Plant Health 2019 Abstracts of Special Session Presentations

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Radiative transfer retrieval of physiological plant traits for pre-visual detection of biotic stress via airborne hyperspectral imaging

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Advances carried out in the last 20 years in imaging spectroscopy and thermal imaging for the retrieval of plant traits in the context of biotic and abiotic stress detection will be discussed. In particular, new developments in sensor miniaturization and physical modelling techniques have enabled a fast progress in the early detection of biotic and abiotic stress as part of precision agriculture efforts. The radiative-transfer based methodologies required for the pre-visual detection of stress using manned and unmanned vehicles carrying hyperspectral and thermal cameras will be described for the particular case of *Xylella fastidiosa* (*Xf*) outbreak in Europe. Results obtained using high-resolution hyperspectral and thermal airborne cameras revealed subtle spectral changes related to the *Xf* infection before the symptoms were visible in the field. We found that spectral plant traits calculated from the blue region, solar-induced chlorophyll fluorescence and remotely sensed tree crown temperature were the most statistically-significant indicators for the pre-visual detection of *Xf* symptoms. The use of these physiology-based spectral plant traits along with machine- and deep-learning algorithms will be described, discussing the existing gaps for the operational use of these surveillance methods at larger scales.

High throughput sequencing for clean plant certification in practice

T. HO, Driscoll's Inc, Watsonville, CA, USA

Driscoll's uses a comprehensive testing regime that includes graft indexing, ELISA and PCR to make sure our berries are virus-free. We are studying high throughput sequencing as a new diagnostic technology as well as to meet future international export requirements. As an industry member, we will discuss the advantages, the challenges, and the future needs when using HTS for virus discovery.

Ionomes of plants infected with vascular pathogens: *Xylella fastidiosa* as a case study

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Recruitment and distribution of metals is fundamental for cell survival, and the mechanisms to maintain the appropriate metal concentrations have been highly conserved. Therefore, an evolutionary arms race exists between organisms to acquire the correct metal. In pathogenic microbes, a tug-of-war for available metals can define pathogenicity and host disease physiology. Plant vascular system forms a highway for the transport of critical nutrients including metals. *Xylella fastidiosa*, which is found in the xylem of numerous plants, concentrate metals from the xylem sap to facilitate growth, biofilm formation, and movement. *X. fastidiosa* infections results in changes in the total content of mineral elements in the host plant, the snapshot of mineral content is termed the ionome. Changes in the ionome of infected plants directly correlate with the severity of disease. Our most recent investigations have focused on *X. fastidiosa*

outbreak in olive trees in the Salento region of Italy. In the outbreak area the 'Leccino' variety of olives has proven to be more resistant than the 'Ogliarola salentina' variety. We sampled both cultivars from infected field, classified the samples as symptomatic and asymptomatic and measured the ionome of leaf samples. These analyses reveal that 'Leccino' variety has increased manganese levels compared to 'Ogliarola salentina'. The transition from asymptomatic to symptomatic also showed further remodeling of the ionome. The differences in the two cultivars and the implications for disease will be discussed.

Replicability in science: Concept and status

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Obtaining consistent results in independent studies is one of the goals of science. A study is called replicable if the results are consistent in independent repetitions of an experiment. Although studies that failed to be replicated are not necessarily wrong, this is far from desirable. There is ongoing concern in multiple fields about the "replicability crisis in science", which is the common problem of the increased number of studies that have failed to be replicated (when attempted). Fields such as psychology, statistics, medicine, and social sciences have an engaged community of scholars who are dedicated to the investigation of the replicability crisis, with proposals for solutions. Such proposals include better-informed interpretation and use of P-values and other statistics, improved experimental approaches, research-method transparency, and proactive attempted repetition of published studies (with reports on the negative and positive findings). As with any multidisciplinary debate, communication can be challenging because terms frequently have different meanings in different disciplines or with different authors, such as replicability and reproducibility, sometimes confusingly used as synonymous. In this presentation, I will address the concepts and terms related to replicability, and give an overview of the status of the debate in other fields of science.

Stomata-mediated plant immune response against microbe invasion

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The leaf epidermis represents a barrier that protects the plant against microbial invasion. Of particular importance in this tissue, are the natural pores, such as stomata, that provide ports of entry for pathogens allowing the establishment of endophytic colonization. However, stomata-mediated immune response reduces microbial internalization of leaves. Our recent research has provided evidence that this mechanism of plant resistance is employed towards a broad range of pathogens, including the phytopathogen *Pseudomonas syringae* and the human pathogens *Salmonella enterica* and the enterohemorrhagic *Escherichia coli* O157:H7. Interestingly, *P. syringae* and *S. enterica*, but not *E. coli*, can overcome stomatal defenses in Arabidopsis and lettuce. The mechanism by which they do so will be further discussed. Understanding stomatal defense mechanisms against phytopathogens and enterics may facilitate the implementation of control measures to decrease plant diseases as well as the contamination of edible leaves.

Rise of SDHIs and current status of resistance development

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SDHIs form a highly attractive group of fungicides regarding their history of use, chemical innovations and spectrum of targeted diseases. The mode of action of SDHIs is the inhibition of succinate dehydrogenase in the respiration chain. Along with the broadening of their disease control palette, the structural complexity of the SDHIs has been increased. Current SDHIs share common chemical features, suggesting a similar binding to the target as demonstrated by three-dimensional alignment and computational docking experiments. Different target site mutations (TSM) have been found in field isolates of various fungal species. The acquired SDHI resistance by TSM is highly complex. Some species like *Pyrenophora teres* came up with more than 10 different TSM in the SDHI subunits conferring SDHI resistance, while others have so far shown up only with a small number of TSM, e.g. *Phakopsora pachyrhizi* or *Sclerotinia sclerotiorum*. TSM have been found in gene regions highly conserved over various species, but also in variable regions even on an intraspecific level. Modelling studies indicated that some of the target site mutations conferring resistance to SDHIs have a direct impact on the binding behavior of SDHIs, whereas others influence SDHI binding by long-range structural rearrangement in the transmembrane region of complex II. A diverse and complex picture has also been seen regarding the effects of TSM on the sensitivity towards various SDHIs in various pathogens. Some TSM cause a sensitivity reduction to all currently commercialized SDHIs to a more or less extent, but there are also TSM where no complete cross resistance has been found. This led to intensive discussions on SDHI cross resistance and on appropriate resistance management strategies.

