

Abstracts of Special Session Presentations

Biology of Plant Pathogens

Collaboration Between Industry and Researchers to Improve Management of Viral Diseases of Ornamentals - A Model for Other Crops

Prevention of viral diseases in ornamental production environment. M. J. Klopmeier (1) and J. Van der Meij (2). (1) Ball Horticulture Company; (2) Ball FloraPlant, West Chicago, IL 60185. *Phytopathology* 97:S131.

In the past 20 years, the ornamental industry has observed significant growth in the production and sales of vegetatively propagated herbaceous ornamentals such as geraniums, New Guinea impatiens, petunias, verbenas, perennials and many more. This production has moved "off-shore" to Central America and Africa to take advantage of favorable growing climates and lower production costs (i.e. greenhouse structures and hand labor). Contrary to seed production, vegetative production of these annuals and perennials provides an ideal environment for the introduction and spread of bacterial, fungal and viral pathogens. The planting, maintenance and harvesting of cuttings from these stock plants offers multiple pathways for sap transmissible viruses. Also, the semi-tropical growing environment for these mother plants is also conducive to year-round pressure from insect vectors. A fully integrated clean plant production program is required for successful production and sales. This includes initiating production with pathogen-free stock, excellent sanitation programs for exclusion and prevention of pathogen spread and employee training programs assuring execution of sanitation protocols. The most common viral pathogens affecting these crops include the tospoviruses, tobamoviruses, potexviruses, carmoviruses and viroids. Rapid identification and elimination of these pathogens from commercial varieties along with sanitation practices will be discussed.

New and emerging viruses of ornamental plants—When is virus infection a problem? J. Hammond and R. Jordan. USDA-ARS, U.S. National Arboretum, Floral and Nursery Plants Research Unit, Beltsville, MD 20705. *Phytopathology* 97:S131.

New viruses continue to be detected affecting ornamental crops as new crops are introduced and grown alongside industry standards. Some of these viruses may exhibit little to no symptoms in one host, but cause significant losses in another host. There are good serological reagents and tests for the 'common' viruses that affect multiple hosts (such as CMV, TSWV, INSV and TMV) and for broad-spectrum detection of potyviruses, which allow detection and exclusion of these viruses from initial stocks of new crops or cultivars. In contrast, there are not good serological reagents for broad-spectrum detection of potexviruses, carlaviruses, carmoviruses, tymoviruses and ilarviruses, and newly reported viruses in these groups have emerged in diverse ornamental crops. In collaboration with industry and other research partners, we have combined virus group-specific PCR testing, broad-spectrum serology, electron microscopy, bioassay, purification, and biophysical characterization with genomic cloning and sequencing, in order to identify newly emerging viruses and to produce reagents for their detection in ornamental production. For example, we have identified new potexviruses in phlox, portulaca and *Tricyrtis*; novel carlaviruses in phlox; two novel ilar-

viruses in *Bacopa* and pansy; a new carmovirus infecting *Angelonia* and *Verbena*; three pelarspoviruses in *pelargonium*; an ophiovirus in *Lachenalia*; and novel potyviruses in *Omphalodes*, *Tricyrtis*, *Impatiens*, *Spiranthes*, and phlox.

New and emerging viral diseases of perennial plants. B. Lockhart. University of Minnesota, St. Paul, MN 55108. *Phytopathology* 97:S131.

During the past several years a number of previously undescribed viruses have been associated with disease symptoms in perennial ornamentals. Several of these viruses have been identified and characterized and their pathogenicity verified, while a number of others are currently under investigation. Perennial ornamentals in which these new and emerging viruses occur include abutilon, ash, *Buddleia*, coleus, *Columnnea*, *Cyrtomium*, geranium, hackberry, hosta, hydrangea, lamium, oxalis, penstemon, peony, phlox, roses, and spiraea. Information on disease symptoms, methods of virus detection and current status of virus characterization will be presented.

Management of tobamovirus in ornamental production. S. Adkins (1) and D. J. Lewandowski (2). (1) USDA-ARS, Fort Pierce, FL 34945; (2) The Ohio State University, Columbus, OH 43210. *Phytopathology* 97:S131.

Tobacco mosaic virus (TMV) and other tobamovirus species infect a wide range of ornamental plants. Many tobamoviruses cause similar symptoms in certain hosts, yet cause symptomless infections in other hosts and/or cultivars. Tobamovirus virions are extremely stable and can remain infectious for years. To investigate petunia-tobamovirus interactions, four petunia cultivars were mechanically inoculated with one of six tobamoviruses [TMV, Tomato mosaic virus (ToMV), Pepper mild mottle virus (PMMoV), Tobacco mild green mottle virus (TMGMV), Tropical soda apple mosaic virus (TSAMV) and Hibiscus latent Fort Pierce virus (HLFPV)]. All but HLFPV are known to infect many solanaceous species. No symptoms were observed with TMV, ToMV, PMMoV, TMGMV or TSAMV systemic infections. However, local lesions were observed on inoculated leaves of some petunia cultivars inoculated with ToMV and TSAMV. These same four petunia cultivars were mechanically inoculated with sap from field collected petunias showing obvious symptoms of virus infection (serologically shown to be a tobamovirus). Symptomatic systemic infections resulted in three of four cultivars. Various treatments are now being tested for their ability to reduce the rate of tobamovirus transmission during typical ornamental production protocols.

Detection and characterization of a new Tymovirus of ornamental plants. D. M. Mathews and J. A. Dodds. Department of Plant Pathology, University of California, Riverside, CA 92521. *Phytopathology* 97:S131.

A tymovirus was detected in 2003 which tested positive for *Scrophularia mottle virus* (ScrMV) using ELISA, but was negative for ScrMV using RT-PCR and host range tests. Purified virions were used to produce a polyclonal antibody and over 200 plants, primarily *Diascia*, *Verbena*, and *Nemesia* spp., have tested positive for the virus using ELISA. In a survey of ornamental plants from 5 counties in California, 78% were positive for the tymovirus. In 2005 *Nemesia ring necrosis virus* (NeRNV) was reported as a new tymovirus in Europe which also cross reacted with ScrMV in ELISA. Only 5 of 30 virus isolates from CA produced an amplicon using NeRNV specific primers in RT-PCR, while all 30 were positive using generic tymovirus group primers (Agdia, Inc). Sequence analysis showed significant changes for most in the second strand priming region for NeRNV, explaining the variable RT-PCR

result. Sequence analysis of the entire genome of selected CA isolates showed that they are strains of NeRVN and not a novel tymovirus. A high percentage of plants eventually tested positive for NeRVN after having undergone meristem culture, thus many infected plants make it to the propagation level

without being detected during initial screening. Continuing studies include optimization of early detection to ensure elimination of infected plant material, the threat of the virus to agriculture in general and the effect of mixed infections on detection.

New Approaches to Elucidating the Mechanisms of Seed Invasion and Transmission

Elucidating mechanisms of watermelon seed infection by *Acidovorax avenae* subsp. *citrulli*. R. R. Walcott. Department of Plant Pathology, The University of Georgia, Athens, GA 30602. Phytopathology 97:S132.

Acidovorax avenae ssp. *citrulli* (*Aac*), the causal agent of bacterial fruit blotch of cucurbits (BFB) is a seedborne phyto bacterium, and infested seed represent an important primary inoculum source. Despite efforts to produce pathogen-free seed, costly BFB outbreaks continue to occur worldwide. To improve BFB management, a better understanding of seed infection is needed; hence the role of female blossoms in seed infection was investigated. In greenhouse studies the application of $10^7 - 10^9$ *Aac* CFU/female watermelon blossom led to production of infested seeds in asymptomatic fruits. *Aac* rapidly colonized the stigmas of female blossoms, reaching 10^8 CFU/blossom by 96 h after inoculation. Additionally, as little as 10^3 CFU/blossom led to seed infection within symptomless fruits. A strong linear relationship $R^2 = 0.94$ was observed between blossom inoculum dosage and seed infestation. Using a constitutive green fluorescent protein mutant, *Aac* was observed to penetrate through styles via transmitting tract tissue. By 7 days after inoculation *Aac* penetrated the ovary but did not colonize immature ovaries. These data suggest that female watermelon blossoms play a role in seed infection under field conditions. Based on this assumption it was demonstrated that biocontrol blossom protection could limit seed infection.

Detection, quantification and visualization of the bacterial spot pathogen on seeds and transplant seedlings. D. A. Cuppels. Agriculture and Agri-Food Canada, London, ON, Canada. Phytopathology 97:S132.

The primary inoculum source for the tomato disease bacterial spot is thought to be seed. Our objective was to develop quantitative, qualitative and microscopic methods for following the pathogen on seeds and seedlings. The basis for detection and quantification was both conventional and quantitative PCR (qPCR). The primer sets BSX1/2 and BSX9/10 were derived from the sequence of the bacterial spot diagnostic DNA probe KK1750. The sensitivity of the seed assay was enhanced significantly by first germinating the seed. The detection limit with qPCR for a 20,000-seed sample was ~20,000 bacteria. To visualize the pathogen and follow *hrp* gene expression during seed germination and seedling development, we tagged strains with constitutively-expressed *gfp*, constructed transcriptional fusions and performed RT-PCR. Expression of *hrp* genes was detected within 4 hours of bacteria being exposed to a seed suspension. GFP-tagged strains were observed colonizing intercellular spaces of symptomless cotyledons recently emerged from infested seed. These methods should be useful in studies of the development and spread of bacterial spot inoculum, particularly as it pertains to seed.

Investigating seed-to-seed transmission of *Fusarium* species in maize using fluorescent protein markers. G. P. Munkvold. Department of Plant Pathology and Seed Science Center, Iowa State University. Phytopathology 97:S132.

Seed-to-seed transmission of *Fusarium verticillioides* and other *Fusarium* species involves a sequential process of seed-seedling transmission, symptomless systemic infection of developing plants, and plant-to-seed movement of the pathogen. Tracking the development of these infections has been problematic due to the ubiquitous nature of *Fusarium* species in the maize production environment and the existence of multiple pathways for infection of maize plants. We used green fluorescent protein (GFP)-marked strains of *F. verticillioides* and *F. subglutinans* to investigate the transmission of these fungi from seeds to plants and from plants to seeds. The effects of temperature on seed-to-plant transmission and systemic development of *F. verticillioides* were investigated, and results showed that these phenomena occur over a wide range of temperatures but may be favored by temperatures higher than long-term averages for Iowa. Experiments with GFP-marked *F. subglutinans* demonstrated the occurrence of seed transmission and symptomless systemic infection of maize by this fungus for the first time. GFP-marked *F. graminearum* strains have been used by other researchers to characterize seed infection in wheat. Fluorescent protein markers with different spectral properties now offer rich opportunities for investigating multiple-pathogen interactions in seed pathosystems.

Symplastic pathways to virus seed transmission. A. J. Maule. John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK. Phytopathology 97:S132.

The transmission of viruses in infected seed is widespread affecting many crop species. Although it may occur at relatively low frequencies in a genotype (often <1% infected seed) its impact is disproportionately high in the next generation through creation of many foci of infection in the crop. Except for rare instances of de novo infection of seedlings from contaminated seed coats, seed transmission is dependent upon infection of the embryo. This can occur via two routes, transmission of virus into the zygote by infected gametes, or direct infection of the developing embryo post-fertilization. The former, more common route depends upon the virus infecting the gametic mother cells before symplastic isolation during gametic maturation. The latter route is counterintuitive since it implies the existence of a symplastic pathway for virus passage from the maternal to the filial tissues of a developing seed. Such a macromolecular pathway has not been identified previously. We have studied *Pea early browning virus* (*Tobraviridae*) and *Pea seed borne mosaic virus* (*PSBMV*; *Potyviridae*) as exemplars of the former and latter pathways, respectively, in virus seed transmission in pea. Using *in situ* hybridization and electron microscopy we identified the symplastic routes to seed transmission in each case. For PSBMV, our data show that the maternal and filial tissues of the developing pea seed need not be completely symplastically segregated.

Systematics and Phylogeny: The Tree of Life, Dorylaimia, Triplonchida and the Origin of Nematodes

Roots and shoots: Rooting Nematoda with credible outgroup taxa and replicating internal structure with multigene datasets. M. Blaxter. University of Edinburgh, Edinburgh, EH9 3JT, United Kingdom. Phytopathology 97:S132.

To date, analyses of the phylogenetic structure of Nematoda have used few genes (and usually only one, the nuclear small subunit ribosomal RNA gene, or SSU). Some known artifacts of phylogenetic inference using SSU data could significantly affect the reliability of some parts of the trees, and in particular, placement of the root remains problematic. Independent confirmation of the structure of Nematoda could be gained by using multiple genes in analyses, but generation of these large datasets for targets other than SSU is difficult. Using the NEMBASE database of expressed sequence tags (ESTs) from over 40 species from four of the five major clades, it is possible to identify sets of genes that have good representation across nematode diversity. These multigene datasets can now include sequences from Priapulida,

Tardigrada and Onychophora, as ESTs have been generated for species from these ecdysozoan phyla. Using these data I will illustrate congruence and conflict between different genes and the effects of having more robustly sampled outgroup taxa on nematode phylogeny.

Phylogeny and biogeography of Triplonchida. M. Holterman (1) and O. Holovachov (2). (1) Wageningen University, Wageningen, The Netherlands; (2) Department of Nematology, University of California Riverside, Riverside. Phytopathology 97:S132.

A phylogeny of the Triplonchida and Enoplida was constructed using nearly full-length SSU rDNA sequences. Within Clade 1 (Holterman et al. 2006), a well-supported sister relationship was found between orders Triplonchida and Enoplida. The position of some genera did not correspond to current nematode classification: the genera *Trischistoma* (Triplonchida) and *Rhabdolaimus* (Plectida) were nested in the Enoplida, and the genus *Bastiania* (Plectida) was placed in the Triplonchida. Within the Triplonchida, the Prismatolaimidae and Bastianiidae together constituted a sistergroup to the Diphtherophorina, while the Tripylidae and Tobrilidae occupied a basal position. Surprisingly, *Odontolaimus* was positioned in the Diphtherophorina. Two basal Triplon-

chida families are typical inhabitants of terrestrial and freshwater habitats. Although the resolution at the base of the Enoplida was poor, monophyly of virtually all suborders could be confirmed except for Ironina. The molecular phylogeny did not always correspond to current classification, but in most cases supporting morphological features were identified. Triplonchida and Enoplida have a cosmopolitan distribution, and both orders include marine, terrestrial and freshwater representatives. Although SSU rDNA is effective in revealing the phylogenetic relationships within the most basal nematode clade, it does not give us a clue about the habitat in which the common ancestor of all nematodes arose.

Molecular phylogeny of Dorylaimia. P. Mullin (1) and M. Holterman (2). (1) Unaffiliated; (2) Wageningen University, Wageningen, the Netherlands. *Phytopathology* 97:S133.

Relationships within the Dorylaimia have been explored using small subunit (SSU) and large subunit ribosomal DNA (LSU rDNA). The resulting phylogenies depart from the currently accepted hypotheses of relationships in several key aspects. The Mermithida are placed within the Mononchida, and these orders form the sister group to the animal-parasitic Trichinellida and Dioctophymatida. In contrast to previous studies, Dorylaimida are positioned at the base of the Dorylaimia. *Campydora*, formerly considered a member of Dorylaimida, is placed in Enoplida, while *Isolaimium*, another genus traditionally placed in the Dorylaimia, clusters with members of Chromadoria. SSU rDNA analysis confirms the monophyly of Dorylaimida but fails to recover most relationships within the Dorylaimida. Reconstructions using the more variable LSU rDNA distinguish 12 trophically homogeneous subclades within Dorylaimida, but these bear only a passing resemblance to classical Dorylaimida systematics. Good resolution within the Mononchida allowed the development of subclade-specific SSU rDNA primers and these were successfully tested using real-time PCR on samples containing plasmid vectors with the SSU rDNA fragments of target and close non-target species. This constitutes proof-of-principle that in at least some cases nematode (sub-)families can be identified and quantified using SSU/LSU rDNA.

Dorylaimia, Triplonchida and the Nematode Tree of Life project. P. De Ley. University of California-Riverside, Riverside, CA 92521. *Phytopathology* 97:S133.

The NSF-funded "Assembling the Tree of Life" project on nematode phylogeny is entering its final stages. Thanks to rapid developments in sequencing and genomics technologies, our approaches have evolved to place even greater emphasis on coordination of collective effort, infrastructure and new methodologies. The number of published small subunit rDNA sequences from nematodes has more than quadrupled in the past five years, and will continue to skyrocket as more research teams join SSU sequencing efforts and release as yet unpublished data. In several nematode clades, efforts to obtain broad coverage with large subunit rDNA sequences are helping to disentangle polytomies left unresolved by SSU analyses. The subclass Dorylaimia is the last major clade to be the subject of a Tree of Life symposium and workshop. Discussion of diversity and current phylogenetic hypotheses in this clade completes our exploration of taxonomic diversity within the phylum, and leads us to consider methods for resolving the earliest divergences within the nematode tree. As in other nematode clades, molecular phylogenies match previous morphological analyses in some respects, while differing substantially in others. The independent origins of the trichodorid odontostyle and the dorylaimid odontostyle are confirmed by molecular data. Trichodorids are firmly placed within subclass Enoplia in the order Triplonchida, a group that may be of special relevance to the earliest evolution of nematodes.

The who, what, and where of Longidoridae and Trichodoridae. W. Decraemer (1) and R. T. Robbins (2). (1) University of Arkansas, Fayetteville, AR 72701; (2) Royal Museum of Natural History, Brussels, Belgium. *Phytopathology* 97:S133.

Longidoridae and Trichodoridae are the only plant-parasitic Nematoda that transmit plant-viruses. Both families are polyphagous root-ectoparasites. Taxonomically, they are distantly related; Longidoridae are classified Dorylaimia while Trichodoridae belong to Enoplia. Their associated viruses belong to different virus families, nepoviruses for Longidoridae and tobnaviruses for Trichodoridae. Longidoridae includes the Longidorinae (247 species) within six genera (*Longidorus*, *Longidoroides*, *Paralongidorus*, *Australodoris*, *Paraxiphidorus*, *Xiphidorus*) and Xiphineminae (240 species) with one genus (*Xiphinema*). *Longidorus*, *Paralongidorus*, and *Xiphinema* are virus vectors. Trichodoridae includes 99 species classified in five genera (*Trichodorus*, *Paratrichodorus*, *Monotrichodorus*, *Allotrichodorus*, *Ecuadorus*). The two largest genera in number of species *Trichodorus* and *Paratrichodorus* are didelphic and occur worldwide. The monodelphic genera, *Monotrichodorus*, *Allotrichodorus* and *Ecuadorus* have fewer species and have only been recorded from Central America and South America. Only the didelphic genera have virus vector species. The biogeographic distribution

at species level will be presented with reflection on the evolution of the species.

Morphological cladistics and classification of Dorylaimia. A. Coomans (1) and R. P. Santiago (2). (1) Ghent University, Ghent, Belgium; (2) Universidad de Jaén, Jaén, Spain. *Phytopathology* 97:S133.

Recent molecular data have provided new hypotheses on evolutionary relationships of Dorylaimia with their relatives as well between its subgroups. In some cases the new proposals do not fit the traditional (morphology-based) ideas, making the characterization of taxa problematic. The search and analysis of morphological features according to the principles of phylogenetic systematics (cladistics) should bring further evidences and hopefully more solid support to the proposal of a new classification of Dorylaimia. A series of autapomorphies are here proposed to identify the subclass Dorylaimia and its higher subdivisions. The common ancestor of the Dorylaimia was probably characterized by having five pharyngeal glands whose nuclei and outlets were located posteriad (far behind the nerve ring), and a protractor/retractor system from somatic muscular origin, with six labial muscles and eight stomatal muscles. Other relevant features to trace the evolutionary pathways of the group, and having consequences for its classification, are: stoma structure (including presence/absence of tooth-teeth/odontostyle), the relative position of pharyngeal gland nuclei and outlets, pharynx morphology (cylindrical, flask-shaped), cardiac glands (presence/absence), caudal glands (presence/absence), etc.

Methods of culturing predatory nematodes. A. L. Bilgrami. Cape May County, Cape May Court House, NJ 08210. *Phytopathology* 97:S133.

Culturing predatory nematodes is relatively simple, but tricky as the success depends upon parameters such as duration of life cycle, food type, feeding habit, culturing conditions etc. Some predatory nematodes such as mononchs have specific requirements and feed exclusively on prey nematodes. Others are biphasic and switch over to feed on bacteria in the absence of prey nematodes (e.g., diplogasterids). Stylet bearing predators feed on fungi and bacteria besides prey nematodes. Starting and maintaining cultures is labor-intensive, especially if more than one species is to be grown. Some species are easy to grow (diplogasterids) whereas others are nearly impossible to cultivate (some species of mononchs). Contamination is always a problem that affects entire predatory nematode culture. Predatory nematodes may adapt to artificial selection of the medium used at any time resulting in the improvement of culture conditions. In general, predatory nematodes can be cultured in three phases. 1) Seed culture (starting up one or more from a soil extract, which contain mixtures of all kinds of soil organisms as well as relatively low numbers of predatory nematodes). 2) Mass culture (from a successful seed culture, in order to have sufficient specimens for standardizing culture conditions without undue fear of losing the strain). 3) Stock culture (kept in fairly standardized conditions to minimize sudden decline due to unpredictable changes in storage condition).

Systematics and phylogeography of Mononchida. A. Zullini (1) and P. Mullin (2). (1) Università Milano-Bicocca, Milan, Italy; (2) Unaffiliated. *Phytopathology* 97:S133.

Mononchida have long been of interest for their predaceous behavior, their potential as biological control agents, and their utility as environmental indicators. Relationships within the group have been elucidated primarily based on morphology of the stoma and pharynx, and, to a lesser extent, the caudal region. Based on these characters, the order contains two suborders (Bathyodontina and Mononchina), four superfamilies, and six families, in which 41 genera have been arranged. A phenetic analysis has been made based on the stomatal features of the Mononchina genera. A multivariate analysis of the location of the pharyngeal openings shows a coherent distribution at the family level. Investigations of biogeography have suggested some trends, but the data for many taxa are sparse or lacking. Molecular phylogenies based on large and small subunit ribosomal DNA sequences find good support for some morphologically-based taxa (e.g., Bathyodontidae and Mylonchulidae), but taxon sampling has to date been insufficient to draw robust conclusions for some groups. Of interest, however, is the apparent paraphyly of Mononchida; strong support is found, using SSU sequence data, for the inclusion of the entomoparasitic Mermithida within this order.

Comparative mitochondrial genomics and molecular phylogeny of the Mermithida. B. C. Hyman. University of California-Riverside, Riverside, CA 92521. *Phytopathology* 97:S133.

Signature features of Neoplean nematode mitochondrial DNAs (mtDNAs) include lengthy sequence duplications, inversions, and a striking absence of gene order conservation. Within the family Mermithidae, comparative mitochondrial genomics has enabled modeling the molecular evolution of diverse mtDNA architectures within a more confined taxonomic unit. Rolling

circle mtDNA amplification facilitated determination of complete nucleotide sequences for eight mermithid mitochondrial genomes including three *Romanomermis* and two *Thaumamermis* congeners. Diverse mitochondrial genome organizations were observed at the subfamily, genus, and species levels indicating a rapid rate of mtDNA rearrangement is occurring in the mermithid lineage. The first molecular phylogeny for the Mermithidae was constructed using nuclear 18S rDNA sequences for the purpose of mapping mtDNA rearrangements onto a framework that may help identify ancestral mtDNA forms. The topology of the molecular framework was reminiscent of hypothesized affinities provided by Gafurov (1997) based on morphology and life history traits. This molecular phylogeny has also proven useful for characterizing new mermithid parasitism of terrestrial arthropods.

Systematics and phylogeography of Mermithida. G. O. Poinar Jr. (1) and E. G. Platzer (2). (1) Oregon State University, Corvallis, OR 97331; (2) University of California Riverside, Riverside, CA 92521. *Phytopathology* 97:S134.

Diseases of Plants

Biology and Management of Dollar Spot in Turf

An overview of Sclerotiniaceae. L. M. Kohn. University of Toronto Mississauga. *Phytopathology* 97:S134.

The Sclerotiniaceae in the broad sense of Whetzel (1945) is now two families represented by monophyletic clades, the Rutstroemiaceae and Sclerotiniaceae (Holst-Jensen, Kohn and Schumacher, 1997). The Rutstroemiaceae includes genera with substrate-incorporating stromata and, according to the Outline of Ascomycota (2006) comprises the genera *Lambertella*, Höhn., *Lanzia* Sacc., *Poculum* Velen., *Rutstroemia* P. Karst. and, (perhaps) *Scleromitrella* S. Imai. *Sclerotinia homoeocarpa* is in the Rutstroemiaceae based on molecular phylogenetics. Since little is known about their life histories, which I will discuss, we cannot reject the possibility that the Rutstroemiaceae includes pathogens, even of monocots, in addition to *S. homoeocarpa*. The Sclerotiniaceae, with tuberoid sclerotia, includes 34 genera, including copro- or lignophiles, obligate or facultative plant biotrophs and necrotrophic plant parasites. Again there are taxa with cryptic life histories, although the models, *Sclerotinia sclerotiorum* and *Botryotinia fuckeliana*, are necrotrophs. *S. homoeocarpa* may not be an anomaly in the Rutstroemiaceae.

Demystifying Sclerotinia pathosystems: Lessons from Sclerotinia blight of peanut. B. B. Shew, D. L. Smith, J. E. Hollowell, and T. G. Isleib. North Carolina State University. *Phytopathology* 97:S134.

Sclerotinia blight caused by *S. minor* is a serious disease of peanut, *Arachis hypogaea*. Soilborne sclerotia germinate and infect myceliogenically. All parts of the plant are susceptible. Together with field studies, detached leaf, stem, and whole plant inoculation methods were used to understand the ecology and epidemiology of Sclerotinia blight. In controlled studies, sclerotia germinated at relatively high temperatures (up to 30°C), whereas disease developed most rapidly at lower temperatures (18 to 22°C). Similarly, in field studies, rapid disease increase in cool, wet weather often followed initial disease outbreaks in hot weather. Disease control was most effective when fungicides were applied at disease onset. High levels of partial resistance have been identified in the germplasm line N96076L and several high-yielding advanced breeding lines with N96076L parentage are resistant to Sclerotinia blight in field and greenhouse tests. Isolates of *S. minor* differ in aggressiveness, but no specificity to peanut lines has been found. *S. minor* can persist in infested fields following 4-yr rotations to non-hosts. Infection of annual weeds during winter fallow may account for persistence of the pathogen. Fifteen MCG's of *S. minor* have been found on peanut or weed hosts in North Carolina fields; groups do not appear to exhibit specialization on peanut or weed hosts.

Role of oxalic acid as a pathogenicity factor in Sclerotinia. R. Hammerschmidt. Michigan State University. *Phytopathology* 97:S134.

