacteria) are both resident members of the plant microbiome and a potential tool for mitigating bacterial diseases. While phages have been shown to be important modulators of bacterial communities in other environments, they have been relatively understudied in association with plants. In this talk, I will briefly review what is currently known about bacteria-phage interactions in plant-associated microbiomes, as well as the successes and challenges of phage-based biocontrol. I will also present original research demonstrating the impact of the plant environment on bacteria-phage ecological and evolutionary dynamics. Using the bacterial pathogen *Pseudomonas syringae* pathovar *tomato*, we have shown that growth in tomato leaves alters the costs and benefits of phage resistance, which consequently leads to low rates of resistance evolution relative to evolution in laboratory culture media. We then followed up this work by leveraging experimental evolution to test whether *P. syringae* biofilm formation impacts phage contact rates with bacterial cells. By beginning to address the numerous open questions in this area of research, we hope to better understand the role of phages in shaping plant microbiomes and be able to design robust phage-based biocontrol strategies.

Enriching microbial communities to suppress bacterial speck disease in tomato H. EHAU-TAUMAUNU (1), K. HOCKETT (1,2), (1) The Pennsylvania State University, University Park, PA, USA; (2) The Huck Institutes of the Life Sciences, Penn State University, University Park, PA, USA

Suppressive soils have been observed to reduce the growth or limit the severity of soilborne pathogen(s) over multiple years and are linked to the development of a microbial community or specific populations. Despite many suppressive soil examples, our knowledge is limited for foliar diseases managed by a suppressive phyllosphere community due to limited community transfer between seasons. Therefore, we investigated whether a foliar microbial community could be developed through artificial selection to suppress disease using the model pathosystem of *Pseudomonas syringae* pv. *tomato* (*Pto*) and tomato. Tomato seedlings were inoculated with a field phyllosphere metacommunity and then challenged with *Pto* or MgCl₂ buffer, and measured disease progression for 8 days. Microbial

material was then collected from plants showing the least amount of disease or at random to inoculate naïve plants for the next passage. Overall, disease severity initially increased to a peak at passage 4 or 5 followed by a sharp decline, reaching low severity levels by passage 10. Hence, the repeated passaging resulted in the selection of communities suppressive towards *Pto*. For one passage line, disease severity of the field phyllosphere community was significant compared to the control ($F = 2.58$; $P = 0.0087$). Current amplicon (16S/ITS) and shotgun metagenomic sequencing will examine how the community changed over time and potential taxa contributing to *Pto* suppression; however, further studies into their roles will be required. We believe this microbial community approach can potentially provide robust and consistent disease management.

Challenges of mitigating postharvest losses: An applied perspective from industry

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While it is true that there is much food loss in the postharvest arena, the reasons are quite varied and, in some cases, even deliberate. In situations where losses can be controlled, there are practical hurdles present that must be overcome in order to achieve loss prevention. This presentation will outline some of these obstacles, such as application mechanics and economic/return-on-investment considerations. Insight into some of the nuts and bolts of postharvest processes will also be offered. It is the presenter's goal to provide food for thought to the research community on perhaps not so obvious factors to consider when conducting research aimed to reduce food losses, with the end goal of successful real-world adoption of the research outcomes.

Mycotoxigenic *Fusarium* genotypes associated with stink bugs and field corn

J. OPOKU, H. L. Mehl, USDA Agricultural Research Service, Tucson, AZ, USA

Recent studies in the mid-Atlantic U.S. have identified an association between increasing populations of invasive and native stink bugs in corn fields and increasing severity of mycotoxin concentrations in harvested grain. Characterizing the ecological role of stink bug–*Fusarium*–corn interactions is important for understanding the etiology and epidemiology of mycotoxin contamination. In a survey of various stink bugs species collected from corn fields in the mid-Atlantic, *Fusarium* spp. were isolated from 35% of the stink bugs. A majority (74%) of *Fusarium* isolates belonged to the *F. fujikuroi* species complex, which includes the fumonisin-producing species *F. verticillioides* and *F. proliferatum*. The ability of brown stink bug (*Euschistus servus*) to transmit *F. verticillioides* to non-infected corn ears after feeding on *F. verticillioides*-infected corn was demonstrated in a laboratory experiment, and *F. verticillioides* was isolated from both frass and external and internal body parts of the insects. Results indicate that fumonisin-producing fungi can survive within and be disseminated by stink bug species that frequently infest corn fields. Thus, stink bug–*Fusarium* associations may benefit fumonisin-producing *Fusarium* species as stink bugs help to spread fungal propagules to new infection courts. Factors contributing to the close association between stink bugs and fumonisin-producing *Fusarium* spp. versus other fungi and the potential role of fumonisins in stink bug–*Fusarium*–corn interactions will be discussed.

Geminiviral replicons for plant-based expression of antibodies and immune complex vaccines

H. MASON (1,2), A. Diamos (1), M. Pardhe (1), (1) Arizona State University, Tempe, AZ, USA; (2) Biodesign Institute at ASU, Tempe, AZ, USA

Bean yellow dwarf virus (BeYDV) is a member of the genus *Mastrevirus*, family *Geminiviridae*, and has a single-stranded DNA genome encoding four genes. We developed an *Agrobacterium*-delivered replicon vector system for robust transient expression in plants using elements from the BeYDV genome linked to expression cassettes for target genes. The system is readily modified to co-express immunoglobulin heavy and light chains with separate cassettes in a single replicon, as well as heavy chain fusion proteins that enable the production of recombinant immune complexes (RIC). We optimized the replicons to enhance expression of difficult proteins by limiting the expression of viral Rep/RepA and thus attenuating replication efficiency.