Applications of HTS for enhanced pathogen detection: A vegetable industry perspective

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In healthcare, a revolution is occurring as the lab moves to the bedside. Today, a doctor can make informed decisions about their patient supported by sequence-based diagnostics. Can we do the same thing in agriculture? At Bayer Crop Science, our motto is "Science For A Better Life". Nowhere is this more relevant than in agriculture: the future our farmers face is full of uncertainty. With an increasing global population, rapidly changing climate, and increasing crop movement over broad geographical areas, our crops constantly face novel and unprecedented disease threats. Our vision of plant health for the future is actionable diagnostics that can help our customers make decisions that are better for both their crops and the environment. High-throughput sequencing can help bring this vision to life. Instead of looking for diseases one at a time, new sequencing technologies allow us to broadly scan for thousands of pathogens simultaneously. With rapid, on-the-spot sequencing we believe we can proactively protect fields and greenhouses before they face infection. With new technologies like rapid on-the-spot sequencing, we imagine a future where we can bring accurate, timely and

informative diagnostic information to our grower customers and help them keep their crops as healthy as possible, no matter what challenges they face. Our journey into the future of plant pathology has only just begun- but this talk will focus on our quest to bring “precision medicine” to the farmer’s field.

Versatile applications of CRISPR/Cas9 mediated genome editing in fungal and oomycete pathogens

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CRISPR/Cas9 systems have been shown to be a useful tool for modifying the genomes of various fungal and oomycete pathogens. Previously, we developed an oomycete-optimized CRISPR/Cas9 system which has been used for efficient targeted mutagenesis and gene replacement in the model oomycete pathogen *Phytophthora sojae*. Here, we show several additional cases in which a simplified and generalized “all-in-one” CRISPR/Cas9 expression plasmid has been efficiently used for generating mutants of different targets and purposes in *P. sojae*. We constructed a GFP-tagged endogenous CENP-A (a kinetochore protein involved in cell division) allele by gene replacement, and conducted ChIP-seq to define the nature of centromeres in oomycetes. All the identified *P. sojae* centromeric regions are rich in transposons and show low transcription. In addition, we developed a dual CRISPR/Cas9 method in the basidiomycetous fungus *Cryptococcus neoformans*. This approach was successfully used for simultaneous genome editing of two difficult targets and for allelic exchanges. We generated a double mutant lacking the Rad51 and Rad52 proteins that are required for homologous recombination, which would not be possible to isolate by conventional sequential single gene deletion. Rad51 and Rad52 are required for quelling in *Neurospora crassa*, in contrast, we found they are not required for RNAi-mediated mitotic-induced silencing (MIS) in *C. neoformans*. Using the dual CRISPR system, we also tested by allelic exchange the functions of the essential ribosomal genes *RPL22a* and *RPL22a* encoded by the mating-type locus.

Assessing the risk of resistance towards SDHI fungicides in France

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Succinate dehydrogenase inhibitor (SDHI) fungicides have been registered since 2006 on a large number of crops in France and resistance has been detected in nine fungal species, including *Botrytis cinerea*, *Ustilago nuda*, *Helminthosporium teres*, *Zymoseptoria tritici* and *Sclerotinia sclerotiorum*. In most cases, resistance is associated with a wide variety of mutations in SDHI subunits B, C, or D. Depending on the mutation and the mutation location, contrasting levels of resistance and cross-resistance spectra are observed for SDHIs. Moreover, contrasted resistance costs (e. g. in *B. cinerea*) were also measured, depending on the mutation. In *Z. tritici*, the pathogen responsible for septoria leaf blotch on wheat, resistance is associated with two distinct mechanisms, not yet combined in populations. Increased efflux (leading to multidrug resistance or MDR) generates low levels of resistance to several modes of action, while target site-based resistance is associated with low to high resistance factors. Crosses between isolates carrying the two distinct mechanisms revealed semi-isogenic progeny that we used to characterize resistance. Progeny with both mechanisms of resistance had increased resistance factors and limited fitness penalty with regard to sexual and asexual traits. Population studies showed that resistance based on mutations in SDHI subunits is emerging in French populations and that MDR also evolves rapidly. This ultimately increases the risk of natural recombination by sexual reproduction between isolates carrying the two distinct mechanisms. Field trials identified treatment strategies most favorable for the selection of each mechanism and thus allowed for the design of specific resistance management strategies with regard to SDHI use.

Tracing the production of young plants from stock plants to rooting of cuttings

R. G. LOPEZ, Michigan State University, East Lansing, MI, USA

Over 4 billion stem tip or terminal vegetative cuttings are harvested annually from stock plants for the international floriculture industry. These stock plants are grown in 400 ha. of greenhouses primarily located in Central and South America, Israel, and Africa. In order to prevent the risk of pathogen transmission, the industry begins with true-to-type plants that become the clean and elite stock. This requires extensive efforts to test, identify, and prevent pathogen infection and insect exposure during the build-up and production phases of stock plant management and cutting production and harvest. Time of harvest and post-harvest protocols, proper packaging, cold-chain management, and timely USDA-APHIS port-of-entry inspections are essential for effective international transportation of cuttings, which typically takes 48 to 72 hours. Upon receipt, the unrooted cuttings are rooted in propagation greenhouses to produce rooted liners. Root initiation typically begins in 1 to 3 weeks, and fully rooted cuttings are ready to be shipped to other greenhouse growers or transplanted after 2 to 8 weeks depending on the species.

