between SCN and *F. virguliforme*, the causal agent of sudden death syndrome on soybean (SDS), are unknown. We used green fluorescing protein (GFP) producing strains of *F. virguliforme,* to shed light on this interaction. The fluorescence of these strains allows us to visually assess the ability of the fungus to infect and colonize roots, and provides a means to assess the extent of root colonization. Moreover, in addition to its ability to fluoresce, one of these GFP-producing strains has impaired ability to infect and/or colonize roots. These fungal strains allow us to determine how SCN is assisting the fungus in causing SDS. Greenhouse experiments were conducted using soybean cultivars with varying levels of resistance to SCN, root knot nematode and SDS. Each cultivar was challenged with a GFP-expressing aggressive fungal strain or a GFP-expressing non aggressive fungal strain. Fungal strains were also co-inoculated with root knot nematode or SCN. The use of root knot nematode helped determine whether the *F. virguliforme*/SCN interaction is unique in enhancing SDS, or if other nematode pathogens could facilitate this interaction. The importance of the role of either nematode in facilitating fungal infection and colonization of roots were assessed further by using a fungal strain that is impaired in its ability to penetrate soybean roots.

Using real-time PCR to quantify aster yellows phytoplasma in its insect vector; relationship of infectivity to transmissibility in the aster leafhopper

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The aster yellows (AY) index is used to prescribe insecticide sprays that target the vector of the aster yellows phytoplasma (AYp) *Macrosteles quadrilineatus*, or aster leafhopper (ALH). The AY index is the product of the proportion of infective ALHs and the relative ALH population size at a location. Recently, polymerase chain reaction (PCR) has become used to determine the proportion of potentially infectious AYp-infected ALHs, but limited information exists to determine the proportion of PCR-positive inoculative individuals. As a persistent and propagative pathogen, our current hypothesis predicts a relationship between the population size of AYp in individual ALHs and the frequency at which individuals transmit AYp. To address this hypothesis, we have developed a quantitative, real-time PCR protocol to quantify AYp DNA in ALHs. The elongation factor TU gene was used as a target for AYp and the ALH CP6 wingless gene was used as a target for insect genomic DNA. Target sequences were chosen because they are likely present as single copies in their respective genomes and can be related in a 1:1 ratio. The ratio of AYp DNA to insect DNA is useful to avoid variation due to the DNA extraction procedure. In the insects examined to date, the ratio of AYp genomes to insect genomes range from 0.832 to 0.006. This new methodology will improve the accuracy of the AY index by providing a tool for accurate determination of infective individuals generated following acquisition and inoculation bioassays.

Refining the aster yellows index in Wisconsin: Developing sustainable control tactics for susceptible vegetable crops

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The population magnitude and infectivity associated with migratory *Macrosteles quadrilineatus,* or aster leafhopper (ALH), in early spring influences aster yellows epidemics in Wisconsin carrot. In years when the numbers of spring migrants are noted as trivial, native ALH populations can drive disease cycles by acquiring aster yellows phytoplasma (AYp) from local plant sources and subsequently transmitting it into carrot. The focus of this project is to evaluate the relative epidemiological importance of inoculum sources present in field edges compared with the estimated inoculum in-bound, in migratory ALHs. In 2007 and 2008, total DNA was extracted from ALHs collected on their spring migratory path and ALHs collected in 10 geographically distinct carrot fields. Total DNA was also extracted from symptomatic carrot plants from each of the locations. PCR analyses were used to identify AYp strain types present in the infected ALHs and the carrots. To date, the relative abundance of AYp strain types extracted from carrot and ALHs varied by location. The AYp genotypes present in fields were associated with specific disease symptoms. By combining AYp strain data with vector phenology and infectivity data, we will improve our understanding of where ALHs acquire and when they spread AYp to carrot. This integrated approach will help to refine our current management tactics ultimately contributing to the development of a comprehensive control strategy for Wisconsin carrot growers.

