Abstracts of Special Session Presentations

Biology of Plant Pathogens

Airborne Mycotoxigenic Fungi in Plant and Human Disease


This presentation summarizes current knowledge of the aerobiology of Gibberella zeae ascospores. Ascospores are discharged from mature perithecia on overwintered cereal debris in response to environmental cues that are only partially understood. Ascospores are discharged only cm distances in still air, but are readily transported through and above crop canopies in turbulent air. Vertical atmospheric mixing, during daylight hours, uplifts some spores into the planetary boundary layer (PBL) from which they may be transported long distances. Using remote-piloted aircraft, we have found viable spores of G. zeae in the PBL at all times of day under a wide range of environmental conditions during the period of wheat flowering. Some spores were detected over a lake at 1 km from land. Deposition on cereal spikes of spores from the PBL occurs by spore-laden raindrops or by gravitational settling at night. Atmospheric populations of G. zeae ascospores are potentially a significant regional inoculum source for Fusarium head blight epidemics.

Disease control via understanding molecular determinants of sexual reproduction. B. G. TURGEON (1), D. W. Brown (2), S.-H. Yun (3), R. D. Plattner (2), T. Lee (4), R. Dyer (2), and A. E. Desjardins (2). (1) Cornell University, Ithaca, NY 14853; (2) USDA, Peoria, IL 61604; (3) Soonchunhyang University, Asan, Korea 336-745; (4) Seoul National University, Suwon, Korea 441-744. Phytopathology 92:S93. Publication no. P-2002-0002-SSA.

Gibberella zeae (anamorph Fusarium graminearum), a self-fertile (homothallic) ascomycete, causes wheat head blight and corn ear rot, destructive disease that impose a serious economic toll on North American farmers. Damage includes both yield loss due to kernel rot and reduced quality resulting from mycotoxin contamination. Among pathogenic Fusarium species associated with wheat and corn, G. zeae is the only one that undergoes massive sexual reproduction during the disease cycle. We have recently demonstrated experimentally that sexual spores are a primary inoculum source leading to blight in the field. The experimental proof was made possible by the cloning of the G. zeae MAT (mating type) locus, the master regulator of sexual development. Cloning allowed construction of G. zeae MAT deletion strains that fail to form sexual spores. A preliminary field study conducted in Illinois subsequently demonstrated that MAT deletion strains cause less disease than wild type: these data support the hypothesis that sexual spore load is directly related to disease severity and suggest that interference with sexual development will reduce disease in the field. Each gene or gene product in the sexual reproductive pathway is a potential target for disease control.

Stachybotrys chartarum and human health: questions and concerns. G. A. KULDAU (1), N. Jada (1), I. Yike (2), and D. Dearborn (2). (1) Dept. of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (2) Dept. of Pediatrics, Case Western Reserve Medical School, Cleveland, OH 44106. Phytopathology 92:S93. Publication no. P-2002-0003-SSA.

Stachybotrys chartarum is a commonly encountered fungus found in soil and on decaying plant material. It is known for the production of highly toxic macrocyclic trichothecces and other immunosuppressive metabolites. Skin or mucous membrane contact with S. chartarum results in a painful dermatitis in humans and domestic animals. Because optimal growth conditions for S. chartarum include a high water activity and high cellulosic substrate, this fungus thrives on water-soaked building materials, wet straw and grass, and horticultural products made from recycled plant fibers. There is a high level of public concern and debate within the medical community regarding the effects of inhalation exposure to S. chartarum. Reported incidents of illness in buildings containing spores of this fungus have raised many questions about levels and measurement of exposure, and physiological response to inhalation of S. chartarum spores. This paper will discuss current knowledge regarding exposure and health effects of S. chartarum inhalation.

A case for the potential for aerosol exposure to ochratoxin. J. L. RICHARD (1), G. C. Smiley (1), R. D. Plattner (2), and R. H. Tisdell (3). (1) Romer Labs, Inc., Union, MO 63084; (2) USDA/ARS, NCAUR, Peoria, IL 61604; (3) Toxicology Litigation Consultants, Temple, TX 76505. Phytopathology 92:S93. Publication no. P-2002-0004-SSA.

Exposure of farm workers to aerosols of dust containing fungal propagules and possible mycotoxins has been a concern for some time. Exposure has been documented in a case of ochratoxin-contaminated wheat being handled by farm workers. Disease referable to ochratoxicosis was produced in rabbits exposed to aerosols of particulates generated from the contaminated wheat. We have examined dust collected from a household where signs resembling ochratoxin poisoning occurred in the household pets. Two samples of dust collected from the heating ducts were examined by HPLC and had over 1500 ppb and 306 ppb of ochratoxin, respectively. Ochratoxin A was confirmed by LC-MS and was also evident in the samples by TLC analysis. Subsequently, others have found ochratoxin A in airborne dust and fungal conidia collected by vacuum from carpeting in three additional houses in the United States, which suggests that dust may be a potential source of airborne ochratoxin exposure.


Fusarium head blight (FHB) of wheat (Triticum aestivum) and barley (Hordeum vulgare) is incited by several fungal species in the genus Fusarium. In the United States, Fusarium graminearum (teleomorph, Gibberella zeae) is considered the principal pathogen. Epidemics of FHB have plagued wheat and barley crops throughout the USA over the last decade. In addition to devastating yield losses, the quality of infested crops has also been reduced, largely because of the contamination of grain with Fusarium produced mycotoxins. The upsurge of FHB likely resulted from wet weather following anthesis, the planting of small grains in rotation with corn (Zea mays), the widespread adoption of reduced-tillage, and the unwitting use of highly susceptible wheat cultivars. Although identifying the causes of FHB is not difficult, the management of the disease has proven challenging and it seems unlikely that disease control will be achieved without the use of host resistance in conjunction with chemical, biological and cultural control practices. Understanding the role of mycotoxins in the saprophytic and pathogenic stages of Fusarium should aid development of improved control strategies.
Chestnut Blight: A Ten-Year Study of Disease Management Using Hypoviruses


Located 600 km west of its natural range and nestled in the driftless area of western Wisconsin is a 36 ha forest stand dominated by American chestnut. This stand originated in the 1880’s from 9 trees planted as a fence row. Over the last 120 years, American chestnut has replaced native oak and hickory and is currently the dominant species. This stand lived blight-free until 1987 when 4 trees infected with Cryphonectria parasitica were observed. Early disease management efforts focused on eradication. Analysis of isolates from these early infections revealed the presence of only one vc type which set an ideal stage for management with a hypovirus. In 1990, a survey and management project was initiated that included surveying annually for new diseased trees, mapping all healthy and diseased trees with a GPS to follow disease movement, inoculating cankers with a hypovirus and sampling new and old cankers to determine movement of the hypovirus.


Chesnut blight continues to be a devastating disease of American chestnut trees in eastern North America even though a biological control called hypovirulence appears responsible for survival of chestnut trees in Italy and Michigan. Chestnut tree survival occurs when hypoviruses present in the pathogen population interfere with the full expression of sporulation and pathogenicity in the fungus. Tree survival occurs as wound tissue begins to close cankers on stems and branches of blight-infected trees. Much speculation has been generated for the success of hypovirulence in Italy and Michigan and its lack of success in controlling blight in the eastern forest. The release of hypoviruses into the Cryphonectria parasitica population of the Wisconsin stand may provide for a better understanding of the requirements for successful biological control of chestnut blight in North America. Success of hypovirulence needs to be measured in short and long-term increments as recovery of chestnut trees from chestnut blight in Italy and Michigan appeared to improve through time.


Vegetative incompatibility is known to be a significant barrier to the horizontal transmission of fungal viruses. However, the West Salem population of C. parasitica appeared to be ideal for releasing hypoviruses because initially only a single vegetative compatibility (vc) type was observed. During the course of this study, several new vc types were found, and recently this number has increased rapidly. For many years DNA fingerprints correlated perfectly with vc types, indicating a strictly clonal population structure. Fingerprints indicated that the two dominant clones resulted from independent introductions of C. parasitica. However, later clones may have arisen by recombination because both mating types were present in this population. Some isolates contained both mating types, and sexual reproduction via self-fertilization has been observed. The potential for sexual reproduction and outcrossing signals a shift in population structure from clonal towards random mating.

Hypovirus deployment, establishment and spread: Results after six years of canker treatment. M. L. DOUBLE and W. L. MacDonald. Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV. Phytopathology 92:S94. Publication no. P-2002-0009-SSA.

The stand of American chestnut at West Salem, WI presented an ideal setting for releasing hypoviruses as biological control agents, because all Cryphonectria parasitica infections initially were clonal, eliminating barriers to hypovirus transmission imposed by vegetative compatibility. Between 1992-1997, two hypoviruses were deployed by treating infections with hypovirus-infected inoculum of the resident strain. Hypovirus introductions were halted in 1998. Hypovirus establishment and dissemination were assessed by removing multiple bark plugs from cankers and comparing morphologies of the resulting colonies. Each hypovirus imparts a unique isolate morphology that is distinguishable from virus-free isolates. Hypovirulent colonies were most commonly recovered from infections on treated trees. Dissemination of hypoviruses to non-treated trees has been poor. When cankers were subjectively rated for disease remission based on callus production, cankers on treated trees produced more callus. Both hypoviruses continue to disseminate, but at a much lower rate to cankers on non-treated trees.

Spatial patterns of blight and hypovirus spread within the West Salem chestnut stand. A. M. JAROSZ (1), S. E. Dahir (2), and M. L. Double (3). (1) Depts. of Plant Biology & Plant Pathology, Michigan State University, East Lansing, MI; (2) Wisconsin Dept. of Natural Resources, Madison, WI; (3) Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV. Phytopathology 92:S94. Publication no. P-2002-0010-SSA.

In order to be successful biological control agents, hypoviruses must spread effectively through a Cryphonectria parasitica population. We analyzed among tree spread of the County Line and Euro7 hypoviruses relative to the spread of two major vegetative compatibility groups of C. parasitica in 1998 and 1999. From 1992 to 1997, hypovirus was introduced into all new cankers that could be reached. Introductions were suspended after 1997 when it became logistically impossible to treat all new cankers. Therefore, the 1998-99 census data represent the first two years where C. parasitica and the hypoviruses were allowed to spread naturally within the chestnut population. Rates of pathogen spread are greater than hypovirus spread, suggesting that some spatial pattern of hypovirus deployment may be needed to enhance the effectiveness of biological control.

Evaluation of recovery at the West Salem chestnut stand: A demographic analysis. A. L. DAVELOS (1), A. M. Jarosz (2), S. E. Dahir (3), and J. E. Cummings Carlson (3). (1) Dept. of Plant Pathology, University of MN, St. Paul, MN; (2) Depts. of Plant Biology & Plant Pathology, Michigan State University, East Lansing, MI; (3) Wisconsin Dept. of Natural Resources, Madison, WI. Phytopathology 92:S94. Publication no. P-2002-0011-SSA.

A transition matrix model is being utilized to determine if hypovirus infections of the chestnut blight pathogen, Cryphonectria parasitica, allow American chestnut trees to truly recover with regard to tree growth, health, and reproduction. The degree of recovery at a population level is being assessed by comparing the disease-free, epidemic (new infections not treated with hypovirus), and old epidemic (hypovirus application) areas of the West Salem population. If hypoviruses are acting as an effective biological control agent, transition matrix models should predict similar population growth rates and size structures within the disease-free and old epidemic areas.


The restoration of the American chestnut will be successful if current rates of progress are maintained in chestnut breeding, ecology, and biological control of chestnut blight. Breeding strategies include the transfer of genes for blight resistance of the Chinese chestnut, Castanea dentata, and incorporate them into regional breeding programs. Ecological and silvicultural studies have shown that even low levels of blight resistance may be adequate for effective biological control of chestnut blight under certain forest conditions.
Forces that Shape Microbe Populations in Forest Ecosystems

Forces shaping pathogen population structure in crop ecosystems: Relevance to forest ecosystems? C. C. Mundt. Dept. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331. Phytopathology 92:S95. Publication no. P-2002-0013-SSA.

Our knowledge of pathogen population structure in annual crops has advanced substantially in recent years, owing in particular to the use of selective, central genetic markers. For example, population genetic studies have provided clear descriptions of the impact of clonal versus sexual reproduction on pathogen population structure, and varying degrees of insight into the roles played by migration, drift, and mutation. Interpretations of such data are not always clear, however, due to model assumptions and the fact that most data sets utilized are observational, rather than experimental. Further, there has been a tendency to infer the degree of variation for selectable traits (e.g., pathogenicity) from the degree of variation for neutral markers. The most useful information is attained when molecular and phenotypic data are considered simultaneously. Examples from annual crop systems will be presented, and the degree to which these patterns are expected to be similar to or different from that of forest ecosystems will be discussed.

Crawling through the botryosphaerial mire: Species definition as a prelude to population studies. G. R. STANOSZ (1), D. R. Smith (1), and S. Zhou (2). (1) Dept. Plant Pathology, University of Wisconsin-Madison, WI 53706; (2) Laboratory for Molecular and Computational Genomics, University of Wisconsin-Madison, Madison, WI 53706. Phytopathology 92:S95. Publication no. P-2002-0014-SSA.

The detection and interpretation of the variation and patterns within a fungal species depends on some meaningful definition of that species. The identification, study, and management of plant pathogenic Botryosphaeria species and related anamorphic fungi has been complicated by a lack of distinctive morphological characters and confusing taxonomic literature. It has been difficult to discern whether particular putative species represent a continuum of variability or discrete populations, much less understand what factors might have contributed to these characteristics. Researchers analyzing molecular markers and reevaluating morphological characters recently have made progress in more clearly defining species within this group. The stage now is set for investigation of the structure of populations of some important tree pathogens, and the factors that influence variation and patterns within these species.

Genetic variation and potential for adaptation and gene flow in Cronartium ribicola. P. J. ZAMBINO (1), R. Hamelin (2), and G. I. McDonald (1). (1) USDA Forest Service, RMRS, 1221 S. Main St., Moscow, ID 83843; (2) Natural Resources Canada, Canadian Forest Service, Laurentian Research Centre, Sainte-Foy, Quebec G1V 4C7. Phytopathology 92:S95. Publication no. P-2002-0015-SSA.

Genetic variation in North American (NA) Cronartium ribicola (CR) originates from 1) dispersal from a diverse Asian CR complex to Pinus strobus and Ribes in Europe; 2) introduction to NA locations on European nursery stock; and 3) ongoing dispersal and adaptation to NA habitats and hosts. Differences in RAPD and codominant DNA markers from eastern, midwestern, and western populations suggest that founder effects have not been overcome by inter-regional interbreeding. Virulence occurs to major gene resistance (MGR) in sugar pine (race V1), western white pine (V2), and both hosts (V1V2), but spread of V1 and V1V2 races has been minimal. CR variants having differential development and/or reduced urediniospore production on different clones of Ribes hudsonianum var. petiolaris or differences in teliospore germination at different temperatures have been tentatively identified, but factors affecting spread and persistence of CR variants and potential for life cycle variations need further investigation.

