
Rhizoctonia solani isolates were obtained from creeping bentgrass exhibiting brown patch symptoms in two locations within North Carolina, Sandhills Research Station (SRS; 59 isolates) and Country Club of North Carolina (CCNC; 41 isolates). All isolates belonged to AG-2 based on nuclear status and anastomosis reaction. Analysis of ITS sequences placed all isolates from CCNC in AG-2-IIB. Isolates from SRS formed a clade within AG-2-2 that was distinct from all known subgroups. SRS isolates produced colonies that were medium brown with brown sclerotia in concentric circles, while AG-2-IIB colonies were dark reddish brown with dark brown sclerotia produced at the center and periphery of the plate. SRS isolates exhibited reduced growth rate at 12°C and increased growth rate at 36°C compared to AG-2-2-IIB. AG-2-IIB isolates caused higher disease incidence (70-80%) on creeping bentgrass seedlings at 25°C than SRS isolates (50-63%). We propose that the SRS isolates represent a novel subgroup of R. solani designated AG-2-2-V.

Advances in the integration of morphological and molecular characterization in the genus Phytophthora: The case of P. niederhauseria sp. nov. Z. G. Abad (1,2) and J. A. Abad (2). (1) Plant Pathogen Identification Laboratory-PPIL; (2) Dept. Plant Pathology, NCSU, Raleigh, NC 27695. Phytopathology 93:S1. Publication no. P-2003-0002-AMA.

Phytophthora is one of the most important Genera of plant pathogens. Morphological identification to species level remains a major challenge due to constraints of variability, heterothallism, and overlapping of morphological characters. Using an integrated system of morphological and molecular characterization developed at the PPIL, a non-papillate heterothallic species has been identified from Arbutusae and English Ivy plants grown in greenhouses in NC. Morphological characters of the anamorph and teleomorph do not fit the descriptions of the 75 reported species. Phylogenetic analysis of the ITS rDNA region sequences with other 70 species (PPIL data base) positions P. niederhauseria in the same cluster with P. sojae. Other close species are: P. cajani, P. vignae, P. melonis, and P. pistaeae. Both, the unique morphological characters and the phylogenetic analysis identified this organism as a new solid species. The name of Phytophthora niederhauseria has been coined to honor Dr. John S. Niederhauser, a notable Plant Pathologist.

Morphological and molecular characterization of Pythium festivum sp. nov. isolated from corn. Z. G. Abad (1,2), J. A. Abad (2), G. Olaya (3), and C. Watrin (3). (1) Plant Pathogen Identification Laboratory; (2) Dept. Plant Pathology, North Carolina State University, Raleigh, NC 27695; (3) Syngenta Crop Protection, Vero Beach, FL 32967 and Greensboro, NC 27409. Phytopathology 93:S1. Publication no. P-2003-0003-AMA.

Phytophthora festivum sp. nov. isolated from corn roots in Iowa during the summer of 2002, a putative new Pythium species (PPNS) has been identified. The morphological characters include: spherical terminal and intercalary sporangia and unique dichinous multiple long antheridia with twisting serpentine forms, spherical and obureinate oogonia and crescent aplerotic oospores. Most morphological features do not fit with descriptions of any of the over 200 described taxa. The phylogenetic analyses of the ITS rDNA region strongly supports that this organism is an undescribed Pythium species. The Neiborn-joining phylogenetic tree with 100 bootstrap supports that this organism is an undescribed Pythium species.

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Diversity of the Iris yellow spot virus N gene in the USA. J. A. Abad (1), J. Speck (1), S. K. Mohan (2), and J. W. Moyer (1). (1) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695; (2) University of Idaho, 29603 U of I Lane, Parma, ID 83660. Phytopathology 93:S1. Publication no. P-2003-0004-AMA.

Iris yellow spot virus (IYSV), a member of the Family Buvaviridae and Genus Tospovirus, has been observed to cause severe disease in onion and chive crops in the western U.S. This study examined the genetic diversity of IYSV isolates obtained from symptomatic plants from Colorado (CO), Idaho (ID) and Utah (UT), using RT-PCR with specific primers to amplify the N gene. The amplicons were cloned and sequenced. Phylogenetic analysis showed two distinct groups. The ID isolates grouped with IYSV from the Netherlands and Israel, whereas the UT isolates were more closely related to IYSV from Brazil. Interestingly, the chive isolates from ID, although grouped with the Brazilian sub-clade, formed a distinct subgroup and exhibited the least similarity (81% at the amino acid level) when compared with the IYSV type strain (Dutch isolate), suggesting that it may be a different virus. The high variability observed in the N gene of IYSV is probably the result of a host-virus, and possibly vector, interaction in addition to the diverse geographic origin of the isolates.


The 2002 TSWV epidemic in North Carolina severely affected tobacco, tomato, pepper, potatoes and other crops. In nature, this Tospovirus exists as a highly heterogeneous population with a great capacity for genetic variation. Sixteen samples were selected from 72 isolates collected in 30 counties. The isolates were from tobacco, tomato, potato, pepper, and cabbage, respectively. For phylogeny, a 0.9 kb fragment from the L RNA, the entire G1/G2 gene (3 kb) from the M RNA and the NSs (1.5 kb) and N gene (0.9 kb) from the RNA were sequenced. Phylogenetic analysis showed that NSs and L RNAs are more conserved than G1/G2, and the N gene. Sequence similarity ranges are: 97-99% for L, 90-99% for G1/G2, 98-99% for NSs and 96-99% for the N gene. All isolates have a single monophyletic origin with the exception of an isolate from cabbage. The 2002 epidemic isolates exhibit...
a few but potentially significant differences at the nucleotide and amino acid levels in G1/G2 and N genes. This is the first report in North America for TSWV in potato and cabbage.


Fire blight, caused by Erwinia amylovora, is a very serious disease on pome fruits, controlled mainly by antibiotics. The use of antibiotics on plants is banned in many countries, moreover, highly resistant pathogen strains to streptomycin, the most effective antibiotic used, have emerged in Lebanon and elsewhere. Plant extracts have a good potential as alternatives to antibiotics and are environmentally safe biocontrol agents. Fifty-nine plant extracts were tested in vitro, in agar diffusion tests, for their efficacy against E. amylovora. Seven extracts, all essential oils, gave promising results with minimum inhibition concentrations (MIC) ranging from 40 to 0.32 µl/mL, compared to streptomycin with MIC of 100 µg/mL. Two plant extracts, tar oil and clove oil, with in vitro MICs of 0.32 and 0.64 µl/mL, respectively, were tested in vivo on inoculated apple and pear plantlets. The in vivo tests showed that the plant extracts significantly decreased the disease severity index in comparison to that of the non-sprayed check.

Studies on induced resistance against fire blight (Erwinia amylovora) with different bioagents. K. Abo-Elyourr (1), W. ZELLER (1), M. A. Sallam (2), F. Laux (1), and M. H. Hassan (2). (1) Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Pflanzenschutz, Heinrichstrasse 243,D-64287 Darmstadt; (2) Faculty of Agriculture, Department of Plant Pathology, Assiut University, Egypt. Phytopathology 93:S2. Publication no. P-2003-0007-AMA.

Three different bioagents (BION®, ethic oil from Thymbra spicata and the antagonistic bacterium Rahneella aquatilis Ra39) were tested on their efficacy against fire blight (Erwinia amylovora) and on their resistance induction activity. The experiments were carried out under controlled climatic conditions in the greenhouse. For the studies M26 apple rootstocks were used as host plant. Moreover as a marker of resistance in physiological studies the total phenol content and enzymatic activity PPO were estimated. The treatments with BION®, ethic oil and Ra39 resulted in a reduction of the disease index of up to 63.7, 30.8 and 58.6% respectively. This was correlated with a decreasing effect on the growth of bacteria up to 64.2, 49.5% and 63.8% respectively during the course of infection. In physiological studies on apple rootstock shoots significant changes in the total phenol content and activity PPO were found after infection. In physiological studies on apple rootstock shoots significant changes in the total phenol content and activity PPO were found after infection. In physiological studies on apple rootstock shoots significant changes in the total phenol content and activity PPO were found after infection.

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Management options for false smut in upland rice in Edo, Nigeria. M. O. AHONSI (1) and A. A. Adeoti (2). (1) ETHZ, LFW, CH-8092 Zurich; (2) UNAAB, PMB 2240 Abeokuta, Nigeria. Phytopathology 93:S3. Publication no. P-2003-0013-AMA.

False smut (FS) induced by Ustilaginoidea virens (Cke) Tak. is a common inflorescence disease of rice in Edo, Southern Nigeria. FS could be severe especially in years with high and extended rainfall, causing serious reduction in grain yield and contamination at harvest that reduces the market value of harvested grains and loss of income by farmers. Field experiments were conducted at two farm sites in two consecutive years under rain-fed upland rice conditions to investigate sowing date, host-plant resistance, and fungicide sprays as possible management options for FS. Rice, S. T. Lagoke (4). (1) ETHZ, LFW CH-8092 Zurich; (2) USDA-ARS 1301 Ditto Av. Detrick, MD 21702-5023; (3) IITA, PMB 3112 Kano Nigeria; (4) UNAAB, PMB 2240 Abeokuta Nigeria. Phytopathology 93:S3. Publication no. P-2003-0014-AMA.

Exploiting soil suppressiveness and beneficial rhizobacteria for Striga hermonthica control in Africa. M. O. AHONSI (1), D. K. Berner (2), A. M. Emechebe (3), and S. T. Lagoke (4). (1) ETHZ, LFW CH-8092 Zurich; (2) USDA-ARS 1301 Ditto Av. Detrick, MD 21702-5023; (3) IITA, PMB 3112 Kano Nigeria; (4) UNAAB, PMB 2240 Abeokuta Nigeria. Phytopathology 93:S3. Publication no. P-2003-0015-AMA.

Striga hermonthica (Del.) Benth. (SH) is a serious constraint to cereal production in Africa. Effects of soybean-maize rotation, N, P fertilizers and ALS-herbicide use on natural soil suppressiveness to SH in maize were evaluated. Flourescent pseudomonads (GSP) that suppress SH seed germination were selected from suppressive soils and tested for ability to control SH in maize. Potential of ethylene-producing pseudomonads (EP) in combination with N2-fixing legumes selected for suicidal germination of SH seeds were evaluated. Results showed that soil suppressiveness to SH is biotic and soil treatments that increased fertility, including preceding soybean crop, application of P to preceding soybean, and N to current maize crop enhanced suppression. Six of 15 GSP reduced SH counts and increased maize yield. Reduction in SH parasitism in maize preceded by a cowpea or soybean crop was increased when the crop was inoculated with EP and Bradyrhizobial strains. A SH control approach combining selected legumes and GLP treatments that increased fertility, including preceding soybean crop, application of P and N to current maize crop is suggested to avoid damage from FS. Use of Cupravit may be justified when flowering of large field of high yielding susceptible variety is in the usual frequent rains of August/September.


Stagonospora convolvuli strain LA39, an effective bio-control agent of Convolvulus arvensis (L.) (field bindweed) and Calystegia sepium (L.) R.Br. (hedge bindweed), produces toxic metabolites, leptothesphaeroidine and elsinochrome A which may limit its acceptability for use. Another strain, 214Caa produced the same toxins, cercosporin. Therefore, 30 Stagonospora sp. strains including LA39 and 214Caa, were characterized for pathogenicity on both weeds, and production of the three metabolites. Ten strains were more aggressive than LA39 on either weed. Twelve strains produced leptothesphaeroidine, 20, elsinochrome A, and four, cercosporin. Cercosporin producers produced neither leptothesphaeroidine nor elsinochrome A, and were among the weakest pathogens. All 10 leptothesphaeroidine producers also produced elsinochrome A. Except one strain which produced only elsinochrome A, all 10 most pathogenic strains produced both leptothesphaeroidine and elsinochrome A. Elsinochrome A-negative strains did not cause significant disease, suggesting that elsinochrome A production may be linked to pathogenicity, and that selecting elsinochrome A-negative strains to reduce potential risk of using Stagonospora sp. strains for bio-control of bindweeds may be difficult. Assessment of the potential risk of elsinochrome A and probably leptothesphaeroidine is necessary.

Genetic and pathogenic diversity of Fusarium pseudograminearum and F. graminearum causing head blight of wheat in Australia. O. A. AKINSANMI (1), V. Mitter (1), S. Simpfordorfer (2), D. Backhouse (3), D. Yates (4), and S. Chakraborty (1). (1) CSIRO Plant Industry, CRC for Tropical Plant Protection, University of Queensland, 4072; (2) NSW Agriculture, Tamworth; (3) University of New England, Armidale; (4) Department of Botany, University of Queensland, Australia. Phytopathology 93:S3. Publication no. P-2003-0016-AMA.

Epidemics of Fusarium head blight (FHB) have increased in Australia in recent years due to above average rainfall and conservation tillage practices. Monosporidial isolates of races 1 and 2 of the fungus, has been identified by others as a significant cause of FHB in Australia. FHB epidemic levels have been low in different cropping histories in Queensland and northern New South Wales (NSW) were evaluated using species-specific PCR assays. Isolates were quantitatively evaluated for aggressiveness and specificity on wheat cultivars with varying levels of resistance to FHB in plant infection assays. Genotypic diversity of the isolates was evaluated using AFLP. There were significant differences in aggressiveness of isolates within and between species. Variations in aggressiveness were not related to the source of isolates and DNA fingerprints. Although there were significant differences in aggressive-ness among cultivars tested, there were no significant cultivar and isolate interactions. The results indicated that distinct genetic groups exist within F. pseudograminearum and F. graminearum isolates in Australia. Results also showed that isolates within each group have different biological properties in relation to their pathogenicity on wheat.

Novel Pyrenophora triticeti-repentis isolates from Arkansas wheat. S. ALI (1), R. Cartwright (2), T. Friesen (3), J. Rasmussen (1), and G. Milius (2). (1) North Dakota State Univ., Dept. of Plant Pathology, Fargo, ND 58105; (2) University of Arkansas, Dept. of Plant Pathology, Fayetteville, AR 72701; (3) USDA-ARS, Red River Valley Ag. Res. Center, Fargo, ND 58105. Phytopathology 93:S3. Publication no. P-2003-0017-AMA.

Pyrenophora triticeti-repentis, causal agent of tan spot of wheat, occurs in monoculture as multiple races (one ability to cause necrosis and chlorotic (ch1) lesions on wheat differentials. Tan spot occurs in Arkansas, but the race structure in that population has not been investigated. Fifty-one single-spore isolates, obtained from diseased leaves in 2002, were identified to race. Twenty-eight isolates were race 1 ( nec+ ch1+), but 23 isolates produced a novel race consisting of nec- ch1- on ND495, Katepawa, and 6B365, and avirulence on Glenlea, Salamouni, and M-3. Additionally, 17 of the 101 wild isolates lacked ToxA. The 23 isolates appear to form a new race. The data indicate that P. triticeti-repentis in Arkansas has similarities to and differences from races in other regions of the US. Additional collections from Arkansas wheat will be made in 2003 for further testing.


Pyrenophora triticeti-repentis is the causal agent of tan spot, a major foliar disease of wheat in the US Great Plains. Ptr ToxA, a host selective toxin produced by races 1 and 2 of the fungus, has been suggested by others as a potential screening tool for developing tan spot resistant germplasm. Between 1998 and 2002, a total of 309 hard red spring (HRS) and durum wheat breeding lines were evaluated for reaction to purified Ptr ToxA and to inoculation with conidia of race 1. Up to 27% of the HRS and 68% of the durum wheat genotypes were susceptible to the fungus but insensitive to Ptr ToxA toxin. These results indicate that reliance on Ptr ToxA as a screening tool may lead to development of toxin-insensitive, disease-susceptible germplasm. Therefore, we conclude that breeding programs should evaluate germplasm with spore inoculations.


Seeds of broomrape Orobanche cernua were exposed to 0, 25, 50, 75, and 100 milli molar (mM) NaCl solutions during their preconditioning period (14 days of moisture) under laboratory conditions and induced to germinate by synthetic germination stimulant (GR24). There was significant reduction in seed germination along with the increasing salt concentration resulting in 35.2%, 32.5%, 23.6%, 14.3% and 9.2% germination, respectively. Similar results of 29.4%, 21.3, 20.5 and 17.4% germination, were also obtained when O. cernua seeds were exposed to 0.0, 1.0, 1.25 and 1.5 Molar (M) levels of NaCl for 9 hours respectively. With no salt preconditioned seeds showed heavier protein profile bands at the range of 6.5 – 14.2 Kilo Dalton (KDa) than dry seeds. Seeds from the 0.75 M NaCl treatment showed similar...
profile to the water preconditioned ones, plus an extra band at the range of 29 – 36 kDa. However, the protein profile of 1.0 and 1.5 M NaCl treated seeds showed weaker bands with the absence of the 29 – 36 kDa band. At 75 mM NaCl treatment tomato plants showed no differences in their growth values in both the Orobanche-infested and non-infested soils, and extracted tomato roots showed no Orobanche infection. On the contrary, the control (tap water only) showed 11.2 Orobanche attachments per plant root.

Extracted tomato roots showed no Orobanche infection. On the contrary, the values in both of the Orobanche-infested and non-infested soils, and mM NaCl treatment tomato plants showed no differences in their growth respectively.

### Ralstonia wilt of geranium: A test case for the new agro-bioterrorism regulations


In February 2003 many US greenhouses reported wilting geraniums (*Pelargonium spp.*). Immunological and PCR tests determined that they were infected with *Ralstonia solanacearum* Race 3, imported in lately infected geranium cuttings from Kenya. A 1999 outbreak derived from Guatemalan cuttings caused little damage. However, in 2002 *R. solanacearum* Race 3 was listed under the Agricultural Bioterrorism Protection Act. Scientists and officials were caught between strict new quarantine and biosecurity regulations and were forced to grow crops from open asymptomatic cuttings from possibly latently infected plants. Legal restrictions on possession of the bacteria forced a single APHIS lab to identify all samples to race, resulting in backlogs. Limited data on pathogen epidemiology on geranium hampered efforts to develop policy for outbreak containment. Based on our experience with the disease in Wisconsin greenhouses, we will propose streamlined diagnostic tools throughout the Colombian cassava-growing areas of Valle, Cauca, Eastern Plains, and North Coast, where yield losses reach almost 90%. We detected and confirmed that a phytoplasma was associated with the FSD by using a nested PCR assay with the specific primers R16fmF2/R16mR1 and R16F2m/R16R2. To classify the phytoplasma, we used the universal primers P1/P7 and R16F2nR2 to amplify the 16S ribosomal DNA gene. Fragments of 1.2 kb were amplified only from samples collected from symptomatic plants. Sequence analysis of the cloned fragments showed that the phytoplasma was similar to *Cissium* white leaf phytoplasma (Genbank acc. no. AF373106, 16SIII X-disease group), with a 100% sequence homology. This study is the first to report a phytoplasma in association with FSD in cassava.

### Detecting the phytoplasma-frogskin disease association in cassava (*Manihot esculenta* Crantz) in Colombia


Frogskin disease (FSD) is a major pest of cassava roots that is spreading throughout the Colombian cassava-growing areas of Valle, Cauca, Eastern Plains, and North Coast, where yield losses reach almost 90%. We detected and confirmed that a phytoplasma was associated with the FSD by using a nested PCR assay with the specific primers R16fmF2/R16mR1 and R16F2m/R16R2. To classify the phytoplasma, we used the universal primers P1/P7 and R16F2nR2 to amplify the 16S ribosomal DNA gene. Fragments of 1.2 kb were amplified only from samples collected from symptomatic plants. Sequence analysis of the cloned fragments showed that the phytoplasma was similar to *Cissium* white leaf phytoplasma (Genbank acc. no. AF373106, 16SIII X-disease group), with a 100% sequence homology. This study is the first to report a phytoplasma in association with FSD in cassava.

### Detecting SSR markers associated with resistance to cassava bacterial blight (CBB) in Colombia


Four, half-sib, BC1 families of cassava (*Manihot esculenta* Crantz) were evaluated for their reaction to CBB, caused by *Xanthomonas axonopodis pv. manihotis*, under natural disease pressure in Villavicencio, Colombia. Of the four families, which shared a recurrent, resistant, male parent (M Nga19), GM 315 was chosen for its wide segregating response to CBB. To identify markers linked to CBB resistance, highly resistant (R) and susceptible (S) individuals were selected for bulked segregant analysis, and the R parent and bulks evaluated, using 486 SSR primers. Seven SSR markers detected polymorphism between R bulk/parent and S bulk, with the marker SSRY65 being putatively associated with CBB resistance genes. The potential of this marker for breeding CBB-resistant cassava is discussed.

### Interaction of *Xylella fastidiosa* with different varieties of *Nicotiana tabacum*: A comparison of colonization patterns

E. ALVES (1), S. F. Pascholati (1), E. W. Kitajima (1), and B. Leite (2). (1) Univ. of São Paulo, P.O. Box 9, CEP 13418-900, Piracicaba, SP – Brazil; (2) Univ. of Florida, 155 Research Rd., Quincy, FL 32351. Phytopathology 93:S4. Publication no. P-2003-0023-AMA.

After the indication of *Nicotiana* spp. as an experimental host for *Xylella fastidiosa* (XI), it became essential to understand how the interaction evolves and how investigational conditions alter symptomatology. Varieties TNN, Havana and RPI had their symptoms monitored and correlated with the presence of bacteria inside leaf petiole vessels. Scanning electron microscopy and image analysis were used as tools for comparisons. The colonization efficiency was established by calculating the ratio between colony forming units per gram and percentage of colonized vessels. No statistical differences were found. We also observed that leaf symptoms could be reversed with pruning, but not with mowing. Leaves with lesions on the substrate where plants were growing. In addition, pruning was effective in delaying symptom development. These results show that: colonization patterns were similar in tobacco varieties, fertilization may affect Xf symptom expression and pruning may be used as an aid to diminish XF advance.

### Relationship between leaf symptoms and the proportions of xylem-colonized vessels of plum, coffee and citrus colonized by *Xylella fastidiosa*

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Symptoms caused by *Xylella fastidiosa* (XI) in host plants are often attributed to vessel obstruction. Proportion of vessels colonized by Xf and titters of bacteria in leaf petioles of plum, coffee and citrus were correlated with symptomatology. Leaves exhibiting mild (MS) and severe (SS) symptoms were sampled from Xf-inoculated plants. Scanning electron microscopy was used to examine vessels. Bacterial titer was determined by isolation and confirmed by PCR. The percentage of Xf-infected vessels was influenced by the degree of symptom expression, host, and symptom expression*host interactions. Percentage of colonized vessels in petioles of coffee was higher than in petioles of plum and citrus whether trees were exhibiting MS or SS. Bacterial titer did not vary statistically among the different symptoms of symptoms. We consistently correlated with a higher proportion of colonized vessels in coffee and plum, but not in citrus. Citrus was the lowest colonized host.

### Pyrenophora tritici-repentis race 8 identified in North America


*Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat, produces multiple host selective toxins (HSTs), including Pt ToxA, Pt ToxB, and Pt ToxC. The specific complement of HSTs determines the race of a particular isolate. Race 8 (Lamari et al. in press) isolates, recently collected from Syria, are characterized by the production of necrotic lesions on the wheat cultivar Glenlea (ToxA) and spreading chlorotic lesions on the wheat cultivars 6B662 (ToxB) and 6B365 (ToxC). Inoculation of these host differentials with two *P. tritici-repentis* isolates designated PT82 and HV00, collected from two different fields in Kansas, resulted in the race 8 disease phenotype. These results were confirmed by PCR, Southern, and Western analyses. Thus, to the best of our knowledge, the *P. tritici-repentis* isolates PT82 and HV00 from Kansas are the first report of race 8 in North America. Identification of the races of *P. tritici-repentis* responsible for disease development on wheat cultivars grown in various regions is essential to the management of tan spot of wheat.

### Effects of cultural practices on disease in lowbush blueberry in Maine


Lowbush blueberry fields in Maine were extensively surveyed from 1999 to 2002 for stem and leaf spot diseases. More disease was found in bearing fields than in non-bearing fields in each year. Cultural practices used by growers varies greatly in lowbush blueberry production, ranging from nominal inputs to the extensive use of fertilizers, pesticides, and irrigation. The effects of some of these practices on disease were examined using reports of grower’s practices in surveyed fields and field studies studying individual inputs. Pruning method and irrigation do not have consistent significant effects on leaf spot or stem diseases. However, less stem disease was observed in burned fields than mowed fields in most years. Fertilizer treatments do not appear to have any significant effects on stem disease. Pesticide treatments applied during late bloom significantly decreased the level of leaf spot but did not significantly increase yield. Future experiments will examine the effects of cultural practices during the non-bearing year on disease symptoms in the subsequent bearing year.

### Absence of induced thermodurability in *Radopholus similis*

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Conditioning heat treatments induce thermotolerance and reduce phytotoxicity to subsequent quarantine heat treatments in vegetable, fruit, and cut flower commodities. If Radopholus similis acquires thermotolerance under conditioning treatments, the efficacy of subsequent disinfection regimes will be reduced. Screening cassava genotypes against Cassava mosaic disease (CMD), O. A. ARIYU (1,2), E. B. U. Anderson (2), B. M. Koerbler (2), A. A. O. Dixon (2), L. S. Waterman (2), and S. W. Winter (1). (1) DSMZ-Plant Virus Division, Messeweg 11/12, Braunschweig, Germany; (2) IITA, PMB 5320, PO Box 1938, Ibadan, Nigeria. Phytopathology 93:SS. Publication no. P-2003-0030-AMA.

An inoculation method viz, “biological method” has been evaluated for screening cassava germplasm for resistance to CMD. All major begomoviruses causing Cassava mosaic disease in Africa and India (ICMV) were sequenced and cloned. Infections in Nicotiana benthamiana were induced by biolistic inoculations using the Helios gene gun and by agro-inoculation. Similarly, cassava genotypes with different levels of resistance to CMD were inoculated with infectious virus clones and total DNA extracts from N. benthamiana plants infected with respective viruses. Using inoculation with EACMV-Ug/Ke (UgV) DNA extracts, 80% of the plants from highly susceptible TME 117 and the new improved cassava genotype 96/1039 became infected. Infection rates in 96/1089A, TME 3 and TME 4 were low (16%) with plants showing initial virus symptoms recovering. Similar results were obtained for EACMV. Inoculation of the resistant genotypes with ICMV in single and mixed infections with EACMV showed a severe synergism in TME 117, while the resistance status in 96/1089A, TME 3 and TME 4 was not affected. The biolistic delivery of begomovirus clones to cassava was proven but not efficient to reach a high number of infected plants compared to biolistic delivery of DNA extracts from virus-infected N. benthamiana plants.

Expression profiles of Xanthomonas axonopodis pv. citri genes associated with pathogenicity and virulence. G. ASTUA-MONGE (1), J. Fretzin-Astua (1,2), S. A. Carvalho (1), and M. A. Machado (1). (1) Lab. de Biotecologia, Centro APTA Citros Sylvio Moreira, Cordeirópolis, SP, Brazil 13490-970; (2) EMBRAPA, Cordeirópolis, SP, Brazil 13490-970. Phytopathology 93:SS. Publication no. P-2003-0032-AMA.

Citrus canker, caused by Xanthomonas axonopodis pv. citri (Xac), is one of the most devastating diseases of citrus in the world. Based on the entire genome sequence of this important plant pathogenic bacterium, we have constructed macroarrays containing 279 Xac genes potentially involved in pathogenicity and virulence. Such arrays have been used to compare the expression profiles of Xac growing in NB medium, the hrp-inducing medium XVM2, and in planta. XVM2 is known to induce hrp and avr genes in several species of the genus Xanthomonas. Data indicate that several genes were upregulated by XVM2, while only a few were repressed. Among those induced by XVM2, several avr, rrf, hrc, and secretion-associated genes were identified. A correlation between in vivo and in vitro expression profiles is also presented.

Association of a PR-10 protein in the resistance of Cornus florida to powdery mildew. F. J. AVILA (1), M. T. Mambage (1), and E. F. Howard (2). (1) Tennessee State University, Cooperative Agricultural Research Program, Nursery Crop Research Station, McMinnville; (2) Vanderbilt University, Department of Biochemistry, Nashville TN. Phytopathology 93:55. Publication no. P-2003-0033-AMA.

This study was conducted to determine if the resistant mechanism of dogwood, C. florida to powdery mildew caused by Microsphaera pulchra was associated with Pathogenesis Related (PR) proteins. Detached leaves of resistant and susceptible genotypes were inoculated with conidiospores and total protein was extracted at 0, 24, 48 or 72 h after inoculation. Proteins were analyzed using two-dimensional isoelectric focusing/SDS-polyacrylamide gel electrophoresis. Changes in protein patterns were found between inoculated and non-inoculated leaves in two of the resistant genotypes and in the susceptible genotypes. An intense protein spot (‘c’) with isoelectric point 7.5 + 0.5 and a molecular weight 18 + 2 KD detected in one of the resistant genotypes. An intense protein spot (‘c’) with isoelectric point 7.5 + 0.5 and a molecular weight 18 + 2 KD detected in one of the resistant genotypes. The expression of the ‘c’ protein was analogous to PR-10 proteins reported in other plant species. Our results suggest that the ‘c’ protein is related to PR-10 proteins and is induced during the resistance response.


The efficacy of a weather-based disease-warning system for management of sooty blotch and flyspeck (SBFS) on apples was evaluated in six apple orchards in Illinois in 2001 and 2002. This system was developed in North Carolina and Kentucky and was modified in Iowa. The second cover spray was applied to a block of 10 trees after 175 hours of leaf wetness duration after the first cover spray, while the rest of the orchard received the conventional sprays (at 10- to 14-day intervals). Leaf wetness was measured with the watchdog leaf wetness/temperature logger (Spectrum Technologies, Inc., Plainfield, IL) placed beneath the apple tree canopy. The system saved...
Antagonisms among microorganisms are strategies that maintain both inter- and intra-specific competition, which is particularly important among those microorganisms that are ecological homologues. A biocontrol bacterium, *Bacillus mojavensis*, is patented as an endophytic biocontrol agent of plant diseases. However, field use of this bacterium indicated that equally successful endophytes, such as *Fusarium verticillioides*, are superior in colonizing maize in the presence of this bacterium. It was determined that fusaric acid accounted for the reduction in bacterial growth and resulting decrease in biocontrol activity. Fusaric acid, at concentrations as low as 22 μM, accounted for a 41% reduction in the growth of this bacterium. It is also toxic to this bacterium. Fusaric acid-resistant mutants of *F. verticillioides* were ineffective in colonizing *B. mojavensis*-infected maize, suggesting that the biocontrol bacterium must be modified to resist fusaric acid before its use under field conditions.

Effect of weather conditions on the dynamics of airborne inoculum of grape powdery mildew. R. Bacon (1), B. G. Talbot (2), and O. CARISSE (1). (1) HRDC, Agriculture and Agri-Food Canada, 430 Gouin Blvd, Ste-Jean-sur-Richelieu, QC J3B 3X6, Canada; (2) Department of Biology, Sherbrooke University, 2500 Université, Sherbrooke, QC J1K 2R1, Canada. Phytopathology 93:S6. Publication no. P-2003-0039-AMA.

Grape powdery mildew, caused by *Uncinula necator*, is an important disease in many viticultural areas. Production of conidia is a key factor in disease development. Airborne conidia concentration (ACC) can be measured with spore samplers. However, the effect of the environment on the dynamics of ACC has never been modeled. In 2000, 2001 and 2002, 0.3 ha experimental plots were established on a commercial site. Spores were trapped using a 7-day continuous spore trap throughout the course of the experiment. This study revealed that ACC followed a diurnal pattern. Time series analysis showed that the weather variable most affecting ACC was the temperature on the day of sampling. A model based on degree-day was developed to describe seasonal ACC. This model could be used as an indicator to initiate airborne conidia sampling.


Significant challenges including increased trade and movement of people and the threat of bioterrorism clearly indicate the need for responsive and proactive strategies to support exotic plant pest exclusion efforts. A single repository of readily accessible pest information and an early warning system of emerging pest threats is needed. The North American Plant Protection Organization’s Phytosanitary Alert System (PAS) provides reports of significant emerging plant pests at www.pestalert.org. It is intended to facilitate awareness, detection, prevention and management of exotic species in North America. PAS also serves its users on an international level by facilitating awareness, detection, prevention and management of exotic species under field conditions. In the spring of 2002, horseradish roots collected from a commercial field were also infected with root-knot nematode. Female and eggs of nematode were extracted from the galls on the roots. The sets (roots) planted in both of the fields had been propagated in southern Missouri where root-knot is a common disease on several crops. Morphological and molecular techniques are being employed to determine the species of the nematode.

Improved disease control and yields with combinations of biocontrol and resistance inducing fertilizers applied to vegetable crops. P. A. BACMAN (1) and C. Dorman. Dept. Plant Pathology, Penn State University, Univ. Park, PA. Phytopathology 93:S6. Publication no. P-2003-0036-AMA.

Experiments were developed to determine the effects of individual and multiple small effect treatments on disease control and yield in broccoli, tomatos, and squash. Treatment used alone and in combinations included: a root inoculating/ISR-inducing biocontrol treatment (BioYield, incorporated in transplant medium), a calcium silicate fertilizer (enriches structural resistance, incorporated in transplant medium), a phosphorous acid foliar fertilizer (a probable ISR inducer), and/or an antagonistic leaf inhabiting *Bacillus cereus*. Each treatment or treatment combination was replicated 10 times to a non-treated control. Each treatment is mildew on squash, and early blight and bacterial canker on tomato were significantly suppressed by treatments that combined biocontrol and fertilizers. In broccoli and cucumber, diseases were at low levels, but combinations enhanced yields. These data indicate that combinations of several small-effect treatments can enhance disease control and yields above those achieved with individual treatments.

Sharpshooter feeding behavior in relation to inoculation of Pierce’s Disease bacterium, *Xylella fastidiosa*, in grape. E. A. BACKUS (1), F. Yan (2), and J. Habibi (2). (1) USDA-ARS, Parlier, CA 93648; (2) University of Missouri, Dept. of Entomology, Columbia, MO 65211. Phytopathology 93:S6. Publication no. P-2003-0037-AMA.

The recent introduction of the glassy-winged sharpshooter into southern California has caused a great increase in PD disease incidence in many grape-growing regions. Although host plant resistance to the bacterium and/or vector is being sought, research is hampered by lack of detailed knowledge of the transmission process. The purpose of our project is to characterize the feeding behavior of sharpshooters on grape, and to begin to identify the precise stylet activities that permit inoculation of PD bacterium during feeding. To do this, we are using electropenetration graph (EPG) monitoring of feeding, videomicrography of stylet movements, and histology of salivary sheaths. Correlation experiments show that EPG waveforms represent specific stages of stylet penetration. Inoculation experiments have narrowed the range of waveforms that could be responsible for the permissive inoculation behavior. The ultimate goal of our research is to develop a Stylet Penetration Index which could provide a rapid means of screening grape varieties for host plant resistance.


The oxidative burst (OXB) is the earliest response during establishment of plant immunity. Virulent pathogens elicit a single burst of H₂O₂ in plants. Avirulent pathogens also elicit a second burst that is associated with hypersensitive cell death and establishing downstream plant defense responses. Bacillus mycoides isolate Bac J (BmJ), a systemic resistance-inducing biological control agent, elicited a biphasic OXB in sugar beet. BmJ and avirulent strains of Erwinia carotovora pv. betavasculorum both elicited OXBS that were identical in timing, with a primary peak at 15 min and a secondary peak at 2.5 h after syringe infiltration. However, 100-fold more BmJ cells elicited 2-fold less H₂O₂ production during the second burst. Both resulted in equivalent pathogenesis-related protein production and systemic disease control of Ceratospora beticola, but only the avirulent Erwinia isolates caused hypersensitive cell death. This is the first account of a biphasic OXB being elicited by a biological control agent.


A 2000 field study investigated the inoculation period of sooty blotch and flyspeck (SBFS) fungi on apples at two locations (Pella and Gilbert) in central Iowa. Apples were enclosed in Fuji bags at the start of fruit development (mid-June). The bags were removed form groups of apples for two week intervals, then replaced for the remainder of the season. In September, bagged apples were harvested and examined for SBFS colonies. With With this method, we could determine when inoculum was present in a location. The distribution of mycelial types among locations varied. The study also provided evidence that SBFS inoculum is present throughout the season, and that it first appears earlier than expected. Differences were found in mycelial type with respect to amount of inoculation in different exposure intervals. Incidence of mycelial type was compared to rainfall, leaf wetness, and temperature events that occurred during apple exposure. The results of the study show the behavioral diversity existing among members of the disease complex.


In response to requests for more crop-specific information (e.g., turf diseases for those interested in turf careers, diseases of ornamentals for those in landscape horticulture, etc.), students in my undergraduate plant pathology course were asked to study (individually or in small groups) disease identification and management for crops important to them, and prepare a “notebook” on this information. Literature on a wide variety of diseases is available, interviews with crop specialists are arranged whenever possible, and students are encouraged to find samples and bring them to lab. Following several “checks” in the course of the semester, each student’s notebook is individually discussed and evaluated in a 30-minute interview. This approach of working with individuals and small groups is demanding of the instructor’s time, but most students respond very positively, and find it a motivating and useful project, allowing them to learn specific, customized information, and to become familiar with sources of practical information.

A pyramid of near-isogenic lines of cotton with 0, 1, 2, or 3 genes for bacterial wilt resistance. M. B. Bayles (1), M. ESSENBERG (2), and W. E. Fry (1). (1) Cornell University, Dept. of Plant Pathology, Ithaca, NY 14853; (2) Cornell University Long Island Horticulture Research & Extension Center, Riverhead, NY 11901. Phytopathology 93:57. Publication no. P-2003-0047-AMA.

Late blight has been well studied on potato and tomato, but very little is known about the disease as it affects petunia. In this study we addressed the effects of temperature and moisture on pathogen establishment, incubation period, latent period and sporulation of P. infestans on petunia as compared to tomatoes. Results show that petunia are much less susceptible than tomatoes, resulting in diminished pathogen growth and sporulation on petunia compared to tomatoes. However, the temperature and moisture optima for each process studied were similar on both hosts. Temperatures ranging from 18-23°C were generally optimal for establishment. Two hours of free moisture enabled some pathogen establishment, while most establishment occurred within 6 hours. Incubation period was shortest at 23°C and 28°C. Late period was shortest at 18°C and 23°C. Sporulation was greatest (per cm²) from 13-18°C, but declined with higher temperatures and was nearly absent at 28°C.


Endophytic Embellisia sp. isolated from locoweeds species in the genera Astragalus and Oxytropis produce the mammalian toxin swainsonine. The lack of reliable sporulation in culture has made comparison between isolates of the endophytes difficult. RAPD-PCR analysis using several primer sets were carried out on endophytes isolated from different plants and different locations. Comparison of polymorphic banding patterns show distinct groupings of these fungi. Isolates from the plant genera Astragalus differed from those isolates from Oxytropis locoweeds using several primer sets. Primers OP-2 and OP-12 have also differentiated isolates according to the location from which they were collected. These results suggest that there is variability among different isolates of endophytes depending on the locoweed species and the location from which they were collected. The findings also suggest that RAPD-PCR analysis is a reliable method for studying variability among endophyte isolates that display inconsistent sporulation in culture.


‘Paymaster 1220’ and RR/GR’ cotton plants inoculated with Agrobacterium tumefaciens isolate 25A were subjected to various temperatures, photoperiods, and fertilizers to determine optimal conditions for expression of bronze wilt. Greatest symptom severity was obtained using 450 g pasteurized sand:clay (3:1) mixture (pH 8.0), 150 mg/week of Peter’s 15:5:25 fertilizer, microelements, a 14-hour photoperiod, and temperatures of 30°C days and 25°C nights. Under these conditions plants developed bronzing and severe leaf and phloem necrosis during boll development. More than 80% of the plants suffered death of shoot terminals. Embryo development in seeds was disrupted, resulting in high percentages of light seed. Symptoms were proportional to boll load. Reducing night temperature to 20°C or the photoperiod to 12 hours significantly reduced disease severity.


Grapevine Yellows, a lethal disease of winegrapes, causes leaf yellowing, shoot dieback, fruit abortion and eventually, death of infected vines. Two strains of phytoplasmas cause grapevine yellows in Virginia. An insect vector is suspected of spreading grapevine yellows in Virginia because many phytoplasma-caused plant diseases are transmitted by phloem-feeding insects, such as leafhoppers, planthoppers or psyllids. In 2002 and 2003, we used sweep nets and yellow sticky traps to survey the populations of potential vector species in and around infected vineyards. Captured potential vectors were assayed by polymerase chain reaction (PCR) for the presence of target phytoplasmas. Because non-vector species can test positive for phytoplasmas, we performed transmission experiments with several known vector species to determine which vector species could acquire grapevine yellows. Membrane feeding trials also were performed to determine which insect species introduced target phytoplasmas into susceptible plants during feeding. Possible vector species have been identified.


Twenty-four permanent plots of *A. fraseri* (Fraser fir) with at least one disease focus caused by *P. cinnamoni* per plot were established over five counties in western NC in 1999. Plots ranging from 227 to 1070 trees each were assessed biannually for symptoms and mortality due to Phytophthora root rot over the next 3 yr. In 2002, mortality across plots was 8.9% and ranged from 2.3-19.2%. Plot aspect but not slope, tree age or annual rainfall was correlated with mortality. North and east facing plots had the greatest mortality. The monomolecular model described mortality best but neither fit disease incidence data. Only five of 22 plots had a significant effect of disease incidence over a 2 or 3 year period. Phytophthora root rot has been spreading in NC during the past several years. Factors can induce similar symptoms. Drought conditions in the region for the past several years may have limited disease development over years in the majority of the plots.


Eight-week-old rooted cuttings of Freedom Red or Angelica White poinsettia were transplanted to Fafard no. 4P potting mix with or without composted swine waste at 4 or 8% (v/v) in Apr 2002. A Pesta formulation of BNR621 or P2023, two binucleate *Rhizoctonia* fungi (BNR), was amended at 0.5% (v/v) to potting mix with or without swine compost. Poinsettias in 15-cm diam, pots were transplanted into nine rice grains colonized by *P. aphanidermatum* or *P. ultimum* either 3 days or 1 day after transplanting. Controls included untreated, mefenoxam drenched, and non-infested treatments with five replications. Severity of root rot in the infested control was similar over cultivars for the two *Pythium* spp. after 60 days. The combination of compost and BNR amendment resulted in a 30 and 36% increase in top weight for Freedom Red and Angelica White, respectively, compared to compost or BNR alone. Likewise, root rot was less in combination treatments. Apparently, the BNR fungi utilized the compost as a food base to antagonize *Pythium* and/or the effect of beneficials in the compost was additive to the effect of BNR fungi.


A new organic alternative, kaolin, is now commercially available as a potential replacement for certain insecticides that manage key apple insect pests. Kaolin is a clay that has been previously used as an insect additive in the food industry. When sprayed onto the tree, kaolin forms a white, physical barrier on the surface of the leaves and fruit (i.e., the tree turns white) which repels insect pests or makes the feeding, egg-laying, or colonization site unrecognizable and/or unsuitable. However, it appears that kaolin may have other effects on the tree. A three-year project was initiated on ‘McIntosh’ trees to determine potential non-target horticultural and disease impacts of kaolin. First year results indicate that kaolin did have a negative effect on apple scab incidence on foliage and fruit compared to a non-fungicide treated control, however, the resulting incidence was not commercially acceptable. There was no apparent interaction of kaolin with a standard fungicide program.


*Rhizoctonia solani* is an important pathogen of cowpea (*Vigna unguiculata*) worldwide and is the predominant cause of seedling damping-off. The ideal control measure would be the utilization of resistant cultivars. Single seeds of Charleston Greenpack and White Acre cowpea and Kentucky Wonder-191 were 4.9, 4.7, and 4.1, respectively. The 0.4 cm × 0.33 g treatment should be effective for evaluating cowpea germplasm for resistance to *R. solani*.


*Xylella fastidiosa* (Xf) is the xylem-limited bacterium that causes Pierce’s disease of grapevine. Detection of this pathogen prior to symptom development is critical for improved management of the pathogen. ELISA and PCR are currently used for routine detection of the pathogen; however both detection methods are limited by low titer or patchy distribution of the pathogen. In this study we found a significant effect of Xf on disease ratings for White Acre, Charleston Greenpack, and Kentucky Wonder-191 were 4.9, 4.7, and 4.1, respectively. The 0.4 cm × 0.33 g treatment should be effective for evaluating cowpea germplasm for resistance to *R. solani*.


All grapefruit grown in South Africa is cross protected to enable production of marketable fruit because of severe strains of CTV. For many years, Nartia
was the universal cross protecting strain. In the USDA Ft. Detrick quarantine facility a Nartia isolate recently collected from the original field source, a Nartia isolate maintained under protected conditions for 25 yrs, and another mild isolate, Mouton, were used as sources for single aphid transmissions using *Toxoptera citricida*. The resultant subisolates were analyzed by ELISA and RT-PCR of the coat protein gene and a region on the 5’ end of the CTV genome which was amplified by a pair of universal primers. The RT-PCR amplified products were screened by the heteroduplex mobility assay to quickly detect genotype differences. Four genotypes were identified by analyses of sequence diversity.

**Improved ELISA tests for the detection of barley yellow dwarf virus (BYDV) and cereal yellow dwarf virus (CYDV) isolates in infected plants and aphid vectors.** W. O. BLISS, K. Blum, and M. D. Bandia. Agdia Inc., 30380 CR 6, Elkhart, IN 46514. Phytopathology 93:S9. Publication no. P-2003-0058-AMA.

A group ELISA test was developed and optimized for the detection of cereal yellow dwarf virus (CYDV) and barley yellow dwarf virus (BYDV) infections in plants and aphids. This group test detects CYDV-RPV and BYDV-MAV, BYDV-PAV, BYDV-RMV and BYDV-SGV. These tests offer higher sensitivity than has been previously reported. The tests detected BYDV infections in artificially infected plant tissue that was diluted in healthy tissue up to 4000 times. Tests that specifically detect only BYDV-MAV, BYDV-PAV and BYDV-SGV were also developed from the same biomaterials. Several renowned institutions in the United States of America and in Russia provided the antibodies used in these tests. The virus specific tests do not cross react with other phenolic compounds. Phenolic compounds have been commonly observed in earlier ELISA tests for BYDV. Non-specific cross-reactivity was observed in the test for BYDV-barley. This may be due to contamination of the test solution with different plant material.

**Fungi associated with a stem disease of ararntah and pigweed weevil infestation.** J. T. BLODGETT (1,2), W. J. Swart (2), and S. vdM Louw (2). (1) USDA-Forest Service, 1730 Sanco RD, Rapid City, SD 57702; (2) Univ. of the Free State, Dept. Plant Sciences and Dept. Zoology & Entomology, Bloemfontein 9300, South Africa. Phytopathology 93:S9. Publication no. P-2003-0059-AMA.

Tissue decay in branches, stems, and root collars of *Amaranthus hybridus* was observed in plots near Bloemfontein, South Africa. Examination of stems revealed larval galleries of the pigweed weevil (*Hypoloxa haerens*). The most common fungal species isolated from discolored tissues near insect galleries was *Fusarium subglutinans* (42%); from weevil larvae was *F. subglutinans* (29%); from adult weevils was *Alternaria tenuissima* group (31%); and from cankered stems was the *A. tenuissima* group (40%). Three of the seven most common fungal species produced cankers following inoculation, with *F. sambucinum* and *F. oxyssporum* being the most aggressive. Although fungal species compositions differed (P < 0.01) among the two plant parts and the two insect stages listed above, all four had the same major pigmented species, suggesting the pigweed weevil acts as a vector for the two *Fusarium* spp. There is significant potential for disease loss affiliated with this insect-fungal association.

**Fertilization decreases resistance of red pine to the Sphaeropsis canker pathogen.** J. T. BLODGETT (1,2), P. Bonello (2), and D. A. Herms (3). (1) USDA-Forest Service, 1730 Sanco RD, Rapid City, SD 57702; and Ohio State Univ. (OSU/OARDC); (2) Dept. of Plant Pathology, Columbus, OH 43210; (3) Dept. of Entomology, Wooster, OH 44691. Phytopathology 93:S9. Publication no. P-2003-0060-AMA.

The Sphaeropsis shoot blight and canker pathogen, *Sphaeropsis sapinea*, causes extensive damage throughout the world on trees predisposed by stress. Fertilization is often recommended to increase resistance. In a controlled field study, we examined the effects of fertilization on *S. sapinea* canker development, and on induced lignification and accumulation of soluble phenolic compounds in red pine (*Pinus resinosa*). Wounded branches were inoculated with agar plugs colonized by the pathogen; noncolonized plugs were used for controls. Fertilization increased canker size (P = 0.048) and nitrogen content (P < 0.001), and decreased the C:N ratio (P < 0.001), the induction of lignin (P = 0.014), and total soluble phenolic accumulation (P = 0.004), compared with no fertilization. This suggests that fertilization decreases resistance of red pine to *S. sapinea*, and that lignin and soluble phenolic compounds may be involved in host defense.

**Multiplex real-time PCR detection of toxigenic *Fusarium* species.** B. H. BLUHM (1), M. A. Cousin (2), and C. P. Woloshuk (1). (1) Dept. of Botany and Plant Pathology; (2) Dept. of Food Science, Purdue University, West Lafayette, IN 47907. Phytopathology 93:S9. Publication no. P-2003-0061-AMA.

Several species of *Fusarium* produce mycotoxins in addition to causing diseases of cereal crops. The objective of this study was to develop a fast and sensitive assay to detect *Fusarium* species in cereal grains, and specifically distinguish *Fusarium* species that produce trichotheccenes and fumononis. Toxigenic species and their genes were amplified by PCR and fluorogenic probes were used to screen from conserved regions of rDNA, TRB6, and FUMI. Real-time PCR conditions were optimized for consistent amplification of the three products in a single, multiplex reaction. The detection limit of the multiplex assay was 5 pg of purified genomic DNA from both *F. graminearum* and *F. verticillioides*. No cross reactivity was observed with genomic DNA purified from 34 non-*Fusarium* fungal species. When applied to contaminated barley and corn samples, the assay reliably detects multiple *Fusarium* species. The speed, sensitivity and accuracy of the multiplex assay make it well suited for use by food processors as well as plant pathologists.

**Identification and characterization of pseudomonads causing basal glume rot of cereals in Russia.** V. K. BOBROVA (1), I. A. Milyutina (1), A. V. Troitsky (2), E. V. Muravieva (2), A. N. Ignatov (3), and N. W. Schaad (4). (1) Moscow State University; (2) RRI Phytopathology, Moscow, 143080; (3) Bioengineering, Moscow, 117312, Russia; (4) USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD 21702. Phytopathology 93:S9. Publication no. P-2003-0062-AMA.

Basal glume rot is a wide spread disease of cereals in Russia. It is thought to be caused by *Pseudomonas syringae* pv. atrofaciens. To confirm the identity of the causal organism, 46 strains isolated from wheat, barley, and rye, and type strain of *P. syringae* (a LMG5095) were characterized by biochemical tests (LOPAT), pathogenicity, BOX-PCR, PCR-RFLP and DNA sequencing of SyrB locus and 16s-23s ITS. The strains were identified as *P. syringae* [23], *P. cichorii* [9], or *P. tolaasi* [14] according to LOPAT results and were pathogenic on original host plants. Despite this, only 11 strains could be typed as *Psa* by PCR-RFLP and ITS sequencing. ITS of other strains showed more similarity to *P. tolaasi* (80%). For these strains, primers for SyrB resulted in a PCR product with low similarity to *SyrB* gene (<80%). BOX-PCR and PCR-RLFP analysis revealed variation within this group. The results suggest that other pseudomonads besides *Psa* cause basal glum rot in Russia.


Dispersal of citrus canker bacteria (*Xanthomonas axonopodis pv. citri*) in wind driven spray and splash was investigated in several experiments. Storm conditions were simulated using electric blowers to generate turbulent wind (c. 40-90 kph) and sprayer nozzles to simulate rain. The rain was fed into the wind stream 1 m upwind from an inoculum source of canker-infected trees. Samples were taken using panels (0.47 m2) placed 1 m downwind at 0, 0.5, 1, 3, 5, 9, 14, 19, 24, 27, 30 and 52 h. Up to 12000 bacteria ml-1 spray sampled were dispersed at 0 h. This number declined over the first 4 h and <400 bacteria ml-1 were subsequently dispersed from 5 to 52 h. Bacteria were collected at all distances sampled (1, 2, 4, 6, 8, 10 and 11 m). Wind speed ranged from 17 to 7 and 5 kph at 1, 6 and 10 m, respectively. The majority of the bacteria were recovered at 1 m (< bacterial ml-1), with an exponential decline with distance resulting in <58 bacteria ml-1 beyond 6 m. The results suggest that citrus canker bacteria are dispersed in large numbers in wind driven rain over prolonged periods of time.


Different devices (Burdark cyclone samplers, filter samplers, and rotordisks) were tested to sample airborne propagules of *A. flavus* in an irrigated area of southwest Arizona. Both cyclone and filter samplers caught propagules of *A. flavus*. Although there was no significant difference in the number of propagules caught by the cyclone (7.6-713.8 x 103 of air sampled) and filter samplers (2-1414.2 m-3) over a 2 h period, the catches were not correlated. Cyclone samplers were also operated continuously for 168 h and collected a dry sample that was ideal for plating and enumerating, characterizing fungal isolates. Rotordisks collected conidia of *A. flavus* under controlled conditions, but failed to collect *A. flavus* in the field. Rotordisks did catch propagules of other fungi in the field, but the rotordisks became overloaded with dust particles if operated for more than 2 h. Where isolate culture and characterization is required cyclone samplers are ideal for long-term
monitoring of air borne propagules of A. flavus. Cyclone and filter samplers can also be used for sampling up to a few hours.


Stem damage on winter wheat due to the eyespot pathogens (Tapesia yallundae, TY and T. acuformis, TA) causes yield loss in some locations and years in the UK. The timing and development of stem infection for each species is not fully characterized. Using data from field experiments at Rothamsted, England (1986/87, 1987/88) thermal time (accumulated degree-days) was related to the progress of TY and TA on stems. In both years the severity of stem lesioning was positively correlated with thermal time. In 1986/87 there was no significant difference in severity between TY and TA. In both seasons the predominant species isolated was initially TY but TA became predominant later in the season, indicating that TY had infected the stem earlier. A controlled environment experiment showed no difference between stem incidence or severity of lesions between TY and TA when stems were inoculated directly. Thus TY may infect stem earlier than TA, indicating events in the leaf sheaths (or leaf sheath/stem interface), such as time of infection or growth rate may differ between TY and TA.

**Survival of Tilletia indica teliospores in soils.** M. R. BONDE (1), D. K. Berner (1), S. E. Nester (1), G. L. Peterson (1), M. W. Olsen (2), B. M. Cunfer (3), and T. Sim IV (4). (1) USDA-ARS, Ft. Detrick, MD 21702; (2) Univ. of Arizona; (3) Univ. of Georgia; (4) Kansas Dep't of Agriculture. Phytopathology 93:S10. Publication no. P-2003-0066-AMA.

Karnal bunt of wheat, incited by *T. indica*, typically results in small reductions in grain quality and yield. However, because of its international quarantine status, it has the potential to cause large economic losses to wheat-exporting countries. To determine the potential for *T. indica* to survive in the U.S., a teliospore longevity study was initiated in Kansas, Maryland, Georgia and Arizona field plots. Soil from each plot was artificially infested and placed in mesh bags, and bags placed in the respective soils within special PVC pipes. The pipes then were buried vertically in the plots. Bags were also placed outside pipes in Arizona, and infested soil from each plot and pure spores were maintained dry in a laboratory. During the first 2 years, viability declined more rapidly in the field in pipes than outside pipes, and more rapidly in fields in Kansas and Maryland than Georgia or Arizona. In the laboratory over 3 years, viability decreased more rapidly in dry soil from Kansas and Maryland than Georgia or Arizona, while pure spores remained the same.


Early leaf spot reductions in peanut-maize intercrops may be due to effects on dispersal or infection phases in the life cycle of the causal agent, Cercospora arachidicola. Infection-related mechanisms were evaluated in 11 × 11 m plots near Asheville, NC in 3 experiments in 2000 and 2 in 2001, arranged in a randomized complete block design with 3 blocks and 5 treatments: peanut monocrop, strip intercrop (4:4 peanut and maize rows, respectively), and patterns of 1:1, 2:1, and 3:1. In each experiment, 5 randomly-placed peanut plants were inoculated with *C. arachidicola* conidia in each plot and evaluated for disease severity after approximately 21 days. Severity was not affected by intercropping in the third inoculation of 2000 nor in the 2001 trials. Where an effect was seen in 2000 (*P < 0.01*), severity was highest in strip intercrops and lowest in the 1:1 intercrop, with monocrops intermediate. The results suggest that alterations in the infection phase of the pathogen’s life cycle do not have a major role in overall disease reductions seen in other work.


Field grown tomato plants were treated with water or Oxycorn, which contains salicylic acid (SA) and a hydrogen peroxide generator. Treatments began 48 hours before transplantation of seedlings into the field and continued every 14 days through out the growing season. Young leaves were harvested 2 days and 10 days after treatments for quantification of defense gene expression using probes for PR1a and a peroxidase. Effects of the treatments on plant performance were evaluated by monitoring growth, fruit production, and disease. Potential effects on insect visitations were measured by weekly visual inspections during the growing season. SA treatments were found to have no significant detrimental effect on fruit yield. It was observed that SA treatments significantly reduced the time to first fruit. Insect visitations to tomato treated with SA were found to be significantly lower for insects with sucking/piercing mouthparts. Results from northern blots for PR1a and peroxidase transcription accumulation revealed variable effects of the SA treatments on defense gene expression at the time points measured.