GmRAR1 and GmSGT1-2 participate in various modes of soybean immunity against microbial pathogens

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RAR1 and SGT1 are important components of defense signaling pathways induced by structurally diverse resistance (R) proteins. RAR1 and SGT1 are thought to modulate R protein stability serving as co-chaperones of HSP90. Similar to their counterparts from several other plants, soybean RAR1 and SGT1 proteins interact with each other and two related HSP90 proteins. However, resistance induced by two different soybean R proteins requires RAR1 and SGT1, but not HSP90. *Rsv1*-mediated extreme resistance to Soybean Mosaic Virus and *Rpg-1b*-mediated resistance to *Pseudomonas syringae* were compromised in plants silenced for *GmRAR1* and *GmSGT1-2*, but not *GmHSP90*. This suggests that RAR1-/SGT1-dependant signaling may not always be associated with a dependence on HSP90. Unlike in Arabidopsis, SGT1 in soybean is also required for resistance signaling against the bacterial pathogen *P. syringae*. Plants silenced for *GmHSP90s* or *GmRAR1* show altered morphology suggesting that these proteins also contribute to developmental processes. Silencing *GmRAR1*and *GmSGT1-2* impaired resistance to virulent strains of *P. syringae* and systemic acquired resistance (SAR) in soybean as well. Since the Arabidopsis *rar1* mutant also showed a defect in SAR, we conclude that RAR1 and SGT1 serve as a point of convergence for basal, *R* gene-mediated and systemic immunity in diverse plants.

Detection of chromosome rearrangements in *Gibberella zeae*

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Chromosome rearrangements between fungal strains may reduce fertility in sexual crosses and result in progeny that are inviable or have reduced fitness. Such rearrangements act as a post-zygotic barrier to gene flow and may contribute to speciation. *Gibberella zeae* (anamorph: *Fusarium graminearum sensu lato*) is a homothallic species that can reproduce sexually by self-fertilization or outcrossing. *G. zeae* is composed of multiple phylogenetic lineages that are morphologically, pathologically, and toxicologically similar, but contain DNA sequence polymorphisms whose distribution suggests reproductive isolation between the lineages. Genetic and cytological methods for detecting chromosome rearrangements are difficult and/or laborious in *G. zeae*. For that reason, we investigated counts of viable ascospores per ascus in *G. zeae* outcrosses as an indicator of chromosomal rearrangements. Ascospores can be observed in rosettes of asci extruded from crushed perithecia or by observing unordered tetrads that are ejected from mature perithecia. Individual asci can be assigned to the following four classes: 8, 6, 4, and 2 ascospores. Crosses between different male strains and two female tester strains often produced significant frequencies of asci with 6, 4, or 2 ascospores per ascus, which indicates the presence of one or more rearrangements. These results suggest that this method will be useful to survey populations of *G. zeae* for chromosome rearrangements.

Two separate phage genomes appear associated with citrus greening (Huanglongbin)

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Citrus Huanglongbin (HLB), also known as citrus "greening" is a lethal disease of citrus that is now widespread in Florida. HLB is caused by *Candidatus* Liberibacter asiaticus (Las), which has not been cultured; consequently Koch's postulates have not been confirmed. The recent availability of a draft Las genome derived from infected psyllids (GenBank accession NZ_ABQW00000000) reveals that prophage DNA appears in some of the available contigs, but phage have not been previously been associated with HLB. Using dodder transmission, we have continuously curated HLB from a single infected Florida citrus tree for three years. We developed a DNA extraction protocol from dodder that enriched for Las and greatly reduced chloroplast and mitochondrial DNA contamination (as determined by PCR) and used multiple displacement amplification (MDA) to obtain sufficient DNA to create a fosmid library with an average insert size >32 kb. This fosmid library was found to be surprisingly biased towards phage DNA inserts; the phage DNA was confirmed associated with HLB. Based on shotgun library assembly, fosmid walking, and direct PCR cloning and sequencing, two related, but distinct phage partial genomes were assembled.