Toward defining Armillaria populations and determining relationships to ecological behavior. M.-S. KIM (1), N. B. Klopstein (2), J. W. Hanna (1,2), and G. I. McDonald (2). (1) Dept. Forest Resources, University of Idaho, Moscow, ID 83844; (2) USDA Forest Service, RMRS, 1221 S. Main St., Moscow, ID 83843. Phytopathology 92:S95. Publication no. P-2002-0016-SSA.

Ecological behavior, such as pathogenic or saprophytic tendencies, of Armillaria genets and associated environmental data are being recorded from Armillaria located in Inland Northwest. Genetic markers generated by amplified fragment length polymorphism and intergenic spacer region sequence polymorphism are being used to characterize Armillaria species, populations, and genets (vegetative clones). Genetic markers will be analyzed for relationships to environmental attributes and ecological behavior. This approach may allow an interpretation of environmental adaptation and gene flow among Armillaria genets and populations. The integration of genetic marker data with geographic information systems may allow prediction of Armillaria distribution and behavior at the landscape level. Information derived from these studies should provide new tools and approaches to manage Armillaria root disease.


Swiss needle cast disease of Douglas-fir currently affects over 52,000 ha of forested lands in western Oregon. The pathogen, Phaeocryptopus gaeumannii, is endemic to the region and until recently was considered an insignificant forest pest. Recent levels of disease have caused unusually high defoliation, growth loss, and, in severe cases, tree mortality. Research since 1995 has sought to understand the underlying causes of the current epidemic. Increasing acreage of Douglas-fir plantations in the coastal fog belt zone where climatic factors are most favorable for pathogen development may have enabled P. gaeumannii to increase above historically normal levels, leading to increased disease pressure. SSCP and microsatellite analyses of P. gaeumannii indicate two reproductively isolated, sympatric lineages. The abundance of one lineage was positively correlated with disease severity in the epidemic area.

Life in the woods and in wood products: Genetic tales from the ophiostomatoid front. L. Bernier. CRBF, Université Laval, Québec, QC, G1K 7P4. Phytopathology 92:S95. Publication no. P-2002-0018-SSA.

The genus Ophiostoma includes a large number of species that cause sapstain, a cosmetic problem which nevertheless causes millions of dollars in losses to the forest products industry worldwide. It also includes aggressive pathogens, such as Ophiostoma ulmi and O. novo-ulmi which are responsible for two successive Dutch elm disease pandemics. Abundant production of asexual and sexual fruiting bodies, adaptation to transportation by vectors such as bark beetles, and heterothallic mating systems are various features that favour dissemination and recombination of genotypes within and among populations of several Ophiostoma species. In recent years, cases of interspecific recombination have also been documented, notably between O. ulmi and O. novo-ulmi. Thus, there appears to be high potential for adaptation and gene flow in Ophiostoma spp. The presentation will focus on a few saprophytic and pathogenic Ophiostoma species that are closely related phylogenetically. I will discuss the population dynamics of these organisms with regard to their potential impact on tree health, wood quality and development of successful control strategies.

Forest clearing and fire exclusion and their impact on microbial populations: Examples from tropical and temperate forests. M. GARBELOTTI (1), W. Otrosina (2), I. Chapela (1), and G. Gilbert (3). (1) Dept. of ESPM Ecosystem Sciences Division, U.C., Berkeley, CA 94720; (2) US Forest Service, Athens, GA 30602; (3) Environmental Studies Dept., U.C., Santa Cruz, CA 95064. Phytopathology 92:S95. Publication no. P-2002-0019-SSA.

A few cases are discussed in which forest management practices have had either a direct or an indirect impact on fungal populations. The establishment of tree plantations in a complex has been shown to be directly correlated with the creation of stumps in new studies from Italy and California. Results also indicate that the outcome of such practices is not uniform, but rather dependant on other ecological factors. Fire exclusion appears to have significantly altered forest structure and composition, further allowing for shifts in the genetic makeup of Heterobasidion populations at the inter- and intra-specific level. Another byproduct of logging, openings in the tree cover, may affect microbial populations. For instance, the population structure of the mangrove decay fungus Coriolopsis caperata appears to be directly correlated to the presence of significant gaps in the distribution of mangroves.

Phenotypic plasticity (PP) and ecotypic adaptation: Responses of microbial populations to environmental (E) and host variation through time and space. G. I. McDonald. USDA Forest Service, Rocky Mt. Research Station, Moscow, ID 83843. Phytopathology 92:S95. Publication no. P-2002-0020-SSA.

Forest ecosystems are highly heterogeneous in time, space, and biologi- cal composition. Surviving populations have evolved diverse and powerful
The Interaction Between Endosymbiotic Bacterial and the Circulative Transmission of Viruses


Plant viruses from the genera Luteovirus, Polerovirus, Enamovirus and Begomovirus avoid degradation in the hemolymph of their aphid or whitefly vector by interacting with Buchnera GroEL. The GroEL homolog is produced by endosymbiotic bacteria (Buchnera spp.) and is abundantly present in the insect’s hemolymph. Buchnera GroEL is highly homologous to molecular chaperones of the heat shock protein HSP60 family. Mutational analysis of full-length cDNA clones of two poleroviruses has demonstrated that the minor coat protein, which protrudes from the surface of a virus particle, contains the determinants for GroEL binding. Mutational analysis of Buchnera GroEL has shown that the ability of the virus to infect the vector coincides with reduced capsid integrity and loss of infectivity.


Mt CoI sequence analysis for the species Bemisia tabaci (Genn.) has revealed phylogenetic groupings based in extant geographical origin. New World (NW) haplotypes represent three distinct clades at <2-6% divergence, whereas, Old World B. tabaci comprise seven clades which diverge amongst themselves and NW B. tabaci by ~8-14%. Whiteflies harbor prokaryotic symbionts, some of which provide nutritional needs, whereas, others may be nonessential or deleterious. However, little is known about the extent to which endosymbionts are associated with B. tabaci, or whether they influence biotype formation and evolution. Examination of symbionts for B. tabaci from different plant hosts and geographical locations revealed a diverse array of microflora. Phylogenetic analysis of eubacterial 16S rDNA sequences indicated that all B. tabaci harbored a primary symbiont, however, the 16S rDNA phylogeny was not strictly concordant with the whitefly mt COI tree. Secondary symbionts, representing two Enterobacteriaceae clades, were identified for 13 of 20 collections, and at least 33% of B. tabaci harbored Wolbachia.


The propagative persistent transmission of the corn stunt spiroplasma (CSS), Spiroplasma kunkelii, by Dalbulus maidis was studied at an electron microscopic level. CSS infection reduces fecundity and longevity of most Dalbulus species, but improves survival of D. maidis in the absence of its major food and reproductive host during maize-free winter seasons. CSS enters D. maidis gut cells by endocytosis and moves through gut cells into spaces between plasmalemma and basal lamina. CSS predominantly replicates in gut muscle cells, and degrades the basal lamina to move into the hemolymph. Helical spiroplasmas accumulate in the canalculus of type III salivary gland cells, but not in those of other salivary gland cell types. Whereas CSS accumulates at large numbers in D. maidis, the leafhopper is not negatively affected by the infection. CSS does not degrade essential organs, such as fat body, neurons, gut and salivary glands, and apoptosis or necrosis of CSS-infected cells was not observed. CSS may produce metabolites advantageous for D. maidis explaining the mutual beneficial association.

Transmission of Tomato yellow leaf curl geminivirus by its whitefly vector Bemisia tabaci depends on the interaction between the virus and the insect endosymbiotic GroEL. H. CZOSNEK, S. Morin, and M. Ghanim. The Hebrew University of Jerusalem, 76100 Rehovot, Israel. Phytopathology 92:596. Publication no. P-2002-0024-SSA.

TYLCV is transmitted by the whitefly B. tabaci in a circulative manner. Virus particles taken up through the stylets enter the esophagus and filter chamber, cross the gut wall into the haemolymph, reach the salivary glands, are released into the salivary canal and injected into the plant during feeding. In aphids a 63 kD GroEL homologue protects luteoviruses from degradation in the insect haemolymph. A similar protein is involved in the transmission of TYLCV by B. tabaci. Anti GroEL antibodies recognized a ~70kD B. tabaci protein and labeled the the whitefly coccoid endosymbionts. Feeding whiteflies with anti-GroEL antiserum prior to acquisition of virions reduced TYLCV transmission by more than 80%. We have isolated a GroEL gene from B. tabaci and used a yeast two-hybrid assay to show that the coat protein (CP) of TYLCV was able to bind efficiently to GroEL. Hence we propose that GroEL-CP interaction in the haemolymph is a necessary condition for circulative transmission of TYLCV.

Spatial Scale and Phyllosphere Biology


Processes such as nutrient consumption by bacteria on leaf surfaces differ in magnitude and are discontinuous over very small spatial scales. Cells of Erwinia herbicola harboring a gene fusion conferring fructose-dependent green fluorescence used as a biosensor for this important carbon source occur. Yet population sizes of Ps were markedly affected by the numbers of immobilization. When Ps was sprayed on established bean plants, little spread of the marked strain occurred. Yet when Ps was applied to seed at planting, spread to other plants immediately after emergence was rapid, and when conditions were appropriate for growth, extensive. When comparable numbers of two strains of equal fitness were applied to the same seeds, mean population sizes of the strains remained equal throughout the season, but ratios of the two on individual leaves varied by 100 fold or more. These results suggest a model in which the frequency and timing of relatively infrequent immigration events interact with rapid growth on very young leaves to shape the eventual populations that develop on those leaves.


Phyllosphere microorganisms (biological control agents, pathogens, resident saprophytes,etc.) can spread simultaneously in time and space. The nature of this spatiotemporal increase has important implications for understanding ways to compete along alternative paths of adaptation (ecotypes) and PP (the ability of one genotype to form different phenotypes in differing environments). PP is common in plants and animals and rarely investigated in phytopathology. Vegetative, reproductive and life history traits can be anticipatory (responsive to E cues) or passive (proportionally responsive to E stimuli) and each can be either non-adaptive or adaptive. Examples of PP in microbes are color morphs in Candida, rhizomorph initiation in Armillaria, and perhaps shortened life cycles in rusts. Induced resistance is known in conifers, hardwoods, and herbaceous plants and may be plastic or even epigenetic. Several cases of transgenerational induction of defensive responses in plants are known. Application of new techniques such as cDNA-AFLP and microarrays will facilitate sorting out the competing factors and responses characteristic of forest ecosystems.
microbial population dynamics and for developing strategies for the management of plant disease. I will present data for wheat stripe rust as a model of a phyllosphere microorganism, under the assumption that disease levels are proportional to pathogen population size for an obligate plant parasite. Traditional theory has held that plant diseases increase in time and space as waves of constant velocity. Others, however, have suggested that, for microbes dispersed in the daytime, turbulent atmosphere, velocities will increase in time and space. Field studies with wheat stripe rust indicate that epidemic velocity increases in both time and space, and that the effect of scale may be influenced by the reproductive capacity of the pathogen. Thus, the dynamics of microbial spread may depend strongly on the spatial and temporal scales at which it is studied.


Surface-Interactions and Biofilms of Plant-Associated Microbes


Epiphytic bacteria, including the phytopathogen Pseudomonas syringae are highly spatially aggregated on leaves. While many bacteria are solitary on leaves, a majority of bacteria occur in aggregates of more than 100 cells. The behavior of cells of differing states of aggregation is very different. Upon drying of leaf surfaces most solitary cells succumb while the fraction of cells that tolerate drying increases with increasing aggregate size about above 64 cells/aggregate. Cells in aggregates are intrinsically more tolerant of stresses since cells in aggregates removed from leaves are more tolerant of drying than solitary cells in a uniform environment on membranes. Since mutants of P. syringae deficient in n-acyl homoserine lactone production have reduced stress tolerance on leaves it appears that cell density-dependent traits expressed within cell aggregates contribute to epiphytic fitness.


Water availability is thought to fluctuate repeatedly and rapidly on leaf surfaces. However, the extent to which limited water availability is a driving factor in the dynamics and distribution of bacterial populations on leaves is unknown. A water deprivation-responsive bacterial biosensor was used to quantify water availability to the common bacterial saprophyte Pantoaea agglomerans on leaves. Although bacterial exposure to water deprivation increased rapidly following inoculation onto plant leaves, the rate of this increase was influenced by the relative humidity while the steady-state exposure level between 3 and 4 h after inoculation was not. This observation, along with a lack of evidence for heterogeneity in water availability among microsites on leaves during this period, suggests that in the 4 h following inoculation, the bacteria remain in sites that are uniformly hydrated. In the subsequent 1 to 5 days, cellular aggregation on the leaf surface appears to measurably decrease bacterial exposure to water deprivation.


Pantoaea stewartii subsp. stewartii causes Stewarts’s vascular wilt of sweet corn and maize. Virulence depends on at least three gene systems. First, a Hrp (type III) secretion system promotes plant cell membrane dysfunction and development of water-soaking (Wts) lesions during early infection. Second, the cps gene cluster encodes functions for the synthesis of a capsular polysaccharide (CPS), which blocks the xylem and causes the plant to wilt. Third, the esal/esaR genes encode a quorum sensing (QS) regulatory system to direct the cell density-dependent synthesis of the CPS. Mutants in esal are repressed for CPS synthesis and have a strong surface attach phenotype, while esaR mutants with a deregulated, hypermucoid phenotype are attachment-deficient. In contrast, the wild type strain attaches only to a limited degree during the early stages of growth, and the onset of CPS production at higher cell density interferes with further adhesion. The CPS-repressed and hypermucoid strains are greatly limited in their ability to move on artificial surfaces and in planta. We predict that the developmental stages of biofilm formation, i.e. attachment, movement, microcolony formation and biofilm maturation, are key steps in pathogen invasion and dissemination. We present data using epifluorescence microscopy with GFP-tagged strains to evaluate this hypothesis.


Agrobacterium tumefaciens causes crown gall neoplasia on plants. The DNA transfer event resulting in crown gall is relatively well studied, but less is known regarding the initial events, including attachment, leading to plant association. Large populations of saprophytic agrobacteria are associated with soils and are typically more numerous than those associated with plants. We have studied the overlap between those functions that facilitate inert surface interactions and those involved in host tissue association. A. tumefaciens forms dense, complex biofilms on inert surfaces. A range of functions important for colonization and biofilm development on inert surfaces have been identified, including motility, chemotaxis and cell surface polysaccharides. Several regulatory systems are also implicated during surface interactions, affecting exopolysaccharide synthesis and anaerobic metabolism. Some of these functions have also been identified as important for attachment to plants. Conversely, many plant attachment functions are also required for inert surface interactions.