Hot dry conditions favor aflatoxin development and accumulation in peanut. Drought periods during the last 3-4 weeks before harvest are especially conducive to the accumulation of aflatoxins. Other field conditions or factors may increase the risk of aflatoxin contamination to a lesser extent. Reduced soil calcium levels, for example, have been shown to increase the risk of contamination. A number of factors were characterized in peanut production fields, including soil calcium and other soil nutrient levels, soil type, slope, weed competition, and populations of nematodes in soil samples. Pod samples collected within 2 weeks of inversion were assayed for aflatoxin levels. Spearman correlation coefficients were calculated between aflatoxin levels and data on other characteristics. Aflatoxin contamination was positively correlated to soil calcium and nematode numbers, but only significant (*P < 0.10*) for nematode numbers.


Phytophthora capsici is a major pathogen of economical crops in both temperate and tropical climates worldwide. Isolates that are pathogenic on tropical crops have been described as *P. tropicalis* through morphometric analysis. The research objective was to determine if isolates of *P. capsici* causing black pod on cacao in Brazil were genetically similar to isolates causing Phytophthora blight on peppers in the U.S. AFLP analysis was used to evaluate genetic diversity. Cluster analysis of AFLP results identified two distinct clusters, consisting of either all temperate isolates or all tropical isolates. PCR analysis with PCAP and ITS1 primers and subsequent restriction of the amplicon with *MspI* also differentiated temperate and tropical isolates. Sequence analysis of the PCR product revealed a 166-bp segment with a single base change at the restriction site. Results support the hypothesis of separate populations within the *P. capsici/P. tropicalis* group. Additional data on mitochondrial gene phylogenies will be presented.


The spruce-fir ecosystem throughout the Southern Appalachians has been known to be in decline for the last 40 years, with recent decline rates believed to be increasing. Extensive surveys of disturbances in high elevation red spruce (*Picea rubens* Sarg.) and Fraser fir (*Abies fraseri* Pursh) forests exist, however recent quantifiable research is lacking. In 2002, 19 spruce-fir forest plots established in the Black Msx. of North Carolina during the 1980’s were resurveyed to observe changes in forest structure parameters and tree mortality. Results show an overall increase in live basal area and stem density at all elevations for spruce and fir populations. Data suggests vigorous regeneration is occurring at many sites, especially in areas of former severe mortality. High rates of decline in crown condition continue to manifest in survivors of previous surveys. Causal factors behind the current trend in decline remain unknown; no correlation to balsam wooly adelgid (*Adelges piceae* Rathbrug) or other biotic diseases was found.

Flax (Linum usitatissimum) production in North Dakota (ND) increased from 32,375 ha in 1996 to almost 300,000 ha in 2002. Pasmo disease, caused by Septoria liniola, reduces flax yield in the Canadian provinces of Manitoba and Saskatchewan, but little is known about its distribution and effect on yield in ND. A field survey for pasmo was conducted in 74 flax fields across 19 ND counties in August 2002. The survey indicated that pasmo was present in 17 of 19 counties surveyed, and county mean incidences ranged from 0 to 21%. Two field trials were conducted in 2002 at Langdon, ND to evaluate fungicides for pasmo control. The fungicides azoxystrobin, chlorothalonil, mancozeb, and pyraclostrobin reduced the progression of leaf necrosis compared to the untreated control in one trial, but fungicides had no effect in the other trial. Fungicides did not affect yield.

**Validation of fire blight prediction systems for Georgia apple production.** M. P. BRANNEN (1) and J. Garner (2). (1) Dept. Plant Path., Univ. of Georgia, Athens, GA 30602; (2) Mountain Research and Education Center, Blairsville, GA 30512. Phytopathology 93:S11. Publication no. P-2003-0073-AMA.

Fire blight, caused by Erwinia amylovora, is a major disease of apples in Georgia. Blossom infections are highly correlated to temperature and moisture conditions during bloom. Predictive systems, such as MaryBlyt (a computerized system) and Cougar Blight (a non-computerized system), have been developed to time application of antibiotics for fire blight control. While these systems have been tested elsewhere, they had not been validated in Georgia, where conditions are often ideal for fire blight development. Both MaryBlyt and Cougar Blight were tested for two years on a Rome Beauty variety. In 2001, 93 antibiotic applications were applied to the conventionally protected plots, while each of the predictive systems was utilized seven applications. In 2002, eleven applications were applied with conventional and Cougar Blight programs, while MaryBlyt called for ten applications. Disease control was equivalent for all programs in both years. Following validation, both predictive systems are now being utilized by county extension personnel in the apple-producing region of Georgia.


Fire blight, caused by Erwinia amylovora, affects members of the Rosaceae including apple (Malus domestica Borkh.) and raspberry (Rubus idaeus L.). In apple, it has been postulated that sorbitol is both required for pathogenesis and conversely restricts disease development. Interestingly, raspberry does not produce sorbitol. In this study, we examined differences in raspberry and apple isolates of *E. amylovora*. We confirmed earlier reports that raspberry isolates do not cause fire blight in apple while apple isolates cause restricted fire blight symptoms in raspberry. Apple and raspberry isolates grew equally well with sucrose, but with sorbitol, raspberry isolates grew more slowly than did the apple isolates. SDS-PAGE profiles of extracellular proteins and polysaccharides of raspberry and raspberry isolates grown with sucrose or sorbitol were similar within isolates but different between apple and raspberry isolates regardless of the carbon source. We speculate that the differences in protein and polysaccharide profiles represent differences in pathogenicity factors which delimit the host range of *E. amylovora isolates*.


The IR-4 Project was established in 1963 to provide pest management solutions to growers of fruits, vegetables, and other minor crops. IR-4 became involved in the registration of Bt products in 1970 and in 1982, a biological control program was established. The primary functions of the Biopesticide Program are to assist registrants with the regulatory process and to promote the development of new products by funding research through a competitive grants program. The registration assistance involves consulting and petition preparation for submission to the Biopesticides and Pollution Prevention Division (BPDD) of EPA. The IR-4 Project also is involved in the submission of Experimental Use Permits (EUP=s) for products prior to complete registration. IR-4 has worked with 45 different companies and industry organizations (such as the Biopesticide Industry Alliance or BPIA) and is involved in partnerships with EPA the Pest Management Regulatory Agency (PMRA Canada) and the California Department of Pesticide Regulation (CDPR). Since 1995, the IR-4 Project has funded over 2 million dollars in biopesticide research with $427,000 in 2003 alone. In 2003, The IR-4 Project funded 48 research projects out of 108 requests. Out of the projects funded, 27 were biofungicides, 4 were bioinsecticides, 4 pheromones, 8 bioceramicides and 8 were plant growth regulators. Projects included food use projects and non-food use (turf, ornamentals, greenhouse, etc.).


**Fusarium oxysporum, F. solani, and Gaumannomyces graminis var. tritici (Ggt), are soilborne pathogens. Decomposing Brassica spp. release glucosinolates which inhibit microorganisms. Our objectives were to evaluate Brassica spp. for fungistic and fungicidal activity against Fusarium and Ggt, and to identify glucosinolates involved in inhibition. The test was a factorial with 6 fungal isolates (3 of Ggt, 1 each of F. solani, F. oxysporum, and F. graminarum) and 6 mulches (B. juncea ‘Indian Mustard’ mulch, B. juncea ‘Indian Mustard’ meal, B. napus ‘Dwarf Essex Rape’, B. napus canola, wheat, and no mulch) in an RCB with 3 replicates. Plant tissue was placed in jars covered by inverted Petri dishes containing potato dextrose agar with a fungal spore suspension. Colony diameters were measured three times and the mean calculated. Growth of all fungi was inhibited by *B. napus* treatments and wheat mulch, but inhibition was fungistic. Inhibition was fungicidal with *B. juncea* mulch and meal, and no growth was recorded. Identification of glucosinolates from ‘Indian mustard’ responsible for fungicidal activity will be discussed.**


Four applications of tebuconazole (0.23 kg/ha) were applied to peanut cv. Georgia Green by either airplane or conventional ground equipment in a total volume of 28 to 47 or 94 to 112 l/ha, respectively, in five trials. Air and ground sprayers were to be believed equal in one day to the day of spray, and there were up to four times in greater yield. At harvest, there were no differences between treatments in levels of leaf spot (*Cercospora arachidochila*) or stem rot (*Sclerotium rolfsii*) in four of the trials where disease pressure was low. Pod yields were consistently high (4630 to 6006 kg/ha) and similar between treatments except one of the four tests where plots sprayed by air had higher yields. At the fifth site, both diseases were severe. Disease control was superior for the ground-sprayed plots and pod yields were higher. Aerial applications are convenient and generally effective, but where disease pressure is intense, at least some applications by ground may be advisable.


Sustainable control of Rhizoctonia disease of potato, which reduces tuber yield and quality, was evaluated using rotation crops and biocontrol. Potatoes treated with *Lactarisar arvallis, Trichoderma virens*, or *Bacillus subtilis* were planted as subplots following rotation crops of barley and ryegrass, barley and clover, or potato at two locations in Maine. Treatment effects on disease levels, tuber yield, and soil microbial communities were determined. The ryegrass rotation significantly reduced stem canker severity and increased tuber yield. Fungicidal treatments varied by rotation and location, with *L. arvallis and T. virens* reducing black scarf severity and incidence in some rotations. Fatty acid profiles and soil digestion plating demonstrated distinct differences in microbial community characteristics among rotation crops and biocontrol treatments. Significant crop and biocontrol interactions were observed indicating that biocontrol can be enhanced within beneficial rotations, leading to greater reductions of Rhizoctonia disease of potato.


The biocontrol agent *Pseudomonas fluorescens* PF-5 produces a suite of antibiotics that antagonize seed- and root-rotting plant pathogens. These antibiotics include pyoluteorin (PLT), pyrrolnitrin (PRN) and 2,4-diacylphloroglucinol (PHL). Using HPLC and transcriptional fusions to assess Pf-5 expression in *Pseudomonas fluorescens* strain 97330, we found that PLT and PHL each undergo positive autoregulation while repressing the other's production. Antibiotics include pyoluteorin (PLT), pyrrolnitrin (PRN) and 2,4-diacetylphloroglucinol (PHL). Using HPLC and transcriptional fusions to assess Pf-5 expression in *Pseudomonas fluorescens* strain 97330, we found that PLT and PHL each underwent positive autoregulation while repressing the other’s production. This phenomenon occurred i) at low (nanomolar) concentrations for both compounds, and ii) at the transcriptional level, for PLT. PRN amendments also repressed PLT biosynthetic gene transcription. We further characterized...
the potential signaling role of PLT in Pf-5 by demonstrating PLT autoinduction in vivo, with cross-feeding experiments on seeds and roots in pasteurized soil. Our experiments suggest i) crosstalk among antibiotics of host-related groups of the conifer shoot blight pathogen conigenus Pasteurized soil. Our experiments suggest i) crosstalk among antibiotics of autoinduction the T group (mostly from conigenus diameter growth and morphology on potato dextrose agar were compared for six isolates. Isolates of the P group (mostly from Picea and Pinus hosts) tended to grow more rapidly and were substantially different in colony color and texture than isolates of the T group (mostly from Tsuga). Conidia of the P group isolates tended to be longer and had greater ratios of length to width than those of T group isolates. Morphological differences support separation of these groups, which has implications for those involved in diagnosis, management, or research of the serious diseases these fungi can cause.


Comparative analysis of genomes provides opportunities to understand functional differences and evolutionarily conserved features between organisms. Past comparative genomic approaches have handled small data sets that are notional differences and evolutionarily conserved features between organisms. Past comparative genomic approaches have handled small data sets that are put comparative analysis places large demands on computing resources. De!CIFR is being developed to handle multi-species comparative and functional genomic analysis of genomes using computational grids. The system has been designed to fulfill the need of basic genome annotation, high volume analysis and data mining as well as providing a framework for analyzing partially sequenced genomes in the context of well characterized genomes. The software foundation was derived from the EnsEMBL core system has been designed to fulfill the need of basic genome annotation, through 4 generations. Seed from S4 lines were investigated for aflatoxin accumulation using a laboratory assay. Lines with the same parental background but differing in aflatoxin levels (near-isogenic) were then subjected to proteome analysis to identify constitutively-expressed proteins associated with resistance. Previous work has shown enhanced constitutive protein expression to be a differentiating factor between resistance and susceptibility. Results of these investigations will be discussed.

Determining causes and control measures for an almond replant disease. G. T. BROWNE (1), J. H. Connell (2), and S. T. McLaughlin (1). (1) USDA-ARS, Dept. of Plant Pathology, Univ. of Calif., Davis, CA 95616; (2) UCCE, Orovil, CA 95965. Phytopathology 93:S12. Publication no. P-2003-0084-AMA.

Almond orchards replanted without pre-plant fumigation at old almond sites have occasionally failed to establish in Northern California. The replant disease (RD) is associated with necrosis of developing roots on Marianna 2624 rootstock (MR, not graft compatible with all almond cultivars). In field trials, incidence of severe RD on MR was prevented by pre-plant broadcast soil injection with chloropicrin (Pc) or tree site injection with Pc, 1,3-dichloropropene (1,3-D), or methyl bromide (MB)(Pc:75:25); but MB or 1,3-D broadcast shank injections were ineffective. French prune on MR (known graft compatible) was highly susceptible to RD as was almond on MR. Agra production was assessed on mycelial plugs - transmissible. Apparently, MR is susceptible to RD, but not because of its marginal compatibility with almond. In greenhouse tests, soil treatment with fludioxonil fungicide or autoclaving prevented RD symptoms, but difenoconazole, mefenoxam, and chlorothalonil + streptomycin had no measurable effect on the disease. Determinations of fungal and bacterial roles in RD are underway.


Phytophthora ramorum has emerged as a new, lethal pathogen on oaks in the U.S. Further, numerous cultivated and wild plants support growth of this pathogen. Little is known about the environmental parameters which influence infection, dissemination, and the influence of inoculation temperatures and illumination regimes on growth and sporulation at two research facilities. Cultures were incubated on clarified V-8 agar with sterol, at temperatures ranging from 4-30°C, under 12-hr diurnal light (cool white and near-UV) or darkness. Colony diameters were measured periodically, and number of chlamydospores were counted after 14 days. Mycelial growth was assessed on mycelial plugs - transmissible to autoclaved soil extract and subjected to various temperatures and light regimes. Results with American isolates of P. ramorum, from Lithocarpus densiflorus, Quercus agrifolia, and Rhododendron sp. were compared with those obtained using the type culture isolate (Germany).


Armillaria species are responsible for significant losses of woody plants worldwide, including North America, where ten pathogenic species have been identified. Very little is known about the inter- and intra-species genetic diversity in these fungi. The internal transcribed spacer regions 1 and 2 from ten pathogenic North American Armillaria species. Amplification products were cloned, and the ITS1-5.8S-ITS2 region from six clones were sequenced. Our results indicate that most isolates were heterozygous. Surprisingly, some diploid isolates had more than two alleles, indicating that the mechanism of concerted evolution does not seem fully operational. Because concerted evolution occurs most frequently during sexual recombination events, our results indicate that such events are rare in Armillaria species. Implications of these findings will be discussed.


Myxobacteria are soil dwelling gram-negative gliding bacteria, which produce resistant myxospores. They produce a wide range of antibiotics and lytic enzymes which are used to prey on microorganisms. Although they seem to be ubiquitous in soil, their role in agroecosystems is unknown. In order to understand what role myxobacteria play in regulation of bacterial plant pathogen populations in soil, the relative sensitivity of bacterial plant
pathogens to myxobacterial lysis in vitro was evaluated. Plant pathogens including Pseudomonas syringae pv. syringae, P. s. pv. alisalensis, P. s. pv. tomato, Xanthomonas campestris pv. vitians and Erwinia caratovora subsp. caratovora, were lysed to the same extent as Escherichia coli. As comparisons, lysis of bacterial biological control agents was evaluated. Most biological control agents tested were not significantly lysed. However, Erwinia herbicola C91 was lysed to the same extent as E. coli. Lysis of these organisms in soil is currently being evaluated. The extent to which different myxobacteria lyse plant pathogens was also evaluated in vitro. Myxobacteria collected from agricultural soils differed in the extent to which they lysed foliar plant pathogens.


Aspergillus ear rot of corn (Zea mays L.) produced by Aspergillus flavus Link:Fr is of economic concern due to the production of aflatoxin B1. Our objectives were to determine the usefulness of Oh516 as a source of resistance to Aspergillus ear rot and aflatoxin production by determining the types of gene action for low levels of bright greenish yellow fluorescence (BGYF) aflatoxin, and ear rot. We have hypothesized that further inoculating lines based on low levels of BGYF as a method to indirectly reduce the concentration of aflatoxin in grain. In 2001 and 2002, grain from the resistant inbred Oh516 (P1), the susceptible inbred B73 (P2), and the F1, F2, F3, BC1P, BC1P2, and BC2P2S1 generations were evaluated for severity of Aspergillus ear rot, aflatoxin production, and levels of BGYF following inoculation. Dominance is important for low levels of BGYF and low concentrations of aflatoxin in grain. The correlation coefficients between aflatoxin concentration and BGYF in 2001 and 2002 were \( r = 0.67 \) and \( r = 0.57 \). The F1 generation had significantly lower toxin values than the average of the two parents also indicating dominant gene action for resistance.


Polymyxa graminis, a flagellated protozoan, is presumed to vector Wheat streak streak mosaic virus (WSSMV) as well as numerous other soilborne viruses of grasses. In this study, we baited potential vectors from WSSMV-infected dried roots and found that two ciliated protozoans, Colpoda cucullus and Colpoda steineii, harbor WSSMV internally. Direct sequencing of RT-PCR-amplified WSSMV RNA from six-month old monoxenic subcultures of Colpoda revealed that WSSMV replicates in Colpoda. Furthermore, preliminary confocal microscopy of immunostained Colpoda cultures detected the presence of both structural and non-structural viral proteins within the protozoan. To our knowledge, no ciliated protozoan has ever been shown to parasitize plants, making Colpoda a mysterious player in the disease cycle of WSSMV and potentially other soilborne viruses.


Major gene resistance is the most effective tactic for managing Wheat streak streak mosaic virus (WSSMV) and Wheat soilborne mosaic virus (WSBMV) on small grains. However, assessing resistance to WSSMV and WSBMV is complicated by disease gradients within field plots, the long periods between transmission in autumn to symptom expression in spring, and the statistical interaction between disease incidence and planting date for individual genotypes. Choosing the proper tools and techniques for detecting incidence depends on the mechanism of resistance and the correlation between dominance in resistance to symptoms. In addition to describing our obstacles and strategies in resistance assessment, we describe a novel, facile statistical analysis for objectively determining relative susceptibility based on logistic regression.


Assessments were made for vegetative compatibility of 55 isolates of Cercospora kikuchii from three fields in Louisiana and three isolates from other locations. Nitrilotriazone (nit) mutants were generated from chlorate medium. One nitl and one NitM were chosen from each isolate, and these were paired with each other in all possible combinations. Fifty-six isolates were self-compatible, of which 16 were assigned to six vegetative compatibility groups (VCGs). The other 40 isolates were compatible only with themselves. Of the six VCGs, three included isolates from different locations. Only one VCG included isolates from both soybean leaves and seeds, while two and three VCGs included isolates only from leaves and seeds, respectively. This suggests that populations of C. kikuchii from soybean leaves and seeds are genetically different.


Nineteen isolates of Cercospora kikuchii were used to evaluate six commercial soybean cultivars. Hornbeck 5588, Asgrow 5701, DeltaPine 5806RR, Terral TV59R85, SSRT 6299N, and DeltaPine 6880, for resistance to Cercospora leaf blight. Soybean plants at the V2-V3 stage were atomized with spore suspensions (200,000/ml) and grown for 1 week in moist chambers built on greenhouse benches with transparent plastic sheeting. Moist chambers were opened for about 6 hours every day around noon. Leaves that emerged after inoculation, were removed every 3-5 days so that PAI nucleated leaves were exposed to direct sunlight. Disease severity was examined 4 weeks after inoculation. Resistant reactions were those in which symptoms covered one percent or less of leaf area. Asgrow 5701 and Terral TV59R85 were resistant to three and two isolates, respectively. However, these cultivars were not completely resistant to any isolate. Isolates from soybean leaves and seeds caused comparable levels of disease on these cultivars.


Streptomyces turgidiscabies, S. scabiei and S. acidiscabies are gram-positive filamentous bacteria that produce thaxtomin, a phytotoxin, and cause potato scab. The genes encoding the thaxtinom biosynthetic pathway are located on a mobile pathogenicity island (PAI), the PAI is horizontally transferable in vitro from S. turgidiscabies to the nonpathogen S. lividans. Here we present the genetic organization of a portion of the greater than 400 kb PAI in S. turgidiscabies. Open reading frames (ORFs) have been identified using third position codon bias and putative functions have been assigned based on homology to known proteins. Thaxtinom biosynthetic proteins and Necl1, a secreted virulence protein, are encoded at opposite ends of the PAI. Other ORFs include the fad operon from Rhodovococcus fascians and a gene with homology to the tomatinase protein in Fusarium oxysporum. There are other ORFs with putative functions consistent with a PAI, including transposable elements, ABC transporters, and transcriptional regulators. Characterization of putative pathogenicity-related ORFs is ongoing.


The H1 gene conveys chlorotic lesion resistance against avirulent races of E. turcicum, which causes northern corn leaf blight (NCLB). Four populations of sweet corn with different frequencies of H1 were selected for general resistance to E. turcicum race 1 which is virulent against H1. Each population was advanced from cycle 0 to 5 by mass selection with parental control. At least 600 plants of each cycle were inoculated; and the next cycle population was advanced from cycle 0 to 5 by mass selection with parental control. At least 600 plants of each cycle were inoculated; and the next cycle population was advanced from cycle 0 to 5 by mass selection with parental control. At least 600 plants of each cycle were inoculated; and the next cycle population was advanced from cycle 0 to 5 by mass selection with parental control. At least 600 plants of each cycle were inoculated; and the next cycle population was advanced from cycle 0 to 5 by mass selection with parental control.


Coconut coir dust, a waste product from coconut fiber processing, has been shown to be a good alternative to peat in growing media.
experiments were conducted to determine if coconut coir dust can also suppress soilborne plant pathogens by using tomato damping-off caused by *Phytophthora capsici, Phytophthora nicotianae, Pythium aphanidermatum*, and *Pythium ultimum* as model pathosystems. Tomato seeds cv. ‘Bonnie Best’ were infested and grown on media 5 x 5, 2.5-ml plastic plug trays. Growing media were infested with two-week-old cultures of the pathogens in V8 juice-peat medium at the rate of 5 × 5, 2.5-ml plastic plug trays. Growing media were infested with two- and five-pathogen mixtures. The isolates showed a similar growth trend with respect to temperature peaking at 25°C. Distinct polymorphic differences were found among the isolates based on the RAPD-PCR banding patterns. Effects of amending soil with cover crop, compost, and manure on soil microbial community during the growing season on field grown tomatoes. L. M. CARRERA, J. S. Buyer, A. A. Abdul-Baki, L. J. Sikora, B. T. Vinyard, and J. R. Teasdale. United States Department of Agriculture, Agricultural Research Service, Beltsville, MD. Phytopathology 93:S14. Publication no. P-2003-0099-AMA.

Sustainable production systems aim to improve soil quality by use of soil amendments and cover crops. Characterization of the soil changes triggered by the use of such amendments and cover crops on a soil microbial community was investigated in a tomato (Lycopersicon esculentum Mill.) field production system that utilized poultry manure compost, poultry manure, and hairy vetch (Vicia villosa Roth.) cover crop as nitrogen sources. Field plots were located at the Agricultural Research Center, Beltsville, MD. A randomized complete block design experiment with 4 replications was used. Treatments consisted of each of 4 mixtures (1) control, (2) control + poultry manure compost (10 t/ha), (3) amended with compost-HV mixture of HV and poultry manure compost (10 t/ha), and (4) control + poultry manure compost (5, 10, 20 t/ha), and two levels of poultry manure (2.5, and 5 t/ha). Soil samples were taken at 5 different times during the tomato-growing season. Communities were characterized by soil fatty acid analysis. Data were analyzed using canonical variates MANOVA analysis. Significant seasonal variations in microbial community structure were found, suggesting effects of the tomato and poultry manure. Fungi were strongly affected by the treatments, but Eubacteria, Gram-positive bacteria, and actinomycetes populations were not affected. The microbial community in the soil amended with compost-HV mixture was significantly different from the other treatments. These data showed significant effects of soil amendments to the soil microbial communities. Demonstrate the effectiveness of an IPM program for the management of leaf blight diseases of carrots in New York. J. E. CARROLL, P. Chen, and G. S. Abawi. Dept. of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456. Phytopathology 93:S14. Publication no. P-2003-0100-AMA.

Leaf blight diseases caused by *Alternaria dauci* and *Cercospora carota* are of common occurrence on carrots, impacting production costs and yield. Results obtained from experimental plots over several years have confirmed the utility of the Canadian Alternaria blight threshold of 25% disease incidence as triggering the first fungicide spray, with subsequent sprays timed according to conducive weather and disease progress. Results have also demonstrated that carrot varieties differ considerably in their susceptibility to each of the two pathogens. In 2001 and 2002, large-scale validation of an IPM program based on the 25% threshold level and varietal reaction was conducted in collaboration with carrot growers. A total of 26 fields, each planted to one to four cultivars, were sampled weekly by variety and a copy of the scouting report was given to the grower. This management strategy was effective against both diseases and consistently delayed the first fungicide spray, resulting in as much as 50% reduction in fungicide applications while maintaining plant health and yield. Role of *Phomopsis vaccinii* in upright dieback of cranberry. N. J. CATLIN and F. L. Caruso. University of Massachusetts, Cranberry Station, East Wareham, MA 02538. Phytopathology 93:S14. Publication no. P-2003-0101-AMA.

Upright dieback disease of cranberry (Vaccinium macrocarpon) has been reported from all areas of cranberry cultivation and is commonly a recurrent problem in the cranberry beds in which it is present. The typical symptom is tip dieback of the cranberry upright, or stem, that results in a pattern of diseased uprights dispersed among healthy uprights. An estimated 25 percent of uprights will show symptoms across severely affected areas. *Phomopsis vaccinii* has been assumed to be a causal agent due to its frequent recovery from diseased uprights and its role in twig blight and canker diseases of blueberry (Vaccinium corymbosum). Koch’s Postulates have been successfully completed for *P. vaccinii* on tissue-cultured cv. ‘Early Black’ and cv. ‘Stevens’ cranberry plants in the lab and on rooted cuttings of the same cultivars in the greenhouse. Symptom development, mode of infection, and infection courts will be discussed. Development of an integrated management strategy for Angular leaf spot (*Phaeosoropsis grisela*) on snap beans in Ontario, Canada. M. J. CELETTI (1), M. S. Melzer (2), and G. J. Boland (2). (1) Ontario Ministry of Agriculture and Food, University of Guelph, Guelph, Ontario; (2) Department of Environmental Biology, University of Guelph, Guelph, Ontario. Phytopathology 93:S14. Publication no. P-2003-0102-AMA.

Angular leaf spot (ALS), caused by *Phaeosoropsis grisela*, was confirmed in several fields of snap bean (*Phaseolus vulgaris L*) in Ontario, Canada during 2000. Pathogen survival, variety response and fungicide efficacy studies were conducted to develop a strategy for managing ALS in Ontario.
To determine if the pathogen can overwinter in Ontario, diseased pods and leaves were air-dried and placed on the soil surface or buried 5 and 25 cm below the soil surface in the fall of 2001. Diseased plant material was retrieved the following spring and fall, washed, incubated at high relative humidity, and macerated with a toothpick on the surface of 20-day-old snap bean plants var. Gold Mine. ALS lesions developed on plants sprayed with diseased leaf material retrieved from the soil surface, indicating P. griseola can overwinter in Ontario. Sixteen varieties of snap beans were susceptible to P. griseola in two field and three growth room studies, however, some varieties had fewer or smaller lesions on leaves or pods and appeared less susceptible than others. Pyraclostrobin (100 g.a.i/ha), thiamethoxam (55 g.a.i/ha) and vinclozolin (750 g.a.i/ha) were applied to snap beans var. Strike at the 10-30% or 50-70% bloom, or both, to determine the timing and efficacy of these fungicides for disease control. Vinclozolin did not significantly reduce the severity or incidence of ALS compared to the untreated check. However, pyraclostrobin and thiamethoxam reduced both disease incidence and severity. Results suggest that ALS could be managed in Ontario by growing the least susceptible varieties, applying efficacious registered fungicides, and deep plowing infested crop residue.


Fusarium species have been causing major world-wide cereal crop diseases such as corn ear rot and Fusarium head blight, resulting in tremendous economic loss to producers. The use of microbial antagonists of Fusarium is a sustainable approach to attenuate disease damage by suppressing the pathogen. Of the bacterial antagonists, diverse Bacillus sp. strains are often known to be active against many soil-borne fungal and bacterial diseases by antibiotic. Soil bacteria were isolated and screened for antagonistic activity against F. graminearum. One of these, D1/2, showed high in vitro activity against its mycelial growth and that of several other common fungal phytopathogens. Comparative rDNA sequence analysis identified it as a strain of B. subtilis. Its cell-free culture filtrate at different concentrations inhibited macroconidium germination and hyphal growth of F. graminearum in liquid medium. Under these conditions, the culture filtrate induced swollen hyphal cell formation in the fungus. Inoculating alfalfa seedlings with D1/2 reduced them from damping-off fungus disease when challenged with F. graminearum macroconidia. The isolate, designated B. subtilis str. D1/2, is therefore applicable to controlling plant diseases caused by Fusarium species.


Nitric oxide (NO) is an important defense signal both in plants and animals. Here we report the identification of a nitric oxide synthase like enzyme in plants, which is responsible for the dramatic increase in NO production following pathogen infection of resistant but not susceptible tobacco and Arabidopsis. The protein was purified ~33,000 fold and identified by mass spectrometry. Its sequence corresponds to a variant form of the P protein of the glycine decarboxylase complex (GDC). Proof that the variant P is the pathogen-inducible NO synthase (INOS) of plants was provided by the demonstration that inhibitors of the P protein of GDC blocked NO synthesis, and that the Arabidopsis variant P produced in E. coli and that of several other common fungal phytopathogens. Comparative rDNA sequence analysis identified it as a strain of B. subtilis. Its cell-free culture filtrate at different concentrations inhibited macroconidium germination and hyphal growth of F. graminearum in liquid medium. Under these conditions, the culture filtrate induced swollen hyphal cell formation in the fungus. Inoculating alfalfa seedlings with D1/2 reduced them from damping-off fungus disease when challenged with F. graminearum macroconidia. The isolate, designated B. subtilis str. D1/2, is therefore applicable to controlling plant diseases caused by Fusarium species.


Tobacco mild green mosaic tobamovirus (TMGMV) causes a lethal hypersensitive reaction in tropical soda apple (TSA) and is considered a potential bioherbicide for this noxious weed. To assess its nontarget risks, TSA species in 41 families were screened for susceptibility to TMGMV. Symptoms visual, confirmed by ELISA, developed in commercial tobaccos (Nicotiana tabacum) and peppers (Capsicum annuum, C. frutescens), but not in tomatoes (Lycopersicon esculentum) and eggplants (Solanum melongena). The following methods were tested for application of TMGMV in TSA-infested fields in Florida: 1) manual inoculation; 2) spraying intact plants or 3) moving and spraying throughout TSA var. Strike at the 10-30% or 50-70% bloom, or both, to determine the timing and efficacy of these fungicides for disease control. Vinclozolin did not significantly reduce the severity or incidence of ALS compared to the untreated check. However, pyraclostrobin and thiamethoxam reduced both disease incidence and severity. Results suggest that ALS could be managed in Ontario by growing the least susceptible varieties, applying efficacious registered fungicides, and deep plowing infested crop residue.

Field trials of South Dakota soybean varieties for susceptibility to northern stem canker. T. E. CHASE. Plant Science Department, South Dakota State University, Brookings. Phytopathology 93:S15. Publication no. P-2003-0108-AMA.

Pathogenicity of Diaphorthe phaseolorum var. caulivora (DPC) isolates was assessed in 2001. Plots were inoculated at the R3-R5 stage by insertion of DPC-infested toothpicks into soybean (Glycine max) stems. Canker development was rapid and resulted in girdling lesions and wilting within three weeks, typical of northern stem canker. Controls inoculated with sterile toothpicks showed no symptoms. Infection efficiency ranged from 32% to 85% among the five test isolates. The most pathogenic isolate (DPC00-126) was used to screen soybean varieties entered into SDSU crop performance testing (CPT) trials in 2002. Varieties within maturity groups 0, I and II (including conventional and glyphosate-resistant) were inoculated (ten stems each plus two checks). Within conventional groups, 50%-76% of varieties were susceptible and within the glyphosate-resistant group 72%-12% of varieties were shown to be susceptible, although one group could not be assessed because of maturation effects. These results demonstrate a significant degree of susceptibility to northern stem canker in South Dakota adapted germplasm.
Plant defensins have been shown to play a role in protecting plants from pathogen invasion. We have previously shown that a defensin encoded by a mungbean cDNA exhibited insecticidal activity against bruchid. To address whether naturally occurring defensin in mungbean also has similar biological activities to the bacteria expressed mungbean defensin (vCGRP), we isolated a defensin (Vrd) in a soybean resistant line from a bichromatic state with a brachitic isogenic line of mungbean Vigna radiata VC6089A by a procedure involving CM-Sepharose chromatography and Superdex Peptide HR10/30 gel filtration in FPLC system. The purified defensin was identified by Western blot analysis using anti-vCGRP antiserum and N-terminal amino acid sequencing. The purified Vrd was shown to inhibit the growth of a soil-borne pathogenic fungus, Rhizoctonia solani and exhibited in vitro insecticidal activity against Callosobruchus chinensis. Artificial mungbean seeds containing 0.2% Vrd reduced the percentage emergence of C. chinensis from 54.4% to 21.3%. Vrd also showed inhibitory activity on in vitro protein synthesis. Thus, Vrd showed biological activities similar to that of vCGRP.


IR-4 conducts about 100 residue studies per year, of which approximately 30% are with fungicides. IR-4 utilizes crop groups to obtain residue tolerances on a large number of crops and is working with EPA and other interested parties to expand the crop-grouping scheme as identified at the Crop Group Symposium held in Washington, DC during October 2002. Crop groups allow IR-4 to obtain 300 to 400 clearances with about 100 data activities. This has greatly saved time and money for the growers registering new or existing product. Efficacy data is needed to support registration of this vast number of uses and for prioritizing projects. IR-4 is conducting limited efficacy studies on crops and pests of high interest and has established a “data mining” program for collecting pesticide efficacy data developed outside of IR-4. We encourage researchers and growers to communicate with us about fungicide efficacy data that identify useful tools for the control of diseases or indicate concern for adequate efficacy or phytotoxicity. Researchers and growers should consider representative crops of the crop groups when submitting project clearance requests to IR-4.

Characterization of a Xylella fastidiosa field strain on the genomes basis. J. CHEN (1), E. Civerolo (1), R. Jarret (2), M. A. Van Sluys (3), and M. C. de Oliveira (4). USDA-ARS, (1) Parlier, CA 95668; (2) USDA-ARS, (3) University of California, Davis, CA 95616; (4) Instituto de Biociencias, Univ. de Sao Paulo, 05508-900 Sao Paulo, Brazil. Phytopathology 93:S16. Publication no. P-2003-0111-AMA.

Five randomly amplified polymorphic DNA (RAPD) fragments from a Florida strain of Xylella fastidiosa (Xf) causing Pierce’s disease (PD) in grapevine were sequenced and used to search the GenBank database including the genome sequences of four Xf strains causing PD (PD-Temecula, almond leaf scorch (ALSD-Dixon) and oleander leaf scorch (OLSD-Dixon-1) from California, and citrus variegated chlorosis (CVC-9a5c) from Brazil. Five RAPD fragments were most similar to 43-bp sequence at the 3' end of PDX3-1 was almost identical to part of the iRNA-lys gene, which could be the att site for DNA integration.


A survey was conducted to determine soil biological and chemical variables associated with iron-deficiency chlorosis (IDC) of soybean in 15 Minnesota soybean fields in late June through mid-July, 2002. Samples were collected from 15 locations along a transect that ran from green through chlorotic soybeans in each field. Sampling area at each location was a 150-cm-long soybean row. Chlorosis was visually scored. A soil sample was taken along the soybean each field. Sampling area at each location was a 150-cm-long soybean row.


Previous studies of chickpea Ascochyta blight used different sets of chickpea differentials and different fungal isolates which has made it difficult to compare results across studies. This study was designed to characterize the virulence of differentials using a defined set of isolates. Nineteen differentials were tested using six isolates of A. rabiei with a mini-dome technique. Two wk-old seedlings were inoculated with conidia (10^{5} spores ml^{-1}), and immediately covered with plastic cups for 24 hours. Disease severity was rated 14 days after inoculation using the (1) no disease to (9) (dead plant) rating scale. All the differentials were either resistant or susceptible to Promise isolates, whereas their reactions to pathogen isolates were highly variable with disease ratings ranging from 2 to 9. Results showed that many of the differentials reacted similarly to A. rabiei inoculation which means that the number of differentials can be significantly reduced without sacrificing accuracy in describing virulence of A. rabiei.


Stripe rust, caused by Puccinia striiformis f. sp. tritici, was monitored by field surveys and trap plots. The disease was widespread in the U.S. Pacific Northwest in 2002. The epidemic affected about 70% of the spring wheat and 2.5% of the winter wheat acreage (20% of the total wheat acreage). In major winter wheat cultivars, resistances including durable high-temperature, and short-life (HTAP) resistance were found in breeding programs conferred by various genes were still effective. The warmer winter of 2001–2002 and lower temperatures in May 2002 favored survival of the rust pathogen and development of the disease. A group of races that were more virulent on spring wheat cultivars and new to the region were predominant. A dramatic increase in spring wheat cultivars that do not have adequate HTAP resistance, especially ‘Zak’, contributed to the epidemic. Use of foliar fungicides, at 12 to 30 million dollars in Washington alone and 125 million dollars in the Southwest, North Carolina and Virginia. Traditional breeding practices have produced few cultivars with moderate disease resistance. Introduction of anti-fungal genes into peanut germplasm through genetic engineering offers an alternative method of control of Sclerotinia blight and other fungal diseases. Transgenic peanut plant lines containing anti-fungal genes have been produced from somatic embryos of the susceptible cultivar Okrun and have been tested for S. minor resistance under greenhouse and field conditions. The results presented here are from a three year field trial study in which these transgenic peanut lines were subjected to high disease pressure with no application of fungicide for S. minor control. Disease incidence ranking was consistent over the three year study period with transgenic peanut lines averaging 32% less disease than the non-transgenic susceptible control. Three transgenic plant lines demonstrated disease resistance comparable to or greater than that of the non-transgenic resistant control while retaining excellent product yield and grade.


Fungal diseases of peanut, such as Sclerotinia blight caused by Sclerotinia minor, are responsible for increased production costs and yield losses of up to 50% for peanut producers in the Southwest, North Carolina and Virginia. Traditional breeding practices have produced few cultivars with moderate disease resistance. Introduction of anti-fungal genes into peanut germplasm through genetic engineering offers an alternative method of control of Sclerotinia blight and other fungal diseases. Transgenic peanut plant lines containing anti-fungal genes have been produced from somatic embryos of the susceptible cultivar Okrun and have been tested for S. minor resistance under greenhouse and field conditions. The results presented here are from a three year field trial study in which these transgenic peanut lines were subjected to high disease pressure with no application of fungicide for S. minor control. Disease incidence ranking was consistent over the three year study period with transgenic peanut lines averaging 32% less disease than the non-transgenic susceptible control. Three transgenic plant lines demonstrated disease resistance comparable to or greater than that of the non-transgenic resistant control while retaining excellent product yield and grade.


Soybeans are the most important legume produced in the United States, used for food, feed, and fuel. A devastating soybean rust pathogen, Phakopsora pachyrhizi, is found worldwide except North America and Europe. Currently, there are no resistant cultivars commercially available in the US. Glycine max cv. Komata is an Asian cultivar that shows resistance to several soybean rust isolates. In order to prepare for the arrival of the soybean rust pathogen, it is crucial to understand the molecular mechanisms involved in resistance. As an initial step, a real-time PCR-based approach and a northern blot analysis were used to assess the expression of known soybean defense-
related genes during a susceptible and a resistant interaction using two different soybean rust isolates. The defense-related genes used in this study included acidic and basic chitinases, beta 1,4-glucanase, and phenylalanine ammonia lyase (PAL). In the resistant interaction, differences in the transcription levels of these genes were observed within the first 12 hours after inoculation.

Indole derivatives produced by the fungus Colletotrichum acutatum. K.-R. CHUNG (1), T. Shults (1), and Ü. Ertürk (2). (1) CREC, University of Florida, Lake Alfred, FL 33850; (2) Heat and Cold Laboratory, Agricultural Research Center, Gdańsk, Poland. Phytopathology 93:S17. Publication no. P-2003-0117-AMA.

C. acutatum infects citrus flowers, causing blossom blight and yellow fruit abscission. The affected flowers accumulated higher levels of indole-3-acetic acid (IAA) than healthy flowers. Production of IAA isolates was investigated. The production of indoles by C. acutatum was dependent solely on tryptophan (Trypt). Totally, 14 isolates tested were capable of synthesizing indoles. HPLC analysis and color reactions using various chromogenic reagents after a fluorescence TLC separation unambiguously identified IAA, tryptophol (Tol), indole-acetaldehyde (IAAld), indole-acetamide (IAM), indole-pyruvic acid (IPA), and indole-lactic acid (ILA) from cultures amended with Trp. The data suggest that C. acutatum may synthesize IAA using various pathways. Increasing Trypt concentrations drastically increased the levels of TOL and ILA, but not IAA and IAM. The ability of C. acutatum to produce IAA and other indoles may in part contribute to the increased IAA levels in citrus flowers after infection.

Genomic analysis of white mold resistance in soybean. S. J. CLOUGH (1,2), T. D. Vuang (2), and G. L. Hartman (1,2). (1) USDA-ARS; (2) University of Illinois, Dept. Crop Sciences, Urbana, IL 61801. Phytopathology 93:S17. Publication no. P-2003-0118-AMA.

Sclerotinia sclerotiorum is an important pathogen of soybean producing the disease White Mold. Partial resistance to this pathogen has been reported, however understanding of the molecular basis of resistance is limited. The recently developed cDNA microarray technology provides a promising tool to aid our search for genes involved in disease resistance. The power of this tool lies in its ability to measure the expression of tens of thousands of genes simultaneously at any specific time point and to compare that directly to a control. The susceptible cultivar Williams 82 and the resistant plant introduction P1194639 were inoculated by applying agar plugs containing a fresh culture of S. sclerotiorum to freshly cut stems. Stem tissue was collected at 0, 3, 18, and 48 hours post inoculation and immediately frozen in liquid nitrogen. Total RNA was fluorescently labeled to determine gene expression profiles using microarrays containing 146 genes. Several gene representatives that show strong correlation with resistance will be converted into molecular markers to determine if they are associated with known QTLs.

Evaluation of tank mixtures of fludioxonil and azoxystrobin for post-harvest disease control on citrus. A. COCHRAN (1), A. Tally (2), and E. Telford (3). (1) Syngenta Crop Protection, Visalia, CA 93292; (2) Syngenta Crop Protection, Basle, Switzerland. Phytopathology 93:S17. Publication no. P-2003-0119-AMA.

Fludioxonil (50WP) and azoxystrobin (208SC) applied solo and in combination were evaluated for post-harvest control of green mold on Valencia oranges. Oranges were surface sterilized using a 0.525% sodium hypochlorite solution, wound inoculated using a probe (3x2nm) dipped in a spore suspension of Penicillium digitatum (1 x 10^6), and then incubated at 68°F for 24 hours. Fruit were then dipped for 30 seconds in either water or various treatments, air-dried, and then stored in a high humidity environment on open trays for the trial duration. Disease incidence was assessed at regular intervals and the area under the disease progress curves (AUDPC) were calculated. Azoxystrobin and fludioxonil applied solo significantly reduced disease incidence and AUDPC compared to the untreated check but were not equivalent to imazalil. However, the combination of both fludioxonil and azoxystrobin provided control equal to that of imazalil.


Phytophthora root rot of citrus in Florida is caused by P. nicotianae and P. palmivora. A naturally occurring mutant of P. nicotianae was identified as hypovirulent on citrus and reduced root rot caused by virulent isolates. The induction of resistance genes in the host was investigated as a possible mechanism of resistance. Sour orange trees were exposed to zoospores of the hypovirulent P. nicotianae isolate, and to virulent isolates of P. nicotianae and P. palmivora for 24 hours. The control was exposed to water. RNA was then extracted from the roots and was probed with sequences of putative resistance genes using Northern blot analysis. A CDNA encoding a putative PR-4 protein, clone 60, was differentially expressed. No expression was seen in the control but expression was markedly increased in the three treatments. Expression was highest for the hypovirulent isolate treatment. The nucleotide sequence of clone 60 had high homology to a PR-4 protein in tobacco. RACE (rapid amplification cDNA end) was used to obtain the full sequence of the CDNA 60 gene. Real time PCR will be used quantify the level of the PR gene expression based on the various treatments.


A study was conducted in 2001 and 2002 to determine if growing pumpkins (Cucurbita pepo) in a no-till cover crop would reduce disease severity and result in improved efficacy of novel spray regimes including Messenger (harpin protein) and Serenade (Bacillus subtilis). The effect of ground cover, cultivar resistance, and unconventional fungicide programs on severity of pumpkin diseases was evaluated. The main plots of the split-split design were no-till Vicia villosa plus Secale cereale or conventional tillage. Sub-plots were a factorial design of cultivar (‘Magic Lantern’ and ‘Wizard’) and chemical program (grower standard program, two programs employing reduced risk products, and untreated plots). In 2001 Southern blight (Sclerotium rolfsii) was lowest in no-till plots than in conventionally tilled plots (6.5% vs. 5.1%). Powdery mildew (Podosphaera xanthii) was highest in unsprayed, intermediate in plots sprayed with the unconventional programs and lowest in conventionally sprayed plots in both years. Disease reductions due to cover cropping were low and did not improve the effectiveness of alternative programs.


Cowpeas (Vigna unguiculata [L.] Walp) are an important food legume. Rhizoctonia hypocotyl rot caused by Rhizoctonia solani AG-4 is a major soilborne disease of cowpeas, and a constraint to cowpea production by reducing plant stand, root biomass, and yield. We evaluated the effects of biological seed treatments Kodiak® (Bacillus subtilis GB3) Gunstafsson LLC, Plano, TX 75093, composted cow manure, and pine bark in suppressing Rhizoctonia hypocotyl rot. Treatments were arranged in a complete randomized design with five replications; 10 seeds per pot and grown in the greenhouse for 10 days at 28°C. Composted cow manure, and Kodiak® (B. subtilis GB3) significantly (P < .0001) reduced Rhizoctonia hypocotyl rot of cowpeas. Organic amendments and biological seed treatments are a sustainable method of suppressing soilborne plant pathogens.


Rates of the disinfectants, chloramine-T, hydrogen dioxide, hydrogen peroxide, iodine, quaternary ammonium (dimethyl benzyl and dimethyl ethylbenzyl ammonium chlorides), and sodium hypochlorite, were sprayed on 2.25 to 4 cm² pieces of polyethylene (ground fabric, solid disc), metal (galvanized, stainless steel), and pine (natural, pressure-treated, latex-painted) that had been inoculated 18 hrs earlier with a suspension of Botrytis cinerea conidia. Several hours after a disinfectant had dried, substrates were inverted and then stored in 100% relative humidity for 7 days. The next day percent germination was counted for greater than 100 conidia. Lethal dose of the disinfectant resulting in 50 percent mortality (LD₅₀) and slope of the dose curve were calculated by Probit analysis. Substrate affected disinfectant activity (LD₅₀’s). In general, LD₅₀’s were highest on natural and pressure-treated pine and lowest on latex-painted pine and stainless steel. Results show that the material being disinfested affects the rate of a disinfectant.

Survival of the Fusarium head blight biocontrol agent Cryptococcus nanaeodatis OH128.9 on wheat. A. B. CORE (1), D. A. Schisler (2), T. E. Hicks (1), P. E. Lipps (1), and M. J. Boehm (1). (1) Ohio State University/ORDC, Dept. of Plant Pathology, Columbus, OH 43210; (2) USDA-ARS, NCAUR, Peoria, IL 61604. Phytopathology 93:S17. Publication no. P-2003-0124-AMA.
Cryptococcus nodaensis OH182.9 is a naturally occurring wheat anther colonist. Application of OH182.9 to wheat at anthesis reduced Fusarium head blight (FHB) severity by 56% and doubled 100-kernel weight in field trials. Confirmation of the ability of OH182.9 to survive on wheat heads prior to anthesis would support the possibility of applying OH182.9 earlier in crop development without sacrificing to efficacy. OH182.9 was produced in liquid culture and applied to field and greenhouse grown wheat just prior to and during early anthesis. Populations of OH182.9 on extended anthers were monitored for 10-12 d after application and on kernels harvested from treated heads. Significant populations of OH182.9 were recovered from all treated plants indicating that OH182.9 is able to survive in the absence of anthers. A significant increase in OH182.9 populations 4-8 d after application was observed suggesting that OH182.9 reproduced on the head. OH182.9 was recovered on kernels of treated wheat. Results of studies will be presented.


Resistance breakdown to rice blast caused by Pyricularia grisea occurs after one to three years of cultivar release in Colombia. For developing cultivars with durable resistance we have analyzed the genetic structure of blast pathogen populations using MGRDNA and rep-PCR fingerprinting and studied the diversity and frequencies of avirulence/virulence genes in the fungus. P. grisea in Colombia is mainly clonal. Some resistance genes are effective against all isolates of a lineage. Avirulence genes vary in frequency, suggesting that these genes could be associated with pathogenic fitness. Therefore, the resistance genes corresponding to those avirulence genes would be more relevant for breeding durable resistance. We are identifying and predicting the durability of resistance gene combinations based on frequencies of avirulence genes. We have identified the possible resistance genes present in our commercial rice cultivars and initiated a backcrossing program incorporating desired resistance genes in rice varieties of Latin America through marker assisted selection, controlled inoculations, and field evaluations.


Reactions between complementary nit (nitrate non-utilizing) mutants of Aspergillus flavus were influenced by the composition of the medium on which vegetative compatibility analyses were performed. Vegetative compatibility groups were found to differ in their sensitivity to medium composition. Medium components that influenced complementation included simple sugars and complex polymers. Complementation was inhibited by sucrose, fructose and reduced agar concentration (regardless of medium hardness), and stimulated by starch and several other polymers. An improved medium for vegetative compatibility analysis of A. flavus was developed. The medium contained the following per liter: 3 g NaNO₃, 1 g K₂HPO₄, 0.5 g MgSO₄ 7 H₂O, 0.5 g KCl, 36 g glucose, 20 g starch (Difco), 13 g agar (EM), and 12 g agar (Sigma). The medium was adjusted to pH 7.0 prior to autoclaving. In addition to support of faster analyses, the improved medium helped to resolve ambiguous reactions, simplify vegetative compatibility grouping, and expand the number of A. flavus VCGs that could be tracked with vegetative compatibility analyses.

Mixtures for simultaneous management of tan spot and leaf rust. C. M. COX (1), K. A. Garrett (2), R. L. Bowden (2), and A. K. Fritz (3). (1) Dept. of Plant Pathology; (2) USDA-ARS; (3) Dept. of Agronomy; Kansas State University, Manhattan, KS 66506. Phytopathology 93:S18. Publication no. P-2003-0127-AMA.

Mixtures are more desirable if they simultaneously reduce the severity of multiple diseases caused by pathogens with different life histories. The objective of this research was to determine the effectiveness of a wheat cultivar mixture on management of the residue-borne disease tan spot, alone and in the presence of leaf rust, and to compare the relative effectiveness of mixing for tan spot vs. leaf rust. Winter wheat cultivars Jagger and 2145, which have complementary resistance to leaf rust and tan spot, were mixed in proportions of 0, 25, 50, 75, and 100%. Field plots were inoculated with each pathogen alone, both pathogens, or treated with a fungicide. For both diseases, the gradual increase in the proportion of the susceptible cultivar decreased. Leaf rust and tan spot severities on the susceptible cultivar in 50:50 mixtures were reduced by at least 32% and 16%, respectively, compared to monocultures. Mixtures were significantly more effective at reducing leaf rust relative to tan spot.

Characterization of Armillaria spp. from southeastern peach orchards using fatty acid methyl ester (FAME) profiling. K. D. COX (1), H. Scherr (1), and M. B. Riley (2). (1) Plant Pathology, University of Georgia, Athens, GA; (2) Plant Pathology & Physiology, Clemson University, Clemson, SC. Phytopathology 93:S18. Publication no. P-2003-0128-AMA.

Armillaria tabescens and A. mellea have been implicated as etiological agents of Armillaria root rot in southeastern stone fruit orchards, but it is unclear which species is more prevalent. Despite considerable efforts to develop molecular tools to distinguish Armillaria species, ITS and IGSP heterogeneity among individuals of A. tabescens and A. mellea from this geographical region has led to the pursuit of alternative identification methods. Whole-colony FAME analysis was conducted on wood block cultures of regional isolates of Armillaria species and other wood-rotting basidiomycetes commonly found on peach. Armillaria species were differentiated on the presence of fatty acids with molecular weights less than 18 carbon chains. For individual species, there were differences in FAME profiles among the secondary growth states (mycelial sheets, sclerotial plates, and rhizomorphs). Our results indicate that species identification from unprocessed infected material using FAME analysis is possible.


Field and greenhouse studies are being conducted to evaluate the survival of a Longidorus sp. in bare fallow soil. At a Georgia nursery, the population density was 45 (range: 20 to 88) Longidorus/100 cc soil in the upper 15 cm of 10 fallow field plots in April. The density decreased to 2 (range: 0 to 3) Longidorus/100 cc soil by August, and Longidorus was not detected in soil of any plot after 9 months. In a growth chamber study, Longidorus population densities were initiated at 1 to 3 Longidorus/fallow containers and containers with loblolly pine seedlings. The initial population density was 68 (range: 53 to 77) Longidorus/100 cc soil. The density in fallow containers decreased to 4 (range: 1 to 6) after 128 days at 24°C, and after 234 days, there were 2 (range: 0 to 5) Longidorus/100 cc soil. In containers with seedlings, the population density was 26 (range: 17 to 40) Longidorus/100 cc soil after 128 days. Ten additional seedlings were planted in each of this treatment at day 128, and by day 234 the density was 65 (range: 48 to 78) Longidorus/100 cc soil. Bare fallow may be an important strategy for managing Longidorus in infested fields.


Black spot disease, caused by the necrotrophic toxin producing fungus Alternaria brassicicola is a major pre and post harvest problem on cultivated Brassica species. No satisfactory level of resistance has been identified among cruciferous crop plants, although resistant and susceptible ecotypes of A. thaliana have been reported. We initiated a functional genomics based approach for elucidating the mechanisms of pathogenicity in the fungus and defense responses in resistant and susceptible Brassicaceae hosts. The goals of the project are to use random and targeted mutagenesis strategies to produce fungal mutants, sequence ESTs generated from a suppression-subtractive cDNA library of cabbage tissue infected with wild-type fungus, and characterize global transcriptional patterns using microarrays. We will present results obtained thus far from our mutant screen and sequence and analysis of ESTs.


Anthracnose disease of turfgrass, caused by the mitosporic fungus Colletotrichum graminicola, has reached near epidemic proportions in many regions of North America over the last decade. Our ITS sequence and DNA fingerprint studies with fungal isolates from Pennsylvania golf courses suggest that there are at least two major subgroups of isolates associated with the disease in that area. Using the same approaches, we are extending these studies to examine samples from New Jersey and other parts of North America. To examine differential pathogenicity of isolates representing these two distinct clades in controlled inoculations, the fungus has been transformed for GFP expression. Finally, several classes of fungicides with different modes of action are used to control these pathogens in the field. We
discuss evidence for differential fungicide resistance in vitro in the context of these genetically distinct fungal populations.

**Distinguishing Pelamigatus christiei from other plant-parasitic nematodes associated with warm-season turfgrasses.** W. T. Crow (1) and N. R. Walker (2). (1) Entomology and Nematology Department, University of Florida, Gainesville, FL 32611; (2) Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078. Phytopathology 93:19. Publication no. P-2003-0132-AMA.

Plant-parasitic nematodes are common and destructive pests of both warm and cool-season turfgrasses. *Pelamigatus christiei* has been associated with warm-season turfgrasses in Florida for many years. The authors have recently identified *P. christiei* associated with bermudagrass in California, Mississippi, and Oklahoma. *P. christiei* may be confused with common turfgrass parasitic nematodes. Yields were higher and TSWV incidence lower for NC 71 compared to K-326. TSWV incidence tended to be less and yields higher in plots treated with fenamiphos compared to 1,3-D fumigated plots. However, this difference was negated when plants were pre-treated with imidacloprid and ASM. ELISA positive reactions were up to 100 percent higher than percent incidence, but followed the same trend as percent incidence.


Fusarium Head Blight (FHB), incited by *Fusarium graminearum*, damages wheat by reducing yield and contaminating grain with deoxynivalenol (DON). The influence of irrigation on the accumulation of DON was studied in three spring wheat cultivars (Alsen, Pioneer 2375, Wheaton) inoculated at anthesis with *F. graminearum* at two inoculum concentrations (Low: 2.5 × 10^5; High: 1 × 10^6 spores/ml). FHB severity at early dough ranged from 26 to 30 (Low) and 50 to 70 percent (High). Irrigation treatments were imposed from early dough until harvest (1.38 mm/day; NI, no irrigation). Spikes were sampled for DON analysis at early dough, hard dough, kernel hard and harvest. Across all other variables, DON accumulation was significantly (P < 0.05) lower under 1 compared to NI (1, 13.5 ppm; NI, 20.9 ppm). Under both irrigation treatments, DON in kernels decreased significantly from the first to the last samplings and between the intervals from early dough to kernel hard in the irrigation treatment. Models forecasting the accumulation of DON may need to consider the effects of excessive rainfall during grain development.