Oxalic acid has been implicated as a pathogenicity factor in *Sclerotinia sclerotiorum* and other necrotrophic pathogens. This simple organic acid has a variety of toxic effects on plant cells, suppresses the oxidative burst associated with early host defenses, and is required for normal pathogen development. The role of oxalate and its biosynthesis will be reviewed. Because of studies on related pathogens, it is likely that *Sclerotinia homoeocarpa* also produces oxalic acid during pathogenesis. In vitro studies demonstrated that *S. homoeocarpa* produces oxalic acid under the same conditions as *S. sclerotiorum*. Infection of bentgrass leaves with *S. homoeocarpa* or *S. sclerotiorum* resulted in a localized increase in oxalic acid at the infection site. Oxalic acid in tissues decreased in lines of bentgrass expressing resistance to *S. homoeocarpa* and in all lines inoculated with *S. sclerotiorum*. Oxalic acid

The Mermithida represent a widely distributed group of invertebrate parasitic nematodes found in the marine, freshwater and terrestrial habitats. The typical life cycle involves an infective second stage juvenile that emerges from the egg and enters the invertebrate host, a parasitic third stage juvenile that develops in the host, then emerges and molts twice (usually simultaneously) to become an adult. Descriptions of the majority of mermithids have been based on relatively few postparasitic juveniles and/or adult forms. Since mermithids are important biological control agents of crop pests and disease vectors, descriptions based on both morphological and molecular characters are required. Phylogeography is an important component of systematics and we propose the use of *Agamermis*, a parasite of orthopterans, *Romanomermis*, a parasite of mosquito larvae and *Heleidomermis*, a parasite of biting midges, as models for mermithid biodiversity and analysis of biogeographic factors affecting the evolution and distribution of mermithid species. Molecular studies with members of the Tetradonematidae are also proposed in order to determine their relationship with Mermithidae and with the other nematode families now placed in the subclass Dorylaimia.

decreases were correlated with an increase in oxalic acid oxidase activity. The bentgrass enzyme activity was partially purified and compared to known oxalate oxidases. Treatment of bentgrass leaves with potassium oxalate or methyl jasmonate induced oxalate oxidase activity and resistance to *S. homoeocarpa*.

Sclerotinia homoeocarpa: From F.T. Bennett forward. B. J. Horvath. Virginia Polytechnic Institute and State University. *Phytopathology* 97:S134.

Dollar spot is caused by *Sclerotinia homoeocarpa* F.T. Bennett, and the disease is one of the most important turfgrass diseases on creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*) in North America. The pathogen was first described in 1937 in the United Kingdom. Only reports from the UK have observed sexual or asexual spore production, and attempts elsewhere have not been successful. As a result, accurate identification and classification of this pathogen has been difficult, and attempts to resolve the classification of this pathogen has relied on the use of molecular techniques. Early research on dollar spot focused on the development of cultural, biological, and chemical management of the disease. Once fungicides were identified as being efficacious on dollar spot, their widespread use resulted in the development of fungicide resistance to many of the classes used for disease control. Fungicide use and availability has become more restricted, and therefore, recent research has sought to develop a better understanding of the biology and epidemiology of this important turfgrass pathogen so that other control measures are more effective. Disease resistant cultivars have also become more prevalent as chemical use has been restricted, and recent efforts to improve creeping bentgrass has resulted in traditionally developed varieties that are much less susceptible to dollar spot. Transgenic varieties expressing resistance have also been tested and show promise. Dollar spot continues to be a challenge to control, and understanding the history of research into the management of this disease will be critical to identifying new avenues of research on this important pathogen.

Biology of Sclerotinia homoeocarpa and epidemiology of dollar spot. M. Boehm. The Ohio State University. *Phytopathology* 97:S134.

Dollar spot, caused by *Sclerotinia homoeocarpa*, is typically referred to as the most widespread and economically important disease of golf course turfgrass. Compared to Sclerotinia species that attack other crops, relatively little is known about the biology, ecology and epidemiology of *S. homoeocarpa*. Turfgrass pathologists aren't sure what to call this pathogen let alone how or where it survives, whether the fungus reproduces sexually or how it attacks turfgrass. We don't know much about the environmental parameters associated with the activity of *S. homoeocarpa*, its population dynamics in turfgrass or the molecular and biochemical basis of how *S. homoeocarpa* parasitizes turfgrass. Such information may ultimately lead to the development of more environmentally-sound and effective dollar spot management strategies. This presentation will provide an overview of what has been published regarding the biology, ecology and epidemiology of *S. homoeocarpa* and will highlight potential areas of future research related to this important turfgrass pathogen.

Taxonomic clarification of the dollar spot pathogen: Sclerotinia homoeocarpa. J. F. Powell and J. M. Vargas, Jr. Michigan State University. *Phytopathology* 97:S134.

The taxonomic status of the dollar spot pathogen, *Sclerotinia homoeocarpa*, has been in question since the 1940's. It has been well-documented that this

organism does not belong to the genus *Sclerotinia*, but should be placed within the genera *Rutstroemia*, *Lanzia*, or *Moellerodiscus*. ITS1 sequences from *Sclerotinia homoeocarpa* isolates from North America and Australia were compared with those of isolates from Britain, the original culture used to describe the species *S. homoeocarpa*, and representative members of the genera of *Rutstroemia*, *Lanzia*, and *Moellerodiscus*. Parsimony analysis identified that *S. homoeocarpa* clustered within the genus *Rutstroemia*, indicating that its generic taxa should be *Rutstroemia* rather than *Sclerotinia*. The teleomorphic strain of *S. homoeocarpa* used to describe the species exhibited closer relations to *Rutstroemia cuniculi* and *R. henningsianum* than to isolates responsible for causing dollar spot disease. The species epithet *homoeocarpa* should be applied to the strain previously identified as the teleomorphic strain of *S. homoeocarpa* and not apply to the pathogen responsible for dollar spot symptoms. The remainder of the isolates responsi-

ble for dollar spot formed a distinct clade. However, the ITS1 and ITS2 sequences of isolates from North America expressed sequence divergences from isolates from Britain of 16 and 15 bases, respectively. Differences in mycelial morphology, stromatal morphology, and temperature tolerances between these groups also exist. Based on these data and the fact that the species epithet *homoeocarpa* cannot be applied to these fungi, new names need to be applied. It is proposed that the dollar spot pathogens of British origin be identified by the epithet *Rutstroemia festucae* to denote the limited host range of this organism in the British Isles. The dollar spot pathogens from North America, including isolates from Australia and the Netherlands, are proposed to be identified as *Rutstroemia floccosum* to denote the woolly/fluffy mycelial growth habit of this organism in culture and as part of the infection cycle.

Free Trade: Challenges to Plant Health

Regulatory scrutiny, plants for planting, and free trade. S. Wager-Pagé. USDA-APHIS, Riverdale, MD. *Phytopathology* 97:S135.

Globalization and free trade agreements contribute to the increasing volume and diversity of plants for planting (P4P) imported into the United States annually. P4P can originate from regions where the pest risk is not well-known. APHIS participates in the development of international standards that provide guidance for the application of phytosanitary measures. These standards provide for use of emergency and/or provisional measures when a new, previously unknown pest is identified; however, a precautionary approach enabling countries to take action on unknown pests has not been adopted. APHIS regulates all P4P imports in order to safeguard U.S. plant health. Current regulations require that P4P with the exception of those identified as high risk for introduction of quarantine pests are enterable with a phytosanitary certificate if inspected and found free of pests. Unlike fruit and vegetable imports, APHIS does not require a pest risk analysis (PRA) in order to establish P4P import requirements; therefore, APHIS is revising the code of federal regulations to increase the level of phytosanitary security. APHIS will propose a regulatory mechanism to restrict importation of some P4P that are a pathway for quarantine pests until a PRA is developed.

Dilemma of safer and freer trade in Florida. W. N. Dixon. Bureau of Entomology, Nematology and Plant Pathology - Gainesville, FL. *Phytopathology* 97:S135.

Florida, a high-risk sentinel state for exotic invasive pests, is the poster child for the condition where the global marketplace and increasing importation of agricultural products and numbers of international travelers is overwhelming the capacity of our safeguarding systems to effectively deter establishment or achieve early detection. Our safeguarding technology, regulatory protocols and available manpower are not adequate in light of Florida's hospitable environment of host plants, climate and geography. Significant economic impacts have resulted from exotic diseases such as citrus canker, leatherleaf fern anthracnose and tomato yellow leaf curl virus. The level of damage from huanglongbing is yet to be realized, but it will be far more than citrus canker. Introduced invasive pests have cost Florida over one billion dollars over a five-year period from 1995 to 2000. The challenge and the dilemma of safer and freer trade in Florida is magnified by nearly 30 ports of entry; over 85% of the plants imported to the U.S. go through Miami; the climate gradient in Florida matches that of temperate, tropical, and subtropical; over six million tons of perishable cargo enters Florida each year; and that nearly 50 million people visit Florida each year, a 20% increase in the last 10 years.

Perspective on free trade from a trading partner. M. Gilkey. USDA-APHIS - Mexico City, Mexico. *Phytopathology* 97:S135.

Canada to the north is our largest trading partner and Mexico to the south is our second largest trading partner. The United States is the largest trading partner for each of the other two countries. Over 300 million border crossings took place between the US and Mexico (US DOT-BTS 2003). Even with significant increases in inspection personnel at every border crossing, the proportion of vehicles and shipments inspected will remain at approximately 2% for harmful organisms to US agriculture and ecosystems. The U.S. and Mexico collaborate on many programs to limit and eradicate harmful pests to agriculture. More specifically, on a bilateral basis both countries have allocated considerable resources to the control and eradication of economically significant fruit flies and other organisms of economic impact. The departments of agriculture in both the United States and Mexico manage

these unwanted plant pests to meet phytosanitary requirements for movement of commodities and to prevent introductions of non-established fruit fly species. Even with extensive exclusion and inspection activities at major US/Mexico ports of entry, strict regulatory procedures, anti-smuggling programs, and carefully designed export/trade work plans, the United States and Mexico continue to spend millions of dollars each year detecting and eradicating incursions of these fruit flies and other pests.

Diagnostics - Are we prepared? J. Stack. Kansas State University - Manhattan, KS. *Phytopathology* 97:S135.

Advances in transportation and communications technologies have led to the dissolution of ecological barriers that in the past naturally regulated the dispersal of biological organisms (i.e., people, animals, plants, microbes, and insects). The rapid movement of pests and pathogens has greatly reduced the time to prepare for emerging diseases and pestilence. Consequently, outbreaks may occur before the development and deployment of appropriate diagnostic tools to facilitate early detection, accurate diagnosis, and rapid response. Over the last few years, a partnership for preparedness for natural and agricultural plant systems was established among USDA (CSREES, APHIS, ARS), state departments of agriculture, the National Plant Board, the IPM Centers, and the National Plant Diagnostic Network. Diagnostic technology is being developed, validated, deployed nationally, and diagnosticians are being trained. Several challenges exist including: 1) the need for greater international cooperation in identifying threats for which diagnostics are required, 2) high throughput diagnostic capability deployed at least regionally, 3) greater emphasis and investment in research on microbial genomics research to enhance diagnostic technology and in research on sampling strategies and logistics, and 4) continued development of the emerging fields of forensic plant pathology and forensic entomology. At present, we are only partially prepared. The magnitude of negative impacts from introductions over the last century is tremendous. In light of globalization and free trade, the potential negative impacts of future introduction are staggering. If we do not adequately prepare, our standard of living and social stability will decline markedly.

Global trade and plant health. D. Avery. Hudson Institute - Churchville, VA. *Phytopathology* 97:S135.

Plant science today faces its biggest challenge since one billion Asians were threatened with famine in the 1960s. Plant science overcame that threat, saved 16 million square miles of forests, and earned a Nobel Peace Prize. The new challenge: provide for a peak human population of 8-9 billion by 2050, plus their pets, without taking more wildlands for crops. Biofuels will add importantly to demands for higher crop yields. China, India, and Indonesia are under intense pressure from dense populations and rising affluence. Brazil is the only country with much underused land. African plant productivity has been stymied primarily by bad governance. Science and trade are the most powerful tools we have to meet the new challenge. Both are under activist attack, but organic farming needs too much land to grow nitrogen and produces weak yields. Local production loses massive comparative advantages. Trade may increase the danger that foreign pathogens and pests will cross national boundaries, but trade and specialization are vital to higher yields. Plant science's recent gains against potato late blight, African witchweed, and Black Sigatoka disease of bananas show how international scientific cooperation can produce plant health solutions. Biotechnology and plant protection chemistry will both be necessary to achieving conservation and a sustainable, higher-yield future.

International Movement of Ornamental and Forestry Diseases

Movement of pathogens on forestry plants shipped internationally. S. Cela. Canadian Forestry, Canada. *Phytopathology* 97:S136.

The trade in plants for planting is an important economic driver internationally. The value of this trade is \$478 million between Canada and the U.S. and \$1.71 billion between the U.S. and other countries. Numerous authors have documented the movement of both invasive plants and insects associated with the trade in plants for planting. Only recently however are regulatory authorities and the scientific community recognizing and documenting the movement of serious invasive plant pathogens on plants moving in international trade. Often these pathogens may have little or no direct impact to the plants upon which they move, but may result in serious impacts to the Canadian environment or to other industries as a result of the imposition of quarantine restrictions or non-tariff trade barriers. This presentation will provide an overview of the importance of the plants for planting pathway in pathogen movement, some examples of pathogens that have moved on the pathway and the impacts these are having to the Canadian environment and economy.

Case study 1: *Ralstonia solanacearum* on geranium. M. Klopmeier. Ball Horticulture Co. *Phytopathology* 97:S136.

In 2003, *Ralstonia solanacearum* Race 3 Biovar 2 (RsR3B2), a select bioterrorism agent and quarantine pest, was accidentally introduced on geranium cuttings imported into the U.S. from Africa and Central America. This initiated a cavalcade of events involving state and Federal phytosanitary officials, basic research scientists and industry stakeholders (growers and geranium propagators) resulting in new pest management procedures (domestically and internationally) to prevent further accidental introductions. The impact of these new procedures on the geranium industry will be discussed.

The U.S. ornamental horticulture industry perspective on international plant pathogen movement and steps to mitigate spread - greenhouse crops. L. Schmale. SAF. *Phytopathology* 97:S136.

The movement of plant pathogens movement is a global problem – and we must increase our international efforts to address it. Of course, each nation reserves the right to protect its own agriculture and its own environment against pathogens introduced as a result of trade. However, with increased

global trade, there tends to be an increase in the spread of pathogens (and other pests). Quarantines often penalize our domestic growers, and sometimes fail in their protection goal, as well. Simple border inspection is not usually adequate to prevent introductions. Improved diagnostic tools can help – but can also “create” new problems. Moving to a “systems approach” can be effective, particularly in combination with a true clean stock program. There is no one easy answer – but the answers will only be found in increased scientific knowledge and more inter-industry and international cooperation. One good, albeit early, example of that kind of cooperation involves *Bemisia tabaci*, a pest in its own right, and also a vector of several significant viruses. This cooperative effort, if extended to the international level, could provide one new paradigm for helping stop or mitigate the spread of plant pests. USDA-APHIS is revising the Quarantine 37 regulations to try to bring protection of U.S. agriculture and resources into the 21st Century. That effort may provide new insights, as well. In the final analysis, of course, any success will depend upon the quantity and quality of the underlying science.

The U.S. ornamental horticulture industry perspective on international plant pathogen movement and steps to mitigate spread - nursery crops. M. Teffeu. ANLA. *Phytopathology* 97:S136.

With the increased globalization of trade, the U.S. nursery industry has become an unfortunate victim of the introduction of foreign plant pathogens, resulting in millions of dollars of crop loss on the producer and retail level. One of the consequences of these introductions is that Federal and State plant inspection authorities have become unavoidable “partners” with industry in the production and marketing of nursery crops. Using *Phytophthora ramorum* as an example, USDA APHIS has had to develop “Certified Nursery Protocols” to deal with this quarantined pathogen to eradicate it from crop production sites and prevent its introduction into other areas of the U.S. Whether through known or undetermined channels, the presence of current and potential non-native, foreign introduced plant pathogens has resulted in the nursery industry re-evaluating its response to these pests throughout the entire crop production, shipping and retailing chain. Currently under review is the development “systems approaches”, based on the HACCP concept, to prevent the introduction of foreign plant pathogens into production systems and to all the nursery industry to respond in a rapid and proactive basis. These approaches need to be supported by appropriate research and evaluated for effectiveness. In addition, there is discussion within the industry as to how to respond to possible changes in Quarantine 37 regulations, NAPPO RSPM 24 and related guidelines.

Stem Rust: A Threat to Global Wheat Production

The global threat posed by Ug99. R. Ward. CIMMYT, Mexico. *Phytopathology* 97:S136.

Stem rust race Ug99 (a.k.a. TTKS), first formally observed in 1998 in Uganda, was initially noticed because it defeated the widely used major gene *Sr31*. By 2005 Ug99 colonized the wheat fields of Kenya and Ethiopia and is now known to be in Yemen. Ug99 has also spawned a mutant that is virulent to *Sr24*. The predicted immediate path of Ug99 to South Asia covers a region which produces 19 percent of the world’s wheat (ca. 117 million tons) with a population of one billion people. North Africa, Central Asia, China, Australia, Europe and the Americas also lay exposed to resurrection of stem rust as a persistent threat to wheat. Ug99 and its derivatives are a strategic threat to world wheat production- and therefore the livelihood and food security of millions of poor people- as well as the overall health of the global economy. Collaborative research under the umbrella of the CIMMYT-ICARDA led Global Rust Initiative (GRI), initiated and Chaired by Dr. Norman E. Borlaug, has established that Ug99 defeats most race-specific resistance genes used in commercial varieties grown throughout the world. The GRI (see <http://www.globalrust.org>) seeks to mobilize global scientific assets to systematically reduce the threat of Ug99 while maintaining and increasing resistances to leaf and yellow rusts.

Progress in the identification and utilization of adult-plant resistance to *Puccinia graminis tritici* race Ug99 (TTKS) in CIMMYT spring wheats. R. P. SINGH (1), Y. Jin (2), J. Huerta-Espino (3), P. Njau (4), and R. W. Ward (2). (1) Internacional Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600, Mexico, DF; (2) USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN; (3) INIFAP-CEVAMEX, Apdo. Postal 10, 56230, Chapingo, México; (4) KARI-NPBR, P.O. Njoro, Kenya. *Phytopathology* 97:S136.

The spread of race Ug99 (TTKS) of *Puccinia graminis tritici*, the causal organism of stem (or black) rust, from East Africa to Arabian Peninsula poses a major threat to wheat production worldwide due to the susceptibility of a

majority of current cultivars and breeding materials. Testing of wheat seedlings in the greenhouse and adult plants under high disease pressure in the field have identified wheat materials that carry high to moderate but adequate levels of adult-plant resistance (APR). Among these materials are included old-tall Kenyan cultivars, 4-5 minor, additive genes carrying APR wheat stocks developed by D. R. Knott in late 1970s, older semidwarf CIMMYT-derived cultivars, and new high-yielding materials. The best APR wheat line from CIMMYT displayed a very low disease score (5MR) during both years of field testing. A common feature of all the above APR lines was the presence of known durable slow rusting gene *Sr2* in combination with other unknown number of minor genes referred as the “*Sr2*-Complex”. Because complex APR is expected to be durable, a breeding scheme that includes shuttling of segregating populations between Mexico and Kenya has been implemented to enhance the incorporation of APR in a wider range of popular wheat cultivars and promising wheat germplasm. Identifying APR genes and linked molecular markers are also being pursued to aid selection of APR in the presence of effective race-specific resistance genes.

Evolution and adaptation within *Puccinia graminis*. L. J. Szabo. USDA ARS Cereal Disease Laboratory, St. Paul, MN. *Phytopathology* 97:S136.

Puccinia graminis is a macrocyclic, heteroecious rust fungus with a broad host range including more than 50 genera of cereals and grasses. This species is commonly subdivided into six to eight formae speciales, based on telial host range. Molecular and genomic tools are currently being applied to understand the evolution of this species. *P. graminis* f. sp. *tritici*, the causal agent of wheat stem rust, is the most extensively studied form of *P. graminis*. Race surveys have been performed to monitor populations of *P. graminis* f. sp. *tritici* and determine the effectiveness of stem rust resistance genes. Changes in race structure are commonly observed as the rust adapts by over coming resistance genes. A recent example of this is the discovery of the isolate Ug99 in Uganda with virulence to *Sr31*. Simple sequence repeat (SSR) marker analysis indicated that Ug99 (race TTKS with virulence to *Sr31*, TTKS v*Sr31*) represents a distinct genetic lineage from the race clusters found in North America. In addition, the a new race with virulence to *Sr24* and *Sr31* (race TTKS v*Sr24vSr31*) found in Kenya in 2006 has an identical SSR

genotype. These results indicate that this new race (TTKS vSr24vSr31) represents an adaptation within Ug99 lineage rather than occurrence of a new genotype.

New virulence within race TTKS (Ug99) of the stem rust pathogen and effective resistance genes. Y. JIN (1), Z. A. Pretorius (2), and R. P. Singh (3). (1) USDA-ARS Cereal Disease Laboratory, University of Minnesota, St. Paul, MN; (2) Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa; (3) International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600, Mexico, DF. Phytopathology 97:S137.

Stem rust resistance gene *Sr24* in wheat is a valuable gene, effective against most races of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*)

worldwide. It is highly effective against race TTKS (or Ug99), a recently emerged race in Eastern Africa that is considered to be a serious threat to wheat production worldwide. In 2006, moderately susceptible to susceptible infection responses were observed on wheat lines and cultivars carrying *Sr24* in a field stem rust screening nursery planted in Njoro, Kenya. Pure cultures derived by single-pustule isolation were tested on seedling plants of the stem rust differentials and tester stocks carrying various stem rust resistance genes. Isolates were identified to be race TTKS with virulence on *Sr24*, indicating the occurrence of a new variant within race TTKS. Genes effective against this new variant include: *Sr13*, 22, 25, 26, 27, 28, 29, 32, 35, 36, 37, 39, 40, 44, *Tmp*, and stem rust resistance present on the 1A.1R translocation. The combination of virulence on *Sr24* and *Sr31* has substantially increased the vulnerability of wheat to stem rust worldwide.

Epidemiology/Ecology/Environmental Biology

Addressing Today's Critical Issues in Disease/Pathogen Assessment

To honor Horsfall-Barrett and repeal their scale. F. W. Nutter, Jr. Iowa State University, Ames, IA. Phytopathology 97:S137.

The accuracy and precision of disease severity assessments might be improved if there was a better understanding of how the laws of psychophysics actually relate to the theory and practice of phytopathometry. In 1945, Horsfall and Barrett proposed that rating scales to assess disease severity should be logarithmic. They claimed (without experimentation) that "visual acuity is proportional to the logarithm of the stimulus when visually assessing disease severity". Horsfall and Barrett further claimed that raters could not accurately discriminate among disease severity levels between 25 and 50% because, according to the Weber-Fechner Law. Using the method of comparison stimuli to test this claim, it was demonstrated quite conclusively using two different pathosystems (wheat leaf rust and grapevine downy mildew) that raters could discriminate among different levels of severity, and that while Weber's law was found to be applicable when visually assessing disease severity, Fechner's law did not apply. In both pathosystems it was found that raters could discriminate among disease severity levels between 25 and 50% according to a linear model (not logarithmic). Therefore, based upon actual experimentation, it is proposed that rating scales should be linear and not logarithmic, as has been the theory and practice for more than sixty years.

Assessing the severity of phoma stem canker in canola. J.-N. Aubertot. INRA, Paris, France. Phytopathology 97:S137.

Phoma stem canker (caused by *Leptosphaeria maculans*) is one of the main diseases that affect canola world-wide. Sound assessment of phoma stem canker symptoms is required for plant breeding, as well as epidemiological, physiological and agronomic studies. The disease is usually characterised by the visual assessment of the severity of cankers created by the pathogen at the crown level of the plants according to a rating scale based on the percentage severity estimates of cross-section cankered crowns. Observations are summarised according to a disease index. This presentation will introduce i) a mathematical tool to help define the sample size as a function of the precision required on the disease index; ii) an innovative computer-aided training program to guide raters. A relationship between the coefficient of variation of the disease index and the sample size was established, under the hypothesis that the symptoms follow a multinomial distribution (severity grades). Predicted coefficients of variation were compared with observed coefficients of variation of experimental field data. This validated the proposed relationship which was then exemplified for typical severity distributions. Like many other rating systems for disease severity, rater bias effects have been identified when assessing the severity of phoma stem canker on canola. A computer-assisted training program, named Phomadidacte, has been developed to guide raters on how to use the rating scale. The basic principle of this program consists in displaying pictures of cankered cross-sections of canola previously rated by a panel of eight independent experts and comparing the two sets of assessments. The efficacy of Phomadidacte was tested in an experiment which compared the grades given by two groups of ten raters who had been trained either with or without using Phomadidacte with those given by a panel of three experts (using diseased field samples). Of all of the participants, only three raters, all from the Phomadidacte group, succeeded in having a percentage of agreement with the experts greater than 75%, whereas none of the raters in the other group succeeded in achieving this threshold. The generic methods described in this paper can certainly be applied with success to other pathosystems.

Standard area diagrams: Do they improve the accuracy and precision of disease assessment data? S. J. Michereff. Universidade Federal Rural de Pernambuco. Phytopathology 97:S137.

Standard area diagrams (SAD) are representations of a series of plants, a plant or parts of the plant with symptoms in different severity levels. The SAD are important auxiliary tools in the disease assessment, because they serve as visual guide for the raters. After the elaboration, SAD should be submitted to a rigorous validation process for analysis of the accuracy and precision levels of the estimative. Accuracy refers to the proximity of an estimate to a real value of disease amount, while precision refers to the variation or repeatability associated with an estimate. As example, of 28 SAD elaborated for quantification of the disease severity in tropical crops for my research group, 25% were reproved in the validation phase, because the accuracy and precision levels didn't differ significantly of the evaluations without the use of SAD. The validation should not only be accomplished by experienced raters, because sometimes those raters accomplish the estimates intuitively and they ignore the SAD. Besides, the estimates in field not always they are accomplished by experienced raters and developed SAD cannot capture the perception difference. Even after a rigorous validation process, several factors can interfere in the obtaining of improvements in the estimates of disease severity, such as plants presenting leaves with different morphologic types (okra), different cultivars with variations in the leaf morphology (cowpea) and in the type of disease symptom (sugarcane), it defoliation due to the plant physiology (yam) or due to the disease (peanut). Therefore, the simple use of SAD doesn't guarantee the improvement of the accuracy and precision levels of the estimative of disease severity, should be considered several other factors during the elaboration, validation and utilization phases.

Detecting and quantifying the temporal and spatial dynamics of plant pathogens using GPS, GIS and remote-sensing technologies. K. Ahmad. Iowa State University, Ames, IA. Phytopathology 97:S137.

The goal of this research project was to test the hypothesis that plant pathogens and pests differentially impact the temporal and spatial patterns of healthy (green) leaf area index and that by measuring percentage reflectance (intensity) in the near-infrared, temporal and spatial patterns unique to each plant pathogen and pest can be used as the basis to correctly identify the cause(s) of plant stress in crops. To test this hypothesis, IKONOS satellite imagery (1 m resolution) was obtained from soybean fields located in Argentina (2005) and South Africa (2006). Images were rectified, processed and analyzed using IIMAGINE and ArcGIS softwares. Disease gradients of Asian soybean rust (change in image intensity with respect to distance from a local area source of inoculum) were detected and quantified by fitting Gregory's ($\ln X - \ln Y$), Kiasow and Shiyomi's (linear $X - \ln Y$), or Fry's (linear $X - \logit Y$) disease gradient models. These models explained up to 89.9, 93.6, and 86% of the variation in disease intensity (R²), respectively. Temporal and spatial (area, shape) changes in image intensities (representing changes in green leaf area index) indicated that focal expansion rates of Asian soybean rust foci can be used to differentiate Asian soybean rust from other diseases of soybean, thus providing a GIS, GPS, remote sensing-based method to correctly diagnose the cause(s) of crop stress.

Pathogen threat assessment is predictive plant pathology. D. G. Luster. USDA/ARS Ft. Detrick, Frederick, MD. Phytopathology 97:S137.