Both were confirmed to be associated with HLB on citrus. The idea that phage DNA may be over-represented in infected plants but not in psyllids raises the possibilities that Las is a lysogenic host and that phage may contribute to HLB disease.

Viability of *Phytophthora nicotianae* oospores in North Carolina tobacco populations

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Phytophthora nicotianae, the causal agent of black shank of tobacco, occurs in all tobacco-producing areas of North Carolina. A recent state-wide survey revealed multiple fields in which both the A1 and A2 mating types were present. Very little is known about the contribution of sexual sporulation to pathogen variability and black shank epidemiology. A1 and A2 isolates that originated from the same fields were paired on soft carrot agar amended with 10 ppm cholesterol to identify the potential for sexual compatibility and reproduction. A subset of compatible isolates from within fields was further examined to assess the percentage of oospores from pairings that were viable. After 4-wks incubation, oospores were extracted from the agar and stained with tetrazolium bromide. Viable oospores were identified microscopically based on a specific color reaction as described by Sutherland and Cohen in 1983. Viability of the within-field pairings ranged between 20 and 48%. Germination of oospore progeny is being investigated to determine if sexual reproduction serves as a source of pathogen variability. Understanding the biology of sexual sporulation in *P. nicotianae* will help elucidate how variability develops within populations and how it influences short and long term durability of resistance genes in tobacco.

Search of a "DNA barcode" for identification of species of the genus *Fusarium*

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"DNA barcoding" is a tool that aims to become a taxonomic method using a short standard genomic sequence, present in all the taxons of interest and showing sequence variation, enough to discriminate among species. The members of genus *Fusarium* are recognized as important plant pathogens, human pathogens and saprophytes and its routine taxonomic identification relies on macro and microscopical characteristics and molecular methods. However, identification can be difficult due to the lack of some structures in culture or to the lack of enough polymorphism in ribosomal sequences. Barcoding could provide an easy and reliable method to overcome these problems. This study evaluated sequences of cox (mitochondrial cytochrome oxidase subunit 1) and aox (alternative oxydase) as potential DNAs barcodes for identification of *Fusarium* species. DNA was extracted from 12 *Fusarium* isolates previously identified by traditional methods, including *F. solani, F. oxysporum, F. proliferatum*, and *F. moniliforme*. For the amplification of aox, primers were designed by our group showing amplification of a region of 800 bp approximately in all *Fusarium* species but not in *Alternaria* sp. or *Pestalotia* sp. Amplicons were sequenced and analyzed using MUSCLE alignment software. So far, our results suggest that the combination of these two genes may provide a method for identification of the species tried in this work. Future work will include a higher number of species and isolates from each species.

GFP expression from a biologically active minireplicon of Sonchus yellow net virus

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Sonchus yellow net virus (SYNV) is the most extensively characterized plant rhabdovirus that replicates in the nucleus. The minimum infectious unit of SYNV is a ribonucleoprotein core complex, which consists of the nucleocapsid (N) protein, the phosphoprotein (P) and the polymerase (L) protein complexed with the negative-strand genomic RNA. We have constructed a SYNV minireplicon (MR) that functions in planta to permit reverse genetics studies of the core N, P and L proteins, and the trailer and leader regions of SYNV RNA. The MR presents a new approach to express the core complex in plants via agroinfiltration into Nicotiana benthamiana leaves. The MR has been engineered to generate a transiently expressed antigenomic (ag) RNA that is processed by ribozymes flanking the leader and trailer regions. The transcript consists of the SYNV leader sequence, the 5'UTR of the N gene, a GFP reporter gene, a gene junction sequence, a DsRed or CAT reporter gene, the 3'UTR of the L gene and the SYNV trailer sequence. To assess the biological activity of the MR, leaves were agroinfiltrated with plasmids expressing the agRNA, the N, P and L proteins and suppressors of gene silencing. At about 5 days after infiltration, numerous GFP fluorescent foci that persisted for up to three weeks were observed within the infiltrated leaves. Mutagenesis results reveal that the N, P and L genes, and elements of the leader and trailer sequences are required for MR replication.