**Mapping of quantitative trait loci associated with pathogenicity and aggressiveness of Gibberella zeae (Fusarium graminearum) causing head blight of wheat.** C. J. R. Cumagun (1), R. L. Bowden (2), J. E. Jurgenson (3), J. F. Leslie (2), and T. Miedaner (1). (1) State Plant Breeding Institute (720), University of Hohenheim, D-70593 Stuttgart, Germany; (2) Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502; (3) Department of Biology, University of Northern Iowa, Cedar Falls, IA 50614. Phytopathology 93:19. Publication no. P-2003-0135-AMA.

*Gibberella zeae* is the major fungal pathogen of head blight, a destructive disease of wheat and other small grains worldwide causing losses in quantity and quality of grain yield. Deoxynivalenol (DON) and nivalenol (NIV) mycotoxins produced by the fungus pose health hazards to humans and animals. This study was conducted to identify quantitative trait loci (QTL) associated with pathogenicity and aggressiveness in a crossing population of *G. zeae* (Z-3639 × R-5470), using amplified fragment length polymorphism (AFLP) markers. Aggressiveness data were collected for two years in the greenhouse. An AFLP linkage map of 468 loci with an average interval of 2.8 map units between loci was used for mapping. The crossing population segregated qualitatively for pathogenicity with 38 progenies and one parental strain being non-pathogenic. The remaining 61 progenies significantly varied in their disease-producing capacity i.e. aggressiveness. For pathogenicity, two large QTLs near TOX1 (amount of toxin) and 4P were detected on linkage group 4 that explained 82% of phenotypic variation. The second QTL only contributed about 5% more. When the two QTLs were considered together, might just be detected because the first QTL due to an inversion breakpoint in this region. Aggressiveness QTLs were detected in one linkage group. Two major QTLs near TR1 (trichodien pathway) and EAMTTG0665K explained together 75% of the phenotypic variation of fungal aggressiveness in this cross. These QTLs had complementary gene effect on aggressiveness. Pathogenicity and pigmentation seemed to be monogenically inherited while aggressiveness appears to be quantitatively inherited. All NIV chemotypes of the mapping population were about two times more aggressive than the NIV chemotypes. In conclusion, the rather simple inheritance of both pathogenicity and aggressiveness might be a hint that *G. zeae* can adapt non-specifically to higher host resistance levels.

**Spatial pattern analysis of Sharka disease (PPV-M strain) in peach orchards in southern France.** S. Dallot (1), T. Gottwald (1), G. Labonne (2), and J. B. Quot (2). (1) USDA-ARS-USHL, Fort Pierce, FL 34945; (2) ENSA-INRA, Montpellier, 34060 France. Phytopathology 93:19. Publication no. P-2003-0136-AMA.

individual Plum pox virus strain M infections were monitored visually during one to 3 years in 18 peach plots. The spatial pattern of the disease was investigated using binary data directly or after parsing the data in quadrats of 4, 9 or 16 trees. Ordinary runs demonstrated minimal aggregation of adjacent symptomatic trees. Beta-binomial analysis indicated overdispersion of incidence for all quadrat sizes in 15 plots and the binary power law showed that intensity of aggregation was a function of incidence. Spatial relationships among quadrats were evaluated by spatial autocorrelation and SADIE analysis. The two methods were in good agreement and showed significant aggregation in a majority of data sets. Local areas of influence of PPV spread and relationships at longer distances were further investigated. Combined results indicated a wide range of spatial patterns of PPV-M infected trees. Factors that may have influenced such patterns, like the mechanisms of primary disease introduction, the diversity of aphid species and the structure of the orchards will be discussed.

**Glassy-winged sharpshooter transmission of Xylella fastidiosa, causal agent of citrus variegated chlorosis.** V. D. Damsteegt (1), R. H. Bralney (2), P. A. Phillips (3), and A. Roy (2). (1) FDWSRU, 1301 Ditto Ave, Ft Detrick, MD 21702; (2) UFL, CREC, Lake Alfred, FL 33850; (3) UC Coop Ext Service, Ventura, CA 93003. Phytopathology 93:19. Publication no. P-2003-0137-AMA.

Citrus variegated chlorosis (CVC), caused by Xylella fastidiosa (Xf), is a serious disease of citrus in Brazil and Argentina. The pathogen is transmitted by several sharpshooter leafhopper species, including *Oncometopia nigricans* (Bransky et al., Plant Dis. 86:1237-39, 2002). The glassy-winged sharpshooter (GWS), *Homalodisca coagulata*, is a serious pest in California, where it transmits Xf strains to several crops including grapes, alfalfa, and almonds. Transmission studies over a three-year period at the USDA BSL3-P containment facility at Ft Detrick, MD, utilizing CA field-collected GWSW, a Brazilian strain of CVC, and sweet orange seedlings, have shown consistent, though inefficient transmission of CVC. Test plants were observed for CVC symptoms, analyzed by ELISA, by membrane entrapment immunofluorescence (MEIF), and by real-time PCR using species-specific primers for Xf. No Xylella were detected in field-collected GWSS but were detected in GWSS following feeding on CVC-infected source plants.

**Control of Phytophthora cryptogea in the hydroponic forcing of wilted choy with synthetic and biosurfactants.** K. de Jonghe, I. De Zerbi, W. T. Crow (1) and N. R. Walker (2). (1) Entomology and Nematology Department, University of Florida, Gainesville, FL 32611; (2) Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078. Phytopathology 93:19. Publication no. P-2003-0138-AMA.

Currently, the use of pesticides is still the most spread control measure against diseases in agricultural crops. Environmental restrictions and public awareness have made research for alternatives very important. One option is the use of surfactants to control zoosporic plant pathogens. Synthetic and biosurfactants controlled sporangia formation and had a direct lethal effect on zoospores. Good control of Phytophthora cryptogea foot rot was obtained in a hydroponic system with wilted choy (Cichorium intybus var. foliosum). At a concentration of 20 µg/ml the surfactant synthetica MBA1301 gave complete protection against the pathogen. PRO1, a rhamnolipid biosurfactant extracted from a Pseudomonas aeruginosa strain, significantly reduced the disease level. Application of surfactant producing Pseudomonas fluorescens bacteria also lowered the disease level significantly.
PCRF detection of specific 2,4-diacetylphlorogucinol-producing Pseudomonas fluorescens genotypes. L. DE LA FUENTE (1), D. V. Mavrodi (1), L. S. Thomason (1,2), B. B. Landa (3), and D. M. Weller (1,2). (1) Washington State University, Dept. of Plant Pathology, Pullman, WA 99164; (2) USDA-ARS, (3) Dept. de Agronomia, Univ. de Cordoba, 14000 Cordoba, Spain. Phytopathology 93:S20. Publication no. P-2003-0139-AMA.

Strains of fluorescent Pseudomonas spp. that produce 2,4-diacetylphlorogucinol (DAPG) provide biological control of diverse soilborne pathogens. Seventeen distinct genotypes (A-Q) of these strains have been distinguished by rep-PCR and RFLP analysis of phlD, a key gene in DAPG synthesis. The phlD gene is highly conserved, with DNA sequence identity among genotypes ranging from 77 to 99%. We used differences in the phlD sequence to design specific PCR primers for the detection of the A, B, D, K, and P genotypes. Strains of genotypes D and K are highly aggressive colonizers of the rhizosphere by budding and form both bacterial and slime-like structures that contribute to disease severity. Diverse genotypes in the rhizosphere of wheat grown in natural soil even when the population density of one genotype was 200-fold less than that of the other. PCR-specific detection is a rapid, reliable approach to study interactions between genotypes without the use of antibiotic resistance-marked strains.

Genetic diversity and activity of Trichoderma stromaticum, a mycoparasite of the cocoa witches’ broom pathogen. J. T. DE SOUZA (1), J. H. Bowers (2), G. J. Samuels (2), P. K. Hebbar (3), and A. W. Pomella (4). (1) University of Maryland, Wye Research and Education Center; (2) USDA, ARS, Beltsville, MD; (3) Mars Inc., Hackettsport, NY; (4) Almirante Cacau, Iaujupe, BA, Brazil. Phytopathology 93:S20. Publication no. P-2003-0140-AMA.

Witches’ broom, caused by Crinipellis perniciosa, inflicts severe damage in cocoa plantations in Central and South America. In Brazil, a commercial formulation of Trichoderma stromaticum is being used in the field. However, performance has been inconsistent. One strategy to improve biocontrol is to exploit the diversity of microorganisms belonging to the same species. We collected 95 T. stromaticum isolates from Bahia and the Amazon region. Diversity was studied using AFLP and RAPD analyses. All the isolates collected from Bahia were genetically similar and related to the commercial isolate. Isolates from the Amazon region were more diverse, Mycelial growth at different temperatures and on saline media, and sporulation at 30°C and on brooms varied greatly among the isolates. Several isolates showed superior activity in vitro when compared to the commercially available one. Field studies to relate in vitro activity to biocontrol efficacy are ongoing.

Control of Verticillium wilt with microbial antagonists, crop residues or lignin incorporation in soil. J. DEBOSE, D. Claeyts, and M. Hölte. Ghent University, Laboratory of Phytopathology, B-9000 Ghent, Belgium. Phytopathology 93:S20. Publication no. P-2003-0141-AMA.

Verticillium dahliae var. longisporum is an important soilborne pathogen of several crops and forms persistent survival structures, the microsclerotia. The main objective is to control this wilt disease on crucifers with the help of antagonists. To test the potential of antagonists, we developed a microtiter assay. Nylon filter discs containing microsclerotia were exposed to several antagonists, including Talaromyces flavus, Pseudomonas spp. and Serratia plymuthica. The potential of crop residues or lignin was tested by incorporating them in naturally infested soil. After incubation, viability of the microsclerotia in soil and lignin- and melamin degrading potential of soil was assessed.


The incidence of anthracnose fruit rot in highbush blueberry, incited by Colletotrichum acutatum, depends mainly on the amount of inoculum from overwintering infections on the bush. Dormant flower buds were recently shown to harbor the majority of these infections. Whether infectious propagules survive on or within the bud will influence efforts to manage the disease by reducing overwintering inoculum. Eighteen dormant ‘Bluecrop’ twigs bearing 149 flower buds were sonicated and washed, then plated on water agar. Half were surface-sterilized before plating. No propagules were found in the tissue wash and there was no significant difference in the number of infections in sterilized and unsterilized buds, suggesting that the pathogen survives within the bud, between layers of bud scales. Bud scales were removed from 100 ‘Bluecrop’ flower buds, fixed, and mounted on slides to examine the location and nature of infectious propagules. Another 100 buds were dissected and plated on water agar to ascertain how deep into the layers of bud scales C. acutatum spore masses appeared.


Citrus blight (CB) is a disease of unknown cause that can be transmitted experimentally by root grafting. We have recently found strains of citrus tristeza virus (CTV) in CB-susceptible rootstocks reported to be resistant to CTV. citrus sudden death (CSD) is a new disease in Brazil. Failures to control CSD with acceptably low costs of control can be attributed to the lack of understanding of the disease’s nature and cause. Two questions: Are there strains of CTV that predominate in roots? And, are any of these associated with CB or CSD? RT-PCR with CTV based primers and a Clontech PCR-Select cDNA Subtraction Kit were used with RNA and dsRNA from roots to produce numerous clones with nucleotide similarities of zero to 100% with known CTV strains. Conclusions: Based on sequence analysis, citrus trees in Florida and Brazil, including some on CTV-resistant cultivars, contain a wide variety of CTV strains in their roots. A strain of CTV was found that appears to be implicated as the cause of CSD. Research on the possible association of root infecting strains of CTV with CB continues.

Soil fumigation and oxamyl drip applications for nematode and insect control in vegetable plasticulture. J. DESAEGER (1), A. Csinos (1), P. Timper (2), G. Hammes (3), and K. Seebold (1). (1) University of Georgia, Dept. of Plant Pathology; (2) USDA, Coastal Plain Experiment Station, Tifton, GA 31793-0748; (3) DuPont Co., Cherrylog, GA 30522. Phytopathology 93:S20. Publication no. P-2003-0144-AMA.

The effect of oxamyl in combination with the soil fumigants 1,3-D, metam sodium and methyl bromide on nematode management and fruit yield in vegetables was investigated in Tifton, GA, for three fall and two spring crops, from 2000 until 2002, using four vegetables: squash, cucumber, pepper and eggplant. Soil fumigation alone, irrespective of application method or formulation, gave significant control of southern root-knot nematode (Meloidogyne incognita) in all but one test. Oxamyl by itself did not control root-knot nematode, but reduced insect populations on eggplant. Oxamyl in combination with pre-plant soil fumigation gave small but consistent reductions in root galling. Greatest reductions in galling due to oxamyl were found when fumigation provided less than optimal nematode control. Oxamyl galling suppression significantly reduced the impact of nematode infection. Crop yields following fumigation were often significantly greater when oxamyl was added.


MARYBLYT (MB) and Cougarblight (CB) are forecasters used to identify infection periods for blight of apple and pear. MB uses flowering, bacterial population (EIP), wetting, and average daily temperature, whereas CB uses flowering, wetting, a 4-day temperature window, and a measurement of inoculum pressure as parameters for predicting blossom infection. All possible action thresholds of both models were evaluated using historical weather and disease incidence data collected from several regions using ROC analysis (a procedure used to identify optimal thresholds for decision making). Areas under the ROC curves were equivalent based on a Wilcoxon-type statistic suggesting that the two forecasters performed similarly in their ability to predict blossom blight. However, preliminary results showed that MB was a better predictor of disease when disease was present, but tended to overpredict disease when it was not present, whereas the opposite was true for CB. Further analysis is needed to determine the appropriate balance between the two costly errors.


Models that predict the probability of Fusarium head blight (FHB) based on weather variables observed prior to and during the flowering growth stage of wheat were developed using data collected in OH, ND, MO, and KS. Two of
the models. Model 1 and Model 2, were able to predict FHB epidemics with 70 and 84 percent accuracy, and are being used to forecast FHB epidemics in the U.S. In this analysis, the accuracy of these models was evaluated with 23 cases not used during model development (with 9 epidemics), and errors used to identify variables that influenced model performance. Model 1 and Model 2 correctly predicted 65 and 74 percent of the cases. All 8 errors made by Model 1 and 5 of the 6 errors made by Model 2 were false negative predictions (incorrectly predicting low disease). Most of the cases misclassified by the models were associated with the presence of corn residue or the highly susceptible wheat variety Norm. Current modeling efforts will attempt to incorporate residue type and variety susceptibility into model predictions.


Secretary gland in the esophagus of the soybean cyst nematode *H. glycines* are the main sources of secretions involved in plant parasitism. The products of eleven candidate parasitism genes from *H. glycines* that express different gland secretions were selected for antibody production. Two synthetic peptides were generated to the predicted amino acid sequence of each gene and conjugated to carrier molecules for immunizations. Polyclonal sera to six parasitism gene peptides bound to proteins of predicted size on Western blots of total proteins extracted from mixed parasitic stages of *H. glycines*. Two polyclonals bound to secretory granules within the two subventral esophageal gland cells of parasitic stages of *H. glycines*, and four others bound within the esophageal gland cell of *H. glycines* parasitic stages. Immunofluorescence microscopy. In total, polyclonal antibodies specific for as many as seven parasitism genes were produced. The polyclonal sera will be used to detect when and where *H. glycines* secretes the different gene products during parasitism of soybean roots.


Different chemicals have the potential to manage white rust ( *Albugo occidentalis* ) on spinach (*Spinacia oleracea*), and their application may have an impact on nontarget entomopathogenic fungi. Experiments were conducted where spinach was sprayed with pyraclostrobin weekly or according to a model based on temperature and leaf wetness. In a second experiment pyraclostrobin, acibenzolar-S-methyl and Naiad were applied weekly or according to the model. Both acibenzolar-S-methyl initialized at the second true leaf stage, and pyraclostrobin reduced white rust incidence compared to nontreated plots. This reduction occurred in plots sprayed according to the model and plots sprayed weekly. Plots where Naiad was applied weekly had significantly fewer leaves that were infested with aphids than nontreated plots. There were no differences in the percent of aphids infected with entomopathogens among treatments. Chemical applications scheduled according to the model may be effective in managing white rust in Maryland.


The infection process by *Colletotrichum acutatum* on almond was studied using digital analysis of montage images generated from a series of sequential, partially focused light micrographs. Color depth maps and line profiles allowed rapid depth quantification of fungal colonization in numerous tissue samples. On leaves and petals, penetration was initiated from appressoria. Colonization occurred as a combination of subcuticular, intramural, and intracellular fungal growth. On petals, colonization was at first subcuticular, followed by intracellular or intramural invasion. After penetration, colonization between 24 to 48 h resembled a bioticrop host-pathogen interaction on both tissues based on the presence of healthy host cells adjacent to fungal hyphae. Later the interaction became necrotrophic with collapsed host cells. The results demonstrate the complex interaction between *C. acutatum* and almond where the interaction changes from symptomless to symptomatic infection in both petals and leaves during a several-day process.


In laboratory and greenhouse experiments, the efficacy of copper alternatives was tested for control of bacterial spot disease of peppers caused by *Xanthomonas campestris pv. vesicatoria*. Treatments included bactericides ( dibromonitrilopropionamide - DBNPA, and hydrogen dioxide - Zerotol), systemic-acquired resistance compounds (SAR - Actigard, and harpin protein - Messenger), an antibiotic ( oxolinic acid - Starner), and a natural product (QST 713 strain of *Bacillus subtilis* - Serenade). DBNPA at 500 or 1000 mg/L was the treatment that provided best disease reduction (>95%) compared to the control. It had low phytotoxic effects and was more effective than Kocide when copper-resistant strains were used (>80%). Starner, Serenade, and Messenger did not significantly reduce disease, whereas Zerotol, Milasana, and Actigard provided intermediate disease reduction of 60 to 80%. All treatments had some phytotoxic effects. Thus, DBNPA represents the best alternative compound to copper-based treatments.


Similarity searching, using programs such as BLAST, has become the backbone of sequence annotation. With the advent of high throughput sequencing, management and analysis of the resulting BLAST reports have become a non-trivial problem. This is especially true for smaller research organizations which do not have the infrastructure to handle the massive amounts of data produced by high throughput methods. Alkahest NuclearBLAST has been developed as a free, open-source, web-based solution to these problems. Its user-friendly PHP web interface allows researchers to specify batch BLAST requests and provides access to the returned BLAST results. These results can then be browsed using point and click navigation or searched by keyword using a standard web browser. The system automatically handles the execution of BLAST similarity searches and deposition of the results into a MySQL relational database. The MySQL database is structured in such a way as to enable simple execution of more advanced data mining. More information including the program can be obtained from http://www.alkahest.org.

Characterization of a gene ( sml ) encoding a protein from Trichoderma reesi involved in plant associated interactions. S. DION (1), R. C. Howell (2), and C. M. Kenerley (1). (1) Texas A&M Univ. Dept. Plant Path. & Microbiol., College Station, TX 77843; (2) USDA, College Station, TX 77845. Phytopathology 93:S21. Publication no. P-2003-0152-AMA.

The biocontrol agent, *T. virens*, induces the production of plant defense-related compounds (terpenoids) in the roots of cotton seedlings. We have identified and cloned a gene, *sml*, which encodes a protein of 138 amino acids (aa) with a predicted molecular mass of 14.4 kDa, an 18 aa signal peptide and a pl of 5.76. Several potential post-translational modifications are predicted from the sequence. This protein (SM1) contains four cysteine residues and shows high similarity to SPL from *Phaeosphaeria nodorum* and *Asp f 13* from *Aspergillus fumigatus*. The sml gene is expressed during growth in minimal media supplemented with different carbon sources and in simulated parasitism (*Rhizoctonia solani* cell walls as carbon source). We hypothesize that SME is responsible for terpenoid induction in cotton radicals as direct application of SM1 to radicals stimulated terpenoid synthesis as detected by HPLC analysis. To test this hypothesis we are preparing PCR-mediated gene disruption constructs to generate mutants of sml for plant assays.


Members of the *Araceae* are susceptible to the bacterial pathogen *Xanthomonas axonopodis pv. dieffenbachiae* which is capable of causing crop losses up to 100 percent. When 187 strains isolated from nine Aroid hosts were subjected to Rep-PCR, six genetic clusters were generated. One cluster was found to represent only strains isolated from *Syngonium spp.* Forty strains isolated from *Anthurium, Difenbahcia* and *Syngonium* were subjected to Amplified Fragment Length Polymorphism (AFLP) and tested for pathogenicity on five Aroid hosts. AFLP data correlated well with Rep-PCR data. Based on pathogenicity tests the *Syngonium* strains were selectively pathogenic on *Syngonium*. None of the strains from other hosts caused significant disease on *Syngonium*. DNA from ten representative strains was amplified by PCR using primers to the ITS and hrp B. Phylogenetic analysis of sequenced PCR products revealed that the *syngonium*
strains were a distinct group from other members of *X. axonopodis* pv. *diesenbachiae* and support the use of *pv. syngnii* for strains isolated from *Syngonium*.


*Magnaporthe grisea* is an ascomycetous fungus and the causative agent of rice blast disease, a major limiting factor in rice production in many parts of the world. Because of the socio-economic impact of this disease, pinpointing fungal genes involved in pathogenicity will be important in designing novel disease control strategies. To achieve this goal, NCSU, along with five other universities, implemented a high-throughput screening and functional genomics approach to identify pathogenicity-related genes. We designed a high-throughput screen to analyze 50,000 random insertion *M. grisea* mutants for pathogenicity on rice seedlings. Mutants that cause no or fewer disease symptoms compared to wild type are promoted to secondary and tertiary screens for phenotype confirmation and further characterization. To date, we have analyzed over 9,000 mutants and confirmed a ‘low’ pathogenicity phenotype for about 0.1%. Their characterization, as well as our screening methods, mutant storage and tracking system, will be discussed.


Alternaria blight (*Alternaria dauci*) and Cercospora blight (*Cercospora carotae*) incite disease on carrot leaves and petals. A disease-forecasting model, Tom-Cast, was tested at thresholds of 10, 15, 20 disease severity values (DSV) to time fungicide sprays using a copper-based fungicide (copper hydroxide), a reduced risk fungicide (azoxystrobin), and a standard commercial fungicide (chlorothalonil), alone and in alternation. Based on AUDPC data, disease severity of petiole and leaf blight was similar for plants treated with fungicide every 7 days or according to Tom-Cast 10 or 15 DSV. The number of spray applications were reduced by 7 (2001) or 8 (2002) with treated with fungicide every 7 days or according to Tom-Cast 10 or 15 DSV. The rate of disease progress was reduced in ST compared to NT and CT systems, which were increased by growth in the host on nutrient broth and purified by dimensional confocal laser scanning microscopy in wheat var. Polk within 12 hours of spray inoculation with an incompatible strain of the pathogen. Callose was deposited in walls of cells that lined sub-stomatal chambers, and was restricted to portions of walls of individual cells that faced the chamber. This host cell response was not observed in a compatible interaction between the same fungal strain and wheat cv. Riband, where host cell autofluorescence was not evident until eight days after inoculation during formation of pycnidia.


Phytophthora crown and root rot (PCRR) of pepper caused by *Phytophthora capsici* is a recurrent problem able to incite severe losses over large acreages. In field trials, No Till (NT) systems were associated with increased AUDPC values compared to Conventional Till (CT) and Strip Till (ST) systems. The rate of disease progress was reduced in ST compared to NT and CT systems, but yields were superior for CT. In fungicide efficacy field trials, Ridomil Gold provided control in fields where sensitive strains were dominant. Other systemic or protectant fungicides were not effective. Regional trials for 3 years demonstrated that host resistance limits crop losses. For example, cultivars Paladin, Aristotle and Conquest had significantly lower plant loss incidence (12, 20, and 23% respectively) than the commercial susceptible standard King Arthur (48%). Cultural practices and fungicide applications play a role in PCRR management but host resistance is the most effective.


*Stemphylium botryosum* and *Cladosporium variable* are endemic pathogens of spinach seed crops. In February 2002, volunteer spinach and woody spinach debris were collected from fields in Washington that were cropped to spinach the previous season. Pseudothecia of *Pleospora herbarum* observed on the debris developed into *S. botryosum* when the debris was surface-sterilized and the pseudothecia placed on agar media. Isolations from lesions on leaves of volunteers yielded *C. variable*. Pathogenicity of 13 isolates of *S. botryosum* and 1 isolate of *C. variable* was verified on spinach in the greenhouse. Assays of spinach seed lots from the US (3) and EU (8) showed all were infected with *S. botryosum* (0.3 to 86.0% incidence) and *Cladosporium spp.* (0.2 to 27.3% incidence). Seed samples from one lot were soaked in 1.2% NaOCl for 0, 10, 20, 30, or 40 min, rinsed, and assayed. Pseudothecia of *P. herbarum* developed on 54.8, 23.3, 16.8, 19.0, and 18.3% of the seed, respectively; and *C. variable* developed on 49.0, 0.3, 0.0, 0.0, and 0.0% of the seed, respectively. Pathogenicity of seed isolates of both fungi was confirmed on spinach.


There are no reports of Verticillium wilt in fresh/processing spinach, even though *Verticillium dahliae* (Vd) can be seedborne in spinach. In 2002, a hybrid spinach seed crop in Washington developed late-season wilt symptoms. Assays of the harvested seed and stock seed of the male and female parents revealed 59.5, 44.0, and 1.5%, respectively, were infected with *Vd*. Inoculation of 10 spinach seed lots from the US and EU revealed 8/10 were infected with *Vd*, at 0.8 to 74.8%. Five spinach seed isolates of *Vd* were tested for pathogenicity on three spinach cultivars, by dipping transplants into a spore suspension of each isolate or in sterile water. At inoculation, plants of Cv. A were 2 wk old, and plants of cvs. B and C were 9 wk old. All inoculated plants of cvs. B and C, and 10/20 inoculated plants of Cv. A developed wilt symptoms. None of the control plants developed wilt symptoms. Vd was reisolated from roots and stems of each isolate-by-cultivar treatment, and was found on 26.4% of the seed from 7 of 11 inoculated plants that bolted (47/178 seed), but on 0.0% of the seed from 6 control plants that bolted (0/205 seed).


A confocal laser scanning microscope study of a compatible interaction between wheat and the Septoria tritici leaf blotch pathogen, *M. graminicola* (ana. *Septoria tritici*), was reported previously. Although there are known host resistance genes in this pathosystem, specific resistance mechanisms have not been clearly elucidated. We report here two host responses during an incompatible interaction that may represent manifestations of resistance. Host cell autofluorescence and callose deposition were detected by three-dimensional confocal laser scanning microscopy in wheat var. Polk within 12 hours of spray inoculation with an incompatible strain of the pathogen. Callose was deposited in walls of cells that lined sub-stomatal chambers, and was restricted to portions of walls of individual cells that faced the chamber. This host cell response was not observed in a compatible interaction between the same fungal strain and wheat cv. Riband, where host cell autofluorescence was not evident until eight days after inoculation during formation of pycnidia.


Crown gall, caused by *Agrobacterium tumefaciens*, is a problem in many crops, particularly walnuts. There is no satisfactory control measure. The objective of this research was to test the disease control efficacy of bacteriophage of *A. tumefaciens*. Bacteriophage were isolated from soils by enrichment on nutrient broth with cultures of *A. tumefaciens*. Enrichments were purified by chloroform treatment, tested for ability to form plaques on bacterial lawns, and singly plated three times for homogeneity. Host range tests were performed, and two isolates selected for efficacy testing. Phage were increased by growth in the host on nutrient broth and purified by chloroform and PEG methods. Phage preparations were applied to roots of 2-year-old walnut trees in cold storage, and trees planted in three field trials. Treatments were two phage strains, a combination of the two strains, and a sterile buffer control. Each trial included 7 replications, and 10 trees per plot. After seven months roots were excavated and observed for galls. In two of the trials, one treatment had significantly fewer galls than the controls. Bacteriophage are a potential biological control for crown gall.


Crown and petiole rot caused by *S. rolfsii* var. *delphinii* is a recurring problem in landscape plantings and nursery production fields in the Midwest U.S., therefore, a better understanding of overwinter survival in the soil will aid in development of more effective management recommendations. Sclerotia were produced on strips of inoculated cotton placed on moistened sand in a crisper. After one week at 27°C, sclerotia were easily removed for experimental use. Twenty-five sclerotia of *S. rolfsii* var. *delphinii* were added.
to each of 218 sealed nylon mesh bags. The bags were buried at three depths (surface, 15 cm, and 30 cm) at two locations in Iowa (representing loam and sandy-loam soils) in early Sep 2001. Incidence of sclerotial germination was quantified in Oct and Nov 2001 and in Apr, May, Jun, and Jul 2002 by placing recovered surface-sterilized sclerotia on carrot agar amended with antibiotics. Survival was ~50% at the surface in Jun and Jul, but was highly variable at the 15- and 30-cm depths. This is the first documented evidence that S. rolfsii var. delphini is overwinter in the Upper Midwest U.S. long enough to resume activity in a subsequent growing season.


Pathogenicity tests were conducted on Pulmonaria and Astilbe species as potential hosts of S. rolfsii var. delphini. Both studies were on potted plants in a greenhouse at 25 to 30°C. Inoculum was produced by transferring a plug from a culture of S. rolfsii var. delphini on PDA to an autoclaved carrot disk. After 2 days of incubation at 25°C, three mycelium-infested carrot disks were placed on the soil surface at the base of each plant. All pots were enclosed in plastic bags to maintain high humidity at the inoculation site. Pulmonaria and Astilbe were repeated once. All inoculated plants developed characteristic signs and symptoms of S. rolfsii var. delphini infection within 10 days, whereas non-inoculated control plants remained symptomless. A preliminary report has been published on petiole rot of Pulmonaria longifolia. To our knowledge, this is the first report of petiole rot caused by S. rolfsii var. delphini on Astilbe arnoldii.


Both Phytophthora nicotianae (Pn) and P. palmivora (Pp) attack ornamental plants, but they can be difficult to differentiate due to similar morphologies. 146 papillate, heterothallic isolates of Phytophthora isolated from herbaceous and selected woody ornamental crops in South Carolina from 1995 to 2001 were compared using morphological, physiological, and molecular methods. Isolates of Pn (108) and Pp (38) fell into two distinct groups. Pn isolates were recovered primarily from a broad range of herbaceous plants and Buxus spp. Colony morphologies primarily were ro saceous, and sporangia were mostly non-caducous. Both A1 and A2 mating types were present, and isolates grew at 35°C. Pp isolates primarily were recovered from Hedera helix and Fatsia japonica. Colonies were uniform, and sporangia were caducous with long digitations. Isolates primarily were A1 and did not grow at 35°C. Ribosomal internal transcribed spacer regions (ITS1 and ITS2) from representative isolates of each group were amplified and digested with Alul; patterns matched those published for P. nicotianae and P. palmivora.


To examine the role of rain splash in dispersal of Gibberella zeae (anamorph of Fusarium graminearum), the cause of Fusarium head blight of wheat, rain splash water was collected during rain events at two heights (0, 30 cm) above the soil surface in a reduced tillage wheat field with corn residue during the 2001 and 2002 growing seasons. The collectors consisted of sheltered funnels and flasks. Splashed rain was collected for three rain events in 2001 and 6 rain events in 2002. Spore levels were determined for each rain episode by transferring 1-ml of rain splash water to replicated petri plates with Komada’s selective medium to detection of the presence of G. zeae. Numbers of colony forming units per ml of rain ranged from 0 to 5, and from 0 to 3, in 2001 and 2002 respectively. Colony number was positively correlated with mean intensity of rain (mm/hr) with r = 0.85, and p < 0.0001. Results indicate that rainfall may disseminate Fusarium spores under natural conditions.


To examine the Fusarium species associated with head blight, viable propagules of Fusarium sp were sampled daily from wheat spikes in two fields, and from the air using a Burkard cyclone sampler with an air throughput of 16.5 L per min in three fields, in 2001 and 2002. To recover spores from spikes at each sampling time, five spikes were vigorously shaken for 2 min in 50 ml of distilled deionized water. A 1-ml sample from both the Burkard sampler and spikes washing was transferred to replicated plates of Komada’s selective medium. Fungal isolates were identified on carnation leaf agar. Disease assessments were made daily for incidence and severity at 30 locations per field. F. graminearum was found to be the most prevalent (60%), followed by F. culmorum (25%) and F. equiseti (4%). Mean disease ranged from 1 to 59% for incidence, and from 0.3 to 35% for severity. Air borne inoculum level estimated from Burkard sampler ranged from 0 to 480 cfu per day, and recovered from spikes ranged from 0 to 380 cfu per spike per day. Inoculation tests indicated that F. graminearum and F. culmorum isolates were pathogenic.


To determine the relationship between incidence (I; percent diseased heads) and severity (S; percent diseased spikelets per head) for Fusarium head blight and to determine if severity could be predicted reliably from incidence data, disease assessments were made visually at multiple sample sites (ranging from 30 to 100 per field) in artificially and naturally inoculated research fields and production fields in 2001 and 2002. Incidence and severity data for each assessment date and field were analyzed using linear regression analysis. Ten different, but interrelated, models were fitted to the data and models were compared based on coefficient of determination (R^2), mean square error and residual plots. Mean disease incidence and severity varied among data sets, ranging from 18 to 85% for incidence, and from 2 to 52% for severity. The best fitting model was based on the complementary log-log transformation of I and S (R^2 ranged from 0.69 to 0.91). The functional relationship was consistent among years.


Heterodera glycines, the soybean cyst nematode, depends on a structure of fused plant cells, the syncytium, as its sole source of nutrition. The nematode produces secretory proteins in its esophageal glands, which then are injected into host tissue through the nematode’s stylet. These stylet-secreted proteins, which we termed parasitism proteins, most likely are involved in the formation of the syncytium. Several parasitism proteins of H. glycines contain nuclear localization signals (NLS). We hypothesized that these proteins are imported into host plant nuclei after injection into the cytoplasm through the nematode stylet. Translational fusions of parasitism proteins with GFP and GUS were expressed in plant cells, which demonstrated that H. glycines secretory proteins are imported into plant nuclei. We further hypothesize that these proteins are in gene expression changes in the host plant that lead to an enlargement and fusion of adjacent cells to form the syncytium.

Screening tobacco germplasm for resistance to Rhizoctonia solani, P. E. ELLIOTT (1), H. D. Shew (2), and J. S. Levin (1). (1) Dept of Crop Science; (2) Dept of Plant Pathology, North Carolina State University, Raleigh, NC. Phytopathology 93:S23. Publication no. P-2003-0168-AMA.

Rhizoctonia solani causes stem rot and target spot of greenhouse-produced tobacco seedlings. No fungicides are registered for control of these diseases, so sanitation is the primary disease management strategy. Seedling resistance to R. solani has not been characterized in current tobacco germplasm. The objective of this study was to screen seedlings of a diverse array of accessions, including several classes of tobacco cultivars and related Nicotiana species for resistance to a stem rot isolate (AG 4) and a target spot isolate (AG 3) of R. solani. Experiments were conducted in environmentally controlled growth chambers. Two weeks after seeding, rice grains colonized by R. solani were placed on the surface of the growth medium contained in polystyrene trays floating on a nutrient solution. Symptoms, including death, stem lesions, and target spot lesions were observed over 42 days for stem rot and 56 days for target spot. A wide range of disease incidence was observed across accessions for both diseases, indicating that useful levels of partial resistance may exist for use in future breeding efforts.


The aim of the study was to compare vegetative compatibility tests with assays using primers specific to Fusarium oxysporum f. sp. basilici (FOB) for...
their reliability in identifying pathogens recovered from soil dilution plates. Ninety-five monosporic isolates of *F. oxysporum* were recovered from soil from a field where Fusarium wilt of basil was high. Pathogenicity tests on basil seedlings in the greenhouse identified 12 FOB isolates. Nitrate non-utilizing mutants were selected from test isolates on chlorate agar and paired with tester strains of FOB. Vegetative compatibility (VC) tests correctly identified the 12 FOB isolates and did not identify any nonpathogens as belonging to the FOB VC group. DNA was extracted from each isolate and amplified with known primers in PCR tests (Plant Dis 83:576-581). PCR assays correctly identified the 12 FOB, but also identified 4 nonpathogenic isolates that did not sector on chlorate media. PCR assays were faster, but not as reliable as VC tests. Additional DNA primers are being tested.


*Pea enation mosaic virus* (PEMV) can present a serious problem in pea growing areas of the United States. There is currently no natural source of resistance to this virus incorporated into commercial lines. PEMV-Cp transgenic peas were tested over three successive years for resistance to PEMV under field conditions. Two types of field experiments were conducted; one to determine if the transgene was the cause of resistance and the other to evaluate agronomic characteristics of transgenic lines. Agronomic tests were done using a Random Complete Block design four replicates for seven genotypes. PEMV resistance tests used a 2X8 Factorial arranged in a split-split plot design with cultivar as the main plot and virus infection as a subplot, with four replicates per test. Some PEMV-Cp lines had excellent resistance to PEMV, and had agronomic characteristics that were not significantly different from healthy controls. These results indicate that resistance is effective in the field, and presence of the transgene has no negative impact on the agronomic characteristics of selected lines.


Experiments were conducted to obtain peas with resistance to multiple virus diseases, using a dsRNase gene from *Schizosaccharomyces pombe* (pac1) and an enzymatically inactive mutant (E251K) of pac1. We hypothesized that the enzyme would digest dsRNA, providing a multi-virus resistant phenotype. Transgenic peas were inoculated with 5 viruses and resistance was obtained to three of these (CMV, PEMV, PeSV). However, the enzymatically inactive mutant also provided resistance to these same viruses. Neither transgene had any effect on the potyviruses PSbMV or BYMV. Therefore, to determine if the transgene was the cause of the disease and to test the ability to evaluate agronomic characteristics of transgenic lines. Agronomic tests of pac1 were done using a Random Complete Block design four replicates for seven genotypes. PEMV resistance tests used a 2X8 Factorial arranged in a split-split plot design with cultivar as the main plot and virus infection as a subplot, with four replicates per test. Some PEMV-Cp lines had excellent resistance to PEMV, and had agronomic characteristics that were not significantly different from healthy controls. These results indicate that resistance is effective in the field, and presence of the transgene has no negative impact on the agronomic characteristics of selected lines.


Spores of *Phaeacronemum spp.* and *Phaeoacremonium clamydosporea* have been successfully trapped in California vineyards. These fungi have been shown to cause infection by water splashing onto susceptible pruning wounds. Various grapevine tissues, soil samples and standing water were collected from different vineyards to test for the epiphytic presence of these fungi. All samples were washed in sterile distilled water and filtered before culturing them onto plates of potato dextrose agar amended with tetracycline. *Pa. clamydosporea* was isolated most commonly from the surface of spurs, cordon, trunks and old lignified tendrils. *Phaeoacremonium spp.* were also commonly isolated from the surfaces of spurs, surfaces of roots, leaves, clusters and in soil. It is possible that many of the positive isolations were from tissues previously infected and diseased. However, since many of the tissues were asympomatic or were not positive for these fungi when surface sterilized and cultured, it is speculated that these fungi may have the ability to survive epiphytically on grapevines.


Esca has been a problem on both wine and table grape varieties in California for over 70 years. The erratic nature of symptoms in infected vineyards has resulted in sporadic research efforts to understand disease biology. Symptom expression of esca in vineyards less than 10 years of age has been rare until recently, when we observed esca symptoms in many vineyards less than 3 years of age. The fungus *Phaeoacremonium clamydosporea* has been identified as the primary causal agent of esca in California vineyards with species of *Phaeoacremonium* also playing a role. Data show that weather conditions appear to greatly influence with esca occurrence. In years with high rainfall and warmer temperatures, fruitlets and leaf symptoms of the esca were abundant. Spore trapping data and some speculation seemed to indicate that symptom expression occurs in the year when new infections occur. However, vineyard surveys over the past two years, indicate that symptom expression may not occur until the year following infection. Correlations between symptom expression, rainfall and temperature will be presented.

**Survey of alternative hosts for Pantocea stewartii, causal organism of Stewart's disease in Iowa.** P. D. ESKER (1,2), J. Aalsburg (1), and F. W. Nutter, Jr. (1). (1) Department of Plant Pathology; (2) Department of Statistics, Iowa State University, Ames, IA 50011. Phytopathology 93:S24. Publication no. P-2003-0174-AMA.

A field study was conducted in 2002 to determine the epidemiological importance of alternative hosts for *P. stewartii*, the bacterial pathogen that causes Stewart's disease of corn. At Chinton, Crawfordville, and Johnston, IA, 50 to 100 tillers or stems were collected from each of the following species: *Avena fatua*, *Hordeum jubatum*, *Phalaris canariensis*, *Glycine max*, *Amaranthus palmeri*, *Calyx tenuis*, *Calyx tenuis*, *Setaria lutescens*. Tillers and stems were examined for the presence of corn flea beetle (Chaetocnema pulicaria) feeding scars; if present, 0.10 to 0.13 g of plant tissue surrounding each feeding scar was excised, ground in extraction buffer, and tested for the presence or absence of *P. stewartii* using ELISA. The incidence of tillers or stems with feeding scars that had corn flea beetle feeding scars ranged from 9 to 100%. *Pantocea stewartii* was detected only in yellow foxtail, with 3.3% of samples testing positive. These results indicate the potential epidemiological importance of alternative hosts in the Stewart's disease pathosystem.


Aflatoxins are known carcinogens produced by *Aspergillus flavus* upon infection of several crops. Our previous research has shown the involvement of the 14-3-3 homolog, *maf1* (modulator of aflatoxin), in the biosynthesis of aflatoxin. The 14-3-3 proteins are ubiquitous in their distribution and have functions ranging from regulating primary metabolism in plants to controlling trafficking in cells. Some of these proteins are believed to coordinate the allocation of metabolites among different metabolic pathways. Therefore, a differential approach was used to identify and characterize the role of MAF1 in the regulation of aflatoxin biosynthesis. First, DNA microarray analysis was used to investigate the effect of maf1 disruption on the expression of a group of genes linked to aflatoxin biosynthesis. Disruption of *maf1* affected the expression levels of several genes including those directly involved in aflatoxin biosynthesis, such as *vor1*, *omtA*, and *norA*. Two-Hybrid approach is being used to identify protein interactions utilizing MAF1 as bait. Results obtained from these two approaches are presented in this poster.


A polymerase chain reaction (PCR) assay employing species-specific primers was developed to differentiate *Erysiphe (=Uncinula) necator* from other powdery mildews common to the Pacific Northwest, United States. Conidia, cleistothecia or mycelium were collected from grape leaves using a Burkard cyclone surface sampler and their DNA extracted. Primer pairs, Uncin144 and Uncin511, were developed by aligning internal transcribed spacer 2 (ITS2) and species-specific primers. PCR amplification of DNA products of *E. necator*, but not from powdery mildew species collected from 35 disparate hosts. The appearance of a single 367 base pair fragment by gel electrophoresis was considered evidence of successful detection. Amplification products were sequenced to verify the specificity of *E. necator* primers. This PCR-based test could enable the detection of *E. necator* in field samples with hours of collection.

**Morphological and molecular characterization of *Pythium spp.* causing cavity spot of carrot in California.** J. P. FARRAR (1), S. Van Tuyl (2), and R. M. Davis (2). (1) California State University, Plant Science Dept., Fresno, CA 93720; (2) University of California, Plant Pathology Dept., Davis, CA 95616. Phytopathology 93:S24. Publication no. P-2003-0177-AMA.
Recent changes in the *Pythium* spp. isolated from carrots during disease diagnosis prompted an examination of *Pythium* spp. causing cavity spot in California. Carrots with cavity spot lesions were collected from June 2001 to March 2003. *Pythium* spp. were isolated and pathogenicity tested on carrot. Twenty pathogenic isolates were characterized for temperature, cardinal temperatures, sporangia formation, oogonium and oospore diameter, plerotic or aplerotic oospores, hyphal width, DNA sequence of the ITS region, and mefenoxam resistance. Sixteen isolates were identified as *P. sulcatum*, three as *P. irregularis*, and two as *P. ultimum*. Two irregular and two sulcatum were resistant to mefenoxam based on growth at 50 PPM. *P. sulcatum* isolates did not form sporangia on grass blades or carrot matchsticks in water and corn meal agar or V8 with and without betanicotinocerol flooded with soil-water extract. These may be a new variety of *P. sulcatum* that lacks sporangia.

A combined agar absorbent and BIO-PCR assay for rapid, sensitive detection of *Xylella fastidiosa* (Xf) in grape and citrus. M. Fatmi (1), V. D. Damsteegt (2), and N. W. SCHAAD (2). (1) Inst. Agron. Hassan II, Agadir, Morocco; (2) USDA-ARS, Foreign Disease-Weed Science Research Unit, Frederick, MD. Phytopathology 93:S25. Publication no. P-2003-0178-AMA.

Application of PCR for disease diagnosis has been limited by the presence of PCR inhibitors. Inhibition can be overcome and sensitivity increased with BIO-PCR by enriching bacteria on agar media, however, Xf grows slowly. We have developed an efficient agar absorbent BIO-PCR assay for detecting Xf in grape and citrus plants. By spotting symptomatic grape petioles and citrus leaf veins onto PD2 agar, we found 43% of the grape and 100% of the citrus samples were positive after a 1 or 4 h absorption treatment. All grape samples were positive by 5 days with BIO-PCR. Two of 6 citrus and none of 12 grape samples were positive by direct PCR conducted at spotting. Viable Xf were recovered from all samples after 14 d growth on PD2. For routine assays, we recommend touching tissue to the bottom of a microfuge tube for direct real-time PCR and to each of four PD2 agar microfuge tubes. After one h, add 50 ul of PCR water to two tubes, vortex, and use 5ul for real-time PCR. If negative, assay the other two tubes after 5 days enrichment.


Soybean mosaic virus (SMV; Genus *Potyvirus*; Family *Potyviridae*) is one of the most widespread viruses in soybean (*Glycine max* [L.] Merr). Isolates of SMV are classified as pathotypes G1 through G7 based on the differential reactions on resistant soybean cultivars. Most SMV isolated in the USA is of the G1-G3 pathotypes. The cultivar Hutcheson, released in Virginia in 1988, carries an *Rsv1* allele for resistance to G1-G3, and is widely used in the Middle-South region. SMV isolates that overcome that resistance of Hutcheson are of particular interest for their biological and genomic diversity. Greenhouse experiments showed that 20 field isolates of SMV collected in Virginia and other states between 1998-2002 have pathogenicity characteristics of G5 and G6 pathotypes, and produce distinctive symptoms on Hutcheson. Seven Virginia other states between 1998-2002 have characteristics of G5 and G6 experiments showed that 20 field isolates of SMV collected in Virginia and South region. SMV isolates that overcome that resistance of Hutcheson are seed germination, in the susceptible Essex (*G. max*), and *Rsv1* were shown in field tests to decrease plant yields, seed quality, and the most widespread viruses in soybean (*G. max*), not Essex-

**Characterization of soybean resistance genes and alleles to soybean mosaic virus by tracking virus movement**. A. C. FAYAD and S. A. Tolin. Dept. of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Phytopathology 93:S25. Publication no. P-2003-0180-AMA.

**Resistance to Soybean mosaic virus (SMV) in soybean (Glycine max [L.] Merr.) is controlled by single dominant genes at three distinct loci, based on genetic studies and responses to SMV pathotypes. The mechanisms of resistance at the *Rsv1*, *Rsv3*, and *Rsv5* loci were investigated in differentially responding lines and isolines of a single cultivar containing specific gene(s).**

Greenhouse-grown plants of each line were inoculated with different SMV pathotypes to monitor events during the infection process. Leaves were sampled over time and tested for SMV presence by immuno-printing. Number of infection sites, their size, and proportion of leaf area infected were determined. These data show a continuum of SMV-soybean interactions, ranging from complete susceptibility, local and systemic necrosis showing restriction to virus movement both cell-to-cell and long distance, to extreme resistance with no detectable virus.

**Intra-specific evolution of Rhizoctonia solani AG-1 IA from soybean in Brazil based on polymorphism at the ribosomal DNA ITS1-5.8S-ITS2 operon**. R. C. Fernille (1), M. C. Meyer (1), M. B. Ciampi (1), E. E. Kuramae (1), N. L. Souza (1), and P. C. CERESINI (2). (1) UNESP – Universidade Estadual Paulista, Campus de Botucatu, SP 18610-970; (2) Campus de Ilha Solteira, SP 15385-000, Brazil. Phytopathology 93:S25. Publication no. P-2003-0181-AMA.

**Rhizoctonia solani anamorph group 1 IA (AG-1 IA) is a major pathogen infecting soybean in Brazil, causing the soybean leaf blight.** Despite the fact that *R. solani* AG-1 IA is amongst the most important soybean pathogens, little information is available on its genetic diversity and evolution. For inferring haplotype diversity and inter-specific evolution, polymorphisms at the ribosomal DNA ITS1-5.8S-ITS2 operon were determined and networks of haplotypes were built (Posada & Crandall 2001. Trends in Ecol. & Evol. 16(1):37-45). Sequences from a worldwide outgroup sample represented by rice isolates of AG-1 IA were included in the analysis. Higher haplotype diversity was observed for the Brazilian soybean sample of AG-1 IA in contrast with the rice sample. A total of 27 polymorphic sites were detected on the Brazilian AG-1 IA soybean sample while only 2 were observed on the rice sample. Haplotypes of AG-1 IA from Maranhão (Northern Brazil) occupied the tips of the network, indicating recent origin. The putative ancestral haplotypes detected in the network had Mato Grosso (Central-western) origin.


Root diseases of strawberry limit productivity and are a key focus in our methyl bromide alternatives program. Plant sources were sampled prior to planting, after establishment at important phenological stages to evaluate root rot symptoms and pathogen incidence. In replicated field trials at 3 locations (Clayton and Plymouth NC and Vidalia GA), during 2 growing seasons (2000-2002), the pathogen complex isolated was impacted by plant source, location of experiment, and pre-plant soil treatments. Fungi and stramenopiles most frequently isolated pre-plant and after field establishment were *Fusarium* sp., *Rhizoctonia fragariae*, *Pythium irregularare*, other *Pythium* species, and *Phytophthora cactorum*. Farming systems and soil treatments implemented influenced incidence and crop yield. Pathogens associated with transplants also must be managed, particularly those associated with emerging diseases such as *P. cactorum*.

**Contrasting genotypic diversity in soil borne vs. aerial Phytophthora infestans populations from central Mexico.** S. P. FERNANDEZ-PAVÍA (1), N. J. Grünwald (2), E. Garay-Serrano, C. Belmar-Díaz (4), M. Díaz-Valasis (5), M. Cadena-Hinojosa (5), and G. Rodríguez-Alvarado (1). (1) HAF-UMSNH, Morelia, Mex; (2) USDA-ARS, Prosser, WA; (3) IFIT-CP, Montecillo; (4) PICTIPAPA, Metepec, Mex; (5) INIFAP, Chapingo, Mex. Phytopathology 93:S25. Publication no. P-2003-0183-AMA.

Phytophthora infestans oospores have been found in different soil types in central Mexico. While genotypic diversity has been extensively studied in foliar populations, soil borne populations have not been characterized. Genotypic diversity was compared between *P. infestans* populations from soil borne vs. aerial inoculum according to mating type and isozyme genotype (*Gpi* and *Pep*). Isolates were obtained from soil (oospore populations) and diseased foliage collected in potato fields in central Mexico. Both mating types were present in all populations, but the A1 mating type was more frequent in soil populations. Frequencies of multilocus genotypes varied among regions and between soil borne and foliar inoculum forming oospore areas. Novel genotypes were found in all soils. Preliminary data confirms the hypothesis that genotypic diversity is higher in populations derived from oospores than from aerial inoculum.


Commercial production of high quality papaya in Puerto Rico has been declining due to severe losses caused by *Papaya ringspot virus* (PRSV). Presently, there is no information as to which weeds are alternate hosts of PRSV on the island. Replicated plots were established in which half the papaya plants were weeded regularly and the other half in which weeds were allowed to grow. PRSV infection was allowed to occur naturally. A succession of weeds was collected and indexed with DAS-ELISA for the presence of PRSV. Results indicate that the same weeds test consistently positive for the virus, in all the replicates of the experiments. A total of 38 species were tested. The following weeds were positive to PRSV:
Parthenium hysterophorus, Chamaesyce nutans, Poinsetia heterophylla, Sida carinata, Ipomoea turcicus, Phaseolus lunatus, Indigofera spp., Tridax procumbens, Amaranthus dubius, Wedelia trilobata and Emilia sonchifolia, and Synedrella nodiflora.


In nature bacteria are often organized in biofilms. Biofilms on inert surfaces have been studied in depth using confocal scanning laser microscopy (CSLM) with a variety of fluorescent probes. In contrast, there have been few studies of biofilms on living surfaces including plants. CSLM in combination with the LIVE/DEAD BacLight Viability Kit (Molecular Probes, Inc.) were used in a survey of biofilms on retail alfalfa, clover and mug bean sprouts. Biofilm area and depth were quantified by use of a 20X dry lens due to its large free working distance and field of view. Correction factors were determined using spherical fluorescent beads of known diameter to correct for distortion in z-axis measurements. Biofilms were readily observed and consisted primarily of five bacteria. Average areas for biofilms for the sprout samples were 201 um² to 470 um² (range of 32 to 7283 um²). The average depth of the observed biofilms was from 2.0 to 6.3 microns (range of 1.7-12.6 microns) indicating only a single to a few layers of cells. Despite a lack of considerable depth, such biofilms on sprout cotyledons may harbor plant and human pathogens making their eradicating more problematic.


The incorporation of biological and abiotic suppressive factors into potting media may augment disease management strategies. The efficacy of aluminum (Al)-amended potting medium containing 20% composted swine waste (CSW) was assessed for control of Phytophthora parasitica. Steamed and unsteamed medium was amended with no Al or at 0.0079 g Al/g medium as an Al₂(SO₄)₃ solution at pH 4 or 6. Amended media were placed into Buchner funnels maintained at 2.5 kPa soil moisture tension and then infested with leaf disks colonized by P. parasitica. Leaf disks were buried for two-day durations beginning on day 0, 6, 13, and 21. Leaf disks were removed and the sporangia on the edges of each leaf disk counted. Medium treated with the pH 4 solution reduced sporangia production by 60% on day 0. Exchangeable Al levels decreased over time, and abiotic suppression was only observed at >2 µM Al/g medium. Sporangia production in unsteamed media was reduced by 50% on leaf disks buried on days 6, 13, and 21, but not day 0. Al-amendment of a 20% CSW potting medium with the LIVE/DEAD Light Viability Kit (Molecular Probes, Inc.) were used to test the pathogenicity of transformants on S. sapinea. Using an Agrobacterium-mediated transformation protocol, S. sapinea was sensitive to a range of monomeric Al species, whereas the pH dependant suppression developed.


Aluminum (Al) is toxic to Thielaviopsis basicola and Phytophthora parasitica. Al fungitoxicoc occurs over a wide pH range, so multiple species of Al may be responsible for suppression. The goals of this work were to quantify the toxicity of monomeric Al species to P. parasitica (sporangia) and T. basicola (chlamydospores) and to determine if Al accumulates in the pathogens. Al levels ranging from 0-100 µM and pH levels between pH 4-6 were modeled using GEOCHEM-PC. Colonies were grown in 5% carrot and enhanced suppression of P. parasitica (sporangia) and occurred before biological suppression developed.

Host range and impacts of Claviceps purpurea (Fr.) Tul on native and invasive Spartina spp. in West Coast estuaries. A. J. FISHER. Dept. of Vegetable Crops, University of California, Davis, CA. Phytopathology 93:S26. Publication no. P-2003-0188-AMA.

The fungal pathogen Claviceps purpurea is present on populations of native Spartina foliosa in the San Francisco Bay, California. In the San Francisco Bay area, the invasive Spartina alterniflora has hybridized with the native Spartina foliosa producing a hybrid that is also highly invasive. Three years of field sampling in the San Francisco Bay area suggest that the native S. foliosa is more heavily impacted by C. purpurea than S. alterniflora or invasive Spartina hybrids. Under controlled conditions, both the native and the invasive Spartina are equally susceptible to the pathogen. Using seed production as a measure of fecundity, results from three years of experiments show that infected inflorescences produce fewer seed than uninfected inflorescences. Seed from infected inflorescence weigh less than seed from uninfected inflorescences. Using RAPD nuclear DNA markers, analysis showed little genetic diversity in C. purpurea isolates collected throughout the San Francisco Bay Area. Sampling in Humboldt Bay showed no C. purpurea on the invasive S. densiflora and sampling in Willapa Bay, Washington produced very low rates on the invasive S. alterniflora.


Cercospora zeae-maydis (Tehon and Daniels), the fungal pathogen causing Gray Leaf Spot (GLS) of Maize (Zea mays L.), overwinters as black stromata in corn residue. An understanding of this overwintering mechanism is important in controlling the disease. Production of stromata in culture morphologically and functionally similar to those produced in the field is critical these studies. Pine tip blight is a serious disease worldwide of many conifers, especially exotic pine species grown in managed plantations. Successful genetic transformation of the causal agent, Cercospora zeae-maydis, has not been reported. Various transformation protocols utilizing polyethylene glycol or Agrobacterium tumefaciens were tested for their efficacy with S. sapinea. Using an Agrobacterium-mediated transformation protocol, S. sapinea is more heavily impacted by C. zeae-maydis grown on V-8 agar were transferred to flasks containing potato dextrose broth and placed on a shaker for 4-6 days. Aliquots of the broth medium containing discrete black stromata were transferred to sterile glass petri dishes lined with filter paper. Black stromata were removed and transferred to new sterile glass petri dishes lined with filter paper which were sterilized. Laboratory produced stromata were morphologically more uniform than stromata from diseased leaves in the field; however, functionally they are similar both in conidiophore and conidia production. This is an effective method for increasing stromata, in quantity, for epidemiological experiments.