Detecting the causal agent of beech leaf disease through microbial profiling

C. FEARER, The Ohio State University, Columbus, OH, USA

Beech leaf disease (BLD) is an emerging tree disease affecting beech species, including American (*Fagus grandifolia*), European (*Fagus sylvatica*), and Oriental (*Fagus orientalis*), in northeast North America. The recently described nematode *Litylenchus crenatae* ssp. *mccannii* (LCM) was detected in BLD affected foliage and may be associated with the disease. However, speculation on the direct role of the nematode in infection still remains. In this study we profiled the microbial communities associated with asymptomatic, symptomatic, and naïve (control) American beech foliage by using a high throughput sequence-based metabarcoding analysis of fungi, bacteria, phytoplasmas, and nematodes. We then used both a differential abundance analysis and indicator species analysis as well as several diversity metrics to attempt to discover microbes associated only with symptomatic foliage. To do so we amplified the organism-specific phylogenetic informative regions of the 16S, 18S, and internal transcribed spacer (ITS1) regions using Illumina MiSeq. Our results detected the amplicon sequence variant (ASV) associated with LCM, but in all symptom types. However, four ASVs associated with the bacterial genera *Wolbachia*, *Erwinia*, *Paenibacillus*, and *Pseudomonas* and one ASV associated with the fungal genus *Paraphaeosphaeria* were detected only in symptomatic samples. In addition, we identified significant differences based on symptom type in both the alpha and beta diversity indices for the bacterial and fungal communities. These results suggest that LCM may not be fully responsible for BLD, but rather that other microbe(s), may be contributing to the syndrome. This project provides the foundational steps to determining the etiology of BLD.

LAMP and the advance of field-portable molecular diagnostics in forest pathology

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Surveillance of diseases in forest systems has never been more important, both because of the increased arrival of introduced invasive species and the resurgence of native ones due to a changing climate. Though traditional isolation-based diagnostic methods are still a valuable tool for confirmatory tests, they are time-consuming and do not fit the need for rapid and on-site diagnostic responses. The technology and capability of the molecular diagnostic toolkit have advanced greatly in the last couple of decades, and field-portability has been one of the directions in which a great deal of improvements has been achieved. Loop-mediated isothermal amplification (LAMP) is one of several approaches that has allowed scientists to utilize the specificity and flexibility of molecular diagnostics directly at point-of-care. LAMP reactions occur under isothermal conditions, removing the need for complex equipment, and are less sensitive to inhibitors, thus tolerating the use of crude extracts, which can be obtained in the field. Finally, the use of LAMP technology is fairly easy and requires minimal training, which is a key feature for the accessibility of its wide application. Here we describe some recent successful applications of LAMP technology in forest pathology, comparing the different visualization methods that can be adopted, touching base on the challenges that can occur, and discussing the approaches that we have used to solve them. The presentation will conclude with some considerations on the versatility of the technology and its potential application beyond diagnostics.

Plant virus-based COVID-19 vaccine candidates

N. STEINMETZ, University of California, San Diego, CA, USA

Nanoscale engineering is revolutionizing the way we detect, prevent, and treat diseases. Viruses are playing a special role in these developments because they can function as prefabricated nanoparticles. We utilize and build-upon the high-precision assemblies of the viral capsids and utilize them as platform technologies, engineered and repurposed for a desired function. More specifically, we have developed a library of plant virus-based nanoparticles, and through structure-function studies, we are beginning to understand how to tailor these materials appropriately for applications targeting human, veterinary, and plant health. In this presentation I will discuss our efforts to develop COVID-19 vaccine candidates using our plant virus-based vaccination technology. While COVID-19 vaccine roll-out has begun, emergence of new and more contagious variants, as well as the cold chain requirements of the newly approved vaccines bear significant challenges to achieving global vaccination. The plant virus-based vaccination platform offers highly suitable attributes to serve as a vaccination platform technology: the plant virus nanoparticle serves as an adjuvant. While non-infectious toward mammals, plant viruses mimic the molecular patterns associated with pathogens, making them highly effective adjuvants. The technology is a plug-and-play display technology and the plant virus nanotechnology can be engineered to display epitopes derived from SARS-CoV-2 or other pathogens offering rapid generation of vaccines from identification of target virus sequence. The product is stable outside the cold chain, and plant virus-based nanotechnologies can be integrated into polymer device technology to achieve slow-release of the active ingredient, allowing for continuous boosts, thus requiring only single-dose administration.

Climate change and plant disease: Adaptation strategies for tropical and temperate regions

K. A. GARRETT, University of Florida, Gainesville, FL, USA

Weather has major effects on the risk of crop losses to disease and arthropod pests, so climate change and variability are key risk factors. Disease management in temperate regions has been “easier” in some respects. Hard winters destroy many pathogens and pests, or at least reduce their numbers. Breeding for resistance is faster for short-season crops, allowing faster adaptation to new threats, and the absence of these crops during winter can break green bridges for pathogen survival. These helpful buffers on pathogen population growth probably tend to make regional disease management success in temperate areas less dependent on having a high percentage of growers make good disease management choices. That is, without these buffers supplied by cold winters, the success of individual farmers will likely tend to be more dependent on the success of their neighbors. Decision support tools can aid in regional management strategies, such as scenario analysis to determine the likely outcomes of policies intended to support adaptation to climate change. For example, impact network analysis (INA) is a framework for evaluating how successful regional disease management strategies are likely to be, and what sorts of adjustments are necessary to adapt to global change. Three important examples of such impact networks will be discussed: regional management of new invasive diseases, seed systems which may or may not reduce the spread of disease through seed, and crop breeding networks that determine how limited pools of disease resistance genes are deployed across landscapes.

Utilizing regionally coordinated nematode-protectant seed treatment trials and partnerships to enhance applied nematology research and extension

K. BISSONNETTE, University of Missouri, Columbia, MO, USA

For more than a decade, soybean cyst nematode (SCN) has been estimated to be the most yield limiting nematode pest of soybeans in the United States and Ontario. As SCN is present in most soybean producing regions of North America and continues to be detected in new geographies, efforts to reduce SCN populations, increase yield, and educate growers are imperative. Seed-applied nematode-protectants are relatively new tools developed for managing SCN. In 2019 and 2020, small-plot field trials were established in 12 states and 1 Canadian province to test untreated seed, a fungicide base, and nine nematode-protectant seed treatment products for their efficacy in controlling SCN across multiple environments. By utilizing a large geographic footprint, diverse cropping systems were evaluated while integrating collaborators in plant pathology and nematology. These trials have become a foundation for standardization of applied nematology trials for SCN in the region. As a result, data generated has provided a broad range of farmers with relevant information about nematode-protectant seed treatments in their area and has multiplied the impacts of local Extension and the SCN Coalition. The data generated allows farmers to make more informed management decisions for the respective operations. As SCN has

grown and adapted to survive in North America for more than 65 years, applied nematology research and Extension efforts must also adapt to changing agricultural practices and problems. Collaborative applied nematology research will continue to drive change in the North American agricultural sector to create a more sustainable, resilient future for North American farmers.