Exploring microorganisms from plant, soil, and other environments for biocontrol potential against crop pathogens and pests

M. TWIZEYIMANA, AgBiome, Inc, RTP, NC, USA

Every year an estimated 10–16% of global food production is lost to plant diseases and pests despite tremendous improvement in management practices over the years. The control of most important crop diseases and pests has heavily relied on the use of synthetic chemicals and resistant cultivars. Despite good efficacy of chemicals against most pathogens and pests, intensive use may result in resistant populations. Similarly, high variability in pathogen or pest may speed up the process of pathogen or pest populations overcoming the genetic resistance of selected plant cultivars. The discovery of biological products that can effectively control pathogens and pests may result in improved integrated pest management (IPM) programs. At AgBiome, we are building a core collection of

sequenced microbes from plant-related environmental sources. We employ both the microbes and their sequences in the discovery of new biological products and traits for disease and pest control in crops. For biological products, each microbial isolate is screened in bioassays against a broad range of fungal pathogens, insect pests, and plant-parasitic nematodes. Microbial candidates with confirmed activity proceed to the fermentation and formulation processes before they are evaluated in the greenhouse and field.

Deep learning for image-based detection of northern leaf blight in maize

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In the United States and parts of Canada, northern leaf blight (NLB), a fungal foliar disease of maize caused by *Setosphaeria turcica*, has progressively become more severe in the past 5 years. In maize, breeding for genetic resistance is the most effective and economical approach for control of NLB. When breeding for NLB resistance, the visual scoring of gray-brown necrotic NLB lesions at multiple time points throughout the growing season is essential, but this effort is very time-consuming and prone to discrepancies between different raters. In that light, efforts are ongoing to develop a non-destructive, image-based phenotyping system that allows for rapid and accurate detection of NLB lesions (presence/absence) under field conditions. In the first iteration of this system, an image analysis pipeline that integrated convolutional neural networks (CNNs)—deep learning models used for image recognition and classification—was developed through training on a large manually annotated image data set, then deployed to detect the presence/absence of NLB lesions in diverse field images. Overall, the system attained an accuracy of 95% and greater on test set images held out from training.

Fusarium wilt of cotton in Alabama

J. J. COLEMAN, Auburn University, Auburn, AL, USA

Over 125 years ago, Fusarium wilt of cotton was first described on infected plants from fields in Alabama. Since then the causative agent, *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), has been isolated from all major cotton production areas worldwide. Despite the prevalence of this pathogen, little is known on a molecular level how the fungus is able to cause disease. In an effort to understand the virulence factors required to colonize cotton, the genomes of five *Fov* isolates were sequenced including a representative of the highly virulent race 4 genotype. Comparative genomic analysis between these isolates and other members of the *F. oxysporum* species complex revealed that large portions of the *Fov* lineage-specific chromosomes share syntenic regions. Interestingly, the genome of the race 4 isolate was significantly larger than the other *Fov* genomes and ~25% of the genome was identified as lineage-specific. Several genes of interest that could be involved in pathogenicity on cotton are currently being investigated, including putative small secreted proteins that may serve as effectors and secondary metabolites. One example is a copper specific extracellular superoxide dismutase encoded by *FoSOD5* which is induced by plant carbon sources, confers tolerance to reactive oxygen species, and is required for full virulence on cotton. To aid in evaluation of potential *Fov* virulence factors for cotton, we have developed a CRISPR/Cas9 ribonucleoprotein mediated transformation system that can be used to generate mutants and facilitate gene editing.

A chrysanthemum industry built on clean stock principles

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Healthy plants are saleable plants and are key in a lucrative flower business. Clean stock programs are foundational to successful flower enterprises. Since flowers bring aesthetic pleasures to humanity and the beauty is enhanced by health, then disease-free production is even more important. Chrysanthemums are sold as cut flowers, as potted flowering plants, or as garden plants. During the first half of the twentieth century, as the chrysanthemum industry was being developed, devastating diseases had to be overcome in order for the business to survive. Methods had to be developed to detect systemic fungal and bacterial pathogens and later expanded to include virus and viroid pathogens in order to deliver clean plant products to customers. Visual inspection could not adequately detect vascular pathogens or virus/viroid pathogens that did not express symptoms until flower. The procedures developed by specialist propagators combined with fungicides have been so effective that fungal diseases such as *Ascochyta*, *Septoria* and *Verticillium* no longer cause major losses and many growers today do not recognize diseases that were a constant menace in the past.

Microbes as biocontrol, biofertilizer and biostimulant products: The pathway to regulatory approval

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Historically, regulations have focused on microbial biological control products and regulations for newer products such as biofertilizers and biostimulants are not as well developed. The international community, (IPPC) has published a regulatory standard (ISPM 3) that establishes guidelines for export, shipment, import and release of biological control agents and other beneficial organisms (May 29, 2017). The USA handles biological control organisms at the federal level through USDA APHIS under the Plant Protection Act 2000. A 526 permit is required for the importation, interstate movement and environmental release of biological control organisms of plant pests and weeds. Each state also has the authority to establish state regulatory statutes regulating biological control organisms. Biofertilizer microbes are governed under fertilizer regulations for use in the USDA National Organic Program. Biostimulant products are the latest products to come to market. The USA Farm Bill (December 2018) published the first legal definition as “a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield”. This is a first step toward developing a regulatory framework for this product category.