A. tumefaciens forms biofilms on root surfaces covering the epidermis, sloughed epidermal cells, and root hairs. The root tip and the shoot are relatively sparsely colonized by this bacterium. The biofilm is initiated by polar attachment of individual bacteria. Additional bacteria bind to the previously attached bacteria and the attached bacteria divide to form the large bacterial clusters present in the mature biofilm. Genes required for biofilm formation on roots include those required for attachment to plant surfaces (att genes) and those for the synthesis of some exopolysaccharides including cellulose. Other genes required for bacterial virulence such as vir genes are not required for biofilm formation on roots.


We have evaluated the ultrastructural properties of Pseudomonas putida biofilms and the transcriptional regulation of genes that are expressed during biofilm development under conditions of matric stress (low water content), solute stress, and when water is not limiting. Biofilms that formed under a matric stress produce significantly more exopolysaccharides and confocal scanning laser microscopy (CSLM) of gfp-tagged cells with the extracellular polysaccharide (EPS) stained with calciofleur revealed that most of the EPS was localized at the air-biofilm interface rather than distributed uniformly through the biofilm. CSLM also revealed that biofilms that develop under matric stress growth conditions are taller and more compact than those that form under solute stress conditions or when water is not limiting.
Furthermore, we have identified numerous transcriptionally regulated genes whose expression is increased or decreased under matric stress conditions. These results suggest that a unique suite of adaptive traits are required for biofilm growth under low water content conditions and that they differ from those required for biofilm growth under solute stress conditions or when water is not limiting.

Biofilm formation on abiotic surfaces by a fluorescent pseudomonad. S. HINSA (1), M. Espinosa-Urgel (2), and G. O’Toole (1). (1) Dartmouth Medical School, Hanover, NH 03755; (2) Estacion Experimental del Zaidin CSIC, Spain. Phytopathology 92:S98. Publication no. P-2002-0035-SSA.

Pseudomonas fluorescens WCS365 is an environmental isolate that has been shown to have biocontrol activity. In order to persist in the environment this bacterium must attach to a variety of surfaces. We are investigating the requirements for attachment to abiotic surfaces. Previously, mutants were isolated that were defective for biofilm formation on a 96 well polyvinylchloride plastic plate. Characterization of these mutants led to the identification of an ABC transporter and a large outer membrane protein (OMP) required for biofilm development. The genes encoding the ABC transporter and OMP appear to be conserved among soil Pseudomonads, but not by plant or human pathogenic strains of Pseudomonas. Biofilm formation by the mutants was analyzed in both static and flow systems. We have determined that these mutants are impaired in their ability to irreversibly attach to a surface.

The cep quorum-sensing system of Burkholderia cepacia is a regulatory checkpoint for biofilm development. L. Eberl. Dept. of Microbiology, Technical University of Munich, 85350 Freising, Germany. Phytopathology 92:S98. Publication no. P-2002-0036-SSA.

Burkholderia cepacia is capable of colonizing a wide variety of surfaces in water, soil, and the rhizosphere of plants. To analyse the genetic mechanisms underlying surface colonization random insertion mutants of B. cepacia strains defective in biofilm formation were isolated. A quantitative analysis of the biofilm structures formed by wild type and mutant strains revealed that the isolated mutants are impaired in their abilities to develop a typical three-dimensional biofilm structure. Molecular investigations showed that the genes required for biofilm maturation fall into several classes: (i) genes encoding for surface proteins, (ii) genes involved in the biogenesis and maintenance of an integral outer membrane, and (iii) genes encoding regulatory factors. Three of the regulatory mutants produce greatly reduced amounts of N-octanoylhomoserine lactone (C8-HSL). This compound serves as the major signal molecule of the cep quorum-sensing system. Using GFP-based monitor strains it is shown that bacteria communicate with each other in the rhizosphere of tomato plants by the aid of homoserine lactone signal molecules.

Epidemiology/Ecology/Environmental Plant Pathology

Creating the Right Environment for Biological Control


Biological control is a viable disease management strategy with increasing importance as part of integrated pest management, particularly in sustainable and organic systems. One of the main constraints to more widespread and larger-scale application of biocontrol is its often times variable performance relative to chemical pesticides or other disease control measures. This Symposium takes an holistic view of the diverse environmental conditions that influence biocontrol. Each speaker presents unique approaches for improving efficacy and reliability by selecting or creating more favorable conditions in order to realize the full promise of biocontrol.

Matching the right strain for particular host genotypes. K. P. Smith. Dept. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN. Phytopathology 92:S98. Publication no. P-2002-0038-SSA.

Despite all that is known about the genetics of host-pathogen interactions that result in disease, relatively little is known about comparable interactions between hosts and biocontrol agents that result in disease suppression. If certain host genotypes better support disease suppression by a biocontrol strain, then it should be possible to exploit the host for improving biocontrol. Understanding host effects on disease suppression by biocontrol agents will lead to a better understanding of the mechanisms involved and to strategies for improving disease suppression. It may be possible to select host genotypes that enhance disease suppression or identify combinations of host genotypes and biocontrol strains that result in superior disease suppression. One important factor for biocontrol that is influenced by plant genotype is the population size of antagonists. Other factors influenced by the host include enhancing antibiosis by the biocontrol agent or inducing host resistance. This talk will review evidence for host effects on disease suppression and address challenges for advancing our understanding of these interactions and exploiting host genetics to improve biocontrol.

Manipulating host plant nutrition to alter biocontrol activity. W. H. ELMER (1) and D. M. Huber (2). (1) Connecticut Agric. Expst. Sta., P. O. Box, 1106, New Haven, CT 06504; wade.elmer@po.state.ct.us; (2) Dept. Botany and Plant Pathology Dept., Purdue Univ., West Lafayette, IN 47907. Phytopathology 92:S98. Publication no. P-2002-0039-SSA.

Manipulating the nutritional status of the root and rhizosphere can alter biological control of root diseases. Both nitrogen and chloride amendments have been demonstrated to increase the efficacy of biological control agents. A common mechanism underlying many of these interactions involves increasing the availability and function of Mn in the rhizosphere. These amendments can act directly on the microbes in rhizosphere or indirectly through altering the quantity and quality of root exudates that, in turn, favor disease-suppressing microbes. The efficacy of biocontrol agents applied to wheat to suppress take-all can be dependent on fertilization practices. For example, fertilizers that inhibit nitrification and increase Mn enhance the biocontrol suppression of Gaeumannomyces graminis. Sodium chloride suppresses asparagus crown rot. When NaCl was applied to one side of a split-root asparagus root system, there were reductions on disease severity, root colonization by Fusarium spp. and increased numbers of fluorescent pseudomonads and Mn-reducing bacteria in the rhizosphere.

Identifying and manipulating soil factors that influence biocontrol. B. H. OWNLEY (1) and B. Duffy (2). (1) Dept. of Entomology and Plant Pathology, Univ. of Tennessee, Knoxville, TN 37996; bhowley@agmail.ag.utk.edu; (2) USDA-ARS, Albany, CA 94710. Phytopathology 92:S98. Publication no. P-2002-0040-SSA.

Identification of factors that influence disease suppression is essential to improving biological control. Certain root-associated bacteria and fungi have been shown to effectively protect crops against soilborne fungal pathogens. However, commercial success of these microorganisms for biocontrol has been hampered due in large part to inconsistent performance between field locations. Variability has been attributed to many different causes, including soil characteristics. Soil pH and certain minerals have been evaluated individually for their impact on biocontrol, but in general, analysis of the overall abiotic soil environment and its influence on biological control is lacking. In this overview, the status of research on the influence of soil physical and chemical properties on biocontrol of soilborne pathogens with model systems using Pseudomonas, Bacillus and Trichoderma will be reviewed, and the methods employed to arrive at these conclusions will be discussed. The results of manipulation of edaphic factors to influence biocontrol will be included.

An holistic approach towards improving biocontrol of nematodes. R. A. Sikora. Soil Ecosystems, Phytopathology and Nematology, University of Bonn, Bonn, D-53115 Germany; rskora@uni-bonn.de. Phytopathology 92:S98. Publication no. P-2002-0041-SSA.

Achieving effective biocontrol of plant parasitic nematodes in the rhizosphere of host plants is directly related to detecting the optimum site for the antagonist-nematode confrontation. Targeted selection of microbial antagonists using what is termed the ‘inside-out / outside-in’ approach has revealed unique modes-of-action. Predetermined screening programs based on intricate host-parasite interactions has significantly improved nematode control. Recognizing vulnerable stages in nematode life cycles aid in isolation of effective antagonists occupying specific niches in the root system. This improves chances for successfully controlling such highly developed parasites. Application of rhizosphere and endophytic micro-

S98 PHYTOPATHOLOGY
Detected and managed foodborne human pathogens on fruits and vegetables


Greater consumption of plant products has coincided with recent increased illnesses associated with these foods. FDA estimates that over 17% of investigated outbreaks between 1996-2001 were associated with plant-derived foods. Although not the source of contamination, about 4% of imports and 1% of domestic produce samples tested contained Salmonella or Shigella. Response initiatives include: more inspections and surveillance, industry guidance, training and outreach, consumer education, and coordination with federal and state agencies. A research initiative is directed to improve detection methods; prevent pathogen uptake, growth, and survival; and link agricultural practices with pathogen reduction. Plant pathologists could use modern laboratory and field techniques to study plant-human pathogen interactions. To exert the most benefit to human health and food safety, research goals need to target development of validated contamination prevention practices.


Numerous outbreaks of human pathogens have been associated with fresh produce, and their detection has become of the utmost importance for epidemiological, control, and experimental studies. Detection of human pathogens on fresh produce relies on the sensitivity of available methods and is constrained by the biology of the target organism. Sample preparation of produce may influence success of pathogen detection. The ultimate objective of the organisms' detection dictates use of one or more available methods: enrichment, polymerase chain reaction, immunoassay, or direct plating. However, each of these methods is limited by its utility in particular situations, due to the biology of the organism or inherent sensitivity of each method. Epidemiological and ecological studies require alternative detection methods to food safety microbiology. Use of the green fluorescent protein (gfp) has provided insight into understanding pathogen colonization of fresh produce by visualizing single cells in situ. Method validation is essential to determine an appropriate detection assay for each situation.


Consumers increasingly prefer lightly processed, more natural products without chemical preservatives. Driven by consumer demand, the number of fresh and processed fruits and vegetables has been increasing. However, recent outbreaks involving fruits and vegetables suggest that fresh-cut produce can be a reservoir for foodborne pathogens. When the protective barrier of the peel is removed during the processing of fresh-cut fruits and vegetables, they become vulnerable to microbial contamination and colonization, especially at abusive temperatures. Therefore, it is important to investigate the survival and growth kinetics of major foodborne pathogens on fresh-cut produce and to develop novel approaches and combinations of treatments to effectively reduce the contamination of fresh-cut produce with pathogenic bacteria. Lytic bacteriophages, bacteriocins, and the competing microflora in combination with low pH and temperature may provide an attractive alternative for decontaminating fresh-cut fruits that may contain various bacterial pathogens.


Fresh fruits and vegetables that are contaminated with human pathogens usually cannot be successfully decontaminated without losing freshness. To prevent product contamination, produce handlers wash and handle fresh produce with water systems that have been treated with oxidizing chemicals. These chemicals kill microbes while dissipating due to reactions with the microbes and other reducing agents. The most common treatment has been the addition of chlorine, either as chlorine gas or hypochlorite ion, to the water used to handle or wash the product. Because water chlorination has been associated with hazardous disinfection by-products, alternative treatments are under consideration. These include water treatment with hydrogen peroxide, ozone, chlorine dioxide, acidified sodium chlorite, a peroxyacetic acid based product, vapor chlorine dioxide, or ozone decontamination.

Microbial communities in agricultural soils are extraordinarily complex. Hundreds or thousands of microbial species inhabit the same amount of soil as a single crop plant, and their populations fluctuate dramatically over time in response to environmental variables. While functionally significant populations of pathogens and their antagonists exist in most soils, little is known about the degree of site specific adaptation and diversity of these microbial populations. It is thought that differences in soil type, management practices and environmental conditions affect microbial communities in ways that limit successful introduction of biocontrol agents at different sites. Introductions of individual species also impact microbial community structure in the rhizosphere. Such perturbations may be transient or long lasting, and they likely reflect the introduction method as well as the biological activity of the introduced microorganism. Only by clearly defining the ecological relationships between indigenous and introduced populations can we improve the success of different biocontrol strategies.

Foodborne illness associated with the consumption of fresh fruits and vegetables has increased during the past decade. Crops may become contaminated when grown in fields irrigated with contaminated water. Sites of association on the plant, surface or subsurface, of the pathogen following in-field contamination have not been delineated. Postharvest, produce is often sanitized, but efficacy is limited, and is likely dependent on accessibility of the organisms. The transmission of *E. coli* O157:H7 from irrigation water, applied directly to soil (avoiding contact with the edible portion of the plant), into lettuce plants was demonstrated using epifluorescence microscopy, laser scanning confocal microscopy and recovery of viable cells from the inner tissues of plants. *E. coli* O157:H7 persisted in plants for 20 d following irrigation, and immersion of harvested lettuce heads in a 200 ppm chlorine solution did not eliminate all *E. coli* O157:H7. Experiments demonstrate *E. coli* O157:H7 can enter the lettuce plant through the root system and migrate throughout the edible portion of the plant; thereby protected from the action of sanitizing agents.

Managing Risk to Minimize Crop Loss

Development of an infection risk forecaster for hop powdery mildew. W. F. MAHAFFEE (1), C. S. Thomas (2), W. W. Turechek (3), C. M. Ocamb (4), and W. D. Gabler (5). (1) USDA-ARS-HCRL Corvallis, OR; (2) FieldWise, Yubba City, CA; (3) Cornell Univ, Geneva, NY; (4) Oregon State University, Corvallis, OR; (5) University of California, Davis, CA. Phytopathology 92:S100. Publication no. P-2002-0050-SSA.

In 1997, hop powdery mildew (*Sphaerotheca macularis* (syn. *S. humuli*)) was introduced into Yakima Valley and subsequently spread to the growing regions in Oregon and Idaho. In order to rapidly assist growers in reducing the cost associated with the prevent fungicide program, the Gabler-Thomas grape powdery mildew risk infection model was adapted for hops. A combination of growth chamber and field surveys were used to identify critical temperatures and add rules to account for impact of supra-optimal temperatures on infection frequency and host susceptibility. Weather networks were established or accessed for temperature and rainfall data. These data were georeferenced and interpolated into a map showing the risk index over the Willamette and Yakima Valleys in OR and WA. Contour maps were posted daily to the web. Growers using the model in 2001, used 1 fewer application of fungicides for mildew control and had 60% less cone (fruit) incidence.