Pine tip blight is a serious disease worldwide of many conifers, especially exotic pine species grown in managed plantations. Successful genetic transformation of the causal agent, Sphaeropsis sapinea, has not been reported. Various transformation protocols utilizing polyethylene glycol or Agrobacterium tumefaciens were tested for their efficacy with S. sapinea. Using an Agrobacterium-mediated transformation protocol, S. sapinea is more heavily impacted by C. zeae-maydis grown on V-8 agar were transferred to flasks containing potato dextrose broth and placed on a shaker for 4-6 days. Aliquots of the broth medium containing discrete black stromata were transferred to sterile glass petri dishes lined with filter paper. Black stromata were removed and transferred to new sterile glass petri dishes lined with filter paper which were sterilized. Laboratory produced stromata were morphologically more uniform than stromata from diseased leaves in the field; however, functionally they are similar both in conidiophore and conidia production. This is an effective method for increasing stromata, in quantity, for epidemiological experiments.


Recent Phytophthora blight epidemics in New Jersey pepper fields with a history of mefenoxam use for vegetable production resulted in the need to evaluate the sensitivity of Phytophthora capsici populations in this region for mefenoxam sensitivity. Using global positioning equipment, plants exhibiting crown rot symptoms were collected from four counties, and isolations were made onto selective media. Isolates were grown for three days on clarified V8 agar amended with 0, 5, and 100 ppm Roidmil Gold 4E (4 lb/gal mefenoxam). Linear growth measurements for each rate were averaged, percent growth as compared to the unamended controls (0 ppm) were determined, and isolates were designated as sensitive, intermediate, or insensitive to mefenoxam. A high percentage of isolates from Salem and Gloucester counties were insensitive or intermediate in both 2001 and 2002; however, a high percentage of isolates collected from Atlantic and Cumberland counties in 2001 were sensitive but a greater proportion of isolates collected in 2002 were intermediate.


Chipped, non-composted mulches may contain materials derived from forest residues and invasive Spartina hybrids. Under controlled conditions, both the native and the invasive Spartina are equally susceptible to the pathogen. Using seed production as a measure of fecundity, results from three years of experiments show that infected inflorescences produce fewer seed than uninfected inflorescences. Seed from infected inflorescence weigh less than seed from uninfected inflorescences. Using RAPD nuclear DNA markers, analysis showed little genetic diversity in C. purpurea isolates collected throughout the San Francisco Bay Area. Sampling in Humboldt Bay showed no C. purpurea on the invasive S. densiflora and sampling in Willapa Bay, Washington produced very low rates on the invasive S. alterniflora.
of contaminating urban landscapes and gardens. An experiment was conducted to determine if Vd-infested wood chips could serve as a source of inoculum for woody hosts such as amur maple, redbud, and green ash. Infested wood chips were incorporated into a potting mix and stem sections from trees planted in this mix were assayed for Vd. To date, Vd has been recovered from 20% of amur maples. To determine the effect of composting on recovery of Vd, compost piles of three different compositions were constructed. Infested wood chips were placed inside the piles, and were assayed for Vd after 4 and 8 weeks. Vd was recovered from infested wood chips composted for 4 and 8 weeks, but there were no significant differences in recovery amongst the three compost treatments at either time point. Eggplant, treated with 8-wk-old composted wood chips became infected with Vd. This work suggests that non-composted and improperly composted wood chips can be potential sources of Vd in urban settings.


_Gaeumannomyces graminis var. tritici_ (Ggt) causes take-all disease of wheat. During infection the fungus produces melanized hyphae, which cluster to form mycelial mats. G. graminis _var. tritici_ (Ggt) was transformed with a plasmid containing the hygromycin resistant gene (_hph) and a 1-kb fragment of a polyketide enzyme synthase gene (PKS1). Polyketide synthase is the first enzyme in the melanin biosynthesis pathway. Of 1,000 hygromycin resistant transformants generated, only 25 putative transformants were resistant to hygromycin and able to produce melanin. A polymerase chain reaction and southern hybridization analysis verified integration of the _hph_ gene and PKS1 partial sequences into the transformants’ genomes. Pathogenicity tests are underway with non-melanized transformants to study the role of melanin in take-all development in wheat.


_A Longidorus_ sp. has been associated with stunted lobolly pine seedlings at a Georgia nursery. Population densities averaged 38 _Longidorus_1/100 cc soil in March 2002 prior to application of treatments. Plots were treated with dazomet (560 kg/ha), metam sodium (380 kg/ha) or oxamyl (4.5 kg/ha) and treated with dazomet (27 cm) and metam sodium (22 cm) soil. Seedling heights in plots treated with dazomet (27 cm) and metam sodium (22 cm) were greater than those in oxamyl (12 cm) or the control (10 cm) plots. Soil fumigation with dazomet or metam sodium may have limited use for long-term control of this _Longidorus_ in pine production areas.


To identify genes involved in biocontrol of Fusarium wilt of tomato by nonpathogenic Fusaria, 415 isolates of _F. oxysporum_ were screened for pathogenicity and biocontrol ability. Biocontrol ability ranged from low to high. Differences in gene expression were studied with differential display analysis by extracting RNA from 2 pathogenic and 2 biocontrol isolates. cDNA was made from RNA by reverse transcription and was amplified using anchored and random primers. The resulting products were separated by gel electrophoresis and fragments visualized with a fluorescence scanner. Twenty-two cDNA bands with potential differential expression between the pathogen and biocontrol were identified. Excised amplicons and the Bands with high homology to known sequences included an ATP synthase, an ABC transporter, and a lysine permease. ABC transporters can function in detoxification of toxic compounds and have not been previously reported in Fusarium. Studies continue with additional isolates to determine whether these genes are present in the different phenotypes (but expressed differently), as well as studies on the regulation of the ABC transporter.


Early blight is a common disease of potato and tomato. E. G. Simmons asserted that two morphologically and culturally distinct species were responsible: _Alternaria tomotaphila_ on tomato, and _Alternaria solani_ on potato. We studied 24 living isolates and 4 herbarium samples and used Simmons’ characteristics to confirm identity: mycelium color, amount and distribution of spores, length and width of the beak, and the number of 2-beaked spores. To determine if these morphological differences were supported by DNA sequences and physiology, leaf inoculations and fungicide response tests were performed. Isolates were sprayed at standardized concentrations onto attached tomato and potato leaves and the presence and severity of disease was measured after 7-8 days. _A. tomotaphila_ was more virulent on tomato, while _A. solani_ was more virulent on potato. Spores of all isolates were subjected to the commonly used fungicides chlorothalonil and azoxystrobin and the percent germination was determined. Results showed no segregation of fungicide response by species, but did reflect the spraying history of fields.

**Use of coalescent-based methods to infer rates of intraspecific recombination in RNA plant viruses.** R. FRENCH and D. C. Stenger. USDA-ARS, University of Nebraska, Lincoln, NE. Phytopathology 93:S27. Publication no. P-2003-0197-AMA.

Phylogenetic analysis of sequences from multiple isolates of plant viruses often reveals a radial tree topology with little bootstrap support for internal branches. These phylogenetic trees based on nucleotide sequences of 49 U.S. isolates of wheat streak mosaic virus (WSMV) was partly due to homoplasy, which may result from recurrent mutation or a history of recombination. Under the coalescent theoretical framework one may calculate the relative likelihood and rate of recombination given the observed number and sample frequencies of biallelic polymorphic sites. The inferred recombination rate for _WBSV_ was much higher than the sequence divergence rate. For comparison, recombination rates were estimated for sample sets of coat protein gene sequences from GenBank for 34 barley yellow dwarf disease WYM isolates, 67 cucumber mosaic virus (CMV) isolates, and 26 yam mosaic virus (YMV) isolates. Apparent recombination rates for YMV and CMV were similar to WSMV while that of _WBSV_ was at least 10-fold lower. These results suggest that recombination may be an important factor affecting the genetic structure of plant RNA virus populations.

**Phytophthora capsici associated with weeds in conventional vegetable farms of southeast Florida.** R. D. FRENCH-MONAR (1), P. D. Roberts (2), and J. B. Jones (1). (1) Plant Pathology Department, University of Florida, Gainesville, FL 32611; (2) Plant Pathology Department, SWFREC, Lake Alfred, FL 34458. Phytopathology 93:S27. Publication no. P-2003-0198-AMA.

Weeds may play an important role in the survival of Phytophthora _capsici_ from one growing season to another. Between May 2001 and March 2002, weed samples were collected in Palm Beach County from conventional vegetable farms. Most weed samples were common ragweed (_Ambrosia artemisiifolia_), fall panicum (_Panicum dichotomiflorum_), purple nutsedge (_Cyperus rotundus_), yellow nutsedge (_C. esculentus_), common purslane (_Portulaca oleracea_), Carolina wild geranium (_Geranium carolinianum_), and black nightshade (_Solanum nigrum_). Root tissue was disinfested and plated on a medium semiselective for _Phytophthora_ spp. and on PDA amended with antibiotics for isolation of general fungi. Based on PCR, morphological characterization, and pepper bioassays, _Phytophthora_ spp. isolated from _P. oleracea_, _G. carolinianum_, and _S. nigrum_ were identified as _P. capsici_. Fourteen other fungal genera were isolated from root tissue. Weed species may harbor inoculum of _P. capsici_ during and between vegetable growing seasons.

**Relationships between plant maturity, disease and yield loss due to Verticillium wilt.** K. E. FROST (1), D. I. Rouse (1), and S. H. Jansky (2). (1) UW-Madison, Dept. of Plant Pathology, Madison, WI 53706; (2) UW-Stevens Point, Dept. of Biology, Stevens Point, WI 54481. Phytopathology 93:S27. Publication no. P-2003-0199-AMA.

The assessment of Verticillium wilt of potato is difficult because clone maturity may confound disease assessment. To characterize possible relationships between plant maturity, disease symptomology and yield loss due to _Verticillium dahliae_ (Vd), 150 clones of tetraploid progeny from 2x x 4x and 4x x 2x crosses of (2x _Solana tuberosum_-wild species hybrids with
(4x) potato were planted in a Vd screening field and clean field. Plant vigor, vine maturity, disease and yield were evaluated for each plot. In the Vd screening field, area under the disease progress curves (AUDPC) averaged 1353 ± 629 units greater, yields averaged 46% (±31%) lower, and maturity scores averaged 0.82 ± 0.59 units greater. There was no correlation between difference in maturity and difference in AUDPC or difference in maturity and yield loss. There was a significant correlation, \( r^2 = 0.277 \) (\( P = 0.005 \)), between the difference in AUDPC and yield loss. Stem and root colonization, PCR and ELISA are being used to detect Vd to identify possible improved assessment methods in potato.


The developmental expression of cellulase genes in *H. glycines* was determined using quantitative real-time RT-PCR. These studies revealed: i) \( hq\)-eng-2 was highly expressed in parasitic second-stage juveniles (J2) and adult males, ii) \( hq\)-eng-4 and \( hq\)-eng-4 were highly expressed in eggs containing J1/J2, iii) \( hq\)-eng-5 was mainly expressed in pre-parasitic J2, and iv) \( hq\)-eng-6 was mainly expressed in parasitic J2. All cellulase genes had relatively lower expression levels in late parasitic stages. All six recombinant HG-ENGs degraded CM-cellulose, and optimum enzyme activity ranged from 100 to 1650 U/mg protein. Treatment with 1 mM of Co\(^{2+}\), Mg\(^{2+}\), Fe\(^{2+}\) did not affect enzyme activity, while Zn\(^{2+}\), Cu\(^{2+}\) and Mn\(^{2+}\) inhibited enzyme activity from 23% to 73%. In tests with 12 different substrates, enzyme activity was restricted to substrates with beta-1,4 linkages. HG-ENG-5 and HG-ENG-6 also had relatively high activity on xylan. HG-ENG-5 and HG-ENG-6 slightly degraded microcrystalline cellulose suggesting that these cellulases might directly degrade plant cell walls.


Field experiments were conducted in Marin, Alameda, and Santa Cruz counties to evaluate the effectiveness of chemical treatments for controlling Sudden Oak Death. Nursery-grown saplings as well as native populations of mature coast live oak (Quercus agrifolia) and tanoak (Lithocarpus densiflora) were tested. Trees were infected by placing a small amount of mycelium of *Phytophthora ramorum* under the bark. A variety of commercially available chemicals that have been used on other Phytophthora species were evaluated in at least 10 different trials. Application methods included trunk injections, soil drench applications, and foliar sprays. Treatments with phosphonate compounds significantly and consistently reduced lesion size in both oaks and tanoaks. Injecting the chemicals into the trunk of the tree was found to be the most effective method. Treatment of the tree prior to infection was found to be significantly more effective at controlling the disease than treatment after infection. Phosphonate treated trees remained resistant to new *P. ramorum* infections for at least three months. However, mature trees may require a longer period following chemical treatment to become resistant to infection.

*Phytophthora ramorum*: An emerging forest pathogen. M. GARBELLOTTO (1), D. M. Rizzo (2), J. M. Davidson (2), K. Ivors (1), P. E. Maloney (2), D. Hüberli (1), K. Hayden (1), T. Harnik (1), and S. T. Koike (4). (1) Department of ESPM, 151 Hilgard Hall, University of California, Berkeley, CA 94720; (2) Department of Plant Pathology, 1 Shields Ave., University of California, Davis, CA 95616; (3) Pacific Southwest Research Station, USDA Forest Service, P.O. Box 245, Berkeley, CA 94710; (4) Cooperative Extension Service, University of California, 1432 Abbott St., Salinas, CA 93901-4507. Phytopathology 93:S28. Publication no. P-2003-0203-AMA.

Aided initially by taxon specific PCR primers, we have determined that *Phytophthora ramorum*, cause of Sudden Oak Death, is not restricted to oaks, but has a host range encompassing at least 11 families and 18 plant species, including dominant tree species such as redwood and Douglas-fir, as well as understory shrubs, and herbaceous plants. This pathogen can infect all of the dominant woody species in coastal woodlands of Central California. While forest *Phytophthora* species are commonly known to cause root decay and/or canker, P. *ramorum* appears to have a novel adaptational feature that causes above ground disease symptoms, and can be dispersed asexually. Analysis of North American isolates of *P. ramorum* using amplified fragment length polymorphisms (AFLPs) indicates largely a clonal population with no subdivisions based on geography or host of origin. The AFLP data also indicate that the European and North American populations are quite distinct. The finding of *P. ramorum* on many host species in a relatively short timeframe, underlines the power of DNA-based diagnostic approaches, and has important consequences for determining the epidemiology, ecological impact and management of this important new forest pathogen.


A low, uniform level of disease may be indicative of a large number of initial pathogen propagules. To evaluate how reliable this inference might be, we considered a range of parameter values describing dispersal in a simulation model of the spatial developmental of plant disease epidemics. Though it was possible to generate a uniform pattern of low disease levels from a small number of initial propagules, this was an unlikely result under the assumptions of our model for parameters describing *Phytophthora infestans*. In contrast to temperate regions, infection by *P. infestans* in Ecuador and Peru is often uniform, implying a large number of initial propagules. Disease management practices that depend only on reducing *P. infestans* spore production within a field may be less useful in the highland tropics because of high levels of external inoculum.


Although *Pythium aphanidermatum* and *P. irregularare* are important plant pathogens their population genetics have received little attention. In order to assess the genetic diversity of these species we analyzed their internal transcribed spacer (ITS) region sequences and performed Amplified Fragment Length Polymorphisms (AFLP) analysis. These species have distinct patterns of genetic variation. The ITS sequence of *P. aphanidermatum* is identical in all the isolates sampled and analysis of AFLP dominant markers indicates largely a clonal population with no variability in fungicide resistance and geographic distribution. Analysis of the ITS region sequences as well as AFLP analysis supports the presence of a highly divergent population or cryptic species structure within *P. irregularare*, with at least two subgroups that overlap in their geographic distribution.


Osmotins are a family of proteins associated with the survival capability of a plant to biotic and abiotic stresses. Osmotins have been implicated in broad-spectrum pathogen resistance as well as drought, salt, and cold tolerance. Although osmotin genes have been characterized from wild *Solanum* species, very little is known about osmotin genes from cultivated *S. commersonii* osmotin sequences was conducted on genomic DNA of thirty-three potato cultivars. A single 0.5 kb nucleotide amplification product was noted for each. Cultivars could be differentiated as having 0, 1, 2, or 3 of the osmotin gene products. Gel
purification of all PCR products was conducted followed by complete sequence analysis on both strands. Sequence alignment revealed a range of 91-99% similarity to S. commersoni osmotin genes. A phylogenetic tree was generated for cultivar comparison based on osmotin gene amplified products. Osmotin gene family members may be differentially expressed during various types of stress and studies are ongoing to address this.


Xanthomonas leaf blight of onion, caused by Xanthomonas campestris pv. allii, is an economically important disease in many onion producing regions. Resistance to Xanthomonas leaf blight has been identified in two short day onion cultivars, but these cultivars are no longer available commercially and resistance in long day cultivars has not been reported. Gmemplasm accessions and cultivars of Allium cepa L. and A. fistulosum L. were screened in growth chamber assays to identify new sources of resistance to Xanthomonas leaf blight. Plants were pin-pricked at five equidistant locations on each leaf with a sterile needle bearing a bacterial mixture of X. campestris pv. allii from five day-old nutrient agar plates. Plants were subsequently incubated at 26°C and 100% relative humidity for 14 days and lesion expansion from each pinprick was measured to quantify disease severity. Differences in susceptibility to Xanthomonas leaf blight were observed and are discussed in relation to an overall integrated pest management program.


Xanthomonas campestris pv. allii (Xca) population dynamics on leaves of various pulse crops and weeds were monitored in growth chamber assays. Epiphytic populations of the spontaneous rifampcin mutant Xca strain RO177 were recovered and enumerated by leaf rinsing and subsequent spiral plating onto rifampcin-amended media. Populations increased on all pulse crops evaluated and several weeds when maintained at 26°C and 100% relative humidity, but disease symptoms were never observed. In other studies, population dynamics of strain RO177 in pulse crops were monitored in planta. Strain RO177 was pressure infiltrated into leaves, and leaf discs were removed and homogenized before plating onto rifampcin amended nutrient agar. Populations of RO177 increased in planta in all pulse crops evaluated when infiltrated into leaves, but disease symptoms were absent. Field studies are currently evaluating survival of Xca on pulse crops and weeds and implications for Xanthomonas leaf blight of onion management.

**Alternatives to methyl bromide for calla lily production.** J. S. GERIK (1), S. S. Vail (1), C. L. Elmore (2), and I. D. Greene (3). (1) USDA-ARS, Parlier, CA 93648; (2) U.C. Davis, Davis, CA 95616; (3) Golden State Bulb Growers, Moss Landing, CA 95039. Phytopathology 93:S29. Publication no. P-2003-0209-AMA.

A trial was established in Marina, CA to test alternatives to methyl bromide for calla lily production. The pest targets were weeds and soilborne pathogens. Chemicals were applied through the drip irrigation tape in either water used to apply the fumigants may be important. Yield and quality losses due to Karnal bunt (KB) in the U.S. have been insignificant. However, international quarantines have made KB an important trade issue. To evaluate the relative susceptibility of U.S. spring wheat to KB, 140 cultivars representing all market classes were screened for resistance during at least three seasons. For each entry and year, 10 spikes from each of 2 planting dates were injected at awns emerging stage with an aqueous suspension of sporidia. The mean percent infected kernels/spike from each entry, planting date, and year, was calculated for a final disease rating. Among 56 hard red, 3 hard white, 5 soft red, 15 soft white, and 26 durum wheats to KB, 140 cultivars representing all market classes were screened for resistance during at least three seasons. For each entry and year, 10 spikes from each of 2 planting dates were injected at awns emerging stage with an aqueous suspension of sporidia. The mean percent infected kernels/spike from each entry, planting date, and year, was calculated for a final disease rating. Among 56 hard red, 3 hard white, 5 soft red, 15 soft white, and 26 durum wheats, the percent infected kernels ranged from 0 to 40.6%, 3.8 to 42.0%, 1.8 to 25.3%, 2.0 to 36.4%, and 0 to 29.4% respectively. The cultivar ‘ Thatcher’ is in the pedigree of many of the most resistant hard red cultivars, including ‘Chris’ that carries the dominant resistance gene Kb1. This suggests Kb1 might be contributing to disease control in the most resistant hard red wheats. ‘Emore’ is a common parent in the most resistant durum cultivars, suggesting that a component of this cultivar is contributing to resistance.

**Influence of bacterial populations on leaf spot development in resistant and susceptible lettuce cultivars.** P. H. GOLDSMITH (1), S. T. Koike (2), E. Ryder (1), and C. T. Bull (1). (1) USDA-ARS, Salinas, CA; (2) UCCE, Salinas, CA 93950. Phytopathology 93:S29. Publication no. P-2003-0214-AMA.

Bacterial leaf spot, caused by Xanthomonas campestris pv. vitians (Xcv), is an important disease on California lettuce. In greenhouse and field studies, we found significant levels of resistance to Xcv in some commercially available cultivars. This indicates that there is potential for use of resistance as a disease management tool in California. We examined the influence of...
bacterial concentration on development of leaf spot in cultivars that we had classified as resistant and susceptible. In field studies bacterial leaf spot severity was greater at high applied concentrations (10^5 cfu/ml) of bacteria than at moderate application levels (10^3 cfu/ml) on a susceptible lettuce cultivar. At application levels below 10^2 cfu/ml, lettuce did not develop disease in either field or greenhouse studies. In preliminary greenhouse experiments, the relationship between applied bacterial concentration and disease severity was linear in resistant varieties and exponential in susceptible ones. We are examining additional susceptible and resistant cultivars to determine if this difference is consistent.

Molecular evolution of Phytophthora infestans. L. GOMEZ (1), J. L. Thorne (2), and J. B. Ristaino (1). (1) Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695; (2) Dept. of Statistical Genetics, North Carolina State University, Raleigh, NC 27695. Phytopathology 93:S30. Publication no. P-2003-0215-AMA.

A phylogenetic approach is being used to understand the molecular evolution of P. infestans. We asked the question whether mitochondrial or nuclear DNA sequence evidence justifies the specific hypothesis that a common ancestor of P. infestans originated in South American or Mexican populations. Isolates of P. infestans were obtained from various locations including Brazil, Bolivia, Ecuador, Peru, Costa Rica, Mexico, USA, and Ireland. One isolate of P. mirabilis and one isolate of P. cinnamomi were also included as outgroups. Portions of seven genes were sequenced including three mitochondrial genes Nad4 (P2 region), Rpl5 (P3 region), and Cox1 (P4 region) and four nuclear genes, including beta-tubulin, translation elongation factor (Tef1), Ras, and 18S rDNA. Preliminary phylogenetic analysis, using maximum parsimony and neighboring analysis is underway and indicates variation among isolates. Concordance among genealogies is currently being analyzed.


The mechanisms by which the bacterial wilt pathogen Ralstonia solanacearum causes disease are subtle yet sufficient to cause drastic crop losses. A variety of secreted compounds, including plant cell wall degrading enzymes, type III effectors, and extracellular polysaccharide, collectively contribute to full-blown bacterial wilt virulence. Many of the known secreted factors move out of the cell via Sec-dependent translocation through the inner membrane. Using the recently available R. solanacearum GM1000 sequence we have chosen a reverse genetics approach to identify Sec-dependent secreted proteins by mutating tatC, a gene encoding the twin arginine translocation (TAT) secretion pore. Bioinformatic analysis of the GM1000 genome using the algorithm TATFIND identified 71 likely TAT-dependent secreted proteins. Some of these were independently identified as highly expressed during bacterial growth in tomato by in vivo expression technology (IVET). We will discuss the virulence of a tatC mutant of R. solanacearum, as well as the possible function of TAT-dependent secreted proteins in bacterial wilt pathogenesis.


Isolates of Colletotrichum spp. and G. cingulata from symptomatic fruit and leaves from the US and Brazil were characterized based on morphology, mtDNA RFLPs, VCGs, and the sequence of a 200 bp intron of the glyceraldehyde phosphate dehydrogenase gene. Pathogenicity on leaf and fruit was also determined. Isolates of C. gloeosporioides, C. acutatum and G. cingulata were distinguished based on colony color, conidial shape and the ability to produce perithecia in culture. Diversity among the isolates. Brazilian isolates of each species could be distinguished from the US isolates. All isolates tested were pathogenic on fruit. Over 150 leaf isolates belonged to 3 of 13 VCGs and all leaf isolates tested for mtDNA RFLPs (64) were in 3 of 10 mtDNA haplotypes. All leaf isolates from the US belonged to a single VCG and a single mtDNA haplotype.

Potato virus Y (PVY) has reemerged as a serious threat to seed potato production in areas of the northeastern United States. This reemergence may partially be linked to recently introduced, asymptomatic potato varieties where disease incidence is frequently underestimated. Research plots were established in 2001 and 2002 at University Park and Presque Isle, ME to monitor the efficiency of tuber infection among potato varieties Atlantic, Shepody, and Russet Norkotah relative to the timing of PVY infection in the growing season. Early season infections of PVY (pre-bloom developmental stage) resulted in the highest proportion of infected tubers compared to infections which arose during and following the bloom developmental stage. Throughout the growing season, PVY infection of the late-season maturity cultivars Norkotah and Shepody was found in the highest proportion of developing potato tubers (80%) followed by medium maturing varieties Shepody (25%) and Atlantic (24%). Knowledge of PVY movement patterns into and distribution within developing potato tubers will aid in the reliability and accuracy of detecting PVY in dormant tubers.


Fusarium root rot, caused by Fusarium solani f. sp. pisi, is one of the most important fungal diseases of pea (Pisum sativum) and is found in most pea growing areas around the world. Currently, no commercial cultivars are resistant to this pathogen. Availability of new sources of partial resistance could provide another tool for managing Fusarium root rot. Three hundred eighty-seven accesses from the Pisum core collection were evaluated for resistance to Fusarium root rot in two independent experiments. Nonparametric analysis of variance conducted on ranks of disease severity for each accession indicated that the two experiments corresponded well. Forty-four PI lines with a disease severity rating of 2.5 or less on a 0 to 5 scale were selected as serving as good sources of partial resistance. Resistance was quantitative in nature and immunity to Fusarium root rot was not found. Comparison of disease resistance data for Aphanomyces root rot and Fusarium root rot showed a weak, but significant and positive correlation. The 44 PI lines selected are currently being evaluated in field trials to validate results from this study.


The orchid Spiranthes cernua grows in moist areas in the southeastern US and cultivated varieties are now being used as flowering perennials. We have previously reported Dasheen mosaic virus (DoMV) infection in commercially-grown plants exhibiting chlorotic blotching and/or mosaic symptoms. Other similarly symptomatic plants were, however, ELISA negative using DoMV polyclonal antisera, yet gave a positive reaction with the resistance Fusarium root rot in two independent experiments. Nonparametric analysis of variance conducted on ranks of disease severity for each accession indicated that the two experiments corresponded well. Forty-four PI lines with a disease severity rating of 2.5 or less on a 0 to 5 scale were selected as serving as good sources of partial resistance. Resistance was quantitative in nature and immunity to Fusarium root rot was not found. Comparison of disease resistance data for Aphanomyces root rot and Fusarium root rot showed a weak, but significant and positive correlation. The 44 PI lines selected are currently being evaluated in field trials to validate results from this study.


Leprosis caused by citrus leprosis virus (CiLV), a plant rhabd-like virus, is the most important viral disease of citrus in Brazil at the present time. This disease has been prevalent in South America for many years, but recently the disease has appeared in Venezuela and Panama, with unconfirmed reports in Guatemala, Costa Rica and Honduras. A study of citrus leprosis from Panama was initiated using transmission electron microscopy and by characterization of the viral genome. Bacilliform virus-like particles enclosed in vesicles associated with the endoplasmic reticulum were observed in infected tissues. Some similarity with both animal and plant rhabdoviruses.

S30 PHYTOPATHOLOGY
Mature grapevine decline is an increasingly serious problem for vineyards in Pennsylvania. The symptoms of decline include reduced shoot growth, sparse yellow foliage, necrosis and stuntng of the roots, reduced yield and inferior fruit. A recent study that surveyed Pennsylvania vineyards found that Cylindrocarpon destructans was isolated repeatedly from the roots of declining grapevines. In an effort to evaluate environmentally sustainable management practices, the efficacy of several types of compost on the suppression of Cylindrocarpon destructans was examined. In growth chamber studies, the population of C. destructans was monitored over time in soilless mixes amended with 0, 10, 25 and 50% compost using serial soil dilution plating. An increase in C. destructans population as the amount of compost increased from 0 to 50%. Several microorganisms isolated from these composts have also demonstrated antagonism toward C. destructans in vitro.


Root knot nematodes (Meloidogyne spp.) are microscopic endoparasites that invade root tissue and complete their life cycle. Meloidogyne incognita infects a wide range of economically important host plants causing the formation of root galls. The nematode initiates development of multinucleate giant cells, which serve as permanent feeding sites within the root and provide nourishment for the developing worm to complete its life cycle. Plant cell surface rearrangement occurs at the plasma membrane and cell wall during the increased changes which are monitored using proteomic and microscopic analyses. Medicago truncatula were inoculated with J2-phase Meloidogyne incognita and root tissue, representing a developmental time series, was collected for analysis. Root plasma membrane proteins were isolated and then separated using 2D-polyacrylamide gel electrophoresis. Roots from each sample date were examined using a novel microscopy technique to track the progression of the nematode infection process.


The gram-negative, fastidious bacterium Xyella fastidiosa (Xf) was successfully transformed with two RSF1010 derivative plasmids belonging to the incompatibility group IncQ using electroporation. pXf004 and pXf05 were found to be present as autonomous, structurally-unchanged DNA molecules when propagated in Xf. However, neither pXf004 nor pXf05 was able to transform Xf with antibiotic selection. When plasmid DNAs were isolated from Xf or plasmid DNAs isolated from E. coli supplemented with a TypeOne™ Inhibitor, TRI, the frequency of transformation was increased by 13 or 5 fold, respectively. Plasmid pXf05 was also used to transform one additional grapevine strain of Xf.


The viral NSm protein has been implicated in the movement of Tomato spotted wilt virus (TSWV) within infected plants. To obtain sufficient specific antibody to confirm these reports, a polyclonal antibody was produced against a NSm recombinant protein. The NSm gene of TSWV was cloned in an expression vector, and a recombinant protein produced in E. coli. This recombinant protein was purified and used to inject rabbits for production of polyclonal antibody. Upon confirming that the serum collected was reactive with the recombinant protein, the IgG fraction was purified using Protein A columns and confirmed by Western blotting to bind with both the recombinant NSm protein and the native NSm protein found in extracts of infected plants. Thin sections from diseased tomato leaf tissue and tissue from healthy control plants were fixed according to the standard protocol used for thin sectioning. The sections were then stained with NSm antibody and the reaction visualized through immunocytochemistry. Staining was observed only in cells at the edge of the diseased tissue lesions. Consistent with the report of NSm being a movement protein, the staining was localized near plasmodesmata in diseased tissues. The utility of recombinant protein in eliciting antibodies that can be used in the visualization of the native protein, and in subsequent studies, including the attempts to identify plant proteins reacting with movement protein of the virus, is demonstrated.

Impact of phytoalexins and lesser cornstalk borer damage on resistance to aflatoxin contamination. B. Z. Guo (1), V. Sobolev (2), C. C. Holbrook (3), and R. E. Lynch (1). (1) USDA-ARS, Crop Protection and Management Research Unit, Tifton, GA 31793; (2) USDA-ARS National Peanut Research Laboratory, Dawson, GA 39842; (3) USDA-ARS, Crop Genetics and Breeding Research Unit, Tifton, GA 31793. Phytopathology 93:39. Publication no. P-2003-0226-AMA.

In peanut, the mechanism of resistance to Aspergillus flavus has been reported as the capacity to synthesize phytoalexins, the antibiotic secondary metabolites. The lesser cornstalk borer (LCB) is one of the most destructive insects in peanut production area. Penetration of peanut pods by insects enhances infection of pods by A. flavus/parasiticus and aflatoxin contamination in peanut. Water activity is a measurement of the energy status of the water in a system, indicating how tightly water is bound. We use water activity in a pod to explain the drought stress placed on the plants and drought tolerance. Field experiments were carried out in the rainout shelters to study the influence of phytoalexins on resistance to aflatoxin formation in peanut lines and determine if damage to the peanut by lesser cornstalk borer compromises the resistance. We compared two peanut cultivars, Georgia Green (popular commercial cultivar) with a small root system and Tifton 8 (drought tolerance) with a large root system. Rainout shelter was moved over 90 cm over cover crops in a 2x2 design and their responses to metalaxyl were determined on medium amended with metalaxyl. Results showed that metalaxyl-resistant isolates were readily found in these places. Also, the vegetative growth of some resistant isolates and the oospore formation were stimulated by metalaxyl. Results showed that metalaxyl-resistant isolates were readily found in these places. Also, the vegetative growth of some resistant isolates and the oospore formation were stimulated by metalaxyl. Among 37 isolates with different levels of resistance, 13 out of 25 isolates with typical A11-type formed oospores without pitting and the remaining 12 isolates did not form oospores. Results suggested that a considerable amount of normally heterothallic isolates can form oospores in vitro under the influence of this fungicide.


Black shank control failures have been observed in fields where multiple disease management practices were used. Tests in these fields also showed unexpected responses to fungicide treatments. To determine if black shank symptoms can be associated with infections by fungi other than Phytophthora parasitica var nicotianae, broad spectrum and specific fungicides were applied alone and in combination. Root samples were collected and cultured for Rhizoctonia, Pythium, and Phytophthora through the season. Mefenoxam sensitivity was determined for P. parasitica isolates using mefenoxam treated plates. Broad-spectrum fungicides were more effective in controlling disease than mefenoxam. In addition, nine Phytophthora isolates, not typical of P. parasitica, were isolated from soil and roots. Koch’s postulates proved these Phytophthora spp. cause root rot on tobacco. The detection of Phytophthora isolates insensitive to mefenoxam and the detection of other pathogenic Phytophthora species may explain additional control failures in tobacco fields with histories of black shank and mefenoxam use.

Chitosan, a polyacetylene molecule, provides a positive effect when combined with fungicides utilized to control Potato Late Blight. Although “chitosan only” treatments at levels near 1 mg/ml can provide beneficial effects, in these tests chitosan (Vanson, Inc, Redmond, WA) was successfully utilized at 25-50 μg/ml in combination with a processed copper sulfate pentahydrate (Bondtech Inc. Muncie, IN) at concentrations of 14-28 μg/ml. This combination was often associated with low disease symptoms and was consistently associated with less yellowing of the leaflet. The use of these low levels in the control of Potato Late Blight should reduce both grower in-put costs and the level of residual fungicide in the acreage cropped.


The impact of cropping frequency on the severity of leaf spot (LS) diseases and southern stem rot (SSR) in peanut was evaluated. Peanut was grown after 1, 2, 3 yr of cotton, and 1 to 5 yr of bahiagrass were among the patterns evaluated. Highest LS ratings were seen in plots in a peanut monoculture. Disease severity, where peanut was planted by cloning using random primers and bahiagrass, was lower than where the plots maintained a peanut monoculture. In 1 of 2 yr, LS ratings were higher in the peanut monoculture compared to those where peanut was cropped after 1 yr of corn. Incidence of SSR was similar and sometimes higher, where peanut followed 1 or 2 yr of another crop, than in the peanut monoculture. Peanut cropped after 1 yr of corn suffered less SSR damage compared to those grown after 1 yr of cotton. Pod yield often increased as the interval between peanut crops lengthened. When compared to the peanut monoculture, yield was often higher when peanut was grown after 2 or 3 yr of corn, 3 yr of cotton, and 3 or 4 yr of bahiagrass.


The objectives of this study were to characterize an unknown virus associated with decline in black raspberry (Rubus occidentalis) in Oregon. Mechanical and aphid transmissions from symptomatic R. occidentalis to Nicotiana benthamiana and aphid transmission to virus-free R. occidentalis have been successful. DrRNA extraction from symptomatic R. occidentalis revealed two major bands of about 8 and 9kb, suggesting a bipartite genome structure. RNA was cloned using random primers, and a RT-PCR detection method was developed that enabled viral detection in several symptomless hosts, including R. idaeus, R. lacinatus, R. armeniacus, and ‘Marion’ blackberry (a R. sp. complex). This virus may be Black raspberry necrosis virus (BRNV), originally described in 1955 but poorly understood. However, grafting and aphid transmission do not induce the characteristic tip necrosis seen with BRNV. Partial sequence analysis of the genome reveals homology to the strawberry mottle virus (SmMoV).

A single amino acid substitution in the sixth leucine-rich repeat of barley protein 1, 2, 3, and 4 yr of cotton, and one to 5 yr of bahiagrass were among the patterns evaluated. Incidence of SSR was similar and sometimes higher, where peanut was cropped after 1 yr of corn. Pod yield often increased as the interval between peanut crops lengthened. When compared to the peanut monoculture, yield was often higher when peanut was grown after 2 or 3 yr of corn, 3 yr of cotton, and 3 or 4 yr of bahiagrass.

Induced expression of Sarcoptoxin IA enhances host resistance against Egyptian broomrape (Orobanche aegyptiaca Pers.). N. HAMAMOUCH (1), R. Alli (2), and J. B. Towner (1). (1) Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, 406 Price Hall, Blacksburg, VA; (2) Newe Ya’ar Research Center, Ramat-Yishay, Israel. Phytopathology 93:S32. Publication no. P-2003-0234-AMA.

Parasitic weeds such as broomrape are difficult to control because they are closely associated with the host root and are concealed underground for most of their life cycle. These parasites are not controlled effectively by traditional cultural or herbicidal methods, and the best long-term strategy for limiting damage by broomrape is the development of resistant crops. Our approach to engineer broomrape resistance is based on parasite-induced expression of a selective toxin. Sarcoptoxin IA is a bactericidal peptide from the flesh fly (Sarcophaga peregrina). The gene encoding this toxin was fused to a promoter taken from a defense-related isogene of 3-hydroxy-methylglutaryl coenzyme A reductase (HMGR). The HMGR gene is expressed specifically at the site of parasite ingress, thereby concentrating spatial and temporal inclusions of the toxin in the parasites affected by the transgene. The Sarcoptoxin IA gene construct was used to transform tobacco using Agrobacterium-mediated transformation. Transgene integration into the plant genome was confirmed by PCR, and its expression demonstrated by reverse transcriptase (RT)-PCR, respectively. Host response to parasitism was assayed in polyethylene bags and in soil inoculated with Egyptian broomrape seeds. When challenged with the parasitic transgenic plants, (1) exhibited greater biomass accumulation and reduced levels of parasitism as compared to non-transgenic plants. Moreover, the frequency of tubercle necrosis and death was higher on transgenic vs. non-transgenic plants. Sarcoptoxin IA expression had no significant effect on host growth and development in the absence of broomrape, as transformed and non-transformed plants attained similar stature and biomass. We hypothesize that sarcoptoxin IA is toxic to the parasitic plant, rather than the host, and is affected by the parasite's position immediately around or inside the parasite, which acts as a strong sink on the host.


A potexvirus was isolated from Phlox stolonifera Cv. Sherwood Purple, which showed chlorotic mottle and general chlorosis. A virion preparation from Nicotiana benthamiana revealed a coat protein of apparent MW 22,000, which was reactive on Western blots with antisera to papaya mosaic virus (PMV), yellow mosaic virus, plantain virus X, potato virus X and potato aucuba mosaic virus. Diagnostic PCR using universal potex primers (Agdia Inc.) yielded a product with high homology to known potexviruses; the remainder of the genome was then cloned and sequenced. The 3'-terminal 947nt share 94% identity with the available sequence of the Staphylococcus pullorum virus, reported by Geering and Thomas (1999, Arch. Virol. 144:577-592) to be related to, but distinct from, PMV. The c. 6.7kb phlox potexvirus sequence shows closer homology to PMV than to other available potexvirus sequences. The phlox potexvirus may therefore be regarded as a strain of the Alternanthera potexvirus, and related to, but distinct from, PMV.

Identification of host components involved in the pathogenicity of Potato spindle tuber viroid on tomato. R. W. HAMMOND and Y. Zhao. USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705. Phytopathology 93:S32. Publication no. P-2003-0236-AMA.
Potato spindle tuber viroid (PSTVd) causes a serious disease of potato and is subject to quarantine regulations. Infection of the experimental host, ‘Rutgers’ tomato, with the small, circular RNA of PSTVd results in stunting, abnormal development of root and vascular tissues, and leaf epinasty and deformation. These are the symptoms that the farmers understand for the disease.

Intraspecific and intragenomic variation of \textit{Armillaria} spp. was investigated in various ways. The genetic diversity was further analyzed using Parsimony and Neighbor-Joining methods for regions analyzed, with the exception of the 5.8S rDNA. Variation will be found in all rDNA sequences. Specific hybridization. Intragenomic variation was verified by visual analysis of sequence chromatograms and PCR with specific internal primers. Specific AFLR molecules that will trigger RNAi in the fungus, use of AFLR-silenced strains were well separated from the other isolates of \textit{Armillaria} spp. (\textit{A. mellea}, \textit{A. calvescens}, \textit{A. sinapina}, \textit{A. ostoyae}, \textit{NABS X}, and \textit{A. cepistipes}) clustered together, despite their previous separation based on in vitro compatibility and/or morphology. A more detailed phylogenetic analysis and an examination of hybridization among \textit{Armillaria} spp. are underway.

Characteization of North American \textit{Armillaria} species: Phylogenetic relationships among \textit{A. mellea}, \textit{A. tabescens}, \textit{A. gallica}, \textit{NABS X}, and \textit{A. cepistipes} clustered together, despite their previous separation based on in vitro compatibility and/or morphology. A more detailed phylogenetic analysis and an examination of hybridization among \textit{Armillaria} spp. are underway.

Intraspecific and intragenomic variation of \textit{Armillaria ostoyae} within the western United States. J. W. HANNA (1,2), N. B. Klopfenstein (1), M.-S. Kim (1), G. I. McDonald (1), and J. A. Moore (2). (1) USDA Forest Service-RMRS, Moscow, ID 83843; (2) University of Idaho, Dept. of Forest Resources, Moscow, ID 83844. Phytopathology 93:S33. Publication no. P-2003-0239-AMA.

Intraspecific and intragenomic variation of \textit{Armillaria ostoyae} were observed through sequencing of ribosomal DNA (rDNA) including nuclear large subunit rDNA (nLSU) regions. Phylogenetic trees were generated using Neighbor-Joining analysis. The trees methods include engineering plants to produce molecules that will trigger RNAi in the fungus, use of AFLR-silenced strains in biocontrol and other methods that may deliver AFLR-specific RNA activation molecules to crop-infesting \textit{Armillaria} spp. are underway.

Characterization of North American \textit{Armillaria} species: Phylogenetic relationships among \textit{A. mellea}, \textit{A. tabescens}, \textit{A. gallica}, \textit{NABS X}, and \textit{A. cepistipes} clustered together, despite their previous separation based on in vitro compatibility and/or morphology. A more detailed phylogenetic analysis and an examination of hybridization among \textit{Armillaria} spp. are underway.

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\textit{Fusarium oxysporum} causes wilt or yellows on a large number of crops. On sugar beet, Fusarium yellows reduces root yield as well as succrose percentage and purity in the root. The primary causal agent is \textit{F. oxysporum} f. sp. betae (FOB), although \textit{F. acuminatum} also has been reported to cause the disease. We examined the species of \textit{Fusarium} isolated from sugar beet with yellows symptoms that could cause symptoms on beet. \textit{Fusarium} species: Phylogenetic relationships among \textit{A. mellea}, \textit{A. tabescens}, \textit{A. gallica}, \textit{NABS X}, and \textit{A. cepistipes} clustered together, despite their previous separation based on in vitro compatibility and/or morphology. A more detailed phylogenetic analysis and an examination of hybridization among \textit{Armillaria} spp. are underway.


A luxR homolog in \textit{A. vitis} strain F2/5 was recently shown to be associated with a hypersensitive response on tobacco and necrogenesis on grape. A second luxR-like gene (R1320) has now been identified by Tn5 mutagenesis. The insertion in this ORF results in loss of the HR and a reduction in grape necrosis. R1320 was compared with other LuxR transcriptional regulators from related species within Rhizobiaceae. TLC-biosensor generated profiles of N-acyl-homoserine lactone autoinducers suggest that there are no differences between the R1320 mutant and F2/5. Eleven ORFs within a region of about 14 kb flanking the Tn5 insertion were identified and aligned with homologous genes in \textit{A. tumefaciens} strain C58 showing high identity to corresponding genes in \textit{A. tumefaciens} strain C58. The R1320 mutant was fully complemented mutant with a corresponding cosmide clone (approximately 35 kb). When the luxR-like gene alone was cloned and used in complementation experiments, corresponding phenotypes were not restored. Site-directed mutagenesis was performed to check whether other candidate genes may be related to hypersensitive response and necrosis.


Various soil fumigants, plastic mulches, and plant varieties were tested for their effects on the population of \textit{Verticillium dahliae} in soil and wilt disease of strawberry in the field. Soil fumigants (applied as main plots in a split-split plot design) were methyl bromide/chloropicrin (MBC), chloropicrin at 4 rates (112, 168, 224, and 336 kg/ha), Telone C35 at 2 rates (317 and 476 kg/ha), and a non-treated check. Standard plastic and virtual impermeable films (VIF) were applied in subplots. Five strawberry varieties were planted as sub-subplots. In summary, all the fumigants were effective in reducing \textit{V. dahliae} in soil and disease incidence, and in increasing plant growth and yield compared to the non-fumigated soil. Chloropicrin rates lower than 336 kg/ha, however, was less effective than MBC or Telon C35. VIF plastic reduced disease and increased yield in most fumigated treatments. Telone C35 or MBC with VIF, depending on the variety, gave the largest yield increase. The relative susceptibility of strawberry varieties to Verticillium wilt was (high to low) Ventana, Camarosa, Diamante, Aromas, and Camino Real.

A rapid PCR-based method for the detection of \textit{Magnaporthe oryzae} from infected perennial ryegrass. P. F. HARMON (1), L. D. Dunkle (2), and R. Latin (1). (1) Department of Botany and Plant Pathology; (2) U.S. Dept. of Agriculture, Agricultural Research Service, Purdue University, West Lafayette, IN 47907. Phytopathy 93:S33. Publication no. P-2003-0243-AMA.

Gray leaf spot caused by \textit{Magnaporthe oryzae} is a serious disease of perennial ryegrass. Because turf managers must select appropriate fungicides for perennial ryegrass, accurate and timely identification of the pathogen is essential for efficient and effective disease management. We developed a PCR-based method to detect \textit{M. oryzae} in infected perennial ryegrass tissue. The method utilizes a commercially available kit that is used for isolation and amplification of plant DNA from leaf tissue. The kit protocol was modified and found to be reliable for the extraction of \textit{M. oryzae} DNA from barley. Primers were designed to amplify a 687 bp fragment of the Pot2 transposon that is found in multiple copies in the genome of the pathogen. The protocol amplified amounts of purified DNA as low as 5 pg and consistently and specifically detected \textit{M. oryzae} in single diseased leaf blades as well as in field samples of infected perennial ryegrass.
Detection of viral plant pathogens using PCR group test primers. A. M. HARNESS (1), B. P. Kulemeka (1), S. K. Zaviev (2), and M. D. Bandia (1). (1) Agdia, Inc., 30380 County Road 6, Elkhart, IN 46514; (2) Laboratory of Molecular Virology, Institute of Agricultural Biotechnology, Moscow 127550, Russia. Phytopathology 93:S34. Publication no. P-2003-0244-AMA.

We have developed and optimized seventeen PCR group tests that offer a sensitive diagnostic method for plant viruses with broad detection capabilities. The viral PCR group test primers are based on conserved genomic regions and can detect unidentified virus and isolate members of the same group. These tests can be used as an aid for identification of viral etiology of unknown plant diseases and test for known viruses when other tests are not available. The PCR group tests can also be used to confirm ELISA results, used in association with ELISA via immunocapture RT-PCR, and assist in diagnosis in cases where ELISA results are complicated by host tissue reactivity. Power and utility of the PCR group tests in plant diagnostic will be discussed using specific case studies.

Evaluation of fungicide dip treatments for control of Colletotrichum acutatum on strawberry in Florida. T. L. HARP (1), D. R. Tory (1), L. Lavenderie (2), and A. Tally (3). Syngenta Crop Protection, (1) Vero Beach, FL 32967; (2) Newport Park, CA 91320; (3) Greensboro, NC 27409. Phytopathology 93:S34. Publication no. P-2003-0245-AMA.

Strawberries are cultivated on nearly 7,000 acres in Florida, and have a current value approaching $200 million. One of the biggest disease problems for strawberry growers is fruit anthracnose, a fungal disease caused primarily by Colletotrichum acutatum. The pathogen is thought to be introduced into Florida on infected transplants obtained from Canada, thereby removing the possibility of preventative control. The purpose of this study was to evaluate the efficacy of dip treatments of various fungicides (Switch, Quadris, and Captan) for control of anthracnose in pre-inoculated transplants. Transplants obtained from infected planters were allowed to incubate for 4 days, and then planted into the field. Fungicide treatments were made by dip application to transplants for 5 minutes immediately prior to planting. Plants that were not dipped became severely infected within 2 weeks, resulting in approximately 80% transplant death. By contrast, some of the dip treatments provided almost complete control. Transplants that received a dip application followed by a foliar fungicide program had a lower incidence of fruit anthracnose than those plants that received no foliar program.

Common weeds serving as alternate hosts for pathogens of dry-edible beans and sugar beets in the Nebraska Panhandle. R. M. HARVISON, University of Nebraska, Panhandle Research and Extension Center, Scottsbluff, NE 69361. Phytopathology 93:S34. Publication no. P-2003-0246-AMA.

During the 2001 and 2002 cropping seasons, a survey was conducted in the Nebraska Panhandle looking for weeds exhibiting symptoms of root disease or leaf blights. Among the weeds observed was red root pigweed (Amaranthus retroflexus L.), common lambsquarters (Chenopodium album L.), and fireweed (Koehia scoparia L. Schrad.). The sugar beet pathogens identified from at least one of the three weeds included Cercospora beticola, Rhizoctonia solani, Fusarium oxysporum, Pythium spp., Polynymyx betae and Nacobbus abernans. The bean pathogens found infecting weeds included R. solani, and Pythium. Disease symptoms observed on weeds were very similar to those on beets and beans, including foliar wilting and/or yellowing from root pathogens. Root symptoms consisted of lesions coalescing to larger rotted areas (Rhizoctonia), vascular discoloration (F. oxysporum), and a tip rot of taproots or feeder roots (Pythium). Cercospora and Nacobbus produced symptoms on weeds identical to those on sugar beets. Results show the potential role that infected weeds can play in crop disease by nullifying the effects of rotation.

Nature of Capsicum annuum cv Avelar’s resistance to systemic infection by Pepper mottle virus. W. L. HATCHER III (1), R. R. Dute (2), and J. F. Murphy (1). Departments of (1) Entomology and Plant Pathology; (2) Biological Sciences, Auburn University, AL 36849. Phytopathology 93:S34. Publication no. P-2003-0247-AMA.

We have shown that systemic infection of Capsicum sp. by viruses in the genus Potyvirus involves movement down the stem in external phloem with rapid movement up the stem to young tissues in internal phloem. Pepper mottle virus-FL (PepMoV-FL, Florida strain) does not move through internal phloem of C. annuum ‘Avelar’ plants which restricts infection to lower portions of the plant. We conducted microscopy studies to determine whether PepMoV-FL occurs in external and/or internal phloem of the petiole of the inoculated leaf of Avelar plants. Our hypothesis was that the lack of virus in internal phloem of the stem resulted from an inability of PepMoV-FL to load into internal phloem in the inoculated leaf. Immuno-fluorescence labeling revealed that PepMoV-FL occurred in external and internal phloem of inoculated leaf petiolar of Avelar plants (similar to susceptible controls) suggesting that Avelar’s resistance is not a block in entrance into internal phloem in the inoculated leaf but appears to occur within the stem.


Burkholderia cepacia, currently the most important bacterial pathogen of onions in New York, infects at the leaf axil causing bacterial canker and, as infection advances into the bulb, causes sour skin. Onion field surveys spanning four consecutive seasons showed a transient rise in B. cepacia populations as each season progressed, establishing baseline levels and a trend for comparison. Some surveys of soils harbor to non-onion crops, and to onions rotated with other crops, found lower levels of B. cepacia. Surveys of onion fields where winter cover crops had been planted suggest that some species may be useful in suppressing B. cepacia populations. We have utilized a micromculture procedure to screen for additional crop species that influence the rhizosphere population of B. cepacia in naturally infested organic soils. Some crops impacted bacterial population due to the hypothesis that cultivation of non-onion crops or winter cover crops reduces soilborne inoculum, and represents a useful strategy for disease control.

Effect of lesion nematode (Pratylenchus penetrans) on establishment of pyrhythm (Tanacetum cncineriifolium) in Australia. F. S. HAY (1) and G. P. Stirling (2). (1) T.I.A.R, University of Tasmania, P.O. Box 447, Burnie, Tas., Australia 7320; (2) Biological Crop Protection, 3601 Moggill Rd., QLD, Australia 4070. Phytopathology 93:S34. Publication no. P-2003-0249-AMA.

Pyrhythm is grown in Tasmania, Australia for the production of insecticidal pyrethrins. The effect of P. penetrans on establishment of pyrhythm was determined in 60 field plots, sown in August 2000. In February 2001, 20 plants and surrounding soil were collected from each plot. Foliage was dried overnight (100°C) and weighed. Washed roots were cut into lengths (1-5 cm). Nematodes were extracted from roots and from 400 ml soil by Whitehouse tray technique over 4 days and counted. Roots were dried and weighed as for foliage. Significant (P < 0.05) negative correlation coefficients were obtained between the number of P. penetrans/g dry weight of root and the dry weight (g/plant) of foliage (r = -0.43), roots (r = -0.39) and plants (r = -0.43). The relationship between the number of P. penetrans/g dry weight of root (X) and the dry weight (g) per plant (Y) at 6 months after sowing was described by the regression equation Y = 12.5 - 0.0001X. The lowest and highest mean population density of P. penetrans/g dry weight root was 666 and 12,469 respectively, with mean dry weight of 3.65 and 0.56/g/plant respectively. P. penetrans had no effect on plant density.

Lipid A mutant of Rhizobium leguminosarum affects root nodule development in pea. J. G. HAYNES (1), V. Tasdem (2), E. Kammerbech (2), R. W. Carlson (2), and J. D. Sherrir (1). (1) University of Delaware, Newark, DE; (2) University of Georgia, Athens, GA. Phytopathology 93:S34. Publication no. P-2003-0250-AMA.

Lipopolysaccharides (LPSs), present in Gram-negative bacteria, are crucial components of both plant pathogen and symbiotic nitrogen-fixing bacteria (rhizobia). The LPS of R. leguminosarum (Rv) contains many structurally unique features that are thought to have a role in symbiotic infection and nodule formation on pea roots. One such feature is a very long chain fatty acid, 27OHC28:0, found in the lipid A region of rhizobial LPS. An acyl carrier protein (ACP) is required to transfer this 27OHC28:0 to the lipid A of the Rv LPS. An LPS mutant was made by interrupting acp-XL, thus preventing transport of the 27OHC28:0 moiety to its LPS. The mutant bacteria were studied to determine the effect of this mutation on bacterial growth and nodule formation in an attempt to elucidate the function of 27OHC28:0 in normal nodule development. Growth of wildtype and mutant bacteria in liquid culture was compared. Peas were inoculated with either wildtype or mutant strains and nodule development was followed using light and electron microscopy, nodule counts and nodule measurements.

Effect of low temperature on populations of Xylella fastidiosa in naturally infected sycamore. T. HENNEBERGER (1), K. L. Stevenson (1), and K. O. Britton (2). (1) University of Georgia, Department of Plant Pathology, Athens, GA 30602-7274; (2) USDA Forest Service, 320 Green St., Athens, GA 30602-2044. Phytopathology 93:S34. Publication no. P-2003-0251-AMA.

To determine the effect of low temperature on populations of X. fastidiosa in naturally infected sycamore (Platanus occidentalis), root and
shoot samples were collected monthly for 13 months from two locations in Georgia. Soil and air temperatures were recorded at each site and used to generate cumulative hours below temperature thresholds ranging from −5°C to 10°C. To estimate bacterial populations, sap extracted from each sample was inoculated onto Pseudomonas inoculated onto agrose plate medium. Bacterial populations in shoots were negatively correlated with cumulative hours below an air temperature of −5°C (r = 0.96), but in roots, bacterial populations were only weakly correlated with cumulative hours below soil temperature thresholds from 0°C to 10°C (r = 0.61). Our results indicate that air temperatures below −5°C are associated with limiting bacterial multiplication in sycamore shoots and that factors other than low soil temperature may influence multiplication of X. fastidiosa in sycamore roots.

Studies on the etiology and epidemiology of bull’s eye rot of ears, J. L. HENRIQUEZ (1), D. Sugar (1), and R. A. Spotts (2). (1) Southern Oregon Research and Extension Center, Oregon State University, Medford, OR 97502; (2) Mid-Columbia Agricultural Research and Extension Center, Oregon State University, Hood River, OR 97831. Phytopathology 93:S35. Publication no. P-2003-0252-AMA.

Species-specific PCR-based identification was performed on a collection of 470 isolates of fungi causing bull’s eye rot of ears. Results indicated the presence of Neofabraea perennans, N. alba and a putative new species as the causative agents of the disease in Oregon and Washington. Cankers developed on pear tree branches following monthly inoculations with N. perennans and N. alba. Cankers were only produced when inoculations were performed from September through April. Conidial production on cankers occurred the year after the year of inoculation at the end of summer and during the fall. Copper sulfate reduced conidial production on cankers of N. alba, while trifloxystrobin and ziram did not. None of the fungicides tested suppressed conidial production on cankers by N. perennans. Natural fruit infections in the orchard occurred from May to September, with a relatively higher incidence from mid August until harvest, coincidental with a greater conidial production in cankers.


We tested efficacy of six dipping treatments (200 ppm and 500 ppm sodium hypochlorite, 1 ppm and 5 ppm chlorine dioxide, KleenUp soap solution, and a non-dipped control) and four brushing times (15, 30, 60, and 90 seconds) for post-harvest removal of sooty blotch and flyspeck (SBFS) colonies. Percent diseased area was determined for Honey Gold apples harvested from two orchards in Iowa and Wisconsin. After dipping and brushing treatments were applied, percent diseased area was rated again, and the post- and post-treatment ratings for each apple were compared. Chlorine dioxide (5 ppm) and KleenUp were most effective, regardless of brushing time. Increasing the brushing time increased SBFS removal. The most effective treatment was KleenUp soap followed by 90 seconds of brushing (93% removal).