The American Society of Plant Pathologists has maintained a formal effort to prioritize threatening and emerging crop pathogens for over 70 years, and the APS Emerging Pathogens and Diseases Committee is continuing the process. In order to accomplish prioritization in a rigorous fashion, criteria must be developed to assess critical aspects of a pathogen's threats, risks and costs, in

advance of an introduction. While the development of criteria addressing pathways of introduction, economic costs, and social issues is relatively straightforward, the generation of criteria encompassing all biological factors involved in the introduction of a pathogen is more complex. Criteria development for biological factors is based upon the disease triangle, in that criteria are designed to help predict whether a new pathogen genotype could establish, reproduce and spread in a new climate on susceptible host plants.

Moreover, in order to be useful in assessing the relative risk posed by an introduction, quantitative scales must be developed to serve as metrics for the individual biological factors. The quantification of critical biological factors is complicated by differences in pathogen taxonomy, life cycle environment, and host. This presentation will address recent efforts by the APS EPDC to develop robust, quantitative criteria for pathogen prioritization.

Approaches for Predicting Establishment and Expansion of Exotic Invasive Forest Pathogens

Principles of invasion biology: A foundation to predict pathogen invasiveness. R. C. Venette. USDA Forest Service - Northern Research Station, St. Paul, MN. Phytopathology 97:S138.

General principles have emerged from numerous cases of exotic species invasions that explain why certain taxa may (not) invade. These principles have been most rigorously tested for plants and animals, yet should apply to pathogens as well. Factors contributing to invasion success include the number of propagules introduced into an area; the capacity for propagule production and persistence; climatic similarity between source and recipient areas; history of successful invasion; and taxonomic dissimilarity to resident taxa. Extrinsic factors such as the abundance and density of susceptible host plants, pathways to move pathogens over major geographic barriers, the economic and environmental value of affected are also critical to predict the likelihood and impact of pathogen invasion. Unfortunately, for many taxa (not only pathogens), these factors are not known precisely. Nevertheless, even qualitative descriptions can be integrated within a multicriteria decision making framework to rank exotic pathogens from most to least threatening. Such a framework will be described for exotic fungi and nematodes.

Disjunct plants between North America and eastern Asia: A potential source of invasive pathogens. Q. Guo. USDA Forest Service-Southern Research Station, Asheville, NC. Phytopathology 97:S138.

Although invasive pathogens can cause enormous, long-term damage to diverse forest ecosystems, efforts to predict sources of potentially invasive forest pathogens are quite limited. Because of similar climates, geographical settings, and floras, eastern Asia (EA) and North America (NA) share ca 120 genera of paleo-disjunct plants, and 1,567 reciprocally introduced plants, which comprise potentially invasive, neo-disjunct plants. Although infection by an introduced pathogen seems most likely to occur on similar hosts under similar environments as its origin, these relationships can be better defined to predict potentially invasive pathogens. Regional plant diversity is about 1/3 higher in EA than in NA, which may limit or promote pathogen establishment and spread. The likelihood that a suitable host will occur increases with diversity, but reduced host prevalence also reduces chances of pathogen establishment and spread. Predictions of potentially invasive pathogens can be based on comparisons of richness and performance of pathogens and hosts in both native and exotic ranges in EA and NA. We can incorporate concepts of origin, host-specificity, enemy release, and distribution to help predict areas where potentially invasive pathogens could be exchanged, so that early detection/prevention efforts can be prioritized.

Predicting potentially invasive pathogens by phylogenetic analyses of intercontinental relationships among plant hosts and pathogens. B. A. RICHARDSON, M.-S. Kim, N. B. Klopfenstein, and P. J. Zambino. USDA Forest Service – RMRS, Moscow, ID. Phytopathology 97:S138.

Since the break up of Laurasia, geographic separation of Asia and North America has allowed disjunct plant and pathogen communities to follow different evolutionary paths. As a result, some plants in Asia coevolved as hosts of different pathogens than related plant species in North America, and vice versa. Phylogenetic relationships among disjunct host plant taxa may provide a basis for predicting exotic hosts for some pathogens. For example, sister taxa of plant hosts existing in North America and Asia may represent potential sources for introductions of invasive pathogens between continents,

if other conditions are conducive for pathogen establishment. In addition, previous studies suggest that introduced pathogens can become more aggressive or virulent if permitted to hybridize and introgress with related species, races, or populations. Here, we discuss phylogenetic relationships among Asian and North American populations of the white pine blister rust fungus, *Cronartium ribicola*, and some of its hosts. Phylogenetics of this pathosystem will be used to explore concepts for identifying source populations, evaluating risks of introductions, and identifying potential hosts in North America.

Application of phylogeographic analysis to predict sources of potentially invasive forest pathogens. M.-S. KIM, N. B. Klopfenstein, J. W. Hanna, P. J. Zambino, and B. A. Richardson. USDA Forest Service, Rocky Mountain Research Station, Moscow, ID. Phytopathology 97:S138.

Determining the distribution of forest pathogen species and genetic variants across geographic and evolutionary scales is essential for 1) identifying species with potential to act as invasive plant pathogens in other settings, 2) detecting their arrival, and 3) assessing their potential for interacting with native species. In addition, hybridization among variants and/or closely related species represents an additional risk for generating novel invasive pathogens. Phylogeographic analyses of endemic/established forest pathogens provide baseline information to determine whether invasive pathogens are the result of introduction, changing environments, or hybridization. Unfortunately, global phylogeography of plant pathogens is largely undetermined. The utility of phylogeographic analysis has been demonstrated in preliminary studies on *Armillaria* spp. Phylogenetic and phylogeographic studies of the endemic pathogen *Armillaria ostoyae* show significant genetic variation among North American regions, and allow comparisons with global distributions. Interspecific and intraspecific hybridization are both evident in *Armillaria* species from North America. A more complete survey of genetic variation within *A. ostoyae* and closely related species is needed for Asia, Europe, and North America to assess potential risks of introductions and subsequent risks associated with hybridization and introgression.

Phylogeography and ecology of pine rusts: A basis for insights into invasive forest pathogens. M.-M. Chen. University of California-Berkeley, Berkeley, CA. Phytopathology 97:S138.

Pine rust fungi cause costly diseases in nurseries, plantations, and forests of the Northern Temperate zone. These taxonomically complex fungi are often heteroecious, having coevolved with specific aecial and telial hosts of diverse distributions in North America (NA), East Asia (EA), and Europe (E). Of 3 species recently reviewed (Chen 2006), *Cronartium ribicola* parasitizes 12 white pine species and 24 *Ribes* or *Pedicularis* in NA, EA, and E; *C. quercuum* infects 27 pine species and 28 *Quercus* species in NA and EA; and *C. coleosporioides* is known to utilize 7 pine species and several Scrophulariaceae alternate hosts in NA. Theories for relationships among pine rust fungi and hosts are provided by Chen (2004). White pine blister rust was putatively introduced to NA from an external source, but the source lineage remains unidentified. Determining sources of invasive pathogens is difficult, because invasiveness depends on complex interacting factors, e.g., ecosystem imbalances, disturbances, host maladaptation/susceptibility, and lack of biological control. Phylogeographic analyses based on molecular evolutionary genetics, natural history, population biology, paleontology, and speciation processes provide a means to predict potential sources of invasive rust pathogens. This information can help prioritize efforts to prevent introductions and spread of pine rust pathogens.

Cross Domain Bacteria: Emerging Threats to Plant, Humans, and our Food Supply

***Escherichia coli* O157:H7 on spinach and lettuce; environmental investigations in the Salinas region of pre-harvest contamination.** M. B. Cooley, D. Chao, and R. E. Mandrell. USDA, ARS, WRRC, 800 Buchanan St., Albany, CA 94710. Phytopathology 97:S138.

Escherichia coli O157:H7 (EcO157) is an enteric pathogen that can contaminate fresh produce. Several recent outbreaks associated with lettuce and spinach have focused attention on Salinas, CA and the surrounding region. Fifty seven MultiLocus Variable number tandem repeat Analysis (MLVA) EcO157 types were recovered from the Salinas watershed. Phylogenetic analysis indicated that related EcO157 types were generally isolated in the same proximity. Furthermore, a point source of EcO157 contamination was

discovered. During a low stream flow situation, closely related pathogens were only detectable up to 139 m downstream from the point source. Nevertheless, during flooding events, identical strains were collected from the watershed at points 24 km apart, indicating that long-distance transport may be possible under these circumstances. During the baby spinach outbreak investigation (2006), samples taken on or near 2 implicated farms yielded 32 MLVA types and these were again clustered spatially. Furthermore, Eco157 strains identical to the outbreak strain were isolated from several sample types (cattle, water, sediment, dust and feral pig) within a few miles of one implicated field. These data suggest that MLVA typing is an effective high-throughput investigation tool of the transport of Eco157 in agricultural environments, especially following pre-harvest contamination events.

Foodborne outbreaks related to fresh produce: The public health challenge of detection and response. R. V. Tauxe. Division of Foodborne, Bacterial and Mycotic Diseases, National Center for Zoonotic, Vectorborne, and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30306. *Phytopathology* 97:S139.

Recent nationwide outbreaks of infections with *E. coli* O157:H7 linked to leafy green vegetables and of salmonellosis linked to fresh tomatoes highlight a growing trend. Since the 1970's, the foodborne outbreaks linked to fresh produce have increased in number, size, and in the proportion of all foodborne outbreaks for which they account. In the 1970's, a mean of 2 such outbreaks were reported each year, with an average size of 21 cases of illness apiece. By the 1990's (through 1997) this had increased to 16 outbreaks per year, and an average size of 43 cases. In 1998, surveillance for foodborne disease outbreaks was enhanced, resulting in an increased number of reports of all foodborne outbreaks, including those from produce. The proportion of outbreak-associated illnesses from outbreaks of known food source that was accounted for by fresh produce typically consumed raw increased from 0.6% in the 1970's to 12% the 1990's to 14% for the period 1998–2004. This contribution includes only the outbreaks caused by a single produce item – the contribution would be higher if complex foods that include produce were included. For the period 1998–2004, 384 produce-associated outbreaks and 15,384 associated cases were reported to CDC, with a mean size of 42 cases per outbreak. Among the 190 for which the etiology was reported, 53 were caused by *Salmonella*, 19 by Shiga toxin-producing *E. coli* O157, 73 by norovirus, 8 by Hepatitis A virus, and 5 by the parasite *Cyclospora*. The most frequently implicated foods were leafy greens, sprouts, tomatoes, melons, juice and green onions. The raw produce-associated *E. coli* O157 outbreaks were most often linked to leafy greens, and the *Salmonella* outbreaks were often linked to tomatoes. The introduction of Hazard Analysis–Critical Control Point methodologies to the fruit juice industry was followed by a decrease in those outbreaks. In many of the recent bacterial outbreaks, the most likely point of contamination was pre- or peri-harvest. Recent observations of the behavior of enteric bacteria in and around domesticated plants indicate that they can readily contaminate the edible plant tissues before, during or after harvest, that they appear to be well-adapted to persist in on and inside food plants, and that eliminating them after contamination has occurred is difficult. As some of these foods are often eaten without further cooking, preventing pre- or peri-harvest contamination is the key to decreasing human illness. Better understanding of the dynamics of the interactions between bacteria that are pathogenic for humans, the plants we eat, and the environments in which they are grown and processed is needed to clarify points of intervention or control that will reduce the risk of foodborne disease.

Human bacterial pathogens on fresh produce, what we know and research needs. J. D. Barak. USDA, ARS, WRRRC, Produce Safety and Microbiology Research Unit, 800 Buchanan St., Albany, CA 94710. *Phytopathology* 97:S139.

Salmonella enterica and pathogenic *E. coli* cause the highest proportion of fresh produce-linked epidemics. However, large differences exist among these

enteric pathogens in their ability to attach to and colonize plant surfaces. Enteric pathogens utilize specific molecular mechanisms to attach to and colonize plants, some of which have a role in animal pathogenicity and virulence. Enteric pathogens, animal and plant, share environmental niches, water, insects, and plants. Furthermore both utilize similar mechanisms for plant colonization. *S. enterica* can grow on plant surfaces and reach high populations; however, the presence of disease or plant-associated bacteria in the absence of disease multiplies the *S. enterica* populations. The utilization of plants as vectors for enteric animal pathogens between hosts causes a unique public health concern as removal of the bacteria following colonization has been unsuccessful and decontamination strategies damage fresh produce. The separation of pathogens by their ecological niche, animal or plant, now appears to be artificial and future research will benefit from a shift of paradigm regarding the biology of these pathogens.

***Burkholderia*: “A Jack of all trades”.** C. F. Gonzalez (1) and J. J. LiPuma (2). (1) Department of Plant Pathology & Microbiology, Texas A&M University, 2132 TAMU, College Station, TX 77843; (2) Department of Pediatrics and Communicable Disease, University of Michigan Medical School, Ann Arbor, MI 48109. *Phytopathology* 97:S139.

The genus *Burkholderia* is comprised of more than 40 species that occupy an array of ecological niches. We have traditionally thought of *Burkholderia* species as plant pathogens, owing to the description of *Burkholderia* (*Pseudomonas*) *cepacia* as the causative agent of soft rot of onions more than 50 years ago. However, it has become clear that the interactions of members of this genus with a wide variety of hosts range from beneficial to pathogenic to host dependent. Furthermore, the interaction of *Burkholderia* with their hosts is complex and can cross domains. These observations suggest that *Burkholderia* are environmental “generalists” capable of expanding their host range and niche in the environment.

Niche adaptation by *Serratia marcescens*: A versatile enterobacterial pathogen of many hosts. J. Fletcher. Oklahoma State University, Stillwater, OK. *Phytopathology* 97:S139.

Cucurbit yellow vine disease (CYVD) is a serious disease in many cucurbit production areas of the U.S. The causal bacterium was identified as the Enterobacteriaceae *Serratia marcescens* (Sm), based on 16S rDNA, *groE* sequence and DNA-DNA hybridization. However, strains of Sm from a variety of ecological niches (water, soil, humans, animals, insects, plants) show considerable phenotypic heterogeneity. CYVD strains have identical Rep-PCR patterns regardless of cucurbit host, geographic location, and year of isolation, and were clearly differentiated from other strains. Pathogenicity tests on a variety of plant species showed that differences in symptoms and in percentage of plants infected correlated with bacterial strain. Our results demonstrate that Sm strains from non-plant niches can cause symptoms in plants, but their interactions with the plant host differ from those of CYVD strains. CYVD strains of Sm have been associated with the host phloem and are transmitted from plant to plant by the squash bug, *Anasa tristis*. When the CYVD pathogenic strain, ZO1-A, and a non-pathogenic rice endophytic strain, RO2-A, were tagged with green fluorescent protein (GFP), labeled bacteria could be recovered from plants 5 wk after inoculation. Fluorescence and confocal microscopy revealed that Sm dropped onto the leaf surface did remained on the surface, while bacteria forced into the intercellular spaces by vacuum infiltration remained in the vicinity of the stomata through which they entered, apparently not moving to, or entering, the vascular bundles. In contrast, labeled bacteria introduced into squash stems via an inoculating fork were seen within xylem vessels above and below the inoculation sites. The colonization and translocation of Sm in squash plants may be dependent upon how and where the bacteria were introduced. Whether the same would be true of other Enterobacteriaceae species, such as *E. coli* O157:H7 in leafy greens, is a question of great interest.

Information Technologies for Multi-Scale Disease Forecasting and Surveillance Systems

Architecture of Pest Information Platforms: Past, present and future. S. Isard. Pennsylvania State University, State College, PA. *Phytopathology* 97:S139.

The USDA Soybean Rust Information System was constructed in 2005 to serve as the cyberinfrastructure for the USDA Coordinated Soybean Rust Framework, and transitioned into the Pest Information Platform for Extension and Education (PIPE) the following year. The PIPE is people connected by computers using advanced IT to add value to field observations. The goals of

the system are to enhance support for IPM decision making and provide information for documenting good management practices for crop insurance purposes. Currently the platform is structured to enhance the efficiency of the six tasks (field observation, data management, modeling, integration of information, interpretation, and dissemination) required to provide effective decision support for pest management. It is anticipated that the field observation task will become increasingly mechanized while the data management, modeling, and integration of information tasks will be collapsed into a single component (engine) over the next few years. However within the near future, people will remain the key component of the PIPE, devoting a greater proportion of their time to interpretation and dissemination of information.

Field and diagnostic data collection: (Part 3) University sources. J. Stack. Kansas State University. *Phytopathology* 97:S140.

Universities are an important source of organized and informal forecasting and surveillance data for plant-based production systems. The Land Grant University-based National Plant Diagnostic Network (NPDN) has become a significant and credible source of plant diagnostic data and routinely contributes to the national plant disease and pest database. Most NPDN labs are well equipped and staffed by well trained diagnosticians. Samples submitted to NPDN labs come from intensive production field, fruit, and vegetable crop systems, glass-house ornamental, fruit, and vegetable production systems, and backyard gardens. Informal forecasting and surveillance data are routinely collected and disseminated locally by plant breeders, extension specialists, and researchers. Breeders often conduct disease assessments on a wide array of plant genotypes in many states and in other countries. These data are not often used outside of the breeding program. Extension personnel routinely conduct disease surveys and make informal, and often accurate, forecasts. These and other informal data sources are rarely captured for analysis. Challenges to the exploitation of formal and informal data sources include the need for appropriate policies regarding access to data, ownership of data submitted to NPDN labs, data quality and verification, and incentives for submitting data to a centralized database.

Field and diagnostic data collection: (Part 2) Industry sources. W. Dolezal. Pioneer Hi-Bred International, Inc, Johnston, IA. *Phytopathology* 97:S140.

Accurate monitoring of the plant health of American agriculture necessitates the involvement of all agricultural segments: governmental agencies, universities and the agricultural industry. Many members of the industry conduct: 1) scouting of research and seed production fields for pests; 2) phytosanitary field inspections; and 3) provide disease diagnostics testing for their customers. Much of the private sector pest monitoring & diagnostic activities are not being utilized in current government pest monitoring programs. The USDA PIPE program proved to be an excellent vehicle for fostering collaborative efforts between the public and private sector in monitoring for Asian Soybean Rust (*Phakopsora pachyrhizi*). The American Seed Trade Association's Phytosanitary Committee has developed a draft proposal for a similar PIPE type program, for a narrow list of new and emerging pests for corn, soybeans and watermelon. Adding private industry pest data into a USDA central database can provide: i) useful pest distribution information to state extension specialists; ii) more resources at no or little cost to APHIS in monitoring for emerging & exotic pest threats; and iii) a conduit to build greater public & private pest monitoring collaborations. This collaborative pest monitoring database will provide improved pest distribution records for state and federal regulators to enhance their decision making.

Field and diagnostic data collection: (Part 1) Government sources. R. Magarey. North Carolina State University, Raleigh, NC. *Phytopathology* 97:S140.

The Pest Information Platform is a good example of how universities, government, and industry can share survey and diagnostic data as well as pest data sheets, model output and management recommendations. USDA-APHIS played a key role in creating the soybean rust PIPE but the challenge is to identify how it can continue to interact as the PIPE develops. One of the key roles for APHIS is as a data provider in the area of exotic pest detection. For example, the Cooperative Agricultural Pest Survey collects survey data on exotic pests in all 50 states. PPQ has data on many important exotic pests, including biological data sheets, survey methods, risk analyses, pest models, and US port interception data. It is possible that some of this data may be shared with stakeholders if adequate data sharing protocols could be developed. Including high risk exotic pests in future pest information systems would be mutually beneficial to all parties involved. This is especially true for building a pest platform that links information sources for multiple stakeholders proactively, rather than immediately after a high risk exotic pest detection.

The operational deployment of models: Why some fails and others succeed. E. De Wolf (1) and S. Isard (2). (1) Kansas State University, Manhattan, KS; (2) Pennsylvania State University, State College, PA. *Phytopathology* 97:S140.

During the past 12 years, more than 50 refereed reports were published documenting research efforts to develop disease prediction models and evaluate their potential role in disease management. However, relatively few of these models were deployed as part of operational disease forecasting systems. The success of a forecasting system depends on the importance of the disease to crop production, frequency of epidemics, and probability of an efficacious management response. Recent advances in information technology allow for unprecedented amounts of weather observations to be stored,

processed and the results displayed as part of disease forecasting systems. Yet, technology alone does not translate into success. The modeling results are most useful when accompanied by the interpretation of an extension specialist or other disease expert. The future of disease forecasting is bright if plant pathologists can continue to leverage emerging technologies and provide useful tools for plant disease management.

The interpretation of model output: Making sense of it all. A. Mila. North Carolina State University, Raleigh, NC. *Phytopathology* 97:S140.

The development of new Internet technologies has enabled the creation of more complex disease forecasts, which can be distributed to stakeholders with increasing ease. As it becomes easier to create model output, interpretation becomes an increasingly important consideration. In this presentation, the effect of model assumptions and the importance of knowledge of the biology of a pathosystem on interpreting model outputs will be discussed. Is there any model output that answers all questions? Or is the output a result of the question asked? Model output, from an ANOVA analysis to a Bayesian hierarchical model, may create disagreement among plant pathologists. Why is that? In some cases because of the model assumptions used, in some others because the output is not well supported by available knowledge on biology and epidemiology of the system that is modeled. The objective of this presentation is to stimulate discussion on how model interpretation is directly related to model development (i.e. data collection, assumptions on model structure etc) and how limitations in interpretation may be overcome. Two examples will be presented: prevalence of Sclerotinia Stem Rot of soybean in 5 states of North-Central US and distribution of races of *Phytophthora nicotianae* in North Carolina.

Dissemination to stakeholders (Part 1). D. Hershman. University of Kentucky, Princeton, KY. *Phytopathology* 97:S140.

Prior to the first occurrence of soybean rust (SBR) in the US (November 2004), SBR information was provided to stakeholders through diverse, but disconnected, outlets. A more coordinated information system was needed to deal with SBR information needs. Thus, the SBR Coordinated Framework was developed by USDA-APHIS, which led to the establishment of the SBR-Pest Information Platform for Extension and Education (PIPE) in 2005. The SBR-PIPE provides a means for integrating, summarizing, and posting near real-time SBR data for use by a range of stakeholders. Data are collected by state extension specialists from a large network of sentinel and transient plots. Specialists then use the SBR-PIPE to develop and refine SBR recommendations, which are then distributed to stakeholders via a web-based public interface. This interface provides a map showing the extent of SBR monitoring efforts and current (and past) distribution of SBR. State SBR situation commentaries, developed by state specialists, are also made available, as are a host of additional documents, such as state fungicide spray guides. The platform also provides access to numerous links and tools with direct application to SBR education and management. The SBR-PIPE is unique in that stakeholders can gain access to a large amount of SBR information from 35 states and Canada through a single website. Additional crop and pest PIPEs are under development.

California systems to disseminate disease modeling information. J. Strand. University of California, Davis, CA. *Phytopathology* 97:S140.

When California began a concerted effort to promote the use of models to improve plant disease management, the UC Statewide IPM Program and industry began programs to verify models in the field and to put weather data, forecasts, and model results into the hands of end users. Use has been by growers or consultants who make on-farm crop protection decisions, rather than for identification of trends or disease migration. Distribution of model results for disease management has been through subscriptions operated by agricultural service companies and by advertising-funded, publicly available commercial services. University Web sites also supply model results at small numbers of weather stations, but their primary purpose is for extension personnel to use in training growers and consultants. While the Web is the primary delivery mechanism for model outputs, some growers take advantage of improved results they can gain from monitoring data in their own orchard, vineyard, or field. Challenges include developing common ways of distributing and displaying results from different models to support learning and how best to convey to users the risks associated with various components of the modeling system.

Challenges and opportunities for internet-based disease information systems. G. Bergstrom. Cornell University, Ithaca, NY. *Phytopathology* 97:S140.

The preceding speakers have outlined the latest developments on components of contemporary, internet-based, pest information systems. One model system, the USDA Pest Information Platform for Extension and Education (PIPE),

delivers useful disease and insect pest information to U.S. producers of soybean and vegetable legumes and is being adapted for information delivery on other crops and pests. Grower and consultant interface systems like PIPE could be enhanced by effective integration with pest databases from private industry as well as databases on exotic pests from government regulatory agencies. The identity and needs of primary and secondary users should be considered in information system content and design. Disease forecasters must collaborate to provide harmonized model output and interpretation. Various

models exist for provision and funding of pest information systems, from government-financed and free public access to private, subscription-based services. To sustain collaborative pest information systems, plant pathologists and other specialists from universities, private industry, and government must be enabled, recognized, and supported for the time they allocate to data collection, input, and interpretation for end-users. As these systems mature, technologies or protocols that minimize the time demands on these contributors should be developed.

Interkingdom Encounters in the Phyllosphere

Phages don't have it easy. B. Balogh (1), F. B. Iriarte (1), A. Obradovic (2), M. T. Momol (3), and J. B. Jones (1). (1) University of Florida-IFAS, Gainesville, FL; (2) University of Belgrade, Belgrade-Zemun, Serbia; (3) University of Florida-IFAS, Quincy, FL. *Phytopathology* 97:S141.

Bacteriophage-based biological control of tomato bacterial spot is a relatively well-studied area, and tomato bacterial spot is presently the only plant disease in the US against which a commercial bacteriophage product is available. Experiences with this pathosystem highlighted a major problem that compromised effective disease control. Phage residual activity is extremely short in the field: phage populations can plummet with as much as a hundred fold reduction per hour after application and can fall to undetectable levels in a day. The major cause of this reduction is sunlight radiation (UVA+B). Evening and dawn phage applications attenuated the sunlight effect resulting in increased persistence of high phage populations and subsequent better disease control. Formulations that provided partial protection from sunlight also enhanced phage survival rates and contributed to better disease control. Differences in persistence of individual phages have been observed and it is possible that application of more UV tolerant phages will lead to better control. Phages also vary in their ability to multiply in the phyllosphere. Preliminary data suggest that the more prolific phages are also more effective in disease control. Thus, applying the right phage at the right time and with the right formulation may get the job done.

***Pseudomonas syringae* exploits programmed cell death in fungi for competitive gain and modulation of its epiphytic behavior by plants.** S. E. Lindow, G. Wichmann, and G. Dulla. University of California, Berkeley, CA. *Phytopathology* 97:S141.

Many *P. syringae* strains harbor a protein, which we term Phc, that has significant homology to fungal Het-c proteins involved in heterokaryon incompatibility (HI). Transformation of *phc* from *P. syringae* B728a or DC3000 into wild type *Neurospora crassa* strains resulted in transformants with the classic HI phenotypes of drastically reduced growth rates, lack of conidial production, and cells undergoing a process known as hyphal compartmentation and death. *P. syringae* strains are able to colonize and move along fungal hyphae and multiply on the surface of such fungi, particularly at sites of dead hyphal cells. *P. syringae* may thus induce HI in fungi via delivery of Phc to overcome nutrient limitations on leaves. The majority of cells of *P. syringae* are found in relatively large cell aggregates on the surface of healthy bean leaves, facilitating quorum sensing (QS) via its production of 3-oxo-hexanoyl homoserine lactone. QS, which is directly related to levels of ferric iron, enhances epiphytic fitness via extracellular polysaccharide production but suppresses swarming motility and thereby invasion of leaves. Plant tannins released onto leaves sequester iron and thus suppress QS in *P. syringae*. Thus cross talk between plants and *P. syringae* involving iron chelators can greatly influence the behavior of this pathogen.

Very small sheep: How Tydeid mites interact with powdery mildews and plant hosts. D. M. Gadoury (1), R. C. Seem (1), W. F. Wilcox (1), G. Loeb (2), A. P. Norton (3), and H. Melidossia (4). (1) Dept. Plant Pathology, (2) Dept. Entomology, Cornell University, NY; (3) Colorado State University, Fort Collins, CO; (4) Pace International, Rochester, NY. *Phytopathology* 97:S141.