How to interpret a positive identification. L. G. Brown. Plant Epidemiology and Risk Analysis Lab, USDA/APHIS/PPQ, Raleigh, NC 27606. Phytopathology 92:S100. Publication no. P-2002-0051-SSA.

When a plant pest is positively identified then USDA-PPQ considers the pest as a potential threat to ecosystems and agriculture. A plant pest is anything injurious to a plant or plant product, i.e. other plant(s), plant pathogens, arthropods, nematodes, or mollusks. The New Pest Advisory Group (NPAG) conducts the assessments of exotic plant pest introductions into the USA. Non-invasive pests have not been determined, therefore, may not be tracked in reference. An invasive pest can become established, but not pose a threat; in contrast, an invasive and threatening pest is one that becomes established, and causing economic injury to ecosystems and/or agriculture. Pest mitigation is recommended when a pest is invasive and threatening. Interpretation of a pest’s ability to become established is based on the scientific literature and opinion. Interpretations and recommendations are made with considerable uncertainty, due to the knowledge gaps with rarely studied pests in remote geographic areas. An appreciation for the culture, framework, and uncertainty that accompany recommendations and how to interpret positive pest identifications will be presented.


The maximum pest limit (MPL) concept was developed as a practical method of maintaining quarantine security against the import of invasive pest species of plants. The MPL itself is simply a threshold upper limit, above which the pest species in question is deemed capable of establishing a population if imported in a consignment of fruit or vegetables. This limit depends on various biological and ecological characteristics of the pest species in question. Important aspects of implementation relate to how treatment and sampling may be combined to reduce the risk that the MPL will be exceeded. If a specified level of treatment efficacy is required (for example, probit nine level), then choice of an appropriate sample size becomes the main problem for regulatory authorities seeking to maintain quarantine security.


One measure of the effectiveness of a disease predictor is to examine the probability of disease occurrence before and after using the predictor. Bayes’ Theorem can be a useful tool to examine how a disease forecast (either positive or negative) affects the probability of occurrence. The sensitivity and specificity of the forecast are key quantities needed for a Bayesian analysis. The other component necessary is how often the disease occurs (i.e., the prior probability). For diseases where little or no prior information on occurrence is available, most forecasts will be useful in that they will increase or decrease the probability of disease occurrence. For extremely common or extremely rare diseases, the specificity or sensitivity is seldom of sufficient quality to substantially affect the probability of disease occurrence.


International disciplines for managing sanitary and phytosanitary (SPS) risk in global trade aim to strike a balance between a country’s right to protect itself from injurious pests and diseases and the obligation that it do so in the least trade restrictive manner practicable. As such, the WTO-SPS Agreement requires governments to develop and implement regulations, including import restrictions, commodity treatments, and other border control measures, in a transparent, non-discriminatory fashion. Whereas import tariffs and other trade policy instruments are grounded in political-economic considerations, SPS measures are to be based on scientific assessments of potential biological risks associated with trade -- whether these risks adversely affect agricultural production by introducing crop pests or the environment with invasive species. Given this mandate, greater emphasis has shifted to the role of risk assessments that underlay risk abatement regulations. Determining risk and what should be done about it -- while also seeking to minimize the trade impact -- are at the center the SPS Agreement and national regulatory decision-making.

Risk assessment, concept, terminology, development and future opportunities. X. B. Yang. Iowa State University, Ames, IA. Phytopathology 92:S100. Publication no. P-2002-0055-SSA.

Farming is a high-risk business and plant disease is a major risk factor in agriculture. The need to analyze and quantify disease risk is increasing perhaps due to changes in agricultural technology, climate, and consumer demands. Risk assessment models vary according to users who can be grouped as risk handlers, risk advisers, and risk managers. Users in each group require different kinds of risk assessment information including short- (in a season), mid- (pre-season), and long-term (years/decades) temporal scales or farm, region, or continent spatial scales. The presentation will discuss the concepts, development, and future opportunities in disease risk assessment.

New Applications of Statistical Tools in Plant Pathology


Epidemiological questions are often phrased in terms of occurrence and timing of events within populations. Examples include germination in a population of spores, disease onset in a population of plants, defoliation in a population of defoliable leaves, death in a population of sclerotia, etc. The resulting data are generally analyzed using logistic regression or discriminant analysis after deciding upon a suitable assessment endpoint at which the
population is divided into two groups, e.g., diseased and healthy. Failure time analysis (survival analysis) is a more powerful way of analyzing such data; it considers the entire frequency distribution of time to event occurrence (e.g., of disease), not merely disease status at the assessment endpoint. Failure time analysis has the added advantage of accommodating censored data, i.e., “missing” data for which only a minimum time during which disease did not occur is known. This presentation will emphasize the use of proportional hazard models that describe the probability of being diseased at time \( t \) when still healthy at \( t-1 \) in relation to one or more constant or time-dependent covariates. Specific examples and software applications will be discussed.

Use of linear mixed models for analyzing data obtained in designed experiments. L. Madden. Ohio State University, Dept of Plant Pathology, Wooster, OH 44691. Phytopathology 92:S101. Publication no. P-2002-0057-SSA.

It is traditional to use fixed-effects analysis of variance (ANOVA) to determine if experimental factors (e.g., treatments) significantly affect the results. However, it is now becoming more common to use linear mixed models (LMMs) to analyze data from planned experiments. LMMs formally handle experiments with both fixed (e.g., fungicide treatment) and random (block, location) effects, in that tests for the effects of factors and their interactions, as well as the standard errors for means and other contrasts, are calculated correctly. Although computationally intensive, it is straightforward with LMMs to directly analyze experiments with two or more source of variation (e.g., split plots); quantify the effects of variable heterogeneity (e.g., variation dependent on factor levels) on responses; properly test for the effects of repeated measures (such as time during the growing season); and account for the correlation of spatially-referenced data. With programs such as PROC MIXED of the Statistical Analysis System, maximum likelihood can be used to estimate parameters and test for effects.


A large number of non-parametric tests are available for the analysis of data. Non-parametric tests are found in virtually all fields of statistics, from experimental design to time series analysis, and are often used in place of their parametric counterparts when certain assumptions about the underlying population are questionable. For example, the Wilcoxon Mann-Whitney test is the non-parametric counterpart to the two sample \( t \)-test. These tests are often more powerful in detecting population differences when certain assumptions are not satisfied. Non-parametric statistics are also useful when the sampling distribution of a descriptive statistic or index is unknown. Using ranks rather than the observed data, and the use of procedures such randomizations or the jackknife, are commonplace among non-parametric tests. In this talk, I will introduce and discuss the advantages of some non-parametric tests that I have used to characterize epidemics of single and multiple diseases.

Using multivariate statistics in phytopathological research. S. SANOGO (1) and X. B. Yang (2). (1) Dept. of Entomology, Plant Pathology, & Weed Science, New Mexico State University, Las Cruces, NM; (2) Dept. of Plant Pathology, Iowa State University, Ames, IA. Phytopathology 92:S101. Publication no. P-2002-0059-SSA.

To disentangle the nature of a pathosystem or a component of the system such as disease epidemics for descriptive or predictive purposes, measurement is conducted on several variables of the physical and chemical environment, pathogenic populations, and host plants. For instance, it may be desired (i) to distinguish pathogenic variation among several isolates of a pathogen based on disease severity; (ii) to identify the most important variables that characterize the structure of an epidemic; and (iii) to assess the potential of developing regional scale versus site-specific pest management schemes using weather and site variation. In all these cases, a simultaneous handling of several variables is required, and entails the use of multivariate statistics such as multivariate analysis of variance, discriminant analysis, principal component analysis, and correspondence analysis. A succinct overview of when and how to use these tools will be presented along with illustrative examples.


Artificial neural networks (ANNs) comprise a group of analytical tools for empirical modeling. ANNs extract patterns from datasets, which may contain subtle and poorly understood relationships, without preconceived assumptions about model form. Dozens of models belong to the ANN family; some correspond to statistical models either directly or as special cases. Accuracy is maximized through nonlinear transfer functions and, in some networks, a flexible, manipulable architecture. Representative input and target data are required to assure model fit to new cases. Final model form is determined by minimum error of predicted output, by minimum generalization error estimated from validation data, or by similar criteria. So far, ANNs have been equal or superior to comparable statistical models in epidemiological applications such as disease forecasting. However, a rigorous search for the optimal ANN may be laborious and prediction accuracy over statistical models may be improved only slightly, which may not be enough to justify the effort. Thus, ANNs should be applied to moderately or highly complex problems that cannot be modeled adequately by statistical methods.


Evidence-based medicine practiced by clinicians requires an integrated assessment of the available evidence, and associated uncertainty, but there is also an emphasis on decision-making, for individual patients, or at other points in the health-care system. Here we focus on the application of decision theory in evidence-based disease management. The decision-making process consists of identifying the decision maker, quantifying the important risk factors, characterizing the possible actions and their consequences, and determining the context of disease management, risk factors, and objectives relating to the pathogen, the host and the environment are combined into a statistical prediction rule, or the environment are combined into a statistical prediction rule, or indicator. The sensitivity and specificity of the indicator characterize its accuracy and have important applications in evidence-based decision-making. Sensitivity and specificity are combined with information on the prior probability of disease, using Bayes’ theorem, to calculate the conditional probabilities of disease, given the evidence related to risk factors.


Quantitative studies of plant diseases can be improved significantly when prior information is incorporated into the analysis. The Bayesian approach permits drawing inferences for quantities of interest by combining information from the observed data and available prior knowledge into a probability model. The ability to incorporate prior information (sometimes subjective) is seen as an advantage as well as a disadvantage of the Bayesian approach. The recent focus on complex modeling of scientific phenomena and advances in computationally intensive numerical methods have facilitated the application of Bayesian methods in several fields. A Bayesian analysis typically involves three steps: a) build a full probability model, the joint probability distribution for all observable and unobservable quantities, b) calculate the posterior distribution, or conditional probability distribution of the unobserved quantities, using the observed data, and c) evaluate the fit of the model and the implications of the resulting posterior distribution. These steps will be demonstrated through examples.

3rd IE Melhus Graduate Student Symposium: New Thesis Research Contributions to Plant Disease Epidemiology

Epidemiology of downy mildew of oilseed poppy. J. B. SCOTT (1), F. S. Hay (1), C. R. Wilson (1), P. J. Cotterill (2), and A. J. Fist (3). (1) TIAR, University of Tasmania, Burnie, TAS Australia, 7320; (2) GlaxoSmithKline, Latrobe, TAS Australia, 7307; (3) Tasmanian Alkaldoids, Westbury, TAS Australia, 7307. Phytopathology 92:S101. Publication no. P-2002-0063-SSA.

Downy mildew (Peronospora arborescens), has become the major disease affecting oilseed poppy (Papaver somniferum) since its first record in Tasmania, in 1996. Two field trials conducted in 2000 and 2001 studied the progression and spatial distribution of downy mildew epidemics. The logistic and exponential models best described the progression of disease incidence.
and severity, respectively. Incidence and severity increased rapidly following canopy closure. In 2001, incidence increased from 0.16 percent, prior to canopy closure, to 100 percent at late flowering (40 days). Spatial analyses of epidemics were conducted by fitting the beta-binomial and binomial distributions, median runs analysis and the spatial analysis by distance indices analyses technique. All analyses demonstrated that the distribution of incidence and severity was strongly spatially aggregated from canopy closure until at least late flowering. These results suggest that secondary spread from a few primary infections is the dominant force in epidemics.

The influence of environment and host growth for improved fungicide applications for control of southern stem rot of peanut. S. L. RIDEOUT (1), T. B. Brenneman (1), and K. L. Stevenson (2). (1) Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793; (2) Department of Plant Pathology, University of Georgia, Athens, GA 30602. Phytopathology 92:S102. Publication no. P-2002-0064-SSA.

During 1999, 2000, and 2001, peanut plants were periodically and destructively sampled for frequency of plants showing visible signs or symptoms of the pathogen, Sclerotium rolfsii from set row lengths at four locations for each year. Southern stem rot temporal development was found to be consistent within a given growing season across the four different locations. However, great variability was noted in epidemics across growing seasons as stem rot disease progress data was best described by different models across the three years. The monomolecular model provided the best fit for frequency of infections in 1999 (0.60 < R² < 0.72). However, in 2001, the Gompertz model provided the best fit (0.76 < R² < 0.82). In addition, area under disease progress curve values were significantly different (P < 0.05) across the three years. Soil temperature (5 cm depth), canopy temperature and relative humidity, precipitation, and host growth were recorded for all 12 trials. Environmental conditions across the three growing seasons were markedly different. Correlations between the environmental and host growth parameters were conducted to determine which factors promote southern stem rot epidemics.

**Molecular/Cellular Plant-Microbe Interactions**

**Functional Genomics of Plant-Pathogen Interactions**


Communication between microbe and plant is mediated by components of communication circuits (signal transduction pathways) that start with or lead to production of signaling effectors, some of which are small molecules elaborated via the enzymes of secondary metabolism. Cochliobolus spp. and members of a sister genus, Alternaria, as well as Gibberella spp., are notorious for their abilities to produce vast numbers of small molecules, some of which are known already to have roles in pathogenesis. Our genomics program focuses on filamentous ascomycete plant pathogens, representing wide phylogenetic distribution, with a particular concentration on the maize pathogen C. heterostrophus, a fungus readily manipulated by both conventional and molecular genetic procedures. Genomics technologies assist in gene discovery and experimental validation of candidate genes, including genes such as polyketide synthases and nonribosomal peptide synthetases responsible for synthesis of small molecules. The end result is expected to be a set of fungal genes that function in concert to effect plant parasitism.


Plant pathogenic microbes have the remarkable ability to manipulate biochemical, physiological and morphological processes in their host plants through a diverse array of virulence factors. Oomycetes, such as Phytophthora, form a unique branch of eukaryotic pathogens and are arguably the most devastating pathogens of dicot plants. Structural genomic studies of Phytophthora are well under way and the challenge in the post-genome era is to link a sequence to a phenotype using computational tools for data mining and robust high throughput functional assays. The overall objective of this research is to use the technology of virus-mediated gene expression to carry out high throughput functional screening of Phytophthora genes in plants. This strategy allowed us to unravel a battery of novel Phytophthora genes that trigger a variety of cellular and molecular responses in plant cells and to establish functional connections between Phytophthora genes and plant processes.

**Pseudomonas syringae pv. tomato DC3000: Genomics and phytopathogenecity.** A. COLLMER (1), J. R. Alfano (2), A. M. Baldo (3), C. R. Buell (4), S. Cartinhour (3), A. K. Chatterjee (5), T. P. Delaney (1), S. G. Lazarowitz (1), G. B. Martin (1), D. J. Schneider (3), and X. Tang (6). (1) Cornell University, Ithaca, NY; (2) University of Nebraska, Lincoln, NE; (3) USDA-ARS, Ithaca, NY; (4) The Institute for Genomic Research, Rockville, MD; (5) University of Missouri, Columbia, MO; (6) Kansas State University, Manhattan, KS. Phytopathology 92:S102. Publication no. P-2002-0068-SSA.