An intact cuticle in distal tissues is essential for the induction of systemic acquired resistance in plants

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Systemic acquired resistance (SAR) is initiated upon recognition of specific microbial effectors by cognate plant resistance proteins and immunizes distal tissues of plants against secondary infections. SAR involves the generation of a mobile signal at the site of primary infection, which then translocates to and activates defense responses in the distal tissues via unknown mechanism(s). We have recently shown that an acyl carrier protein, ACP4, is required for the processing of the mobile SAR signal in distal tissues of Arabidopsis. Although acp4 plants generated the mobile signal, they were unable to respond to this signal to induce systemic immunity. The defective SAR in acp4 plants was not due to impairment in salicylic acid (SA)-, methyl SA-, or jasmonic acid-mediated pathways but was associated with the impaired cuticle of acp4 leaves. Other genetic mutations impairing the cuticle or physical removal of the cuticle from wild-type plants also compromised SAR. This cuticular requirement was only relevant during the time of mobile signal generation and translocation to the distal tissues. Together, these results demonstrate a novel role for the plant cuticle as the site for SAR-related molecular signaling.

Biological control of Sclerotinia stem rot with an endophytic *Bacillus* sp. strain on oilseed rape

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Stem rot, caused by *Sclerotinia sclerotiorum*, is one of the most important diseases of oilseed rape in China. The antagonistic activities of sixty-three endophytic bacteria strains, which were isolated from leaves, stems and roots of winter wheat, were tested against *S. sclerotiorum in vitro*. The study showed that fourteen trains had a remarkable inhibitory effect to the growth of mycelia, production and germination of sclerotia. The evaluation of the antagonistic strains against stem rot of oilseed rape indicated that the strain Em7 reduced disease incidence. In greenhouse experiments, Em7 had 97% control effect against stem rot of oilseed rape when it was sprayed to leaves before 24 h of inoculation *S. sclerotiorum*. Em7 was primarily identified as *Bacillus* based on its morphology and physiology. Microscopic studies showed that *Bacillus* sp. Em7 induced diverse morphological alterations in pathogens hyphae. Hyphal morphological alterations include cytoplasm exosmosis, deformation, swelling of apex.

First approach to the characterization of *de novo* pyrimidine biosyntheis pathway in *Phytophthora infestans* as a target for pathogen control

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The oomycete *Phytophthora infestans* is the causal agent of the tomato and potato late blight, causing high economic losses worldwide. Current control strategies are far from being adequate and an interesting and unexplored alternative for control could be based on the inhibition of the *de novo* pyrimidine biosynthetic pathway. Indeed, inhibitors of some of the enzymes in this pathway have been proposed as therapeutic agents for a wide range of human parasites and some plant pathogens. Bioinformatic analyses of the enzymes in the *P. infestans* pathway were performed, in order to select the best targets for enzymatic inhibition. Based on the similarity to host enzymes, different predicted subcellular localization, architecture, predicted 3D structure and phylogenetic relations, the last two enzymes of the pathway, orotate phosphoribosyltransferase and orotidine-5-monophosphate decarboxylase, were selected as the most promising targets. Nevertheless, enzymes 3 and 4 dihydroorotase and dihydroorotase dehydrogenase cannot be ruled out. Key aspects of their metabolic inhibition were also determined for future virtual screening of a compound library using molecular docking. These enzymes are being cloned, expressed and purified in their recombinant form. This will allow their future biochemical characterization. To our knowledge, this is the first study of the pyrimidine biosynthesis in oomycetes.