Bringing the lab to the field: New monitoring method for forest pathogens

R. C. HAMELIN, University of British Columbia, Vancouver, BC, CANADA

The world's forests face unprecedented threats from invasive insects and pathogens, driven by global trade and climate change. Early detection and surveillance activities are essential to monitor the environment and promote early interventions. DNA detection using the polymerase chain reaction (PCR) has become an integral part of this process. One of the biggest challenges is that biosurveillance of forest enemies is often done in remote locations where laboratory facilities and equipment are not available. We have developed a point-of-use real-time PCR system using a crude buffer-based DNA extraction protocol and lyophilized, ready-to-use reactions for point-of-use applications. We demonstrated the use of this approach with a broad spectrum of forest pathogens such as white pine blister rust and sudden oak death. DNA can be obtained within a few minutes from a variety of tissues, including infected leaves, pathogen spores. The assays were accurate and yielded no false positive nor negative. The shelf-life of the lyophilized reactions was confirmed after one year at room temperature. Successful tests conducted with portable thermocyclers and disposable instruments demonstrate the suitability of the method, named in situ processing and efficient environmental detection (iSPEED), for field testing. The kit required to conduct this method fits in a backpack and can be carried to remote locations for point-of-use accurate and rapid detection of pests and pathogens.

Gene silencing, overexpression, and editing in plants using plant viruses

S. P. DINESH-KUMAR, U. Nagalakshmi, N. Meier, J. Y. Liu, University of California, Davis, Davis, CA, USA

Over the last 3–4 decades, plant viruses have been engineered for gene function studies in plants. Specifically plant viral vectors have been developed to downregulate gene expression through virus-induced gene-silencing (VIGS) approach. Furthermore, virus-mediated overexpression (VOX) vectors have been developed to overexpress host or foreign proteins or peptides or small RNAs/microRNAs to study specific biological processes in plants or for crop improvement. More recently, plant viral vectors have been engineered to deliver gene-editing components such as single guide RNAs (sgRNAs) and CAS nucleases. I will discuss our efforts towards development of RNA viruses for VIGS, VOX, and genome editing in dicot and monocot plants.

Virus species nomenclature: Current issues and possible solutions

J. KUHN, NIH/NIAID/DCR/IRF, Frederick, MD, USA

Names of species in botanical, fungal, zoological, and prokaryotic taxonomies have a uniform, Latinized binomial, format originally introduced by Carl Linnaeus more than 250 years ago. This format is not currently in use in virus taxonomy. Indeed, virus species nomenclature is not currently standardized at all, resulting in a wide diversity of formats. The potential need for a standardized virus species naming format has been recognized and discussed at the level of the International Committee on Taxonomy of Viruses Executive Committee (ICTV EC) over the past few years. Practical difficulties associated with current practices in species naming, along with possible solutions for implementation of a standardized nomenclatural format, similar to other biological taxonomies, will be presented in this talk. This work was supported in part through Laulima Government Solutions, LLC prime contract with the US National Institute of Allergy and Infectious Diseases (NIAID) under Contract No. HHSN272201800013C. J.H.K. performed this work as an employee of Tunnell Government Services (TGS), a subcontractor of Laulima Government Solutions, LLC under Contract No. HHSN272201800013C. The views and conclusions contained in this document are those of the author and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the US Department of Defense, US Department of Health and Human Services, US Department of the Army, or of the institutions and companies affiliated with the author.

Understanding the host and the environment to manage the soybean cyst nematode, *Heterodera glycines*

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Managing soybean cyst nematode (SCN) has been a challenge and despite best efforts, almost all counties growing soybeans in the US are increasingly infested with SCN. However, SCN numbers decreased in a long-term soy-corn rotation experiment, sans interventions. We investigated 6,000 SCN cysts from these field plots, to find a steady buildup of a specific mycobiome made up of only 14 different genera, relatively undisturbed by crop rotation and time. Several isolates of these 14 genera were good parasites of SCN cysts and produced nemato-toxic metabolites in vitro. Further greenhouse screening identified a few key isolates that could be effective commercial biocontrol agents. Our results suggest strong roles for both the host plant and SCN in selecting this mycobiome. Host resistance, the most effective tool to manage SCN is mostly derived from a single source, PI 88788. This resistance conferred by a three-gene quantitative trait locus (QTL), *rhg-1b*, is being overcome by many field populations of SCN. The most prominent alternative source of resistance is multi-QTL involving the *rhg-1a* haplotype and the *Rhg4* QTL, from soybean line Peking. Introducing an extra copy of the α -SNAP gene from *rhg1-b* or *rhg1-a* into a variety with the Peking background bolstered the resistance of the Peking variety. But, an extra copy of α -SNAP in a variety with the PI 88788 background did not provide any benefits. We are investigating the

molecular basis of this enhanced resistance. Together, stacking multiple resistance types and compatible microbial communities will help improve SCN management.

Canines for detection of oak wilt

K. L. SNOVER-CLIFT, Cornell University, Ithaca, NY, USA

Oak wilt, caused by the fungus *Bretziella fagacearum*, was first discovered in upstate New York in 2008. Five years later (2013), it was identified for the second time in the same Glenville, NY, neighborhood as the original find. Concerned with possible spread, the New York State Department of Environmental Conservation (NYSDEC) and Cornell University, Plant Disease Diagnostic Clinic (CU-PDDC) collaboratively began surveying disease-favorable sites across the state and educated community members, arborists and nursery owners, and county extension staff to be on the look-out for additional cases. In 2016, surveys found two additional locations, Long Island and the Canandaigua area, each at least 180 miles apart from the original site and from each other. The primary oak wilt response has been incorporating early detection methods. In 2020, a canine project focusing on an invasive insect pest and weed introduction was underway in New York. The oak wilt team members requested a proof-of-concept exercise to determine if canines could provide an early warning system and detect early tree infections. This presentation will review the process of the 5-day training of the canine team and their human handlers on oak wilt detection and the outcomes of the proof-of-concept study. Additionally, the use of another early warning tool, the implementation of a beetle vector molecular analysis procedure targeting the oak wilt pathogen, will be reviewed.