Transcriptome responses of sweet potato to the infection of SPCSV and/or SPFMV

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Sweet potato (*Ipomoea batatas*) ranks among the top 10 most important crops in the world and has played an important role in providing nutritional and economic sustainability for subsistence farmers in Sub-Saharan Africa. Viral disease has been a constant and ever-growing concern for sweet potato production. The most important viral pathogens of sweet potato are Sweet potato chlorotic stunt virus (SPCSV) and Sweet potato feathery mottle virus (SPFMV). Co-infection of sweet potato by SPCSV and SPFMV has been dubbed sweet potato virus disease (SPVD) and can cause upwards of ~90% yield loss in infected plants. Current understanding of sweet potato responses to SPCSV and SPFMV at the molecular level remains very limited. Using deep transcriptome sequencing, we have generated genome-wide mRNA and sRNA profiles of three sweet potato cultivars (with varying degrees of SPVD resistance) inoculated with either SPCSV or SPFMV alone, or both, at three different time points post-inoculation. Comprehensive analysis of these expression profile data allowed us to identify viral responsive mRNAs, which were enriched with those involved in response to biotic stimulus, photosynthesis and primary and secondary metabolisms. Furthermore, viral responsive microRNAs (miRNAs) and phased small-interfering RNAs (*phasiRNAs*) were also discovered and their corresponding targets were analyzed. Our results provide a better understanding of regulatory networks underlying the sweet potato responses to the infection of SPCSV and/or SPFMV.

The use of SDHI fungicides in premixtures; successes and concerns

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SDHI fungicides have largely been used in form of FRAC 7/11 premixtures for disease control in specialty crops. The argument being that the two active ingredients help each other delay resistance development and that the efficacy spectrum is broadened. Using two case studies we show that for some crops the combination has proven to be an effective, long-term strategy for control of the target disease giving only occasional rise to outbreaks of secondary pathogens due to resistance development. The other case study discusses how the combination led to dual resistance in the target pathogen and perhaps accelerated selection of resistance in non-target organisms. Specifically, for peach growers in the southeastern United States the rotation of FRAC 3 and FRAC 7/11 combinations applied preharvest has resulted in excellent pre- and postharvest brown rot control over the last decade despite traditionally high disease pressure. A few peach growers, however, did experience outbreaks of secondary diseases, including *Alternaria* and *Anthraco* fruit rots, associated with yield loss and fungicide resistance. For strawberry growers the application of FRAC 7/11 combinations for gray mold control has become a risky endeavor due to widespread resistance to FRAC 11 fungicides and location-specific resistance to FRAC 7 fungicides. Thus, the ‘internal’ resistance management strategy argument for the use of the mixture for gray mold control is no longer applicable. The continued use of these mixtures for primarily gray control may have likely contributed to the selection of FRAC 11-resistant *Colletotrichum acutatum* associated with recent outbreaks in strawberry anthracnose disease.

Biological product formulation and production processes and challenges

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Marrone Bio Innovations (MBI), based in Davis California, is an industry leader in developing natural biostimulants and biopesticide products. For the past 11 years, the company has developed and commercialized a broad portfolio of products that includes Fungicides, Insecticides, Nematicides and Plant health products. These bio- and plant- based products are approved for virtually every cultivated crop, from fruits and vegetables to cereal grains and nuts, from turfgrass and flowers to alfalfa and cotton. These products are organic-compliant and when incorporated into an IPM (Integrated Pest Management) program, they can be an effective solution to pest control problems in conventional, transitional or certified organic production systems. The formulation effort not only involved foliar and soil application but also seed treatment for protection against pests as well providing plant growth and yield enhancement. This presentation will discuss the complex multidisciplinary approach involving microbiology, chemistry, fermentation, formulation and plant sciences to screen bioactive ingredients and develop them into products through scale up and manufacturing. Various case studies involving liquid and solid formulations will be presented along with the challenges associated with stability, processing, efficacy and delivery of the product. Application studies involving the effects from field such as pH, water hardness, temperature, adjuvant and chemical tank mixes and their impact on the performance of the product will be discussed.

Using systems genetics to uncover molecular mechanisms that integrate nutrient and defense responses

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Plants are exposed to a fluctuating environment in which they need to respond to diverse changes of biotic and abiotic factors. For instance, roots that forage the soil for nutrients and water, are faced with water, mineral, and microorganismal distributions that fluctuate spatially and temporally in the soil. To function efficiently, roots therefore need to determine the biotic and abiotic properties of local soil environments and whether the optimal response in given environments is to sustain growth or instead to initialize defense programs. We are interested in how responses to abiotic and biotic factors are encoded in the genome, and how the thresholds at which responses are elicited are changed via natural genetic variation to allow for local adaptation. For this, we use the root of *Arabidopsis thaliana*, a plant species that has successfully colonized large parts of Eurasia; a range displaying stark variations of climates and soils. Using a systems genetics approach that integrates high throughput phenotyping, genome wide association mapping and functional genomic approaches, we identify genes and their variants that make roots respond differently to the same cues and study their relations to environmental variables. Among the most notable mechanisms that we found is a leucine-rich-repeat-receptor-kinase, which is involved in determining root growth responses to low iron levels, as well as to flagellin. Our data suggests a model in which iron availability and the presence of microbes are integrated by this and other receptor kinases.

Characterization of early *Phytophthora infestans* infection in potato with aerial, field-based hyperspectral imaging

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In vivo foliar spectroscopy offers the capacity to rapidly and non-destructively characterize plant physiological status which can be used to detect the effects of necrotizing pathogens on plant condition prior to visual symptoms. Dead tissue yields relatively consistent changes in leaf optical properties that challenges the ability to identify causal pathogen identity. Contact hyperspectral reflectance systems have been established as effective tools for rapid, early, real-time disease detection in crops, and for detecting physiological differences that enable pathogen discrimination. Here, we test the capacity of aerial, hyperspectral imaging spectroscopy to detect physiological effects of *Phytophthora infestans* (causal agent of late blight) over the course of disease development in the field. We previously have shown that hyperspectral reflectance can be used to identify both *P. infestans* and *Alternaria solani* (early blight) infected plants with 90% accuracy 2–4 days before symptoms appear. We measured continuous VSWIR reflectance (400–2500 nm) on four potato genotypes with two fungicide treatments in East Lansing, MI over the period of one month with a HYSPEX imaging spectrometer. We found this method could detect pathogen incidence and severity as low as 5–10%, and that deteriorating plant health could be detected within a week of inoculation. SWIR wavelengths (>1300 nm) were important for this detection. This offers great potential for use in early disease detection in field agricultural systems with the suite of hyperspectral satellites planned for the next decade.