Generation and evaluation of tobacco lines transformed with fungal genes PDX1 and PDX2 involved in vitamin B6 biosynthesis and resistance to the toxin cercosporin, S. HERRERO, S. A. Denislow, and M. E. Dubb, North Carolina State University, Raleigh, NC 27605. Phytopathology 93:S35. Publication no. P-2003-0254-AMA.

Many members within the genus Cercospora produce and are resistant to cercosporin, a phytotoxin that generates singlet oxygen when irradiated by light. Our laboratory has identified two genes, PDX1 and PDX2, from C. nicotianae that confer cercosporin autoresistance and are involved in the synthesis of vitamin B6, a quencher of singlet oxygen. Our laboratory is investigating if PDX1 and PDX2 will also provide resistance when expressed in plants. Haploid tobacco plants were transformed with PDX1 and/or PDX2 using Agrobacterium tumefaciens strain EHA105. In vitro selection and PCR analysis revealed that 13 lines were transformed with PDX1, 27 lines with PDX2 and 10 lines harbored both genes. Depending on the line, the number of insertions ranged from 1 to 3 for PDX1, and from 1 to 4 for PDX2. We used a midvein culture technique to generate double haploid lines homozygous for the insertions, and are presently evaluating selected lines for resistance to cercosporin and to C. nicotianae as well as for resistance to other biotic and abiotic stresses.

Soil variables and fine root histology from loblolly decline sites in central Alabama, N. J. Hess (1), C. H. Walkinshaw (1), E. A. Carter (2), and A. J. Goddard (3). (1) USDA Forest Service, Pineville, LA 71360; (2) USDA Forest Service, Auburn, AL 36849; (3) National Forest in Alabama, Montgomery, AL 36107. Phytopathology 93:S35. Publication no. P-2003-0255-AMA.

The study objective was to associate soil characteristics with host symptoms and to define fine root damage using histology techniques. Soil samples were taken in Michigan, Wisconsin, Illinois, Indiana, and Ohio. These data were then used to estimate the host response to loblolly decline. Using this approach, we found that soil pH and organic matter were significantly correlated with loblolly decline sites and control plots. A sub-sample of plots was used for fine root histology of loblolly pine trees. Soil from decline plots had higher bulk density values and lower total porosity compared to control plots, but these were not considered to be growth limiting factors. The soil pH ranged between 4.0 and 5.7. The ratio between exchangeable calcium and aluminum may be a contributing factor to loblolly pine decline but additional research is needed. The histological findings revealed significant wounding of the fine roots and other measured root variables correlated to reduce stem radial growth.


The prevalence of canker, caused by the fungus Godronia cassandrae, has been increasing in lowbush blueberry fields throughout Nova Scotia in recent years. Symptoms of Godronia canker appear in early spring midway up the stems as light brown colored lesions surrounded by a purplish border that encircles vascular buds. Buds and stem tissue above the lesion die back. Vegetative buds below the lesion grow vigorously so that by early July severely infected fields can look deceptively healthy. A survey of 54 random fields in Nova Scotia in 2002 showed that 91% had some disease. Within these fields, the incidence of infected stems ranged from 0.5% to 5% and averaged 2%. Since lesions usually girdle the stems, the incidence data can be regarded as crop loss figures. Geographic region (coastal or inland), origin of fruit (pasture or woodland) or age did not correlate with disease level, but pruning practice did. Lowbush blueberries are pruned biennially by felling mowing or by fire with oil burners or straw. Fields that were burned had substantially less disease.


Citrus tristeza virus (CTV) is the most prevalent and destructive viral pathogen of citrus. Genome sequences of CTV isolates from different locations and with different biological properties have a highly conserved nucleotide sequence in the 3-prime eight kilobases of the genome, but a higher level of nucleotide sequence diversity in the 5-prime eleven kilobases. Conserved nucleotide sequences were identified from the alignment of the complete genomes of the pathogenically and genetically distinct T36, T39, VT and SY568 CTV isolates, and used to design primers for the polymerase chain reaction mediated cloning of sequences from CTV isolates of unknown genetic complexity. Overlapping primers designed to amplify a complete CTV genome were successfully used to amplify portions of the genomes of the Florida isolates T3 and T66, and from isolates from Peru, New Zealand and Japan. Sequence analysis of clones of T3 and T68 indicated these isolates were genetically distinct from those known, and can be considered new strains. The results suggest that this experimental approach can provide DNA for sequence analysis from genetically diverse CTV isolates.

Variability in green stem incidence among soybean cultivars. C. B. HILL (1), H. A. Hobbs (1), and G. L. Hartman (1,2). (1) Dept. of Crop Sciences, University of Illinois, Urbana, IL 61801; (2) USDA-ARS. Phytopathology 93:S35. Publication no. P-2003-0258-AMA.

Green stem is a disorder of soybean (Glycine max Merr.) that when prevalent in a soybean field, complicates harvesting by making the stems harder to cut. The cause of green stem is unknown. Incidence of green stem (percentage of plants with green stem) in several hundred commercial cultivars was evaluated in Illinois state yield trials during 2000-2001. Data was collected from trials at Urbana, Illinois in 2000 and 2001. There were highly significant differences among cultivars for green stem incidence (P < 0.001). In 2002, data was collected at three locations: Dekalb, Monmouth, and Urbana, Illinois. Mean incidence was 30% at Monmouth, 19% at Dekalb. and 9% at Urbana. Again, differences among cultivars for green stem incidence were highly significant (P < 0.001), indicating that there was genetic variability for resistance to green stem among the cultivars. Public cultivars with high green stem incidence included Macon, Maverick, Pana, and Williams 82, while Dwight, Loda, and Savoy had low incidence. A summary of the incidence of green stem in commercial cultivars is online at http://web.aces.uiuc.edu/vips.
A reovirus of the fungus Cryptococcus parasitica that is infectious as particles and related to the Colivirus genus of animal pathogens. B. L. HILLMAN (1), S. S Yupany (2), H. Kondo (2), and N. Suzuki (2). (1) Rutgers University, Dept. of Plant Biology and Pathology, New Brunswick, NJ 08901, (2) Research Institute for Biosources, Okayama University, Kurashiki, Okayama, Japan. Phytopathology 93:S36. Publication no. P-2003-0259-AMA.

A reovirus has been purified from a morphologically distinct hypovirulent isolate of Cryptococcus parasitica, the filamentous fungus that causes chestnut blight disease. Virus particles isolated from infected mycelium contain 11 segments of dsRNA and show physical characteristics typical of the family Reoviridae. Sequences of the largest three segments of the virus reveal that it is closely related to the Colivirus genus within the family, which includes the human pathogen Colorado tick fever virus. Introduction of purified particle preparations into protoplasts made from virus-free isolates of the fungus resulted in newly infected colonies with the same morphological characteristics and virus composition as the original virus-infected isolate. This represents the completion of Koch’s postulates for a true dsRNA virus from a filamentous fungus and the description of a definitive fungal member of the family Reoviridae.


Seashore paspalum (Paspalum vaginatum) has great potential for use in salt affected turf sites. Because of its tolerance to drought and high salinity irrigation, use of this grass on golf courses, athletic fields, and lawns in coastal areas may aid in conservation of fresh water resources. Plant-parasitic nematodes are damaging pests of turfgrasses in Florida, particularly sting (Belolololaimus longicaudatus) and lance (Hoplobolaimus galeatus). It is unknown how these and other plant parasitic nematodes impact seashore paspalum. Therefore, experiments were performed to compare the susceptibility of ‘SeaSle 1’ seashore paspalum and ‘TidDwair’ bermudagrass to B. longicaudatus and H. galeatus. Plugs of either grass were planted into USGA root zone mix in 15 cm diameter clay pots. Two weeks after planting, 16 pots of each grass were inoculated with 100 B. longicaudatus, 100 H. galeatus, or remained uninoculated. Nematode population and root length analysis were performed 60 and 120 days after inoculation. Both nematode species reproduced on both grasses, but only B. longicaudatus caused root-length reductions. Population densities of B. longicaudatus were higher (P < 0.05) than bermudagrass after 60 days than on seashore paspalum, but not after 120 days. B. longicaudatus caused root-length reductions of 35 to 40% after 120 days on both grasses, but reductions were not significantly different (P < 0.05) between grasses. This indicates that damage thresholds used for diagnosis of B. longicaudatus on bermudagrass may be used for seashore paspalum as well.

Root lesion nematode management in field crops in southeastern Australia. G. J. HOLLAWAY (1), S. P. Taylor (2), and V. A. Vanstone (3). (1) Dept of Primary Industries, Victoria, Australia; (2) South Australian Research and Development Institute, South Australia; (3) Dept of Agriculture Western Australia. Phytopathology 93:S36. Publication no. P-2003-0261-AMA.

Root lesion nematodes (Pratylenchus thornei and P. neglectus) are common in field cropping soils in southeastern Australia. Studies during the previous 12 years have shown these nematodes to be important pathogens to intolerant crops, especially wheat. Yield loss caused by these nematodes was related to infecting density. Nematode population and root length analysis were performed 60 and 120 days, 40 to 50 cm, from the center of clay pots. Two weeks after planting, 16 pots of each grass were inoculated with 20,000 nematodes. Inoculation with B. longicaudatus resulted in newly infected colonies with the same morphological characteristics and virus composition as the original virus-infected isolate. This represents the completion of Koch’s postulates for a true dsRNA virus from a filamentous fungus and the description of a definitive fungal member of the family Reoviridae.

Comparative disease reactions of Cycle 0 and Cycle1 alfalfa following inoculation with Phoma sclerotioides. C. R. HOLLINGSWORTH (1), R. W. Groose (2), and F. A. Gray (1). (1) Univ. Minn., NW Res. and Outreach Ctr., Crookston, MN 56716; (2) Univ. Wyo., Dept. of Plant Sciences, Laramie, WY 82071. Phytopathology 93:S36. Publication no. P-2003-0262-AMA.

Brown root rot (BRR) of alfalfa, caused by P. sclerotioides, is associated with widespread winter mortality when environmental factors are conducive for disease. The objective of these studies was to determine if field-grown plants surviving six years of disease pressure by P. sclerotioides could reproduce progeny with traits for BRR resistance. Disease severity ratings (DSR) of six Cycle 0 (C0) alfalfa entries not exposed to BRR disease pressure were compared with multiple Cycle 1 (C1) half-sib families of the same six entries that were bred in the greenhouse from field-plants exposed to BRR for six years. Experiments included C0 and C1 entries, cv. Peace (Canadian BRR resistant check), cv. Multiplier (U.S. BRR susceptible check) and ‘WY-BRR’ (a Wyoming BRR resistant experimental line consisting of a composite of half-sib family lines). Overall, five of six C1 entries had less severe DSR than C0 entries. Cycle 1 Heinrichs was the only exception, with C0 exhibiting excellent BRR resistance. WY-BRR also exhibited resistance.


Forty isolates of Sclerotinia minor obtained from 10 winter weed species growing in Northeastern North Carolina were examined for their mycelial interactions. The weeds came from fields previously planted in peanut and with a history of Sclerotinia blight. Isolates were paired on DS medium in all combinations and mycelial interactions were scored as either compatible or incompatible. Fifteen mycelial compatibility groups (MCGs) were identified and eight MCGs represented approximately 82% of the isolates examined. The largest MCG included 12 isolates while seven MCGs consisted of a single isolate. All self pairings (control) were compatible. Five single ascospore isolates obtained from laboratory-produced apothecia of 10 randomly selected isolates were also examined for their mycelial interactions on DS medium. No ascospore isolates produced the same MCG. Local populations of S. minor were composed of phenotypically different isolates. Isolates from a particular area or weed host were heterogeneous according to MCG, colony morphology, and aggressiveness on peanut.


Recycled irrigation water is a significant source of inoculum for Phytophthora disease epidemics in ornamental nurseries. At least 10 species of Phytophthora have been isolated from components of nursery recycling irrigation systems. Recovery of individual species has been much greater in runoff water collected at entry points to retention ponds than in water existing ponds collected at irrigation risers. In this study we investigated the horizontal distribution of Phytophthora populations in a retention pond. Rhododendron leaf disks in mesh bags were placed in the pond for 24 h at 0, 10, 20, 30, 40, and 50 meters from a runoff water entry point. Then leaf disks were removed, rinsed in pond water and sterile distilled water, and plated in petri dishes containing PARPH agar. The disks were examined daily for a week and the numbers of developing Phytophthora colonies were compared among the six distances. The recovery of Phytophthora spp. decreased with increasing distance from the runoff entrance. This result implies that location of the pump house can be an important factor in managing Phytophthora pathogens in irrigation water.


Proper identification of Alternaria spp. is complicated by difficulty in the use of morphological characters, which can be highly variable depending on culture conditions. To assist in taxon identification, we have constructed restriction maps of the IGS region from 10 representative species of Alternaria for 14 restriction enzymes. IGS fragment size varied from 2.0 to 2.7 kb among isolates. A. brassicicola and A. minicula of the brassicicola species-group had identical restriction maps. Similarly, two species of the alternata species-group, A. alternata and A. tenaisinna, had identical restriction maps. However, two other species of the alternata species-group, A. gaisen and A. longipes, had significantly different restriction maps. Three species of porri species-group, A. crassa, A. dauci, and A. porri, had different restriction maps with few shared restriction sites. From the restriction mapping study, it was concluded that the IGS region of Alternaria is highly variable and may have sufficient information to elucidate phylogenetic relationships among closely related taxa. Full-length sequencing of the IGS region is underway.

Eleocharis kurogawii is distributed widely and has been caused weed problem in rice production area in Korea. Although Epicroccosorus namatosporus is selected as a potential mycoherbicide agent, but it should not be used effectively in field before resolving the safe bio-carrier of this fungus. The studies and application of plants is often of great importance in effecting biological weed control. The purpose of this research was to determine: 1) the cultural condition for melanization and sporulation of alginate pellets; 2) the effect of inert fillers on melanization and conidiation; and 3) the weed efficacy of the sodium alginate pellet in field. Melanin production of E. namatosporus was affected by striking frequency. Percentage of melanized beads was increased to 80.6% at higher rpm up to 180. The melanized pellets were produced conidia with abundant mucilage after illuminating 4,500 lux of fluorescence light at 28° for 48 h. When wheat bran and rice polish were amended and produced the conidia with 65.4 and 68.4 mg per 100 pellets, respectively. When 1 l of conidial suspension of 6.0 x 10⁵ conidia per ml was applied on 30-day-old plants in a plot, 74.5% of the plants were killed within 20 days, whereas its melanized sodium alginate pellets killed 57.8% of the plants in the same period. Number of tuber formation of Eleocharis kurogawii in the untreated plots were 128.5t, but those of the plots treated with conidial suspension and melanized pellets were 22.1 and 39.7 at the end of the season. Results of this study showed that melanization of mycelia mixed sodium alginate is an important sporulation factor in E. namatosporus as a mycoherbicide.


This study was conducted to determine the efficacy of the Epicroccosorus namatosporus for control of Eleocharis kurogawii and to evaluate the meteorological factors which affect the weed efficacy in field condition. The best time to control the E. kurogawii with E. namatosporus for biological control of field would be during July when the temperatures were 20.4-23.4° during the surface wetness duration of 12.6 -16.1 h and application time of 6:00 PM and 8:00 PM; weed efficacy was 81.90%. On June 10 in Milyang area, where the field experiments was performed, mean temperature was 15.6° with 11.3 h of dew duration, and on Aug 20, the temperature was 15.4 h of dew duration. In these periods, the weed efficacy was recorded at 61.8 and 60.8%, respectively. Time required for complete plant death was 25.8 and 25.6 days at the application times of June 10 and Aug 20. At the time of application of July 7, 18, and 27, mean temperature was 20.4-23.4° with 12.6-16.5 h of dew duration. Weed efficacy of these periods were very high with 81.4-90.8%. Three years of field observations showed that infection in field can occur at any time through the summer season, although total infection rates vary between months and between years. Disease progress was slowly from 24.4% on 30 June to 49.2% end of growing season. Results of this studies indicate that Epicroccosorus namatosporus has potential as a mycoherbicide for controlling the rice weed Eleocharis kurogawii but has limited by a meteorological factors.

Field control of bacterial fruit blotch of watermelon with a plant defense activator. D. L. HOPKINS. Plant Pathology Department, Mid-Florida REC, University of Florida, Apopka. Phytopathology 93:S37. Publication no. P-2003-0268-AMA.

Once bacterial fruit blotch (BFB) of watermelon, caused by Acidovorax avenae subsp. citrulli, gets into a field, the only control is multiple applications of a copper-containing fungicide. This is usually effective, but might fail to provide control in hot, humid, and rainy conditions. In this study, Acibenzolar-S-methyl (Actigard), a plant activator, was evaluated alone and in combination with copper for control of BFB. Acibenzolar-S-methyl provided excellent control of BFB in the transplant house and was effective in the field when applications were initiated prior to transplanting or immediately after emergence of direct-seeded watermelons. Acibenzolar-S-methyl alone did not provide adequate field control of BFB. Its effectiveness decreased late in the season. The most effective and consistent control was obtained with a combination of acibenzolar-S-methyl early in the season and a copper material, such as cupric hydroxide, added 1-2 weeks prior to anthesis. Acibenzolar-S-methyl, when combined with copper materials, can play an integral role in the management of BFB of watermelon in the field.

Suppression of powdery mildew and botrytis blight of begonia induced by Trichoderma hamatum 382 in peat and compost-amended potting mixes. L. E. HORST (1), C. R. Krause (1), L. V. Madden (2), and H. A. J. Hoitink (2). (1) USDA, ARS-ATRU, Wooster, OH 44691; (2) The Ohio State University, Dept. of Plant Pathology, Wooster, OH 44691. Phytopathology 93:S37. Publication no. P-2003-0269-AMA.

Composts may induce systemic resistance (ISR) in plants against root as well as foliar diseases. Unfortunately, this effect is highly variable in nature. For example, less than 2% of several different types of comports tested naturally induced ISR in plants. The research reported here shows that inoculation of Trichoderma hamatum 382 (T382) into a light Sphagnum peat mix significantly reduced the severity of powdery mildew and of Botrytis blight of begonia. This plant dry weight yield also was significantly. Amendment of the potting mix with a batch of composted cow manure (5%: v/v) that naturally induced ISR in plants also increased plant quality and significantly decreased the severity of these diseases. T382 and the naturally ISR-active compost suppressed Botrytis blight and powdery mildew and improved plant quality as effectively as foliar sprays with Daconil and Pipron, respectively.


Pre-emergence damping-off of cotton seedlings by Pythium spp. and Rhizopus oryzae is initiated by the production and release of compunds from the germinating seed that induce resting structures of the pathogens to germinate and grow. Cotton cultivars are quite variable with respect to the timing of seedling emergence and release of inducer compounds. The release of these compounds is highly susceptible to disease, while those that do not release inducers are virtually immune. Once pathogen germination has been induced, all cultivars are susceptible. Seed treatment of susceptible cultivars with biocontrol preparations of Trichoderma virens prevents pre-emergence damping-off in cotton. When pathogen inducers present in seed exudates are degraded by propules of T. virens in culture, their capacity to induce germination of pathogen propagules is destroyed. Inducer compounds exposed only to culture filtrates from T. virens are not adversely affected. Inducer compounds are not extractable from seed exudate with immiscible organic solvents, indicating that they are not the fatty acid compounds previously reported.


A novel subtractive strategy was designed to clone candidate parasitism genes expressed in the esophagial gland cells of M. incognita: (i) separate mRNA pools were isolated from contents microaspirated from 53 esophageal glands of parasitized or unparasitized roots; these isolates that release parasitism inducers are highly susceptible to disease, while those that do not release inducers are virtually immune. Once pathogen germination has been induced, all cultivars are susceptible. Seed treatment of susceptible cultivars with biocontrol preparations of Trichoderma virens prevents pre-emergence damping-off in cotton. When pathogen inducers present in seed exudates are degraded by propules of T. virens in culture, their capacity to induce germination of pathogen propagules is destroyed. Inducer compounds exposed only to culture filtrates from T. virens are not adversely affected. Inducer compounds are not extractable from seed exudate with immiscible organic solvents, indicating that they are not the fatty acid compounds previously reported.


Beef curl top virus (BCTV) causes unpredictable disease problems for chile pepper in New Mexico. Surveys of southern New Mexico chile fields and the weeds surrounding those fields were carried out during 2001 and 2002 to assess the incidence of BCTV. Weeds are also being assessed during 2003. Leaf samples were tested by PCR using a primer set which amplifies a portion of the coat protein gene to detect any BCTV infection and with strain-specific primers to determine the virus strain present. Both Worland and CFH strains were detected in chile and weeds in 2001, while only Worland was detected in 2002. Curl top incidence was much higher in 2001 (30-50% in chile and 1.3% in weeds) than in 2002 (0.5-1.0% in chile and 0.06% in weeds). Variability among BCTV isolates was compared among 10 chile fields in 2001 and among fields in 2002 using PCR and sequence analyses. In chile, CFH and Worland were found singly or in combination in plants. Genetic variability within a BCTV strain was found even within a single chile field.

The broad host range pathogenicity of *S. sclerotiorum* has been attributed to the synergism between oxalic acid and a comprehensive arsenal of cell wall degrading enzymes (CWDEs). We are investigating the relative roles of ambient pH and carbon catabolite repression on the regulation of genes encoding CWDEs in this fungus. So far, we have identified a number of putative members of the carbon catabolite repression regulatory pathway from the DNA sequence database and from a collection of cDNA expressed sequence tags. Expression analysis of these genes by Northern hybridization has determined that (i) they are expressed during plant infection, (ii) acidic ambient pH enhances transcription accumulation of several of these genes, and (iii) carbon-catabolite repression may be involved in their expression. The search for genes encoding molecular chaperones which mediate the carbon catabolite repression regulatory pathway have been previously identified from *S. sclerotiorum* (GenBank Accessions: 2293071 and 28411234). We are currently working to functionally disrupt the snf1 gene to determine its involvement in CWDE gene regulation and pathogenicity.

Accelerated breakdown of aldicarb in Alabama cotton field soils. J. L. Hutchinson (1), K. S. MCLEAN (1), Y. Feng (2), C. Burmester (2), and G. W. Lawrence (3). (1) Dept. of Entomology & Plant Pathology; (2) Agronomy & Soils Dept., Auburn University, Auburn, AL 36849; (3) Dept. of Entomology & Plant Pathology, Mississippi State University, Miss State, MS 39762. Phytopathology 93:S38. Publication no. P-2003-0274-AMA.

Accelerated degradation of aldicarb was examined in soil from two cotton fields. In field 1, aldicarb was effective at reducing *Rotylenchulus reniformis* numbers, while in field 2, aldicarb appeared to have no efficacy. Treatments were arranged in a 2x2 factorial with soil as the main factor and the addition or absence of 0.85 kg a.i./ha of the sub factor. Setten ina, planted in each pot and samples collected at planting and every 3 days thereafter for 43 days. Concentrations of aldicarb and metabolites were measured using HPLC. At planting, aldicarb was detected at 304 and 318 ppm in fields 1 and 2, respectively. Aldicarb levels dropped to 64.5 and 3.5 ppm in fields 1 and 2 at 15 and 9 DAP. Aldicarb sulfone was not detected in field 2 soil but was found at 3 to 43 ppm in field 1 soil. Complete degradation of aldicarb occurred within 12 DAP in field 2 while the aldicarb metabolites were present at 43 DAP in field 1. Rapid degradation of aldicarb occurred in field 2 where the nematicide had no effect on *R. reniformis*.

The role of defense-related genes and oxidative burst in the establishment of systemic acquired resistance to *Xanthomonas campestris* pv. *vesicatoria* in *Capsicum annuum*. B. K. HWANG and S. C. Lee. Laboratory of Molecular Plant Pathology, College of Life and Environmental Sciences, Korea University, Seoul 136-701, Korea. Phytopathology 93:S38. Publication no. P-2003-0275-AMA.

Inoculation of primary pepper leaves with an avirulent strain of *P. sinaloa* caused bright and yellow mosaic spot. Although there are some resistance variety to the virus, most of them are break down when it comes to infected. When using RT-PCR, identification of *Pepper golden mosaic virus* resistance in barley varieties, Mokkuseko3(rym,rym5) and Eas2(rym3) were represented resistance repone but Morihadaka (rym2), Iukushirazu (rym3), Franka (rym4), Misato Golden (rym5), Amakunjo (rym6), Bulgarian347 (rym9) became to infected with this virus. With RAPD markers, bulk segregate analysis method was used for Linkage analysis, it was mapped 30.3 cM genetic distance to the genomic region between OPE 18 to rym, rym5 in Mokkuseko 3 and 30.2cM genetic distance to the genomic region between OPM 14 to rym3 in Gangbori. These results can be used for resistance screening of *BaYMV* to barley varieties.


Bymoviruses (BaYMV, BaMMV) and soilborne wheat mosaic virus are the most severe diseases to barley field in Korea. The obligate parasite transmitted soil borne fungus *Polymyxa graminis* and its symptoms are the yellow mosaic spot in early spring. The farmer’s field of south-east part of Korea were surveyed from 1999 to 2001. The rate of infection to BaYMV was 11.3% and 20% in Cheongdo and Kyungi, which were grown with covered barley, and 52.4%, 62.9% in Kosung and Saseong of southeastern part of Korea, which were growing malting barley. The disease incidence of the transmitted virus were occurred in different farmer’s field. The south-eastern region of Korea were infected with BarYMV (barley yellow mosaic virus), BaMMV (barley mild mosaic virus), SBWMV (soilborne wheat mosaic virus) but others, for example, Kyungsopk infected with BaYMV. The rate of infection was different among the barley type, the malting barley was the most severely infected about 35% but the infection of covered barley was 11% and the infection of naked barley was 9%, respectively.


Three isolates of *Pepper golden mosaic virus* (PepGMV) differ in symptom expression on pepper: PepGMV-Sinaloa (S; formerly SGMV) caused bright golden mosaic, PepGMV-Mosaic (Mo) caused yellow-green mosaic, and PepGMV-Distortion (D) caused mosaic and foliar distortion. The bipartite genome of each isolate was sequenced and compared to two other PepGMV isolates (-Tam and -CR). Nt sequence identity for the five isolates ranged between 92.2-96.0% (DNA-A) and 87.6-99.6% (DNA-B). Cloned DNA-A and DNA-B components of PepGMV-S, -Mo, and -D were infectious by inoculation into pepper, and each isolate’s distinct symptoms were reproduced. PepGMV-S and -Mo were transmissible between pepper and tomato by Bemisia tabaci biotype B whereas, PepGMV-D was not. However, PepGMV-D DNA-A was whitely transmissible when co-inoculated to pepper with either DNA-B of PepGMV-S or -Mo. These results indicate that PepGMV exhibits significant variation in both genotype and phenotype, such that PepGMV may be viewed as a species complex.

DNA polymorphism among strains of *Xanthomonas translucens* in cereals in Russia. A. N. IGNATOV (1), E. V. Matveeva (1), S. V. Tsyganova (2), V. Politko (1), and N. W. Schaad (3). (1) RRI Phytopathology, Moscow, 143083; (2) Centre Bioengineering, Moscow, 117312, Russia; (3) USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD 21702. Phytopathology 93:S38. Publication no. P-2003-0280-AMA.
X. translucens (Xt) is harmful bacterial pathogen of cereals in Russia. We determined the genetic relationships among 45 Xt strains isolated from wheat (10), rye (9), oats (12), and barley (14) in Russia along with Xt type strain 962 (LMG876) and X. campesiris (Xc) NCPPB528 by rep-PCR, ISSR-PCR, primer-terminated reverse-line PCR (PTRLP), and PCR-RFLP using 10-23 ITS region. Based on qualitative differences in PCR profiles, the strains could be divided into four subgroups (SG); 1 (22), 2 (8), 3 (8), and 4 (5). ITS of strains in SG 1 and 2 showed a 98% and 70% similarity to Xt 962 and Xc 528 whereas strains of SG 3 and 4 showed a 69% and 99% similarity to Xt 962 and Xc 528, respectively. The strains of SG 3-4 possessed homologous avrBs2 loci. All strains of SG 1-2 were isolated from wheat or rye and barley, respectively. Strains of SG 3-4 originated from wheat, oats or rye. Thus, considerable genetic variation occurs in strains of Xt in cereal crops in Russia.


Dollar spot, caused by Sclerotinia homoeocarpa, is a common and persistent disease of turgrasses. Our objective was to examine the genetic diversity and pathogenic aggressiveness among isolates collected from two cool-season and five warm-season grasses over a wide geographic range within United States. These isolates clustered into three groups based on similarity of their rDNA ITS sequences. However, isolates were not grouped according to the host or geographic location from which they were collected. For example, isolates from different hosts and/or geographic locations sometimes had identical ITS sequences. Other isolates from the same host and turfgrass sward were placed in different ITS similarity groups. Isolates also varied in pathogenicity to Kentucky bluegrass following inoculation. On average, isolates from one ITS group caused more disease (P < 0.01) on Kentucky bluegrass following inoculations than isolates from the other groups. These results suggest that differences in pathogenicity may be associated with the ITS groups.

Peronosclerospora sorgi resistant to metalaxyl treatment of sorghum seed in Texas. T. ISAKEIT (1), G. Odvody (2), R. Jahn (3), and L. Decanini (1). (1) Texas A&M University, College Station; (2) Texas A&M AREC, Corpus Christi; (3) TCE, Wharton. Phytopathology 93:S39. Publication no. P-2003-0282-AMA.

Sorghum downy mildew (SDM), caused by Peronosclerospora sorgi, appeared in several fields in Wharton county during 2001 and 2002, in spite of metalaxyl or mefenoxam seed treatment. A field trial was established in 2002 to identify fungicide resistance of P. sorgi from this area. There was no reduction (P > 0.05) in systemic SDM in eight hybrids treated with the highest labeled rate of mefenoxam (0.31 g a.i./kg), as compared with the controls (4-26% SDM). Oospores from leaves were used to inoculate mefenoxam-treated seed inoculated with these isolates ranged from 40-76%, which was not different (P > 0.05) from the controls, 30% and 41%, respectively. Sporangia from plants taken from five locations were used to inoculate Kentucky bluegrass following inoculation. On average, isolates from one ITS group caused more disease (P < 0.01) on Kentucky bluegrass following inoculations than isolates from the other groups. These results suggest that differences in pathogenicity may be associated with the ITS groups.

Clavibacter michiganensis subsp. sepedonicus complete genome sequencing project. C. A. ISHIMARU (1), S. E. Brown (1), D. L. Knudson (1), D. M. Francis (2), and J. Parkhill (3). (1) Colorado State University, Dept. of Biogticultural Sciences and Pest Management, Fort Collins, CO 80523; (2) Ohio State University, Dept. of Horticulture and Crops Sciences, Wooster, OH 44691; (3) Sanger Institute, Wellcome Trust Genome Campus, Pathogen Sequencing Unit, Hinxton CB10 1SA, UK. Phytopathology 93:S39. Publication no. P-2003-0283-AMA.

Clavibacter michiganensis belongs to the high GC gram-positive bacteria and contains several major phytopathogens of monocots and dicots. Little is known of the molecular basis of its pathogenicity or host specificity. The complete genome of the type strain of a representative subspecies, C. michiganensis subsp. sepedonicus, is being sequenced by a whole-genome shotgun strategy. The shotgun-sequencing phase of the project has been completed with 61,043 reads, giving 32.6 Mb of sequence for a theoretical genome size of 3.4 Mb. The GC content is 72.2%- and typical of the species. Progress on closure and annotation will be presented.


Phytophthora blight, caused by Phytophthora capsici, is one of the most serious threats to processing pumpkin (Cucurbita moschata) industry in Illinois. In the past four years, we observed differences in severity of disease and aggressiveness among fields. These differences raised the question whether the isolates of P. capsici in different fields vary in aggressiveness. This study was conducted to investigate genetic diversity and pathogenic variation among the processing pumpkin-infecting P. capsici isolates. Fifteen isolates of P. capsici from 15 different processing pumpkin fields in different locations were included in this study. DNA fingerprinting using seven random amplified polymorphic DNA (RAPD) primers (10-base oligonucleotide, Operon Technologies, CA) was employed to assess genetic variation among the isolates. RAPD analysis clustered the isolates into six groups irrespective of mating types. Inoculation of pumpkin seedlings in greenhouse revealed that isolates belonging to distinct genetic groups differ significantly in their pathogenicity.


Development of fungicide resistance is now one of the major problems in plant disease control. Important factors contributing to the potential for evolution of resistance in the pathogen may include the availability and rates of mutations conveying resistance and their associated genetic mechanisms and fitness costs. As a model for fungicide resistance, we have evolved resistance in experimental populations of Basidiomycetes, a rich system for forward and reverse genetics and functional genomics. Results of these experiments will be presented. These studies will be extended to experimental and field populations of two important plant pathogenic filamentous fungi, the genetically tractable Botrytis cinerea and its close relative, Sclerotinia sclerotiorum, in which field resistance has been observed.


Aspergillus flavus is the main causal agent of aflatoxin contamination of cottonseed. A. flavus can be divided into two strains, S and L, based on physiological, morphological, and genetic criteria. Strain S isolates produce greater quantities of aflatoxins than L strain isolates in cottonseed. Aflatoxin contamination of cottonseed can be severe in South Texas. The structure of A. flavus communities associated with South Texas cottonseed was determined by analyzing 186 truckloads of commercial cottonseed sampled at the Valley Co-op Oil Mill in Harlingen, TX from 1999 through 2001. The quantity of A. flavus (CFU) and the percent of A. flavus composed of the S strain were both correlated with aflatoxin contamination of South Texas cottonseed. CFU differed between both regions and seasons, while percent S only differed between regions. The Rio Grande Valley had significantly lower CFU and S strain than the Coastal Bend and Upper Coast regions. Cottonseed produced in 1999 had significantly more A. flavus than that produced in either 2000 or 2001. Results suggest that the S strain may be an important causal agent of aflatoxin contamination in South Texas.


Pine wilt, caused by the pinewood nematode Bursaphelenchus xylophilus, is a lethal disease of Scots pine throughout the central and eastern United States. We tested tree injection with the insecticide/nematicide abamectin to prevent the disease. Scots pine trees (10 cm dbh) were left untreated or were injected in May 2002 with 30 ml water or 2% abamectin using pressurized systemic tree injection tubes. Liquids were successfully injected into all trees within 1 h with no leakage or excessive resin production. On 20 Jun, wounds on two branches on each tree were inoculated with a water suspension containing nematodes. By December, 14/18 of the untreated, 8/20 of the water-treated, and 4/20 of the abamectin-treated trees had major branch dieback or were dead from pine wilt.

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another study, non-inoculated, mature Scots pines (32-86 cm dbh) were either injected with abamectin in April 2002 or were not treated. By Jan 2003, 7/50 of the non-injected trees, but none of the 50 abamectin-treated trees, had died from pine wilt.


Labor shortages in the U.S. necessitate development of a mechanical approach to harvest apples. A new harvester which uses a rapid displacement actuator on main stalks to remove apples from trees with narrow-inclined trellises has shown good potential. With this technique, loss of stem during harvesting is reduced to 57%, depending on cultivar. In particular, apple skin is removed together with the stem, flesh tissue is exposed creating a potential point of entry for pathogens. We evaluated the susceptibility of the stem area, with and without stem pulls, to blue mold decay on 11 cultivars of mechanically harvested apples, and the effectiveness of P. syringae (used in BioSafe 110) to control these defects. On fruits with stem pulls inoculated with P. expansum decay incidence ranged from 0% on Jonagold, Pink Lady and Spitzen to 41% on Envy. Between cultivars it ranged from 1.7 to 8.3%. On fruit with the stems, decay did not exceed 3.3%, except on Gala (6.6%). P. syringae reduced decay on fruit with stem pull to 3.3% on Empire and Gala, and below that on other cultivars with 7 cultivars having no decay.

Identification of defense response genes involved in the interaction between rice and rice blast (Magnaporthe grisea). C. JANTASURIYARAT (1), G. Lu (1), M. Gowda (1), B. Zhou (1), J. Hatfield (2), D. Kudrna (2), R. Dean (3), C. Soderlund (2), R. Wing (2) and G.-L. Wang (1). (1) Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43210; (2) Arizona Genomic Institute & Computational Laboratory, University of Arizona, Tucson, AZ 85721; (3) Fungal Genomics Laboratory, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695. Phytopathology 93:S40. Publication no. P-2003-0289-AMA.

To identify defense genes up or down-regulated in rice blast interaction, a large-scale EST sequencing approach has been initiated. Eight cDNA libraries reflecting different aspects of the disease resistance were constructed. Two libraries were made from resistant reaction in different time points after inoculation, two libraries from susceptible reaction, two libraries from partial resistant reaction, one library from a lesion mimics mutant, and one control library. About 7000 clones from each library were randomly picked up and up to 30 clones from each library had been othercultivars or cultivar. ESTs were analyzed and posted at AGI database. This large collection of rice ESTs will be a valuable resource for functional genomics in rice and other cereals.


The mechanisms by which rootstocks exert their influence on scions are not well understood. This study represents a comprehensive comparison of gene expression patterns in apple (Malus domestica cv Gala) scions grafted to either M.7 EMLA or M.9 T337 rootstocks. The M.7 EMLA rootstock is semi-dwarfing and reduces susceptibility of the scion to Erwinia amylovora. In contrast, the M.9 T337 rootstock is dwarfing and does not alter fire blight susceptibility of the scion. Expression was determined using cDNA-AFLP coupled with silver staining of the gels. A total of 92 unique differentially amplified bands were identified. Scions grafted to M.9 T337 rootstock exhibited greater expression of a number of photosynthesis-related, transcription/translation-related, and cell division-related genes. Scions grafted to M.7 EMLA rootstocks showed increased expression of a number of stress-related genes. The increase in stress-related gene expression in scions grafted to M.7 EMLA rootstocks may play a role in the greater degree of tolerance to various stresses observed with scions grafted to M.7 rootstocks.


The rice blast fungus, Magnaporthe grisea, is one of the most studied plant pathogenic fungi. Whole genome sequence of this pathogen along with automated annotation and ORF prediction was released in June 2002. About 12,000 genes are predicted in the genome of M. grisea. EST alignment has been an essential tool to confirm gene expression, and contributes to the annotation. In addition, cDNA clones are useful for functional genomics, particularly proteome analysis. However, the EST projects often suffer from redundancy within cDNA libraries, which imposes a restraint in gene finding efforts. Various cDNA normalization strategies have been reported. We generated full-length cDNA library using SMART (Switching Mechanism At 5’ end of the RNA Transcript) technology. This technique relies on the intrinsic TiD activity of reverse transcriptase to create full-length cDNA. The SMART technology can be integrated into recombinational cloning system such as the GATEWAY technology. We will discuss the use of SMART and GATEWAY technologies to construct a normalized full-length cDNA library from the appressorial stage of the rice blast fungus.


Fusarium solani f. sp. glycines (Fsg) has been reported to produce at least two phytotoxins. Li and her collaborators (1999) have earlier shown that cell-free culture filtrates of Fsg develop sudden death syndrome (SDS) symptoms in soybean leaves. We have shown that cell free extracts prepared from two Fsg isolates produced SDS symptoms in leaves of the susceptible cultivar Williams 82. Both 1-D SDS PAGE and 2-D SDS-PAGE were applied to test the protein profiles of diseased and healthy leaves. A ~56 kDa protein is degraded in diseased leaves following feeding of soybean seedlings with Fsg cell-free extracts. MALDI-TOF MS was applied to determine the mass fingerprint of this protein. Data base search using this fingerprint revealed that the peptide is indeed Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit, that was shown to be degraded by the victorin toxin produced by the fungal pathogen Cochliobolus victoriae in oats (Navarre and Wolpert, 1999).


Immunofluorescent localization studies of six isolates of Tobacco ringspot virus in the nematode Xiphinema americanum were conducted. These viruses were originally isolated from various hosts (soybean, blueberry, blackberry, and grape) from different areas of the United States, and the isolates caused a range of symptoms and disease severity on selected hosts. Populations of X. americanum acquired virus from cucumber infected with each of the six TRSV isolates. The viruliferous nematodes were recovered and used in immunolocalization and transmission studies. In parallel comparisons of transmission efficiency, the blueberry isolate was consistently transmitted at higher levels, while an isolate from blackberry was transmitted with lower efficiency. Immunofluorescent localization studies of TRSV isolates in viruliferous nematodes demonstrated similar sites of retention for all six TRSV isolates. The major site of retention was the style extension and all isolates had a low frequency of retention in the esophagus and esophageal bulb.


Fourteen Rhizoctonia solani (rice sheath blight) isolates from five counties in Arkansas, the major rice-growing state in the United States, were characterized by anamorphosis, nucleotide sequence of the internal transcribed spacer (ITS) region and pathogenicity assays. Imperfect hyphal fusion with an AG1-IA tester indicated that all 14 isolates belonged to the AG1-IA group. Phylogenetic analysis of nucleotide sequence of rDNA-ITS revealed three classes of the pathogen isolates with minor nucleotide substitutions. Sequences of a major class of rDNA-ITS (9 isolates) were identical to the sequence of rDNA-ITS of isolates from Vietnam and the Philippines. Virulence of the pathogenic isolates toward rice cultivars was evaluated by a detached leaf inoculation method. The second youngest leaves of greenhouse-grown rice plants were detached and inoculated with potato dextrose agar plugs containing hyphae and inoculated in petri dishes. Most virulent, moderate virulent and least virulent isolates were determined three days after inoculation. Differential responses of rice cultivars toward the isolates were also detected by this method. No correlations between nucleotide sequences of rDNA-ITS and virulence of the pathogen isolates were evident. Characterized R. solani isolates should facilitate the development of rice cultivars that are more resistant to sheath blight.
Stepwise evolution of *Fusarium oxysporum* f. sp. *ciceris* races inferred from fingerprinting with repetitive DNA sequences. M. M. Jimenez-Gasco (1), M. G. Milgroom (2), and R. M. Jimenez-Diaz (1,3). (1) Dept. of Crop Protection, Institute of Sustainable Agriculture, CSIC, 14080 Cordoba, Spain; (2) Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853; (3) University of Cordoba, Spain. Phytopathology 93:S41. Publication no. P-2003-0295-AMA.

*Fusarium oxysporum* f. sp. *ciceris*, the causal agent of Fusarium wilt of chickpea, consists of two pathotypes (yellowing wilt) and eight races (0, 1B/C, 1A, 2-6) of diverse geographic distribution. The genetic variability and evolutionary relationship among races were investigated by DNA fingerprinting with repetitive sequences. The inferred phylogeny of races based on DNA fingerprinting shows that each race forms a monophyletic clonal lineage. By mapping virulence to each differential cultivar onto the inferred phylogeny, we show that virulence has been acquired in a simple stepwise pattern, in which mutations to virulence accumulate sequentially in clonal lineages, resulting in races capable of overcoming multiple host plant resistance genes or multiple resistant cultivars. We propose that these changes in virulence occurred over the last 9,000 years in association with the spread of chickpea agriculture.


*Magnaporthe grisea* (anamorph *Pyricularia grisea*) causes blast in rice and gray leaf spot (GLS) in turfgrass. Much work has been done to elicit the genetic mechanism of blast resistance in rice. Gray leaf spot resistance in turfgrass has been documented but little is known about the genes responsible for this resistance. To investigate if homologues of rice defense genes are conserved in turfgrass and involved in the defense response to GLS infection, we used genomic DNA from St. Augustinegrass to hybridize to 47 rice defense response genes originally identified by subtractive suppression hybridization (SSH). St. Augustinegrass DNA strongly hybridized with thirteen SSH rice genes. Northern blot analysis is underway to determine the expression of these genes in GLS resistant and susceptible cultivars of St. Augustinegrass. Cross infection assays of different *M. grisea* isolates on rice and turfgrass will be presented.

Cultivar susceptibility to apple scab: variation among inoculum sources. T. JOBIN (1), P. Neumann (2), and O. Carisse (1). (1) HRDC, Agriculture and Agri-Food Canada, 430 Gouin Blvd, Ste-Jean-sur-Richelieu, QC J3B 3E6, Canada; (2) Dep. Sciences Biologiques, Université de Montréal, C.P. 6128, Montréal, QC, H3C 3J7, Canada. Phytopathology 93:S41. Publication no. P-2003-0297-AMA.

Most studies on cultivar susceptibility to apple scab, caused by *Venturia inaequalis*, were conducted using conidia isolated from the cultivar MacIntosh. Variation among inoculum originating from different cultivars was studied. Conidia from MacIntosh, Cortland, Empire, Spartan and Paulared were collected from scabbed leaves. The same 5 cultivars were inoculated with each of the collected inoculums and with an inoculum made from a mixture of conidia from all 5 cultivars. Disease assessment was made every 2-3 days until 22 days after inoculation. Disease incidence, severity, conidia production, leaf area diseased and infection efficiency were evaluated. For each combination of inoculum-cultivar, electron-microscopy was used to observe the interactions between conidia and cultivar 24 hours after inoculation. Both the cultivar and the inoculum significantly influenced the number of lesions per leaf. Conidia production per lesion was related to the inoculum but not to the cultivar.


The establishment of exotic plant pests within the United States (US) poses significant risk to US agricultural and environmental resources. The New Pest Advisory Group (NPAG) is the process within the USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) that provides a systematic process to rapidly evaluate new and potential pest introductions. Pests may include arthropods, plant pathogens, nematodes, weeds and mollusks. This assessment includes data collection, synthesis and dissemination. The data may be analyzed by an *ad hoc* panel comprised of the APHIS and University experts. The result of this assessment is a recommendation to PPQ management as to how the agency should respond to the pest situation.


*Globodera tabacum solanacearum* (tobacco cyst nematode – TCN) is one of the most serious disease problems of flue-cured tobacco in Virginia. However, TCN reproduction is strongly FCR (cultivar resistance) specific. The *Ph* gene for resistance to *Phytophthora parasitica var. nicotianae* (black shank) is an important tool to help reduce nematicide use and increase economic returns.

Development of rice cultivars with improved blast resistance using marker assisted selection. V. JOHNSON (1), J. Gibbons (1), K. Molenhauer (1), Z. Wang (1,2), and Y. Jia (2). (1) UA RREC, Stuttgart, AR 72160; (2) DB NRRC, Stuttgart, AR 72160. Phytopathology 93:S41. Publication no. P-2003-0300-AMA.

Marker assisted selection is being employed in rice breeding to accelerate the development of cultivars with improved rice blast *Magnaporthe grisea* resistance. A dominant gene marker was used to monitor the incorporation of the rice blast resistance gene *Pi-ta* in 2,600 genomic DNA samples of *F₂* plants. F₂ plants carrying specifically a resistant allele of the *Pi-ta* gene were analyzed by agarose gel electrophoresis. In one group of 2,109 samples of 7 *F₂* populations, about 33% were homozygous for the resistant allele, 17% were homozygous for the susceptible allele, and 50% were heterozygous as determined by the *Pi-ta* marker. This marker was used to verify the results of pathogenicity tests. When seedlings were inoculated with isolates containing the corresponding avirulence allele, 50% were resistant to infection. Seedlings determined to have the susceptible allele were susceptible to infection. Use of *Pi-ta* marker data increases the efficiency and speed of cultivar development.

Molecular analysis of Cytochrome P-450 genes from the pine stem rusts. D. L. JOLY (1,2), L. Bernier (2), S. F. Covert (3), and R. C. Hamelin (1). (1) Centre de recherche en biologie forestière, Université Laval, QC, Canada; (2) Centre de recherche en biologie forestière, Université Laval, QC, Canada; (3) Warnell School of Forest Resources, University of Georgia, GA. Phytopathology 93:S41. Publication no. P-2003-0301-AMA.

Cytochrome P-450 (CYP) genes are known to be involved in the oxidative metabolism of endogenous and xenobiotic compounds. A single amino acid change can often result in the ability or the inability to use or degrade a substrate. P-450s are involved in phytoalexin detoxification in some host-pathogen interactions. Molecular analysis of P-450s in *Cronartium* and *Peridermium*rusts showed a ratio of nonsynonymous to synonymous substitutions four times higher than in other coding regions, suggesting positive selection. Intra-specific polymorphisms were also observed in some cases. For the pine blister rust, *C. ribicola*, we found a Single Nucleotide Polymorphism that causes an amino acid change. In eastern North American populations of *C. ribicola*, two alleles, Cyp157 and Cyp142, were present but western populations were fixed for one allele. This contrasts with the loci analyzed so far, where both populations shared the same alleles but different in allele frequency.

Susceptibility of creeping bentgrass to isolates of *Sclerotinia homoeocarpa*. J. D. JONES and A. M. Manbhum. University of Illinois, Dept. of Natural Resources and Environmental Sciences, Urbana, IL 61801. Phytopathology 93:S41. Publication no. P-2003-0302-AMA.

Dollar spot, caused by *Sclerotinia homoeocarpa*, is a major fungal disease of creeping bentgrass (*Agrostis palustris* Huds.) in the United States and Canada. While six vegetative compatibility groups have been identified within this species, virulence effects of these fungi on creeping bentgrass varieties are unknown. The objective of this research was to determine if variability in resistance exists among creeping bentgrass varieties when inoculated with a collection of *S. homoeocarpa* isolates. Fifteen commercial bentgrass varieties representing diverse genetic backgrounds were inoculated with 25 isolates collected from the Midwestern U.S., Arkansas, Florida, and Rhode Island. Plants were assessed for percentage of leaf area displaying
dollar spot symptoms and discriminant analysis was used to examine variation. Detecting the existence of races will lead researchers to identify mechanisms of resistance, select resistant varieties, estimate the longevity of resistance, and develop more comprehensive fungicide trials.

Fine mapping of the resistance gene Fom-2 in melon using SSR and STS markers derived form BAC end sequences. T. JOOBEUR (1), J. J. King (2), S. J. Nolin (1), C. E. Thomas (3), and R. A. Dean (1). (1) Department of Plant Pathology, Fungal Genomics Laboratory, North Carolina State University, Raleigh, NC 27695; (2) Seminis Vegetable Seed 37437 State Highway 16, Woodland, CA 95695; (3) USDA-ARS, US Vegetable Laboratory, Charleston, SC 29414. Phytopathology 93:342. Publication no. P-2003-0303-AMA.

The soil-borne fungus Fusarium oxysporum f. sp. melonis causes significant losses in commercial melon worldwide. Isolates of a non-toxigenic strain were cured with control measure available is the use of resistant cultivars. In melon, resistance to F. oxysporum races 0 and 1 is conferred by a single dominant gene; Fom-2. Two codominant PCR based markers FM and AM were found to flank Fom-2 at 0.7 and 4 cm, respectively. Using a chromosome walking approach, a single contig of BAC clones covering the genetic interval encompassing Fom-2 was constructed. The BAC end sequences of these clones were successfully used to develop SSR/STS markers and delimit the position of Fom-2 to two BAC clones. In order to narrow down Fom-2 physical interval and identify candidate genes, the two BAC clones are being subjected to shotgun sequencing. Sequence data and results of annotation will be presented.


The gene, cpmk2, encoding a mitogen-activated protein kinase (MAPK) of C. parasitica was cloned and examined the biological function of cpmk2. The sequence comparison of cpmk2 shows the highest homology to pmk1 of Magnaporthe grisea and reveals that it belongs to the yeast extracellular signal-regulated kinase (YERK1) subfamily. The E. coli-derived CpMK2 showed the basal level of kinase activity indicating the gene product is a catalytically active protein kinase. The cpmk2 disruption resulted in several phenotypic changes such as impaired pigmentation, reduced conidiation, hydroporosopy, and growth defect on solid plates. The reduced laccase production, acid-supplemented plate, and conidiation, displaying a dramatic reduction in conidial area, by the cpmk2-null mutant were may be due to the reduced growth on solid media rather than the transcriptional down-regulation of the tannic acid-inducible laccase and the results from impaired virulence factor(s), respectively. A molecular symptom of the down-regulation of mating pheromone gene Mf2/1 was also observed in the cpmk2 mutant. Comparing with other previously known disruption mutants, almost all the phenotypic changes in the cpmk2-disruptant, the major Gshp, protein gene of C. parasitica, are phenocopied in the cpmk2-null mutant, which suggests that, in addition to cAMP pathway, CpMK2 pathway appears to function downstream of a CPG1-dependent signal.


Fusaria of the Liseola section were isolated from 1996 to 1998 corn survey samples (162, 104 and 111 samples respectively) and tested for fumonisin contamination. The incidence of isolates belonging to mating population A (F. verticilliodes) ranged from 70.2 percent to 89.5 percent. In the 1996 to 1998 samples, from 63 percent of the isolates, were contaminated with fumonisin B1 from 0.1 to 91 mg/kg, with fumonisin B1 from 33.5 to 33.5 mg/kg. Pearl millet samples were collected from 1996 to 1998. 119 isolates were paired with mating population A (F. verticilliodes), D (F. proliferatum) and F (F. thapsinum) tester strains. No successful crosses were obtained with mating population F, 50.4 percent were fertile with mating population A and 10.1 percent crossed with mating population D. 39.5 percent did not form any perithecia. We isolated Fusarium pseudogriseum Link and believe that many of the isolates that were infertile belong to this species. This finding was confirmed by DNA sequence comparisons with known isolates from Nigeria. Fumonisins FB1 and FB2 were not detected in any of the 81 analyzed grain samples.


Aflatoxins are natural carcinogens found in the environment and are of great interest from a food safety standpoint. They are secondary metabolites produced mainly by Aspergillus flavus Link and Aspergillus parasiticus Speare. Volatiles metabolites from A. flavus NRRL 3357 (aflatoxigenic) and A. flavus NRRL 1579 (non-aflatoxigenic) cultured on corn were collected by purging and trapping headspace. Volatiles were absorbed by thermal desorption (TD) columns. Columns were then placed in a thermal desorption unit which injected the volatiles into a GC/MS for analyses and identification. In a preliminary study (after 1, 3 and 5 days) we were able to distinguish volatiles that were associated only with A. flavus (toxicigenic) but not with A. flavus (non-toxicigenic). Volatiles that were associated with toxicigenic A. flavus included ethyl acetate, 3-methyl-1-butanol, 1-pentanol, 1-hexanol and nonanal, and with non-toxigenic A. flavus 2-methyl-1-propanol, 1-penten-3-ol, 2-pentanol, and 1-octene-3-ol, were identified.

Dazomet as an effective alternative to methyl bromide in northern USA forest nurseries. J. ZUWIK and J. Pokorny, USDA Forest Service, St. Paul, MN. Phytopathology 93:S42. Publication no. P-2003-0307-AMA.

Dazomet (Daz), a chemical fungitd for soil-borne pests, is a potential alternative for bare-root forest nurseries historically reliant on methyl bromide (MBR) fumigation. Experimental and observational field trials were conducted (1994 – 2000) in North Central nurseries to 1) refine guidelines for Daz use within nursery soil management regimes, and 2) compare Daz to MBR in the reduction of soil-borne pests and subsequent effect on seedling quality. Guideline-related studies identified cover crop residue management, soil treatment, control, and tillage management, and identified pre-fumigation factors to consider. These factors subsequently impact soil fungal populations, root disease development and seedling quality. Important Daz application factors identified were incorporation depth and implement selection, chemical rate, and soil moisture levels. In comparative trials, Daz performed as well or better than MBR in reducing selected soil fungi and in producing quality seedlings. Acceptance of Daz as an MBR alternative will depend on grower willingness to consider multiple factors and to use it within an integrated soil management regime.


A putative tobanamovirus, Florida hibiscus virus, was recently isolated from hibiscus landscape plantings in Florida. Accurate diagnosis of this virus is essential to prevent clay. We compared indirect and double-antibody sandwich (DAS) chain reaction (IC-RT-PCR) as compared to evaluate their usefulness for diagnosis of this virus. Both indirect and double-antibody sandwich (DAS) ELISA were more sensitive than DBIA with hibiscus bark and leaf extracts. Both ELISA and DBIA were 2-8 times more sensitive than DAS-ELISA with hibiscus bark and leaf extracts. Both ELISA and DBIA were able to detect < 1 pg of virus in partially purified preparations and was 2-8 times more sensitive than DAS-ELISA with hibiscus bark and leaf extracts. Both ELISA and DAS-ELISA techniques and DBIA are applicable to large numbers of samples because of their simplicity. IC-RT-PCR could be a useful alternative to ELISA and DBIA for virus detection in cases where sample size is limited or virus concentration is low.


Sclerotinia sclerotiorum is widely distributed on potato and other crops in the Columbia Basin of Washington (CB). Previously, populations of S. sclerotiorum in other regions were found to be clonal. Population analyses of 207 isolates from potato and other crops in CB and 16 isolates from other regions using 11 microsatellite markers revealed high genotypic variability among isolates. Differences were found between potato fields, especially in comparisons between collection years. But no significant differences were found among crops in CB or with populations in other geographic regions. Substantial outcrossing rates were inferred from the detection of multiple mycelial compatibility groups (MCG) within progeny of single apothecia. No significant differences were found among isolates for in-vitro response to
4 fungicides and 4 temperatures, and no lesion size differences were observed in aggressiveness tests on potato stem. No correlations were found between microsatellite haplotypes and MCG, or any of the phenotypic characteristics.


Stem rot, caused by Sclerotinia sclerotiorum, has historically been difficult to manage on potato in the Columbia Basin of Washington. Fungicide applications are currently recommended prior to row closure. Ascosporas were captured by exposing a semi-selective medium over potato canopies and by placing harvested blossoms onto agar medium over two years in ten fields. Ascospore numbers peaked around initial full bloom. Nearly all collected blossoms at full bloom were contaminated with S. sclerotiorum. Infection occurred in commercial fields when contaminated blossoms fell on the plant canopy, or when stems came in contact with colonized blossoms fallen on the ground. Disease incidence was significantly reduced in replicated plots where flowers were removed or registered fungicides were applied at initial full bloom. No significant differences were found between iprodione, dichloran and fluziram in preventing stem rot occurrence in greenhouse experiments. Initial fungicide application should be scheduled to cover blossoms at initial fluazinam in preventing stem rot occurrence in greenhouse experiments.