Powdery mildews are a unique class of biotrophic fungal plant pathogens whose thallus, with the exception of haustoria in epidermal cells, is wholly external to their host. As such, they are especially susceptible to grazing by mycophagous mites in the family Tydeidae. One Tydeid species (*Orthotydeus*

lambi) provides partial control of grape powdery mildew (*Uncinula necator*). Field and laboratory experiments on ten different grape cultivars provided evidence of a tritrophic interaction, in which *U. necator* served as a food source for *O. lambi*, the host plant benefited from suppression of disease, and *O. lambi* benefited from the presence of a complex arrangement of leaf trichomes in vein axils (domatia), which provided refuges from predators. *O. lambi* substantially reduced powdery mildew on grapevine foliage and fruit, although the magnitude of disease suppression was greater on some cultivars than others, depending on mite density and innate susceptibility to grape powdery mildew. Maintaining dense and effective populations of *O. lambi* required careful selection of pesticides applied against powdery mildew and other members of the pest complex on grapevine. The potential of *O. lambi* for biological control of powdery mildews and inherent limitations of the system for commercially relevant disease suppression will be discussed.

***Lysobacter-Magnaporthe* interactions: A model for bacterial pathogenesis of fungi.** D. Y. Kobayashi. Rutgers University, New Brunswick, NJ. *Phytopathology* 97:S141.

The genus *Lysobacter* is comprised of predatory gliding bacteria capable of antagonizing a broad range of microbial hosts. Strains of the species *L. enzymogenes* have been characterized as biological control agents for numerous diseases, including many caused by foliar fungal pathogens. Several of the mechanisms associated with *Lysobacter* antagonism towards fungi, including production of cell wall degrading enzymes and antibiotics, have also been linked to biocontrol activity. Recently, direct interaction studies between *L. enzymogenes* and *Magnaporthe* spp. have indicated the bacterium is capable of establishing more complex, pathogenic associations with fungal hosts compared to simple parasitic associations resulting from the activity of enzymes and antibiotics. The bacterium colonizes fungal mycelia in a distinctive manner that can differ between strains of *M. oryzae*, as well as between other fungal taxa, and is capable of colonizing fungal hyphae both externally and internally. Experiments using mutant derivative strains indicate *L. enzymogenes* utilizes several antagonism mechanisms, including enzymes, secondary metabolites and a type III secretion system, possibly in a stage specific manner to successfully colonize its fungal hosts.

Protozoa and enteric pathogens: More than a grazing game. M. T. Brandl (1), P. Gourabathini (2), and S. G. Berk (2). (1) USDA, ARS, Albany, CA; (2) Tennessee Technological University, Cookeville, TN. *Phytopathology* 97:S141.

Protozoa are ubiquitous in water, in soil and on plants, where they graze on bacteria. We have investigated the interaction of ciliated protozoa with various bacterial species, with emphasis on enteric pathogens that have caused outbreaks of illness linked to vegetables. Several species of protozoa have been isolated from lettuce and spinach leaves, including *Glaucoma* sp., *Tetrahymena* sp., and *Colpoda steinii*. During grazing on GFP-*Salmonella enterica* and -*E. coli* O157:H7, *Tetrahymena* and *Glaucoma*, but not *Colpoda*, released large numbers of vesicles containing viable bacterial cells. In contrast, most *L. monocytogenes* cells were digested after phagocytosis, suggesting a relative specificity in this interaction. *Tetrahymena* produced vesicles also during feeding on *Pseudomonas chlororaphis*, a common epiphyte. The survival rate of *S. enterica* in *Tetrahymena* vesicles was significantly higher than that of cells remaining free in suspension. Additionally, *S. enterica*-containing vesicles were observed on leaves colonized by this pathogen and then inoculated with the protist. The presence of bacteriophagous protozoa in the phyllosphere and their sequestration of bacteria as small aggregates in vesicles, which may offer protection against environmental stresses, indicate a new role for these organisms in the ecology of bacterial colonists on plants.

Rhizosphere Communities and Plant Health

Impact of soil health management on nematodes and soilborne diseases. B. K. Gugino and G. Abawi. Department of Plant Pathology, NYSAES, Cornell University. *Phytopathology* 97:S141.

Growers and agriculture service providers have observed and documented a reduction in crop quality and yield as related to a gradual degradation in soil health. The latter is evident by the occurrence of compacted layers, poor aggregate stability, low organic matter and increased pressure from pathogens and other pests. The new concept of soil health employs a holistic approach

for integrating and optimizing the physical, chemical and biological properties of soil to enhance soil functions including plant health and productivity in an economic and environmentally sustainable manner. Sustainable management practices for improving soil health include reduced tillage systems, alternative crop rotations, cover crops and organic matter amendments. Research results have demonstrated that all these practices significantly impact soil biology and especially the population of plant pathogens, epidemiology of resultant diseases and crop damage. The effect of soil management practices on the major soilborne vegetable pathogens prevalent in New York and the northeast will be described. The challenge will be to develop sustainable IPM programs for managing soilborne pathogens that are compatible and integrated with long-term sustainable soil management.

Nematode assemblages as indicators for plant health. H. Ferris. Department of Nematology, University of California, Davis. Phytopathology 97:S142.

Soil nematode assemblages include representatives that obtain food from higher and lower plants, bacteria, fungi, or from other soil organisms. Feeding on higher plants may be considered detrimental if the plants have economic or aesthetic value; it may be beneficial if the plants are considered undesirable. Generally, a preponderance of herbivores in the nematode assemblage is an indicator that recent soil management has diminished functional diversity. Together with other soil organisms, nematodes perform important ecosystem services in healthy and productive soils; they enhance mineral cycling, transport bacteria and fungi to untapped resources, enhance microbial turnover, provide resources to other organisms, and regulate opportunistic species. Absent continued input of mineral fertilizers, a healthy and productive soil requires an active soil food web to provide resources for plants and to regulate populations of root herbivores. Nematodes participate in activities at most functional levels in well-structured soil food webs. Consequently, analysis of the nematode fauna indicates the extent to which important ecosystem services are being performed by nematodes and by their functional counterparts in other groups of soil organisms. The management challenge is to enhance the diversity and abundance of beneficial soil organisms so that the ecosystem services are in concordance with the needs of healthy plants.

Manipulation of rhizosphere bacterial communities to induce disease suppressive soils. M. Mazzola. USDA-ARS Tree Fruit Research Lab, Wenatchee, WA. Phytopathology 97:S142.

The biological nature and operative functional groups have been described for several disease suppressive soils. Transforming this knowledge into effective field-level disease management requires strategies that selectively promote the operative microbial population. Plant cultivation is a viable means to manipulate the composition and function of rhizosphere bacteria for disease suppression. Several examples exist of suppressive soils developing in response to crop monoculture, and in several cases disease control corresponds with specific transformations in composition of the rhizosphere bacterial community. Efficacy of soil amendments in disease control is commonly attributed to a general increase in microbial activity or, in the case of brassicaceae plant residues, generation of biologically active chemistries. However, suppression of root rot incited by *Rhizoctonia solani* in response to several different brassicaceae seed meal amendments required an active soil microbial community. Disease suppression is associated with elevated *Streptomyces* rhizosphere populations, and individual strains from seed meal

amended soils provided disease control to an equivalent level and in similar manner. These and additional studies demonstrate that management of resident microbial resources holds promise as a means to achieve the biological suppression of soil-borne diseases.

Linking arbuscular mycorrhizal fungi with plant health: Mechanisms and challenges. S. Hu (1) and T. Rufty (2). (1) Department of Plant Pathology and (2) Department of Crop Science, North Carolina State University, Raleigh, NC 27695. Phytopathology 97:S142.

Arbuscular mycorrhizal (AM) fungi colonize roots of terrestrial plants and contribute to plant health through enhancing plant resistance to abiotic and biotic stresses. In low fertility, acid soils, which exist in many parts of the world, AM fungi can enhance plant uptake of nutrients and alleviate aluminium and manganese toxicities. Recent evidence indicates that mycorrhizae also facilitate nutrient acquisition from decomposing organic residues and large-scale transfer of nutrients between plants. Results from controlled environment studies show that AM fungi often induce plant defense responses and suppress plant pests and pathogens. The relationships are difficult to assess in field, so complexities of mycorrhizal fungi and pathogen interactions and their regulation remain unresolved at this time. Enhancement of mycorrhizal fungal populations can provide the greatest benefit to plant hosts in highly degraded soils, where the number and species diversity of native AM fungi are low. And, a recent area of emphasis is manipulation of mycorrhizal populations to assist land sustainability projects attempting to increase crop productivity in alternative agroecosystems or restore areas to native vegetation. An understanding of the relationship between mycorrhizal diversity and function, and mycorrhizal interactions with other beneficial microbes and soil-borne pathogens in the rhizosphere seems prerequisite for development of management practices that optimize the potential benefits of mycorrhizae.

Soil health indicators and the challenge of understanding how organic mulches affect root health of berry crops in the field. T. A. Forge, D. O’Gorman, C. Koch, X. Wu, and A. Muehlchen. Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre. Phytopathology 97:S142.

Root growth of perennial fruit crops is enhanced by organic mulches. This enhanced root growth is the result of interrelated changes in soil physico-chemical properties and several biological processes, including pathogen suppression and enhanced nutrient cycling. We studied the effects of a variety of organic mulches on root growth, soil health indicators (nematode community structure, physico-chemical properties), nutrient fluxes, oomycete community structure, and abundance of root-lesion nematodes, *Pratylenchus penetrans*, in the root zone of red raspberry. Compost and manure treatments with the lowest *P. penetrans* abundance also produced the greatest root biomass. Nematode indicators of enhanced nutrient cycling were positively correlated with fine root biomass. While actual nutrient fluxes and other nematode indicators of soil food web structure were affected by mulch treatments, the changes were not related to changes in root biomass. Hybridization arrays, PCR and greenhouse bioassays indicated the presence of several *Pythium* species that could also be contributing to the observed differences in root growth. Changes in *P. penetrans* abundance explained changes in root growth better than any other biotic or abiotic variable assessed. The relationships of *Pythium* spp. and enrichment opportunist nematodes with root growth deserve additional study.

The Ecological Complexities of Biological Control: Trophic Cascades, Spatial Heterogeneity, and Behavioral Ecology

Spatial variation in top-down control, multiple enemy interactions, and the probability for trophic cascades. R. F. Denno. University of Maryland Entomology Department, University Park, MD. Phytopathology 97:S142.

A key question in biological control is how multiple natural enemies interact to collectively suppress populations of pest herbivores. Interactions among natural enemies can be antagonistic, synergistic, or simply additive, which can alter the strength of top-down control, herbivore suppression, and the probability for trophic cascades. Moreover, spatial subsidies of predators and local habitat structure can moderate or intensify multiple enemy effects and mediate the strength of top-down forces. Thus, from a biocontrol perspective, it becomes essential to critically assess the nature of interactions among natural enemies, how such interactions affect herbivore populations, and how habitat and landscape structure might alter top-down control and its cascading effects to plant resources. We conducted manipulative experiments in an

arthropod-dominated grassland to assess the effects of predator diversity on food-web dynamics. We found a significant negative relationship between predator diversity and the ability of the predator complex to suppress herbivores, a relationship that resulted from extensive intraguild predation. However, increasing habitat complexity reduced intraguild predation by providing spatial refuges for intraguild prey. Associated with dampened intraguild predation was increased top-down control of herbivores and the occurrence of a trophic cascade. Across the landscape, spatial variation in habitat structure results in gradients in the strength of top-down control. The consequences of food-web complexity are compared between arthropod- and nematode-dominated systems.

Constraining complexity to achieve effective biological control of *Diaprepes abbreviatus* in Florida citrus orchards. L. Duncan, R. Stuart, and J. Graham. Citrus Research and Education Center. Phytopathology 97:S142.

The Diaprepes root weevil, *Diaprepes abbreviatus*, is an exotic pest of citrus, ornamentals, other crops and native plants in Florida, Texas and California.

Observations in Florida indicate that weevil population levels and damage to citrus trees is greater in orchards growing on shallow poorly-drained fine-textured sandy soils in the “flatwoods” than in orchards growing on the deep well-drained coarse sandy soils of the “central ridge”. Caged sentinel weevil larvae buried beneath citrus trees on the central ridge are killed by endemic entomopathogenic nematodes (EPNs) at rates as high as 70% per week during certain periods of the year whereas mortality rates in flatwoods orchards rarely exceed 10% per week. Significant spatial and temporal relationships between EPNs and nematophagous fungi (NF) on the central ridge are consistent with the hypothesis that steinernematid EPN populations are directly regulated by NF and that heterorhabditid EPNs are indirectly regulated by the influence of NF on steinernematid competitors. Current laboratory and field studies are assessing these kinds of food web interactions for a variety of sites and soil types in an effort to enhance the effectiveness of weevil biological control programs through habitat manipulation and both augmentation and conservation of EPNs.

Spacial ecology of food webs with entomopathogenic nematodes. D. Strong. Section of Evolution and Ecology, UC Davis. Phytopathology 97:S143.

During last decade a combination of conceptual and technical tools has greatly accelerated science in the rhizosphere. Molecular biology, ecology, and evolution have combined to provide extraordinary insights of the rhizosphere as an environment more viscous and finely heterogeneous than the more familiar aboveground aerial or purely aquatic milieus. We have learned that living roots and foodwebs of soil organisms is an environment in which ecological neighborhoods are smaller, grain is finer, dispersal more restricted, and abiotic factors (gravity, moisture, temperature, and nutrients) vary over shorter distances than the aerial and aquatic realms that we have known quite well for a century or more. Powerful and subtle synergies are coming to light. Rhizosphere mutualisms with mycorrhizae and rhizobia, which provide water and nutrients to plants, have recently been shown to be delicate states, subject to a web of interactions with other microbes and readily ranging into parasitism and pathogenesis. By grazing upon these bacteria and fungi, larger eukaryotes (nematodes, mites, enchytraeid worms, collembola, and detritus shredding insects) are probably also important actors in these foodwebs, either promoting plant growth by releasing mineral nutrients or the opposite by short circuiting nutrient flow. Finally, root pathogens and herbivores have can have hugely harmful effects upon plants, which natural enemies of these harmful organisms can reverse. The key notion of foodweb ecology applies in spades to the interspecific interactions of the rhizosphere, indirect interactions originating several trophic steps away from the root have large influences upon plant fitness. These interactions are ancient, as bacteria, fungi, and other eukaryotes have evolved together for the 400 MY since the origins of roots and their progenitor organs. No longer is the biology of the rhizosphere “out of sight, and out of mind”.

Applications of spatial and temporal ecology to management of plant parasitic nematodes. B. Westerdahl. Department of Nematology, UC Davis. Phytopathology 97:S143.

The spatial and temporal ecology of plant parasitic nematodes has been examined on a number of crops by various researchers. Examples are (1) root-knot nematode on annual crops including potatoes, carrots, sugarbeets, and tomatoes; (2) ring nematode on perennial crops such as peaches, almonds, prunes and walnuts; (3) lesion nematode on walnuts and Easter lilies; (4) citrus, root-knot and dagger nematode on grapes; and (5) spiral, ring, root-knot and *Anguina pacificae* on turfgrass. The results of these studies will be discussed in the context of their contribution to the integrated pest management of plant parasitic nematodes.

Molecular/Cellular Plant–Microbe Interactions

Advances in Bioengineered Resistance to Nematodes

Transfer and enhancement of natural R genes for nematode resistance. I. Kaloshian. University of California, Riverside, CA. Phytopathology 97:S143.

Plant wild germplasms have been a rich source of disease resistance (R) genes. Several nematode resistance genes were first identified in wild relatives of crops and then introduced to cultivated species. However, the introgression process was often slow because of linkage drag of undesirable traits frequently associated with wild plant species. An example is the tomato R gene *Mi-1* that confers resistance to three species of root-knot nematodes,

From trophic cascades to biological control: Does it all come down to how individual parasites make infection decisions? E. Lewis and G. Stevens. Dept. of Nematology, UC Davis, Davis, CA. Phytopathology 97:S143.

Entomopathogenic nematodes (EPNs) are common in soil around the world and they sometimes reduce host insect populations in natural conditions. But natural EPN populations are usually distributed in ways that limit their large-scale impact on hosts. Thus, these natural populations require augmentation to render acceptable levels of pest reduction. How does EPN behavior interact with environmental characteristics to determine their distribution in soil? Our data suggest that infection decisions made by EPN infective stage juveniles (IJs) are part of a multi-component feedback loop that reinforces EPN patchy distributions in soil. When given a choice between an infected host and a healthy uninfected host, IJs of *Steinernema glaseri* will invade the infected one. Given the same choice, *S. carpocapsae* will usually invade the uninfected one. When natural populations of these EPN species are sampled, *S. glaseri* is more highly aggregated than is *S. carpocapsae*. We suggest that infection decisions of individuals relate to population distributions for EPNs in a species-specific manner and that EPN behavior may limit the potential to manipulate their population structure in agricultural systems.

A realistic appraisal of attractants involved in nematode orientation to roots. R. Perry. Rothamstead Research. Phytopathology 97:S143.

The response of plant-parasitic nematodes to various stimuli has been the subject of much research, principally in the context of root location. Such stimuli can be divided broadly into three distance-based classes. ‘Long distance attractants’ enable nematodes to migrate to the root area. Movement to individual roots (using ‘short distance attractants’) and movement of juveniles of endoparasitic nematodes to preferred invasion sites (using ‘local attractants’) depend on gradients set up from the root surface into the soil medium. Such gradients are likely to include volatile and non-volatile chemicals and electrical signals. The techniques used to assess nematode responses have been mainly agar plate or electrophysiological assays and there are only a few studies using three-dimensional methods. However, it is not always possible to translate the results of *in vitro* behavioural bioassays to the reality of the soil environment without knowledge of plant and soil biology. This talk will examine some of the putative attractants in the context of information on plant physiology and the root environment, especially in terms of the temporal and spatial attributes of root-induced gradients.

Trophic phylogeography: A research program for examining ecosystem assembly and functioning within a historical coevolutionary framework. B. Adams and D. Wall. BYU, Dept. of Microbiology and Molecular Biology; Department of Biology and Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO. Phytopathology 97:S143.

A pressing issue facing ecosystem ecologists today is the ability to predict how biodiversity will respond to global changes (land use, climate change). Additionally, little is known of the roles biotic and abiotic factors play in ecosystem assembly over space and time. We suggest that understanding how biodiversity responded to change in the past offers a logical framework for examining the range of potential changes that can be expected to occur in the future. Research programs in historical ecology, evolutionary biology, and molecular systematics have developed analytical tools that can inform the universe of possible future evolutionary responses. We propose a novel method for investigating soil food webs and distribution ecology that treats the players in soil food webs as co-evolving entities in time and space, with additional consideration for geophysical constraints. We suggest that our approach can reveal patterns of soil community assembly over time and space, and serve as a framework to test hypotheses of biotic and geophysical drivers of trophic relationships.

potato aphids, and whiteflies. *Mi-1* was identified in *Solanum peruvianum* in the 1940s but it took over two decades to introgress *Mi-1* into tomato, *Solanum lycopersicum*. Another limitation in this process has been identifying the nature of the R gene. For example, more than a decade was required to clone *Mi-1*. Using virus-induced gene silencing, it is now possible to identify R genes and devise efficient cloning strategies. Once a gene is cloned, it can be quickly transferred into desirable cultivars using stable plant transformation. Such an approach was used to identify *Mi-9*, a heat stable root-knot nematode R gene in *Solanum arcanum*. Approaches used in *Mi-9* discovery will be presented as an example for identification and transfer of R genes into cultivated crops.

Parasitism genes: Novel targets for engineering universal root-knot nematode resistance by RNAi. R. S. HUSSEY (1), G. Huang (1), E. L. Davis (2), and T. J. Baum (3). (1) University of Georgia, Athens, GA; (2) North Carolina State University, Raleigh, NC; (3) Iowa State University, Ames, IA. *Phytopathology* 97:S144.

Root-knot nematode infection and parasitism of plants is mediated by parasitism proteins coded by parasitism genes expressed in the esophageal glands and secreted through the stylet. These parasitism genes provide desirable targets for genetically engineering root-knot nematode resistant plants. Parasitism gene *16D10* encodes a conserved root-knot nematode secretory peptide that stimulates root growth and functions as a ligand for a putative plant transcription factor. RNA interference (RNAi) was used to silence this parasitism gene in root-knot nematodes. Silencing of *16D10* in root-knot nematodes by expressing *16D10* dsRNA in *Arabidopsis* resulted in transgenic plants that are resistant to the four common species of root-knot nematode. Since no known resistance gene has this wide effective range of root-knot nematode resistance, bioengineering crops expressing dsRNA that silence target root-knot nematode parasitism genes to disrupt the parasitic process represents a viable and flexible means to develop novel durable root-knot resistant crops. Indeed, RNAi silencing of parasitism genes could provide crops with unprecedented broad-spectrum resistance to root-knot nematodes.

Nematicidal Bt crystal proteins targeting plant endoparasitic nematodes. X. Li, A. Tan, M. Voegtline, S. Bekele, J. Wei, and R. V. Aroian. University of California, San Diego. *Phytopathology* 97:S144.

Plant-parasitic nematodes (PPNs) cause significant economic losses worldwide. The current control strategies of PPNs include crop rotation, resistant varieties, chemical nematicides, and Integrated Pest Management. Under the Montreal Protocol, a major chemical nematicide, methyl bromide, is to be phased out in the near future. Transgenic plants expressing the environment-friendly and vertebrate-safe insecticidal crystal (Cry) proteins of *Bacillus thuringiensis* (Bt) are one of the modern breakthroughs in crop sciences. This technology has resulted in significant reductions in toxic chemical pesticide use and in significant gains in crop yields for corn and cotton. Our laboratory has demonstrated that Cry proteins present in the Cry5 and Cry6 subclades are

toxic to phylogenetically diverse free-living nematodes. These data suggest that Bt Cry proteins might have utility in controlling parasitic nematodes. We thus set out to test the hypothesis that Cry proteins expressed in transgenic plants might provide protection against plant-parasitic nematodes. We modified the codons of three different nematicidal Cry genes for expression in plants and transfected these three genes in tomato roots using *Agrobacterium rhizogenes*. We succeeded in generating tomato root lines expressing each of these three Cry proteins. We then infected these transgenic roots with root-knot nematode, *Meloidogyne incognita*. Here, we will present the results of these studies that demonstrate the utility of some nematicidal Cry proteins in controlling *M. incognita* and the potential to one day use transgenic Cry-expressing plants to control PPNs.

Optimizing biotech crops for nematode resistance. H. J. ATKINSON and P. E. Urwin. Centre for Plant Sciences, University of Leeds, UK. *Phytopathology* 97:S144.

Transgenic expression of cysteine proteinase inhibitors (cystatins) provides 70–80% crop resistance to many nematodes. This general effect is valuable for crops damaged by more than one nematode species. Enhanced resistance levels result from stacking cystatins with natural partial resistance. Additive resistance of a cystatin with chemoreception-disrupting peptides or RNA interference may be essential for durability. Biosafety requires case-by-case study of the transgene, the crop and where it is to be grown. Cystatins have proven useful for exploring many biosafety issues surrounding uptake of biotech crops for nematode control. They are not a toxic or allergenic hazard to humans. They have an acceptable margin of exposure when expressed in food. Root-specific and nematode responsive promoters help exclude even safe biopesticides from food. Cystatins also lack impact on non-target insects, their parasitoid natural enemies, soil microbes and soil nematodes. Non-target nematodes deserve close study. They are valuable biomonitors of soil health and judged by some environmentalists as being at risk from biotech crops that control plant parasitic nematodes. Our aim is for such a crop to have no more impact on soil than results from crop rotation. A key challenge is deployment in the developing world to enhance food security. Here high pest pressure must be resisted and the level of biosafety and durability must be assured after the simple act of planting.

Contributions of Plant Virology to Biotechnology

Utilization of viral genes and regulatory elements for plant biotechnology. J. Hammond. USDA-ARS, Beltsville, MD. *Phytopathology* 97:S144.

Many of the features of plant viruses also have biotechnology applications. The Cauliflower mosaic virus 35S promoter is widely used to express foreign genes in transgenic plants; promoters from other dsDNA or ssDNA viruses are also used, as are their transcriptional termination signals. Other useful regulatory elements include translational enhancers to increase protein expression levels; ribozymes to process and/or regulate mRNAs; internal ribosome entry sites, ribosomal frameshift and readthrough sequences to allow differential expression of products from a single transcript; and sub-genomic RNA promoters or defective interfering RNAs utilized in plant viral vectors. Genes of value include suppressors of RNA silencing; proteases to process precursor proteins to mature forms; movement proteins to alter plasmodesmal exclusion limits; and replicase proteins to amplify RNAs for high-level protein expression. Viral coat proteins (CPs) are used as carriers for antigenic epitopes for vaccine or antibody production; some CPs can self-assemble, or assemble (in plant or bacterial cells) on RNAs containing an origin-of-assembly sequence. Infectious full-length clones or derivatives are used for transient expression of foreign genes, for virus-induced gene silencing (VIGS), and gene discovery; amplicons may be expressed in transgenic plants or as episomal vectors. Viral transgenes often induce effective resistance against homologous and/or related viruses.

Application of transgenic plants for virus resistance and environmental risk assessment. D. Gonsalves. USDA-ARS, Hilo, HA. *Phytopathology* 97:S144.

About 20 years have passed since the first report showed that transgenic plants expressing the coat protein gene of Tobacco mosaic virus were resistant to that virus. Numerous reports have since proven that the concept of 'pathogen-derived resistance' is an effective approach for controlling many plant viruses. Despite this proven technology, only a handful of virus-resistant transgenic crops have reached the stage of commercialization. These crops include squash, potato, and papaya, with papaya and squash still being produced commercially to any extent. I will focus this presentation on describing the steps that were taken towards testing and commercializing the products, and discuss the important factors that perhaps have contributed towards the

paucity of commercial virus-resistant transgenic crops. The concerns on environmental risks that virus-resistant transgenic plants posed will be summarized in an attempt to develop a consensus on what environmental risks are important to consider. These points on application and environmental risks are presented in the hope that we plant pathologists would develop a better track record in the next 20 years towards the practical application of this extremely powerful approach for controlling plant viruses.

Plant viral-based vectors for production of biomedical. A. V. Karasev. University of Idaho, Moscow, ID. *Phytopathology* 97:S144.

Plant viruses were developed as vectors for expression of various value-added products in plants about 20 years ago. Several factors were important for this development. The high level of expression, the extra-chromosomal nature of the virus replicon, and lack of cross-reactivity with animal proteins, all argued in favor of this type of production. Various types of vaccine candidates and therapeutic proteins and peptides were successfully expressed in plants in different virus vectors. The most successful examples are TMV, PVX, and CPMV. To enhance the vaccine or therapeutic effect of a plant-produced biomedical, several presentation platforms were developed based mainly on virus capsid proteins. This strategy was used successfully to deliver different types of biologically active peptides exposed on virus-like particles. Despite these remarkable technical achievements, development of commercial products lagged behind due to regulatory constraints.

Induction and suppression of RNA silencing in plant-virus interactions. V. B. Vance. University of South Carolina and National Science Foundation, Columbia, SC. *Phytopathology* 97:S144.