Evolution of azole-resistant *Aspergillus fumigatus* in agricultural and clinical environments

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Azoles are used to treat both plants and people with fungal diseases, but antifungal resistance is developing in both clinical and agricultural environments. The environmental use of azoles is thought to be the driving force for much of the clinical resistance in the human pathogen *Aspergillus fumigatus*. Our goal was to determine if azole-resistant *A. fumigatus* was present in sixty agricultural sites in Georgia and Florida where azoles were used, and if so, to identify the molecular mechanism and compare the genetic similarity of resistant agricultural strains with clinical samples. We isolated over 750 *A. fumigatus* from soil or plant debris from azole-treated fields, with approximately 27% of the strains showing resistance to the fungicide tebuconazole at 3 µg/mL. However, susceptibility profiling of 105 of the tebuconazole-resistant strains for minimum inhibitory concentrations (MIC) showed that 100 of the strains had MIC values ≥ 1 µg/mL of itraconazole, yet only 13 of the strains, all with the TR46/Y121F/T289A *Cyp51A* allele, were above the established clinical epidemiological cutoff for itraconazole (≥ 2 µg/mL). We genotyped 180 resistant and sensitive strains from agricultural sites and 60 clinical strains with 9 SSR markers and conducted whole genome sequencing on 100 environmental strains. Overall, there was no genetic clustering of agricultural strains by crop or geographic origin, although an azole-resistant lineage was detected. Preliminary results showing signatures of agricultural origins in azole-resistant strains in both the environment and clinic will be discussed.

Hyperspectral sensors for the detection, quantification and identification of plant diseases – demands, confounders and strengths for field detection

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For the purpose of integrated plant protection, it is of high relevance to include recent and future innovations into plant protection management. A site-specific management of plant diseases will lead to a reduced input of agrochemicals and thus to ecological and economic benefits. Previous research showed that hyperspectral sensors can be used to detect and quantify crop stress that is caused by plant diseases. The majority of these studies were conducted under controlled conditions because hyperspectral sensors demand high standards to the environmental conditions and the measuring system. These high demands make a field approach challenging as there are many parameters to consider to generate high quality, reproducible and comparable field data. This work presents the main factors that affect the quality of hyperspectral field data and shows basic approaches to develop protocols for hyperspectral field measurements. The requirement for a reliable disease detection in the field is to produce comparable field data on different measurement days with the same setup. Therefore, different approaches were tested and used. The gain of knowledge out of hyperspectral measurements on different scales and under differing environmental conditions has to be integrated through scale transfers and recent analysis methods, such as feature selections, supervised classifications and regression analysis. Main goal is the definition of the characteristics for one sensor that fulfills the practice demands for plant disease detection under uncontrolled field conditions.

Dip before you stick: Benefits and risks of cutting dip treatments against arthropod pests

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Starting clean is an essential part of IPM. Despite strict pest control programs at the propagators' facilities, it is relatively common to find low numbers of pests in shipments of floriculture propagative plant material (cuttings) to finishing greenhouses. These pests may be resistant to pesticides due to previous exposure at the propagator, limiting a grower's control options. Even when biological control agents are applied right at the start of the crop, they may have a difficult time "catching up" as pest populations have a "head start". Dipping is a technique where unrooted cuttings are immersed in a suspension before they are stuck in growing media. We tested biopesticides (entomopathogenic fungi) and/or reduced risk materials such as insecticidal soap or mineral oil as a dip against *Bemisia* whiteflies, western flower thrips and two spotted spider mites on poinsettia, chrysanthemum and various bedding plants in research trials and commercial greenhouses. We also evaluated the risk of disease (*Pectobacterium*) transfer through the dipping process in poinsettia. We demonstrated that cutting dips are an effective, economic and safe method to reduce pest infestation levels at the beginning of the production cycle and that biocontrol strategies are more effective thereafter. Furthermore, use of cutting dips in combination with biocontrol mitigates the risk of resistance, so that pesticides can be used as a clean-up spray at the end of production, if needed.

CRISPR/Cas9 genome editing tools for functional genomics and biocontrol of fungal plant pathogens

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The bacterial cluster regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 nuclease (Cas9) system is a powerful genome editing tool due to its simplicity, versatility and broad applicability. To aid in CRISPR/Cas9 genome editing in fungal plant pathogens, we have developed bioinformatics tools and performed genome-wide prediction of highly specific guide RNA (gRNA) spacers in the genomes of important pathogenic fungi. Using *Magnaporthe oryzae* and *Fusarium graminearum* as examples, the polycistronic tRNA-gRNA (PTG) strategy was demonstrated for efficient expression of gRNAs and multiplex genome editing based on the endogenous tRNA processing system. Following the induction of double stranded breaks (DSBs) by Cas9, the efficiency of targeted mutagenesis or gene replacement via non-homologous end joining (NHEJ) or homology-directed repair (HDR) was evaluated in the absence or presence of donor DNA templates. High efficiencies of targeted mutagenesis were achieved in *M. oryzae* and *F. graminearum*, leading to the generation of single, double and triple gene mutants. The CRISPR/Cas9 tools and genome editing strategies derived from this study are expected to facilitate the functional discovery of fungal genes and the potential application of gene drive for biocontrol of devastating fungal plant diseases.