Gene therapy using *Citrus tristeza virus* to manage HLB disease

C. A. EL-MOHTAR, T. Shilts, C. Robertson, A. Levy, W. O. Dawson, University of Florida-CREC, Lake Alfred, FL, USA

In 2005, *Candidatus Liberibacter asiaticus* (Clas), an intracellular gram-negative bacteria and the causal agent of huanglongbing (HLB), was discovered in Florida where the Asian citrus psyllid (ACP) insect vector was established. In a short period of time, the bacteria spread into all citrus producing regions of the state. Currently, almost 100% of the trees are infected, acreage and yield are decreasing, processing plants are closing and the disease is spreading in California and Texas. Citrus is commercially propagated by grafting tissue of a limited number of elite lines on a selected set of rootstocks, and these lines have no tolerance to HLB. To screen therapeutics to manage HLB, we are using a mild strain of *Citrus tristeza virus* (CTV) that localizes to the phloem tissue of citrus where the CLas bacteria resides and ACP feeds, as a bio delivery tool. We screened therapeutics to induce tolerance/resistance in the plant by expressing antimicrobial peptides (AMPs) and downregulating negative regulators of plant defense and susceptibility genes by RNA interference (RNAi). Furthermore, to prevent the spread of the bacteria among trees, we manipulated the phloem diet to target ACP specifically by expressing bacterial modified pesticidal proteins (Mpp) or truncated sequences of psyllid genes to induce RNAi. Our screening process identified AMPs and plant targets that induce tolerance in the plants. ACP reproduction was affected by an Mpp and RNAi targeting of multiple psyllid genes.

Current situation of *Xanthomonas* spp. on common beans in Cuba

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Common bacterial blight (CBB) caused by *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* subsp. *fuscans* (*Xcf*), is a serious seedborne disease in both temperate and tropical bean production zones with crop yield decline of up to 40%. The main goal of this work was to detect and study the phylogeny of *Xanthomonas* spp. isolates from different provinces producing common beans in Cuba. Samples of leaves and pods from naturally infected plants were collected and processed to isolate and identify pathogenic bacteria. Isolation was performed in culture media (YDCA and XCP1) and the pathogen was detected by polymerase chain reaction (PCR) using specific primers. Rep-PCR technique and the sequencing of housekeeping genes were used to molecularly characterize the identified isolates. In addition, the pathogenicity of these isolates was evaluated in the susceptible cultivar *Bat-304*, as well as in resistant and susceptible genotypes from Canada. A DNA fragment was obtained using PCR and the size of the fragment corresponded to the size reported for these *Xanthomonas* species. Molecular and pathogenic characterization reported a high variability among the isolates identified. These findings demonstrate evidence of the diversity of *Xanthomonas* spp. isolates in common bean production areas. Moreover, these results suggest the necessity of more in-depth studies about the molecular determinants related to pathogenic variability.

Transcriptional changes of the root-knot nematode *Meloidogyne javanica* in response to tomato root exudate

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Root-knot nematodes (RKNs) are devastating agricultural pests and cause billions of dollars in annual yield losses. Infective juveniles (J2s) of RKNs are usually attracted to and penetrate at the elongation region of root tips. The exudate from tomato root is highly attractive to J2s of RKNs. Recently, polyamines found in tomato root exudate were shown to be potent attractants of RKNs. However, it is largely unknown how J2s of RKNs response to root exudate at molecular levels. Here we used *Meloidogyne javanica* and tomato root exudate (TRE) as an experiment system to identify RKN genes responsive to root exudate. We treated J2s of *M. javanica* with TRE or water (mock treatment) for 90 minutes and conducted RNA-seq to profile differentially expressed genes in *M. javanica*. Using Tophat and Cufflinks pipeline, we identified 79 genes that were significantly upregulated ($\text{Log}_2\text{Fold-change} \geq 1.5$) and 21 genes that were downregulated ($\text{Log}_2\text{Fold-change} \leq -1.5$) in J2s of *M. javanica* treated with TRE compared with mock treatment. Genes encoding G protein-coupled receptors and neuropeptides are enriched in upregulated genes. These genes are expected to function in nematode nervous system and host finding. In addition, expressions of cell-wall degrading enzyme genes and a group of putative cysteine-rich

effector genes are highly induced. These groups of genes are important in RKN penetration and establishment in roots. In contrast, most of downregulated genes encode proteins of unknown function. Identification of RKNs genes responsive to root exudate can provide new targets for nematode control.

Hyperspectral approaches for monitoring disease progression and resistance in five-needle pines

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Five-needle pines are large, long-lived conifers and are all susceptible to white pine blister rust (caused by the non-native fungal pathogen *Cronartium ribicola*). The pines have a suite of strategies, occurring at low population frequencies, for resisting the disease. Even though genetic resistance occurs at various frequencies, it can be very difficult to identify trees with usable and durable levels of resistance that could be included in breeding for restoration and reforestation. In this study we tested the ability of hyperspectral imaging, using a custom motion control system and machine learning, to identify and track the presence and progress of the disease in order to identify potential resistance seed sources in southwestern white pine. We conducted a greenhouse study on 175 open pollinated seedlings from 10 seed sources selected across the latitudinal range of the species. Seedlings were randomized and half were artificially inoculated with *C. ribicola* spores while the remaining were used as controls. The seedlings were scored for disease symptoms and patterns of growth. A support vector machine was able to automatically detect infection with a classification accuracy of 87% ($k = 0.75$) over 16 image collection dates. Additionally, hyperspectral imaging was able to accurately detect health vigor status (as a proxy for disease progression) using the normalized photochemical reflectance index. The approach suggests a way forward to scale up early disease detection in forestry.