HC-Pro is a plant viral protein that suppresses RNA silencing and causes anomalies in the biogenesis of a variety of small regulatory RNAs, including siRNAs and microRNAs. We examined the role of the four Arabidopsis DICER-LIKE (DCL) proteins in both sense- and hairpin-transgene induced RNA silencing and compared the siRNA profiles with those in plants in which silencing was suppressed by HC-Pro. Our results indicate specific roles for DCL2 and DCL4 in these processes and suggest a dual mechanism of action for HC-Pro in suppression of silencing. To address the role of endogenous proteins in mediating HC-Pro suppression of silencing, we used the yeast two-hybrid system to identify HC-Pro-interacting proteins in tobacco. One of these, nRAV, is a protein related to the Arabidopsis RAV family of putative

transcription factors. Studies in tobacco and Arabidopsis indicate that ntRAV and its Arabidopsis homolog RAV2 are endogenous regulators of RNA silencing that are required for HC-Pro suppression of VIGS, but not for HC-Pro-associated defects in the endogenous microRNA pathway. The presentation will highlight the impact of HC-Pro studies on a variety of biotechnological applications.

Virus-induced gene silencing (VIGS) for gene function studies in plants. S. P. Dinesh-Kumar, T. Burch-Smith, Y. Liu, M. Schiff, Y. Dong, X. Zhu, and P. Mamillapalli. Yale University, New Haven, CT. Phytopathology 97:S145.

Virus induced gene silencing (VIGS) is a plant RNA silencing technique for characterizing the function of plant genes. VIGS uses viral vectors carrying a fragment of a gene of interest to generate the double-stranded RNA which initiates the silencing of the target gene. The phenotype of the plant silenced for a particular gene by VIGS mimics its loss-of-function mutant phenotype. VIGS is rapid (3–4 weeks from infection to silencing), does not require development of stable transformants, allows characterization of phenotypes that might be lethal in stable lines, and offers the potential to silence either individual or multiple members of a gene family. Several viral genomes have been modified to produce VIGS vectors. The most widely used VIGS vectors

are based on the Tobacco rattle virus (TRV). TRV-based VIGS vectors have been used to silence genes in a number of plant species including *Nicotiana benthamiana*, tomato, pepper, potato, petunia, poppy and Arabidopsis. Since TRV can infect the meristem, it has been used to study flowering in *N. benthamiana*, petunia and poppy. In addition, it has also been used to study fruit development in tomato. We and others have used VIGS to perform forward and reverse genetic screens. Examples of functions of many interesting genes that we have identified using VIGS in development and disease resistance will be discussed.

Designer virus particles as materials for bio and nano technology. J. Johnson. The Scripps Research Institute, La Jolla, CA. Phytopathology 97:S145.

A static, icosahedral, 30nm plant virus was used as an addressable “nano block” for molecular electronics, protein immobilization, and novel particle patterning on gold surfaces. DNA virus capsids that exhibit large-scale, pH sensitive, subunit reorganization were recently characterized by biophysics and molecular genetics for the harnessing of these properties for nano-devices. The characteristics of these novel platforms and machines will be presented and applications discussed.

Effector Molecules in Diverse Host–Parasite Interactions

Responses of plants to viral protein and nucleic acid effectors. S. Whitham. Iowa State University, Plant Pathology, Ames, IA. Phytopathology 97:S145.

Plant viruses are obligate intracellular parasites that typically encode four to ten proteins that perform basic functions of replication, movement, encapsidation, and transmission. Each of these proteins is generally multifunctional and can potentially have functions as effectors of host responses to viral infection. Recent evidence also suggests that nucleic acids of viral origin, and in particular RNA, can also serve as effectors of host responses. One manifestation of the activities of these viral proteins and nucleic acids is the dramatic changes in the mRNA accumulation (i.e. the expression) of certain host genes as viruses accumulate. A variety of microarray-based approaches have been used to characterize altered host gene expression in response to viral infection. These studies have provided insight into the different functional groups of genes that are induced by plant viruses as well as mechanisms that control their expression. We have employed a macro-dissection strategy to sample infected and non-infected tissue from the same leaf for global mRNA transcript profiling. This approach has allowed the spatial relationships between sites of Turnip mosaic virus accumulation and the accompanying changes in host mRNA transcript abundance to be determined. The roles of viral proteins and nucleic acids in eliciting changes in host gene expression as well as potential consequences of these gene expression changes will be presented.

Xanthomonas XopD TTSS effector is a repressor of hormone-induced transcriptional responses. M. B. Mudgett. Stanford University. Phytopathology 97:S145.

XopD, a type III effector protein from *Xanthomonas campestris* pv. *vesicatoria* (Xcv), the causal agent of bacterial spot disease on tomato and pepper, encodes a cysteine protease which cleaves small ubiquitin-related modifier (SUMO) precursors and removes SUMO from SUMO-conjugated proteins. After delivery into the plant cell, XopD localizes to subnuclear foci indicating that the major host targets are likely nuclear sumoylated protein(s). To analyze the potential role of XopD in Xcv virulence, we created a *xopD* null strain where the entire ORF was deleted by marker exchange mutagenesis. Interestingly, growth of the Xcv *xopD* deletion strain was significantly reduced in susceptible VF36 tomatoes; however leaf senescence appeared more rapidly in the infected tissue compared to leaves infected with wild type Xcv. Only the wild type gene complemented the Xcv *xopD* mutant phenotype. An inactive catalytic core protease mutant *xopD* (C470A) did not, indicating that XopD protease activity is required to suppress leaf senescence during infection. *Agrobacterium*-mediated transient expression of XopD in *Nicotiana benthamiana* repressed PDF1.2 gene transcription induced by methyl jasmonate treatment revealing that XopD can suppress gene expression *in planta*. We are currently testing whether XopD is altering hormone biosynthesis and/or perception during by directly or indirectly regulating gene expression during infection. We will present the data from these studies and propose possible biochemical mechanisms by which XopD can delay leaf senescence. Our working hypothesis is that XopD may repress host-induced senescence to save nutrients and/or an ecological niche for the growing pathogen.

Functional analyses of putative effectors controlling biotrophic invasion by the rice blast fungus. B. Valent. Kansas State University. Phytopathology 97:S145.

The rice blast fungus *Magnaporthe oryza* is a hemibiotrophic fungus that sequentially invades living plant cells using intracellular invasive hyphae (IH) that grow from cell to cell. Using live-cell imaging, we reported that IH are tightly wrapped in extra-invasive hyphal membrane (EIHM) with distinctive membrane caps at the tips of IH when they first enter the host cell, and that IH seek out plasmodesmata for moving into the next cell (Kankanala et al, The Plant Cell, February 2007). To begin to identify and characterize effector proteins secreted by IH into live plant cells to control host membranes and plasmodesmata, we used whole genome microarrays to identify novel IH-specific secreted proteins. Our initial gene replacement experiments have not shown phenotypes associated with putative effectors. To study effector secretion *in planta*, we fused known blast effectors to green fluorescent protein. Predicted effector signal peptides directed secretion of GFP from the fungus, and fusion proteins accumulated at predictable locations inside the EIHM. Fluorescence concentrated in the EIHM caps and in previously unrecognized structures, Blast Interfacial Complexes (BICs). Fusion proteins accumulated in BICs as long as IH grew in the cell. Correlative light and electron microscopy showed that BICs are complex membrane-rich and vesicle-rich structures between the fungal wall and EIHM. We will discuss a potential role for BICs in blast effector secretion.

Oomycete RXLR effectors: Structural basis of avirulence and virulence in *Phytophthora infestans* AVR3a. J. Bos. Ohio State University, OARDC-Plant Pathology, Wooster, OH. Phytopathology 97:S145.

The oomycete *Phytophthora infestans* causes late blight of potato and tomato and is arguably the most destructive pathogen of solanaceous crops. Tremendous progress has been made recently in understanding the biology of *P. infestans* effectors. Two classes of effectors target distinct sites in the host plant: Apoplastic effectors are secreted into the plant extracellular space, and cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments. The cytoplasmic RXLR effectors are characterized by a conserved motif following the signal peptide. This domain is functionally interchangeable with a malaria host targeting domain and appears to function in delivery into host cells. One member of the RXLR effector family is AVR3a, which exhibits dual effector functions. AVR3a induces hypersensitivity mediated by the resistance protein R3a and suppresses cell death induced by *P. infestans* INF1 elicitor. We utilized extensive structure-function analyses to gain insight in the molecular basis of AVR3a effector activities. We found that distinct amino acids of AVR3a condition R3a hypersensitivity and cell death suppression. These results point to a model that involves the interaction of AVR3a with a host protein and is not consistent with the recognition of AVR3a through an enzymatic activity.

Cyst nematode parasitism: Three glands, many effectors, big effects. T. J. Baum. Iowa State University, Plant Pathology, Ames, IA. Phytopathology 97:S145.

Cyst nematodes feed only after they have ended their migratory phase inside the roots of host plants and, therefore, are referred to as sedentary endoparasites. In contrast to the feeding of migratory parasites, sedentary feeding requires that sophisticated feeding cells are formed in the plant host.

The feeding cells of cyst nematodes are termed syncytia, and their formation most likely is accomplished by nematode effector molecules, so-called parasitism proteins, which are secreted from the cyst nematode mouth spear. Parasitism proteins are the secretory products of parasitism genes, which are expressed exclusively in the nematode's three esophageal gland cells. More than fifty parasitism genes have been identified from the soybean cyst nematode. Although a few parasitism protein functions were recognized from similarities to known functionally characterized proteins, an integrated

approach of molecular techniques is used to decipher the functions of the majority of parasitism proteins. For this purpose, parasitism genes are overexpressed in planta, parasitism proteins are visualized after secretion using antibodies, plant gene expression in response to parasitism protein action is profiled, protein-protein interactions are identified, and parasitism genes are knocked down using RNAi techniques. As a result, functional details of this pathosystem's sophisticated host-pathogen interactions are emerging and novel control mechanisms are being developed.

Oomycete Genomes Come of Age

Genome dynamics in the pathogen/host arms race: Initial analysis of the *Phytophthora infestans* genome. M. C. Zody (1), R. H. Y. Jiang (1,2), R. Handsaker (1), M. Grabherr (1), C. D. Kodira (1), F. Govers (2), P. Birch (3), S. Whisson (3), J. Win (4), H. Judelson (5), J. B. Ristaino (6), W. E. Fry (7), S. Kamoun (4), and C. NUSBAUM (1). (1) Broad Institute of MIT and Harvard; (2) Wageningen University; (3) Scottish Crop Research Institute; (4) Ohio State University; (5) University of California, Riverside; (6) North Carolina State University; (7) Cornell University. *Phytopathology* 97:S146.

Phytophthora infestans, the Irish Potato Famine organism, causes late blight in potato and remains a critical pathogen. Sequence and analysis of the *P. infestans* genome shows it to be dynamic and rapidly evolving. Genome plasticity appears to greatly contribute to the weaponry of *P. infestans*. The *P. infestans* genome is dramatically larger than those of other sequenced oomycetes. This is explained by its very high repeat content. Up to 75% of the genome can be accounted for as repeat, mostly as relics of ancient transposons. At least 35% is recognizable as recently active transposons. We have annotated ~21,000 non-repeat genes, using a combination of ab initio methods and a new comparative gene finder. Orthosearch, tuned specifically for oomycete genomes. Despite the high neutral nucleotide divergence of *P. infestans* from *P. sojae* and *P. ramorum*, the majority of the proteome is highly conserved. In contrast, secreted proteins are rapidly evolving, and among these, genes in the RxLR family of effectors may be the fastest evolving. They are thought to play a significant role in promoting infection. The genome of *P. infestans* contains ~50% more RxLR loci than those of *P. sojae* and *P. ramorum*. Further, preliminary analysis shows a correlation of small secreted genes with retrotransposon activity, suggesting a role for retrotransposition in creating gene diversity.

Genome sequence of the vegetable pathogen *Phytophthora capsici* produced with traditional and next generation technology. K. Lamour. University of Tennessee. *Phytopathology* 97:S146.

The objectives for this project were to (i) produce a *de novo* draft genome sequence for the vegetable pathogen *Phytophthora capsici* using 454 GS20 sequencing technology and to compare this to sequence produced using traditional Sanger sequencing and (ii) to identify heterozygous sites and further develop these into a population resource. A moderately inbred line of *P. capsici* was developed and genome coverage of 23X and 5X was generated using 454 and Sanger respectively. An overview of the sequencing project and the challenges associated with assembly and annotation will be discussed. Comparison of the apparent advantages and disadvantages of the two approaches will be presented and progress towards the development of a population resource for *P. capsici* will be discussed.

Transcriptional networks involved in the development of *Phytophthora infestans* spores. H. S. Judelson. University of California at Riverside. *Phytopathology* 97:S146.

Integral to the pathogenesis of *Phytophthora* species are their abilities to form and germinate spores. The availability of genome sequences for *Phytophthora*, combined with methods for functional genomics, provides the means to comprehensively analyze genes involved in the biology of its spores. Using microarrays, the expression of the *P. infestans* transcriptome was examined during the formation and germination of asexual spores, and during oosporogenesis. Genes showing major shifts in mRNA abundance in spores versus hyphae represent >20% of the transcriptome and encode structural proteins, such as flagellar components, and participants in cellular regulation or metabolism. Many of the *P. infestans* stage-specific genes appear to be oomycete-specific, and many lack homologs in *P. ramorum* or *P. sojae*. Subjecting several genes to homology-dependent silencing revealed pathways needed for sporulation, germination, or appressorium formation. Mechanisms of transcriptional regulation of the stage-specific genes are being explored using comparative genomics and by functionally characterizing mutagenized promoters in transformants. Phylogenetic footprinting often reveals candidates for transcription factor binding sites, and functional studies and EMSA have confirmed their roles in developmental gene regulation.

The pathogenicity effectors of *Hyaloperonospora parasitica*. R. Allen, L. Baxter, R. Baumber, P. Bittner-Eddy, M. Coates, K. Fisher, S. Hall, L. Hughes, S. Lee, J. Meitz, A. Rehmany, L. Rose, and J. BEYNON. WarwickHRI, Warwick University, Wellesbourne, Warwick, CV35 9EF, UK. *Phytopathology* 97:S146.

Hyaloperonospora parasitica is an obligate oomycete and the causal agent of downy mildew on Arabidopsis. Recently we have cloned two pathogenicity effector genes, ATR1 and ATR13, from the pathogen and shown them to code for unique proteins that are under amazing levels of diversifying selection. This implies that they are locked in an "arms race" with the plant's pathogen detection system and consequently these levels of diversity are mirrored in the host resistance genes, RPP1 and RPP13, associated with recognition of ATR1 and ATR13, respectively. We have used this natural diversity to determine specificity in the ATR13/RPP13 interaction and reveal the depth of the complexity involved in these recognition processes. The newly sequenced genome of *H. parasitica* will allow a more global approach to the identification of effector proteins and their analysis.

Groovy times: Structure, evolution, and function of oomycete effectors. J. BOS, C. Bruce, C. Cakir, W. Morgan, S.-K. Oh, J. Song, J. Win, C. Young, and S. Kamoun. Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster, OH 44691. *Phytopathology* 97:S146.

Oomycetes secrete an arsenal of effectors to modulate plant innate immunity and enable parasitic infection. Deciphering the biochemical activities of effectors to understand how pathogens successfully colonize and reproduce on their host plants became a driving paradigm in the field of oomycete pathology. Two classes of effectors target distinct sites in the host plant: Apoplastic effectors are secreted into the plant extracellular space, and cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments. Of particular interest are the RXLR and Crinkler (CRN) cytoplasmic effectors that are characterized by conserved motifs following the signal peptide. The RXLR domain is functionally interchangeable with a malaria host targeting domain and appears to function in delivery into host cells. The availability of five oomycete genome sequences allowed us to build genome-wide catalogues of the effector secretome. Many effector families exhibit hallmarks of positive selection probably as a result of a coevolutionary arms race with host factors. We employed discovered effectors in high-throughput in planta expression assays to screen for effector activities. The perturbations caused by these effectors help elucidate the mechanisms of pathogenicity as well as further illuminate mechanisms of plant defense and innate immunity.

Effector repertoire of *Phytophthora sojae*: Structural and functional genomics. B. M. TYLER (1), D. Dou (1), R. H. Y. Jiang (1,2), X. Wang, S. Kale (1), F. Arredondo (1), and S. Tripathy (1). (1) Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA 24061; (2) Laboratory of Phytopathology, Wageningen University, NL-6709 PD Wageningen, The Netherlands and Broad Institute, Cambridge, MA 02141. *Phytopathology* 97:S146.

The genome sequences of several oomycete plant pathogens, including the soybean pathogen *Phytophthora sojae*, reveal that each of these genomes encodes several hundred proteins with sequence similarity to cloned oomycete avirulence genes. We have identified conserved motifs present in large numbers of these genes, including the *P. sojae* avirulence gene Avr1b-1. We have tested the function of the motifs in Avr1b-1 using *P. sojae* stable transformants and soybean transient expression assays. The RXLR motif, found near the N terminus of most of the effectors, is required for Avr1b-1 to confer avirulence when expressed in *P. sojae*, but not when expressed inside soybean cells, supporting the hypothesis that RXLR is required for Avr1b-like effector proteins to transit into plant cells. The sequences surrounding the RXLR motif are also required, but there is only a weak requirement for the adjacent DEER motif. Avr1b contains two C terminal motifs that occur as a pair in approximately half of all the *P. sojae* effectors, and both are required to confer avirulence. Overexpression of Avr1b-1 increases the virulence of *P. sojae* transformants on susceptible cultivars and we are currently determining the role of the conserved motifs in conferring increased virulence.

Plant Disease Management

Disease Forecasting Models: What Comes Under the Umbrella of Model Validation?

Model validation: What comes under the umbrella of validation concepts for pathogen/disease detection and forecasting models. F. W. Nutter, Jr. Iowa State University. *Phytopathology* 97:S147.

New tools for detecting plant pathogens and predicting pathogen/disease threats (forecasting) are being developed at a rapid pace, yet, well-defined concepts with regards to what constitutes the “validation” of detection tools and models is often vague and misconstrued. For example, with regards to disease forecasting models, the concept of validation might be defined as: (i) the impact of a model on managing a disease, (ii) the impact of a model to achieve attainable yield potentials with reduced management inputs, and/or (iii) documenting a model’s ability to generate a larger net return on investment compared to the conventional disease management program. Thus, it is the impact of using a model that is often “validated”, and not the model itself. The “cost” of conservative disease forecasting models in generating false negatives with regards to the actual disease risk is rarely assessed and weighed against the benefits of reducing the “costs” of false positives. Moreover, long distance pathogen transport models are often “validated” without actually quantifying the concentration of pathogen dispersal units actually escaping (take-off) from a diseased crop (per unit volume of air per unit time) is actually accurate. The number of dispersal units per m² of air during dispersal (flight), and deposition (landing) are also rarely measured for validation purposes. The use of balloons and unmanned aircraft equipped with spore trapping devices offers a means to validate such models. The use of receiver operating characteristic curves and Bayesian statistical approaches, as well as economic models to quantify and evaluate model impact, are all potential tools that can come under the validation umbrella. This symposium will attempt to elucidate what comes under the umbrella of validating disease/pathogen detection forecasting tools and models.

Use of autonomous unmanned aircraft to validate and improve long distance pathogen transport models. D. G. Schmale. Virginia Tech, Blacksburg, VA. *Phytopathology* 97:S147.

Many plant pathogens are transported over long distances in the atmosphere, threatening agriculture in the United States from both inside and outside the borders of the country. An increased understanding of the movement of plant pathogens in the atmosphere is essential for establishing effective quarantine measures, preventing the spread of plant disease, and mitigating potentially damaging events targeted at our nation’s agriculture and food supply. Atmospheric transport models have been used to predict the long distance transport of plant pathogens, but these models often fail to incorporate actual measurements of spore concentrations and spore viability along proposed particle trajectories. We have developed, tested, and implemented an autonomous (self-controlling) unmanned aircraft system to rapidly detect and monitor the movement of plant pathogens tens to hundreds of meters above agricultural fields. These aircraft are equipped with unique spore-sampling devices that are opened and closed by remote control from the ground. Data collected with these aircraft are being used to validate and improve a long distance transport model for the fungal plant pathogen *Gibberella zeae*, causal agent of Fusarium head blight of wheat and barley. This work has important implications for predicting the rapid spread of soybean rust, tobacco blue mold, stem rust of wheat, and cucurbit downy mildew across broad geographic regions.

Validation of weather inputs for disease warning systems. M. L. Gleason. Iowa State University. *Phytopathology* 97:S147.

Weather data are essential inputs to disease-warning systems. However, gathering reliable weather data often poses formidable barriers to implementation of warning systems. Despite the recent advent of low-cost, automated weather-monitoring devices, substantial challenges remain. Leaf wetness duration (LWD) exemplifies the range of issues that can confound reliable operation of warning systems. Although LWD is a key input to dozens of warning systems, there is no accepted standard for LWD sensor calibration. Different types of LWD sensors, and even sensor orientation, can lead to very different assessments of environmental wetness. In tree crop canopies, furthermore, extreme sensitivity to micro-site variations in some climates can create large artifacts in LWD measurements that impact warning system performance. Added to these problems are the usual issues that beset on-farm weather monitoring: cost, convenience, and long-term reliability. Site-specific weather estimation can substitute for or supplement on-site measurements, but accurate estimation of LWD has proven to be challenging. Models have been developed to improve the reliability of site-specific LWD

estimates, but much field validation and model refinement remain to be done. Among the most pressing needs for improved site-specific estimation are more sophisticated spatial interpolation methods.

Bayesian statistics and information theory with regards to model validation. N. McRoberts (1), G. Hughes (2), and L. V. Madden (3). (1) SAC, UK; (2) University of Edinburgh, UK; (3) Ohio State University. *Phytopathology* 97:S147.

Over the last ten or so years plant pathologists have started to use methods from clinical diagnostics to develop and evaluate decision tools for disease management. To date applications in plant disease epidemiology have focussed on situations where disease is assessed in a binary way (e.g. as requiring treatment, or not). This leads easily to evaluation of decision tools through their sensitivities (Se) and specificities (Sp) and the construction of receiver operating characteristic (ROC) curves. The Se and Sp can be used to calculate the likelihood ratios (LRs) for positive and negative decisions and these values used in a straightforward way to allow Bayesian updating of pre- and post-use probabilities of the 2 different disease categories following use of the decision tool in practice. Where disease assessments are made in more than 2 categories Se and Sp do not exist, but LRs can still be calculated and used in decision-making and decision tool evaluation. The LRs also link the statistical evaluation of decision tools to concepts from information theory and economics such as entropy, utility and regret which may be useful both in the evaluation of the performance of decision tools and in analysing their adoption, or not, by their intended user base.

Dollars and cents: Economic validation for disease forecasting models. P. D. Esker. Iowa State University, Ames, IA. *Phytopathology* 97:S147.

Disease forecasting systems are typically developed to provide producers a method to help them determine the need and timing of a specific management tactic. Unfortunately, most plant disease forecasting systems fail to address the economic affects of making false positive (i.e., the recommendation to spray when not needed) and false negative (i.e., the recommendation not to spray when a spray was needed) predictions, making their overall utility limited in some cases. Incorporating economic cost into forecasting models will enable the evaluation of different forecasting models under real-world scenarios that most producers deal with on a daily or seasonal basis. In this talk, the economic evaluation of disease forecasting models will be addressed based on a binary system of events (e.g., spray or do not spray). A theoretical introduction to the economics of plant disease forecasting will be bridged with a practical application of this methodology, as demonstrated for Stewart’s disease of corn. Different proposed forecasting models will be compared under different economic scenarios. Some of the key concepts that will be discussed will include: sensitivity, specificity, receiver operating characteristic analysis, the economic cost ratio, and forecast probability threshold.

Validating methods for regulatory use: Method validation and proficiency testing at the National Plant Germplasm and Biotechnology Laboratory. P. H. Berger, P. Shiel, D. Seaver, L. Levy, and V. Mavrodieva. National Program Leader, Molecular Diagnostics and Biotechnology, USDA-APHIS-PPQ. *Phytopathology* 97:S147.

Method validation is the process to define the analytical requirements, and confirm that the analytical procedures adapted for a specific pest, pathogen or system are suitable for their intended use. This is done by conducting experiments to determine or verify a number of specific performance characteristics that define and quantify method performance. Typically, validation involves four distinct stages: 1) feasibility studies (can the chosen reagents and protocols detect the target); 2) optimization (reagent concentrations, protocol parameters, initial repeatability between replicates established); 3) determination of performance characteristics (accuracy, precision, specificity, sensitivity, robustness, and method uncertainty); 4) monitoring and maintenance of performance (proficiency testing). Each of the four stages and associated performance characteristics will be discussed. Conclusions based on detailed analysis of a validated method and proficiency testing program will be showcased as an example.

National effort to validate risk assessment models for Fusarium head blight (FHB) of wheat. J. E. Molineros (1), E. D. De Wolf (2), and L. V. Madden (3). (1) The Pennsylvania State University, State College, PA; (2) Kansas State University, Manhattan, KS; (3) Ohio State University, Wooster, OH. *Phytopathology* 97:S147.

Concern over FHB has led to the development of risk assessment models for predicting major epidemics. Probabilistic models for spring and winter wheat were deployed for 23 states via an internet delivery system. These models

were developed based on observations of disease, weather, crop growth stage and production practices from locations throughout the region of deployment. Model development is a recursive multistage process, including data collection, quality control, model fitting, validation, calibration, and error analysis. The validation of disease forecasting models over a large geographical area presents unique challenges including the evaluation of weather observations

used to drive the models, as well as verification of model accuracy, and operational success of the forecasting system. Potential sources of error come from unaccounted information, and errors in model development. Model validation involves balancing model flexibility in new environments and the highest attainable accuracy, sensitivity and specificity.

Emerging Technologies for the Detection and Regulation of Mycotoxin Contamination

The multiple challenges of mycotoxin detection and quantification in silages. G. A. KULDAU and M. A. Mansfield. Penn State, University Park, PA. Phytopathology 97:S148.

Silages made from maize or grasses comprise > 50% of dairy cow diets and are also occasionally used for beef cattle feed. Despite wide recognition of the problem of mycotoxins in forages, detection and quantification of mycotoxins in these feeds presents significant challenges. Precise, reliable and rapid methods for screening forages do not exist. Silages are a challenging matrix to work with due to their non-homogeneous nature, and methods that succeed with grain sometimes fail with forages because of interfering compounds. Commercially available ELISA kits are usually not rated for silages and are known to give false positives and negatives for some mycotoxins. More than 15 toxins, produced by species of *Alternaria*, *Aspergillus*, *Fusarium*, and *Penicillium* are known to occur in silages. However, it is not known which are most relevant to animal health. In maize silage, we found that > 50% of the samples studied contained three or more toxins. We observed that fumonisins were the mostly frequently detected followed by roquefortine C and deoxynivalenol. Of these, roquefortine C is not routinely tested by commercial services nor are several other *Penicillium* mycotoxins that are known to co-occur. Advances in toxicology, analysis and silage management in the field and during storage are needed to improve the situation of mycotoxins in silages.

Social and political challenges in the regulation of mycotoxin contamination. R. BANDYOPADHYAY (1), J. H. Williams (2), and P. S. Ojiambo (1). (1) International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria; (2) Peanut CRSP, University of Georgia, Griffin, GA. Phytopathology 97:S148.

Mycotoxins are toxic to humans and animals when present in food and feed above tolerance limits. Reduction in mycotoxin exposure of humans and animals has necessitated regulatory interventions by some national governments and international agencies. Conservative estimates of lost crop revenues, and the cost of research and monitoring activities, range from \$0.5 to \$1.5 billion annually. To achieve current global regulatory compliance in mycotoxin safety levels farmers also have to spend more effort and assume higher risk. Regulatory levels vary between countries and, as a result, mycotoxins act as Technical Barrier to Trade and have led to trade disputes between nations. While food production, processing and marketing systems of developed economies have been largely successful in delivering mycotoxin-safe products, regulations have been ineffective in most less-developed countries due to socio-economic, technical, institutional and policy factors. Agricultural products of the best quality are exported from less developed countries to augment export earnings, leaving poorer quality foods to be consumed locally, compromising the health of local populations. Wherever regulations cannot be implemented the key management options for consumers and farmers are awareness and adoption of simple mycotoxin management practices.