The genomic sequence of Pseudomonas syringae pv. tomato DC3000 (http://www.tigr.org/tdb/mdb/mdbinprogress.html) is being analyzed for virulence factors, particularly effector proteins that are translocated into plant cells by the Hrp (type III protein secretion) system. We used an iterative process involving computational methods and gene expression and protein secretion assays to characterize the Hrp regulon and identify more than 22 Hrp-secreted proteins. Analysis of these proteins has revealed candidate new accessory secretion factors, homologs of animal pathogen effectors, and new insights into mechanisms of pathogenesis.


We are using genomics approaches to elucidate the signal transduction network controlling activation of defense responses and to identify genes required for disease resistance. Our model system consists of Arabidopsis thaliana and the bacterial pathogen Pseudomonas syringae pv. maculicola (Pmm). As a first step, we determined global gene expression profiles for wild type plants and mutant plants with defects in control of disease resistance responses after infection with Pmm. Analysis of the data yielded insights into the organization of the signal transduction network and the sites of action of the genes affected by the mutations. To test the hypothesis that many of Arabidopsis genes whose expression is increased in response to infection are important for disease resistance, we have employed a reverse genetics approach. Plants with loss-of-function mutations in more than 100 pathogen-induced genes have been identified and tested for enhanced susceptibility to infection by Pmm. Many of the mutations were found to compromise resistance to Pmm, demonstrating that this is an efficient method for identification of plant genes with roles in disease resistance.
Programmed Cell Death in Disease and Development


The mitochondria pathway is regarded as a central component of some types of programmed cell death in animal cells where specific signals cause the release of cytochrome c from mitochondria into cytoplasm to trigger a proteolytic cascade involving caspases. However, plant cells lack prototypical caspases, therefore a role for the mitochondria in programmed cell death (PCD) in plant cells is not obvious. Using a primary cell-culture system in which mechanically isolated mesophyll cells terminally differentiate as tracheary elements (TE), we provide evidence supporting the involvement of mitochondria in TE PCD. The matrix becomes electron dense and fine ultrastructure is lost. This morphology is clearly different than that observed when mitochondria swell and burst such as in necrotic death of mesophyll cells or apoptosis in some animal cells. In addition, the mitochondrial membrane depolarizes prior to death and approximately 30% of the total cytochrome c is released from mitochondria at a point in time when PCD occurs during TE maturation. Moreover, cytochrome c release is calcium inducible, consistent with the calcium requirement for TE PCD. Betulinic acid, which induces mitochondrial depolarization, also induced cytochrome c release. These results suggest a role for the mitochondria in TE PCD but do not support the current animal paradigm for a causative role of cytochrome c in triggering PCD.

Dissecting the loss of HR cell death in Arabidopsis defense, no death (ndd) mutants. A. BENT (1,2), G. Jurkowski (1,2), R. Smith (1,2), I.-C. Yu (2), K. Fengler (2), S. Clough (2), and B. Lippok (2). (1) University of Wisconsin, Madison, WI; (2) formerly at University of Illinois at Urbana-Champaign, Champaign, IL. Phytopathology 92:S103. Publication no. P-2002-0076-SSA.

The fully-sequenced genome of bacteriovorous nematode C. elegans encodes 20,000 protein and RNA genes. The largest gene family (approximately 1,000 members) encodes G-protein-coupled receptors, many of which are olfactory receptor, indicative of the sophistication with which nematodes interact with the environment. Unlike lab-reared C. elegans for which many of the genes are dispensable, behavioral and other aspects of their lifestyle render many of these non-essential genes as being essential in parasites. EST-sequencing projects have revealed many orthologs of C. elegans genes in plant-parasites, including some apparently nematode-specific sequences. Some genes expressed by plant parasites are absent from C. elegans, including a cadre of genes apparently acquired from microbes. We are endeavoring to experimentally validate our computational predictions of horizontal gene-transfer, and speculate that such events played crucial roles in speciation of plant-parasitic nematodes.

What does a worm want with 20,000 genes? The evolution of plant-parasitism, and the essential-gene conundrum. D. BIRD (1) and E. S. Scholl (2). (1) Center for the Biology of Nematode Parasitism; (2) Bioinformatics Research Center, North Carolina State University, Raleigh, NC 27695. Phytopathology 92:S103. Publication no. P-2002-0074-SSA.


Rust fungi have developed a complex infection pathway in which the plant host plays a major role. Infection begins with the germination of a spore on the leaf surface, followed by the development of an appressorium. The development of the appressorium depends on a thigmotrophic signal triggered by the specific topography of the host plant surface. An infection peg formed by the appressorium enters the leaf through a stomata, followed by the development of a substomatal vesicle, infection hypha, haustorial mother cell, penetration of a photosynthetic mesophyll cell and the establishment of a haustorium. Recent work has begun to elucidate the genes and proteins involved in this complex infection pathway and the specific role of the haustoria in nutrient transfer. These include proteases and cellulases involved in cell wall degradation as well as amino acid permeases and sugar transporters involved in nutrient uptake. Given the complexity of the infection pathway and the intimate nature of the interaction, a diverse array of genes will be necessary to establish and maintain the obligate-parasitic lifestyle.


Despite the persistence of soilborne fungi and the availability of only a limited number of control strategies that continuously threaten the sustainability of many crops, soilborne fungal pathogens have received limited attention relative to those causing foliar diseases. The main objective of this collaborative project is to enhance our understanding of the molecular basis of soilborne fungal diseases through the use of a new model system, highlighting Arabidopsis thaliana as the main host and Fusarium oxysporum and Verticillium dahliae as the pathogens. Both the host and the pathogens are being subjected to in-depth studies using tools of molecular genetics, molecular cytology and genomics so that the dynamics underpinning their interactions can be studied from both the host and the pathogen perspective.

Genetic requirements for fungal pathogenicity to plants. A. E. OSBOURN (1), K. Bourab (1), N. Diaz (1), M. Dumfesne (2), A. Foster (1), M. Guillenroux (1), and A. Sesma (1). (1) Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK; (2) Laboratoire de Phytopathologie Moléculaire, Centre de Recherche sur les Plantes, Université Paris-Sud, 91405 Orsay Cedex, France. Phytopathology 92:S103. Publication no. P-2002-0073-SSA.

Fungal pathogens will require a core set of determinants that enable them to infect plants and to cause disease, including factors such as the highly conserved PMKJ-related MAP kinases. In addition to these general factors, particular pathogens will have specific genetic requirements that reflect their lifestyles and host range. For example, there are likely to be subgroups of genes that determine the tissue specificity of foliar and root-infecting pathogens. The ability of phytopathogenic fungi to tolerate antimicrobial plant secondary metabolites represents another facet of specific interactions between fungal pathogens and their hosts. Specific and general requirements for phytopathogenicity will be considered, with particular emphasis on the fungi studied in our laboratory.

How Many Genes Does It Take to Make a Plant Pathogen?

How many genes does it take for a human pathogen to become a plant pathogen? L. G. Rahme. Dept. of Surgery, Harvard Medical School, Massachusetts General Hospital and Shriner’s Burns Institute, Boston, MA 02114. Phytopathology 92:S103. Publication no. P-2002-0070-SSA.

We are studying the molecular mechanisms underlying Pseudomonas aeruginosa pathogenesis in mammals. P. aeruginosa is the most common bacterial cause of lung infections and the primary cause of death in cystic fibrosis patients as well as the most common causative organism of sepsis in burn patients and immunocompromised individuals. We have demonstrated that a human isolate, strain PA14, can cause disease in both plants and animals using a shared subset of virulence factors. This important finding provided us with the opportunity to use plants and insects as model hosts, thereby decreasing the use of experimental animals and allowing a high throughput screen for novel bacterial virulence factors relevant to pathogenesis in mammals. Several novel P. aeruginosa virulence-related factors were identified, the majority of which control functions important for the persistence and severity of P. aeruginosa infection in both plants and animals. The use of genetically tractable hosts such as plants and insects to model P. aeruginosa pathogenesis facilitates the study of the complex mechanisms that bacterial pathogens employ to attack the host and of the host responses in limiting disease development.
We have previously reported our isolation of Arabidopsis dnd mutants that carry out gene-for-gene disease resistance responses yet develop little or none of the HR cell death normally associated with those responses. The dnd mutants also exhibit constitutively elevated broad-spectrum resistance. The mutants' dual gene-for-gene and defective R-protein-mediated defense signaling. Progress toward cloning the DND2 gene will be discussed. Elevated resistance in dnd mutants requires salicylic acid, but suppression of HR does not. Suppression of programmed cell death in these mutants apparently extends beyond R/avr interactions, as dnd plants are also less responsive to fumonisin toxin. ROS production in response to pathogens is potentiated in dnd plants, rather than reduced as might be expected of a plant line that exhibits suppressed HR. No elevation of ROS scavenger mRNA species was observed in dnd mutants. Double- and triple-mutant studies are helping to place the functional defects of dnd mutants with respect to other known mediators of plant defense responses, and to reveal new aspects of plant defense signaling. Genetic suppressor screens have also been initiated.


The interaction of pathogens with plants leads to a disruption in cellular homeostasis, often leading to cell death in both compatible and incompatible relationships. Programmed cell death (PCD) is now recognized to play important roles in plant disease and shares many of the morphological characteristics of apoptosis as defined in animal systems. PCD in plants is only now being studied extensively at either the biochemical or genetic levels [Gilchrist, Ann Rev.Phytopath 1998]. Current data suggest that activation or suppression of PCD may underlie diseases in plants as it does in animals. Apoptosis depends on the activity of a number of genes in animal systems, including specific proteases (caspases), which mediate the orderly disassembly of cellular proteins. We recently reported that infiltration of cell permeable peptide inhibitors of caspases blocked PCD in plant tissues and blocked infection by several necrotrophic bacterial pathogens [Richel,PMPP 59:213 2001]. We also observed that transgenic expression of anti-caspase genes, such as baculovirus p35 and human IAP genes, blocks apoptosis in animal cells and in tomato plants induced by pathogens. These anti-apoptotic transgenes segregated as single loci and conferred resistance to a range of bacterial and fungal pathogens. We will describe high throughput functional cDNA library screens for anti-apoptotic genes using both engineered yeast and an Agrobacterium rhizogenes-mediated root screen. The ability to block plant cell death using inhibitors of plant caspase-like proteins define both a basis for plant disease and a target for sustainable resistance, as well as a basis for marker-based breeding schemes.


Viral Expression Vectors

The expression of reporter genes with screenable or selectable phenotypes from RNA viral vectors has increased our understanding of the basic biology of virus replication and plant-pathogen interactions. However, plant RNA viruses have the potential not to just be research tools, but to be vectors for the production of recombinant proteins as well. As the biotechnology industry develops an increasing number of disease therapies utilizing recombinant proteins, including a class of specific vectors are being examined as recombinant protein production tools. Employing these plant pathogen cells as transient expression vectors is, in effect, making an ally from an enemy. The use of virus expression vectors in large-scale agricultural settings to produce recombinant proteins and the technical challenges that need to be addressed by agriculturists and molecular virologists to fully realize the potential of this latest evolution of plant virology will be discussed.

Utility of the beet yellows virus for gene expression in plants. V. V. DOLJA (1) and G. P. Pogue (2). (1) Dept. Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; (2) Large Scale Biology Corp., 3333 Vaca Valley Pkwy, Vacaville, CA 95688. Phytopathology 92:S104. Publication no. P-2002-0081-SSA.

The overall objective of our research is to understand the physiological changes that occur in a plant as a consequence of microbial infection and that result in the expression of either disease resistance or susceptibility. We have focused on the disease, Victoria blight of oats, caused by the host selective toxin-producing fungus, Cochliobolus victoriae. Toxin production is required for pathogenesis. In the host, toxin sensitivity is required for disease susceptibility and controlled by the dominant Vb gene. Furthermore, the Vb gene may be identical to the Pe-2 gene, which confers resistance to the crown rust pathogen, Puccinia coronata. These observations indicate that the toxin may elicit both disease susceptibility and resistance responses. It has been shown that the toxin, victorin, induces a programmed cell death (PCD) response that shares morphological characteristics of apoptosis. In addition, molecular evaluations indicate that the biochemical features of apoptosis are also implicated in this PCD response, including the activation of caspase-like activities and induced changes in mitochondrial permeability. Recently, we have identified victorin sensitivity, conferred by a single dominant gene, in the model plant, Arabidopsis thaliana which will facilitate a genetic dissection of PCD. Understanding the physiological changes induced by victorin should provide insight into both plant disease susceptibility and resistance, and an event perhaps more fundamental, the regulation of plant PCD.


An emerging question in plant biology is whether plants display analogous features of mammalian programmed cell death during development, abiotic stress and defense against pathogen attack. A number of transgenic crop plants that express animal anti-apoptotic genes have been generated. These genes (human bcl-2, chicken bcl-xl, C. elegans ced-9, and insect sfiap) all suppress apoptotic death in animal cells. Recently, we have shown that expression of these genes in tobacco abrogate disease development in plants infected with necrotrophic fungi, including Sclerotinia sclerotiorum, Botrytis cinerea, and Cercospora nicotianae, as well as a necrogenic virus, tomato spotted wilt virus, suggesting that disease development requires host cell death pathways. Plants with null mutations in these transgenes did not protect against pathogens. Apoptotic features occurred in susceptible plants during infection, but not in transgenic resistant plants. Transgenic plants also displayed tolerance/resistance to abiotic stresses (heat, cold, salt and drought). To identify plant genes regulating apoptosis, a number of approaches are being developed including: death on demand transient assays, yeast-based functional screens, bioinformatics, and high throughput caspase screens. Taken together our data indicate that these anti-apoptotic genes function in plants and should be useful to delineate stress/resistance pathways. These genes also have potential as broad-specificity disease/stress resistance genes in economically important crops.

Improving a wheat streak mosaic virus based gene expression vector for cereal crops. R. FRENCH, K. M. Horken, and D. C. Stenger. USDA-ARS, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583. Phytopathology 92:S104. Publication no. P-2002-0082-SSA.

Wheat streak mosaic virus (WSMV) is a mite-transmitted virus in the family Potyviridae infecting many grasses including several important cereals. WSMV can tolerate addition of foreign gene sequences at the Nib-CP junction and translated proteins are excised from the polyprotein if a Nia promoter–polyadenylation signal site is introduced. Expression levels of a BYV-GUS construct were followed over time in Bobwhite spring wheat plants up to eight weeks old at time of inoculation. Tissues expressing GUS 25 days pi

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included leaf, culm, rachis, inflorescence, and seed pericarp. The PI proteinase cleavage site was mapped allowing sequences to be inserted between PI and HC-Pro. Insertions at this site are more stable over time than those at the Nb-CP junction. WSMV vectors with insertions at both PI-HC-Pro and Nb-CP junctions expressed two reporter genes. This will allow expression of a gene of interest with simultaneous expression of a readily detectable reporter gene such as GUS.