Harnessing new technologies to improve management of cucurbit downy mildew

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The obligate oomycete *Pseudoperonospora cubensis* causes cucurbit downy mildew (CDM) on a broad range of Cucurbitaceae host plants. Until 2004, CDM was managed primarily by host resistance in cucumber and modest fungicide applications in other cucurbits in the United States (US). However, since the 2004 epidemic, management relies on frequent fungicide applications, especially in cucumber where fungicide resistance has already been reported for several chemistries. Genetic analysis of pre- and post-epidemic samples revealed that *P. cubensis* is subdivided into two distinct clades with host specialization and differential response to certain fungicides. While Clade I was predominant among pre-2004 US *P. cubensis* samples, post-2004 samples exhibited a mix of Clade I and II. However, *P. cubensis* from international sources exhibited either Clade I or Clade II often from the same host plant. Using this information and comparative genomics approaches we developed a biosurveillance system for CDM that relies on spore traps and provides information about inoculum level, crop risk, and fungicide resistance for precision disease management within a growing season. Our findings not only provide insight into the causes of the 2004 CDM epidemic, but will significantly improve *P. cubensis* management by shifting current practices to crop specific recommendations for growers that account for population dynamics.

Changing ecosystems - Plant succession effects on soil diversity and function

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Climate warming is a key factor driving range shifts of species. Most work has been focused on shifts of aboveground plant communities, but changes in vegetation can trigger parallel shifts in in soil communities with consequences on ecosystem-level nutrient cycle budgets and ecosystem functioning. High alpine ecosystems are particularly sensitive to climate warming because snow, one of the fastest changing climate features, is the main driver of ecosystem structure and function. Consistent with global patterns, long-term snow cover changes (a thinner and less isolating snow cover that develops later and melts earlier) at the Niwot Ridge in the Colorado Rocky Mountains have resulted in a slow but consistent migration of plants into higher elevations generating a mosaic of habitats ranging from originally free of plants to increasingly vegetated. We used this natural experiment to examine climate-driven shifts of plants and soil communities and their role in shifts of soil carbon and nitrogen dynamics. The main hypothesis was that snow cover would be the driving factor of plant communities (less snow – more plants) that in turn drive soil communities (i.e., including nematodes) (more plants – more soil biota) that together drive soil C and N up. In addition, we predicted that positive relationships would be particularly evident for plant pathogens and parasites.

Start clean, stay clean- National Clean Plant Network

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Propagation material is the most important factor productivity and farm sustainability, and this is especially the case in clonally-propagated crops. Plants from any particular cultivar originate from a seedling. The plant is normally evaluated in breeding plots for several years before release in the nursery system as a named cultivar. During this period there is ample opportunity for infection by one or more systemic pathogens which are carried over to its clones. The National Clean Plant Network aims to eliminate all systemic pathogens of importance from breeding accessions before they are released and massively propagated in the nursery system. The process involves traditional and state-of-the-art technologies that aim to identify systemic pathogens of concern but also discover new ones that may pose a threat to the crop. The merits of the process and the linkage to state certification programs will be discussed.

Alternative hosts of *Xanthomonas vasicola* pv. *vasculorum*, causal agent of bacterial leaf streak of corn

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The bacterial leaf streak pathogen *Xanthomonas vasicola* pv. *vasculorum* was reported on Nebraska corn in 2016, the first report in the United States. It is also the causal agent of gumming disease of sugarcane, and capable of infecting several other plant species, including *Sorghum bicolor* and some tropical plants. The diverse host range indicates the potential for this bacterium to infect plants associated with cornfields in the United States. To test this hypothesis, five replicates of various plants found in Nebraska, including

crops, turf and ornamental grasses, prairie grasses, and weeds were spray inoculated in a greenhouse with a 10^8 CFU/mL aqueous suspension of *X. vasicola* pv. *vasculorum*. Twelve symptomatic alternative hosts included oat (*Avena sativa*), rice (*Oryza sativa*), orchardgrass (*Dactylis glomerata*), indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), timothy (*Phleum pratense*), sand bluestem (*Andropogon hallii*), johnsongrass (*Sorghum halepense*), green foxtail (*Setaria viridis*), bristly foxtail (*Setaria verticillata*), and yellow nutsedge (*Cyperus esculentus*). Asymptomatic alternative hosts included downy brome (*Bromus tectorum*), tall fescue (*Festuca arundinacea*), and western wheatgrass (*Pascopyum smithii*). To test the likelihood of infection of susceptible species in the field, asymptomatic alternative hosts were transplanted to a field naturally infested with *X. vasicola* pv. *vasculorum*. From infection by natural inoculum, big bluestem and bristly foxtail developed symptoms.

Stink bug-*Fusarium* interactions and their associations with mycotoxin contamination of corn in the mid-Atlantic U.S.

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Stink bugs, including invasive brown marmorated stink bug (BMSB) (*Halyomorpha halys*) and native brown stink bug (*Euschistus servus*), can severely damage crops including field corn. Frequency and size of stink bug infestations in mid-Atlantic U.S. corn fields have increased, and there is growing concern that this may contribute to reductions in grain quality. A correlation between BMSB damage and fumonisin contamination was identified, and the ability of BMSB to increase *Fusarium* infection and fumonisin contamination in corn was demonstrated in field experiments. Subsequent studies demonstrated a similar synergistic association between native brown stink bugs and fumonisin contamination of corn. Potential for stink bugs to vector mycotoxigenic *Fusarium* spp. was examined, and *Fusarium* was isolated from 34% of stink bugs collected from corn fields. Among these isolates, 78% were fumonisin-producing species including *F. verticillioides* (Fv) and *F. proliferatum*, and 18% were trichothecene-producers. Furthermore, identical SSR haplotypes of Fv were isolated from stink bugs and corn collected from the same field. In laboratory experiments, stink bugs were able to transmit Fv to non-infected corn ears after feeding on Fv-infected corn. Thus, strategies that control both the pest (stink bug) and pathogen (*Fusarium*) must be implemented to mitigate risks of mycotoxin contamination in corn.