Variability and survival of Clavibacter michiganensis subsp. michiganensis (Cmm) in seed. W. S. KANESHIRO and A. M. Alvarez. Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI 96822. Phytopathology 93:S43. Publication no. P-2003-0311-AMA.

Validation of seed assays for Cmm at multiple testing facilities requires the use of uniformly infested seeds to attain reproducible results between labs. Complications arise because Cmm strain characteristics vary and survival rates in seed are unknown. Cmm strains from infested seed lots were characterized to determine pathogen variability. An additional seed lot was generated by inoculation of potato pedicels with a virulent strain to quantify effects of prolonged storage on recovery. Cmm strains exhibited phenotypic and genotypic variability that often prevented consistent pathogen identification. Concentrations of Cmm in individual seeds were highly variable, and showed a general decline after 6 months of storage at room temperature. At 12 and 18 months post-harvest, Cmm was recovered from 13 of 60 seeds using a nonselective medium, but was recovered from only 8 of the 13 seeds when antibiotics were added. Reduced Cmm recovery over time may indicate increased susceptibility of the quarantine cells to antibiotics that would lead to an underestimation of viable pathogen populations in stored seed.

Characterization of an RND transporter located within the 13 seed when antibiotics were added. Reduced Cmm recovery over time may indicate increased susceptibility of the quarantine cells to antibiotics that would lead to an underestimation of viable pathogen populations in stored seed.

A tripartite resistance-nodulation-cell division (RND) efflux system, called the PseABC transporter, was identified at the left border of the syringopine (spy) gene cluster of Pseudomonas syringae pv. syringae strain B301D. The RND efflux system is encoded within a 5.7-kb operon encoding an outer membrane protein (PseA), a periplasmic fusion protein (PseB), and an RND-type cytoplasmic membrane protein (PseC). The RND efflux system encoded by the pseABC operon is homologous to a putative RND efflux system of Ralstonia solanacearum with sequence identities of 48% (i.e., PseA), 51% (i.e., PseB), and 61% (i.e., PseC). Nontypical mutations within the pseABC operon were generated by nptII insertional mutagenesis. Resultant mutant strains were reduced significantly in secretion of both syringomycin and syringopine. Expression of a pseA::uidA reporter fusion was reduced in either a gacA or gacA mutant background. The PseABC transporter is the first RND transporter described for P. syringae, and it appears to have an important role in secretion of lipopeptide toxins.

Plant Pathogen Database: A community resource for archiving and sharing the genetic and phenotypic information of important pathogens. S. KANG (1), I. Makalowska (2), R. Frederick (3), and D. Luster (3). (1) Depts. of Plant Pathology; (2) Biology, Penn State, University Park, PA 16802; (3) Foreign Disease-Weed Science Research Unit, USDA-ARS, Ft. Detrick, MD 21702. Phytopathology 93:S43. Publication no. P-2003-0312-AMA.

Although efforts to characterize the phylogenetic relationship and population structure of plant pathogens using genetic markers have substantially increased in recent years, complementary efforts to archive the resulting data have been limited. As a consequence, it is often difficult to compare the available data for a given pathogen from multiple laboratories. The main goal of this project is to develop a searchable, internet-based database that links the genotypes and phenotypes of important plant pathogens at both the species and population levels. Multiple search tools will be incorporated to allow visualization of the temporal, spatial, and evolutionary dynamics of pathogen populations. The database will also expedite the identification of newly isolated pathogens by those researchers who have limited experience in taxonomy or by regulatory agency scientists who must quickly assess the potential threat of newly isolated strains.


Xanthomonas oryzae pv. oryzae (Xoo) is the causative agent of rice bacterial blight (BBB), a worldwide disease that is particularly destructive in America and Asia. BBB is controlled essentially through the use of resistant varieties. To develop an appropriate disease management strategy, the genetic diversity of the pathogen’s populations must be assessed. Until now, the genetic diversity of Xoo was characterized by RFLP analyses using ribotyping, and plasmid and genomic Xoo probes. We used AFLP (amplified fragment length polymorphism), a novel PCR-based technique, to characterize the genetic diversity of Xoo isolates. 12 Xoo representative strains were tested with 65 AFLP primer combinations to identify the best selective primers. Eight primer combinations were selected according to their reproducibility, number of polymorphic bands and the percentage of DNA that was excluded between Xoo strains. Forty-seven Xoo strains were analysed with the selected combinations. Some primer combinations differentiated Xoo strains that were not distinguished by RAPD analyses, thus AFLP fingerprinting allowed a better definition of the genetic relationships between Xoo strains.


Use of winter cover crops to prevent soil erosion and add organic matter is critical on the sandy soils in the Midlands of South Carolina. However, certain cover crops may increase pathogens or root diseases in subsequent vegetable crops. Nine cover crops, plus a weedy fallow control, were planted in a randomized complete block design in fall 1994 and fall 1995 on a commercial farm in Pelion, SC. Crops were planted in the same plots each year. Population densities of Rhizoctonia and Fusarium were estimated in non-rhizosphere soil samples collected in fall 1994, spring 1995, spring 1996 and summer 1997. Percentage of organic matter fragments colonized by Rhizoctonia was greater following legumes (mean of 37%) than grains (30%) (P < 0.01). Fusarium CPU were consistently greater in cropped soil than in weedy or fallow soil, and greater in 1995 than 1994 (P < 0.01). Collard was planted in the plots in each summer. Despite differences in fungal populations, Rhizoctonia wistem was not detected and occurrence of Fusarium yellows did not differ among treatments.

Isolation and characterization of persistent phytopathogenic bacteria from orchids. L. M. KEITH (1), K. T. Sewake (2), and F. T. Zee (1). (1) USDA-ARS, PBARC, Hilo, HI 96720; (2) CAHAR, University of Hawaii, Hilo, HI 96720. Phytopathology 93:S43. Publication no. P-2003-0316-AMA.

Although phytopathogens can be found in all orchid production areas, Hawaii grower knowledge about the diseases rarely extends beyond recognizing whether the pathogen is viral, fungal, or bacterial. We report the results of a survey that we conducted with local growers to determine the prevalence of bacterial orchid diseases in Hawaii. On various orchid genera, we recorded symptoms ranging from leaf spots with or without water-soaking or chlorosis to soft rots. Bacteria isolated from field and greenhouse samples were identified using API Strip, standard physiological and biochemical tests. Identification was confirmed by bacteria species-specific PCR. We commonly identified Erwinia spp. and Burkholderia spp. from diseased orchid tissue. Testing these isolates for growth on media containing copper or streptomycin showed our isolates to be resistant. Our field survey and laboratory data suggest that Hawaii’s growers may have inadvertently selected for resistant strains of phytopathogenic bacteria that are now persistent in their stock.
Several species of *Brachia*, a pan-tropical grass genus comprising about 100 species, are forges of economic importance in tropical America and elsewhere. *Acremonium implicatum* forms a symbiotic endophytic associ-

ation with at least some of these economically important *Brachia* species. We sought whether endophytic *A. implicatum* could be seed-

transmitted in *Brachia*. Twenty tillers were vegetatively propagated from a single, endophyte-infected mother plant. Ten tillers were treated with the fungicide Follicul® to eliminate the endophyte while the remaining ten tillers were untreated. Seeds were harvested individually from these genetically identical plants, with or without the endophyte. Some of the seeds were germinated and seedlings grown in the glasshouse. A polymerase chain reaction (PCR)-based method developed previously uses a pair of endophyte-

specific primers to amplify a single DNA fragment of about 500 bp. DNA both from remnant seeds and from 2-month-old seedlings was amplified with these primers to detect presence of the endophyte. The diagnostic DNA fragment was consistently amplified in DNA of seeds harvested from the endophyte-infected plants and DNA from seedlings grown from seeds harvested from endophyte-infected plants, but not from seeds or seedlings originating from fungicide treated endophyte-free plants. We conclude that *A. implicatum* can be transmitted through seeds.


An atypical pea disease was observed in Oregon’s Willamette Valley. Electron microscopy of thin sections revealed only cellular disorganization. Antisera to 12 common pea viruses failed to detect any viruses by DAS-ELISA in mechanically maintained isolates. In host range studies 3 legumes and ELISA in mechanically maintained isolates. In host range studies 3 legumes


**Evaluation of Doppler-radar based AU-pnut advisory for disease management in peanut.** R. C. KEMERAIT, JR. (1), T. B. Brenneman (1), and G. Hoogenboom (2). University of Georgia, (1) Dept. of Plant Pathology, Tifton, GA 31794; (2) Dept. of Biological and Agricultural Engineering, Griffin, GA 30223. Phytopathology 93:S44. Publication no. P-2003-0319-AMA.

Fungicides needed to control diseases of peanut are typically applied on a 14-day calendar schedule; however timing can be based on the AU-pnut advisory radar events since the last spray is an important variable. In 2001 and 2002 field studies were conducted in Tifton and Attapulgus, GA to compare severity of early leaf spot and southern stem rot and yield in plots treated on a 14-day schedule to plots where the AU-pnut schedule was determined with Doppler radar. Also, rainfall at 10 sites was measured daily between 1 May and 31 October using automated rain gauges and Doppler radar. In 2001, daily weather information was similar between Doppler radar and rain gauges (90.5%); however radar overestimated rain events 8.8% of the time and did not detect rain events 0.7% of the time. Each year, disease control and yield were similar between plots treated on calendar and AU-pnut schedules. In Attapulgus trials and the 2002 Tifton trial, AU-pnut required 8 applications compared to 7 applications for the calendar schedule.

**Integration of lesion productivity and ontogenic resistance of fruit into a warning system for grape downy mildew (Plasmopara viticola).** M. M. KENNELLY (1), R. C. Seem (1), D. M. Gadoury (1), W. F. Wilcox (1), and P. A. Magarey (2). (1) Cornell University, Dept. Plant Pathology, NYSAES, Geneva, NY 14456; (2) South Australian Research and Development Institute, Loxton, SA 5333, Australia. Phytopathology 93:S44. Publication no. P-2003-0320-AMA.

DMCast, a previously-described model of grape downy mildew (GDM), predicts infection based on weather and inoculum availability. Individual lesions can sporulate many times, but we found that spore production (sporangia/lesion or SP) declines in proportion to the number of repeated sporulation events (SE), as described by: ln(SP) = 4.83 - 0.502*SE. Thus, SP can differ significantly among similarly-aged lesions. In wet weather (high SE), SP may decline to 10% of the maximum within 2 weeks after lesion appearance. In contrast, in dry weather (low SE), SP was 55 to 82% of the maximum after 3 weeks. Most models use time, rather than SE, to reduce SP. This may underestimate inoculum availability and the potential for resurgence of GDM following dry weather. Additional studies confirmed that within 14 days after bloom (DAB), relative susceptibility (RS) of berries is near 0: RS = 154.47 - 9.106*DAB.

**A bacterial nitric oxide synthase functions to nitrate a peptide phytotoxin.** J. A. KERS (1), M. J. Wach (1), S. B. Krasnoff (1), K. D. Cameron (1), R. A. Bukhalid (1), D. M. Gibson (3), B. R. Crane (2), and R. Loria (1). (1) Department of Plant Pathology, (2) Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY, 14853; (3) USDA-ARS, U.S. Plant, Soil, and Nutrition Laboratory, Ithaca, NY 14853. Phytopathology 93:S44. Publication no. P-2003-0321-AMA.

Potato scab disease is caused by soilborne phytopathogenic *Streptomyces* spp. A nitric oxide synthase (NOS) homolog flanks a cluster of genes involved in production of the nitrated phytotoxin thaxtomin, the primary pathogenicity determinant of potato scab disease. Mammalian nitric oxide synthases (NOSs) oxidize L-arginine to nitric oxide for cell signaling and defense. However, biological functions for bacterial NOSs are unknown. Here we describe a NOS homologue in the plant pathogen *Streptomyces scabies* and reveal its role in nitrating the thaxtomin moiety of the phytotoxin thaxtomin A. Thaxtomin production is essentially abolished by NOS disruption and drastically reduced by NOS inhibitors. In vivo incorporation shows the thaxtomin nitrate derives from L-arginine in a manner implicating NOS activity. High similarity among bacterial NOSs indicates a general function in nitrating secondary metabolites.

**Seasonal pattern of dispersal of conidia of *Cercospora beticola* in sugarbeet fields.** J. KHAN (1) and M. F. R. Khan (2). (1) Department of Plant Pathology, North Dakota State University, Fargo, ND; (2) North Dakota State University & University of Minnesota. Phytopathology 93:S44. Publication no. P-2003-0322-AMA.

*Cercospora beticola* is the most damaging foliar pathogen of sugarbeet. Information on the time of primary discharge and dispersal of *Cercospora* conidia, the progressive increase in conidial population, and the eventual exhaustion of conidia dispersal is critical in the management of *Cercospora* leaf blight of sugarbeet. Spore traps were installed in sugarbeet fields in Minnesota and North Dakota in 2002. Conidia were trapped on 645 mm² of the microscope slide that was covered with petroleum jelly. The slides were replaced weekly and examined microscopically to determine the number of spores trapped per week. There was a relationship between the number of *Cercospora* conidia present in the air and the disease severity of the *Cercospora* leaf blight. Highest number of conidia was trapped in late August to mid-September which corresponded to the time that *Cercospora* leaf spot was most prevalent in the sugarbeet fields. Locations that had higher number of conidia in the air had more severe disease.

**Should the recommendation for the *Cercospora* prediction model be modified?** M. F. R. KHAN (1) and J. Khan (2). (1) North Dakota State University & University of Minnesota, Fargo, ND 58105-5758; (2) Department of Plant Pathology, North Dakota State University, Fargo, ND, 58105-5788. Phytopathology 93:S44. Publication no. P-2003-0323-AMA.

According to the *Cercospora* leaf spot prediction model, sugarbeet growers south of the Polk-Norman county line in Minnesota should apply the first fungicide application at first symptoms, a second application 14 days after, and subsequent applications are based on disease severity and daily infection values. Growers north of the Polk-Norman county line should apply the first fungicide application at first symptoms, and subsequent applications are based on disease severity and daily infection values. Research conducted at Breckenridge, MN, and St. Thomas, ND, in 2001 and 2002, showed that it is possible that all sugarbeet growers in Minnesota and North Dakota can reduce fungicide applications and effectively control *Cercospora* leaf spot by adopting the practice of applying fungicides at first symptoms, and subsequent applications based on disease severity and environmental conditions.

**Influence of fungal elicitors on loblolly pine growth, morphogenesis and secondary metabolism in vitro.** N. I. KHAN (1) and B. H. Tisserat (2). (1) Department of Biology, Bradley University, Peoria, IL; (2) Fermentation Biotechnology, NCAUR, USDA-ARS, Peoria, IL. Phytopathology 93:S44. Publication no. P-2003-0324-AMA.

*Pinus taeda* L., loblolly pine, is one of the most valuable and widely cultivated timber species in the southern USA for lumber and pulpwood. Techniques to enhance seedling growth may reduce the nursery time. Fungal elicitors are known to affect the secondary metabolism in plants. We report the effects of various fungal elicitors on growth and secondary metabolism in...
P. taeda. Plants, grown in greenhouse and exposed to a number of fungal elicitors, showed significant increases in fresh weight, leaf root number, and shoot length over control (P<0.05). This effect was not dose dependent, although we have not determined the minimum dose yet. Total secondary metabolite α-pinene, was greater in treated seedlings due to increased fresh weight. Fungal elicitors may improve the growth and secondary metabolism in plants.


Paenibacillus polymyxa PKPB1 was evaluated for its ability to protect cucumber roots against Pythium ultimum in a hydroponic system with re-circulated nutrient solution (NS) in a greenhouse. The bacterium was added to NS, and was challenged with P. ultimum a week later. Data were collected on plant height, number of nodes, root rot severity, shoot weight, root dry weight, water consumption and fruit yield. PKPB1 survived in the nutrient solution, colonized plant roots, and significantly reduced root rot severity of cucumber per plant. As a soil treatment 1 week before planting colonization by PKPB1. Treatment with PKPB1 without Pythium gave significantly higher root dry weight and cucumber yield compared with control treatments. Water consumption, root biomass and cucumber yield were also significantly higher in PKPB1-treated plants than in P. ultimum–inoculated or uninoculated control plants. This study demonstrated that P. polymyxa PKPB1 acts as a protective biocontrol agent in greenhouse cucumbers.


The egg pathogenic fungus Paecilomyces lilacinus (strain 251), is a unique strain with a wide range of activity against the most important plant parasitic nematodes. Due to increased production capacity by solid state fermentation and a new water dispersible granule (WDG) formulation, this biological nematicide can be used in an integrated approach to control plant parasitic nematodes. Dose response studies with the root-knot nematode Meloidogyne incognita on tomatoes using the new WDG formulation demonstrated a clear correlation between rate applied and the degree of control. Best control was achieved by applying the biological nematicide at rates of 2 to 4 times 10⁶ conidia per plant. As a soil treatment 1 week before planting the P. lilacinus population in the rhizosphere revealed a decline after 2 to 3 month which can cause reduced efficacy. Repeated application to maintain the antagonist population at a sufficient level could be used to provide long term control of root-knot nematodes.


Biological control products face many obstacles during the final step of commercialisation, the registration process. Although several biopesticides have already proven their ability to efficiently control pests and diseases, due to increased production capacity by solid state fermentation and a new water dispersible granule (WDG) formulation, this biological nematicide can be used in an integrated approach to control plant parasitic nematodes. The egg pathogenic fungus Paecilomyces lilacinus (strain 251), is a unique strain with a wide range of activity against the most important plant parasitic nematodes. Due to increased production capacity by solid state fermentation and a new water dispersible granule (WDG) formulation, this biological nematicide can be used in an integrated approach to control plant parasitic nematodes. Dose response studies with the root-knot nematode Meloidogyne incognita on tomatoes using the new WDG formulation demonstrated a clear correlation between rate applied and the degree of control. Best control was achieved by applying the biological nematicide at rates of 2 to 4 times 10⁶ conidia per plant. As a soil treatment 1 week before planting the P. lilacinus population in the rhizosphere revealed a decline after 2 to 3 month which can cause reduced efficacy. Repeated application to maintain the antagonist population at a sufficient level could be used to provide long term control of root-knot nematodes.


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Deletion of a hypoviral-regulated cppk1 gene in chestnut blight fungus, Cryphonectria parasitica, results in yeast-like microcolonies. M. J. Kim (1), J. H. Kim (1), S. M. Park (1), Y. H. Kim (2), M. S. Yang (1), and D. H. Kim (1). (1) Institute for Molecular Biology and Genetics, Basic Science Research Institute, Chonbuk National University, Chonju, Chonbuk, Korea; (2) School of Agricultural Biotechnology, Seoul National University, Suweon, Korea. Phytopathology 93:S45. Publication no. P-2003-0331-AMA.

The cppk1 gene encodes the Ser/Thr protein kinase of Cryphonectria parasitica and is transcriptionally up-regulated by the presence of hypovirus CHV1-EP713. To determine the function of cppk1, we constructed a cppk1-null mutant using targeted homologous recombination. The cppk1-null mutant was initially isolated as a heterokaryotic form containing both wild-type and cppk1-deleted nuclei. The pure cppk1-null homokaryon was obtained by the single spore isolation of the heterokaryotic form and was used for phenotypic comparison. While the parental heterokaryon appeared normal, the pure cppk1-null mutant exhibited dramatic changes in colony after fungal incubation showed that the biotrophic primary mycelium was capable of penetrating leaf and callus tissue. Prior to invasion, the hyphal tips were club-shaped with adhesive, filamentous structures, suggesting involvement of mechanised mode of recognition and/or braking of the host barrier. The hyphal interaction progressed through the cuticle and randomly invaded leaf tissue. Cuticular disintegration was observed at the site of penetration. Initial stages of infection showed penetrations were more prolific on the adaxial than the abaxial surface of the leaf. Further incubation for 10 days resulted in complete digestion of tissue and accumulation of fibrous debris. Only at this stage, hyphae showed clamp connections, indicative of a change from primary to necrotrophic secondary mycelial stage. This is in accordance with our previous report that secreted hydrolytic fungal enzymes were capable of digesting cellulose, xylan and pectin. Future studies will focus on gene activation during the host-pathogen interaction.


Because of concern over the introduction of highly virulent Australian strains of Fusarium oxysporum f. sp. vasinfectum (FOV) into California, it was necessary to characterize local strains to document the movement of genotypes into U.S. cotton-growing areas. To accurately describe the strains that are presently occurring in California, various genotypic and phenotypic traits were employed. Based on partial sequences of translation elongation factor, phosphatase permease, and beta-tubulin genes and restriction enzyme digestion of the ITS nuclear r-DNA, Australian isolates of FOV do not exist in California. California isolates fell into four lineages. One group of several isolates consisted of races 1 and 2; a second group was represented by two isolates of race 3; a third group, race 4, was common; and a fourth group, race 8, was represented by two isolates. Race 4 was highly virulent on Pima in greenhouse pathogenicity tests and was considerably less virulent on Acala cotton; isolates belonging to the lineage of races 1 and 2 were relatively virulent on Acala but not Pima. Races 3 and 8 were weakly virulent on both Acala and Pima cotton.


Thielaviopsis basicola is a soil-borne pathogen, causing a disease called black root rot on many crops. Despite the practical significance of the pathogen, little is known about how it colonizes plant, the pathogen’s structure and development, and the effect of nutrition on disease development. The main focus of this work is to study as to how phosphorus levels in the soil influence the development of black root rot disease. Our research indicates that low phosphorus levels in the soil are correlated with increased shoot symptoms, especially in ethylene insensitive etr1-1 petunia. We are employing a culture-based survey using a T. basicola selective medium and cytological observations using a GFP–labeled fungal strain to determine whether the shoot symptom severity is related to an increase in fungai population density and/or a change in fungal distribution within the roots. We have also determined whether these variables interact with ethylene perception in petunia.

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morphology. It showed characteristics of yeast-like microcolonial growth. Neither sporulation nor hyphal differentiation into feeding hyphae, a mycelial mat, or aerial hyphae was observed. Instead of bright yellow colonies, altered, dark brown pigmentation appeared as the culture grew. Hyphae of the cppk1-null mutant were shortened and hyperbranched with globose to bulbous cells. Electron microscopy of the cppk1-null mutant revealed the presence of intrahyphal hyphae, which was the most striking ultrastructural change. Subtle changes in the expression of CpkP1 via the anti-sense strategy of cppk1 resulted in abnormalities in colony morphology and pigmentation, which indicated that cppk1 is important for coordinating growth with development and maintaining cell wall integrity.


Messenger is the first commercialized product from a new class of chemistry called harpin proteins. HarpinEa, the active ingredient in Messenger, reportedly activates the natural stress-defense responses in plants that increase plant vigor and result in better overall plant health. Root-knot nematodes (RKN), Meloidogyne incognita, and reniform nematodes (RN), Rotylenchulus reniformis, are important nematode pests of many crop species including cotton. The effects of HarpinEa were studied on cotton in three growth chamber experiments. Plant height, the number of nodes per plant and top root weight, and total biomass increased in cotton treated with HarpinEa compared with untreated plants. With both RKN and RN, HarpinEa-treated plants averaged lower numbers of nematodes per plant than untreated plants. In addition, the RKN females produced fewer eggs per egg mass on treated plants. The increased plant biomass and the decrease in nematode reproduction in these studies suggest that Messenger may be useful in mitigating cotton damage due to RKN or RN nematodes.


The objective of this study was to examine yield, stalk rot, and lodging in near-isogenic hybrid cotton with the cry1Ab gene, cry3Bb gene, both the cry1Ab gene and the cry3Bb gene (stack), and without the cry1Ab and cry3Bb genes. The plots were manually infested with western corn rootworm (CRW), European corn borer (ECB), and inoculated with a mixture of gray leaf spot, anthracnose, and northern corn leaf blight. Yeld height, the number of nodes per plant top and root weight, and total biomass increased in cotton treated with HarpinEa compared with untreated plants. With both RKN and RN, HarpinEa-treated plants averaged lower numbers of nematodes per plant than untreated plants. In addition, the RKN females produced fewer eggs per egg mass on treated plants. The increased plant biomass and the decrease in nematode reproduction in these studies suggest that Messenger may be useful in mitigating cotton damage due to RKN or RN nematodes.

Evaluation of two seed treatments for control of fumonisin in corn grain. C. E. KLEINSCHMIDT and D. G. White. Dept. of Crop Sciences, University of Illinois, Urbana, IL 61801. Phytopathology 93:S46. Publication no. P-2003-0334-AMA.

Fusarium ear rot of corn caused by Fusarium verticillioides (syn. = F. moniliforme) and F. proliferatum is of concern due to the production of fumonisin mycotoxins. In 2002, 30 commercial white and 3 yellow food-grade dent corn hybrids were evaluated for Fusarium ear rot and fumonisin concentration in the grain. Early inoculations by injection down the silk channel and into the side of the ear one and two weeks after pollination. A competitive direct ELISA was used to determine fumonisin concentration. Hybrids differed significantly for both fumonisin concentration and Fusarium ear rot (P < 0.0001). Spearman’s rank correlation for fumonisin concentration and Fusarium ear rot was r = 0.54 (P = 0.0009). Fumonisin concentration, of individual hybrids averaged over replications, prometh from 4.7 to 59.6 ppm. White and yellow food-grade dent corn hybrids had an average fumonisin concentration of 23.6 and 19 ppm respectively. Both the white and yellow food-grade dent corn hybrids had an average of 6% of the ear rotted.


Pea is non-host to the bean pathogen, Fusarium solani f. sp. phaseoli (Fsp). Two individual signaling compounds, chitoan and Fsp DNase, are released from Fsp, each of which can induce the PR genes and immunity against the true pea pathogen F. solani f. sp. pisi. An array of DNA-specific biotic and abiotic elicitors also activate PR genes, indicating a major role for global action on pea chromatin. This activation is preceded by alterations in nucleo/chromatin and enhanced DNA degradation detectable both in vivo and in vitro. Elicitors suppress reproduction in the pea High Mobility Group (HMG) genes, HMG I/Y, and its protein product. HMG I/Y is an architectural transcription factor with “AT hooks”; HMG I/Y binds two AT-rich regions within the promoter of the pea PR gene, DRR206. The induction of pea DRR206 is temporally associated with the suppression of pea HMG I/Y in pea. Tobacco lines stably transformed with the pea DRR206 promoter using the CaMV 35S promoter driving the pea HMG I/Y gene, the elicitation of the DRR206 promoter is suppressed. HMG I/Y can stabilize or alter chromatin structure. In cells w/o HMG I/Y, PR genes maybe better postured for transcription.


The fungus Ustilago maydis alternates between a saproxylic, haploid budding form and a pathogenic filamentous dikaryon. Genes involved in the MAP kinase pathway controlling mating and morphogenesis had been identified previously by complementation of mutants that suppress the filamentous phenotype of a Ustilago adenylate cyclase mutant. Three of these genes encode homologs of MAP kinase cascade proteins that control mating and morphogenesis in other fungi. Another of these genes, ubc2, is a critical virulence factor that is basidiozyme-specific in overall structure and encodes a protein possessing four protein interaction domains. Site-directed mutagenesis and complementation studies indicate that certain amino acids within these domains are critical for complementation. Targeted yeast two-hybrid assays with Ubc2 as bait indicate that Ubc2 interacts with the MAPKK kinase Ubc4. Screening of a Ustilago two-hybrid fusion library revealed additional interactions with Ubc2. Thus, Ubc2 may function as a novel adapter protein in this MAP kinase pathway.


Whole-plant inoculation tests indicate a range of resistance levels among hosta cultivars to S. rolfsii var. delphini-induced peptide rot (Edmunds et al,

Early blight severity, tomato yield, and plant biomass were assessed in plots amended with 10 compost treatments with or without compost tea extracts. Four replicates of treatments were arranged in a split plot design with compost soil amendment as whole plots and tea treatments as subplots. Compost teas were prepared by passive brewing of the respective composts in tap water for 48 hours, and were applied weekly beginning July 1 with a backpack sprayer. Early blight severity was determined on 10 dates between July 26 and Oct. 5 and tomato yields were determined from six weekly harvests. Yields were significantly lower (P < 0.01), and plants were visibly stunted, in plots amended with hardwood bark compost or blood meal than with compost alone. Early blight severity varied significantly among compost treatments (P < 0.05) and was positively correlated with shoot biomass at the end of the season (P < 0.01). Compost teas had no significant effect on tomato yield or early blight severity.

**Armillaria species on woody understory and small woody debris in *Pinus resinosa* sites.** K. W. KROMROY (1), R. A. Blankenship (2), and D. F. Grigal (3). (1) USDA Forest Service, North Central Research Station, St. Paul, MN; (2) Dept. of Plant Pathology; (3) Dept. of Soil, Water, and Climate, University of Minnesota, St. Paul, MN 55108. Phytopathology 93:547. Publication no. P-2003-0343-AMA.

Reduction of inoculum potential by removing substrates that serve as food bases for the fungus is one approach to managing Armillaria root disease. Roots of woody understory plants, along with woody debris and root fragments (DRF), were collected with a golf cup cutter from 13 *Pinus resinosa* sites, including stands of three age classes in northern Minnesota. Expression of *Armillaria* pathogenicity in single roots of sampled plants, 0 to 60% of 16 cm-deep cores having at least one colonized DRF, and 0 to 9% of the individual roots and DRF in 25 cm-deep cores. Frequencies of *Armillaria* presence decreased with increasing overstory age. *Armillaria* was isolated from 26% of DRF and 60% of plants that were assayed. All but one of 42 isolates were *A. ostoyae*. Results show that woody understory plants and small woody debris are involved in survival of *A. ostoyae* on these sites and suggest that their potential as sources of inoculum be considered in root disease management.


The rice blast pathogen, *Magnaporthe grisea*, produces a specialized infection structure, the appressorium, to infect plant tissues. One of the pathways required for appressorium development and pathogenesis is the cAMP signal transduction pathway. To identify new components within this pathway, we used the yeast two-hybrid system to screen adenylate cyclase (MAC1) and the catalytic subunit of cAMP-dependent protein kinase A (CPKA), against an appressorial cDNA library. The protein phosphatase domain in MAC1, unique to fungal adenylate cyclases, interacted with a MAP kinase kinase and a Ser/Thr protein kinase. These interactions could be a feedback between the signaling pathways. Another MAC1 interacting protein was a putative extracellular membrane protein AC1-1, which may serve as a cell receptor or an adhesion molecule. The glutamine-rich N-terminus of CPKA interacted with a putative transcriptional regulator and two different glycine-rich logdrolases. Phosphorylation motifs in these sequences suggest that they could be CPKA substrates.


Studies during the past decade have rigorously established that salicylic acid (SA) plays a critical, multi-faceted role in plant disease resistance. To help elucidate the mechanisms of SA action, several tobacco proteins which interact with SA have been identified including catalase (SABP), ascorbate peroxidase and carbonic anhydrase (SABP3). In addition to its antioxidative activity, SABP3 also has antioxidant activity and plays a role in a hypersensitive response (HR) mediated by the host resistance gene *Pto* and pathogen avirulence gene AvrPto. SABP2 is another SA-binding protein; this very low abundance protein (10 fmol/mg) has high affinity for SA (Kd=90nM). It was purified 24,000 fold from tobacco leaves and its encoding gene was cloned. Recombinant SABP2 exhibits very high affinity for SA, which can be competed by addition of active but not inactive analogs of SA.
The effects of soil properties on ectomycorrhizae have been investigated.


Increased phytoalexin and peroxidase activity in Botrytis fabae-infected broad bean leaves. J. O. KUTI and H. F. Nawar. Texas A&M University, Div. of Agronomy and Plant Sciences, Kingsville, TX 78363.

Effect of soil type and management on ectomycorrhizal colonization of Betula papyrifera. J. L. THOMAS and J. R. JOHNSON. USDA, ARS, Forest Pest Management Research Laboratory, Ogden, UT 84403.

Sequence data suggests a recombination event between a strain of Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV). R. C. LARSEN (1), P. N. MIKLAS (1), and K. L. DRUFL (2). (1) USDA-ARS, Prosser, WA; (2) Washington State University, Pullman.


The soil treatments had no effect on total root length or mycorrhizal colonization, and number and abundance of ectomycorrhizal morphotypes of strawberry black root rot - lack of association with terbacil application.

Commercially-available biocontrol products were evaluated, alone and in combination with green spraying to promote early emergence, for efficacy in controlling Rhizoctonia and other soilborne potato diseases in field tests in New York, Maine. Two bacterial formulations, Deny (Burkholderia cepacia JB2) and Kadiaki (Bacillus subtilis GB03), and two fungal preparations, RootShield (Trichoderma harzianum T-22) and SoilGard (Trichoderma viride G-21), were tested along with a chemical control. All treatments reduced the incidence and severity of stem canker compared to a pathogen-treated control (25-89% reduction). No treatments consistently reduced black scurf on tubers, although Kadiaki, Deny, and SoilGard reduced scurf severity in some years. Green sprayed seed (GS) reached 95% emergence 7-9 days earlier than nonsprouted seed (NS) and showed fewer emergence problems. Overall, GS reduced stem and stolon canker, black scurf, and common scab compared to NS plants. Interactions between spraying and biocontrol factors were not significant for any parameter, indicating there were no enhanced effects of the combination.


Infection of broad bean leaves with Botrytis fabae (Sard.) resulted in rapid accumulation of phytoalexin tyloxapol and increased in peroxidase activities in resistant and susceptible broad bean cultivars. Rapid accumulation of tyloxapol equal to or greater than 2-fold in leaves of resistant than susceptible broad beans was observed. Peroxidase activity was 10 times higher in infected leaves of resistant than susceptible cultivars and 8 times higher in uninoculated leaves of the resistant than in the susceptible cultivars. Peroxidase activity in infected leaves of resistant cultivars was greater than 2-fold and less than 2-fold in leaves of susceptible cultivars when compared with uninoculated leaves. The ratio of peroxidase activity in infected to uninfected leaves increased over time in susceptible cultivars but remained unchanged in resistant cultivars. Using tyloxapol acid synthesis and peroxidase activity as preliminary markers for resistance of broad bean to chocolate spot disease, caused by B. fabae, is suggested in this study.

Effect of soil type and management on ectomycorrhizal colonization of Betula papyrifera. J. L. THOMAS and J. R. JOHNSON. USDA, ARS, Forest Pest Management Research Laboratory, Ogden, UT 84403.

The effects of soil properties on ectomycorrhizae have been investigated extensively in forest ecosystems, but few studies have addressed urban environments, where disruption of soil structure, texture and chemistry is a common occurrence. To address this deficiency a field study was conducted to examine the effects of two soil types (topsoil, inverted subsoil) in combination with four soil management practices (fertilization, mulching with composted yard waste, mulching with a composted bark/manure blend, and bare soil control) on fine root length, percent native ectomycorrhizal colonization, and number and abundance of ectomycorrhizal morphotypes of paper birch. Preliminary results show that trees in topsoil had greater mycorrhizal diversity and four times the total root length of those in subsoil. The soil treatments had no effect on total root length or mycorrhizal diversity.


SABP2 is a member of alpha/beta fold hydrolase family of proteins. It is a lipase, whose activity is enhanced by SA binding. Its expression is induced by tobacco mosaic virus (TMV) infection of resistant, but not susceptible, tobacco. SA is required for the full TMV induction of its expression but by itself is not sufficient for the induction. Blocking of SABP2 suppresses resistance to TMV and blocks development of systemic acquired resistance.

In summary, this low abundance, SA-stimulated lipase with high affinity for SA plays a role in local as well as in systemic resistance and thus, may be the receptor for SA.

The soil treatments had no effect on total root length or mycorrhizal colonization, and number and abundance of ectomycorrhizal morphotypes of strawberry black root rot - lack of association with terbacil application.
Variable rate applications of the nematicide metasodium were examined for the management of the reniform nematode. The test was sampled on one-half acre grids to determine nematode numbers and to georeference their locations in the field. Nematode numbers were classed, low (0 - 4,000), medium (4,000 - 8,000), high (greater than 8,000) and a prescription map was developed based on the three classes. Treatments included metasodium applied at the single rates of 28, 47, and 76 l/ha and a variable rate application of 28 - 76 l/ha. Aldicarb was applied at 5.7 kg/ha as a standard. The experiment was a CRD with 4 replications. Metasodium was injected into the soil surface floodwater of plots having a continuously maintained 9-inch flood depth. Subsequent greenhouse and laboratory experiments confirmed a positive correlation between lowered root zone oxygen (hypoxia) and a reduction in leaf blast. We confirmed that hypoxia at the surface and within the soil eventually resulted in an enhanced ethylene production in the rice root. Ethylene induced resistance to rice blast was stable and persisted in greenhouse test plants. Our results indicate ethylene is an essential component of disease defense response in rice plants growing under hypoxia.

Rice blast frequently limits rice yield in U.S. production areas. Much empirical and significant experimental evidence indicates blast severity is enhanced in low moisture upland soil and reduced in high moisture flooded rice soil. Cultivar response varies with density of continuous flood and time. In a flood depth-plant growth stage test, dissolved oxygen content was 1.9 ppm in soil surface floodwater of plots having a continuously maintained 9-inch flood depth. Subsequent greenhouse and laboratory experiments confirmed a positive correlation between lowered root zone oxygen (hypoxia) and a reduction in leaf blast. We confirmed that hypoxia at the surface and within the soil eventually resulted in an enhanced ethylene production in the rice root. Ethylene induced resistance to rice blast was stable and persisted in greenhouse test plants. Our results indicate ethylene is an essential component of disease defense response in rice plants growing under hypoxia.


A study was conducted from 2000 to 2002 to examine the horizontal and vertical distribution of the reniform nematode Rotylenchulus reniformis (Linford and Oliveira) in Mississippi cotton. Two naturally infested cotton fields were selected. One field was in a continuous cotton production system and the other was in a cotton-corn rotation system. Each field was 50 hectares in size. Each field was grid mapped with sixteen points on 0.52 hectare grids using a Global Positioning System (GPS). A single core, 5.08 cm diameter, 121.92 cm deep, was collected from each grid intersection and each soil core was divided into eight depths: 0-15 cm, 16-30 cm, 31-45 cm, 46-60 cm, 61-75 cm, 76-90 cm, 91-105 cm, and 106-120 cm. Soil samples were collected in the spring and in the fall. Nematodes were extracted using the gravity screening and centrifugal flotation method and then counted with a stero-microscope. Reniform nematodes were found at each of sixteen sample points. This nematode appeared to have an even horizontal distribution across an established cotton production and cotton-corn rotation fields. The number of the reniform nematodes, however, varied at each sample point. The reniform nematodes were found at each of the eight depths and the nematode numbers varied at each sample depth. In the continuous cotton production field, nematode numbers were higher in the fall than in the spring. Higher nematode numbers were found upper soil profiles in the fall than in the spring. In general nematode numbers decreased by depth. In the cotton-corn rotation field, nematode numbers were higher in the fall than in the spring. When corn was planted, more nematodes were recovered in the lower soil profile. Reniform nematode numbers varied depending on season. Corn and cotton had an effect on reniform nematode numbers and distribution in relation to soil depths. The recovery of the reniform nematode at the lower soil sampling depths may help explain why nematode numbers are capable of reaching high levels after a single year rotation with corn.

Identification of a putative insertion sequence (IS) associated with members of the aster yellows (AY) phytoplasma group. I.-M. LEE, Y. Zhao, and K. D. Bottner. Molecular Plant Pathology Laboratory, USDA-ARS, Beltsville, MD 20705. Phytopathology 93:49. Publication no. P-2003-0359-AMA.

A DNA fragment cloned from an aster yellows (AY) phytoplasma strain AY1 (belonging to subgroup 16SrI-B) was shown to contain a sequence that is highly repetitive in chromosomes of several members of the AY phytoplasma group (16SrI). This DNA fragment contains several open reading frames (ORFs). A BLAST search of the NCBI protein database using the deduced amino acid sequences as queries revealed that one of the ORFs encodes a putative transposase that shares a significant homology (about 37% identity and 56% similarity) with insertion sequences from several bacterial species of low G+C Gram-positive firmicute group. The putative IS element was found to be present in several AY subgroup phytoplasmas. Preliminary phytoplasma genomic DNA blot analyses using the putative IS as a probe revealed that different symptom variants derived from the same parent phytoplasma strain exhibit different hybridization patterns. IS-mediated chromosomal recombination events may play a role in symptom expression/modulation in AY-diseased plants.

The EcoPort website was established in January 2000 with University of Florida, FAO of the United Nations, and the National Museum Natural History, Smithsonian Institution as co-founders. EcoPort established a “knowledge commons” where individual and communities can work and learn together to discover and sustainably access information. Access to information is facilitated through public service that will enable participants to own and update the knowledge created by their collective effort. EcoPort’s information and procedures are available for use as a global public good to provide education to communities and individuals engaged in natural resources management and conservation. Data quality is maintained through peer review, and individual ownership of shared information is preserved and displayed. EcoPort has been endorsed by Nelson R. Mandela, Edward O. Wilson, and Jacques Dion. EcoPort contains a wealth of plant pathological related information including specific information on entities, slide shows, books, glossaries, illustrations, and International Plant Protection Convention documents.


*Phytophthora* spp. that were isolated from infected plants. The primer combination E+AT / M+CTA detected a fragment that is specific in A1 mating type (Mat-A1) of *Phytophthora infestans*. This fragment was cloned. The length of the cloned fragment was determined as 218 bp. On the basis of the sequences of the 218 bp fragment, a pair of primers (INF-1, INF-2) were synthesized and used to differentiate *P. infestans* Mat-A1 from Mat-A2. The Mat A1-specific fragment was detected from a Dot hybridization blot analysis of PCR products with primers INF-1 and INF-2 of *P. infestans* Mat-A1, but not in *P. infestans* Mat-A2 or other *Phytophthora* spp. using the cloned fragment as a probe. A DNA Database search suggested that the fragment was a novel one. Approximately 1.6 kb of a single fragment was detected only in the *P. infestans* Mat-A1 by a Southern blot analysis of the genomic DNAs of *P. infestans*. Because the genomic DNAs were digested with BamHI, AvaI and EcoRI, it was concluded that the cloned fragment exists on the genome of *P. infestans* Mat-A1 as a single copy. Using A1 mating type-specific primers, a unique band was obtained within annealing temperatures of 63°-64°. The development of the primers and probe would make it possible to diagnose the A1 mating type of *P. infestans* from infected leaves, stems or tubers of potato before any symptoms appear on the plant.

**Nutritional effects on colony size and biofilm formation of Xylella fastidiosa growing on simple chemically-defined media.** B. LEITE, P. C. Andersen, and M. L. Ishida. University of Florida, IFAS, NFREC 155 Research Road Quincy, FL 32351. Phytopathology 93:S50. Publication no. P-2003-0362-AMA.

We tested two hypotheses: 1) growth of *Xylella fastidiosa* (Xf) requires a smaller number of compounds than in currently available chemically defined media, and 2) colony size and biofilm formation of Xf are influenced by nutrients. New defined media were developed and evaluated for three strains of Xf. New defined media were compared to PW* medium and to XF-26 (defined medium), in agar and liquid culture. A simple medium consisting of 5 organic compounds and 3 inorganic salts supported growth of Xf. Media were formulated based on the chemistry of the xylem fluid of a PD-susceptible Vitis genotype. The performance of Xf was dependent on the strain, media composition and strain*media interactions. Results support the contention that the chemistry and the xylem fluid of media can affect the behavior of Xf. New formulations increased the capacity of Xf to form biofilm and reduced the number of cells in the planktonic state. One medium contained reduced glutathione as an ingredient. The importance of this antioxidant in aggregation, biofilm formation and survival will be discussed.

**Effect of hydrophobicity and divalent ions on biofilm formation of Xylella fastidiosa.** B. LEITE, M. L. Ishida, and P. C. Andersen. Univ. of Florida, IFAS, NFREC, 155 Research Road Quincy, FL 32351. Phytopathology 93:S50. Publication no. P-2003-0363-AMA.

Pathogenicity of *Xylella fastidiosa* (Xf) is often correlated to the capacity of cells to adhere to the internal walls of xylem vessels, with subsequent formation of biofilm followed by vessel occlusion. The establishment of a biofilm requires: 1) adhesion of cells to a surface and 2) the build up of several layers of cells and cell products. These cells will produce and accumulate exopolysaccharides (EPS), contributing for the biofilm architecture. Cell surface hydrophobicity is considered an important component of cell adhesion. Our results concerning colony formation on chemically-defined media for Xf are consistent with what is known that Xf pathogenicity may be affected by hydrophobicity. Some of the new media are highly hydrophobic, and Xf colonies grown under these conditions produced more biofilm and aggregated into larger colonies compared hydrophilic media. Treatments with cations Ca**+** and Mg**+** increased hydrophobicity whereas NaCl decreased it. Manipulation of media hydrophobicity may elucidate the relationship between hydrophobicity, cell surface chemistry and biofilm formation.

**Comparison between isolation and PCR for detection of Xanthomonas citri** (Xc) from citrus canker lesions in Thailand. U. LERTSUCHATAVANICH (1), N. Thaveechai (1), A. ParadornuWat (1), and N. W. Schaad (2). (1) Dept. PI. Path., Kasetsart Univ., Bangkok, Thailand; (2) USDA/ARS Foreign Disease-Weed Science Research Unit. Ft. Detrick, MD. Phytopathology 93:S50. Publication no. P-2003-0364-AMA.

Citrus canker, caused by Xc, is widespread in Asia. Canker is not established in the US and is highly regulated. The reliability of real-time PCR was compared to classical isolation on agar media for detection of Xc in new lesions on immature leaves and old lesions on mature leaves. New lesions were 1- mm or less in diameter, surrounded by chlorosis, with no visible hyperplasia. The larger, old lesions had hyperplasia. A portion of each lesion was removed, soaked in 50ul water and after 20 min., a loop-full streaked on nutrient-rich agar plates. Duplicate 1 ul samples were acquired by real-time PCR in a Smart Cycler. Of 13 new lesions, 7 (54%) were positive by PCR and 8 (62%) by isolation. With old lesions, 15/26 (58%) were positive by PCR but only 9/26 (35%) by isolation. All suspect Xc cultures were pathogenic. These results show that PCR correlates highly with isolations for new lesions but not for old lesions. Apparently old lesions contain few viable cells and, most likely, contribute less to spread of the disease.

**Control of postharvest decay of apple by combining heat treatment, biocontrol and sodium bicarbonate.** B. LEVERENTZ (1), W. J. Janisiewicz (2), W. S. Conway (1), A. B. Blodgett (1), and R. A. Saffner (1). (1) USDA-ARS, PQSL, Beltsville, MD 20705; (2) USDA-ARS, AFRS, Kearney, NV 82540. Phytopathology 93:S50. Publication no. P-2003-0365-AMA.

‘Golden Delicious’ apples were wound inoculated with either Colletotrichum acutatum or Penicillium expansum then treated with heat (38°C) for 4 hours, sodium bicarbonate, and/or one of two heat tolerant biocontrol agents (yeasts). Following four months storage at 0°C, the apples were left at room temperature for two weeks. Populations of both antagonists were stable throughout the experiment. Each antagonist reduced decay caused by *P. expansum*, whereas heat or heat in combination with an antagonist eliminated decay. Either heat or the antagonists alone reduced decay caused by *C. acutatum*, but a combination of the two treatments had a greater effect. Decay caused by this pathogen. Adding sodium bicarbonate increased the effectiveness of the antagonists in reducing decay caused by *P. expansum*. However, sodium bicarbonate alone had little effect on decay caused by either pathogen. The goal of this research is to combine alternative methods of control to provide an effective substitute for synthetic pesticides.

**Characterization of the coffee wilt pathogen in Uganda.** M. L. LEWIS, S. YVEY (1), S. A. Miller (1), G. Hakiza (2), and D. M. Geiser (3). (1) Department of Plant Pathology, The Ohio State University, Wooster, OH 44691; (2) Coffee Research Institute, Mukono-Kiruza, Uganda; (3) Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802. Phytopathology 93:S50. Publication no. P-2003-0366-AMA.

Sixteen isolates of *Fusarium* were isolated from robusta coffee trees in Uganda with symptoms of vascular wilt and characterized based on morphology and partial DNA sequences of translation elongation factor 1-alpha (ef) and beta-tubulin (bena). *Fusarium* species is not a member of GFC, suggesting that new pathogens. Phylogenetic analyses indicated that these isolates represent a new species lineage in the African clade of the Gibberella fujikuroi species complex (GFC). The species most commonly associated with this disease, *Fusarium xylospirax*, is not a member of GFC, suggesting that new outbreaks of vascular wilt in Uganda may have been caused by a new pathogen.


*Tapesia yallundae* causes eye spot disease in wheat. A single resistance gene, *Pch1*, is used in all resistant cultivars grown in the U.S. Pacific Northwest. Recently, a potential new source of eyespot resistance was reported in SS767
Fusarium solani f. sp. glycines (Fsg) is in the same fungal class as Saccharomyces cerevisiae. To compare the genes of these two fungi, cDNA was synthesized by reverse transcription from total RNA extracted from a culture of Fsg. cRNA was synthesized from cDNA and labeled with biotin by an in vitro transcription. After fractionation, cRNA was hybridized to the Affymetrix GeneChip Yeast Genomic S98 Array containing 9159 probe sets. The array was then scanned in an Agilent GeneArray Scanner and the MicroArray Suite was used to identify ‘Present’ or ‘Marginal’ genes. Approximately 4.8% of the yeast probe sets hybridized to Fsg cRNA indicating that few genes had sufficient homology to hybridize to S. cerevisiae. Using the NetAffx analysis, Fsg cRNAs hybridized with probe sets associated with signal transduction, structural and ribosomal proteins, metabolism, cell division, and transport. In addition, Fsg cRNA hybridized to a gene that is similar to the plant PR-1 class of pathogenesis-related protein genes and three genes involved in multiple-drug resistance.

Analysis of gene expression of Fusarium solani f. sp. glycines and soybean during infection. S. Li (1), A. G. Hernandez (2), G. L. Hartman (1,3), P. A. Schweitzer (2), and L. Liu (2), (1) Univ. of Illinois, Dept. Crop Sciences, Urbana, IL 61801; (2) Univ. of Illinois, W. M. Keck Center; (3) USDA-ARS, Urbana, IL 61801. Phytopathology 93:551. Publication no. P-2003-0372-AMA.

Soybean sudden death syndrome is caused by Fusarium solani f. sp. glycines (Fsg). To study the gene expression during infection, a normalized directionally-cloned cDNA library was constructed from a culture of Fsg and Fsg-infected soybean roots. Fsg and Fsg-infected soybean mRNA were differentially tagged at the 3′-end for sequence identification. Initially, 163 randomly picked clones were sequenced. Analysis of the expressed sequence tag revealed that 88 clones (54%) significantly matched entries in the National Center for Biotechnology Information non-redundant protein database (BLASTX); 21 were homologous with previously identified fungal genes, 59 with plant genes, and four and three with animal and bacterial genes, respectively. Nine fungal genes were involved in metabolism, four were associated with protein synthesis, one with copper resistance protein CRF-1 and seven had unknown functions. Enrichment of either Fsg or soybean genes expressed during infection will be accomplished by appropriate cDNA subtractions.


Although the complete genome sequence of four strains of Xylella fastidiosa (Xi) has been determined, post-genomic research is just beginning. Defined mutants are required to exploit the genomic sequence data. A triparental mating system was employed to introduce a green fluorescent protein (GFP) labeled Mini-Tn5 transposon into the chromosomal DNA of a rifampicin resistant mutant of Xi isolated from coffee. More than 200 putative transconjugants were obtained and confirmed by PCR using simultaneously two pairs of primers, one specific for Xi and the other for GFP. The insertion sites of the GFP labeled transconjugants were identified using a semi-random two-step PCR (ST-PCR) method. Transconjugants with mutations in defined genes are being evaluated in vitro and also in plants. Conjugal matings with E. coli donors combined with our ST-PCR method to define transposon insertions in the recipient, will provide an efficient method to create defined mutants needed for the functional genomic analysis of Xi. GFP labeled mutants will also be useful for localizing the bacterium in infected plants.


Different geographic areas differ in climate patterns, which may result in variation in geographic occurrence of plant diseases. To examine patterns of long-term disease risk in certain geographic regions, modeling approaches were used in which signals of climate variables favorable to a disease were captured from long-term climate data. We studied the geographic risk of wheat stem rust (Puccinia striiformis) in the north central and southwest region and soybean stem rot (Sclerotinia sclerotiorum) in the North Central region. Climate data of PNW from 1881 to 2001 were input in a stripe rust model to calculate disease index (1–10 scale). The 5-year moving average index increased from 6.5 to 7.5 after 1940s in coastal area while the values were about 4 in mountainous area. For stem rot, from 1893 to 2001, cotranslated occurrence index (0–1 scale) increased northward from 0.01 to 0.6. Long-term cyclic patterns for the two diseases were indicated by time series analysis, varying from 4 to longer than 20 years for different areas.
Temporal and spatial development, relative fitness and dynamics of strains of Colletotrichum sublineolum on sorghum. Y. H. LI and D. O. TeBeest. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR. Phytopathology 93:552. Publication no. P-2003-0375-AMA.

Sorghum anthracnose, caused by Colletotrichum sublineolum, is one of the most important diseases of sorghum in the U.S.A. Field and greenhouse experiments were conducted in which three cultivars (B Tx623, Pioneer 8313 and Cargill 888Y) were inoculated with three strains of the fungus (TX430, SS-1 and S54) to quantify disease development and to determine the relationship between infection components and the population dynamics of strains in the field. Disease progress over time and disease gradients were best described with logistic and negative exponential models, respectively. Disease severity remained at low levels until flowering, and then increased rapidly. The slopes of the disease gradients were initially steep across nine rows, some RFLP analyses of isolates from the greenhouse field showed that TX430, which has the highest level of relative fitness, predominated on all cultivars. The results suggest that host selection and infection components have important roles in affecting the overall genetic structure of C. sublineolum populations in the field.


The Wheat streak mosaic virus coat protein gene (WSMV-CP) has been previously transformed into wheat to obtain resistance to WSMV. However, transgene silencing was frequently observed in many transgenic lines. For example, transgenic plants of the T1 generation in one line expressed the coat protein and showed high-level resistance to WSMV infection, but all selfed T2 and T3 transgenic plants lost transgene expression and were susceptible to WSMV. The transgene remained transmitted for WSMV-CP. The gene expression was restored when T2 or T3 transgenic seeds were germinated in the presence of 5-azacytidine, indicating that transgene silencing was at the transcriptional level and was caused by DNA methylation. Restoration of protein expression was concomitant with restoration of resistance to WSMV. Longevity of transgene expression by 5-AzaC was maintained for 15 to 20 days after plants were grown in soil, after which transgene silencing was re-established. This indicated that removal of 5-AzaC permitted transgene re-methylation during the DNA replication process.


A gene cassette, p35S-CNO, was designed to express three gene products driven by a single constitutive CaMV 35S promoter. The individual coding regions were linked to produce a single polypeptide, using spacer sequences encoding specific heptapeptide cleavage recognition site (ENLYFQS) for nuclear inclusion (a Nla) protease of tobacco etch virus (TEV). The Nla protease was included as the second coding sequence of the gene. Gene sequences for two disease related proteins, a Trichoderma harzianum endochitinase and a wheat oxalate oxidase, were used as the first and third unit of the construct, respectively. In order to test if this polypeptide is being properly spliced after translation, and produces functional enzymatic activity in vivo, p35S-CNO was used to transform Arabidopsis. Our preliminary results indicate that transformed plants produce oxalate oxidase and it is the expected post-processing size. Splicing of the chitinase and its activity in transformed plants will be further characterized.

Resistance to Aspergillus flavus in peanut seeds is associated with constitutive trypsin inhibitor and inducible chitinase and beta-1,3-glucanase. X. Q. LIANG (1,2), B. Z. Guo (2), C. C. Holbrook (3), and R. E. Piggott (2). (1) University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793; (2) USDA-ARS, Crop Protection and Management Research Unit; (3) Crop Genetics and Breeding Research Unit, Tifton, GA 31793. Phytopathology 93:552. Publication no. P-2003-0378-AMA.

Peanut is one of the most susceptible crops to Aspergillus flavus invasion and aflatoxin production. The objectives of this research were to study the constitutive expressed protein trypsin inhibitor and inducible antifungal hydrolyase chitinase and beta-1,3-glucanase by Aspergillus infection, which may associate with the resistance in peanut seeds. The purified trypsin inhibitor (TI) in peanut seeds, including two subunits with molecular weight of 10-3.4D and 17-4D, respectively, can inhibit Aspergillus spore germination and growth in vitro in the concentration of 10 µg/ml. The concentration and activity of TI in resistant genotypes were significantly higher than that in susceptible genotypes. The activities of chitinase and beta-1,3-glucanase were increased significantly in the seeds after inoculation with A. flavus. The constitutive activity was considerably higher in the resistance genotypes at 3-4d after inoculation than that in susceptible genotypes. From in-gel (native PAGE) assay, one band and two bands of endo-chitinase isoforms were detected in susceptible and resistant genotypes, respectively. The isoform patterns of beta-1,3-glucanase also showed more bands in resistant genotypes than in susceptible genotypes. Chitinase from PI337494 inoculated with A. flavus was purified with molecular weight 31-KD. The purified chitinase from peanut significantly inhibits spores germination and hypha growth in vitro.


Presence of injured bacteria in processed foods was first recognized almost 50 years ago. Demonstration of metabolic injury and repair in phytopathogenic bacteria has never been reported. The objective of this study was to investigate the lethal and sublethal action of acetic acid (AA) on Erwinia and the resuscitation and infectivity of the injured cells. Upon exposure to AA (0.3%, 6 min), approximately 90% of E. carotovora subsp. carotovora (Ecc) were killed and over 99.9% of the surviving cells were injured. Injured cells were detected and enumerated based on their ability to form colonies on non-selective nutrient agar but not on selective agar such as CVP or PT. Lineage cells were able to restore the ability to grow on selective media after placing them in nutrient broth for 2-8 hr at 20°C, but not at 4°C. The minimum concentration of injured Ecc cells to induce solid rot on green pepper was determined to be 109-1010 CFU/ml as compared to 104-105 CFU/ml for normal cells. This study suggests that direct plating on selective media such as CVP may be inadequate for recovering injured Erwinia cells present in the samples.