A new class of susceptibility gene from one of the oldest known bacterial diseases of plants

Z. DUBROW, Cornell University, Ithaca, NY, USA

Xanthomonas campestris, the causal agent of black rot of crucifers and other diseases of *Brassicaceae* crops, weeds, and ornamentals was one of the first identified species of plant pathogenic bacteria. Despite over 130 years of research, diseases caused by *Xc* remain a major threat to *Brassica* cultivation. Transcription activator-like effectors (TALEs) are a large family of type III secreted effectors present in some *Xanthomonas* species that specifically target and directly upregulate plant susceptibility (S) genes to promote disease development. To find factors involved in cabbage susceptibility to disease, we identified TALE-targeted S genes by investigating TALEs from a large collection of *Xc* strains isolated from New York state which has an \$80 million dollar cabbage industry menaced by black rot of crucifers. By PCR and Southern blotting, we surveyed 128 New York *X. campestris* strains isolated from New York cruciferous weeds and crops and found that 28 (22%) contain TALEs. Sixty *Xc* genomes were sequenced to determine strain relatedness and identify *tal* gene sequences. We found that TALEs were present in a subset of strains from each *X. campestris* pathovar, except raphani and nonpathogenic strains. Inoculations of *B. oleracea* with *tal* gene knockout strains showed that Tal14B is necessary for full virulence. Transcriptome profiles revealed Tal14B specific gene upregulation of a berberine bridge enzyme-like (BBE) gene with a predicted Tal14B binding site in the promoter. Further analysis revealed that eight TALEs from *Xc* strains of multiple pathovars isolated from weed and crop species also have predicted binding sites in the BBE promoter. Additional TALEs upregulate or have binding sites in a second BBE family gene as well. In other pathosystems, conserved TALE targets are important to bacterial pathogenesis indicating a potential for BBE targets to play a broad role in *Brassica* response to *X. campestris*.

North Central Division Winner—Predicting the impact of root-associated bacterial endophytes on plant growth

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Utilizing beneficial microbes for crop improvement is one strategy to achieve sustainable agriculture, but identifying microbial isolates that effectively promote crop growth is a challenge. This study aimed to develop an economical solution to predict the impact of root-associated bacteria on plants. We isolated 183 bacterial endophytes from healthy field-grown roots of domesticated tomato *Solanum lycopersicum* and its wild cousin *S. pimpinellifolium*. Thirty isolates from each genotype were screened for five in vitro functions: auxin or siderophore production, phosphate solubilization, antagonism to a soilborne tomato pathogen, and the presence of DAPG or HCN synthesis genes. Hierarchical clustering of the functional traits suggested classified the isolates into four main groups. To understand how in vitro functional traits of bacteria relate to their impact on plants, we inoculated three isolates from each of the functional groups on tomato seedlings grown in the greenhouse. Isolates from three of the four groups promoted plant growth. To investigate factors that influence the impact of the endophyte on host phenotype, we observed the root colonization behavior of the plant-growth-promoting isolates. Preliminary data suggest that the ability of the isolate to promote plant growth does not correlate with the level of root colonization. Our results indicate that isolates with similar in vitro functional traits perform similarly in binary experiments, regardless of their taxonomic identification or host origin. The strategy we introduced may be helpful for the development of targeted microbial inoculants in future studies.

Using genomic epidemiology to quantify the “hardness” of fitness tradeoffs

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Fitness tradeoffs between environments are widely assumed to limit the spread and emergence of novel pathogens. However, in reality we often observe the evolution of fitness “trade ups” or “no cost” generalists. For example, pathogens with expanded host ranges often emerge without paying any fitness “cost” in terms of performance on ancestral hosts. Using plant RNA viruses as a case study, I will

argue that fitness tradeoffs can be productively classified as either “hard” or “soft” based on how easily evolution can resolve fitness conflicts between environments. However, for these concepts to be usefully applied we need to develop a framework for predicting whether tradeoffs represent “hard” biophysical constraints or “soft” constraints easily overcome by selection. I will then describe how recent advances in genomic epidemiology can be coupled with experimental evolution to study the evolutionary dynamics of pathogen population responding to fitness tradeoffs to gain insights into the mechanisms by which selection can overcome fitness tradeoffs.

Role of *Burkholderia* TAL effector-like proteins for host–microbe interactions

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Transcription activator-like (TAL) effectors are bacterially encoded, DNA-binding, eukaryotic transcription factors secreted through the Type III Secretion System (T3SS) into host cells. TAL effectors are important virulence factors for *Xanthomonas* and *Ralstonia* spp. but have not been widely found outside of these two phytopathogenic genera. Previously, *Burkholderia* TAL-like (*btl*) genes were identified in the *Mycetohabitans* (formerly *Burkholderia*) spp. that inhabit the fungus *Rhizopus microsporus*. While Btl proteins can bind DNA, their role in vivo was unclear due to structural differences with *Xanthomonas* and *Ralstonia* TAL effectors. We found that even though Btl proteins have truncated N- and C- termini, they are still able to be T3-secreted and localize to the nucleus. Despite the requirement of a T3SS by *Mycetohabitans rhizoxinica* to invade *R. microsporus*, a knockout of the single *btl* gene in one strain ($\Delta btl19-13$) did not abolish establishment by that strain. Fungi harboring the $\Delta btl19-13$ strain compared to the wild-type strain underwent transcriptomic changes and were more susceptible to detergent stress caused by sodium dodecyl sulfate. The mechanism by which Btl19-13 and other Btl proteins cause transcriptional changes remains unknown, as Btl19-13 was unable to repress or induce gene expression in typical TAL effector in planta assays. Furthermore, *btl* genes are present across *Mycetohabitans* spp. and vary in sequence and size, raising the question of how conserved their role is across symbiotic partnerships. Functional and phylogenetic analysis of Btl proteins will provide new insight into effector evolution and the diversity of roles TAL-like proteins can play in host–microbe interactions.