Plant virus-based vectors in agriculture and biotechnology, L. G. NEMCHINOV, Y. Zhao, and R. W. Hammond. USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705. Phytopathology 92:S105. Publication no. P-2002-0083-SSA.

Plant diseases are an important component of agricultural systems that affect crop yield and quality, and the development of improved disease control strategies is a continuing and long-term goal of our laboratory. Viral-based vectors are being engineered for the rapid evaluation of target sequences and gene products for plant disease control, for the evaluation of plant gene function in a pathogen background, and for the identification of sequence motifs that can be used to enhance or target gene expression. For example, a Potato Virus X-based vector delivery system is being used to dissect individual domain structures of Potato spindle tuber viroid in order to locate precise nuclear-targeting motifs. Peptide vaccines and improved diagnostic reagents for diseases of livestock and poultry are also being developed by expressing fusion proteins with plant viral capsids. The successful outcome of these applications of viral-based technology will lead to the development of novel disease control strategies and new tools for biotechnology.


HIV-1 is a lentivirus causing AIDS in humans. The disease continues to spread worldwide, severely affecting public health system in developing countries. Currently, the search of the prophylactic vaccine against HIV/AIDs is focused on the envelope protein gp120 and a small regulatory protein named Tat. However, to become suitable for global vaccination the prospective vaccine candidates must pass the test on economics and logistics of its delivery in the poorest and least developed countries in the world. Plants represent an attractive delivery system suitable for harsh conditions in Africa and Southeast Asia. We used plant virus-based vector systems to produce gp120 envelope protein and Tat antigens in tobacco and spinach, and demonstrated applicability of the method for HIV-1 vaccine production. The yield of the HIV-1 specific proteins in plants inoculated with the chimeric viral constructs reached 330 micrograms per gram of tissue. This production system might be very useful to deliver AIDS vaccine throughout the world.

**Plant Disease Management**

**Commercial Biopesticides: Practice and Experience**


Sclerotinia diseases, caused by the pathogens *Sclerotinia sclerotorum* and *Sclerotinia minor*, are major factors affecting the production of vegetable and row crops around the world. Sclerotinia diseases are known to infect more that 380 plant species. Important North American crops impacted by Sclerotinia include soybeans, sunflowers, canola, dry edible beans, snap beans, lima beans, lettuce, cabbage, peanuts and others. Current control techniques are not always effective. Crops losses range from minor plant damage to destruction of entire crops. In 1948, Campbell, et. al. discovered a pathogen of the sclerotia of *Sclerotinia minor* in lettuce production fields of the Salinas Valley, CA (*Coniothyrium minitans*). German scientists have developed a commercially viable method for production of spores of the antagonist. The product label is registered in the US for control of both *Sclerotinia sclerotiorum* and *Sclerotinia minor* in all agricultural soils. It is sold under the brands Intercept*®* W and Contans*®* WG for row crops and vegetable applications respectively. Both brands are exempt from a requirement of tolerance on all crops. Research data from around the world and North America illustrate the effectiveness of this novel biological pesticide.


White mold, caused by *Sclerotinia sclerotorium*, is a major factor in processing bean production in the Midwest. Current control measures include a 3 - 5 year rotation with non-susceptible crops, wide row spacing to promote drying of foliage, precision seeders to avoid clumping of seed, and fungicides (thiophanate methyl, vinclozolin or iprodione) during bloom. The recent registration of Intercept*®* WG (*Coniothyrium minitans*) provides growers with an additional novel and effective method for control. During 2000 and 2001, field trials comparing Intercept*®* alone, Intercept*®* plus fungicide and fungicide alone with an untreated check were conducted each year at three WI locations with a history of disease. Using the three locations as experimental replications, the application of either Intercept*®* shortly before planting, fungicide at flowering or Intercept*®* plus fungicide all significantly reduced disease severity and incidence when compared with the untreated check. Use of Intercept*®* in conjunction with an existing IPM scouting program promises to improve disease management in an environmentally benign manner.


On June 13, 2001, a field plot was artificially infested with naturally produced sclerotia of *Sclerotinia sclerotorium*. On the same day the biopesticide Contans (a formulation containing *Coniothyrium minitans*) was sprayed (4.5 kg/ha) over the surface of the appropriate plots with a single row CO2 backpack sprayer and incorporated to a depth of about 10 cm, and snap beans (cv. Gold Mine) were planted in the treated and control plots. At planting and on October 11, five-500 ml soil samples were bulked from each replicate plot, air dried, and wet sieved to recover the sclerotia. Disease did not develop in the plots because of hot dry weather during the critical infection period between bloom and bean pod maturity. Therefore, no direct measure could be made of the effectiveness of Contans for control of white mold on snap beans. At the October sampling date, statistically fewer sclerotia were recovered from the Contans plots, and all of them were decomposed. Additional trials in commercial fields are planned for 2002.


Investigations on the mode of action of DiTera, a biological nematicide, were undertaken using laboratory bioassays to determine the effects of DiTera on the potato cyst nematode *Globodera rostochiensis*. DiTera did not stimulate hatch of second stage juveniles (J2), but prior exposure to DiTera prevented hatch in the natural hatching medium, potato root diffusate (PRD). DiTera dissolved in PRD irreversibly inhibited hatch by preventing eggshell permeability change, an essential precursor to hatch. DiTera adversely affected movement, and electropharyngeograms of styptorator muscle activity showed that prior exposure of J2 to DiTera significantly reduced stylet thrusting. Electrophysiologest tests demonstrated that exposure to DiTera adversely affected flux of ions across membrane, and electropharyngograms of styptorator muscle activity showed that prior exposure to DiTera significantly reduced hatch of second stage juveniles (J2).

**DiTera recently received U.S. and California EPA registration.** The suspension of use of 1,3-Dichloropropene for nematicide management in California in 1990 stimulated interest in the development of natural products. DiTera has been tested over the past 20 years to determine whether it might be useful as a nematicide. The product has now received U.S. EPA registration that requires the submission of efficacy data. Field trials on carrots and tomatoes demonstrated efficacy against root-knot nematode, *Meloidogyne sp.* Trials on broccoli, cauliflower, Brussels sprouts, and sugarbeets demonstrated efficacy against sugarbeet cyst nematode, *Heterodera schachtii*. Efficacy against lesion nematode, *Pratylenchus vulnus* was demonstrated in trials on carrots. Evaluations included various rates, formulations and treatment timings, and methods of incorporation. In some
trials, a stimulation of growth or a reduction of rotting of galled roots compared to untreated controls or standard nematicides was observed.

**Biological management of postharvest diseases.** J. P. Stack. University of Nebraska, South Central Research & Extension Center, Clay Center, NE 68933. Phytopathology 92:S106. Publication no. P-2002-0090-SSA.

Postharvest handling and storage systems are amenable to the application and successful performance of biologically-based disease prevention products. Management of insect pests and decay-causing microorganisms can significantly reduce postharvest losses from wounds that occur during postharvest handling. Packhouses are configured such that a biological agent can be applied to the wound site, when it’s needed, and at the concentration required for efficacy. Fruit are then stored in regulated environments where extremes of temperature and relative humidity are prevented. The Bio-Save® product line, based on nonpathogenic strains of *Pseudomonas syringae*, is commercially used for decay prevention in pome, citrus, and stone fruits, as well as, potato storage. Storage decays caused by *Penicillium* spp. (e.g., blue mold and green mold of citrus and blue mold of pome fruit) and *Botrytis cinerea* (e.g., gray mold of pome fruit) have been successfully managed with these products since 1995. Market introduction required on-site training of packhouse managers and support industry personnel to facilitate adoption of the new technology. Critical to the continued use of the products was the implementation of an interactive product support service providing information on product application efficiency, active ingredient viability, and expected product performance.

**Control of Fusarium dry rot and silver scurf of potato with *Pseudomonas syringae* containing products Bio-Save 100 and Bio-Save 1000.** B. J. Jacobsen. Dept. Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717-3150. Phytopathology 92:S106. Publication no. P-2002-0091-SSA.

Fusarium dry rot and silver scurf, as the products Bio-Save®110 and Bio-Save® 1000. These products were tested in greenhouse and laboratory trials with *Pseudomonas syringae* (FS) and *Helminthosporium solani* and in commercial storage trials. Treatments included both products, inoculation with thiabendazole sensitive and resistant strains of FS, thiabendazole (Mertect 340F), and combinations of the Bio-Save products with Mertect 340F. In laboratory trials the Bio-save products provided 82-96% control of FS strains sensitive to thiabendazole equal to the uninoculated or Mertect treatments. When thiabendazole resistant FS was used the Bio-save products provided 56-76% control comparable to the uninoculated treatments and >80% control when combined with Mertect 340F. Similar results were obtained in experiments where control of silver scurf was examined. Scanning electron microscope studies revealed that the Bio-save products completely suppressed growth of FS 5 and 12 days post inoculation, whereas Mertect 340 F had active growth when thiabendazole resistant strains were used for inoculation. Results of commercial application trials have provided data similar to the laboratory storage trials.

**Comparison of Molecular Marker Techniques and How They Can Be Used in Breeding Programs**


Molecular markers, defined segments of DNA sequence used to assay genotype, are revolutionizing the analysis and breeding of disease resistance. With DNA markers, resistance genes can be placed on linkage maps, complex forms of resistance can be dissected, resistance genes can be targeted for positional cloning, and relationships between genes in diverse taxa can be revealed. Markers increasingly plays a central role in the genetic analysis of pathogens as well. It is no exaggeration to say that the field of plant genomics owes much of its success to DNA markers. One of the most important applications of DNA markers is marker-assisted breeding. However, biological and technological constraints — especially unreliable and environmentally-sensitive phenotypic assays, effects of genetic background, the need for large populations, and even cumbersome DNA isolation methods — limit the effectiveness of markers in resistance breeding. Techniques such as microarrays, mass spectrometry, and high performance computing address some constraints. Much better experimental design and information management are also essential.

Using candidate ESTs as a marker for disease resistance gene mapping and breeding in rice. G. L. Wang. Department of Plant Pathology, Ohio State University, Columbus, OH 43210. Phytopathology 92:S106. Publication no. P-2002-0093-SSA.

There are many rice ESTs showing a significant similarity to the cloned disease resistance genes and defense response genes in the public and private EST databases. The chromosomal location of 109 rice ESTs in the rice genome was determined using a doubled haploid mapping population. Nine of them were mapped to three regions containing genetically defined resistance genes on chromosomes 6 and 11. Clustering of the ESTs in the rice genome was observed at several chromosomal regions. Some of the clusters were located in the quantitative trait loci (QTL) regions associated with partial resistance to rice blast, bacterial blight, and sheath blight. Northern blot analysis indicated the some of the ESTs induced or suppressed after blast inoculation. These findings demonstrate that the candidate-gene approach is an efficient way of mapping resistance genes or resistance QTLs in rice. Recently, seven cDNA libraries under different blast infection conditions are being constructed for identification of more defense related genes. Analysis of the gene expression profiles in different libraries will be presented.


Resistance gene analog polymorphism (RGAP) is a molecular technique that utilizes high-resolution electrophoresis and sensitive detection of DNA fragments amplified with primers based on conserved domains of plant resistance genes. The technique has possibility of developing direct markers for disease resistance genes. RGAP markers have been used to determine genetic relationships of germplasm in wheat, barley, rice, and other crops. The technique has been successfully used to develop molecular markers for genes in different crops conferring resistance to various diseases. In many cases, RGAP markers completely co-segregated with resistance genes and were mapped on the same chromosomal regions as quantitative trait loci for resistance. Some RGAP markers had high sequence homology with cloned plant resistance genes, suggesting that the technique is useful for efficient cloning of disease resistance genes. RGAP markers specific for resistance genes have been used to determine presence of the genes in germplasm and used in marker-assisted selection for developing disease resistant cultivars.


DNA microarrays are powerful tools to analyze the expression patterns of thousands of genes simultaneously. As part of the NSF-sponsored “Soybean Functional Genomics Program”, we are accumulating a set of unique genes from a larger collection of soybean 5’ ESTs developed by the “Public EST Project for Soybean” (in collaboration with the laboratories of Randy Shoemaker and Paul Keim). For this year, we have prepared a “unigene” set of 13,305 genes from cDNA libraries made to mRNA extracted from developing cotyledons and seed coats, young pods, immature and mature flowers, and 7-day seedling roots. After cluster analysis, we selected each singleton and the 5’ most read of each contig for additional sequencing at the 5’ end. Functional assignments for these 13,305 clones were inferred by matching the blastx matches of the 5’ and 3’ sequences to the best MetaFam superset of proteins. The insertions were amplified from each clone by PCR and were spotted onto glass slides for microarray analysis. One array contained 9,216 genes spotted once and another one contained 4089 clones spotted twice. Several projects with microarrays will be summarized including their use to profile tissue-specific gene expression and developmental changes in gene expression. In another project, we have examined gene expression during the reprogramming of cotyledon cells associated with induction of somatic embryos in soybean tissue culture. The final goal of this portion of the NSF project is to generate a “unigene” set composed of 30,000 genes.

DNA microarrays are recognized as offering considerable power as a means to identify changes in global gene expression. However, conventional microarrays suffer from a variety of drawbacks. Their specificity is limited by cross hybridization occurring between related sequences and motifs. Their sensitivity limits the degree to which individual cells can be analyzed. Their reproducibility raises questions as to the numbers of replications required for acquisition of meaningful datasets. Finally, conventional microarrays do not provide an absolute measure of RNA concentrations within cells and, in an epistemological sense, provide at best a limited description of gene expression. In this presentation, I discuss means to alleviate these problems, and to further extend microarray methodologies to address important issues in plant biology, including the analysis of individuals within segregating populations.

Diagnosis and Management of Nematodes on Ornamental Plants


Plant-parasitic nematodes cause damage to most of the annual and perennial landscape plant species grown in Florida. For a number of years there have been no nematicides available for most ornamental plant uses. This has created a great need for alternative management options for residential landscapes. The void has been filled by many natural products and home remedies, most of which have been disappointing at best. This presentation will cover ongoing research in Florida seeking to develop safe and effective ways to manage plant-parasitic nematodes in landscapes. Soil solarization can be effective in reducing plant-parasitic nematode populations and is well suited to many planting bed situations. Root-knot nematode suppression has been induced by inoculation with the bacteria Pasteuria penetrans. The use of rotation with resistant ornamental plants for management of root-knot nematodes is being explored. The results of recent evaluations of various natural and other alternative products will be presented.