Applying new technologies for the detection of mycotoxigenic fungi. C. Woloshuk. Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN. Phytopathology 97:S148.

Biosecurity requires effective methods for monitoring environmental threats. Whether the source of the threat is in the air over a wheat field, in corn stored in a bin, or in barley entering a malting facility, we must know the potential risk for mycotoxin contamination. At Purdue University, we are testing new technologies that detect and quantify mycotoxigenic fungi. These methods utilize both DNA and fungal metabolite detection technologies. My presentation will focus on application of these methods to better manage stored grain.

Global Diversity of IPM Systems

A global perspective of integrated nematode management innovation. G. W. Bird. Michigan State University, East Lansing, MI. Phytopathology 97:S148.

The presentation will include a history of nematode management, descriptions of recent integrated nematode management innovations, and discussion of proposed research and education priorities. The nematode management history will summarize the pre-synthetic and synthetic nematicide eras resulting in the unexpected consequences leading to the evolution of integrated pest management. Beginning with the US AID International Meloidogyne Project, recent innovations in the processes, strategies and tactics of integrated nematode management will be described using specific examples related to major global food crops and their key phytopathogenic nematode species. Special reference will be given to the nematology components of the USAID IPM CRSP Trainer and Farmer Field Schools in Kyrgyzstan, Uzbekistan and Tajikistan and potential impacts of a bio-economy on infectious diseases caused by plant parasitic nematodes. Emphasis will be placed on the recent use of nematode community structure in soil quality assessment and potential of restoration ecology as a nematode management innovation. Nematology research and education priorities will be discussed in relation to global science and education personnel needs.

Integrated management of Tobacco etch virus in Scotch Bonnet pepper in Jamaica. S. A. Tolin (1), S. A. McDonald (2), and D. Clarke-Harris (3). (1) Virginia Tech, Blacksburg, VA; (2) Ministry of Agriculture and Lands, Bodles, Jamaica; (3) Caribbean Agricultural Research and Development Institute, Kingston, Jamaica. Phytopathology 97:S148.

Scotch Bonnet pepper, *Capsicum chinense*, is the preferred hot pepper in Jamaican cuisine. This habanero-like pepper provides a unique flavor, and has gained market acceptance as a non-traditional export crop for Jamaica. Exports increased markedly in the 1990's, but increased production led to increased incidence of pests and diseases, and a decline in crop yield, quality

and exports. A island-wide stakeholder assessment, done in conjunction with the IPM CRSP, identified viruses as a major constraint in pepper productivity and longevity. Activities of the CRSP identified the major virus to be the potyvirus *Tobacco etch virus* (TEV), and designed experiments that monitored aphid vector phenology and identity in relation to time of infection, and demonstrated that yield losses were greatest with pre-flowering infection times. Field experiments to validate management practices to delay or prevent infection with TEV, using combinations of mulch and stylet oil sprays, and monitoring infection rate by a tissue blot immunoassay, will be described. Management tactics were identified to permit farmers to choose among a combination of tactics to tailor a risk reduction program depending on market conditions, available resources (financial, manpower), and farm conditions. Integration of virus management with management of arthropod pests such as broad mites and a gall midge complex remains particularly challenging.

Strategies for managing bacterial wilt diseases of tomato, potato, and export ornamentals. C. Allen. University of Wisconsin-Madison. Phytopathology 97:S148.

Bacterial wilt disease inflicts large crop losses every year on growers in the tropics and warm-temperate zones. Affected plants are stunted, wilted, and often die soon after showing symptoms. The disease is caused by members of the *Ralstonia solanacearum* species complex, which are widely distributed, highly aggressive, and have a broad host range. The pathogen is usually soilborne but can also be transmitted by irrigation water, equipment, and infected vegetative propagation material. Control of bacterial wilt is difficult, particularly in the developing tropics where the impact of the disease is greatest. *R. solanacearum* can be excluded by planting pathogen-free potato seed tubers or ornamental cuttings. Once soil or irrigation water is infested with the pathogen, the best strategy is planting wilt-resistant cultivars, and there are some moderately resistant tomato lines. Breeding for better resistance in tomato and potato is underway at several locations. Grafting onto wilt-resistant rootstocks has proven effective on the commercial scale in tomato. Rotation with resistant crops is of limited utility and no effective

biocontrol methods exist to date. Soil fumigation is not advisable, but there are some promising soil amendments. Sensitive, rapid, low-tech tests for this pathogen are needed both for clean seed programs and to detect the Race 3 biovar 2 subgroup of *R. solanacearum*, which is a quarantine pathogen in North America.

IPM in vegetable cropping systems in southeast Asia. S. A. Miller (1), A. Baltazar (2), H. Rapusas (3), N. Opina (2), D. Arida (3), E. Gergon (3), G. Norton (4), and E. G. Rajotte (5). (1) The Ohio State University, OARDC, Wooster, OH; (2) University of the Philippines – Los Banos, Laguna, Philippines; (3) Philippines Rice Research Institute, Munoz, Nueva Ecija, Philippines; (4) Virginia Tech, Blacksburg, VA; (5) Pennsylvania State University, State College, PA. *Phytopathology* 97:S149.

Vegetable production in southeast Asia is highly intensive and challenged by a number of disease, pest and weed problems. As in other tropical production areas, many of these problems are very difficult to manage due to misdiagnosis, lack of disease- or pest-resistant varieties and effective pesticides, and overproduction. Further, inappropriate and over-use of pesticides has resulted in reductions in natural enemy populations as well as development of pesticide resistance. Through the Integrated Pest Management Collaborative Research and Development Program (IPM CRSP), integrated, sustainable approaches to pest management were developed. These include grafting bacterial wilt (*Ralstonia solanacearum*)-susceptible eggplant onto wilt-resistant rootstocks, development of a local *Trichoderma* strain to reduce soilborne pathogens, management of eggplant fruit and shoot borer (*Leucinodes orbonalis*) using cultural practices and reduced insecticide applications, and an integrated approach utilizing stale seedbed technique, minimal herbicide applications and reduced hand labor for purple nutsedge (*Cyperus rotundus*)

management. Pest management technologies were transferred to local outreach personnel and farmer-leaders (train-the-trainers).

Integrated management of the invasive pest-plant *Melaleuca quinque-neria* in southern Florida: Special emphasis on the impact of a natural enemy. M. Rayamajhi. USDA-ARS, Ft. Lauderdale, FL. *Phytopathology* 97:S149.

The Australian tree *Melaleuca quinque-neria* (melaleuca), has rapidly invaded the south Florida landscapes at the expense of native plant communities. Hence, an integrated melaleuca management program involving mechanical, chemical, cultural, and biological control methods was developed and is being used by various agencies. Mechanical and chemical methods have been used in eliminating larger trees while cultural and biological control measures reduce seedling recruitment. Natural enemies involved in this program are introduced specialized insects from Australia as well as an adventive pathogen and a scale insect. Their impact on melaleuca populations has been monitored since 1997. The insect-fungus interactions caused chronic defoliation and dieback resulting in additive impacts that inhibited melaleuca stump-regrowth, reduced tree densities, and diminished basal area production due to accelerated tree-mortality. Further spread of melaleuca has ceased as natural enemies killed a large proportion of melaleuca trees and reduced the reproductive potential of surviving trees. By 2005, canopies had opened allowing native species from surrounding areas to colonize the stands. As a consequence, plant species richness and diversity indices were higher compared to 1996 when melaleuca stands consisted of dense monocultures. This phenomenon is expected to intensify as the new and existing natural enemies continue to impact melaleuca monocultures in Florida.

Host Plant Resistance for Growers: From Sequence to the Field

Marker-aided breeding for disease resistance in common bean. J. D. Kelly. Michigan State University, East Lansing, MI. *Phytopathology* 97:S149.

Diseases are regarded as the leading constraint to increased common bean (*Phaseolus vulgaris* L.) production worldwide. The range in variability and complexity among bean pathogens can be controlled with different single gene and quantitative resistance sources. Combining these resistance sources into commercial cultivars is a major challenge for bean breeders. To assist breeders, a major effort to identify molecular markers tightly linked to different genes and/or QTL was undertaken. To date, markers linked to over 20 major resistance genes have been identified, in addition to QTL conditioning resistance to complexly inherited traits such as white mold. The co-localization of resistance QTL with defense response and/or architectural avoidance traits provides a unique opportunity to combine diverse resistance mechanisms. The use of marker-assisted selection (MAS) to incorporate disease resistance into common bean is routine in many breeding programs. Indirect selection of single resistance genes in the absence of the pathogen and the opportunity afforded breeders to pyramid these genes to improve their longevity and retain valuable hypostatic genes will be discussed. Marker-assisted selection for disease resistance in common bean provides opportunities to breeders that were not feasible with traditional breeding methods.

Application of molecular breeding techniques to the development of stalk rot resistance in corn. L. R. ABAD (1), P. J. Wolters (2), T. R. Colbert (3), D. S. Stucker (1), and J. Ho (4). (1) Pioneer Hi-Bred Int'l, Johnston, IA; (2) DuPont, Wilmington, DE; (3) Pioneer Hi-Bred Int'l, Princeton, IN; (4) Pioneer Hi-Bred Int'l, Janesville, WI. *Phytopathology* 97:S149.

Anthrax Stalk Rot (ASR), caused by *Colletotrichum graminicola*, is an important stalk rot pathogen in North America. Early plant death and deterioration of stalks by Anthraxnose leads to loss in yield and increased risk of stalk lodging. A gene conferring ASR resistance, *Rcg1*, was recently fine-mapped and cloned out of MP305-derived lines by Pioneer Hi-Bred in collaboration with University of Delaware scientists. The sequence of *Rcg1* indicates that the gene has homology to the nucleotide-binding site, leucine rich repeat (NBS-LRR) class of disease resistance genes. The *Rcg1* gene sequence, as well as information from the genetic and physical maps of maize in the region flanking the gene, was used to create molecular markers. Using these markers, inbreds containing the *Rcg1* gene were developed. Isogenic inbreds were crossed and the resulting hybrids were evaluated for ASR resistance. Under strong disease pressure, hybrids containing the *Rcg1* gene had significantly less disease development and more yield than the corresponding base hybrids.

From association mapping to marker-assisted selection: Experimental design and application. I. Simko. USDA-ARS, Salinas, CA. *Phytopathology* 97:S149.

The most common method for mapping genes in cultivated plant species is genetic linkage mapping. This approach involves generating populations derived from single crosses and estimating the recombination frequencies between marker loci and the genes of interest. However, such mapping populations sample only a small proportion of all possible alleles, which presents a difficulty when developing universal markers for marker-assisted selection. In contrast to genetic linkage mapping, association mapping is a method that detects relationships between phenotypic variation and genetic polymorphisms in existing cultivars, without the need for developing new mapping populations. The method was originally applied in human genetics, but has recently become a useful tool for mapping genes in plants. This linkage disequilibrium-based method effectively incorporates the effect of many past generations of recombination into a single analysis. The association mapping technique can be used in complementation with the linkage mapping method to effectively locate genes segregating in a population of interest. Here I describe an application of the haplotype association mapping technique for detection of molecular markers linked to the Verticillium wilt resistance gene in potato. Discussed will be the main advantages and drawbacks of the association-based mapping methods and a prospect of their application in plant breeding.

Association mapping of disease resistance in cultivated and wild barley. B. J. STEFFENSON (1), K. P. Smith (2), and G. J. Muehlbauer (2). (1) Department of Plant Pathology, University of Minnesota, St. Paul, MN; (2) Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN. *Phytopathology* 97:S149.

Diseases are one of the most important constraints to barley production in many regions of the world. Information regarding the chromosomal location of disease resistance loci and identification of molecular markers linked to them can greatly facilitate resistance breeding in barley improvement programs. Association mapping (AM) or linkage disequilibrium (LD) mapping is an alternative strategy to standard bi-parental crosses for positioning resistance loci on the genome and is based on statistical assessments for the association between genotypes and phenotypes in reference germplasm sets. AM is being used to map disease resistance loci in cultivated breeding germplasm from the Barley Coordinated Agricultural Project (960 lines from 10 breeding programs per year) and also in an ecogeographically diverse collection of wild barley (318 accessions from 19 countries). Wild barley germplasm was genotyped with over 1000 Diversity Arrays Technology (i.e. DArT) markers and phenotyped for resistance to several races of stem rust. Significant associations for stem rust resistance were detected at eight different locations in the genome, including bin 13 of chromosome 7(5H)

where the *rpg4/Rpg5* complex lies. Some of these associations may represent new stem rust resistance loci, but others could be spurious. Preliminary results suggest that AM may be useful for positioning some resistance genes in wild barley germplasm; however, every effort should be made to validate the associations.

Microarray expression analysis to characterize widely-used, adult-plant rust resistance mechanisms of wheat. S. H. HULBERT (1), L. Perugini (2), C. Yin (1), J. Bai (3), G. Brown-Guedira (2), and R. L. Bowden (3). (1) Washington State University, Pullman WA; (2) USDA-ARS, North Carolina State University, Raleigh, NC; (3) USDA-ARS and Kansas State University, Manhattan, KS. *Phytopathology* 97:S150.

The *Lr34/Yr18* and *Lr46/Yr29* genes of wheat are thought to be race nonspecific because they provide adult-plant slow-rusting resistance to both leaf rust and yellow (stripe) rust. The distal halves of flag leaves are typically

more resistant than the basal halves and both genes can be associated with leaf tip necrosis. Although the genes are becoming widely used around the world, the mechanism of their resistance is unknown. We used microarray analysis to examine expression patterns of more than 50,000 genes between *Lr34/Yr18* isolines. Both the distal and basal halves of isolate flag leaves were compared, both with and without inoculation with leaf rust. The *Lr34/Yr18* gene upregulated the transcription of dozens of genes, typically more in the leaf tips than in leaf bases. Most transcripts upregulated by this gene were upregulated in both inoculated and uninoculated tissue. Most of these transcripts were associated with ABA induction, osmotic stress, cold stress, and/or seed maturation. The results indicate that *Lr34/Yr18* has a novel mode of action compared to most R genes, which regulate gene expression after pathogen recognition. Similar experiments are being conducted with *Lr46/Yr29* isolines. Results with both loci have demonstrated that a high level of isogenicity is necessary for examining the effects of regulatory genes on other transcripts.

Management of Nematodes in Cotton

Cultural practices for management of plant parasitic nematodes on cotton. S. R. Koenning. North Carolina State University, Raleigh, NC. *Phytopathology* 97:S150.

The southern root-knot nematode *Meloidogyne incognita*, reniform nematode *Rotylenchulus reniformis*, and Columbia lance nematode *Hoplolaimus columbus* are the primary pathogenic nematodes affecting cotton. Cultural practices receive limited attention for their ability to suppress pathogenic nematode population densities and thus minimize yield losses in modern cotton production systems. Crop rotation with nonhosts, resistant or antagonistic cover crops, incorporation of organic amendments, and tillage have been investigated for this crop. Selection of rotation crops that are nonhosts for a given species of plant-parasitic nematode can limit the inoculum density of the pathogen. Winter cover crops may be effective in suppressing some plant-parasitic nematodes in cotton, but the efficacy may be limited unless the cover crop is sowed and destroyed before nematodes become active. Incorporation of cover crop residues and (or) animal manures improves water retention in sandy soils and decomposing residues may be toxic to nematodes. Tillage has long been recommended as a means of incorporating crop residue and for destruction of residual roots, but the effects of minimal tillage systems on plant parasitic nematodes is poorly understood.

Novel and conventional strategies for managing in nematodes in cotton with nematicides. T. L. Kirkpatrick (1) and W. S. Monfort (2). (1) University of Arkansas, Hope, AR; (2) University of Arkansas, RREC, Stuttgart. *Phytopathology* 97:S150.

Nematicides are the primary means of nematode management across the U.S. Cotton Belt. Conventional nematicide use strategies fall into three broad categories: i) preplant soil fumigation ii) at-planting application of non-fumigant materials, or iii) post-plant soil or foliar applications of nematicides during the early vegetative growth of the crop. Soil fumigation is expensive and requires specialized application equipment, but efficacy is high. Per hectare costs and ease of application are more attractive with non-fumigants, and these chemicals also may be insecticidal, but they are not as effective against nematodes. Application of nematicides directly to the seed has recently shown promise for limited nematode suppression early in the growing season. While convenience and worker safety are attractive, efficacy is marginal at high initial population densities. A novel recent approach to nematode management has been the use of precision farming technologies for site-specific nematicide placement within individual fields. The potential for improvement in the efficiency of nematicide application appears high with this strategy, but the cost and complexity of the equipment that is necessary may discourage some producers.

Molecular markers and mapping of root-knot nematode resistance in cotton. P. A. Roberts. University of California, Riverside, CA. *Phytopathology* 97:S150.

Host-plant resistance is economic and highly effective for root-knot nematode (RKN) *Meloidogyne incognita* control in cotton *Gossypium hirsutum*. Recently, nematode R gene mapping in cotton has revealed relationships between resistance sources and linked molecular markers. Markers are important for the efficiency of incorporating resistance genes into elite cultivars. Microsatellite markers (SSR) linked to RKN resistance in *G. hirsutum* 'Acala NemX' were identified using segregating progenies and recombinant inbred lines from intraspecific crosses and an interspecific cross with *G. barbadense* Pima S-7. Informative SSR were mapped on the above populations and one co-dominant SSR marker CIR316 was identified tightly linked (2.1 to 3.3 cM) to a major resistance gene (designated rkn1). Additional markers allowed the rkn1 gene to be mapped to cotton chromosome 11. Other markers linked to rkn1 were developed from AFLP screening and converted to CAPS and SNP markers for high throughput screening. Subsequently, a similar location of the major resistance determinant present in the Auburn source of RKN resistance was reported, and additional non-linked minor QTL for resistance identified. Higher levels of resistance in cotton by transgressive segregation were obtained with factors contributed by susceptible parents in intraspecific and interspecific crosses. These gene relationships and use of markers for cotton improvement are discussed.

Progress in development of reniform nematode resistance in cotton. A. F. Robinson. USDA-ARS, College Station, TX. *Phytopathology* 97:S150.

Development of upland cotton (*Gossypium hirsutum*) with resistance to reniform nematode (*Rotylenchulus reniformis*) began in the 1980s, leading to 11 breeding lines adapted to Louisiana or South Texas that combined tolerance and weak resistance to *R. reniformis* with strong resistance to *Meloidogyne incognita*. Evaluation of over 2,000 agronomic and primitive genotypes showed no strong resistance in agronomic *G. hirsutum* and only 12 accessions with weak to moderate resistance. Strong resistance occurs in diploid *G. anomalum*, *G. herbaceum*, *G. longicalyx*, *G. raimondii*, *G. somalense*, *G. stocksii*, and *G. thurberi*, which do not, and in tetraploid *G. barbadense*, which does freely hybridize with tetraploid *G. hirsutum*. Eight projects are introgressing resistance from *G. barbadense* by natural crosses and from *G. arboreum* and *G. longicalyx* via ploidy manipulation using cytoplasm fusion or colchicine. One lab backcrossed high resistance from *G. longicalyx* eight generations into *G. hirsutum*, identified a codominant SSR marker 1 cM from the resistance gene(s), and generated seed with fixed resistance for release in mid 2007. Much progress also was made in other projects. Markers for resistance to *M. incognita* and *R. reniformis* lie on chromosome 11 and compatibility is being explored. Advanced lines with resistance from *G. longicalyx* are being tested at several infested sites in 2007.

Plant Germplasm Collections in the Genomics Age

The National Plant Germplasm System: An overview. P. Bretting and R. Bennet. USDA-ARS National Program Staff, Beltsville, MD. *Phytopathology* 97:S150.

The USDA/ARS leads the U. S. National Plant Germplasm System (NPGS), a federal (USDA/ARS, USDA/CSREES), state (land-grant universities and state agricultural experiment stations), and industry collaboration for conserving a broad spectrum of crop genetic diversity, and promoting its use in research and breeding. The NPGS includes more than 20 different genebanks, located

throughout the U. S. In addition to acquiring germplasm via exploration and exchange, the NPGS maintains and regenerates more than 477,000 different samples. Each year, thousands of these samples are characterized for their genetic diversity and genotypic profiles. Samples are also evaluated for their agronomic or horticultural merit through trials conducted at different sites, over multiple years. The information associated with the genetic resources is also very important, and the NPGS's Germplasm Resources Information Network (GRIN) manages millions of characterization and evaluation data points and thousands of digital images of key crop traits that are accessible via the internet. On average, the NPGS distributes more than 120,000 samples per

year, free of charge and restriction, to researchers worldwide. In addition, the NPGS conducts research aimed at increasing the efficiency and effectiveness of the preceding genetic resource management programs.

Collection of wild and cultivated *Cicer* germplasm and potential use in genetics and breeding. F. Muehlbauer. USDA ARS, Washington State University, Pullman, WA. Phytopathology 97:S151.

Chickpea (*Cicer arietinum*) is a self-pollinating diploid annual cool season food legume crop originating in the Middle East and grown worldwide. Substantial germplasm collections of cultivated and wild species of chickpea are maintained by ICRISAT, ICARDA, USDA-ARS; while smaller collections are held in numerous national genebanks. There is great interest in wild *Cicer* germplasm for breeding disease and pest resistant varieties. Several of the eight annual wild species have shown good resistance to races of *Fusarium oxysporum*, *Ascochyta rabiei* and to various root rots. Also, there is good resistance to cyst nematode and pod borer (*Helicoverpa armigera*) in two of the annual wild species. Use of these germplasm resources for chickpea improvement depends on successful crossing and introgression of the genes to cultivated backgrounds. However, only crosses of cultivated chickpea with *C. reticulatum* and *C. echinospermum* have been successful. Attempts to use embryo rescue to overcome crossability barriers with *C. pinnatifidum*, *C. bijugum* and several wild perennials has been met with limited success. Overcoming these species barriers is currently a major challenge.

Soybean germplasm and disease resistance: The need for change. R. Nelson. USDA-ARS, Urbana, IL. Phytopathology 97:S151.

There are two common problems with germplasm collections: too little data or too much data. In general, germplasm collections suffer from too little data. With large collections, very limited resources, and continually changing needs of users, there is a perennial requirement for additional information. Increasingly, with the USDA Soybean Germplasm Collection, we find ourselves on the opposite side of the dilemma when it comes to certain disease resistance information. We have too much data, at least too much of certain data. Among the largest datasets that we have are reactions to the soybean cyst nematode *Heterodera glycines*, and fungal pathogens *Phytophthora sojae*, and *Fusarium solani* f. sp. *glycines* (synonym: *Fusarium virguliforme*) with over 80,000 soybean accession/pathogen strain interactions recorded. I will use these three pathogens to demonstrate the successes that can come from deploying resistance alleles, the problems associated with over reliance on single sources of resistance, the challenge of making genetic sense from massive amounts of phenotypic data, and the need for new technology in evaluating host plant resistance.

Disease resistance from the USDA National Small Grains Collection - past, present, and future. J. M. BONMAN, H. E. Bockelman, and B. J. Goates. USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID. Phytopathology 97:S151.

The National Small Grains Collection (NSGC) had its beginnings more than 100 years ago with the creation of the USDA Seed and Plant Introduction Office. Since then, the NSGC has grown to include more than 129,000 accessions of cultivated and wild relatives of wheat, barley, oat, rye, triticale, and rice collected from throughout the world. USDA-ARS scientists and cooperators continue to acquire and maintain materials and screen accessions for disease resistance. Through hybridization and selection, new genes have

been identified and deployed from NSGC accessions for resistance to cereal rusts, other foliar diseases, snow mold, and bunts. To further enhance the utility of the collection, the NSGC staff are developing precise information on the geographic origin of resistant accessions and using this information to guide further evaluation and possible exploration. Also, analysis of genetic diversity of accessions using molecular markers has begun. In the future, we anticipate that improved molecular genetic technologies will allow precise identification of r-gene diversity within the collection and provide plant breeders with information on linked markers to enable efficient transfer of resistance from landrace and wild materials.

Pea germplasm collections and their evolution with modern technologies. C. Coyne. USDA-ARS, Washington State University, Pullman, WA. Phytopathology 97:S151.

The genetic diversity in a germplasm collection can be described both phenotypically and genotypically. Combining these two descriptions, meaningful allelic diversity surrounding economic traits may be possible. The four major pea collections in the world, ICARDA, United Kingdom, Australia, and the USDA-ARS, have approached genetic diversity studies using different methodologies. The USDA-ARS focused on a core collection and employed four methods: data mining in the GRIN database; field studies with extensive phenotyping; genotyping using RAPDs and microsatellites; and SNP discovery for allele mining of described pea genes. TAQMAN assays are used for SNP genotyping the pea core collection. These assays may be converted to microarrays thereby increasing the throughput of allele diversity discovery in pea. Other approaches include DART and EcoTilling to increase genotyping throughput. The population structure of the USDA-ARS pea core will be presented and its applications in association mapping studies discussed.

Conservation of crop genetic resources and the Global Crop Diversity Trust. H. Shands. Director Emeritus, National Center for Genetic Resources Preservation, Fort Collins, CO. Phytopathology 97:S151.

Crop diversity is one of the most fundamentally important resources for human life on earth. It sustains life as does the water we drink and the air we breathe—it is food security. Environmental changes, both from natural and manmade causes, are diminishing the genetic resources available to farmers and breeders to combat biotic and abiotic stresses. Conservation is an imperative to retain the crop diversity for future generations on the planet. Conservation activities of all sorts get much printed press but crop diversity gets perilously little support in most countries around the world. We have foods in the markets and most people don't recognize the fine line that divides the haves from the have-nots. The flight to ethanol for fuel has already awakened the people of Mexico to the cost that loss of corn in the market means to tortilla prices. The Global Crop Diversity Trust is focused on ensuring that crop diversity is preserved in multiple collections around the world. Legally enabled by the International Treaty on Plant Genetic Resources for Food and Agriculture, the Trust is one of the funding mechanisms to protect our crop diversity. To identify and secure small, underfunded collections is the primary goal with secondary goals of databasing, characterizing, and distributing the materials to ensure maximum use. Recommendations are being made by crop experts and regional managers and associations. The Trust recently determined that it will support in part the Arctic Global Seed Vault being constructed by the Norwegian government in Longyearbyen, Svalbard.

Potato Cyst Nematode Regulatory Information and Implications for the Future

Current situation of potato cyst nematodes in Idaho. E. Davis, D. McNeal, O. El-Lissy, P. Berger, and R. Bulluck. USDA-APHIS-PPQ. Phytopathology 97:S151.

On April 13, 2007 the potato cyst nematode was discovered in a tare dirt sample from a potato grading facility in Idaho. After the detection, a full-scale incident command system was set up for several months, two new Federal or State laboratories were built and existing laboratories were updated. More than 780 Idaho potato fields were sampled and ~40,000 soil samples were processed in these laboratories. To date a total of 7 fields have been detected with PCN in Idaho. The current plan is to eradicate the nematodes in these fields.

Potato cyst nematode status in the United States. O. El-Lissy, P. Berger, R. Bulluck, E. Davis, and D. McNeal. USDA-APHIS-PPQ. Phytopathology 97:S151.