Distribution of dollar spot fungus in asymptomatic and symptomatic turfgrass

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Dollar spot is one of the most economically important diseases of turfgrasses. The causal agent of dollar spot in turfgrasses belong in the new genus *Clarireedia*. We developed a quantitative real-time polymerase chain reaction (qPCR) molecular detection assay specific to the *Clarireedia* genus. The assay can detect all members of the *Clarireedia* genus tested, quantify the abundance of *Clarireedia* from environmental (field) samples in both asymptomatic and symptomatic turfgrass, and capable of detecting as little as 38.0 fg (3.8×10^{-14} g) of *Clarireedia* genomic DNA. The goal of the project was to determine the distribution of dollar spot disease in asymptomatic and symptomatic creeping bentgrass (*Agrostis stolonifera*) throughout the year using our qPCR assay. The abundances of *Clarireedia* were measured in the leaves and crown tissue of creeping bentgrass fairway turf in May 2019, August 2019, May 2020, and July 2020 from three replicates of 30, 1 cm diam. $\times$ 2.5 cm deep cores spaced 10 cm apart. The number of positive detections for *Clarireedia* ranged from 37% to 69% in asymptomatic turfgrass and 77% to 95% for symptomatic turfgrass. The abundance of *Clarireedia* was measured in leaves, crowns, and thatch throughout 2019 and 2020 from three replicates of 1 cm diam. $\times$ 2.5 cm deep cores of creeping bentgrass maintained as fairway turf. *Clarireedia* was most abundant in leaf tissue, then crown tissue with lower abundances, and then thatch with the lowest abundances.

Evolving moldy murderers: *Aspergillus* as a model for studying the repeated evolution of opportunistic pathogenicity

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Species in the genus *Aspergillus* are saprophytic filamentous fungi that are most commonly found in soil and litter environments of subtropical and warm temperate latitudes. Inhalation of asexual spores produced by *A. fumigatus* and a few other species in the genus cause a group of diseases, collectively referred to as aspergillosis, that affect millions of patients and cause hundreds of thousands of life-threatening infections every year. Since antifungal drugs are not always effective, individuals infected with invasive forms of aspergillosis suffer high morbidity and mortality. Even though a great deal is known about some aspects of *A. fumigatus* pathogenicity, we have only recently begun to examine why pathogenicity varies so dramatically across the entire genus and the traits and genetic elements that contributed to this variation. Addressing this question requires that we consider the fact that pathogenic *Aspergillus* species are not dependent on their hosts for survival and their pathogenic effects are entirely accidental or opportunistic. Thus, understanding the evolution of pathogenicity in the genus requires that we understand how variation in the traits that enable *Aspergillus* species to survive in their natural soil and litter environments has rendered a few of these species capable to establish infections inside human hosts. In my talk, I will present my lab's latest work on the evolution of pathogenicity-associated genes and traits in *Aspergillus*. Given that the ability to cause human disease has repeatedly evolved across the fungal tree of life, and that the vast majority of human fungal pathogens have non-pathogenic close relatives, understanding the evolution of *Aspergillus* pathogenicity can serve as a model for studying fungal pathogenicity in general.

Decision-making under uncertainty

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Does the pathogen always win? Do we have the necessary tools, expertise, and support to identify and manage emerging and re-emerging plant diseases quickly? Are we able to predict when and where an outbreak will occur? Combinations of climate change,

human activities, changing production practices, and movement of vectors and plant material are just a few of the components driving this challenge with emerging and re-emerging plant diseases. The lack of information, or expertise, also makes it rather difficult to inform policy and rapidly respond to epidemics. All of these factors highlight the uncertainty in predicting or developing a sustainable risk monitoring and mitigation program. In this talk, I will describe some of the general aspects of uncertainty, focusing on how this concept influences monitoring from local to global scales. Several examples will highlight potential avenues for improving real-time monitoring, data-sharing, and modeling across these different scales. Lastly, there is recognition that a sustained effort such as this requires a new long-term vision for training, funding, and credit/recognition of such activities.

The dilemma of coordination for huanglongbing management in California

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Plant disease management often requires the coordination of control measures across a region because properties that don't participate can be sources of inoculum for the rest. As individuals cannot be excluded from the benefits of the control efforts of others, they might be tempted to free-ride on their neighbors' efforts while avoiding the costs of control. This dilemma is an obstacle for huanglongbing (HLB) management in California. HLB is an invasive disease of citrus associated with the bacterium "*Candidatus Liberibacter asiaticus*," which is transmitted by the Asian citrus psyllid (ACP). It was first detected in 2012 and to date more than 2,200 HLB-positive trees have been removed from residences in Southern California. To suppress ACP populations and limit HLB spread, citrus growers are being asked to coordinate insecticide treatments, but participation in this area-wide management (AWM) program has been uneven. We studied individual factors influencing decisions to participate, institutional arrangements to promote coordination and the benefits of coordination. Surveys of citrus growers showed that while they believe in the efficacy of AWM, trust that their neighbors will participate is highly variable. Areas with more mandatory institutional arrangements are achieving higher coordination, but this depends on the size of the group, and simulations with an agent-based model have shown that high treatment efficacy and tight coordination windows might be required to effectively prevent HLB spread.

The role of predatory bacteria on the control of pathogenic bacteria

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Soils are estimated to encompass ~25% of global biodiversity by their number of species, and bacteria are the most abundant organisms in soil. Because crops are produced in open fields, bacteria that are pathogenic to plants, animals, and humans may be introduced into crop production systems through a variety of natural and anthropogenic mechanisms. The fate of these organisms is dependent on their ability to survive in the soil or on/in debris. In this perspective, examine the strategies that predatory bacteria use to kill their prey as well as predator survival in the environment could influence predator effectiveness in a predictable way. Soil predators deploy diverse predatory strategies, which can make it difficult for pathogens to develop resistance by simple mutation. A broad variety of bacterial micropredators from different taxonomic levels are involved in predation on bacteria in soil, such as *Bdellovibrio*, *Lysobacter*, and *Myxobacteria*. A better understanding of the abiotic and biotic factors influencing predator performance will allow better prediction of predator impact and lead to strategies for improved plant and human health.