The northern root-knot nematode *Meloidogyne hapla* is an important pathogen of herbaceous perennial ornamental plants in the Northeastern United States. Of particular concern is the fact that this nematode is commonly found in plant propagation material. In field microplots, *M. hapla* infection of several species resulted in root galling but shoot were not affected in the first year. Plant vigor and shoot weights were reduced in the second year. Ninety-eight cultivars representing 96 species in 84 genera were screened for root status to *M. hapla*. Thirty were resistant (no galls or egg masses), and 47 were susceptible (more than 10 galls/plant). Removal of the fine feeder roots from *M. hapla*-infected propagation material greatly reduced or eliminated root knot from plants. Additionally, *M. hapla* populations in soil were reduced below detection levels following six months of rotation with resistant plants such as *Aster* or *Rudbeckia*. An integrated management program involving inspection and sanitation, pruning of feeder roots prior to planting propagation material, and rotation to *M. hapla*-resistant plants has been developed to control this nematode.


Field diagnosis of nematodes in ornamentals depends on knowing symptoms above and below ground, the site’s nematode history, and laboratory assay results. Nematodes rarely cause unique diagnostic symptoms, and going from container nursery to field nursery to landscape increases the difficulty: progressively less uniform planting media, sunlight, watering, other micro-environmental factors; inter-mixing plant species in a landscape: the infamous erratic distribution of nematodes within a very limited area. A hand lens or low-power stereoscope may improve field diagnosis by making nematode signs (e.g., root-knot nematode females in/on roots) more visible. Sampling soil and plant parts correctly, then integrating lab results with other information may affect the plant(s) an integral part of field diagnosis. Presence of a nematode species is not enough to name it a primary pathogen/problem in the landscape. Diagnosticians must understand quantitative relationships of nematodes to disease on specific hosts, and know of other pathogens, pests, and environmental factors that might duplicate and/or exacerbate a suspected nematode problem.


The occurrence of foliar nematodes *Aphelechonoides* sp. in the ornamental industry appears to be on the rise. Although their presence has been documented on various ornamentals for many decades, they have not created much attention until recently. Endemic in many woody and herbaceous perennials, foliar nematodes often remain undetected and are propagated and consequently distributed along with its host. Damage caused by foliar nematodes can also be mistaken for other maladies arising from cultural, nutritional and other disease problems. The recent loss of Oxamyl, a systemic insecticide, from the ornamental market has also played a significant role in this change in pest status. As existing supplies of this chemical are depleted, the ornamental industry is left with no effective chemical means by which to control foliar nematodes. As the ornamental industry strives to improve the quality of product coming to the marketplace, horticultural enterprises are challenged to explore alternative methods of control and eradication of the pest.

Innovations in Bacterial Disease Control Materials


Copper plays a dual role in biological systems. As a trace mineral, copper is essential for proper plant and animal growth. It is a constituent of metalloenzymes and a carrier of oxygen in some organisms. Copper also can be a biocide. The copper ion can be highly toxic and its concentration is an important characteristic for developing use strategies. Bacteria are capable of very rapid multiplication. Thus, copper materials usually are used as part of an integrated management program. Some negative aspects of copper materials are phytotoxicity, reduced copper sensitivity by many bacterial strains, and environmental impacts. These aspects can be reduced through time of applications, tank-mixes, use rate reductions, and the formulation used.


Antibiotics are valuable tools for plant disease management, due to their efficacy and low phytotoxicity. About 25,000 kg of antibiotics are used on plants annually, even though emergence of antibiotic-resistant pathogens has reduced their efficacy in recent years. Streptomycin and oxytetracycline are registered in the US: streptomycin is used primarily to control fire blight of pear and apple whereas oxytetracycline is registered for fire blight on pear and bacterial spot of peach. Gentamicin and oxolinic acid are used to control fire blight in Mexico and Israel, respectively. Concerns focused on the emergence of antibiotic-resistant strains of human pathogens have led to questions about the wisdom of applying antibiotics to plants. Effects of antibiotics on orchard ecology and the health of agricultural workers are understudied, but cited as potential risk factors with this technology. Without evidence that they are detrimental to human health, antibiotics that are currently registered for use on plants will probably continue to be available; however, it is unlikely that additional antibiotics will be registered in the US.

There are relatively few effective bactericides and antibiotics registered for use on plants making control of bacterial diseases notoriously difficult. In general, major agricultural chemical companies are not aggressively developing bactericides for use on plants. Common reasons given for this situation are the development and registration costs and the relative economic returns from antibiotics developed for agriculture as compared to human medicine. This has stimulated efforts to identify alternative controls that are based on disease forecasting and on biological control. The biological controls are usually bacterial strains that are taxonomically related to the pathogen they control. They usually effectively colonize root infections (endophytic and epiphytic) and inhibit the pathogen by niche-exclusion and/or by antimicrobial activity. Probably the most successful biological control of a bacterial disease is strain K84 of Agrobacterium rhizogenes that is effective against crown gall on certain crop species. Potential mechanisms by which K84 and other bacteria suppress disease will be discussed.

Bacterial disease protection with acibenzolar-s-methyl. A. TALLY (1), D. McKenzie (2), and G. Cloud (1). (1) Syngenta Crop Protection, Greensboro, NC 27419; (2) Syngenta Crop Protection, Basle, Switzerland. Phytopathology 92:S108. Publication no. P-2002-0104-SSA.

Acibenzolar-s-methyl (ASM) is the common name for the plant activator known as Actigard, Blockade, or Bion. This product provides a new approach for crop protection by triggering the plant to ward off invading organisms. This general protection can be effective against different types of pathogens: fungi, viruses, and bacteria. It is pathosystem specific and each host/pathogen must be evaluated to determine the utility. ASM has been applied to many different crops where bacterial diseases are an issue. In the U.S., it is currently being used for bacterial spot and bacterial speck protection in tomatoes. Various levels of protection has also been seen on bacterial black spot on mangoes, bacterial spot and angular leaf spot on cucurbits, citrus canker, black rot on Brassicaceae, bacterial spot on peaches, and fire blight on apples. Little protection has been noted on bacterial wilt on potatoes or tomatoes.


Goëmar Laboratories have identified, isolated and industrially extracted from a brown alga, Laminaria digitata, a beta 1-3 glucan, code GL32/GL 33 (“VacciPlant”) that stimulates plant defense reactions and induces resistance to diseases. VacciPlant consists of several units of D-glucopyranosides linked by 1-3 bonds and branches linked by 1-6 bonds. Treatment of tobacco, tomato and wheat cell cultures as well as wheat seedlings under controlled conditions induced metabolic changes typical of defense responses: early events (such as ionics fluxes, phosphorylations, oxidative burst), stimulation of the phenylpropanoid and lipid-delivered pathways (leading to the signals salicylic acid and jasmonates, respectively), and production of PR-proteins (known for their high antimicrobial potential). While VacciPlant had no direct antimicrobial activity, applications at low concentrations (0.5 to 2.0 l/ha) under field conditions protected efficiently apple and pear against Erwinia amylovora and tomato against Xanthomonas campestris pv. vescicatoria. The origin of VacciPlant, its protection potential against important diseases and its mode of action (stimulation of the plant’s natural defense responses) open a new path to plant protection for agricultural practices that have minimal impact on the environment.


Reducing the susceptibility of apple shoot tips with a plant growth regulator illustrates a novel approach to managing fire blight. After an earlier positive test of daminozide and a failure with ethephon, shoot blight suppression by prohexadione-Ca (P-Ca) was discovered in 1994. The suppressive effect began 7-14 days after application. In tests in Virginia orchards in 2001, shoot blight was reduced by 88-96% where P-Ca (Apogee 27.5DF) was applied 3 wk before hail injury, and canker numbers on large limbs and trunks were reduced by 61-96%. Although the main effect was on shoot blight, canker blight frequency was also reduced in one test. A. L. Jones reduced blossom blight with P-Ca one year in a Michigan test. P-Ca, a gibberellin biosynthesis inhibitor, reduces shoot vigor, and growth but shoot blight may be due to factors besides growth status: In experiments where shoot vigor was rated at inoculation, treated shoots were more resistant than non-treated shoots in the same vigor rating category. W. Rademacher et al. suggest that P-Ca may confer resistance to apple by altering the flavonoid metabolism, leading to an increase in flavon-3-ols.

Control of bacterial leaf spot on tomato with bacteriophages. J. B. JONES (1), A. Obradovic (1), B. Balogh (1), M. T. Momol (2), and L. E. Jackson (3). (1) Department of Plant Pathology, University of Florida, Gainesville, FL 32611; (2) University of Florida, NFREC, Quincy, FL 32351; (3) AgriPhi, Inc., P.O. Box 4296, Logan, UT 84323. Phytopathology 92:S108. Publication no. P-2002-0107-SSA.

Bacterial spot of tomato, caused by Xanthomonas campestris pv. vescicatoria, is a serious disease in Florida. For many years, only tank mixes of copper bactericides and mancozeb were used for controlling bacterial spot. Recently, bacteriophages have been deployed to control this disease. The strategy used with bacteriophages for bacterial spot control differed from those used in previous studies with other bacterial diseases. The bacteriophages were applied as a mixture of several different phages so that resistant bacterial strains do not build up. Coupled with this was the use of h-mutant bacteriophage to reduce cross-resistance within the bacterium. We also determined that the timing of bacteriophage application was a critical factor. Initially, applications were made in the mid-morning; however, control was not evident. In later experiments, applications were made prior to sunrise and resulted in significantly reduced disease and increased yield compared to the standard copper-mancozeb treatment. Further improvement of bacteriophage efficacy resulted from modifications in the formulation and timing of application.


As a result of interest from food retailers, processing companies, and growers a method has been developed by the Integrated Pest Management (IPM) Program at Cornell University to define and document the practice of IPM. The method of documentation is based on crop and specific IPM Elements and an associated point system. The elements and point system allow for flexibility in the documentation of IPM practice and incorporation of new research results and technologies over time. Retailers, processors, and growers are using the data from IPM documentation to communicate the practice of IPM to consumers through the use of an IPM logo printed on product labels. The current IPM identifying logo is trademarked and licensed for use by the Cornell Research Foundation (CRF). Among the challenges faced by those using the IPM logo is that, although the number is increasing, relatively few consumers understand the term or concept of IPM. Data from the documentation process provides an accurate source of information to research and extension staff about future programming needs. The potential consumer and retailer demand for IPM products provides an incentive for

IPM Labeling – Has the Time Come?


The Partners with Nature (PWN) IPM certification program was developed in response to grower interest in recognition of their use of IPM. A partnership of the University of Massachusetts, Massachusetts Department of Food and Agriculture, and USDA Farm Service Agency. Early surveys of the consumers, the northeast food industry and growers shaped the structure of the program. Certification was based on a point system, incorporating best management practices associated with soil and nutrient management, cultural practices, pesticide application and record keeping, management of insects, diseases and weeds, and education. Thirteen crops were eligible for IPM certification. One hundred-nine (109) growers participated in the program from 1993 to 1999. A survey of PWN participants showed that the functions of the PWN program most valued were: educating the public; providing IPM information; and improving farm-community relations. An independent survey showed that PWN growers used less pesticide growers than non-certified vegetable growers in Massachusetts.
stronger grower adoption of available IPM practices and creates a demand in the industry for the development of new environmentally sound IPM practices.


In 1994, representatives from Wegmans Food Markets met with the dean and staff members of the College of Agriculture and Life Sciences at Cornell University. Discussion centered on developing a closer relationship between Wegmans and Cornell, and how respective strengths could be combined to better serve agriculture in New York State. The talks focused on Integrated Pest Management (IPM) as an opportunity. The Cornell/New York State IPM program was recognized as a leader in teaching IPM practices to large growers of processing vegetables, but had not had as much success with fresh market producers. Wegmans had a well-established network of local growers supplying fresh market products during the "home-grown" season, which could be used to link the Cornell/New York State IPM program to smaller growers. In 1995, Wegmans became the first retailer in the United States to market IPM grown fresh vegetables with the introduction of IPM sweet corn in one Rochester store. Since then, additional fresh market growers have been trained and more Wegmans stores have offered IPM grown sweet corn, along with sweet cherries and berries. Additionally, Wegmans has worked with Comstock Michigan Fruit to offer processed vegetables under the Wegmans brand that are grown with IPM practices. This presentation will provide an overview of the Wegmans/Cornell/local grower/processor partnership that is bringing fresh and processed products grown with documented IPM practices to consumers and educating consumers about the environmental benefits of IPM production practices.

Plant Diseases Impacting Resource-Poor Farmers in Developing Countries: Can They Be Successfully Controlled?


Most disease control during the 10,000 years that humans have engaged in agriculture has consisted of cultural practices. The practices were generally sustainable and stable, although yields were often low. Some practices ancient farmers used were: adjusting crop density or depth or time of planting, burning, planting diverse crops, fallowing, flooding, mulching, multiple cropping, planting without tillage, using organic amendments, planting in raised beds, rotation, sanitation, and shade manipulations. Most, but not all, of these practices were ecologically sound in the long term. The disease resistance of traditional cultivars or landraces selected over millennia was an immense contribution to mankind. Landraces are usually genetically dependable and stable in that, although not necessarily high yielding, they yield some harvest under all but the worst conditions. Scientists can learn much from history to elucidate principles and practices useful in the future management of plant diseases. Traditional agricultural practices and materials should be studied and conserved before they are lost with the rapid advance of modern agriculture worldwide.


Foliar blight caused by Cochliobolus sativus and Pyrenophora tritici-repentis is the major biotic stress of wheat in the rice wheat system and causes up to 50% yield loss. The continued need for increased food production has to come from a higher and sustainable productivity. It requires a multidisciplinary approach combining novel resistance sources and adapted agronomic practices. Better germplasm resistance has been achieved through recycling genetic resistance sources or wild relatives in high yielding varieties. Germplasm improvement methods centered on regional partnerships are now addressing more specifically the needs of warmer areas. Observations in long-term trials underline the increasing role of soil fertility in reducing disease severity. Studies focusing on the effect of stress conditions on disease development increase the knowledge on the stability of resistance. This makes breeding more efficient and helps recommend more adapted crop management practices. The adoption of reduced tillage methods is also happening. The adoption of new diversified varieties and the resource conserving cropping practices imply farmers' direct participation in the research process.

Management of Begomoviruses by resource poor farmers in the tropics. P. K. ANDERSON (1), F. J. Morales (1), J. P. Legg (2), and P. M. Hanson (3), (1) Centro Internacional de Agricultura Tropical, AA 6713, Cali, Colombia; (2) International Institute of Tropical Agriculture, PO Box 7878, Kampala, Uganda; (3) Asian Vegetable Research and Development Center, PO Box 42, Tainan 741, Taiwan, ROC. Phytopathology 92:S109. Publication no. P-2002-0115-SSA.