Cropping system practices on nematode communities and soil health

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Plant parasitic nematodes (PPN) limit yields in crops in Michigan & USA. Soybean Cyst Nematode (SCN) is considered to be the most damaging pathogen in N. America. Root lesion nematode (RLN) & Northern root knot nematode (NRKN) are common in crops & cause yield losses. The Michigan Applied Nematology lab has conducted trials to evaluate the effect of cropping systems on these nematodes & soil health to provide applied solutions. We have evaluated compost & manures, nematicides & seed treatments, rotations of SCN resistant varieties, surveys of vegetable & field crops, & evaluation of cover crops. A compost blend by Morgan Composting has resulted in death of RLN in lab trials, even in concentrations as low as 5%. An SCN trial identified a reduction of SCN juveniles after the incorporation of two tons/acre of chicken manure ahead of corn. Several nematicides have been evaluated, & results detected for PPN control. Conversely, we rarely observed a reduction of nematodes (*i.e.* SCN or Beet Cyst Nematode (BCN)) with seed treatments with the exception of a trial with Abamectin. We have looked at rotation of resistant varieties. Rotating sources of resistance for SCN resulted in a reduction of SCN & yield increases. We have conducted two state-wide surveys in vegetables & field crops. Some conclusions: NRKN nematode were low in soil from vegetable fields rotated with corn. Cover crops increased beneficials. In the field crops survey, we are correlating tillage & soil type with corn root nematode. Finally, cover crops are being evaluated for host status to PPN & soil health.

Adopting post-harvest diseases into inquiry-based online STEM modules for the next generation of plant pathologists

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Dr. Lou Hirsch is a Lecturer in the Department of Plant Pathology and serves as the Director of Undergraduate Studies for the Agricultural and Medical Biotechnology Program, an interdisciplinary major in the College of Agriculture, Food and Environment at the University of Kentucky. His on-campus responsibilities include program administration and teaching graduate and undergraduate lecture and lab classes, but his scholarship and research focus on the development of standards-based science modules for K-12 STEM programs. To this end, Dr. Hirsch has facilitated partnerships with APS members and STEM teachers through the APS Office of Public Relations and Outreach (OPRO) to enable the publication of laboratory teaching modules in peer-reviewed STEM practitioner journals. Furthermore, he and OPRO recently partnered with PlantingScience.org to create a plant pathology laboratory module on a well-known digital platform frequented by exceptional STEM teachers throughout the United States.

***Arabidopsis* and barley convergently evolved an engineerable pathogen protease detection mechanism**

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Engineering known resistance mechanisms is a valuable approach to combatting plant disease. While modifying NLRs (nucleotide binding leucine rich repeat) that mediate effector triggered immunity is difficult, we can modify NLR-guarded host proteins to alter their specificity. The *Pseudomonas syringae* protease effector AvrPphB activates the *Arabidopsis* NLR RPS5 by cleaving a decoy kinase, PBS1. By exchanging the AvrPphB cleavage site for that of a viral protease, PBS1 can confer resistance to the virus instead of *P. syringae*. Because PBS1-like kinases are highly conserved and involved in pattern triggered immunity, we hypothesized that the

RPS5-PBS1 guard-decoy system would be present in other plants. We found that wheat and barley lines respond to AvrPphB with chlorosis or necrosis. In a genome wide association study of barley, we mapped the response to a single locus with an NLR gene (AvrPphB Response 1; *Pbr1*) that has expression and sequence polymorphism across barley genotypes correlated with AvrPphB response. PBR1 is phylogenetically distant from RPS5, but triggers an HR when transiently expressed in *Nicotiana benthamiana* with AvrPphB. We identified barley PBS1 homologs and showed that they are cleaved by AvrPphB and co-immunoprecipitate with PBR1 in planta. We conclude that barley and *Arabidopsis* convergently evolved NLRs to guard PBS1 kinases. These results suggest that PBS1-based decoy engineering could be deployed in small grain crops like barley and wheat.

New Technology supporting control of airborne pathogens: A start-up company's journey

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Our idea started in human health. How can we dramatically improve quality of life by speciating airborne particles, like pollens and allergens, to enable people to take preventative actions before becoming symptomatic. And then we met the late Dr. Walter Doug Gubler from UC Davis and we realized the agriculture industry had a unique, unmet need for early identification and quantification of airborne disease spores to allow growers to take action to prevent yield losses. But we are not farmers or plant pathologists. We are hardware and software engineers. Scanit will share their story of how startups, who believe their unique technology can benefit the agriculture industry, approach validating their ideas, getting it into the hands of disease experts and growers, and ultimately chase commercialization amidst a challenging AgTech landscape.

Evaluating genetic resistance to downy mildew in *Chenopodium* species for use in breeding programs

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Quinoa is a potential new crop for New England; however, its susceptibility to downy mildew, caused by *Peronospora variabilis* is a key obstacle. The objective of this project was to evaluate differential resistance within the *Chenopodium* genus and identify novel genetic sources of resistance for use in future breeding programs. The long-term goal of this research is to develop a resistant variety of quinoa to be grown in North America. Field trials conducted at UNH evaluated downy mildew disease severity on ten *Chenopodium* accessions representing four species. Accessions were planted in a 2-factor randomized split plot design with three replicate plots per treatment. Disease severity for each treatment was compared and significant differences in disease severity was observed among treatments. *C. berlandieri* var. *macrocalyculum* ecotypes collected from Rye Beach, NH and Appledore Island, ME exhibited the lowest mean disease severity of the season. *P. variabilis* was isolated from each of the 10 accessions and ITS and COX2 sequences were compared; phylogenetic analyses suggest no effect of host species; however, New Hampshire isolates formed a clear cluster when compared with Pennsylvania isolates. These results provide the first step in identifying potential New England native sources of resistance to downy mildew within the genus, and provide preliminary information needed to further investigate resistance at the genomic level in *Chenopodium* spp.