Design and development of a DNA array for early detection and rapid identification of multiple tomato vascular wilt pathogens. B. Lieveens (1,2), M. S. KRUSE (1), M. Brouwer (2), A. C. R. C. Vanachter (1), C. A. Lévesque (3), B. P. A. Cammue (2), B. P. H. J. Thomma (2). (1) Scientia Terraec Research Institute, Sint-Katelijne-Waver, Belgium; (2) Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Leuven, Belgium; (3) Agriculture and Agri-Food Canada, Ottawa, Canada. Phytopathology 93:552. Publication no. P-2003-0380-AMA.

Fusarium wilt, caused by Fusarium oxysporum f. sp. lycopersici, and Verticillium wilt, caused by either Verticillium albo-atrum or V. dahliae, devastate tomato crops worldwide. Monitoring is the foundation of integrated pest management of any disease. However, one of the main limitations in integrated disease management is the lack of rapid, accurate, and reliable means to detect and identify plant pathogens. In this study, we describe a new molecular detection system for rapid and efficient detection of Fusarium and Verticillium wilt pathogens, based on a DNA microarray. We intend to demonstrate the utility of this array for sensitive detection of these pathogens from various matrices such as soil, plant tissue, and irrigation water, collected from both laboratory and commercial settings.

Effect of cyanogenic Pseudomonas aeruginosa on seed germination and seedling growth of plants. Y. H. LIN (1), H. W. Wan (1), Y. C. Lin (1), H. C. Huang (2), S. T. Hsu (1), and K. C. Tseng (1). (1) Dept. of Plant Pathology; (2) Graduate Institute of Biotechology, National Chung-Hsing University, Taichung, Taiwan. Phytopathology 93:552. Publication no. P-2003-0381-AMA.

Among 33 strains of plant-associated Pseudomonas aeruginosa (Pa) tested, 23 (69.7%) strains were cyanogenic. The effects of cyanogenic Pa on seed germination and seedling growth of lettuce were examined in a paired-plate system. It showed that strong cyanogenic Pa strains significantly inhibited seed germination and root and stem growth of lettuce seedlings. The hcnABC genes of strong cyanogenic Pa strain WPFP11r were amplified PCR and further cloned and constructed into a broad-host range vector pBBr1MCS-5. The construct pHg601 (carrying hcnABC) was transformed into a non-cyanogenic P. putida YLFPP4 by electroperoration. The transformant Y44H1 which produced high quantities of HCN was obtained. The inhibition effects of Y44H1 on seed germination and seedling growth of lettuce, cabbage, Chinese cabbage, and tomato were significantly higher than that of non-cyanogenic Y44C1, a transformant with vector only. The results demonstrated that HCN played an important role in inhibiting seed germination and seedling growth of various plants by cyanogenic Pa.

Some strains of plant-associated Pseudomonas aeruginosa (Pa) were strong cyanogenic. The strong cyanogenic Pa inhibited the growth of Ralstonia solanacearum in a paired-plate system and in the effect of cyanogenesis on tomato bacterial wilt caused by Rs was evaluated in a pot experiment. The disease severities of tomato plants treated with a Rs suspension added with strong cyanogenic Pa, were significantly greater than that of plants treated with a suspension containing only Rs. HCN biotransformation genes hcnABC of strong cyanogenic Pa WFP11r had been cloned and transcribed using cyanogenic P. aeruginosa PaLFP4 by electroporation. The transformant Y44H1 which produced high quantities of HCN, also inhibited the Rs in the paired-plate system. The disease severities of tomato plants treated with a Rs suspension added with Y44H1, were also significantly greater than that of plants treated with a Rs suspension added with non-cyanogenic Y44C1, a transformant with vector only. The results demonstrated that HCN play an important role for strong cyanogenic Pa to increase the disease severity of tomato bacterial wilt caused by Rs.


Rhynchosporium secalis causes a leaf disease of cultivated barley (Hordeum vulgare) and rye (Secale cereale) in many parts of the world where conditions are favorable for disease development. It also occurs on many wild grasses including wild barley (Hordeum sp.) and brome grass (Bromus sp.) in Australia. In the past, results from cross pathogenicity tests with barley and rye isolates were inconsistent. We analyzed R. secalis populations from barley, rye, brome grass and barley grass using restriction fragment length polymorphisms (RFLPs) and sequence analysis of the internal transcribed spacer region ITS1 and ITS2 of the 5.8S RNA gene. Multiple RFLP analyses revealed that rye isolates were genetically distinct from isolates of other host species based on a low genetic identity between rye and other host populations (I = 0.01), high population subdivision (average GST = 0.51), and a low number of shared RFLP alleles. ITS sequences revealed that the rye isolates were 1.2 to 1.6% (11-14 single nucleotide differences) different from barley, barley grass, and brome grass isolates. Based on the phylogenetic analysis of the ITS sequences, the rye isolates separated into a clade supported by a 100% bootstrap value, and the rest of the isolates separated into a different clade supported by a 91% bootstrap value on a parsimony tree. Cross pathogenicity tests are necessary to clarify the confusion regarding pathogenicity of barley and rye R. secalis isolates. We propose that R. secalis from barley and rye should be considered as different species.


Rhynchosporium secalis, the causal agent of scald on barley, has always been considered asexual because no teleomorph could be found. In this study we successfully identified partial sequences of the HMG box and alpha domain of the HMG box transcription factor, and transformed into non-cyanogenic R. secalis with strong cyanogenic Pa, were significantly greater than that of plants treated with a Rs suspension added with PaLFP4 by electroporation. The transformant Y44H1 which produced high quantities of HCN, also inhibited the Rs in the paired-plate system. The disease severities of tomato plants treated with a Rs suspension added with Y44H1, were also significantly greater than that of plants treated with a Rs suspension added with non-cyanogenic Y44C1, a transformant with vector only. The results demonstrated that HCN play an important role for strong cyanogenic Pa to increase the disease severity of tomato bacterial wilt caused by Rs.

Variation in Xanthomonas oryzae pv. oryzae isolates adapting to the rice Xa7 resistance gene. K. M. LINHOLM (1), G. Ponziciano (1), K. Garrett (1), J. E. Leach (1), and C. M. Vera Cruz (2). (1) Dept. of Plant Pathology, Kansas State University, Manhattan 66506; (2) International Rice Research Institute, Los Banos, Philippines. Phytopathology 93:S53. Publication no. P-2003-0385-AMA.

Durable resistance conferred by the rice gene Xa7 is a function of the fitness cost of adaptation to virulence by the bacterial blight pathogen, Xanthomonas oryzae pv. oryzae (Xoo). Virulence results from loss of pathogen effector gene function. In a 3 yr study, we observed that complete loss of function of the Xoo effector gene, avrXa7, correlated with a reduction in pathogen aggressiveness and persistence in the population. After 5 yr continuous planting, the Xoo population structure now exhibits a continuum of lesion lengths on rice cultivar IRBB7 (with Xa7 gene), with an increase in isolates that cause moderate lesions on IRBB7. Although this Xoo population exhibits increased virulence to Xa7, disease incidence has not increased on IRBB7. Preliminary sequence analysis of avrXa7 reveals several changes that correlate with moderations in avirulence and fitness phenotypes. These studies confirm that there is a fitness cost associated with loss of avrXa7 function, and that this contributes to durability of Xa7.


Two forecasting models were used to predict the risk of Fusarium head blight (FHB) during 2002. Hourly data from 14 weather stations were used to determine duration of weather variables during pre- and post-anthesis time periods examined by the models. Of 42 risk probabilities calculated, Model 1 predicted 31 with low or moderately low risk and Model 2 predicted 40 with low or moderately low risk. The low probabilities were due to protracted cold conditions (<15°C) during the critical time for inoculum development and infection. Based on these results, growers were informed that the risk of FHB was low to moderately low for the majority of locations and anthesis periods in the state. By 14 to 20 days after anthesis, mean FHB incidence in 159 fields in 30 counties was 4.1%, and ranged from 0% to 48.6%. Over 75% of the fields surveyed had FHB incidence levels below 5%, and only 4% had incidence levels above 15.1%. Results indicated that the models generally predicted the risk of scab adequately for the majority of locations in the state.


Organic soil amendments including cotton gintrash, poultry manure, and a rye/vetch green manure, or synthetic fertilizers were applied to subplots and either tillage or surface mulch was applied to main plots in a field over two seasons. Genetic diversity of the fungal, Archeae, and bacterial communities was determined using multiple GC-clamped primer pairs in a PCR. Multiple 18S rDNA primers were used to amplify 18S fungal rDNA and multiple 16S rDNA primers were used to amplify fungal and bacterial rDNA. Denaturing gradient gel electrophoresis (DGGE) with 18S rDNA primers generally indicated that fungal community structures were similar in soils with the same fertility amendments, but significantly different across amendment types. Bacterial community structure was not greatly affected by tillage or surface mulch treatment. Further spatial statistics will be conducted to determine impact of the treatments on the velocity of spread of P. capsici.


Organic soil amendments including cotton gin trash, poultry manure, and a rye/vetch green manure, or synthetic fertilizers were applied to subplots and either tillage or surface mulch was applied to main plots in a field over two seasons. Genetic diversity of the fungal, Archeae, and bacterial communities in regions ITS1 and ITS2, and the 5.8S rDNA were assessed using DNA directly extracted from soil and amplified with multiple GC-clamped primer pairs in a PCR. Multiple 18S rDNA primers were used to amplify 18S fungal rDNA and multiple 16S rDNA primers were used to amplify 16S Archeae and bacterial rDNA. Denaturing gradient gel electrophoresis (DGGE) with 18S rDNA primers generally indicated that fungal community structures were similar in soils with the same fertility amendments, but significantly different across amendment types. Bacterial community structure was not greatly affected by tillage or surface mulch treatment. Even though there were more species recognized in the soil amended with poultry manure than other amendments in tilled plots. Greatest disease occurred in soils amended with cotton-gin trash. Further spatial statistics will be conducted to determine impact of the treatments on the velocity of spread of P. capsici.
Rhizomonia is one of the most economically damaging diseases of sugar beet. This disease is caused by *Beet necrotic yellow vein virus* (*BNYVV*) and vectored by the soil-borne fungus *Polymyxa betae*. Partially resistant sugar beet cultivars based upon single dominant genes have been developed against this devastating disease. In the summer of 2002, two sugar beet fields with a BNYVV-resistant cultivar in the Imperial Valley of California were observed with severe rhizomonia symptoms, suggesting that resistance had been compromised. Standard soil baiting with sugar beet plants followed by ELISA tests were used to diagnose virus occurrence and reaction. Resistant varieties grown in regular BNYVV-infested soil remained resistant. In contrast, when grown in Imperial Valley BNYVV-infested soil all resistant varieties tested susceptible according to elevated ELISA values. Eight different BNYVV isolates have been isolated from Imperial Valley soil (*IV-BNYVV*) by single local lesion isolation. Three IV-BNYVV isolates contained BNYVV RNA-5 as determined by RT-PCR. Whether IV-BNYVV isolates contain the virulence of P-type remains to be determined.

**Confirmation of dsRNA as a tool for the diagnosis of diseases caused by rhaphido-like viruses transmitted by the mite *Brevipalpus* sp.**


Fungi frequently isolated from declining grapevines in Pennsylvania and New York include *Phaeosphaeria nodorum* (Pch), *Phaeosphaeria sp.* (Pn), *Phialophora* (Phial.) spp., and phialophora-like fungi. Pch and Pn spp. are known causal agents of Petri and Esca disease of grapevines. The internal transcribed spacer, ITS1-5.8S-ITS2, and the partial sequence of reverse polymerase subunit 2 (RPB2) gene regions of the phialophora-like isolates have been analyzed with PAUP using *Phc* and *Phial. sensus stricto* (s.s.) as out group taxa. This data combined with published sequences has shown that *Phialophora* is a polyphyletic genus of several phialophora-like fungi that are found within the Loculoascomycetes (*Phial. s.s. and Pch*), the Pyrenomycetes [*Phial. sensus lato* (s.l) and *Pm* spp.] and the Discomycetes (*Phial. s.l.*). Four *Pfhl. spp.* have been reassigned to the genus *Cadophora*; however the molecular data now shows that this genus is also polyphyletic. The objectives of this research are to assign selected phialophora-like fungi to a proper genus and determine the role of these organisms in vine decline.


We characterized the genetic basis of high level durable resistance to *Pyricularia grisea*, *Sacc.* in the rice cultivar Oryzica Linals 5 (OLS) in F7 RILs from a cross between Fanny (susceptible) and OLS. A linkage map was constructed using 250 molecular markers: SSR, RELP and RGAs. Eleven loci, distributed on chromosomes 2 (locus 1), 3(1), 4(1), 6(2), 8(2), 11(2) and 12(2), were associated with the quantitative expression of one of two resistance traits (lesion type and disease leaf area) to *P. grisea* isolates. QTLs with the largest effects were on chromosomes 8 (LOD 70.0 and explained 30.0% of variance) and 6 (LOD 20.0 and explained 27.0% of variance), and were linked to resistance gene P12 and P9 on chromosome 6, and P11 on chromosome 8 known to confer complete resistance to several *P. grisea* isolates. Other QTLs (LOD 2.0 - 17.41) explained 1.85-10.85% of the variance. The durable, broad spectrum resistance in OLS is associated with major genes inducing hypersensitive reactions and minor genes causing less distinctive phenotypic differences.

**Comparison of gene expression in crown and embryo tissues during resistance to common bunt of wheat.** Z.-X. LU, B. Puchalski, M. Frick, D. Gaudet, and A. Laroche. Lethbridge Research Centre, Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta T1J 4B1, Canada. Phytopathology 93:S54. Publication no. P-2003-0393-AMA.

Common bunt disease can cause serious yield and quality losses in wheat. We employed two wheat lines, “Neepawa” (susceptible) and its near-isogenic sib “BW533” (resistant), and virulent race T1 to study gene expression for common bunt resistance. Two suppressive transcript hybridization (SSH) cDNA libraries were constructed from crown and embryo tissues, and 175 up-regulated and 25 down-regulated clones, and 96 up-regulated and 13 down-regulated clones were identified and sequenced. Moreover, BLAST results indicated that more than two thirds of sequences share their homology to known gene products in available public databases. Sequence data indicated that little was overlapped between crown and embryo mRNA libraries for differentially expressed genes. In crown tissues, eight and one putative LTP genes were identified in crown and embryo tissues, respectively. The results suggested that different genes are differentially expressed in crown and embryo tissues for common bunt resistance in wheat.

**Sensitivity of Alternaria mali from North Carolina apple orchards to pyraclostrobin and boscalid.** Y. J. LI (1), B. B. Shew (1), and H. Ypema (2). (1) Dept. Plant Pathology, NC State Univ, Raleigh; (2) BASF Corporation, Research Triangle Park, NC. Phytopathology 93:S54. Publication no. P-2003-0394-AMA.

Early leaf spot caused by *Alternaria arachidicola* is the main leaf disease on peanut (*Arachis hypogaea*). Eighty-five single spot isolates were obtained from peanut leaves collected from two locations. Field one (NC) did not have a history of strobilurin use; field two (VA) had been exposed to strobilurins. Different cultural conditions were tested for inducing pyraclostrobin-resistant culture (C. arachidica) in vitro. Cultures on clarified V8 agar or peanut oatmeal agar under 12 or 24 h/day of fluorescent light at 24°C produced abundant conidia. Sensitivity of conidial germination to pyraclostrobin (BAS500F) was examined on water agar, amended with serial dilutions of fungicide (0.00025 to 10 micrograms (µg/ml). ED₅₀ were calculated from percentage spore germination observed. ED₉₅ ranged from 0.0003 to 0.003 µg/ml and averaged 0.00129 µg/ml. Natural variation in isolate sensitivity to pyraclostrobin was found at each location, but there was no difference between locations.

Soybean tolerance to *Phytophthora* root rot and damping off is a desirable trait because it is not race-specific and is not overcome as diverse populations of *P. sojae* accumulate over time. The objective of this work was to characterize tolerance to *P. sojae* infection by comparison of pathogen isolates.
colonization within infected tolerant and susceptible seedlings. Real-time quantitative PCR was used to quantify pathogen DNA levels in infected roots at three and six days post-inoculation. Differences in pathogen DNA levels after inoculation, as determined by quantitative PCR, effectively differentiated the tolerant from the susceptible varieties. Significant differences in P. sojae DNA levels were detected among tolerant and susceptible varieties. This assay has utility both as a method of evaluating soybean genotypes for tolerance to P. sojae infection and for further analysis of early colonization events occurring in infected tolerant varieties.

**Disease specific volatile metabolites to discriminate dry and soft rots of potato tubers.** L. H. Lui, B. Pritchiravij, B. A. Vikram, and A. C. KUSHALAPPA. Department of Plant Science, McGill University, Ste-Anne de Bellevue, Quebec, Canada H9X 3V9. Phytopathology 93:S55. Publication no. P-2003-0397-AMA.

Dry rot induced by Fusicarium sambucinum (FSA), and soft rots induced by Erwinia carotovora subsp. carotovora (ECC) and Erwinia carotovora subsp. atroseptica (ECA) are serious diseases in potato storage. Volatile metabolites from potato cv. Russet Burbank water-inoculated or inoculated with the above pathogens were monitored by sampling the headspace at 3 and 6 d after inoculation (dai) using a GC/MS (HAPSTIE). 251 volatiles were detected at 3 dai which increased to 579 at 6 dai. Several disease-specific volatiles were observed: FSA produced 2, 3-butanediol, dinitrate, 2,5-norphornadene and Styrone, whereas, ECC produced diacene, diacene- methyl hydroxy-(1,1-dimethyl-2-hydroxy-ethyl)-amine, Propanone, and 1-methoxy-2-methyl-(1,1-dimethyl-2-hydroxy-ethyl) -amine, and ECA produced, 1, 2-dimethoxy-ethene, acetic acid ethyl ester and dl-2- benzylmecylic acid. The possible use of these differential volatile metabolites in disease detection is discussed.

**Application of EST technology in functional genomics of Arachis hypogaea.** L. M. Luo (1, 2), P. Dang (3), B. Z. Guo (2), C. C. Holbrook (4), and M. Bausher (3). (1) University of Georgia, Georgia; (2) USDA-ARS, Crop Protection and Management Research Station, Tifton, GA 31793; (3) USDA-ARS, U.S. Horticultural Research Lab, Ft. Pierce, FL 34945; (4) USDA-ARS, Crop Genetics and Breeding Unit, Tifton, GA 31793. Phytopathology 93:S55. Publication no. P-2003-0398-AMA.

The genome size of peanut (Arachis hypogaea L.) is about 2800 Mb in comparison with the genome size 128 Mb and 425 Mb of Arabidopsis thaliana and rice (Oryza sativa), respectively, which have been completely sequenced. EST (expressed sequence tag) technology is the most cost-effective route for studying A. hypogaea genome and studying the complicated problem of host resistance and preharvest aflatoxin contamination. We constructed ESTs from sweetpotato leaves and infected sweetpotato varieties using EST databases from soybean and other Legumes. Differential expression of genes clustered in the range of molecular weights between 10-80 kDa across a range of multiple populations of sweetpotato viruses to date suggests that SPPFMV-C and SPPFMV-RC may be distinct viruses.

**Systemic expression of defense responses in Austrian pines induced by Sphaeropsis sapinea.** R. MA and P. Bonello. Dept. of Plant Pathology, Ohio State University, Columbus, OH. Phytopathology 93:S55. Publication no. P-2003-0402-AMA.

The pathogenic fungus Sphaeropsis sapinea causes a variety of diseases including seedling collar rot, tip and twig blight, branch dieback and stem cankers of pines. This study was conducted to test if inoculation of Austrian pines with S. sapinea results in systemic altertations in the expres-

**Sentence:**

The slower the development of fruit rot. A diagram was produced to show whether determination of latent infection is not recommended, recommended, or strongly recommended. These recommendations are also based on whether low, moderate, or high levels of spore inoculum potential exist in a dried plum orchard.


Soybean rust, caused by the Asian species Phakopsora pachyrhizi, has migrated rapidly from its native range in Asia to Africa and South America in recent years. To identify proteins involved in early interactions between P. pachyrhizi and soybean (Glycine max), we conducted a two-dimensional gel electrophoresis analysis of leaf apoplastic and cellular proteins expressed at discrete times during the infection process. Preliminary experiments focused on apoplastic proteins from the soybean line Komata, which contains the Rpp1 gene and exhibits a resistant (immune) response to several P. pachyrhizi isolates. Apoplast proteins from leaves of Komata primarily clustered in the range of molecular weights between 10-50 kDa across a broad range of pl’s from 3-10. Protein profiles are being analyzed from soybeans sampled 24, 72, and 144 h after inoculation. Differentially expressed proteins will be identified by MALDI-TOF mass spectrometry using EST databases from soybean and P. pachyrhizi.


Sweetpotatoes (Ipomoea batatas) are vegetatively propagated tuberous rooted plants grown for food and forage. Virus-free plant stocks are crucial for germplasm exchange. Sweetpotato viruses often occur in complexes, complicating detection by traditional methods. The objective of this research was to identify viruses present in NC sweetpotatoes and describe their genetic diversity using DNA sequencing and phylogenetic analyses, with the future goal of designing a rapid diagnostic system. Amplification by RT-PCR from total RNA extracted from I. batatas yielded several fragments ranging from 0.5 to 1.3kb in size, which were subsequently cloned and sequenced. Comparisons of the C-terminal regions of the NPB protein and the N-terminal regions of the capsid protein indicated infection by multiple potyviruses, including SPPFMV-C, SPPFMV-RC, and SPVY. Preliminary phylogenetic analysis indicates that the FMV strains present are most closely related to 956, C, and the LSU isolates. Sequence analysis of multiple fragments of sweetpotato viruses to date suggests that SPPFMV-C and SPPFMV-RC may be distinct viruses.

**Best time period for determining latent infection of dried plum caused by Monilinia fructicola.** Y. LDU and T. J. Michailides. Dept. of Plant Pathol-

**Alternaria alternata** (AAL), *A. tenuissima* (ATE), and *A. arborescens* (AAR) are the causal agents of Alternaria late blight of pistachio. Isolates of AAL, ATE, and AAR collected from two pistachio orchards with multiple applications of azoxystrobin for more than 2 years were highly resistant to azoxystrobin in vitro; however, none of the 14 wild-type isolates collected from pistachio orchards without a previous history of streubitulin usage was resistant to azoxystrobin. Resistance of AAL, ATE, and AAR to streubitulin was conferred by a single change of glycine to alanine at the codon 143 of the cytochrome *b* (cytb) gene. Based on the sequences of the cytb gene from AAL, ATE, and AAR, species-specific PCR primers were developed to amplify a 226-bp DNA fragment of the cytb containing the mutation site. The restriction enzyme *Fnu*4HI recognized the sequence of GCTGC in the PCR product of resistant isolates only. Digestion of the PCR product with *Fnu*4HI I provided a rapid diagnostic method to detect resistant isolates of AAL, ATE, and AAR.


Low and high levels of resistance to the benzimidazole fungicides, benomyl and thiophanate-methyl, were observed in field isolates of *M. fructicola*, causing brown rot of stone fruit. Low resistant (LR) and high resistant (HR) isolates were also cold and heat-sensitive, respectively, although results from microsatellite DNA fingerprints did not show significant genetic differentiation among the sensitive (S), LR, and HR populations. Analysis of DNA sequence of beta-tubulin gene showed that the LR isolates had only a point mutation at the codon 6, causing substitution of amino acid histidine by tyrosine. The codon 198, which encodes a glutamic acid in S and LR isolates, was converted to a codon for alanine in HR isolates. Based on these point mutations in beta-tubulin gene, allele-specific PCR assays were developed for rapid detection of LR and HR isolates of *M. fructicola* from stone fruit in California.


*Bean pod mottle virus* (BPMV; *Comovirus; Comoviridae*), an important virus of soybean, causes seed coat mottling and loss in seed quality. Although BPMV is not widespread in Virginia, isolates belong to both known subgroups, I and II. A comparison was made of the extent of seed coat mottling caused by three BPMV isolates. Field experiments used isolates S98-1, S98-15 and S01-10 and five soybean cultivars/lines, Bolivar, L78-379, Manokin, Williams and LS97-3004. Plants were inoculated mechanically at different stages of development, V1-V4, V5-V6 and R1-R3, in three replicates. There was more obvious mottling in early inoculated plants (V1-V4) than plants inoculated at V5-V7. Plants inoculated at R1-R3 produced fewer mottled seeds compared to those inoculated at earlier stages. Bolivar had the most extensive seed coat mottling followed by Manokin, Williams and L78-379, whereas LS97-3004 showed no mottling at any of the three stages of inoculation. Seed coat mottling was shown to vary with cultivar, stage of development at the time of inoculation, and BPMV isolate.

The quest for effective resistant genes to wheat stripe rust caused by *Puccinia striiformis* West. in Chile. R. B. MADARIAGA, M. Z. Mellado, and V. V. Becerra. National Institute of Agricultural Research, Quilamapu Regional Center, INIA, Casilla 426, Chillan, Chile. Phytopathology 93:S56. Publication no. P-2003-0406-AMA.

Stripe rust caused by *Puccinia striiformis* West. is the major wheat disease constrain to the 4.3 t ha⁻¹ yield average and the 400 000 has, that are annually cropped. Breeding for disease resistance has been used successfully, however, periodically new cultivar must be introduced to cope with the appearance of emerging virulence. A group of 81 winter and spring wheat genotypes including the 16 Universal - European differential, the Australian - Avocet isogenic lines and Local Cultivars were tested on seven locations during 2000 to 2002. Avocet isogenic lines carrying *Yr*5; *Yr*8; *Yr*10; *Yr*15; *Yr*5k and *Yr*Sp genes as well as several chilean genotypes consistently showed major gene type resistance expressed as absence of disease, whereas Jupateco R was partially effective and Avocet S, Morroco and *Yr*18 / 3. Avocet S showed complete pustules coverage. A significant location x cultivars interaction suggest that there were different virulence inside the pathogen populations in Chile.


The survey, detection and eradication of exotic diseases would be assisted by national scale risk maps created from plant disease forecast models. However, the creation of forecast models is often impeded by the lack of a standardized generic model and the lack of epidemiological data. In this study, a simple temperature response function was investigated as the basis for a disease infection model. The model inputs are the cardinal temperatures and the minimum wetness duration requirement, Wmin. The model was validated with published data from 65 controlled laboratory studies. In most cases, the model performed well and had an average root mean square error of 4.6 h. However, for some pathogens, uncertainty in the input parameters contributed to error. The value of Wmin varied from 1 to 48 h and was unaffected from some genes and others. Pathologists divided into three groups based on their sensitivity to an interruption of wetting by a dry period, another input used in the model. The model will be incorporated into the NAPPPAST system, a developmental risk analysis tool designed for use by the Animal and Plant Health Inspection Service.

NAPPPAST, an internet tool for the weather-based mapping of plant pathogens. R. D. Magarey (1,2), G. A. Fowler (2), T. B. Sutton (2), and C. L. THAYER (2). (1) Animal and Plant Health Inspection Service, Raleigh, NC 27606; (2) Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695. Phytopathology 93:S56. Publication no. P-2003-0408-AMA.

The NAPPPAST system is a new internet tool for pest modeling using weather, climate and soil data. The system is a joint venture between the Animal and Plant Health Inspection Service, NC State University, and ZedX, Inc, an information technology company. The components of the system are an internet-based interface and a 'fill-in-blanks' template. Information from the internet-based graphical user interface allows a user to click on a map and zoom between national and sub-county scales. Risk assessments can be created from historical, climatic or forecast weather data at a 10-km² resolution in the form of maps, tables or graphs. Pathogen models are created from an interactive template using empirical relationships with weather variables or for a generic infection model based on a temperature-moisture response. The template guides the addition of new pests by providing a series of ready prompts and default values. The system will improve the survey, detection and eradication of exotic pests. Its use in risk analysis will be demonstrated with several case studies.

Use of dry chlorine dioxide gas in the treatment of tomato fruit for bacterial soft rot. M. J. MAHOVIC (1), J. A. Bartz (1), and T. Tenney (2). (1) University of Florida, Dept. of Plant Pathology, Gainesville, FL 32611; (2) ICA Trinova, LLC, Forrest Park, GA 30297. Phytopathology 93:S56. Publication no. P-2003-0409-AMA.

Treatment of wound-inoculated *Erwinia carotovora* subsp. *carotovora* tomato fruit for 30 sec with aqueous solutions containing up to 800 ppm free chlorine at pH 7.0 failed to significantly reduce the incidence of bacterial soft rot developing during subsequent storage. By contrast, decay development in similarly inoculated fruit was nearly prevented by a controlled release chlorine dioxide treatment that produced 0.75 ppm w/v chlorine dioxide gas over a 2-h period. Decay incidence was <13% after 3 days storage at 22°C among fruit treated with 0.75 ppm ClO₂ gas produced over a 24-h period and <0.7% after 3 days storage at 22°C among fruit treated with 7.5 ppm ClO₂ gas produced over a 2 or 24-h period. The incidence among inoculated, untreated control fruit averaged 82%, with some individual tests showing 100% decay. All (100%) of the symptom-free wounds contained viable soft rot bacteria at the termination of the storage, whereas <5% or <85% of the wound treated with the 2 or 24-h release treatments, respectively, had viable soft rot bacteria. Phytotoxicity was not observed except for tannin of wound surfaces at higher concentrations.


*Phytophthora sojae* is a common and significant disease of soybean in Illinois. As part of a larger study of *P. sojae*, we examined effectiveness of the common soybean Rps genes available to producers. Seedlings (cv. Sloan) were used to bait 42 isolates of *P. sojae* from soybean fields with a history of seedling diseases in 13 counties across Illinois. Seedlings of 15 cultivars with the Rps genes 1a, 1c, and 1k (five cv. each) from four major seed companies and susceptible ‘Sloan’ were challenged with the 42 isolates using a hypocotyl inoculation method in trials replicated twice. Fifty percent of the isolates were virulent to 1a, 45%
to 1c, and 19% to 1k. Isolates with virulence to 1a, 1c, and 1k originated from 46%, 30%, and 8% of the counties, respectively. Different cultivars with the same Rps gene performed similarly against the isolates. We are testing virulence to 13 Rps gene differentials for these and other isolates from Illinois. We have identified isolates from additional counties that are virulent to Rps 1a, 1c, and 1k. The results verify that none of the common Rps genes are effective in some areas of Illinois.


Identification of mixed populations in Citrus tristeza virus (CTV) infections is very important for the management of CTV including budwood certification, quarantine, eradication, and cross protection. Single aphid transmissions are the best means of separating mixed infections at present. With the objective of characterizing a non aphid-transmissible severe Florida CTV isolate, we attempted to sequence the CTV using the consensus sequencing method. Two of the three CTV populations in the plant belonged to new genotypes which differed from other described sequences as much as 10-30% in the 5’ half of the genome. Sequence analysis indicated that the entire first open frame may be reliable for genotyping purposes, but not some of the 3’ genes. Unknown genotypes may not be detected in plants with mixed infections when specific primer pairs are used in characterization.

A simple heteroduplex mobility assay was developed to facilitate identification of unknown genotypes and for analysis of sequence relatedness between isolates and clones.

A PCR-based screening strategy for detecting deletions in defense response genes in rice. P. MANOSALVA (1), M. Ryba-White (1), C. Wu (1), C. Lei (2), M. Baraoed (2), H. Leung (2), and J. Leach (1). (1) Kansas State University, Manhattan; (2) International Rice Research Institute, Philippines. Phytopathology 93:S57. Publication no. P-2003-0412-AMA.

With the release of the complete rice genome sequence, the challenge for the post-sequencing era is to identify the biological function of the sequenced genes. Our focus is to use rice mutants to understand the functions of defense response (DR) genes in disease resistance. A collection of rice mutants was produced in an IR64 background using diepoxybutane and fast neutron. To identify mutants in DR genes, a PCR-based strategy using DNA pools of the mutant lines was developed. DNA from 8,350 deletion lines was organized into pools of 4,000-7,000 lines and sequenced by single line. Primers corresponding to DR genes were designed and used to amplify pooled DNA by PCR. PCR products were resolved in polyacrylamide gels and polymorphisms were detected by silver staining. We detected an individual mutant line with a deletion in a putative phenylalanine ammonia-lyase gene. The background of the mutant line was confirmed by microsatellite fingerprinting. Phenotypic and segregation analyses are in progress.

Identity of postharvest pathogens of California avocados and their control by thermal conditioning. D. L. MARQUES (1), S. H. De Boer (2), H. Ceri (1), and M. E. Olson (1). (1) Univ. of Calgary, Biofilm Research Group, Calgary, AB, Canada T2N 1N4; (2) Centre for Animal and Plant Health, Charlottetown, PE Canada C1A 5T1. Phytopathology 93:S57. Publication no. P-2003-0414-AMA.

Postharvest pathogens were isolated from avocados from diverse regions of California in 2000 to 2003. Isolations from over 2,200 decayed fruit showed the most prevalent decay fungi were Dothiorrella, Colletotrichum, and Alternaria spp. Dothiorrella incidence was most frequent in the summer and fall. Colletotrichum incidence was lower in spring and similar in frequency at other times. Its incidence became markedly high on fruit harvested during rainy periods. Alternaria was generally constant throughout the year. Postharvest thermal conditioning tests were done with ‘Hass’ avocados from two groves. Fruit were ripened immediately, or stored at 5 or 10°C for 3, 7, or 14 days prior to ripening at 20°C. Stem-end rot (SER) was reduced from 25 to 6%, and from 13 to 3%, in fruit from groves one and two, respectively, when fruit were ripened after 14 days storage at 5 or 10°C. SER reduction at 5°C was superior to that at 10°C after 3 or 7 days storage, though neither of the temperature–time treatments were as effective as the 14 day 5 or 10°C treatments.


Samples of symptomatic tomato leaves were found to be infected with a potexvirus using a potexvirus-group specific primer set in RT-PCR. In a direct ELISA, the samples reacted strongly to rabbit polyclonal antiserum raised against both tomato and pepino isolates of Pepino mosaic virus (PepMV). Viral genome sequences were determined from clones of amplified DNAs generated using group- and virus-specific primers on total RNA extracted from infected leaves. The complete sequence of the genome of this US isolate of PepMV will be presented and compared with known sequences of different tomato and pepper isolates of PepMV from other countries, including Peru, Spain, France and England. In our tests to date, the virus has been detected in tomato samples from Arizona, California, Colorado, Florida, Oklahoma and Texas. PepMV, first described in pepino crops in Peru, is currently the cause of epidemics in tomato in Europe and is also now confirmed in North America.

Evaluation of biofilms formed by Clavibacter michiganensis subsp. sepedonicus. L. L. R. MARQUES (1), S. H. De Boer (2), H. Ceri (1), and M. E. Olson (1). (1) Univ. of Calgary, Biofilm Research Group, Calgary, AB, Canada T2N 1N4; (2) Centre for Animal and Plant Health, Charlottetown, PE Canada C1A 5T1. Phytopathology 93:S57. Publication no. P-2003-0415-AMA.

The gram-positive bacterium Clavibacter michiganensis is a vascular pathogen that develops high cell populations in the lumen of xylem vessels of host plants. Since extrapoly saccharides play a major role in symptom expression, biofilm development may also be an important virulence characteristic. We investigated whether C. michiganensis subsp. sepedonicus, causal agent of bacterial ring rot in potato, also develops biofilms in planta. Scanning electron microscopy of symptomatic ring rot infected potato stems revealed that C. m. sepedonicus produces large bacterial aggregates attached to xylem vessels. The aggregates appear to be embedded in an exopolymeric matrix suggestive of a biofilm structure. The propensity of C. m. sepedonicus to form biofilms was confirmed in vitro using a wood matrix in which C. m. sepedonicus developed very thick biofilms.

Susceptibility of Erwinia carotovora to biofilms. L. L. R. MARQUES (1), S. H. De Boer (2), H. Ceri (1), E. Lee (1), and M. E. Olson (1). (1) Univ. of Calgary, Biofilm Research Group, Calgary, AB, Canada T2N 1N4; (2) Centre for Animal and Plant Health, Charlottetown, PE Canada C1A 5T1. Phytopathology 93:S57. Publication no. P-2003-0416-AMA.

Biofilms are sessile microbial aggregates enclosed in a polymeric matrix and are metabolically distinct from free-living (planktonic) cells. They are important in several animal and human infections as well as in some plant infections. Recently, Pierce’s disease of grape and Mediterranean corrosion of banana caused by Xylella fastidiosa. Since biofilms play a role in pathogenicity and are resistant to antimicrobial agents, they impact on disease control strategies. We analysed strains of Erwinia carotovora subsp. carotovora, which cause decay of vegetable crops, for biofilm formation and susceptibility to antibiotics using the MBECTM device. Biofilms of E. c. carotovora showed remarkably reduced susceptibility to several classes of antibiotics. The minimal concentrations needed to kill biofilm populations were 8 to 512 times higher than concentrations needed to kill planktonic cells.


With the pending phase-out of methyl bromide the California strawberry industry is currently evaluating alternative fumigants and production practices for management of soilborne pests. Remote sensing is being used to assist in these evaluations as well as provide growers with additional tools for making crop management decisions. Data on plant growth (biomass and leaf area), and canopy reflectance was collected at various intervals during the season from replicated subplots within each fumigation treatment and compared to total fruit harvest during the 6 month harvest season in an effort to model the relationship among these parameters. Variation in some canopy reflectance data corresponded with changes in yield. Plant canopy data also was collected from replicates 1-3 acre production blocks for each fumigation treatment and compared to the commercial marketable fruit harvested by the grower. Calibrated, georeferenced aerial images of the production fields collected at 4-6 week intervals were used to calculate vegetation indices and model how changes in a field over time related to crop productivity.
Development of a molecular marker system for detection of *Phytophthora* spp. and diagnosis of species associated with Sudden Oak Death in California. F. N. MARTIN (1), P. Tooley (2), and R. Frederick (2). USDA-ARS, (1) Salinas, CA 93905; (2) Ft. Detrick, MD 21702. Phytopathology 93:S58. Publication no. P-2003-0418-AMA.

A molecular marker system using mitochondrial sequences has been developed for detection of *Phytophthora* spp. in diseased tissue and identification of *P. ramorum* (the causal agent of sudden oak death) and an additional species that is morphologically similar to *P. illis* that is commonly recovered from diseased trees in California. The first multiplex amplification included a primer pair to amplify plant sequences (to serve as a positive control) and a *Phytophthora* genus-specific primer pair. This amplification was diluted and followed by a second amplification with a nested species-specific primer pair. The primers developed for *P. ramorum* and the *P. illis*-like isolates exhibited a high degree of species specificity and did not amplify any of the 27 other *Phytophthora* species evaluated thus far. Some aspects of this marker system have been modified for use with the TagMan real-time PCR system.


Tanox™ is a new fungicide from DuPont containing the active ingredients Fomoxate™ (fomoxadone) and cymoxanil. Fomoxate™ offers broad-spectrum disease control, low use rates, strong residual properties, and short pre-harvest intervals. Fomoxate™-based products control a wide range of economically important plant diseases in cucurbits such as downy mildew (*Pseudoperonospora cubensis*), anthracnose (*Colletotrichum orbiculare*) and Alternaria leaf blight (*Alternaria cucumerina*). Fomoxate™ provides excellent activity against many other diseases caused by *Oomycte* and Ascomycete fungi, and improves control of some bacterial diseases when combined or alternated with copper or EBDC fungicides. Disease management programs containing Fomoxate™-based products in combination or alternating with a companion fungicide are effective, affordable and reduce the risk of resistance development.


Raspberry bushy dwarf virus (RBVD) has become an important problem over the past 15 years throughout raspberry growing regions of the world. Crumby fruit and yield reduction can combine to reduce crop value by more than 50%. RBVD is pollen-borne, making chemical control virtually impossible. Meeker raspberry plants were transformed with the resistance gene or mutated forms of the movement protein gene of RBVD. The presence of these viral genes was confirmed by DNA hybridization analysis and RT-PCR of DNAase digested total RNA preparations. Of 197 transgenic lines planted in replicated blocks in the field under extreme disease pressure, 5 lines remained free of RBVD after four years, all 202 of the wild-type ‘Meeker’ plants in the same plot were infected. Most transgenic lines had 9 of 9 plants infected and a few lines showed partial resistance to infection, with 1 to 6 of 9 plants infected. The 5 lines showing field resistance were also resistant to RBVD when tested by grafting. Fruit evaluations of these 5 lines showed that they contained 5 anthocyanins at the same concentrations as were present in wild type ‘Meeker’ fruit.

Mixed infections of Tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (INSV) in weeds around tobacco fields in Georgia. N. MARTINEZ-OCHOA (1), A. S. Cisinos (1), T. M. Webster (2), and P. Bertrand (1). (1) Dept. of Plant Pathology, University of Georgia; (2) CPMRU, USDA-ARS, Tifton, GA 31793. Phytopathology 93:S58. Publication no. P-2003-0421-AMA.

A survey was conducted in ten Georgia tobacco fields to determine which weeds were infected with TSWV and INSV. In the winter and spring, *Verbena rigida* (stiff verbena), *Cerasium glomeratum* (sticky chicweed), *Stachys floridana* (Florida betony), *Acanthospermum hispidum* (bristly starbur), *Eupatorium capillifolium* (dogfennel), *Gnaphalium cernuum* (crusted), and *Geranium carolinianum* (wild geranium) were found with dual infections of TSWV and INSV. In the summer, *Jacquemontia tannifolia* (smallflower morningglory), *Wahlenbergia marginata* (southern rockbell), *V. rigida*, *A. hispidum*, *Mollugo verticillata* (carpetweed), *Raphanus raphanistrum* (wild radish), *Richardia scabra* (Florida pusley), *Conyza canadensis* (horseweed), and *Desmonium tortuosum* (Florida beggarweed) tested positive for the mixed infections. Since weeds are likely to be the main reservoir for tospoviruses and their thrips vectors, finding TSWV and INSV in weeds could explain recent increases of these dual infections in susceptible field crops.

Characterization of pepper anthracnose isolates from the eastern shore of Virginia and eastern U.S. J. K. MARVEL (1), S. A. Alexander (1), and E. L. Stromberg (2). (1) Dept. of Plant Pathology, Physiology & Weed Science, Eastern Shore AREC, VPI & SU, Painter, VA 23420; (2) Dept. of Plant Pathology, Physiology, and Weed Science, VPI & SU, Blacksburg, VA 24060. Phytopathology 93:S58. Publication no. P-2003-0422-AMA.

The economic impact of pepper anthracnose has increased on the Eastern Shore of Virginia. The species identification of the causal agent(s) is in question. Isolates collected from the Eastern Shore, as well as regionally from pepper, have large differences in colony characteristics. Type cultures from different collections exhibited a high degree of species specificity, and did not amplify any of the 27 other *Phytophthora* species evaluated thus far. Some aspects of this marker system have been modified for use with the TagMan real-time PCR system.


*Ralstonia solanacearum* (Rs) can cause severe losses in potato and tomato in some regions of Russia. To identify the race and biovar (bv), over 100 suspect cultures of Rs were isolated from diseased potato plants from the American Type Culture Collection were also analyzed. Traditional colony characteristics (i.e. color, texture, and size) and conidial characteristics (i.e. shape and size) were used to distinguish isolates. Additionally, Biolog™ profiles and virulence on pepper fruit were used for delineation of isolates. Differences among pepper anthracnose isolates were observed and reported.


Powdery mildew on lettuce, caused by *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*), can appear as early as December and January and develop rapidly during March and April in western Arizona. Few fungicides are registered for control of the disease on lettuce; therefore, the efficacy of several compounds was evaluated in the field from 2000 to 2002. During this 3-year period, disease reduction at crop maturity of at least 90%, compared to nontreated plants, was attained by applications of myclobutanil, quinoxyfen, trifloxystrobin, and wettable sulfur. In the same field trials, acibenzolar-S-methyl, azoxystrobin, Millsana, potassium bicarbonate and pyraclostrobin reduced disease severity from 49 to 70%. Fungicides applied for control of downy mildew, including cymoxanil, cymoxanil+fomoxadone, dimethomorph, fosetyl-Al, maneb, and zoxamide, reduced powdery mildew from 20 to 71%. Alternating applications of azoxystrobin with acibenzolar-S-methyl, but not with potassium bicarbonate, increased the level of disease suppression above that of the individual products alone.


*Pseudomonas fluorescens* spp. that produce 2,4-diacetylphloroglucinol (DAPG) suppress a wide variety of diseases caused by soilborne plant pathogens. Strain Q8r1-96 is representative of certain DAPG producers that aggressively establish and maintain large populations on the roots of wheat and pea. We are evaluating key genes involved in gene regulation and microbe-plant interactions for their role in the unique competitiveness of
Q8r1-96. Three genes, sss recombinase, dsbA, and ptsP, influencing global processes including phenotypic plasticity, secretion, and organic nitrogen utilization, respectively, have been identified in a Q8r1-96 genomic library and are being assessed in vitro and in greenhouse studies for their contribution to the colonization and persistence of Q8r1-96 in the rhizosphere.

In-field detection of citrus canker. V. MAVRODIEVA (1), T. Riley (2), L. Levy (1), and D. Gabriel (3). (1) APHIS, PPQ, CCEP, NPGBL Beltsville, MD 20705; (2) APHIS, PPQ, CCEP, Orlando FL; (3) Plant Pathology Dept., University of Florida, Gainesville, FL 32611. Phytopathology 93:S59. Publication no. P-2003-0426-AMA.

Citrus bacterial canker, one of the most economically important diseases on citrus worldwide, is caused by at least three phylogenetically distinct groups of *Xanthomonas citri*. Since its last introduction in 1995 the disease is under eradication with successful eradication and disease control, and prevention of new introductions require reliable and rapid diagnosis of all citrus causing strains of *X. citri*. Previously we described a pair of universal primers used in a real-time PCR and simple methods of sample preparation for citrus canker detection. Here we report the results of the extensive field testing of the IT 1-2-3.R.A.P.I.D. DNA Purification kit (Idaho Technology). Samples were collected directly in the field by swabbing leaf/fruitleaves and shoots to the diagnostic lab. Following rapid DNA extraction and PCR, Green real-time PCR assay was carried out to detect citrus canker. Samples of different age and condition were tested and citrus canker was detected with very good accuracy and reproducibility.


BAC libraries have been instrumental for physical mapping and understanding the organization of the genome of the bacterial-feeding nematode, *Caenorhabditis elegans*. BAC libraries for plant-parasitic nematodes have not been documented and they could provide immense utility for comparison to *C. elegans* and for investigating adaptations for a parasitic lifestyle. The beta-1,4 endoglucanase genes of cyst nematodes appear to be clustered and are not found in the genome of *C. elegans*. It is unknown if parasitism genes within nematodes exist as “pathogenicity islands” since large clones of genomic DNA nor physical genetic maps of plant-parasitic nematodes exist. We are attempting to construct high-quality (>100 kb average insert) BAC libraries from the cyst nematode species *Heterodera glycines* and *H. schachtii*. A modification of a protocol that digests whole parasites and avoids nucleic preparations to obtain high molecular weight DNA appears to be successful. We are working on developing a rapid DNA extraction and PCR assay to develop high-quality BAC libraries with appropriate genome coverage is being investigated.


We isolated a soybean cDNA whose transcript abundance is increased one day post inoculation (dpi) with the soybean cyst nematode, *Heterodera glycines*, and whose predicted gene product shares high similarity to phosphoglycerate mutases, enzymes in the glycolysis/gluconeogenesis pathway. We designated the gene GmPgm. RNA blot revealed that GmPgm mRNA increased following *H. glycines* infection up to 7 dpi. We identified the cloned gene as *Arabidopsis* gene, AtPgm. Transgenic *Arabidopsis* containing the AtPgm promoter–GUS construct revealed GUS expression only in the shoot and root apical meristems and elevated promoter activity following auxin treatment. The AtPgm promoter also directed GUS expression to the nematode feeding cells induced by *H. schachtii* and *Meloidogyne incognita* up to 14 dpi. Assessment of the Aux1 promoter similarly revealed auxin-induction and feeding cell expression. We suggest that phosphoglycerate mutase plays a role in nematode feeding cells.

Expression of *GmEREBP1* in soybean and *Arabidopsis* induces expression of defense-related genes. M. Mazaire (1), A. A. Elling (1), T. R. MAIER (1), S. R. Rodermel (2), and T. J. Baum (1). (1) Department of Plant Pathology; (2) Department of Botany, Iowa State University, Ames, IA 50011. Phytopathology 93:S59. Publication no. P-2003-0424-AMA.

Ethylene-responsive element binding proteins (EREBPs) are plant-specific transcription factors, many of which have been linked to plant defense responses. Conserved EREBP domains bind to the GCC box, a promoter element found in many pathogenesis-related (PR) genes. We previously reported identification of an EREBP gene from soybean (*GmEREBP1*) whose transcript abundance decreased in soybean cyst nematode-infected susceptible roots, whereas it increased in abundance in infected resistant roots (MPME: 15, 577-586; 2002). Here we report further characterization of this gene. Expression of GmEREBP1-GUS-GFP translational fusions showed that the GmEREBP1 protein was targeted to the plant nucleus. Furthermore, transgenic soybean and *Arabidopsis* plants expressing GmEREBP1 showed elevated mRNA abundance of defense-related gene *PR1* but not *PR5* in soybean, whereas it induced the expression of *PDF1.2* and *Thi2.1* but not *Gib* in *Arabidopsis*. These results suggest that GmEREBP1 functions as a positive trans-acting factor of defense gene expression.

Characterization of *Fusarium oxysporum* f. sp. *lactucae* based upon sequence of the translation elongation factor 1 alpha (EF-1alpha), the mitochondrial small subunit (mtSSU) and the intergenic spacer (IGS) rDNA. G. M. MBOFUNG and B. M. Pryor. Dept. of Plant Pathology, University of Arizona, Tucson, AZ. Phytopathology 93:S59. Publication no. P-2003-0430-AMA.

Fusarium wilt, caused by *F. oxysporum* f. sp. *lactucae*, is a very destructive disease of lettuce and has recently been reported in Arizona and California. In this study, the IGS region, mtSSU and the EF-1alpha gene sequences were determined for *Fusarium* isolates recovered from diseased lettuce plants, other infected crops, and field soil. Phylogenetic analysis of the mtSSU sequences resulted in low resolution among isolates tested. Analysis of the EF-1alpha sequences resulted in higher phylogenetic resolution but grouped the form species *albedinis* with the *lactucae* isolates. Analysis of the IGS sequences revealed that the *lactucae* isolates constitute a closely related monophyletic group distinguishable from the other crop formae speciales and the non-pathogenic soil isolates. The arrangement of repeat subunits within the IGS region and its implication in identifying virulent and pathogenic isolates from lettuce using PCR-based detection methods is discussed.


Forty-three isolates of *Aspergillus caelatus* whose vegetative compatibility groups (VCGs) have been identified were assessed by DNA fingerprinting using a repetitive sequence DNA probe pAF28 cloned from *A. flavus*. Thirteen distinct DNA fingerprint groups or genotypes were identified among the 43 isolates. Twenty-four isolates belonging to VCG 1 produced identical DNA fingerprints and included isolates from the United States and Japan. Four other DNA fingerprint groups had multiple isolates sharing identical fingerprints corresponding to VCGs 2, 3, 12 and 13. Eight of the 13 fingerprint groups corresponding to VCGs 4-11 were represented by a single isolate with a unique fingerprint pattern. These results provide further confirmation that the pAF28 probe can distinguish VCGs of species within *Aspergillus* section Flavi based on DNA fingerprint patterns and that the probe can be used for distinguishing the number of the sample population. Most of the *A. caelatus* isolates produced fewer restriction fragments and weakly hybridized with the repetitive DNA probe pAF28 compared to hybridization patterns obtained with *A. flavus*, suggesting less homology of the probe to *A. caelatus* genomic DNA.


To determine if resistance to strobilurin fungicides was present in the *Podosphaera xanthii* population in a research field planted to pumpkin, azoxystrobin formulated as Quadris was used alone on a weekly schedule for one treatment in a fungicide efficacy experiment in 2002. Other fungicides used with Quadris in a program designed for managing resistance, as recommended for production fields, might provide enough control of powdery mildew to obscure the presence of strobilurin-resistant strains, especially if they were at a low frequency. Control of powdery mildew on upper/lower leaf surfaces based on AUDPC values was 41%/49% for Quadris and 73%/44% for Quadris alternated with myclobutanil (Nova) + chlorothalonil (Bravo). Four of nine isolates obtained from Quadris-treated leaves were highly resistant to strobilurins in a laboratory assay (able to grow within four days at 0 ppm trifloxystrobin). Quadris applied in alternation with Nova + Bravo resulted in better control in previous experiments at this site: 95%/72% in 2001, 100%/98% in 2000, 100%/84% in 1999, and 93%/58% in 1998.

Little is known about the pattern of development of yeast-like fungi on leaves. A model system consisting of detached apple leaves incubated in a moist chamber and GFP-marked A. pullulans, was used to follow colony formation from a single cell placed on mid-veinal or non-veinal sites. On mid-veinal sites, the cell divided 2-fold, and apparent binary fission occurred in about 50% of initial cells. Initial cell(s) budded off >10 blastospores from the poles over 24-30 hours. Colony expansion continued when newly budded cells swelled. After 20 hours, some cells budded off progeny from the poles. Newly formed cells occasionally budded off 1-2 cells. Budding mainly occurred at the margin of the expanding colony. As the colony size remained constant, the colony size at 72 hours averaged 17 cells. At non-veinal sites, the initial cell swelled only slightly and tended to bud off only a few cells from its poles. Only rarely did the secondary cells bud. The colony size at 72 hours averaged 3 cells. These results may explain why colonies on field leaves are found primarily along veins.


Field surveys of hard red spring and durum wheat conducted from 1998-2002 determined previous crop history and prevalence (% symptomatic fields), incidence (% symptomatic tillers) and leaf or head severity (% area symptomatic) of tan spot (Pyrenophora tritici-repentis), the Septoria leaf blotch complex and Fusarium head blight (FHB). Crop rotations were surveyed throughout the growing season. The previous year’s crop was identified as small grains, broadleaf crops, or other. The number of fields surveyed with known previous crop ranged from 500 to 1100 per year. Small grains were the previous crop for wheat in 50 to 58% of the fields each year. Tan spot was the most prevalent disease over all fields and years, and its prevalence and incidence were significantly higher if fields were planted to small grains the previous season. FHB was the most severe disease in 3 of 5 years, and its incidence and severity were higher (P = 0.05 and P = 0.10, respectively) with small grains as the previous crop. The Septoria leaf blotch complex was not impacted by previous crop.


Bacterial fruit blotch of watermelon (BFB) is an important disease caused by Acidovorax avenue subsp. citrulli (Aac). Since the pathogen is seed transmitted, the potential of biological seed treatments to reduce seedborne populations of Aac and seed transmission was investigated. Through in vitro assays for antagonistic activity against Aac, Pseudomonas fluorescens A506, A. avenue subsp. avenue BC2 and Flavimonas oryzihabitans BCM202-3 were selected as potential biological control agents. These bacterial strains were evaluated for their in vitro biocontrol efficacy using watermelon seeds artificially infested with Aac. Of the strains tested, Pseudomonas fluorescens A506 demonstrated the highest level of disease suppression. Similar levels of control were observed for naturally infested watermelon seeds generated by blossom inoculation with Aac. While complete control was not achieved, these findings suggest that biocontrol seed treatments may contribute to an integrated pest management program for BFB.


Aspergillus flavus reduces cottonseed value by producing aflatoxins during seed infection. Xyloglucans are plant-derived polysaccharides critical to the structural integrity of the host cell wall. A. flavus AF36 was found to secrete a xylanase activity when grown on a medium containing xylan as a sole carbon source. Enzyme activity was assayed with xylan conjugated to Remazol Brilliant Blue R. A survey of closely related fungi revealed similar xylanases from A. oryzae, A. parasiticus, A. nomius, and A. tamauri, but not from A. sojae. This xylanase was partially thermostable up to 60°C and tolerant of a wide pH range (5.0-8.0) with no optimum. A concentrated sample of the AF36 xylanase activity was subjected to gel filtration chromatography on a P-60 Superose column. A small peak eluting with a peak of xylanase activity eluted very close to the included volume of the column. Data suggest this hydrolytic activity is associated with a low molecular weight (≤20 kDa) thermostable protein, and is consistent with a highly mobile, stable plant cell wall degrading factor.

Reaction of peanut genotypes to southern blight under field conditions. H. A. MELOUK (1), B. A. Besler (2), W. J. Gričar (2), and R. N. Pittman (3). (1) USDA-ARS, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078; (2) Texas Agricultural Experiment Station, Yoakum, TX 77995; (3) USDA-ARS, Georgia Experiment Station, Griffin, GA 30223. Phytopathology 93:S60. Publication no. P-2003-0439-AMA.

Six peanut (Arachis hypogaea L.) genotypes of a Bolivian origin (PI 339967, 475971, 475972, 497412, 540886, and Bayo Grande) and two runner cultivars (Okrun and Tamrun 96) were evaluated in replicated field plots at Yoakum, TX. For their reaction to southern blight, two release classes were evaluated: (1) plants with a severe southern blight pressure in 2000 and 2001. Sclerotial density of Sclerotinia rolfsii was 2-3 viable sclerotia/225 g soil (Tremona loamy fine sand). There was a treatment by year interaction, and therefore, data from the 2000 and 2001 experiments were subjected separately to analysis of variance. In 2000, all genotypes had significantly (P = 0.05) lower southern blight disease incidence than Okrun, with 13.0 loci/plot. In 2001, PI 339967, 475972, 540886, and Bayo Grande, and Tamrun 96 had significantly (P≤0.05) lower disease incidence than Okrun, with 13.8 loci/plot. These data confirm that at least four genotypes have useful resistance to S. rolfsii.