The Potato Cyst Nematode *Globodera pallida* (PCN) was detected in a tare dirt sample in April, 2006 in the State of Idaho. The infestation in Idaho has been delimited and has been found to date in 7 fields. The Golden nematode, *Globodera rostochiensis* (GN) has been known to occur in the State of New York. For the past 5 years, more than 50,000 samples have been collected throughout the United States and tested for the presence of PCN and GN, with no additional fields detected in any other States. Details on the national PCN survey and eradication plan for the infested fields in Idaho will be outlined.

Role of the technical working group in regulatory processes--PCN as a case study. P. Berger, O. El-Lissy, R. Bulluck, E. Davis, and D. McNeal. USDA-APHIS-PPQ. Phytopathology 97:S151.

The technical working group (TWG) is increasingly becoming a tool of regulatory agencies in the United States and abroad. When faced with a complex scientific issue in which the needed expertise are scattered worldwide, it is important to gather all available expertise so that the right questions are directed to the right individuals. The recent find of the Pale Potato Cyst Nematode, PCN, *Globodera pallida* in Idaho prompted convening

a TWG soon after its discovery. Even before the delimitation of the Idaho infestation was complete, a group of nematologists with worldwide expertise in the PCN pathosystem convened with operational and program regulatory officials (including experts in regulation of the Potato Golden Cyst nematode *Globodera rostochiensis* in New York) to identify potential control and eradication strategies, survey and detection methodologies and review the known science of the PCN pathosystem that can be applied to current data on the Idaho finds. Through the careful, diligent and transparent work of the TWG, an eradication and national survey plans were developed specifically for this situation based on sound scientific concepts and knowledge.

On the morphological identification of pale cyst nematode, *Globodera pallida* associated with potato in the United States. Z. A. Handoo (1), L. K. Carta (1), A. M. Skantar (2), and D. J. Chitwood (1). (1) Nematology Laboratory, (2) Molecular Plant Pathology Laboratory, USDA-ARS, Plant Sciences Institute, Beltsville, MD 20705. *Phytopathology* 97:S152.

Because the initial discovery of *Globodera pallida* in Idaho resulted in immediate regulatory action by several countries, the Idaho State Department of Agriculture and USDA APHIS conducted a detailed survey of Idaho potato fields. From more than 29,000 soil samples processed in Idaho from April to December of 2006, a total of 1,261 suspected potato cyst nematode samples were sent to the ARS Nematology Laboratory in Beltsville, MD for further diagnosis. From these samples we found 5,892 cysts of *G. pallida*, 285 cysts of cereal cyst nematode (*Heterodera avenae*), 37 cysts of clover cyst nematode (*Heterodera trifolii*), and more than 7,000 cyst-like bodies distributed across 151, 59, 1, and 1,050 samples, respectively. After the identity of the nematode from Idaho was established as *Globodera pallida* by morphological examination of mature cysts and second-stage juveniles and molecular analysis of juveniles, we performed a detailed morphological evaluation and morphometric analysis of the Idaho population. Variations in tail shape of this population and its relationship to *Globodera rostochiensis* and the *G. tabacum* complex are discussed. To date seven fields in Idaho have been confirmed positive for *G. pallida*. Surveys to acquire additional information about the distribution of this nematode are underway.

Molecular characterization of *Globodera pallida* associated with potato in Idaho. A. M. Skantar (1), L. K. Carta (2), Z. A. Handoo (2), and D. J. Chitwood (2). (1) Molecular Plant Pathology Laboratory, (2) Nematology Laboratory, USDA-ARS, Plant Sciences Institute, Beltsville, MD 20705. *Phytopathology* 97:S152.

Molecular diagnostic methods were used to positively identify a new population of pale potato cyst nematode *Globodera pallida* (Stone) Behrens. PCR-RFLP of the rDNA ITS region, sequence-specific multiplex PCR, and DNA sequence comparisons all confirmed the identity of the Idaho population as *G. pallida*. The ITS rDNA of the Idaho isolate was identical to sequences obtained for populations from York, England and the Netherlands. Because the tobacco cyst nematode *Globodera tabacum* and the similar golden potato cyst nematode *G. rostochiensis* are also present in the U.S., the ability to distinguish *G. pallida* from these species will be particularly important as more states perform surveys for these cyst nematodes. To prepare for the possibility of mixed field populations, we have designed species-specific primers that can discriminate *G. tabacum* from *G. pallida* and *G. rostochiensis*. Future prospects for additional molecular diagnostics will also be discussed.

Detection of potato cyst nematode, *Globodera rostochiensis*, on potato in Saint-Amable Region, Québec, Canada. F. SUN, S. Miller, S. Wood, and M.-J. Côté. Canadian Food Inspection Agency. *Phytopathology* 97:S152.

Potato cyst nematodes, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *Globodera pallida* (Stone, 1973) Behrens, 1975 are considered the most economically important nematode pests of potatoes worldwide and are the subject of strict quarantine regulations in many countries including Canada. In Canada, *G. rostochiensis* is known to be confined to the Saanich Peninsula of Vancouver Island in the west, and both *G. rostochiensis* and *G. pallida* are present on the island of Newfoundland in the east. In August, 2006, soil and roots of potato plants collected from a 19.2-ha field in Saint-Amable region, Québec, were submitted to the Nematology Laboratory, Canadian Food Inspection Agency, Ottawa. Golden-coloured, spherical-shaped cysts were found associated with the roots, and also extracted from the soil. The nematodes recovered were identified as *G. rostochiensis* by morphological and morphometric analysis and DNA

analysis. The origin of the introduction of *G. rostochiensis* into Saint-Amable is unknown. An intensive soil survey is underway to define the infested area, and strict quarantine measures have been taken to prevent further spread of *G. rostochiensis*.

Regulatory control of PCN: A European perspective. J. Pickup (1) and M. Phillips (2). (1) Scottish Agricultural Science Agency; (2) Scottish Crop Research Institute. *Phytopathology* 97:S152.

When it was adopted by the then six European Union (EU) Member States in 1969, the original PCN Directive (69/465/EEC) became one of the first EU Control Directives for a plant pathogen. Thirty-eight years later, considerable debate has taken place to produce a revised Directive that is appropriate for controlling PCN within the current twenty-seven European Member States. Attempts to bring the Directive into line with other phytosanitary legislation, e.g. the EU Plant Health Directive (77/93/EEC), began over 20 years ago but only relatively recently has the European Commission had sufficient resources to produce a proposal for a new Control Directive. This proposal was drawn up by the Commission, taking advice from a working group of European experts. It was first considered by an EU Council Working Group in May 2005. Since then several Working Group meetings and much discussion and negotiation have taken place, producing many further revisions. This presentation outlines the proposal for the new Directive and considers its potential impact on the control of PCN and on potato production.

Spatial distribution patterns and development of reliable sampling methods for potato cyst nematodes. C. H. Schomaker and T. H. Been. Plant Research International, Postbox 16, 6700 AA, Wageningen, The Netherlands. *Phytopathology* 97:S152.

In 1990 a research program was initiated to develop new sampling methods for the detection with known accuracy of patchy infestations of potato cyst nematodes (PCN) (*Globodera rostochiensis* and *G. pallida*). For detection of PCN two different spatial distribution patterns have to be taken into account. First, the small scale distribution, which originates from the spatial distribution in which host are grown and multiplication on their roots, could be described by a negative binomial distribution. An aggregation factor 'common k' of 70 was estimated and is used to calculate the probability of finding a certain number of cysts when a single core is collected. The medium scale distribution, the result of passive redistribution of PCN by agricultural machinery, was described by a double exponential model providing the size and intensity of any focus with a defined central population density. A computer program, SAMPLE IV, was developed, combining both spatial distribution models, and used to evaluate existing and create new sampling methods. It was also used to develop new improved sampling methods for PCN in seed and ware potatoes. These methods serve as a basis for recommendations on control measures leading to maximum returns (Integrated Pest Management) and legislation, quarantine and export protection by governments.

NemaDecide, a decision support system for the management of potato cyst nematodes. T. H. Been (1), C. H. Schomaker (1), and L. P. G. Molendijk (2). (1) Plant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands; (2) Applied Plant Research, Edelhertweg 1, 8219 PH Lelystad, The Netherlands. *Phytopathology* 97:S152.

A consortium of 8 agro-business and research companies are developing NemaDecide; a decision support system (DSS) that, when complete, will contain all relevant quantitative knowledge about plant parasitic nematodes (cyst nematodes, root knot nematodes and *Pratylenchus* spp.) of some major crops, including potatoes, with emphasis on rotations. The primary task of NemaDecide is to keep nematodes at low, economically acceptable density levels and to prevent the spread of quarantine nematodes. The first phase of the project, the development of a DSS for the management of potato cyst nematodes, has been completed. The interface has been developed in close cooperation with extension workers and was tested on-farm with more than 60 growers. 50 years of nematological research have been structured into stochastic models and integrated in a software package. The quantitative information system provides growers with the possibility to estimate risks of yield loss, population development, detection by soil sampling, calculation of cost/benefit of control measures and provides adequate advice for growers to optimize financial returns. Growers can compare cropping scenarios, ask 'what if' questions and chose from up to 300 different potato cultivars. The DSS answers the top 10 number of questions an extension officer is exposed to.

Potato Viruses and Potato Seed Certification in the 21st Century

Potato breeding and certification in North America. J. L. Whitworth and R. G. Novy. USDA-ARS, Aberdeen, ID. *Phytopathology* 97:S153.

Virus resistance cultivars have been an objective of potato breeders since it was understood that 'degeneration' of seed was caused by virus-infection. Virus resistant cultivars are a component of an integrated pest management strategy. Insecticides, roguing, and seed certification have allowed the use of susceptible cultivars. Traditional breeding methods can take many years to produce resistant cultivars with acceptable agronomic characteristics. Transgenic methods can be used to more rapidly improve potato varieties, but consumer acceptance has limited their adoption. Intra-genetic modification may prove more acceptable to the consumer. Many resources are needed to screen for resistance and determine host symptomatology in new cultivars. New cultivars enter into a certified seed program to produce seed stocks for use by the commercial grower to produce a crop without a significant yield loss due to virus. Seed certification includes maintaining seed lot identity, field inspections, and winter grow-out of seed samples to identify late-season virus infections. Seed certification systems have evolved over the years as new diseases and new technologies have emerged. While the Canadian and United States certification systems are organized under different administrative models, their seed certification regulations are similar and based upon scientific principles.

Evolution of Potato virus Y: Changing pathogenicity. J. H. Lorenzen (1,2), X. J. Hu (1), N. G. Gudmestad (3), and T. Meacham (1). (1) University of Idaho, Moscow, ID; (2) International Institute of Tropical Agriculture, Kampala, Uganda; (3) North Dakota State University, Fargo, ND. *Phytopathology* 97:S153.

Potato virus Y is a well-characterized virus that has been known since the early days of potato seed certification and for which many hundreds of cDNA sequences have been deposited in DNA databases. PVY strains have been described based on symptoms on specific host plants. More recently, new strains and strain variants have been characterized that represent recombinants between PVY^N and PVY^O. Strain variants known as PVY^{NTN} have three recombination junctions and cause symptoms of potato tuber ringspot necrosis disease (PTNRD). Within-strain symptom severity can vary widely. Although it has been claimed that recombination in PVY is common, evidence suggests that the most common recombination-derived strain variants (PVY^{N-W} ~ PVY^{N:O}, PVY^{NTN}) in Europe, North America, and Asia derive from a common recombinational event. We have sequenced new isolates of PVY, and analyzed sequences deposited in GenBank for recombinational events. These analyses have identified new strain variants not yet unreported as unique recombinants. Many of these new strains have been characterized as also causing PTNRD. In this report we will describe some of these new strain variants collected in North America, Europe, and Asia. Monitoring may be appropriate to determine the relative abundance and severity of these new PVY variants.

PVY in the U.S. seed potato crop is changing: Do we blame it on the virus, the vectors or the crop? S. M. Gray. USDA, ARS, Plant Protection Research Unit, Ithaca, NY 14853. *Phytopathology* 97:S153.

A 3-yr survey of the U.S. seed potato crop has revealed the genetic diversity of the *Potato virus Y* population and its geographic distribution. The ordinary strain of PVY (PVY^O) is still the dominant type infecting the U.S. seed potato crop, but other strains of the virus are making inroads in certain geographical regions. Necrotic strains of PVY (e.g. PVY^N and PVY^{NTN}) were found infrequently in most production areas, however a recombinant version of the virus, often referred to as PVY^{N:O}, was widespread. In addition, other variants are emerging, but often localized. The virus strains can differ in their ability to infect tubers and be carried over into the subsequent crop. These differences are influenced by cultivar and environment. Late season infections often do not produce symptoms and virus cannot be detected in the foliage, but there can be an efficient translocation of virus to developing tubers. The most efficient aphid vector, *Myzus persicae*, transmits all strains tested with similar efficiency. Other aphid species that do not colonize potato, but vector PVY are being tested. Clearly, the dynamics of PVY in the seed potato crop is becoming more complex. An expansion of tuber necrotic strains will greatly impact potato production; the impact of much of the other newly discovered genetic diversity in PVY is unknown.

Suppressive Soils: Agronomic Practices to Enhance Biological Control of Plant-Parasitic Nematodes and Plant-Pathogenic Fungi

Managing microbial community evolution to suppress soilborne plant pathogens. L. Kinkel. University of Minnesota, St. Paul, MN. *Phytopathology* 97:S153.

Specific detection of strains and variants of Potato virus Y infecting potatoes. C. Kerlan (1), L. Glais (1), M. Rolland (1,2), and E. Jacquot (1). (1) INRA, Agrocampus Rennes, BiO3P (Biology of Organisms and Populations applied to Plant Protection), Le Rheu; (2) FNPPPT (Seed Potato Growers), France. *Phytopathology* 97:S153.

Continuous changes in populations of PVY require frequent adaptation of detection tools, many of which rapidly become obsolete. Immunoassays using strain-specific monoclonal antibodies are useful tools, although not fully reliable given the serological shift of PVY populations observed during the last two decades. Bioassays on tobacco and potato cultivars bearing resistance genes are time-consuming, but reliably detect the two main strains PVY^O and PVY^N. Bioassays for detecting PVY variants (NTN, NW, N:O) are unavailable. Molecular tests detecting these variants are based either on nucleotide polymorphism or recombination junctions in the genome. We have developed a set of tools combining serology and PCR technique for detecting variants (NW, recombinant and non-recombinant NTN), which are routinely used in seed potato certification laboratories. Besides these tools based on neutral markers, a real-time PCR assay has been based on a single nucleotide polymorphism linked to the necrotic property of PVY on tobacco. This assay distinguishes PVY^N and PVY^O isolates and detects each in mixed infections. A new assay called SNaPshot targets four nucleotides. It allows specific detection in a single test of PVY^O, PVY^N, PVY^{NW} and recombinant PVY^{NTN} isolates. By increasing the number of nucleotides targeted, this innovative tool could be adapted to detect PVY phenotypes not yet described.

Virus detection using array-based technologies. K. L. Perry, B. Agindotan, N. Zhang, and C. Smart. Cornell Univ., Ithaca, NY. *Phytopathology* 97:S153.

As a diagnostic detection method, an array offers the advantage of the simultaneous detection of multiple (hundreds or more) pathogens. Both membrane and glass slide formats for macro- and micro-arrays, respectively, have been described for the detection of plant pathogens. These arrays have been modest in their spotting densities, but the methods and principles demonstrated. We are developing a multipathogen detection system for solanaceous crops, initially focusing on the use of diagnostic oligonucleotides immobilized onto membranes in a microarray format. RNA viruses are detected by amplifying cDNAs from total infected-plant RNAs without the use of pathogen-specific primers. The virus detection microarray has been shown to be effective in detecting 11 potato viruses and a viroid. Oligonucleotides are being designed for an expanded array taking into account the sequences of over 100 viruses of solanaceous plants. In a complementary system, an array has been completed for the detection of 43 fungal and oomycete pathogens of solanaceous crops, including 12 members of the *Fusarium solani* species complex and a majority of the respective pathogens of tomato. Current efforts are to combine these arrays plus one for bacterial pathogens to create a multipathogen detection system.

Methods of seed production for less developed regions in the world. L. F. Salazar. Agdia, Inc., Elhart, IN. *Phytopathology* 97:S153.

Formal seed production programs and their associated seed certification schemes are not common practice in less developed regions of the world. Most countries do not have a system for seed certification, but if they do it is usually adapted from those available in developed countries. Informal seed that can be obtained from ware potato crops, based on the small size of the tubers is planted in more than 95% of the regions. There are several factors conducive to this situation being the most important the scarce knowledge on pathogens and the fact that some countries do not have appropriate environmental conditions or infrastructure to produce good quality seed. Most potato diseases that are found elsewhere in developed countries affect the potato in these regions, with some exceptions. To attempt remedy this situation a seed production system that includes old technology for rapid multiplication and also the modern sensitive technology for pathogen detection was devised some years ago. In this system, named RAPIDSEED, starting with approximately 1000 selected plants (through positive selection) seed of good quality can be produced that after a second generation will be enough to plant more than 170 acres. A training component is required. The system can also be applicable, or it can be made to fit conditions for use in selected locations of the developed world.

Organisms within the genus *Streptomyces* produce a diverse variety of antibiotics. In Minnesota, a naturally-occurring disease suppressive soil supported significantly greater densities, inhibitory activities, and a greater diversity of antibiotic phenotypes than *Streptomyces* communities in an adjacent disease-conducive soil. We hypothesize that density- and frequency-dependant selection are important to generating and maintaining a high intensity and diversity of inhibitory activities within the suppressive soil.

Spatial patterns of pathogen inhibition among *Streptomyces* from the same and different locations in prairie soil provide further support for the hypotheses of density- and frequency-dependant selection, and specifically that local coevolutionary interactions are important to pathogen inhibitory activity in soil. Subsequent research suggests that green manures offer one potential means for managing microbial community evolution in soil, and for enhancing pathogen suppressive activity in agricultural fields. More broadly, these results suggest that long-term management strategies for soilborne plant pathogens should include a focus on maintaining high densities of indigenous soil microbes, and that regular integration of green manures within the cropping system may be an important component of effective and sustainable control.

Soil suppressiveness against the soil-borne disease complex of sudden death syndrome of soybean. A. Westphal (1), L. J. Xing (1), A. Seyb (1), and T. J. Vyn (2). (1) Department of Botany and Plant Pathology, and (2) Department of Agronomy, Purdue University, West Lafayette, IN. *Phytopathology* 97:S154.

Heterodera glycines and sudden death syndrome (SDS), caused by *Fusarium solani* f. sp. *glycines*, combined affect more yield losses to soybean in the U.S. than any other known pathogen. The pathogens form a disease complex in which *H. glycines* synergistically increases the severity of foliar SDS symptoms. Examples of soil suppressiveness to plant pathogens of prokaryotes, basidiomycetes, hyphomycetes, and plant-parasitic nematodes have been described. The objective of this project was to determine whether soils exist that suppress the SDS complex. In soybean monoculture no-tillage infestation trials with *F. solani* f. sp. *glycines* and natural or artificial infestations of *H. glycines*, more severe foliar SDS symptoms were detected in originally fumigated plots than in non-treated plots in the third year after infestation in three of five trials. In most trials, more *H. glycines* cysts at harvest were found in pre-season-fumigated than in non-treated plots, suggesting that the non-treated soils had become suppressive to the disease complex of *H. glycines* and SDS. In long-term tillage plots, *H. glycines* population densities were lower and severity of SDS foliar symptoms was reduced in less intensive tillage systems. Data from this project suggest that suppressiveness against the soil-borne SDS disease complex exists that awaits exploitation for sustainable disease management.

Soil health, oscillations in bacterial populations, and suppression of pathogenic fungi and nematodes. A. H. C. van Bruggen (1), W. J. Blok (1),

E. Kaku (1), A. J. Termorshuizen (1), R. Berkelmans (2), V. V. Zelenev (3), and A. M. Semenov (3). (1) Biological Farming Systems, WUR, Wageningen, the Netherlands; (2) Gebr. Verbeek B.V., Muldersweg 15, 5941 MX Velden, the Netherlands; (3) Lab. Microorg. Cultiv. Proc., Inst. Vaccins Serums, Russian Academy of Medical Sciences, Moscow, Russia, and (3) Dept. of Microbiology, MSU, Moscow, Russia. *Phytopathology* 97:S154.

A healthy soil is defined as a stable soil system with high levels of biological diversity and activity, low available C and N, and resilience to disturbance. This implies that microbial oscillations after a disturbance would be less pronounced and would dampen more quickly in a healthy than in a chronically damaged and biologically impoverished soil. A healthy soil is expected to be suppressive to diseases and pests. Our objective was to relate the amplitude of oscillations in bacterial populations after a disturbance to suppression of root diseases and nematode populations in organic and conventional soils. Daily dynamics of bacterial populations were monitored and disease (Fusarium wilt of flax) suppression was assessed in organic and conventional greenhouse soils with or without application of grass clover residues. Relative peak heights of bacterial populations in amended compared to nonamended soil were lower and Fusarium wilt was more suppressed in organic than conventional soil. Amendment with 1% grass-clover destabilized microbial communities and increased flax wilt temporarily in organic soil. Survival of juveniles of *Meloidogyne incognita* and root knot symptoms on tomatoes were less in undisturbed organic soil than in organic soil that had been steam sterilized in the past. These results are in agreement with the general concept of soil health.

Examining nematode-suppressive soils. J. Borneman and J. O. Becker. University of California, Riverside, CA. *Phytopathology* 97:S154.

Suppressive soils hold considerable potential for managing plant-parasitic nematodes. When the suppressiveness has a biological origin, identifying the causal organisms is the crucial step in realizing this potential. Armed with such knowledge, it may be possible to develop effective and sustainable pest management strategies through application of these organisms or agronomic practices that influence their population densities. This presentation will focus on the development and utilization of a population-based approach for identifying microorganisms involved in nematode suppressive soils. Experiments examining soils suppressive to the sugarbeet cyst and root knot nematodes will be described.

Professionalism/Service/Outreach

7th I.E. Melhus Graduate Student Symposium: Emerging and Changing Viral Pathogens - Biology and Molecular Mechanisms

Methylation as a host defense against geminiviruses. P. Raja, R. C. Buchmann, and D. M. Bisaro. Dept. of Molecular Genetics, Ohio State University, Columbus, OH 43210. *Phytopathology* 97:S154.

Geminiviruses replicate their ssDNA genomes through dsDNA intermediates that associate with cellular histones to form minichromosomes. Like most viruses, geminiviruses are targets of RNA silencing and encode proteins that suppress this adaptive host defense. In addition, geminivirus dsDNA is potentially subject to siRNA-directed methylation, including inhibitory events such as cytosine methylation and histone methylation (e.g. H3K9). We hypothesized that methylation functions as a host defense to inhibit virus replication and/or gene expression and that as a counterdefense, geminiviruses encode proteins that inhibit methylation. We have shown that AL2 protein from Tomato golden mosaic virus (TGMV), and the related L2 protein of Beet curly top virus (BCTV), are silencing suppressors that can inactivate host adenosine kinase (ADK). ADK activity is needed to sustain the methyl cycle that generates S-adenosyl-methionine (SAM), an essential cofactor for most methyltransferases. We have accumulated evidence which indicates that ADK activity and the methyl cycle it supports are required for efficient silencing. Thus AL2 and L2 can suppress silencing by inhibiting methylation. Current work is focused on biochemical studies of viral dsDNA replication intermediates and genetic studies involving inoculation of mutant hosts. Bisulfite sequencing has confirmed that geminivirus dsDNA is targeted by cytosine methyltransferases, and chromatin immunoprecipitation (ChIP) studies have shown that a combination of inhibitory H3K9 and active H3K4 methylation occurs on histone H3 associated with viral dsDNA. This suggests a dynamic balance between virus replication and host defense. In addition, we have found that plant hosts deficient in cytosine or H3K9 methyltransferases, or activities associated with RNA-directed methylation pathways, are exquisitely susceptible to infection by geminiviruses. Cytosine methylation

patterns are reduced or altered on viral DNAs obtained from infected mutant plants. Together, these data strongly support the idea that methylation acts as a defense against DNA viruses, and establish that geminiviruses can serve as models to investigate RNA-directed methylation.

Emergence of tomato leaf curl and yellow leaf curl diseases in West Africa: Identification of a novel begomovirus-satellite DNA complex. L.-F. Chen (1), C. Hagen (1), Y. Zhou (1), M. Noussourou (2), T. Kon (1), M. Rojas (1), and R. L. Gilbertson (1). (1) Department of Plant Pathology, University of California, Davis, One Shields Ave., Davis, 95616; (2) Institut D' Economie Rurale, CRRA de Sotuba BP:262 Bamako, Mali. *Phytopathology* 97:S154.

Tomato leaf curl/yellow leaf curl disease (TLC/YLCD) has emerged as a serious constraint to tomato production in Mali and other countries in West Africa. Surveys of tomato producing regions in West Africa were conducted in 2003–2006, and samples of tomato and pepper plants showing virus-like symptoms were collected. Most of these samples showed symptoms of leaf curl, purple vein and yellowing; although some plants showed a yellow-mottle and leaf crumpling. Squash blot hybridization and/or squash blot/PCR analyses revealed begomovirus infection in most samples. Initial sequence analysis of PCR-amplified fragments indicated the presence of Tomato yellow leaf curl virus (TYLCV) and several potential new species. Sequence analysis of complete genome revealed a monopartite begomovirus composed of TYLCV-mild strain (89%) and recombination event was occurred with Hollyhock leaf crumple virus (11%). Thus, this virus was named: Tomato yellow leaf curl Mali virus (TYLCMLV). A satellite DNA was also associated with TYLCMLV. Infectivity studies established the monopartite nature of the genome; TYLCMLV was infectious in tomato, common bean, *Nicotiana benthamiana*, and other hosts. However, the satellite DNA dramatically increased symptom severity in some hosts, including tomato. TLC/YLCD in West Africa is caused by a complex of locally-adapted begomoviruses and satellite DNAs. The implications of these findings in disease management will be discussed.

Sequence and recombination analysis of potato virus Y. X. Hu. University of Idaho, Moscow, ID. *Phytopathology* 97:S155.

Potato virus Y (PVY) is one of the most economically important plant pathogens. Recognized strains are the ordinary strain PVY^O, the necrotic strain PVY^N and the C strain PVY^C. PVY genome has a high degree of genetic variability and is also subject to recombination. The new recombinants include PVY^{NTN}, PVY^{N-Wi}, PVY^{N-Wi} type B, and PVY^{N:O} reported in many countries. Recently, new recombinant strains of PVY (PVY^{NTN}) causing potato tuber necrotic ringspot disease (PTNRD) spread in North America. Origin of these recombinant strains, and mechanisms driving emergence of tuber necrotic PVY strains are not clear. In this study, we searched for recombination junctions (RJs) among all 42 available whole PVY genome sequences in GenBank. We found 4 main RJs in PVY genomes: RJ1 in the N-terminal region of P1 (nt 498-500 in alignment), RJ2 in the C-terminal region of HC/Pro-P3 region (nt 2392-2416), RJ3 in the N-terminal region of VPg (nt 5827-5889), and RJ4 in the C-terminal region of CP (nt 9169-9381). PVY^{N:O} strain had only RJ2. PVY^{NTN} strain had two groups, one with 3 RJs: RJ2, RJ3, RJ4, and the other with 4 RJs. PVY^{N-Wi} had two RJs: RJ1 and RJ2. Analyzing the PVY RNA secondary structure, we found that many RJs occurred at X or Y shaped regions with extensive secondary structure. Based on this analysis, we hypothesize that RNA secondary structure drives template switching and subsequent recombination in PVY. This relationship will help us to understand PVY evolution and to devise new differentiation methods for screening against tuber necrotic PVY strains.