The future of pathogen diagnostics and disease surveillance (part 1)

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Disease surveillance is the ongoing systematic acquisition and interpretation of plant health data essential to planning and implementing immediate and long-term crop protection options. Disease surveillance involves recording the distribution of pathogen signs and symptoms at the plant population level. Diagnostic tests to detect plant pathogens and sensors to phenotype the host and characterize the environment are currently based on many diverse technologies. With the invention of new technologies, diagnostic tools are evolving rapidly. Next-generation sequencing can be performed in-field by an instrument that weighs about 100 g. The data can be analyzed using an inbuilt database in a laptop system or through online pipelines. Moreover, isothermal technologies allow for results that can be obtained in just 10 min. Automated, sensor-based methods with higher specificity and reliability can improve disease detection and provide a tool to maximize surveillance efficiency. For instance, commercially available sensors have shown the potential to provide helpful information on plant disease epidemics, including identifying early disease symptoms. The data collected through disease surveillance can help develop disease forecasting models with reasonable precision, identify areas at risk, and predict potential losses. Integrating pathogen, host, and meteorological factors will be critical for developing next-generation decision support systems. The integration of technology and analytical techniques and a solid epidemiological understanding of diseases is needed.

Exploring alternative management of plant pathogens of stevia in North Carolina, Mexico, and Paraguay

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Stevia is grown for the production of all-natural non-caloric sweeteners. As demand for stevia sweeteners is increasing worldwide, there is a desire for producing organic stevia in different countries; however, alternative disease management tools are lacking. We evaluated anaerobic soil disinfestation (ASD) for the management of stem rot caused by *Sclerotium rolfsii*. In 2019, experiments using carbon sources for ASD were carried out in microplots at Central Crops Research Station, Clayton, NC, USA. In 2020, field experiments were carried out at Colegio Superior Agropecuario del Estado de Guerrero, Mexico, and Centro de Desarrollo e Innovación Tecnológica,

Paraguay using a factorial arrangement 2×3 where factor A: soil disinfestation (soil amended with wheat bran and molasses vs non-amended) and factor B: foliar fungicide application (control vs azoxystrobin vs pyroligneous acid). In soils amended with wheat bran and molasses as carbon sources for ASD, the viability of sclerotia of *S. rolfsii* was significantly reduced. Soil disinfestation had a significant effect on fresh yield (P value = 0.0443). The application of azoxystrobin (2,658.7 kg/ha) and pyroligneous acid (2,177.7 kg/ha) significantly increased the yield (P value = 0.0007) compared to the nontreated control (1,411.7 kg/ha). These inexpensive organic amendments and botanical fungicides can be alternatives for the management of pathogens in stevia production systems.

Soybean rust: Past, present, and future

C. V. GODOY, EMBRAPA, Londrina, Parana, BRAZIL

Asian soybean rust, caused by *Phakopsora pachyrhizi*, is one of the most severe soybean diseases in Brazil. Epidemics of the disease were common in the country, where the fungus can survive year-round. Management regulatory measures were adopted to reduce the inoculum between crop seasons (soybean-free period) and to avoid late-season sowing. Major resistance genes have been mapped and incorporated into the cultivars. Besides resistance genes, since 2005, the soybean cultivars cycle has been reduced in Brazil, and early sowing allowed a significant increase in the sowing of double summer crops (e.g., soybean-corn, soybean-cotton, and soybean-cover crops). This strategy has contributed to the control of soybean rust by helping the plants to escape the disease. In Brazil, soybean cultivars have been replaced gradually from determined to indeterminate, and the maturity groups, from south to north Brazil, changed from 6.8–9.4 to 4.5–8.2. These characteristics were the main contributions of Brazilian soybean breeding programs to the control of soybean rust, although not targeting the disease as a goal. The use of fungicides is one of the strategies adopted in the management of the disease. Since 2003/04, uniform field trials have been carried out in different producing regions to compare the efficiency of fungicides registered and in the registration phase. Besides the fungicides efficiency, the results followed the fungus sensitivity change to the distinct modes of action over the years, along with bioassays and molecular analyzes. The uniform field trials showed fungicides efficiency reduction for the demethylation inhibitors (DMIs) in 2007, the quinone outside inhibitors (QoIs) in 2013, and the succinate dehydrogenase inhibitors (SDHIs) in 2016. At least six CYP51 mutations (Y131F/H; F120L; K142R; I145F; I475T) and overexpression are involved in the sensitivity reduction towards DMIs. For QoI, the F129L mutation was reported at high frequency (~90%) in 2013/14 isolates and remained stable in the subsequent crop seasons. SDHI fungicides were used on soybean in Brazil for the first time in 2013/14, and *P. pachyrhizi* strains with a lower sensitivity were found in monitoring studies in 2015/16, with a mutation in the subunit *sdhC*-I86F gene. In the subsequent crop season, the mutation C- I86F was found in other regions and is currently the prevalent resistance mechanism for observed sensitivity changes in the field. However, other mutations (C-N88S/D, C-H154R, C-G92R) have been identified at very low frequency. With the *P. pachyrhizi* lower sensitivity to single-site fungicides, the use of multi-site fungicides (mancozeb, chlorothalonil, and copper) has increased in Brazil. The management strategies jointly maintained the disease under control, with an emphasis on the soybean-free period associated with short cycle cultivars, to allow a second summer crop and the use of fungicides for rust control and other diseases.

Emergency response for *Phytophthora ramorum*: What have we learned over the past two decades?

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Over the past several decades *Phytophthora ramorum*, cause of sudden oak death and other plant diseases, has killed over 50 million tanoaks (*Notholithocarpus densiflorus*) and coast live oaks (*Quercus agrifolia*) in California and Oregon urban and rural forests. The pathogen is now known to survive on over 125 plant host species ranging from ferns to Christmas trees. When first discovered in 2000, *P. ramorum* was new to science, so all aspects of a regulatory program had to be developed from scratch including emergency response approaches, diagnostics, survey and inspection protocols, treatments and more. The lack of scientific information made risk assessment and disease forecasting difficult. In 2014 the regulatory program was redesigned to better target risk. By reducing the number of nurseries required to have inspections, the program cost and regulatory burden on the nursery industry were decreased. But quarantine effectiveness and practicality are complicated to assess. In 2004, despite federal and state quarantines, more than two million host nursery plants were inadvertently shipped from a few Southern California and Oregon nurseries that contained *P. ramorum*-infected plants. Potentially infected plants were shipped to over 5,000 establishments nationwide, and infected plants were detected in 176 locations in 21 states. In 2019, “*P. ramorum* in Commerce” shipments from two nurseries resulted in potentially infected plant shipments to 28 states, requiring inspectors to sample over 11,000 plants. Two-hundred fifty positive samples were detected in 14 states. However, *P. ramorum* still has not been detected on forest vegetation in Midwestern, Eastern or Southern forests. An examination of *P. ramorum* risk perception and quarantine response illuminates the challenges involved in invasive species prevention and management in forests and nurseries.