The future of disease control

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Attempts to control plant diseases have been recorded since organized agriculture began. In the 1930s synthetic fungicide development and their use began to expand rapidly. Rachel Carson's 'Silent Spring' in 1962 raised awareness of chemical pesticides and emphasized the need to improve user and environmental safety of crop protection products. R&D investment by the major research-based companies has remained high at approximately 7–10% of annual sales over the last 50 years. This has led to continued improvement in performance and the amount of active ingredient used today is approximately 95% lower than rates used in the 1950s. Many products were withdrawn over the years and the EU's re-registration process (Directive 91/414), has resulted in the removal of more than half of the commercially significant crop protection products. Today, there is a drive for plant protection products with very specific properties, balanced by a favourable regulatory profile. This has led to advances in innovation in amongst others chemical discovery. In addition, modern approaches to biological control, including the use of biologicals, plant extracts and peptides, are becoming more important for a holistic, integrated approach to disease control. These, coupled with new technologies such as digital agriculture and precision application could revolutionise agriculture in the same way as The Green Revolution in the 1950s -1960s which increased agricultural production worldwide.

Impacts of the global emergence of azole resistance among *Aspergillus* spp. on humans in a medical setting

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The environmental mold *Aspergillus fumigatus* causes invasive aspergillosis in humans and has historically been associated with mortality rates as low as 25% and as high as 90% in high-risk patients, including stem cell and organ transplant recipients. Triazole antifungals, such as voriconazole, are first line therapy and have greatly improved survival since their introduction; however, triazole-resistant *A. fumigatus* infections are increasingly reported worldwide and are associated with increased mortality and treatment failure. Resistance markers associated with environmental triazole fungicide use rather than previous patient exposure to antifungals have raised particular clinical and public health concern. Reports of triazole resistant *A. fumigatus* have increased worldwide in the past 15 years, and the first U.S. human isolates of fungicide-associated resistant *A. fumigatus* were described in 2016. Here we review the clinical experience, changing epidemiology, and implications of the two major routes of resistance development in *A. fumigatus*.

Elucidating the virome diversity of grapevine using high-throughput sequencing approaches

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Grapevine is susceptible to many viruses and viroids that often have high economic impact. Until recently, their detection was based on traditional methods (ELISA, RT-PCR), which however do not allow the complete characterization of a plant sample's virome. The last years this was made possible with the application of high-throughput sequencing (HTS), an holistic method that facilitates discovery of diverse and previously unknown viruses/viroids. With the aid of HTS, we attempted to elucidate the virome diversity of Greek vineyards, with an ultimate goal to unveil the etiology of unknown diseases, improve diagnostics and assist the production of high quality propagating material. For this purpose samples were selected, based on their geographic origin, variety, age and symptomatology. Total RNA (from single plants or pooled samples) was subjected to HTS. For the analysis of the obtained data, a grapevine optimized pipeline was developed, which produced large contigs for the majority of the viruses present in each sample. The analysis revealed the presence of many viruses reported for the first time in Greece, as well as a few contigs which may represent novel virus species. Interestingly, different genomic variants of *Vitivirus* species often co-existed in the same grapevine. The high number of viral populations identified, in the small number of grapevines tested, highlight the complex nature of the grapevine virome. Importantly, the obtained data on virus genetic diversity will help to improve diagnostics for the reliable virus indexing in grapevine certification schemes.

Pick your poison: Late flowering *Aspergillus flavus*-resistant maize lines, corn earworm and fumonisin

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A preference in the natural infestation of corn earworm (CEW: *Helicoverpa zea* Boddie) to specific host genotypes was observed in field trials to assess the pathogenicity of *Aspergillus flavus* strains in two maize hybrids and inbreds with contrasting levels of resistance to aflatoxin (AF) accumulation. The resistant hybrid (Mp313E × Mp717) had greater than 14-fold infested ears than the susceptible hybrid (GA209 × T173). Similarly, the resistant inbred (CML322) had greater than 7-fold heavier CEW infestation than the susceptible inbred (B73). These two lines with heavy infestation showed delayed silk emergence, in addition to accumulating seed AF levels that are low and sub-toxic to CEW. Since CEW oviposits directly on silks, the availability of green silk for egg-laying in the late flowering genotypes may be one of the contributing factors to their preferential infestation. The level of CEW infestation had little influence on seed AF levels either in uninoculated ears or in ears manually inoculated with toxigenic *A. flavus* strains. Although no manual inoculation with *Fusarium* species was carried out, the CEW-infested ears showed a significantly greater seed fumonisin (FUM) content compared to uninfested ears. In spite of its superior resistance FUM accumulation demonstrated previously, Mp313E × Mp717 had greater levels of FUM than GA209 × T173 correlating with the level of CEW infestation. In summary, mycotoxin contamination of crops is determined not only by the level of a host resistance to the cognate fungus, but also phenological traits that compromise its resistance.

Abiotic and biotic stresses universally regulate the rice transcriptome

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Environmental stresses, both abiotic and biotic, are large contributors to pre-harvest crop loss. With climate change increasing the incidence of abiotic stresses and the constant pressure of pests and pathogens, it is critical to world agriculture that varieties of plants broadly tolerant to stresses are developed. For this, it is necessary to develop a comprehensive understanding of how plants respond to stress. Here, we present the results of a meta-analysis of publicly available rice stress response transcriptome data. Our results confirm that rice universally down-regulates photosynthesis in response to both abiotic and biotic stress. Rice also generally up-regulates hormone-responsive genes during stress response, most notably genes in the abscisic acid, jasmonic acid and salicylic acid pathways. We identified several promoter motifs that are likely involved in stress-responsive regulatory mechanisms in rice. This study provides a list of candidate genes and pathways to study for improving rice stress tolerance to abiotic and biotic stresses. This study also serves as an important proof of concept that meta-analysis of diverse transcriptome data is a valid approach to develop hypotheses for how plants respond to stress.