Molecular characterization of an emerging citrus virus-Dweet mottle virus and study of the intra-population profile of *Citrus exocortis* viroid. S. Hajeri. University of California, Riverside, CA. *Phytopathology* 97:S155.

Virus and viroid pathogens pose serious threats to the citrus industry. *Citrus leaf blotch virus* (CLBV) is an emerging citrus graft-transmissible agent. It has aroused concerns because of its potential for seed transmission. Molecular characterization of *Dweet mottle virus* (DMV) revealed that it is closely related to CLBV. Immunodiagnostic techniques for DMV will be developed by raising antibodies to the bacterially expressed DMV coat protein. This will allow the in-depth understanding of the DMV and its relationship with CLBV. Viroids are constantly evolving nucleic acid entities due to the error prone nature of the host RNA polymerase. These mutations might have implications on the biology (host range, infectivity, pathogenesis) of the progeny viroids. *Exocortis* is a rootstock disease caused by the *Citrus exocortis* viroid (CEVd)

and is found in all citrus-producing areas of the world. One of the objectives of this project is to study the intra-population profile of the CEVd at the single cell level avoiding the potential bottleneck effects of the organized tissues such as phloem, leaves, or roots of a whole-plant. Citrus and tobacco protoplasts were inoculated with *in vitro* RNA transcripts of a single CEVd cDNA clone. Total RNAs were extracted from the protoplasts and the CEVd progeny RNAs were analyzed through Reverse Transcription-Polymerase Chain Reaction (RT-PCR) with CEVd specific primers and slot-blot hybridization with DNA-DIG labeled probes. The RT-PCR product was sequenced and found to be a sequence variant of CEVd. Randomized RT-PCR products will be cloned and sequences of individual viroid molecules will be used for the genealogical analysis of the viroid progeny population at the cellular level.

Functional and cytopathological analysis of two unique Lettuce infectious yellows virus (LIYV)-encoded proteins: P34 and P26. L. Stewart (1), V. Medina (2), M. Sudarshana (3), and B. Falk (1). (1) Dept. of Plant Pathology, University of California, Davis; (2) Dept. Produccion Vegetal I.C. Forestal, Universitat de Lleida, Spain; (3) Western Institute for Food Safety and Security, University of California, Davis. *Phytopathology* 97:S155.

In recent years, distribution of whiteflies and the plant viruses they transmit has increased throughout the world. Among emerging whitefly-transmitted viruses are members of the genus *Crinivirus* within the family *Closteroviridae*. Lettuce infectious yellows virus (LIYV) is the type member of the genus *Crinivirus*, and provides a system in which to examine gene function and virus biology. This study examines two LIYV-encoded proteins, P34 and P26. P34 is important for efficient replication of RNA2 but not RNA1 of this bipartite virus. The function of P26 is still unknown, but it is associated with plasmalemma deposits, characteristic structures located near plasmodesmata pit fields in infected cells. As part of ongoing functional and mechanistic investigation of these LIYV-encoded proteins, their localizations and effects on cytopathology were assessed using GFP-fused or native proteins expressed from a heterologous Tobacco mosaic virus (TMV) vector. Our results indicate that P34 is not essential for membrane rearrangement that occurs during LIYV infection, and that P26 alone is sufficient to induce the formation of plasmalemma deposits it has previously been associated with. Continuing work is in progress to understand the mechanism by which P34 enhances RNA 2 replication and to determine the role of P26 during viral infection.

Blueprint for Learning - Constructing Courses

Blueprint for learning - Constructing courses. L. Richlin. Claremont Graduate University. *Phytopathology* 97:S155.

The symposium will provide a "Quick Start" for designing courses that facilitate, assess, and document learning, and will offer guidance on such issues as framing questions that encourage discussion, developing assignments with rubrics, and creating authentic tests. It will familiarize participants

with course design elements; enable them to understand themselves as individuals and teachers; know their students; adapt to their own learning environments; design courses that promote deep learning; and assess the impact of the teaching practices and design choices they make. Based on the presenter's recent book of the same name (Stylus, 2006), it will offer an intellectual framework, set of tools, and best practices to enable participants to continually reassess the impact of their teaching on their students' learning.

Faces of the Future in Nematology

The effects of Brassica crops on plant parasitic nematodes, free living nematodes, and soil microbial dynamics when used in combination with reduced rates of synthetic nematicides. E. Riga. Washington State University, Prosser, WA. *Phytopathology* 97:S155.

New and improved Brassica varieties possess both nematicidal and nematode trapping properties and have shown great potential of controlling plant parasitic nematodes. Control of plant parasitic nematodes and enhancement of beneficial free living nematodes is essential to quality crop production and protection of the environment in the USA. In Washington State, plant parasitic nematodes are successfully managed with synthetic nematicides but at a monetary cost that exceeds \$500 per acre. The purpose of this long term experiment was to evaluate the effects of Brassica crops on their own and in combination with reduced rates of synthetic nematicides on plant parasitic nematodes, free living nematodes and soil microbial communities. Our field trials have showed that Brassica trap crops in combination with half the recommended rate of the synthetic fumigant Telone, reduced root knot nematode populations (*Meloidogyne chitwoodi*) from 700 nematodes per 250cc soil to zero, where the economic threshold for *M. chitwoodi* is 1 nematode / 250cc soil; and reduced lesion nematodes (*Pratylenchus penetrans*) and stubby root nematodes (*Paratrichodorus allius*) to below economic threshold. In contrast, the combination treatments did not reduce the

beneficial free-living nematode populations and the non-pathogenic *Pseudomonas*. The cost of growing and incorporating trap crops in combination with reduced rates of nematicides was approximately half the present commercial cost of fumigants.

Sustainable approaches to the management of plant-parasitic nematodes and disease complexes. A. Westphal. Purdue University, West Lafayette, IN. *Phytopathology* 97:S155.

Some soil physical, chemical, and biological factors reduce damage caused by plant-parasitic nematodes. Suppression of damaging populations of plant-parasitic nematodes in soils that experience short crop sequences or sequences of multiple susceptible hosts is most challenging. In southern Indiana, crop sequences of watermelon, soybean and corn do not suppress *Meloidogyne incognita*. In an integrated management approach, several aspects of this watermelon production system can be modified. Small grains as cover crops can be replaced by cover crops with resistance to *M. incognita* or by crops with biofumigation potential. Cash crops with resistance to *M. incognita* can be used to reduce population densities of *M. incognita*. Other approaches to nematode management utilize soil suppressiveness. One-year rotations of soybean with corn neither reduced the soil-borne complex of sudden death syndrome (SDS) nor improved soybean root health over that in soybean monoculture. Reduced tillage combined with crop rotation may reduce the activity of soil-borne pathogens in some soils. For example in a long-term

trial, numbers of *Heterodera glycines* and severity of foliar SDS symptoms were reduced under minimum tillage. Sustainable management strategies require holistic approaches that consider entire production systems rather than focus on a single crop in its year of production.

Nematode parasitism genes as RNAi targets for engineering novel nematode resistant crops. G. Huang (1), E. Davis (2), T. Baum (3), W. Parrott (1), and R. Hussey (1). Depts. of Plant Pathology, (1) Univ. of Georgia, Athens, GA 30602; (2) N. C. State Univ., Raleigh, NC 27695; (3) Iowa State Univ., Ames, IA 50011. *Phytopathology* 97:S156.

Parasitism genes expressed in the esophageal gland cells of plant-parasitic nematodes encode secreted proteins that mediate nematode infection and parasitism of plants. A root-knot nematode (RKN) parasitism gene (*16D10*) encodes a novel secreted parasitism peptide that functions as a signaling molecule to induce root proliferation by specifically interacting with a host plant regulatory protein. This parasitism peptide is conserved in RKN species and appears to mediate an early signaling event in RKN-host interactions. Ingestion of *16D10* double-stranded RNA (dsRNA) *in vitro* silenced the target parasitism gene in RKN and resulted in reduced nematode infectivity on plants. *In vivo* expression of *16D10* dsRNA in *Arabidopsis* and soybean resulted in resistance effective against the four common RKN species. These results validate that this parasitism gene is essential for RKN parasitism of plants and, more significantly, they make available a resistance gene effective against RKN with an effective range of resistance not conditioned by any natural RKN resistance gene. Therefore, targeting RKN parasitism genes through *in planta* delivery of RNAi enables the development of transgenic

crops with effective broad host resistance to RKN and provides a strategy for engineering novel RKN resistant crops for which natural resistance genes do not exist.

Research collaborations can improve the use of organic amendments for plant-parasitic nematode management. I. A. Zasada. USDA, ARS Nematology Laboratory, Beltsville, MD. *Phytopathology* 97:S156.

The concept of utilizing organic amendments to manage plant-parasitic nematodes is not new, but the widespread implementation of this management practice has still not been realized. The use of organic amendments for plant-parasitic nematode management is a complex process requiring an understanding of the transformation and generation of active compounds. As a result, research endeavors to understand and maximize the use of this management practice require a multi-disciplinary approach which draws upon the expertise of nematologists, microbiologists, natural product chemists and soil scientists. Factors that require analysis and clarification include lethal concentration levels of organic amendments necessary to kill nematodes; chemical composition of incorporation material; fate and exposure potential to nematodes of compounds released into the soil; understanding of how the environment (i.e., temperature, microbial community, soil type) influences the activity of organic amendments. Examples of research conducted in a collaborative manner on rye (*Secale cereale*) and brassicaceous cover crops, as well as a biosolid amendment, will demonstrate the power of multi-disciplinary research. Only through collaborative research can consistent and reliable nematode suppression with organic amendments be achieved.

Integrating Diverse Disciplines: Assessing Social & Economic Impact of our Research and Outreach Program

Program evaluation and IPM—Integrating outcome level measures into your program and improving your reporting methods. C. Pilcher. Iowa State University. *Phytopathology* 97:S156.

Integrated Pest Management (IPM) has a long history in research institutions, governmental policies and society. Select efforts have measured adoption, however few research endeavors have focused on the economic, environmental, and health impacts associated with adoption of IPM. With an increasing emphasis on accountability, it is essential to determine the long-term outcomes (especially program impacts) to assess the actual value of the IPM program. A research project was developed assist with the national evaluation of IPM. This program evaluation developed a theoretical framework to evaluate IPM programs, summarized the program accomplishments and assessed the environmental, health, and economic impacts the program made on internal and external stakeholders. The theoretical framework for the National IPM Evaluation included the National IPM Roadmap for IPM, the Logic Model, and the IPM Matrix. A National IPM Evaluation Subcommittee was used to identify key performance indicators to

measure impacts associated with adoption of IPM. Regional IPM Centers were used to further identify key performance indicators and to implement the use of these indicators into their grant and reporting systems. State IPM Coordinators were used to gather existing outcome-level studies to provide resources of state-level, outcome-level evaluations. Logic models have been completed for each cell of the IPM Matrix and have been subjected to expert review. Indicators have been placed into the testing phase for granting and reporting systems. Regional IPM Centers will begin to use these indicators to measure the impacts of IPM on regional level projects (i.e., reduction in environmental risk, reduction in human risks, and cost/benefit analyses). State studies have been indexed into the IPM Matrix and serve as references for existing impact-level studies. At the national level, this research is an important step towards addressing the criticisms of the GAO Report on IPM and determining the true worth of the program. This evaluation articulates the long-term goals (ultimate impacts) of IPM and measures the progress towards these goals. The Regional IPM Centers are integral to the implementation of outcome level indicators on a regional scale. The IPM Centers can then collect data needed to assess the environmental, health and economic impacts associated with adoption of IPM. Finally, this research allows state IPM coordinators to also assess their state programs at the highest level of accountability.

Navigating the Maze of Online Community Resources

Nematode.net: Update on methods for data navigating and comparative genomics of nematodes. M. Mitreva, J. Martin, S. Abubucker, Y. Yin, T. Wylie, J. P. McCarter, and R. K. Wilson. Washington University School of Medicine. *Phytopathology* 97:S156.

The web-accessible resource Nematode.net (www.nematode.net) provides access to genome data specific to free-living and parasitic nematodes. Nearly 300,000 Expressed Sequence Tags and 600,000 Genome Survey Sequences have been generated from 30 nematode species. Nematode.net provides NemaGene EST cluster consensus sequences, NemPep predicted translations and codon usage tables. The cluster pages have a 'cluster hub' which links to a G-browse view of the related EST cluster. The database also provides functional classification: i) a tree-viewer along with a web-based graphical display using the KEGG enzymatic pathway maps; ii) Gene Ontology associations displayed using the Amigo Browser. Efforts were made to integrate it with other nematode-related specialized sites. Nematode.net users are able to link-out to all related *C. elegans* gene pages from each of NemaGene contig pages and Wormbase users can easily link to the proper contig pages within Nematode.net using a redirection enabled by a CGI scripts. The long-term goal of Nematode.net is to provide a useful,

consistent, centralized and lasting database to the worldwide parasitology community.

The Plant Management Network: An electronic resource serving practitioners and educators in applied plant science. D. Gross. Texas A&M University. *Phytopathology* 97:S156.

The Plant Management Network (PMN) is a rapidly expanding electronic resource (<http://www.plantmanagementnetwork.org/>) in applied plant sciences that provides multidisciplinary information to agricultural practitioners, researchers, and educators. PMN resources feature four applied, peer-reviewed journals, namely *Plant Health Progress*, *Crop Management*, *Forage and Grazinglands*, and *Applied Turfgrass Science*. Articles, which are illustrated with an abundance of color images, include applied research reports, management guides, reviews, diagnostic guides, industry news, and perspectives. The PMN image collection contains more than 3,200 items that can be used for diagnostics and for customized training and educational materials. The PMN Plant Science Database accesses thousands of indexed, web-based resources, including all articles published in PMN journals, as well as fact sheets, newsletters, product listings, training materials, and other resources hosted at PMN's university, industry, and non-profit partner sites. Recent additions to PMN include the Soybean Rust Information Center, Arthropod Management Tests, PMN Variety Trials, and Plant Disease Management Reports.

Making WormBase, WormBook and Textpresso truly about nematode worms rather than *C. elegans*. P. Sternberg and the WormBase Consortium. California Institute of Technology. *Phytopathology* 97:S157.

The *Caenorhabditis elegans* community has developed a number of online resources that are highly useful for this "model organism". The WormBase Consortium would like to leverage this progress to facilitate information exchange and research among the broader nematology community. Wormbase.org is a database of information about the genomes, genetics and biology of *C. elegans* and some other congeners. It is updated every 3 weeks with information about genes (expression, function, and interaction) and proteins. Information is extracted from published papers, large-scale data sets (e.g. genome-wide RNAi screens), and contributed by researchers. A community forum and wiki facilitate exchange of information and feedback about Wormbase. Wormbook.org is an open access companion to WormBase, with over 130 chapters on biology and methods, with a few chapters on parasitic nematodes, focusing on those whose genomes are being sequenced. Textpresso.org was developed as a search engine for full text of biological papers using categories. Users can search for individual sentences that have a *C. elegans* gene, a human disease, and a keyword, without specifying the name of each gene. Textpresso systems exist for yeast, *Drosophila* (29,000 papers) and a pilot one for neuroscience (15,000 papers). We plan a system for Nematodes, and seek help developing categories of interest to nematologists (e.g. "soil treatments," "plant diseases caused by nematodes").

NemAToL: Database for the nematode branch of the tree of life. W. K. Thomas. Hubbard Center for Genome Studies - University of New Hampshire. *Phytopathology* 97:S157.

NemAToL is an open relational database supporting the Nematode branch of the Tree of Life and other nematode biodiversity projects. The database is intended to organize and archive morphological, molecular and ecological data in the context of nematode phylogeny and biodiversity. The primary resources include: DNA sequence databases of loci commonly used in phylogenetic analysis (i.e. SSU, LSU, ITS regions of the nuclear rDNA locs, mitochondria l DNA sequences), morphological information (i.e. multifocal video images, still images, TEMs, SEMs, and line drawings), specimen data (localities, maps, associated metadata, and uBio links to distributed information associated with each defined taxon). All of these resources are linked together and organized in the context of nematode evolutionary relationships. The primary access to the database can be achieved by taxonomic queries, BLAST searches with molecular data and by geographic locality. Nematol is easily internet accessible at <http://nematol.unh.edu>.

Three Hundred Years of Nematology: Eelwool to RNAi

The science of nematology revolution. D. Schmitt. University of Hawaii. *Phytopathology* 97:S157.

The mysteries of microscopic organisms were revealed with the development of the microscope. It was some 150 years after this invention before the first plant-parasitic nematode was reported. The importance of soil-borne nematodes as serious pathogens was recognized in the mid-1800's marking the beginning of the nematology revolution. Thereafter, several important plant-parasites were described in rapid succession. Julius Kuhn and Henry Bastian were key researchers who developed a foundation of work that others were able to build upon. About a half century later, N. A. Cobb described many species of nematodes and developed extraction methods that are still used today. Soon after World War II, scientists and farmers alike began to recognize the damage that nematodes were causing to crops. During this same period, the world experienced a population boom never before seen in the history of humankind and this translated into an increased need for sustainable agriculture including a need for qualified individuals to work on nematode induced problems. This issue was partially addressed in the 1950's when the US congress provided funding for developing graduate teaching programs in nematology. Nematode control and systematics received much attention by this group of "new" nematologists. The latest era in the nematology revolution is addressing the science using molecular technology. Thus, in a very brief period of human history, plant nematology grew from the mere recognition of plant-parasitic nematodes as pathogen to a revolutionary science.

Nematicide development, use and detection in the twentieth century. M. McKenry. Dept. of Nematology, UC Riverside, Riverside, CA 92521. *Phytopathology* 97:S157.

Phytophthora Database: A cyber-infrastructure for detecting, monitoring, and managing *Phytophthora*. S. Kang (1), J. Park (4), B. Park (1), J. Blair (1), D. Geiser (1), I. Makalowska (1), S. Park (1), M. Coffey (2), M. Green (3), K. Ivors (3), Y. Lee (4), K. O'Donnell (5), and F. Martin (6). (1) Penn State, University Park, PA; (2) UC, Riverside, CA; (3) NCSU, Fletcher, NC; (4) Seoul National Univ, Seoul, Korea; (5) USDA-ARS, Peoria, IL; (6) USDA-ARS, Salinas, CA. *Phytopathology* 97:S157.

Due to their high virulence and ability to spread rapidly, *Phytophthora* spp. represent a serious threat to agricultural production and ecological systems. Many novel species have been reported in recent years, indicative of our limited understanding of their ecology and diversity in nature. The ability to accurately and rapidly identify *Phytophthora* is crucial for developing effective regulatory and disease management strategies. Systematic cataloging of cultures preserved in historical collections can serve as a baseline for accurate identification, classification, and risk assessment of new *Phytophthora* isolates collected in future surveys. The *Phytophthora* Database (www.phytophthoradb.org) was developed to establish such baseline by cataloging genotypic and phenotypic data in a web-accessible format that can be easily searched, analyzed, and updated. An overview of the organization, functionality, and future directions of the *Phytophthora* Database will be presented.

Comprehensive keys to nematodes at species and genus levels using INTKEY. M. Hodda. Commonwealth Scientific and Industrial Research Organisation, CSIRO (Australia). *Phytopathology* 97:S157.

There are many divergent forces which need to be balanced in designing information systems for biological information: a desire for the broadest possible availability; seeking the benefits of the broadest coverage of taxa, geography and range of information; a strong interest in rapid addition of new information to databases; the needs of compilers for ease of input; the desires of users for ease of output; requirements for data of high quality; and the necessity of accommodating users with different levels of expertise or needs. To balance all these forces within realistic constraints of resources has required compromises and the acceptance that attempts to make biological information available will always have to be regarded as incomplete and works-in-progress. This has been a central tenet of systematics for a considerable period. Despite the many innate difficulties of the task and the taxonomic complications of the Nematoda, it has been possible to design a key to nematodes and similar invertebrates that is simultaneously available, all-encompassing, flexible, and high-quality. The key is available now (<http://www.ento.csiro.au/science/nematode.html>) and has been attracting users from school children to experts. The properties of the program INTKEY have been important in achieving this outcome.

Carbon bisulfide was first applied to soil in the 1880's, chloropicrin in the 1920's. The nematicidal value of these biocides was not apparent because the economic importance of nematodes was unclear. Application equipment gradually transformed from hand-held devices to tractor-drawn implements capable of scratching 15-cm into soil. By 1933 the list of potential nematicidal agents was two-dozen. Most of these early chemicals were waste products from other human endeavors. The first easy-to-apply nematicide was patented in 1943. Subsequent decades brought deeper application equipment and chemicals with greater specificity. Terms such as soil-sickness and replant problem were being vanquished. By the mid 1960's we were monitoring nematicides in soil at parts per million. A decade later interest shifted to monitoring for off-target nematicides at part per billion levels. The off-target appearance of nematicidal agents or their metabolites at part per trillion amounts pointed the way to shorter-lived products, various new application methods, and avoidance of chemicals when possible. America in the 21st century has established million-fold safety factors for pesticides, pollutants in cities are monitored in nanogram amounts and net food imports do not currently exceed 10%. A review of 20th century nematicides can provide new directions for the 21st.

Plant-parasitic nematodes as components of the soil ecosystem. B. Kerry and S. Costa. Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK, Universidade de Coimbra, 3001-455 Coimbra, Portugal. *Phytopathology* 97:S157.

Plant parasitic nematodes affect plant succession in natural ecosystems and nutrient flows in soil, but research on them has centred on their economic importance. Key pest species have been identified, their effects on crop yield quantified and management strategies developed to minimise losses. Nematode damage to crops may be increased by interactions with soil-borne pathogens and reduced by a range of natural enemies. In intensive cropping

systems nematode pests are managed through the integration of crop rotation, plant resistance and nematicides. However, pressures to develop more sustainable methods of nematode management, demand a greater understanding of the role of plant parasitic nematodes in the ecology of soils. Recent research in the semi-natural habitat of sand dunes has demonstrated that the relative significance of top down (natural enemy) and bottom up (plant host) regulation of nematode populations differs between nematode genera; sedentary endoparasites were mostly regulated by bottom up processes and migratory ectoparasites by top down processes. However, individual population densities were small compared to those found under crops. Understanding of these processes is required for future pest management strategies and much may be learnt from research on natural ecosystems.

Nematode biology: 1750 vs 2008. T. J. Bliss. University of Nebraska-Lincoln. *Phytopathology* 97:S158.

As a river widening and narrowing through plains and canyons, nematology has persistently cut its course through the broad landscape of science. From Rudolphi to Chitwood (ca.1750-1950) nematode research was swept broadly forward by the steady current of morphology-based taxonomy. Goeze, Butschli, deMan, Cobb and others made notable advances in the classification of unending roundworm diversity. With the post-1950 discoveries of effective nematicides and nematode-resistant crop cultivars, however, the growing nematological waterway began to funnel into the narrow canyons of plant pathology. For the next 50 years, the swift surge of applied nematology, fed by powerful tributaries of government funding, affected the field in important ways. Economy-driven research on nematode management largely supplanted basic biological inquiry. Recently, the river of applied nematode research slowed as key nematicides were removed from the market and plant pathology shifted to more molecule-driven studies. Today, we are seeing the canyon of applied nematology give way again to the floodplains of basic science. Nematology must continue its confluence with other fields like evolution, developmental biology, soil ecology, genetics and basic plant pathology. If it does, nematode biology will grow into a mighty river flowing strongly into the ocean of scientific knowledge.

When the germ meets the worm: Plant nematology's contributions to plant pathology. A. MacGuidwin. University of Wisconsin. *Phytopathology* 97:S158.

Nematodes were recognized as causal agents of plant disease in the mid 19th century. Studies on the pathogenicity of root-knot and cyst nematodes helped shape the discipline of plant pathology in its formative years. The first agricultural nematology position was established at the beginning of the 20th century and by the 1940's research programs for nematodes were making great progress in understanding and managing nematode diseases. Mounting concern about nematode pests of regulatory importance in the 1940s and 1950s led to a rapid increase in the number of plant pathologists recruited to study nematodes. The duality of nematology as a blend of animal and plant sciences brought to plant pathology unique perspectives on pathogen/host interactions. Research on nematodes have helped advance understanding of the genetics and molecular biology of host/parasite interactions, the epidemiology of soilborne disease, and the role of the soil community in managing plant disease. Nematologists have long appreciated the significance of interactions between nematodes and other pathogens and have contributed to wholistic approaches of studying and managing disease in the field. Today, most plant nematologists in North America work in or are closely associated with departments of plant pathology, but retain a strong identity with the discipline of nematology. The breadth of interests represented by nematological societies, journals, and professional meetings provide nematologists with perspectives that help stir the disciplinary melting pot of plant pathology.

Plant nematology—A bridge between plant pathology and entomology (1). V. Ferris. Purdue University. *Phytopathology* 97:S158.

During my first years at Purdue, I felt like a displaced plant pathologist doing nematology in an entomology department. The course I taught with my husband (John Ferris) was called Fundamentals of Nematology and included free-living nematodes and animal parasites as well as plant nematodes. Insect ecologists soon showed me new ways of looking at nematodes, which, in fact, are tiny animals. A succession of our graduate students collected whole soil and freshwater nematode faunas from agricultural areas, forests, and water systems, and tried new quantitative techniques that entomologists were using to do community ordination. Plant nematode folks told me this was not "real" nematology. Ecology led to systematics, and my first NSF grant was for a nematode collection at Purdue, which has an outstanding museum collection of insects. I was a member of a rebel group of systematists who challenged old ways of doing systematics, and I embraced the methods of cladistics at an early period to infer nematode phylogenies. I explored vicariance biogeography and plate tectonics as explanations for certain extant insect and nematode distributions. This led to the acquisition of new data for molecular systematics. Students in my current course in Molecular Systematics and Evolution have diverse interests, including insects, nematodes, and other life forms.

Plant nematology—A bridge between plant pathology and entomology (2). D. W. Dickson. University of Florida. *Phytopathology* 97:S158.

In the United States there have been at least four Universities where entomology departments also houses plant nematologists, namely University of Florida, Purdue University, Michigan State University, and Rutgers University. In most other universities where plant nematology programs exist plant nematology is aligned with plant pathology. This raises the question, where is the most appropriate home? Only in California has nematology evolved with sufficient strength to form a nematology department. The closes University of Florida could come to realizing a department was having department named entomology and nematology ca. 40 years ago. There are two important points to be raised relative to placement of plant nematology. Most importantly is graduate programs and teaching. Plant nematology graduate program in entomology is not served well by the disconnect from plant pathology, nor is the plant pathology graduate program served well from the disconnect from plant nematology faculty and graduate students. Secondly, with intense competition today for faculty positions within universities the questions arises whether aligning with entomology is a good idea. In each of the four cases mentioned above plant nematology faculty positions have declined at least 50% during the past 10 to 15 years.

Plant nematology—A bridge between plant pathology and entomology (3). W. T. Crow. Dept. Entomology and Nematology. *Phytopathology* 97:S158.

A plant nematologist in a world of entomologists. Being a plant nematologist in an Entomology and Nematology Department has both challenges and rewards. On the negative side, being the smaller of the two disciplines you can get overlooked; "Hello, you've reached the Entomology Department!" On the other hand, it makes it easier to be recognized as being unique from the other faculty; "Oh, your one of the Nematologists." Our department has >60 entomologists and a minority of 7 nematologists. Therefore, when issues come to a departmental vote a unified group of nematologists can easily be out voted by a small percentage of entomologists. As an extension specialist I find that being in the Entomology and Nematology Department has helped my career by allowing me to specialize. When I was an extension plant pathologist at another institution I had to work with all pathogens, and was just another extension plant pathologist. All land grant institutions have at least one of them! However, now I am an extension nematologist, there are much fewer of those around, something unique. This has allowed me to fill a niche and programmatically stand apart from my peers in both plant pathology and entomology.