Across the tropics, resource poor farmers are being devastated by Bemisia tabaci-transmitted begomoviruses. For common beans and cassava continuous crop improvement research over the past 25 years has resulted in the release of numerous cultivars possessing high levels of genetic resistance to begomoviruses, but whitefly management tactics for resource-poor farmers remains a challenge. Tomato, on the contrary, is a high value crop that has not received much attention from plant breeders in the tropics, where begomoviruses have become a major production constraint. The high costs involved in tomato production, forces small-scale farmers to protect their investment with a myriad of pesticides, which only aggravates the whitefly and the absence of begomovirus-resistant tomato cultivars. This presentation discusses the main IPM practices currently recommended to manage begomoviruses in these crops.


Potato late blight (LB) often causes complete loss of the potato crop in the Peruvian highlands. Andean potato farmers are confronting a problem that behaves differently than it did previously (Peruvian populations of the LB pathogen have changed dramatically since the 1980s) and resource-poor Andean farmers have little knowledge of the disease. The disease can be effectively managed using a combination of host resistance and fungicides,
but it is not easy for Andean farmers to obtain and manage appropriate potato varieties, and to effectively and safely apply fungicides. We developed a program of farmer training and participatory research focusing on late blight management in San Miguel, Cajamarca, Peru using the farmer field school (FFS) approach. We developed training materials for FFS facilitators, combining support for adult education on plant disease with support for participatory research on varietal evaluation and disease management. Through the FPR-FFS (farmer field schools with farmer-participatory research), farmers have improved their knowledge and selected new resistant varieties suited to local conditions. The approach is now being extended to a larger number of farmers.


The Cepheid I-CORE thermally-controlled fluorometer module has true 4-color detection capability and is integrated into both the Smart Cycler systems, and the GeneXpert, a system that processes cartridges which integrate sample preparation and PCR. PCR analysis time is 25 minutes or less. The GeneXpert system is a benchtop 4-site instrument that processes 100 FL to 5 mL volume specimens. Any bacteria present are concentrated down to 40 FL, washed, rapidly lysed using a novel ultrasonics technique, mixed with on-board dried-down PCR reagent beads, and transferred to an integrated PCR tube. Sample preparation typically requires 5 minutes or less. Demonstrated applications include anthrax spores from wet aerosols, Group B Streptococcus from swab extracts, and Mycobacteria tuberculosis in buffer, and total time for processing is 30 minutes or less. Advanced technology development is focusing on increasing the optical channels to 8-10 colors and on-board DNA or RNA purification.


Real-Time PCR for Field Diagnosis of Bacterial Diseases


Fluorescent, real-time PCR technologies were developed to overcome limitations of conventional methods used for the detection and/or quantitation of nucleic acids. The results are accurate, precise, and reproducible methods that utilize automation, analytical software, and fluorescent pre-formulated chemistries. Other benefits of these high sample throughput methods include the ability to quantitate and detect nucleic acid over a large dynamic range using closed-tube assays that do not require electrophoresis or post-PCR processing. In this session, I will review the basic concepts behind the Fluorogenic 5’ Nuclease Assay (TaqMan® chemistry) and the SYBR® Green 1 Double Stranded DNA Binding Dye Assay with an emphasis on their use for bacterial detection using the ABI Prism® 7700 Sequence Detection System.


National and international phytosanitary concerns with respect to the sale and export of agricultural products require rapid and accurate diagnosis of plant pathogens under a variety of conditions. The polymerase chain reaction (PCR) has dramatically changed the approach used in detection of microbes because as little as a single copy of DNA can be specifically amplified and detected with a nucleic acid probe. Real-Time detection of PCR with fluorogenic probes characterizes the cycle number at which a signal (fluorescence) is first observed rather than the amount of product that is made. This approach provides unmatched specificity and a quantitative measure of the initial template in the reaction. A multiplexed Real-Time PCR system will be described that employs both Taqman and molecular beacon fluorogenic assays in the ABI Prism® 7700 Sequence Detection System. The results are accurate, precise, and reproducible.
Diagnosing bacterial diseases can be very time consuming. Traditional isolation and pathogenicity tests are very sensitive but require 10-20 days or longer. Serological techniques can reduce the time, but the detection threshold is only $10^2$ - $10^4$ cfu/ml. Classical PCR is 10 times more sensitive than serology but requires confirmation tests. Real-time PCR does not require confirmation by a Southern blot, but the equipment is very expensive. We have developed real-time PCR assays using the portable Smart Cycler SC System (Cepheid, Sunnyvale, CA) for on-site detection of several bacteria including Acidovorax aureus subsp. citrulli in watermelon, Pseudomonas phaseolicola pv. phaseolicola in beans, Ralstonia solanacearum in potato, and Xylella fastidiosa in grape, citrus, and shade trees. All can be detected in under one hour, including sample preparation. If greater sensitivity is more important than time, samples can be enriched for BIO-PCR in liquid or on solid media.

Strobilurins and Turfgrass Disease Management

Methods for assessing sensitivity of fungal pathogens to QoI fungicides. G. Olaya. Syngenta Ag Products, Vero Beach, FL. Phytopathology 92:S111. Publication no. P-2002-0124-SSA.

The mode of action for QoI fungicides is the inhibition of the electron transport in the mitochondria thereby inhibiting fungal respiration. These fungicides have been developed for a broad range of plant diseases. The risk of fungicide resistance is high in this cross-resistance group. Resistance has developed already in certain plant pathogenic fungi. Reliable in vitro and in vivo methods to determine the sensitivity of different plant pathogenic fungi have been developed and implemented. In this paper, several methods to monitor the sensitivity of several target pathogens are described and discussed. A DNA-based test method approach for diagnosis and monitoring of resistance to QoI is also discussed.

Professionalism/Service/Outreach

Application of Quality Assurance and ISO Certification to Plant Pathology

From confusion to compliance: The bumpy road to accrediting a plant health diagnostic laboratory to ISO 17025. C. M. Masters. Canadian Food Inspection Agency, Sidney, BC. Phytopathology 92:S111. Publication no. P-2002-0125-SSA.

Since its inception in 1997, the Canadian Food Inspection Agency (CFIA) has made it a policy for all laboratories under its jurisdiction to be accredited to ISO 17025 (formerly ISO Guide 25). General Requirements for the Competence of Testing and Calibration Laboratories. The Centre for Plant Health initially received accreditation to ISO Guide 25 in January 2001. The accreditation was upgraded to ISO 17025 the following April. Understanding the standard, and how to apply it to the specific needs of a plant health diagnostic laboratory, was essential to the Centre’s successful implementation of its quality system. In addition, it was necessary to have solid support from management, monetary and human resource commitments, support from all staff and adequate quality assurance training.


This presentation will discuss how plant pathologists at Pioneer Hi-Bred International, Inc. utilize the ISO: 9000 system to meet the business need of international seed movements and of their collaborative efforts with personnel from various governmental regulatory agencies in addressing seed borne phytosanitary issues. The development and implementation of quality management programs in laboratory seed testing, disease diagnostics, and phytosanitary field inspection and post entry quarantine will be highlighted.

Extension and Teaching from a Distance

Online core competency training for Kansas County agricultural agents. D. J. JARDINE (1), K. Wright, G. Kepka, G. Snyder, and S. Bales (2). (1) Dept. of Plant Pathology; (2) Dept. of Communications, Kansas State University, Manhattan, KS. Phytopathology 92:S111. Publication no. P-2002-0129-SSA.

Extension Agents are expected to be proficient in areas beyond their formal training. For example, 75% of Kansas agriculture agents have degrees in animal science, yet they routinely dispense general agronomic and home horticulture information. Traditionally, competency in subject matter outside the degree has come over a period of years through in-service training and in-field experiences. Agents routinely complain however, that in-service training programs require too many days out of county. **Diagnosing Plant Pathogens** is a course delivered using the K-State Online system, which is a course management system designed to deliver an interactive web-based classroom to students. Agents can complete the course from their office or home at their convenience. The course consists of a series of modules. Each module contains several lessons and quizzes. Lessons contain symptom descriptions accompanied by appropriate images. At the end of each module there is a comprehensive final exam. Once the course has been completed, the material is available indefinitely as a general reference.


In 1998 Plants, Plagues and People, a popular lecture class offered to undergraduate non-science majors by the Plant Pathology Department at the University of Florida, was adapted to be taught as a web-based distance learning class. Undergraduates from all over the state of Florida can take this class for either biology or humanities credits. One of the motivations for initially offering this class was to introduce the field of Plant Pathology to undergraduate students at the University of Florida. The methodology for accomplishing this goal is not to educate college students about what Plant Pathology is but to give them an understanding of why Plant Pathology and agriculture in general are important to their lives. This is done by teaching basic concepts in the context of biology, evolution, and human history and
The Food Quality Protection Act (FQPA): Expected Impact on Agriculture and the Consumer


This session is planned as an informal discussion of a federal law enacted unanimously in 1996 that may have far-reaching effects on disease management. We have convened a group of experts on its implementation who have first-hand knowledge of its current and expected impact. After this panel of experts answers each of the following questions, the floor will be opened up to comments and further related questions.

1. What is the FQPA and why was it created?
2. Who interprets and enacts the FQPA?
3. What is the status of the FQPA implementation and when will it be completed?
4. What are aggregate and cumulative risk assessments, what models are being used to evaluate them, and how are they being implemented?
5. How is the FQPA affecting fungicide, nematicide, and bactericide registrations and more broadly, disease management strategies?
6. How is the FQPA affecting the way industry does business?
7. How is the FQPA affecting the Cooperative Extension Service?
8. How is the FQPA affecting farmers?
9. What will be the net effect on consumers of agricultural products?
10. How can APS members participate in the implementation of the FQPA?

Following these questions, the audience is invited to ask the panel other FQPA questions or make comments about their experiences with the FQPA.

Non-Traditional or Alternative Careers in Plant Pathology

Regulatory agencies such as Animal and Plant Health Inspection Service offer alternative careers for plant pathologists. S. D. Cohen, USDA-APHIS-PPD, Dept. of Plant Pathology, University of Minnesota, St. Paul, MN. Phytopathology 92:S112. Publication no. P-2002-0136-SSA.

Careers with regulatory agencies emphasize policy, regulatory research, and management activities. Policy support activities may involve developing regulations, writing pest risk assessments, coordinating national and international standards for regulatory activities. Regulatory research activities might include developing new methods for pest risk assessment, evaluating methods for pest detection, and testing quarantine treatments. Management activities may encompass issuing permits, identification of quarantine pests, survey for pests, inspection of import commodities, and regulatory control treatments. Positions with regulatory agencies often require staff to work on multi-disciplinary team projects. Broad training in the sciences is an advantage for these positions as the agency handles issues related to invasive species, insects, diseases, nematodes, snails, weeds, genetically modified organisms, animal health, and wildlife. Competence in foreign languages, statistics, economics, and other areas complementing biological training are also useful.

My job as an educational program specialist in plant pathology-I get to do all the things faculty wished they had time for. K. L. Shelton. Dept. of Plant Pathology, University of Georgia, Athens, GA. Phytopathology 92:S112. Publication no. P-2002-0137-SSA.

After spending the past three years researching Tomato spotted wilt virus of peanuts, I found myself ready for something different yet similar. In
September of 1999 I received the opportunity as I started a new position as an Educational Program Specialist in Plant Pathology. This job allows me the ability to reach out to the public on a daily basis. I have created a summer program for high school students to explore the world of plant science. Teachers also have an opportunity to learn about plant pathology at the Plant Pathology Teacher Workshop held each summer. Throughout the year I travel to schools and career shows/conventions to display information on plant pathology. Contact with high school teachers in the state has become a large focus for outreach. Projects include adapting the exercises to fit the state requirements for middle and high school teachers and developing a partnership between biological science and agricultural science teachers to further the introduction of plant pathology into the school systems. As an Education Program Specialist I spend most of my time doing the things faculty wished they had time to do.

Resources and Funding for Plant Pathology Outreach


The APSnet Education Center is a new (and still growing) website that presents information on plant health and plant diseases (www.apsnet.org/education). It is sponsored by the American Phytopathological Society, is completely free, and includes peer-reviewed publications with many photographs and other resources. There is material for introductory and advanced plant pathology (including disease lessons, labs, topics, and an illustrated glossary). There is also a K-12 section designed for teachers that includes monthly “News and Views,” classroom activities (background information, lesson plans, class handouts), online mentors, resource catalog, and a bulletin board for questions and discussions. “DNA the Easy Way” is one of the K-12 labs from the website and will be demonstrated at this workshop. Students can visualize DNA by lysing bacterial cells on a slide and “stringing up” the DNA with a toothpick in less than one minute. This technique can also be used to study bacteria because only Gram-negative bacteria lyse in 3% KOH; Gram-positive bacteria do not. The technique is equivalent to the Gram-stain reaction, but does not require a microscope or potentially messy stains.


Special Demonstration


Assess is affordable Windows-based image analysis software designed specifically for fast, accurate, and routine disease measurement. The intuitive and user-friendly interface makes Assess a powerful tool for disease measurement, without requiring expertise in computer science or image analysis. In addition to disease quantification, Assess is also optimized for measurement of ground cover, root length, and counting and sizing of objects. The session will cover a short review of concepts and issues encountered in automated plant disease assessment, a live demonstration of the capabilities of the software and a short period of questions and answers. The demonstration will cover, specifically, the measurement of leaf area, foliar diseases (% leaf damage), ground cover, and root length. Object (lesions, seed, etc.) counting, sizing and characterization, as well as other capabilities of the system will also be demonstrated.

Numerous resources are available in a variety of formats that provide cognitive content, background information and practical techniques for the use of living plants and plant products in classroom activities. Electronic and print resources for use by preK-12 teachers will be surveyed. General comments about their age appropriate use and potential role in multicultural classrooms will be included. Suggestions for teachers whose interests range from providing enrichment for their classrooms to those wanting activity intensive or integrative curricula will be offered.


The terms “symptoms” and “signs” are often used interchangeably. For plant pathologists, the terms have unique meanings. Symptoms are external changes of a host plant, such as wilting or necrosis. Signs of a plant disease are the pathogens that are found on a host. Generally, symptoms are always present, while the presence of signs is variable. The ease and confidence of plant disease diagnoses can be related to symptoms and signs. The following situations are listed in order, increasing in ease and confidence for diagnosing a plant disease. 1. General symptoms that are induced by many different pathogens. 2. Symptoms associated with a small number of pathogens. 3. Symptoms that are typical of a specific pathogen. 4. A host with both symptoms and signs. Often, attempts are made to induce the pathogen to produce signs or to isolate the pathogen from the plant tissue. While Webster’s dictionary list symptoms and signs as being synonymous, the distinction is critical in the world of plant pathology.