Management of *Radopholus similis* on banana using banana papers in Sub-Saharan Africa

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Banana is a vegetatively propagated staple food crop in Sub-Saharan Africa (SSA) which production is under the threat of nematode pests, especially *Radopholus similis*. Small scale farming dominates the banana production in SSA, where awareness of nematode diseases is particularly poor among smallholders. Given that agricultural landscape in SSA, developing nematode management in an environmentally sustainable manner that can be adapted to the needs of banana smallholders is in high demand. Our objective was to test the potential of banana papers infiltrated with nematicides in the management of *R. similis* on banana. Experiments were conducted using banana corms of ‘Mbwarzirume’ in Uganda and banana plantlets of ‘Williams’ in Kenya. Banana plants were wrapped with either

fluopyrum infiltrated banana paper, abamectin infiltrated banana paper, or untreated banana paper or without a wrap before planting. These plants were challenged with 5,000 *R. similis* after 3 weeks for corms and with 1,000 *R. similis* after 4 weeks for plantlets. Eight weeks after the nematode challenge, both experiments were terminated, and the number of nematodes in roots was counted. Plants wrapped with nematicides showed approximately 90% reduction in the number of nematodes per plant relative to plants without wrap. This study is currently ongoing, but the results at two locations demonstrate the potential of banana paper as an efficient control option for *R. similis* on banana with nematicides at a micro dosage that is more affordable for smallholders.

Loss of aflatoxin production and impacts to pathogenicity in *Aspergillus flavus*

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Dr. Rebecca Sweany is currently a post-doctoral research plant pathologist working with Kanniah Rajasekaran for the U.S.-Department of Agriculture in the Food and Feed Safety Research Unit at the Southern Regional Research Center in New Orleans, LA. Her research focus involves incorporating novel synthetic antifungal peptides into maize to confer resistance against *Aspergillus flavus* and subsequent aflatoxin contamination. Prior to joining the USDA, Dr. Sweany studied and worked with Dr. Kenneth E. Damann Jr. and Dr. Christopher A. Clark in Louisiana State University's Department of Plant Pathology and Crop Physiology. Dr. Sweany's doctoral and master's research explored the biocontrol mechanisms of non-aflatoxigenic *A. flavus* that lower aflatoxin contamination, as well as *A. flavus*-corn infection specificity. Dr. Sweany will discuss the possible roles for, and advantages/disadvantages of, aflatoxin production by *A. flavus* in plant-pathogen interactions. For example, aflatoxin, a potent carcinogen and mycotoxin to humans and animals, is speculated to confer a competitive advantage to this opportunistic plant pathogen by alleviating oxidative stress from host resistance responses. However, *A. flavus* strains that naturally do not produce aflatoxin readily infect crops, and impact toxigenic isolates when used as a biocontrol to lower aflatoxin contamination. Dr. Sweany will discuss recent research that suggests loss of aflatoxin production may enhance the ability of *A. flavus* to colonize and infect crops.

Free-living protists: Microbiome engineers for sustainable plant growth

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The plant microbiome is increasingly recognized to determine plant growth and health, but remains notoriously hard to manage. This may stem from an approach of the microbiome focusing on bacteria and fungi, but overlooking the bulk of eukaryotic microorganisms—protists. Free-living protists such as amoebae are a core component of the microbiome, yet despite of their high abundance, diversity, and ecological functions, have long been overlooked. Only recent advances in sequencing technologies coupled with enhanced ecological networks models have put the spotlight on these highly diverse organisms encompassing most of the eukaryotic lineages. I will show in this presentation that including free-living protists as part of the microbiome functionality allows for a better prediction of microbiome function. I will then showcase the many plant-relevant functions these microorganisms can carry on and will conclude by demonstrating that protists have the potential to leverage interventions for a stronger effect on the rest of the microbiome.

Type VI secretion system of *Xanthomonas perforans* contributes to the incubation period and is involved in niche adaptation

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Asymptomatic epiphytic survival of the pathogen has been a major concern due to its direct influence on the effectiveness of management strategies. Such epiphytic populations have been a culprit in reducing the efficacy of bactericides as well as impacting the durability of disease resistance. While the majority of studies investigating the mechanistic basis of pathogenesis of *Xanthomonas* have broadly looked at the contribution of virulence factors towards successful apoplastic growth of the pathogen, mechanisms by which this hemibiotrophic pathogen successfully adapts to the phyllosphere and maintains epiphytic asymptomatic colonization are understudied. In this study, we present evidence for the contribution of *i3** during some of the crucial stages of the life cycle of the foliar hemibiotrophic pathogen, seed-to-seedling transmission, transplants, and during epiphytic colonization on mature 4-to 5-week-old plants. Interestingly, in all these stages, *i3** influences a crucial life-history trait of latent infection period. *i3** dictates threshold in planta population, and thus, is determinant of aggressiveness of the hemibiotrophic foliar pathogen, *Xanthomonas*. This study also highlights the importance of *i3** in overcoming osmotic stress during the initial adaptation period to the phyllosphere. In this study, we were also able to identify the evolution and distribution of the T6 clusters in the pathogenic/nonpathogenic *Xanthomonas* species. This study highlights the importance of T6SS-*i3** in overall fitness and niche adaptation of the pathogen by dictating latent infection period as well as offering adaptive advantage at crucial stages of its life cycle, which are considered to be important for mitigation of the pathogen.