Strains of Trichoderma virens over expressing gene pairs for enhanced biocontrol activity. A. MENDOZA-HERRERA and C. Kenerley. Dept. of Plant Pathology & Microbiology, Texas A&M University, College Station, TX. Phytopathology 93:S60. Publication no. P-2003-0440-AMA.

An arginine auxotrophic strain of T. virens was co-transformed with three different pairs of genes that encode for hydrolitic enzymes under control of constitutive promoters. The target genes co-transformed were tv-ech1...
(endochitinase), Tvs-bng1 (beta-1,3 glucanase) and tvsp1 (serine protease) in the following pairs: Tvs-ech1 plus Tvs-bng1; Tvs-bng1 plus tvsp1 and Tvs-ech1 plus tvsp1. Stable transformants were assayed for individual enzymatic activity under non-inducing conditions, and the presence of each introduced gene was confirmed by Southern blot analysis. The expression of each introduced gene pair was demonstrated by Northern analysis following incubation for 6 and 24 hrs in non-inducing medium. Our interest is to determine whether over-expressing gene pairs in a single strain is more effective for biocontrol than the over-expression of individual genes (e.g. Tvs-ech1 plus tvsp1 vs. Tvs-ech1 or tvsp1) or where two strains each over-expressing a single gene are combined as a mixture.


Chemical seed treatments have been widely used to control early season corn fields, experiments were conducted at Crawfordsville and Johnston, IA in 2001 and 2002. Seed insecticides Gauch® or Cruiser®, and the foliar insecticide Warrior® were evaluated. Applications of Warrior® were timed according to corn growth stage, a degree-day model, or an action threshold based on beetle numbers. Combinations of seed treatments with Warrior® reduced disease incidence by 42.0% and 39.9% in 2001 and by 66.7% and 56.3% in 2002, compared to the nontreated control. These reductions, however, were not significantly different than Gauch® or Cruiser® used alone ($p < 0.05$). Therefore, applying foliar insecticides in addition to insecticide seed treatments may not further reduce incidence of Stewart’s disease of corn.


Charcoal rot [Macrophomina phaseolina (Tassi) Goidl] of soybean [Glycine max (L.) Merr.] is a disease of economic significance in the United States causing significant yield losses. In 2002, 24 soybean genotypes in maturity groups 3, 4 and 5 were evaluated using five methods of disease assessments: 1. Internal stem discoloration (PBSD), 2. Colony forming units (CFU), 3. foliar symptoms, 4. Area under disease progress curve for foliar symptoms (AUDPC) and 5. Severity based on the intensity of internal stem discoloration. Linear regression of disease assessment as a function of severity based on the intensity of internal stem severity was significantly correlated ($r^2 = 0.559, P > 0.0001$) with the CFU. However, when all disease measurements were combined the regression trend improved significantly ($r^2 = 0.85, P > 0.0001$). Genotypes that ranked high for resistance as measured by CFU also ranked high using combined measurements of severity, PHSD, foliar and AUDPC. Such methodology for measurement of resistance is more adaptable than quantities used in other studies. Additional studies are planned to conduct field observations and also develop laboratory or greenhouse assays to assess the reaction of soybean genotypes to charcoal rot.

Control of green mold and sour rot of lemons and gray mold rot of grapes by biofumigation with Muscodor albus. J. MERCIER (1) and J. L. Smilanick (2). (1) AgraQuest Inc., Davis, CA 95616; (2) USDA-ARS Parlier, CA 93648. Phytopathology 93:S61. Publication no. P-2003-0443-AMA.

The biofumigant fungus Muscodor albus produces antimicrobial volatiles that inhibited and killed potato dextrose agar colonies of Pantoea stewartii, which cause gray mold and sour rot of citrus, respectively. Biofumigation of lemons with M. albus was attempted to control these diseases. Wound-inoculated fruits were placed in closed plastic boxes at ambient temperature in the presence of rye grain cultures of M. albus for periods ranging from 24 h to 5 days. Biofumigation with M. albus for 24 h provided nearly complete control of green mold among grapes and with $10^5$ conidia/ml, weather fungmination was performed immediately or 24 h after inoculation, while the incidence of green mold among untreated fruits was more than 75%. In addition, sour rot incidence was significantly reduced by biofumigation for periods of 48 h or longer, under moderate or high disease pressure. Fumigation of grapes was also attempted to control gray mold caused by Botrytis cinerea, another fungus killed by M. albus volatiles. Grape bunches sprayed with B. cinerea conidial suspensions and fumigated with M. albus at 2, 5, 10, and 20°C for the duration of the storage period. There was good control of gray mold at all temperatures tested.


The hrp type III secretion and effector genes in Pantoaea stewartii subsp. stewartii are regulated by the HrpX/Y two component regulatory system, the HrpS transcriptional enhancer, the HrpL alternative sigma factor and various regulatory regions. Using genetic approaches we have found that HrpY, HrpS and HrpL act in the order listed at the transcriptional level to form a regulatory pathway. At the end of the cascade, HrpL is required for expression of Hrp box promoters. In this study, we found that purified, unphosphorylated HrpY bound specifically to the regulatory region of hrfS, hrlp and hrlpX in gel retardation assays. In vitro phosphorylation of the receptor domain of HrpY increased its binding affinity to Hrp box promoter fragments, but not for hrlp or hrlpXY promoter fragments. This supports our previous finding that overexpression of HrpY represses the basal level of hrlp. We have now used primer extension to show that the basal expression of hrlp may be due to a sigma-70 promoter located just upstream of the HrpS-dependent sigma-54 box.


Petioles of field-grown ‘Camarosa’ strawberry plants were evaluated for latent infections of Colletotrichum acutatum during the 2000-01, 2001-02, and 2002-03 winter growing seasons in Florida. Petioles were sampled monthly from Oct to Mar in 14- to 20-plant plots sprayed weekly with captan, thiram, or an untreated control. C. acutatum, the causal agent of anthracnose fruit rot (AFR), was detected on petioles in 2001-02 and 2002-03; AFR was observed 1-2 months later during early harvests. In 2001-02, petiole colonization by C. acutatum averaged 15, 40, and 76% in the captan, thiram, and control treatments, respectively, which correlated well with AFR incidence (45, 57, and 81%) in the respective treatments. Similar trends are being observed in the ongoing 2002-03 experiment. C. acutatum was not detected on petioles in 2000-01, and fruit lesions rarely developed. These data suggest that strawberry foliage can be infected by C. acutatum in the nursery, and may be a source of primary inoculum for AFR epidemics in newly planted fields. Foliar (petiole) infections are dramatically reduced by regular applications of captan.

A new species of Moniliaceae infesting Meloidogyne arenaria. S. L. F. MEYER (1), L. K. Carta (1), and S. A. Rehner (2). (1) USDA-ARS Nematology Laboratory, Beltsville, MD 20705; (2) USDA-ARS Insect Biocontrol Laboratory, Beltsville, MD 20705. Phytopathology 93:S61. Publication no. P-2003-0446-AMA.

A nematode-trapping fungus was isolated from cultures of the plant-parasitic nematode Meloidogyne arenaria. The nematode cultures had been maintained on tomato plants in the Beltsville, Maryland, Nematology Laboratory greenhouse, and the M. arenaria population numbers in the pots were lower than expected. Consequently, fungi were isolated from the suppressed nematode population. Fungal isolations from second-stage juvenile containing chlamydospores yielded a new species in the Moniliaceae. The new species produces smooth-walled chlamydospores, stalked adhesive pads, and anastomosing, non-constricting hyphal rings. Conidia are borne on unbranched conidiophores; the conidia each have 3 to 5 septa, and are asymmetrical and spindle-shaped, with one end mamilliform to elongated-conoid and the other end rounded to conoid. Morphological and ITS/DNA comparisons with published species descriptions indicate that this is a previously undescribed species.


Four tree-killing freezes from 1951 to 1989 drastically reduced the commercial citrus acreage in the Lower Rio Grande Valley of Texas. These freezes reduced the incidence of trees with psorosis disease. The development of a new (psorosis-free) grapefruit cultivar, ‘Rio Red’ also helped to reduce the overall incidence of psorosis in new orchards. In the past 6 years, we have observed an increase in field symptoms of psorosis disease. In studies with 2,513 grapefruit trees, obvious psorosis incidence exceeded more than expected. Consequently, fungi were isolated from the suppressed nematode population. Fungal isolations from second-stage juveniles containing chlamydospores yielded a new species in the Moniliaceae. The new species produces smooth-walled chlamydospores, stalked adhesive pads, and anastomosing, non-constricting hyphal rings. Conidia are borne on unbranched conidiophores; the conidia each have 3 to 5 septa, and are asymmetrical and spindle-shaped, with one end mamilliform to elongated-conoid and the other end rounded to conoid. Morphological and ITS/DNA comparisons with published species descriptions indicate that this is a previously undescribed species.
orchiord indicates a possible natural spread of this disease. A related virus is known to be spread by fungus Ophiopsis. Examination and sectioning of feeder roots from symptomatic citrus trees and culturing of soil with baits showed the presence of Ophiopsis-like fungus with resting spores. The Ophiopsis-like fungus of feeder roots of symptomatic citrus trees strengthens the hypothesis of a natural transmission of Citrus psorosis virus. Verification of the virus-vector relationship needs to be established.

Soybean viruses distribution in Ontario. R. Michelutti (1), A. TENUTA (2), T. Anderson (1), and T. Welacky (1). (1) Greenhouse and Processing Crops Research Centre (GPCCR), Agriculture and Agri-Food Canada (AAFC), 2585 County Road 20, Harrow, Ontario, N0R 1G8; (2) Ontario Ministry of Agriculture and Food (OMAF), Main Street, Agronomy Building, Ridgetown College, P.O. Box 400, Ridgetown, Ontario, N0P 2C0. Phytopathology 93:S62. Publication no. P-2003-0449-AMA.

Bean pod mottle virus (BPMV), soybean mosaic virus (SMV), alfalfa mosaic virus (AMV), and tobacco ringspot virus (TRSV) have caused significant losses to producers and the soybean industry in North America by reducing seed weight and quality, increasing costs associated with harvest difficulty, and the involvement of these viruses in the phenomenon known as "Soybean Green Stem". In Ontario soybean viruses often go undetected or are misdiagnosed for other causes. Another concern is the cost and time associated with submitting samples for laboratory analysis limits producer and industry participation. A soybean virus distribution survey was initiated by AAFC and OMAF to increase awareness amongst producers and the Ontario soybean industry of the extent of virus infection. All soybean producers growing soybeans in Ontario were sampled for SMV, BPMV and TRSV in 2001 and 2002 whereas, AMV testing only occurred in 2002. A composite sample of 35 plants from each field was taken. SMV and BPMV were detected in only two breeder nurseries in south-western Ontario in 2001, as compared to 13 grower fields and 20 grower fields respectively in 2002. TRSV was found in two grower fields in 2001 and in 16 and 2 AMV was detected in 15 grower fields in 2002. Although the overall incidence was low in both years, soybean virus infection can have a significant economic impact on food grade and specialty soybean production in the province.


Panicle and shoot blight, caused by Botryosphaeria dothidea, is a destructive disease of pistachios in California. Between 1996 and 1999, the incidence of pistachio blights infected with B. dothidea was recorded in several pistachio orchards in February and March. The number of blighted fruit clusters and shoots was also recorded in the same orchards in late summer before harvest. Four classes of Botryosphaeria risk (low, low to moderate, moderate to high, and high) were defined from the number of blighted clusters and shoots. The incidence of infected buds in early spring, the temperature and precipitation from March through May, were used as independent variables and estimate disease risk in the recorded fields. Uncertainty about the estimated risk was addressed with Bayesian methods. 20 clusters were examined, where p is the number of inputs. Estimated risk using the best fitted model differed from the observed disease risk only in 7% of the fields. The system is currently under validation.


Bacterial spot of tomato, incited by Xanthomonas campestris pv. vesicatoria, previously consisted of three races designated T1, T2 and T3, which represent genotypic groups A, B and C, respectively. T1 strains contain avrXv3 and avrXv4, which interact similarly with tomato plants containing Xv3 and Xv4. In 1998 several tomato strains were isolated which behaved as T2 strains on tomato differentials. The bacterial strains were identified as T3 strains based on PCR analysis of conserved regions. PCR amplification and sequence analysis of the avrXv3 genes resulted in one strain having an in-frame stop codon, and another containing an insertion element. These suscetible-inferior race T4 based on the compatible and incompatible interaction when inoculated onto tomato genotypes containing the Xv3 and Xv4, respectively. Strains collected in 2002 were also identified with two distinct insertion elements in the avrXv3 and avrXv4 and were designated as race T5.


A. tumefaciens Mediated Transformation (ATMT) is an efficient tool for inserting heterologous DNA into the genomes of diverse plants and fungi. For fungi, assexual spores are typically used for transformation. Here we describe an ATMT system designed specifically for Ophiopsis herpotricha, the causal agent of spring dead spot of Bermudagrass. Spring dead spot is one of the most important diseases of Bermudagrass. The anamorph of O. herpotricha does not produce asexual spores in culture and its hyphae are recalcitrant to cell wall degrading enzymes used to generate protoplasts. A modified ATMT system was used to transform mycelial fragments with the hygromycin B resistance gene contained on the Ti plasmid. Transformation was verified via PCR of the hygromycin B resistance gene from genomic DNA. This research exemplifies the utility of ATMT with mycelial fragments as an effective way transform genes into fungi that do not lend themselves to conventional techniques.


Alternaria brassicicola, the causal agent of black spot disease, is a major pest and post harvest problem on most cruciferous crop plants. A NSF funded project was launched to use genomic approaches to elucidate genes that contribute to fungal pathogenicity as well as resistance and susceptibility in A. brassicicola, cabbages and mustard. Using a non-directed approach, we are generating and screening >5,000 fungal mutants via random integration of heterologous DNA to identify genes required for sporulation and pathogenicity. Coordinately, we used a directed design to identify target genes expressed during the host-pathogen interaction by sequencing >6,000 ESTs from a suppression subtractive hybridization cDNA library made from Alternaria infected cabbage tissue. This talk will highlight the analysis and functional binning of the fungal and plant ESTs and list target fungal genes putatively involved in pathogenicity and toxin production.


Late season spray program for reducing primary inoculum in powdery mildew disease management in dogwood. M. T. MMBA2A. Tennessee State University, Nursery Crop Research Station, 472 Cadillac Lane, McMinnville, TN. Phytopathology 93:S62. Publication no. P-2003-0454-AMA.

A 3-year study has shown that early termination of fungicide applications in powdery mildew control in dogwood favor ascospore abundance and source of primary inoculum for the following spring. Applications of propiconazole (Benlate 2.5EC) and thiophanate methyl (Clear Sky 330G F) in late season spray program (September-October) reduced the number of ascospores by 91-92% in 2000; 59-68% in 2001 and 87-90% in 2002 compared to the early termination in August. Household soaps, previously selected for their effectiveness in controlling powdery mildew were also highly effective in reducing ascospore quantity. Ajax reduced ascospore abundance by 59.9-99.8% and Palmolive reduced ascospore quantity by 78-83.9% in 2001 and 2002. Late season spray program that targeted the immature ascospores
A total of 58 commercial ascensions of lilacs were evaluated for resistance to three diseases: powdery mildew (Microsphaera syringae), bacterial blight (Pseudomonas syringae) and alternaria blight (Alternaria sp.) for four-years. Of the 58 cultivars, 41 were of Syringa vulgaris, 4 of S. prestoniae, 4 of S. hyacinthiflora, 2 of S. josifolia, 2 of S. meyeri, 1 of S. reticulata, 1 of S. patula, 1 of S. chinensis, and 1 of S. microphylla. Bacterial blight was restricted to early spring wet and cool weather (April and May), but powdery mildew started in mid-June and spread quickly to cover most of plant foliage, it also persisted throughout to the end of the season. Alternaria blight started in late June and caused severe leaf scorching with tan to dark brown necrotic lesions with a fine powdery appearance. Although the association of Alternaria and species with lilac has been reported, Alternaria spp. are often regarded as secondary pathogens in tree plants. However, the Alternaria sp. in lilac was clearly a primary pathogen causing severe damage to plant foliage and significantly reducing the aesthetic appearance of infected plants. The pathogen seemed to produce a toxin as a mode of action. Cultivars that were resistant to powdery mildew and bacterial blight were often susceptible to alternaria blight. A total of 20 cultivars displayed some resistance to powdery mildew and bacterial blight while 10 were resistant to powdery mildew and alternaria blight; only 8 cultivars displayed resistance to all three diseases. Three cultivars, S. meyeri, ‘Dwarf Korean’, and ‘Palibini’ and S. vulgaris ‘Paul Thirion’ exhibited the highest level of resistance to all three diseases throughout this study.


SVD has recently been successfully applied to a phylogenetic analysis of the family Tombusviridae. This method defines well-conserved amino acid motifs within whole genome protein sequences as linear combinations of short peptides, separated by any number of amino acids. We are analyzing 81 sequences for spherical ssRNA plant viruses. There is substantial agreement between the phylogenetic relationships observed with this method and the current taxonomic classifications for these viruses. For example, the genera Tombusvirus and Diaionovirus within the family Tombusviridae consistently held together within the tree. However, some novel relationships were noted: three necroviruses, beet black scorch, tobacco necrosis D, and leek white stripe, failed to group with the other two necroviruses, tobacco necrosis A, and olive latent-1 in all variations of the method. Viruses of the genera Marafivirus and Tymovirus were seen to form a single clade, and single virus genera were inconsistent in their placement within the tree. Potential reassignments, hypothetical clades, and the results at variance with current taxonomy will be discussed.


Greasy spot, caused by Mycosphaerella citri, produces leaf spots, premature defoliation, and reduces plant vigor and fruit yield. Ascosporae produced in pseudothecia in decomposing leaf litter serve as the main source of inoculum. Mating behavior and pseudothecial ontogeny of the pathogen were investigated. Classical crosses in leaf disks in culture were made among 40 single ascosporae isolates (SAI). Crosses among the same SAI did not produce ascosporae in pseudothecia indicating that Mycosphaerella citri is heterothallic. Among 40 crosses with SAI-01 and with SAI-02, respectively, 17 and 13 crosses produced pseudothecia and viable ascosporae suggesting that the pathogen required two mating types to produce ascosporae. In studies of ontogeny of pseudothecial development, 7 distinct stages such as

stromatic pseudothecial initials, formation of ascogonia, lumen initials, initials of asci and pseudosporophyses, asci with immature ascospores, mature ascus with ascosporae, and pseudothecia after discharge of ascospores were identified. The duration required for the different stages of pseudothecial ontogeny varied based on environmental conditions.


Rind blotch, caused by Mycosphaerella citri, produces a hundreds of minute necrotic, black specks on fruit surface resulting unsightly blemishes. Ascosporae are deposited on the fruit surface where they germinate and grow epiphytically before invasion through stomates. Epidemiology of rind blotch was studied under different environmental conditions in the laboratory and disease were studied. The number of ascosporae deposited on the fruit surface corresponded with the number in the air. Frequent rain and humid conditions in July and August resulted peak epiphytic mycelial growth of the pathogen on the fruit surface. Rind blotch appeared in November. A single spray in either May, June, or July reduced epiphytic mycelial growth, but did not reduce rind blotch incidence and severity. Two sprays, one in June and another in July, reduced epiphytic mycelial growth and rind blotch severity. However, a program of four sprays applied once a month from May to August was the most effective in reducing epiphytic mycelial growth, rind blotch incidence, and severity.


Melanose, caused by Diaportha citri, affects leaves, twigs and fruit of all citrus species, but grapefruit and lemons are more severely affected. Conidia produced in pycnidia in dead twigs serve as the major source of inoculum. In the laboratory, the effect of temperature, wetting and drying, twig age, and melanose severity on infected grapefruit twigs on the production of pycnidia was studied. The optimum temperature for pycnidial development was 24-26°C. Pycnidial production was abundant when melanose-infected twigs were soaked 4 h per day, 3 days per week for 3-10 weeks. Production was greatest on the youngest twigs infected with melanose. The more severe the melanose on twigs, the greater the production of pycnidia. Pycnidial formation was least on twigs of <2 mm diam and twigs of >7 mm diam, whereas its production was highest on twigs of 3-5 mm diam. In field studies, the number of pycnidia produced was highest when infected twigs were either May, June, or July, reduced epiphytic mycelial growth and rind blotch severity. Pycnidial production under field conditions was unrelated to temperature and total precipitation.


Efficacy of Dactylaria higginsi (DH) to control purple nutsedge (PN) in ‘Wizard’ bell pepper, as affected of the surfactants Natrosol (hydroxethyl cellulose, 0.5%) (HEC), PCC 588 (agricultural oil, 2%) (AO), Ivod (nonylphenol ethoxylate, 0.03%) (NEP), LI 700 (soy phospholipids + propionic acid, 0.25%), and Reddy It (polyethoxylated phosphate esters and amines + methylated seed oils, 0.75%) (PEP), was assessed. Weed-crop mixtures (1:5 ratio) were grown season-long (90 days) in 7.5 L-pots in a greenhouse. PN emerged 4 days after transplanting pepper. DH (1 × 10° conidia/ml) was sprayed over crop-weed canopy, 10 and 20 days after PN emergence. When DH was not applied, PN interference reduced crop yield by 85% as compared to weed-free pepper. DH caused 100% disease incidence in all treatments but did not kill PN. PN growth was reduced 53, 44, 22, 20, and 18%, respectively, when DH was applied with SPPA, PEP, AO, NPE, and AO. Crop yield-loss was 27, 30, 51, 54, and 58%, respectively, when DH was applied with SPPA, PEP, HEC, NPE, and AO.

Direct toxicity of fungicides to urediniumspores of daylily and geranium rusts. D. S. MUELLER (1), S. N. Jeffers (2), and J. W. Buck (1). (1) University of Georgia, Griffin, GA 30223 and (2) Clemson University, Dept. Plant Pathology and Physiology, Clemson, SC 29634. Phytopathology 93:563. Publication no. P-2003-0461-AMA.

The recent introduction and rapid spread of rust (Puccinia hemerocallidis) on daylilies suggested a need for treatments that kill spores on plant surfaces.
Azoxyostrobins, chlorothalonil, copper sulfate pentahydrate, iprodione, mancozeb, myclobutanil, propiconazole and triadimenol were evaluated for toxicity to urediniospores. Daylily rust urediniospores were suspended in surfactant, placed on filters on potato dextrose agar amended with each fungicide for 1d, rinsed and tested for germination. Geranium rust (P. pelargonii-zonale) urediniospores were applied dry to filters, sprayed with fungicides, incubated for 2d and tested for germination and ability to infect. Germination and infection of both rusts treated with azoxyostrobins, chlorothalonil, copper sulfate pentahydrate, and mancozeb was <1% compared to non-treated urediniospores.

Temperature effects on systemic infection of maize by Fusarium verticillioides. A. MURILLO-WILLIAMS (1), D. McGee (1), A. Wilke (2), and G. Munkvold (2). (1) Dept. of Plant Pathology, Iowa State Univ., Ames, IA 50011; (2) Pioneer Hi-Bred Int., Inc. Johnston, IA 50131. Phytopathology 93:S64. Publication no. P-2003-0462-AMA.

Fusarium verticillioides (F.v.) is important in maize production as it causes ear rot, stalk rot, and fumonisins accumulation in kernels. In this study we examined the effect of temperature on systemic infection of maize plants and kernels from seeds inoculated with F.v. Growth chambers were assigned temperature regimes (high, average and low) simulating temperatures that occur during spring growing season in Iowa. F.v. colonization, reproductive in stem internodes at all temperatures regimes. Recovery of F.v. declined acropetally from above-ground internodes at all temperature regimes through stage V6, and also at the low and average temperature regimes for stage VT. F.v. was found more frequently in the upper internodes of plants at the high temperature regime than at the low and average temperature regimes.

Proving synergy of co-infection of plant viruses: Cucumber mosaic virus (CMV) and Pepper mottle virus (PpmOV) in bell pepper. J. F. MURPHY and K. L. Bowen. Department of Entomology & Plant Pathology, Auburn University, AL 36849. Phytopathology 93:S64. Publication no. P-2003-0463-AMA.

A synergistic disease response to infection by two or more plant viruses is not uncommon, and we noted such a response in bell pepper to co-infection by CMV and PpmOV. However, while attempting to prove synergism, based on symptoms induced by each virus alone or the mixed inoculum, it was clear that a numerical rating scale could not be developed to accurately compare disease resulting from each of the three types of inocula. We therefore evaluated different parameters associated with each disease to determine if synergy occurred. Plant height (i.e., difference in stem height at harvest relative to initial height) was considered a suitable parameter. The observed response of the co-infection was greater than the expected response according to the Abbot method of estimating interactions, thus proving synergy. Virus titer did not differ between plants infected with each virus alone or CMV+PpmOV.

Selection and characterization of non-adherent mutants and variants of Spiroplasma citri BR3-3X. K. H. MUTAQIN (1), A. Wayadande (1), U. Melcher (2), and J. Fletcher (1). (1) Dept. of Entomology and Plant Pathology; (2) Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078. Phytopathology 93:S64. Publication no. P-2003-0464-AMA.

Evaluation of spiroplasma adherence to their insect vectors would be facilitated by having non-adhering mutants or variants. Electroporation-mediated transposon mutagenesis (EZ-TN™ < DHFR-1 > Tnp Transposome Kit, Epicentre®) was used to obtain mutants of Spiroplasma citri BR3-3X. Transformation efficiency averaged 2.88 × 10⁸ CFUs/µg transposon. PCR employing primers that amplify a gene for dihydrofolate reductase within the transposon indicated the presence of the transposon in the genome of some transformants. A number of putative, naturally occurring binding deficient variants of S. citri BR3-3X were obtained by incubating 10 consecutive passages on a monolayer of cells of the vector, Circulifer tenellus (CT-1). The number of unbound spiroplesma cells declined with each passage until the 8th to 10th passage after which it became constant at about 0.05% of the number of the cells initially applied. Transformants and natural variants will be screened for selection of non-adherent spiroplesmas using CT-1 monolayers.


The lipid envelope membrane of Tomato spotted wilt virus (TSWV) contains two surface glycoproteins G1 and G2. The lectin-binding properties and glycosidase sensitivities of G1 and G2 glycoproteins were compared to determine the oligosaccharides linked to these proteins. The viral proteins were separated by SDS-PAGE and probed by affinity blotting with a battery of biotinylated lectins with specificity for different oligosaccharide structures. Lectins with affinity to glucose, mannose, and N-acetylglucosamine bound to G1, whereas lectins with affinity to sialic acid and N-acetylglucosaminyl sulfate bound to G2. G1, but not G2, was deglycosylated when treated with endoglycosidase H or peptide-N-glycosidase F (PNGase F). Thus, only G1 protein of TSWV appears to be glycosylated with oligosaccharides N-linked to asparagine residues of the protein. Whether these results support a different role of the two glycoproteins of TSWV in virus-vector thrips interactions is being determined.


The Fusarium solani species complex is a highly diverse, cosmopolitan group of species lineages including a wide variety of plant and animal diseases. Tuber rot of caladium is the cause of serious declines in caladium tuber production in recent years. We investigated the evolutionary origins of F. solani isolates associated with caladium tuber rot outbreaks in Florida by using multi-locus phylogenetics. A portion of the beta tubulin (bna) and two clade-specific clade 3 elongation factor 1-alpha (ef1a) genes were sequenced from 69 isolates and a subset of 22 isolates were chosen to sequence the internal transcribed spacer regions (ITS) of the nuclear ribosomal RNA gene. A combined analysis showed two new clades associated with caladium within clade 3, the mostly northern hemisphere clade of the F. solani species complex. Correlation between morphology, phylogeny, and mating type will be discussed.


The reactions of three peanut genotypes (IC-10, IC-34, and ICGV 86388) to TSWV were evaluated by mechanical and thrips inoculation under greenhouse conditions, and compared to the reactions of cultivars CI1-2-39, Sunolec, and Georgia Green. Inoculum for mechanical inoculation was prepared by gridding leaves of TSWV-infected Emilia sonchifolia in phosphate buffer. For thrips transmission, larvae of Frankliniella fusca, were given a 24h access period. After inoculation was visually assessed using an index ranging from 0 (no symptom) to 4 (apical death). ELISA was used to confirm TSWV infection. The differential reaction of the peanut genotypes to mechanical and thrips inoculation will be presented.


Tomato spotted wilt virus (TSWV) significantly impacts peanut production in Georgia, with an estimated crop losses over $100 million. The development of virus resistant cultivars has been the aim for effective disease management. Transgenic peanut lines containing the non-structural protein gene (NSs) of TSWV from a TSWV isolate obtained from peanut were generated. Somatic embryos of peanut cultivar AT-120 were transformed using microprojectile bombardment, selected in liquid medium containing hygromycin and regenerated into plantlets. Plantlets with well-developed root systems were transferred to sterile medium for further development for four weeks, then acclimated to greenhouse conditions. Plants were screened for the NSs gene by PCR using a pair of primers (5' CAT GTC TTC AAG TGT TTA TGA GTC 3' and 5' CAG GAT ATT TGG ACG AAG CAT).
Dip inoculation technique to identify resistance to soybean sudden death syndrome. S. S. NAVI (1), R. Bandyopadhyay (2), R. P. THAKUR (3), X. B. Yang (1) and K. REDDY (3). (1) Iowa State University, Dept. of Plant Pathology, Ames, IA 50011; (2) IITA, PMB 5320, Ibadan, Nigeria; (3) ICRISAT, Patancheru, Andhra Pradesh 502 324, India. Phytopathology 93: S65. Publication no. P-2003-0469-AMA.

A technique to measure relative disease resistance of soybean varieties in greenhouse to Fusarium solani f. sp. glycines (FSG), was developed based on our finding that radicle infection is critical to FSG colonization in taproot. A precise relationship between inoculation and early infection was established. The technique involves: seed germination, dip inoculation of germinated seeds with 1–1/2 cm radicle in FSG spore suspension, transplanting and evaluation. Using this technique three experiments were conducted: (1) planting 2-variety in clay pots, (2) 12 varieties in cone cells and (3) 24 varieties in plastic cups. In all three experiments, inoculated seeds were transplanted in pre-irrigated containers filled with steam sterilized vertisol and a ratio (v/v) and on the top of it one inch height peat mix and organic soil 1:1 ratio (v/v). Plants were incubated in greenhouse at 20-22°C with 12 h light cycle for a month. Mean % incidence and severities differed significantly (P < 0.05%) among varieties. Method demonstrated no variation in symptom expression vs use of containers apart from consistency in repetition.

Effects of dew and post inoculation incubation temperatures on sorghum grain mold infection. S. S. Navi (1), R. Bandyopadhyay (2), R. P. Thakur (3), K. Reddy (3). (1) Iowa State University, Dept. of Plant Pathology, Ames, IA 50011; (2) IITA, PMB 5320, Ibadan, Nigeria; (3) ICRISAT, Patancheru, Andhra Pradesh 502 324, India. Phytopathology 93: S65. Publication no. P-2003-0470-AMA.

Studies were conducted at ICRISAT to determine effects of dew and temperature on inoculation of four major fungi: Curvularia lunata (CL), Cladosporium oxysporum (CO), Fusarium moniliforme (FM), and Phoma sorghina (PS). Panicles of sorghum accession IS 10513 were spray-inoculated separately with CL, CO, FM and PS at soft dough stage and were incubated in dew chambers for 24, 48 and 72 h. After air-drying, panicles were shifted to 3-growth chambers at 15, 20, 25°C night and 30°C day temperature regimes (TR) with 12 h light cycle for 7 days. After another 7-days incubation in greenhouse at 25°C, 50 grains from harvested panicle were plated onto PDA at 28±1°C and grain colonization was recorded. Significant (P < 0.05) effects of dew and TR and their interaction were observed. Infection among fungi varied with change in TR, indicating that individual fungi might have different windows for infection.


Mechanical devices that aid in the inoculation of plants are highly desirable when screening large numbers of plants for resistance to pathogens. We found a Fishman PF-8300 pneumatic dispensing system designed for industrial fluid applications to be highly useful for inoculating large numbers of soybeans with Phythophthora sojae in the greenhouse or laboratory. This unit utilizes various size syringes with Luer lock syringe barrels to dispense a variety of fluids or substances of gel or paste-like consistency. Needles of various sizes can be attached to the syringe and the contents are dispensed by a foot pedal or by a digital timer that activate the air pressure. The timer provides precise metering from 0.04 s to 3 min. Spore suspensions or pre-mashed agar + mycelium of a pathogen can be placed in the syringe, and injected into or onto plants. The air line pressure required to dispense a particular type of inoculum will vary, depending on viscosity and needle size. This system is easy to hold and operate over extended inoculation periods.


Infection of blueberry flowers by Monilinia vaccini-corymbosi (Mv), which causes muffy berry disease, involves conidial germination on the stigmatic surface followed by hyphal ingress into the stylar canal and subsequent colonization of the ovary. The extent to which these events mimic pollen-pistil interactions was investigated. Similar to blueberry pollen tubes, hyphae of Mv adhered selectively to imprints of stylar transmitting tract tissue on nitrocellulose membrane. By contrast, pollen from two other plant species failed to adhere to the imprints, whereas hyphae of M. fruticicola, which is non-pathogenic on blueberry, adhered indiscriminately. Microscopic observation of inoculated pistils showed that similar to pollen tubes, hyphae of Muc tracked the lobes of the stylar transmitting tract without branching, were surrounded by extracellular matrix, and adhered to one another. By contrast, hyphae of M. fruticicola successfully penetrated the style but branched profusely within the lumen with no apparent affinity to specific regions. Putative signals involved in adhesion and conidial guidance are currently under investigation.

Evaluation of a real-time PCR assay to detect Ralstonia solanacearum race 3, biovar 2 (bv2) in geranium plants. E. Nikoleta (1), E. Matveeva (1), and N. W. Schaad (2). (1) Russian Res. Inst. Phytopathology, Moscow; (2) USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD. Phytopathology 93: S65. Publication no. P-2003-0473-AMA.

Ralstonia solanacearum (Rs) race 3, bv2 infects potato and geranium under cool temperatures. The organism is highly regulated in the US and EU. We have developed and tested the sensitivity of a real-time BIO-PCR assay for specific detection of Rs race 3, bv2 in geranium plants. Healthy stems (100 or 1,000) spiked with a single, infected petiole, were soaked in water for 2 h and the liquid used for direct PCR and for BIO-PCR. For BIO-PCR enrichment, 100ul samples were plated onto mSMSA agar for 24-48 h and the plate washings used for PCR. PCR was done in a Smart Cycler using two different sets of primers and probe: 1) Rs race 3, bv2-specific RSC-F, RSC-R primers and RSC-P probe (Ozakman and Schaad, unpublished); and, 2) Rs species-specific RS-1-F, RS-1-R primers and RS-P probe (Weller et al., 2000). None of the samples were positive by a direct PCR assay. With BIO-PCR, both sets of primers and probe detected 1 infected petiole in 100 healthy stems, but only RSC-F, RSC-R primers and RSC-P probe were able to detect 1 infected petiole in 1000 healthy stems (St 36.1).


Sphaerella filum is a cosmopolitan, uredinicolous hyperparasite. However, its reputed non-specificity does not explain its behavior in the field. For example, S. filum is endemic to Melampsora medusae on Populus deltoides in the Southeast, but absent from poplar leaf rust elsewhere in the U.S. In the Pacific Northwest, rust of hybrid poplar and of native Populus trichocarpa is never affected by S. filum even though the latter is present on exotic species at the same location. Whole-plant inoculations with both pathogens confirmed the host specificity observed in the field, whereas detached-leaf assays have not. S. filum isolates from Populus deltoides from the Southeast and from Holcus lanatus and Phalaris arundinacea from the PNW have also been distinguished by morphological and molecular characters. The results indicate a species complex in S. filum that needs further investigation. These findings are of importance, if S. filum is used for biological control of rusts.


In areas of Washington State, U.S.A. and Hokkaido, Japan, winter wheat suffers from speckled snow mold caused by Typhula ishikariensis. Development of a growth chamber method to screen for resistance is desired to accelerate release of new cultivars. Cold hardening is important to the development of resistance and thus, influences of temperature and soil moisture during cold hardening on snow mold resistance were examined. Eight wheat cultivars and lines were subjected to cold hardening temperatures of 2 or 4°C for 1, 2, or 4 wk with soil matric potential of -0.1 or -0.01MPa. After cold hardening, plants were placed on a warm bench at 18°C with 16 h light. Plants were observed for disease development and the percentage of dry plant in each pot was recorded. Significant differences were observed among the treatments. The disease was more severe at colder temperatures, especially in the -0.01MPa treatment. The disease was more severe at colder temperatures, especially in the -0.01MPa treatment. The disease was more severe at colder temperatures, especially in the -0.01MPa treatment.


A disease prediction model was developed based on temperature and wetness-duration requirements for infection of grape leaves and internodes by Phomopsis viticola. Field evaluations were conducted in 2001 and, more extensively, in 2002. The 2002 study consisted of spraying vines with fungicides (thiofanate-methyl or mancozeb) according to a 7-day protective program or when environmental conditions were favorable for infection. For prediction-based treatments, fungicides were mixed with adjuvants (styloil oil
or Regulaid). Sprays were applied from 12-cm shoot growth through 2 wk after bloom; five applications were made in response to predicted infections periods compared to 11 in the protectant program. Analysis of variance indicated that vines sprayed with mancozeb plus oil or Regulaid in response to predicted infection periods had mean disease severity and incidence equal to vines sprayed with a protectant program. Preliminary results indicate that disease forecasting may be feasible for this disease.

**Vegetative compatibility groups of Colletotrichum coccodes**, the causal agent of potato black dot in North America. N. NITZAN (1), L. Tsror (Lahkim) (2), and D. A. Johnson (1). (1) Washington State University, Dept. of Plant Pathology, Pullman, WA 99164; (2) Agriculture Research Organization, Gilat Experimental Station, Dept. of Plant Pathology, M.P. Negev 85280, Israel. Phytopathology 93:S66. Publication no. P-2003-0477-AMA.

Potato black dot, caused by *Colletotrichum coccodes*, is prevalent in most potato production areas in the world, and may damage yield levels and quality. *C. coccodes* belongs to *Deuteromycotina*, where genetic exchange among isolates may occur by means of vegetative compatibility. However, the degree of genetic diversity in the North American population is unknown. Vegetative compatibility of 84 isolates was determined using complementation tests of nitrate non-utilizing (Nit) mutants. The recovery rates of nit and Nit mutants can range from 33.8% and 3.5%, respectively. Based on high anastomosis ability in complementation tests, seven VCG tester isolates were selected. Using these testers 70 isolates were assigned into 5 VCGs at, 21.5, 40.5, 7.2, 4, and 12% in this sample. Fourteen isolates (16.6% of all isolates) could not be assigned to any of the major groups, and showed only self-compatibility.


*Phytophthora sojae* causes soybean phytophthora root rot (PRR). Before 1994, races 1, 2, 3, 4, 13, 15 and 25 were found in Iowa. Race 25 was not considered as an important threat because of its low frequency in Iowa; although it can defeat the widely used resistance gene *Rps* 1-k. Studies were conducted in 2001 and 2002 to determine if new races had appeared and frequencies of previously existing races had changed in the state. A total of 19 PRR isolates were sampled from 144 soy fields across Iowa. Isolations were made from plants and soil using standard techniques, in which Masago medium and leaf disk baiting were used. There were 19 isolates from plants and 33 isolates from soil that were purified and tested on 8 differential lines. Subsequently, the isolates were classified by their virulence patterns. We found 4 races new to Iowa and the percentage of isolates that can overcome virulence patterns. We found 4 races new to Iowa and the percentage of isolates from plants and 33 isolates from soil that were purified and tested on 19 diseased plants and 144 soil samples were obtained across Iowa.


Pecan is an important nut crop throughout much of the southern USA, with GA being ranked as the leading producer. *Melodogynae incognita* and *M. arenaria* are known to attack pecan. In 2002, *M. pyrhythmiae* was first reported on pecan in GA and stress symptoms were observed on exhibiting dead branches in the upper canopy. Host resistance is an effective nonchemical management strategy, however, no data are available on pecan resistance to *M. pyrhythmiae*. Nine pecan rootstocks were evaluated for susceptibility to this nematode. Stocks included seedlings of Apache, Caddo, Curtis, Elliot, Moneymaker, Pawnee, Schley, Stuart, and Wichita cultivars. Seedlings were planted in pots and the soil infested with 1,400 motile J2 per pot. The number of egg masses and root galls per root system, number of eggs per root system, and dry root weight were recorded after 230 days. Resistance rating was based on inhibition of nematode reproduction relative to that on a standard stock (Elliot). Results indicate that all stocks were good hosts to *M. pyrhythmiae*. Further evaluation of pecan germplasm for useful sources of resistance is warranted.


Xanthomonas leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* has been classified 5 races as K1, K2, K3 K4 and K5 in Korea. K1, K2 and K3 are dominant races that have been observed yearly among them and especially K1 race occupied over 60% in rice fields. Recently K1 trend to decrease by spread of varieties with Xa1 and Xa3 genes resistant to K1, however K2 and K3 trend to increase on the contrary. In 2001, 5 isolates, from Jeonnam province were classified K3 race tested by differential varieties as Mileyang23, Changchungbyeo, Pungsanbyeo, Sangangbyeo, Hanggangchalbyeo and Milyang42. These collections showed strong pathogenicity to varieties that have resistant gene to K3 race. These results suggest that the materials collected have more strong pathogenicity than K3 but more weak it than K4 and K5, so we suggest them as a new race, K3a, that showed intermediate pathogenic property comparing to K3 and K4.


When ‘Royal Gala’ apple shoots were inoculated with *Erwinia amylovora* (Ea) and incubated in a growth chamber at 24.5°C, Ea populations dropped from 10% leaf to below detectable limits within 48 hrs. Low Ea populations (<10/leaf) were detected 6 and 14 days after inoculation. Under orchard conditions in June, Ea was detected on leaves after rain events but were short lived. However, in July Ea populations recovered from leaves significantly increased following a bout of rain and days of high relative humidity. Low numbers of Ea were detected 10 July the day after the storm. On 15 July the higher numbers of Ea were detected when leaf washings were plated on media but not when leaves were printed directly onto media, suggest the bacteria were within the leaf. Following 4 cm of rain on 15 July, Ea was detected by both washing and printing. A gfp labeled strain of Ea developed to study epiphytic growth was found to be less fit and virulent than the wild-type parent strain.


An apple disease diagnostic system composed of an array of pathogen-specific oligonucleotide probes based on the rDNA spacer regions of fungi and bacteria causing disease on apple has been designed and tested. Probes specific for apple diseases including fire blight, powdery mildew, apple scab, gray mold, and blue mold were tested for specificity and sensitivity with sample DNA extracted from pure cultures of strains of the various pathogens as well as DNA extracted from strains of closely related non-pathogenic species. Array probes have readily detected various pathogens from inoculated plant tissues including buds, blossoms, leaves and fruit as well as environmental samples including rotation impaction spore traps, ground cover, leaves, flowers and fruit. In field testing, hybridized extractions from spore trap samples correlated directly with apple scab and powdery mildew predictive models for ascospore release.


In October 2001, blighting and a leaf spot were observed on tomato foliage from a transplant producer in Florida. Non-fluorescent bacteria were
consistently isolated. According to fatty acid profiles the strains were most similar to *Pseudomonas huttiensis*. Sequence analysis of the 16S rRNA indicated that the bacterium had 96% homology with *P. huttiensis* (Ph) and *Herbaspirillum rubisubalbicans* (Hr). The tomato, Ph and Hr strains were Gram-negative and oxidase positive, but levan, pectate hydrolysis and arginine dihydrolase negative, and grew at 40°C. With the exception of the Ph strain, both investigated and control Hr strains caused confluent necrosis when infiltrated at high concentrations into pepper and tomato leaves. However, in inoculation tests only the tomato strains were able to produce the symptoms observed on seedlings in the greenhouse. Based on pulsed-field gel electrophoresis, the tomato strains collected in 2001 and 2002 were uniform and variable, respectively, but were very different from Ph and Hr.


Understanding the soil, plant, and pathogen factors which affect potato early drying, caused by *Verticillium dahliae*, is essential to developing effective management strategies. Data were collected on artificially infested field plots (4 replications) to which three plant species were applied as green manures in 2001 at three rates (0, 6, 12 or 24 Mg ha⁻¹). Disease severity (AUSPC) or total yield was regressed on soil pH (ranging from 5.2 to 7.5), nutrients, organic C, inoculum density (ID), and microbial activity. Regression models including soil properties that predicted the severity of potato early drying and yield for Russet Burbank potatoes were compared using Bayesian Information Criteria statistics for goodness of fit. The best predictive model for severity included significant terms for ID, interaction of pH and ID, pH, Na and levels (R² = 0.74, P < 0.001). The best predictive model for yield included significant terms for ID, interaction of pH and ID, NO₃, microbial respiration, and soil moisture (R² = 0.59, P < 0.001).


CNSV is a virus with a bipartite RNA genome, and member of the genus stocks of ornamentals in New Zealand. Based on pulsed-field gel electrophoresis, the tomato strains collected in 2001 and 2002 were uniform and variable, respectively, but were very different from Ph and Hr.

Zoxamide, an active ingredient in Gavel 75DF™ fungicide, has demonstrated robust foliar efficacy against late blight caused by, *Phytophthora infestans*. Field trials were conducted at North Dakota State University in 2000, 2001, and 2002 to characterize the efficacy of Gavel™ in the control of tuber rot caused by *Phytophthora infestans*. Foliar treatments included standard rates of chlorothalonil (Bravo), azoxystron (Quadris), mancozeb (Quadris) and zoxamide (Gavel 75DF) applied at 5 day intervals. Tuber evaluations were made at harvest and at intervals up to 90 days after harvest. In all three years, 3 to 5 applications of Gavel™ resulted in significantly lower levels of tuber infection than the standard protectant treatments. The effect of zoxamide in reducing the production of sporangia and their ability to release mobile zoospores appear to be important factors in the control of late blight tuber rot.

**Identification of fungi involved in natural soil suppressiveness against the beet cyst nematode, *Heterodera schachtii*.** R. O. OLATINWO (1), J. O. Becker (2), and J. Borneman (1). Departments of (1) Plant Pathology; and (2) Nematology, University of California, Riverside, CA 92521. Phytopathology 93: S67. Publication no. P-2003-0489-AMA.

Strains of *Fusarium oxysporum* and *Dactyllula oviparasitica* isolated from a beet cyst nematode suppressive soil were tested for their ability to reproduce soil suppressiveness against *H. schachtii*. These strains were selected because their population levels had been previously shown to correlate with the suppressiveness. Conducive soil (fumigated suppressive soil) was amended with the fungi and sown with Swiss Chard seeds. Four weeks after seeding, the soils were inoculated with second-stage juveniles of *H. schachtii*. After two nematode generations, both fungal treatments decreased *H. schachtii* populations (eggs and cysts) to levels similar to that of the natural suppressive soil. The *D. oviparasitica* treatment resulted in the highest % parasitized eggs and plant yields. These results strongly suggest that these fungi are involved in the natural soil suppressiveness.


The molecular basis of resistance to azoxystrobin has been investigated in an isolate of *Pythium aphanidermatum* able to grow on turf treated with commercial rates of this QoI fungicide. The cytochrome b gene sequence, encoding amino acid residues 73 to 283, has been determined for both the resistant isolate and 4 sensitive isolates since this region includes most previously identified mutations shown to confer target site resistance to QoI fungicides. Previous studies of this isolate has shown that a specific amino acid substitution, G143A, frequently correlates with the QoI resistance phenotype. However, each *P. aphanidermatum* isolate analysed, regardless of QoI inhibitor sensitivity had G at position 143. Instead, the azoxystrobin resistant isolate of *P. aphanidermatum* carries L at position 129, compared with F for the sensitive isolates. Again this amino acid substitution is the result of a single base change. This suggests that the F129L mutation is responsible for the azoxystrobin resistant phenotype of this isolate of *P. aphanidermatum*.


Pink-eye is the only major potato disease in North America without a known cause and it results in significant losses each year. Symptoms include disrupted periderm and bud end rot. Tubers were collected from 13 sites in Wisconsin and bacteria were isolated from over 80 diseased and 25 healthy tubers by soaking tissue sections in water and plating the bacteria on orientation agar to determine if colony types could be correlated with diseased tubers. The genera of representative colonies were determined by sequencing 16S rDNA genes and *Rhizobiun*, *Agrobacterium*, *Bacillus*, *Herbaspirillum*, *Pantoja*, *Variovorax* and *Delftia* were isolated from diseased and healthy tissue and T-RFLP was used to determine if a T-RFLP pattern isolated from bacteria or fungi could be associated with diseased tubers. Pink-eye is associated with storage rot and although most tubers with pink-eye did not rot in moist chambers, diseased tubers from one field were significantly more susceptible to soft rot than healthy tubers when tubers were spray-inoculated with *Erwinia*.
A small 5'-untranslated region of satellite pancreatic mosaic virus determines host-specific movement in millipet plants. R. T. OMAROV (1), W. Qiu (2), and K.-B. G. Scholthof (1). (1) Texas A&M University, Dept. of Plant Pathology & Microbiology, College Station, TX 77843; (2) Southwest Missouri State University, Dept. of Fruit Science, Mountain Grove, MO 65711. Phytopathology 93:S68. Publication no. P-2003-0493-AMA.

Satellite pancreatic mosaic virus (SPMV) is an 824-nucleotide (nt), positive-sense, single-stranded RNA virus that depends on its helper *Pancum mosaic virus* (PMV) for replication and spread. Previous studies suggested that the 5'-untranslated region (5'-UTR) of SPMV is involved in systemic movement in a host-specific manner in millpet plants. To further map this region, we generated a set of SPMV deletion and insertion mutants around a unique BamHI site that was introduced into an infectious clone of wild-type SPMV at nt 78, and investigated their movement phenotypes on proso millet and foxtail millet. Mutants with deletions at nt 78 deleted moved normally in proso millet plants, but failed to spread in foxtail millet plants. The same phenotypes were observed for two insertion mutants with 4 and 18 nucleotides inserted at the BamHI site. These results suggest that at nt 63 to 78 in the SPMV 5'-UTR are crucial for host-specific systemic spread in millpet plants.


Phytophthora ramorum is established in 12 counties in California and has been detected (and targeted for eradication) in Curry County, Oregon. This pathogen infects at least 19 host species in 11 plant families. Many of these hosts are important parts of Oregon’s agricultural commodities. Thus, we have conducted an annual survey for *P. ramorum* in Oregon nurseries, plantations, and other high risk sites since 2001. Following the USDA protocol, hosts were examined and samples collected from symptomatic host species. The samples were processed using standard laboratory techniques and analyzed for the presence of *P. ramorum*. Since 2001, 167 sites have been surveyed with a total of 6,584 samples collected and analyzed. No *P. ramorum* was detected, although other *Phytophthora* species were recovered in both years. As of 2002, Oregon’s agricultural commodities remain apparently free of *P. ramorum*. Surveys will continue in 2003, pending available funding.


The ability of zoospore cysts of *Phytophthora* to infect at least 19 host species in 11 plant families. Many of these hosts are important parts of Oregon’s agricultural commodities. Thus, we have conducted an annual survey for *P. ramorum* in Oregon nurseries, plantations, and other high risk sites since 2001. Following the USDA protocol, hosts were examined and samples collected from symptomatic host species. The samples were processed using standard laboratory techniques and analyzed for the presence of *P. ramorum*. Since 2001, 167 sites have been surveyed with a total of 6,584 samples collected and analyzed. No *P. ramorum* was detected, although other *Phytophthora* species were recovered in both years. As of 2002, Oregon’s agricultural commodities remain apparently free of *P. ramorum*. Surveys will continue in 2003, pending available funding.

The ability of zoospore cysts of *Phytophthora* spp. to germinate by releasing further zoospores (repeated emergence) in irrigation water presents a challenge to managing these pathogens, especially in recycling irrigation systems. Little is known about the effect of water temperature on this phenomenon. The effect of temperature on germination in *P. ramorum* was studied for isolates of *P. parasitica* (GLN 9-3), *P. citrophthora* (GLN 7-23), *P. cryptogea* (FDM51), and *P. cinnamomi* (1D-A). Zoospore suspensions in Eppendorf tubes were encysted by vortex treatment for 70 sec and then incubated at 15, 20, 25 or 30°C. Germination was assessed after 7 hr as either germination by germ tube or by zoospore for 100 cysts. For all four isolates, the optimum temperature for germination by zoospores was 20°C, but the optimum by germ tube was 25°C for GLN 9-3, GLN 7-23, and 1D-A, and 30°C for FDM51. GLN 9-3 showed the strongest tendency to germinate by reemergence. When second generation zoospores were encysted, the resulting cysts had the same temperature optima for germination by germ tube or by zoospores as the first generation cysts.


A study was initiated at the Savannah River Site, New Ellenton, SC, to determine factors involved in decline of longleaf pine associated with prescribed burning. Three years after prescribed burning treatments were initiated, mortality and numbers of symptomatic trees increased in the hot burn plots. Crown symptoms corresponded to tree physiological status determined by cambial sucrose synthase activity. Root pathogenic fungi such as *Leptographium terebrantis*, *L. procera*, and *Heratobasidium annosum* were widespread throughout the study site, regardless of treatment. The *Leptographium* species were found to be pathogenic based upon inoculation experiments and *H. annosum* was involved in root infections and mortality. Histological studies indicated a high fine root mortality rate in the hot burn treatment. The decline syndrome on these sites is a complex of interacting factors and involves root pathogens, soil factors, root damage, and physiological dysfunction.

Detection of *Ralstonia solanacearum* race 3, biovar 2 in asymptomatic potato tubers using a real-time BIO-PCR assay. M. Ozakman (1) and N. W. SCHAAD (2). (1) Plant Protection Central Research Institute, Ankara, Turkey; (2) USDA-ARS, Foreign Disease-Weed Science Research Unit, Frederick, MD 21702. Phytopathology 93:S68. Publication no. P-2003-0496-AMA.

*Ralstonia solanacearum* (Rs) race 3, biovar (bv) 2, is a serious threat to potato production in temperate climates. Canada, US, and the EU list Rs bv 2 as a noxious weed. Although some serological and PCR-based assays are available (Weller et al., 2000), none have detected the organism in asymptomatic tissue. We have designed Rs bv 2 specific real-time PCR primers and probe based on a Rs bv2-specific DNA fragment (Fegan et al., 1998) and a BIO PCR assay using ssMMSA medium for detecting Rs bv 2. All 17 Rs bv2 strains, including five from Germany, were positive whereas 16 potato associated bacteria were negative. Using undiluted potato tuber extract spiked with Rs bv 2, as few as 30 cells/ml extract could be detected. Of 14 naturally infected, asymptomatic potato tubers, two were positive using the real time BIO PCR assay and by isolation on ssMMSA, whereas none were positive with a standard real time PCR assay. This is the first report of the detection of Rs bv 2 in asymptomatic potato tubers by PCR.

Pentathiadecane isolated and identified from the mycelial leachate of shiitake mushroom is a good alternative choice for the control of bacterial wilt diseases. R. P. PACUMBABA (1), C. A. Beyl (1), R. O. Pacumbaba Jr. (1), M. Kobaisty (2), M. R. Tellez (2), and K. K. Schrader (2). (1) Alabama A&M University, Dept. of Plant and Soil Science, Normal, AL 36762; (2) USDA-ARS, Natural Products Utilization Research Unit, University, MS 38677. Phytopathology 93:S68. Publication no. P-2003-0497-AMA.

Pentathiadecane is a natural chemical components of the mycelial leachate of shiitake mushroom (*Lentinula edodes*) that has been recently identified by our cooperative scientists of the Natural Products Utilization Research Unit, Agricultural Research Service, U.S. Dept. of Agriculture, University, MS. This chemical is not available commercially in biochemical and reagents catalogues; however, pentathiadecane can be isolated easily from the mycelial leachate of shiitake mushroom. Since, this chemical is a component of the mycelial leachate, its use as an alternative to other synthetic pesticides for the control of bacterial wilt diseases is very encouraging. Previous test have indicated that the mycelial leachate prevented the growth of *Ralstonia (Pseudomonas) solanacearum* (causal organism of bacterial wilt of tomato and *Curvobacterium flaccumfaciens* pv. *flaccumfaciens* (causal organism of bacterial wilt of beans) in the laboratory. The mycelial leachate, concentrated 10X (autoclaved or not), when applied as a soil drench to soil infested with wilt bacteria, likewise, prevented symptoms development of bacterial wilts of tomato and beans in the laboratory. The chemical apparently appears to be heat stable. At present, we are exploring an excellent delivery system of the mycelial leachate for maximum prevention of symptoms development of bacterial wilt diseases in the field.


*Xanthomomas arboricola* pv. *pruni* (Xap), a Gram negative plant pathogenic bacterium, causes bacterial spot disease on *Prunus* spp. such as peaches and plums. We have identified, cloned and sequenced a specific 943-bp DNA fragment from *Xap* that has proven to be a useful tool for detection and identification of this bacterium. Sequence analysis revealed the presence of an open reading frame (ORF) with the potential to encode a polypeptide of 243 amino acid residues with a calculated mass of 30.26 kDa. Blast searches for amino acid sequences revealed similarity between this ORF and other known genes related to the ABC transporter family of various organisms. The greatest similarity at the amino acid level (39% identity) was manifested with *X. axonopodis pv. citri*. These results suggest the presence of a putative protein possibly implicated in the active movement of substrates across the cellular membrane of this bacterium.

Hilum bleeding as a diagnostic method for estimating percent Soybean mosaic virus or Bean pod mottle virus occurrence in soybean seed. Y. Malaviar as a case study. K. Page (1), R. Unterreiner (1), T. Brumbatch (2), and A. Henn (1). (1) Dept. of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS; (2) Delta Research and Extension Center, Mississippi State University, Stoneville, MS. Phytopathology 93:S68. Publication no. P-2003-0499-AMA.
In 2000 and 2001, four greenhouse tests were conducted to determine if the seed symptom hilum bleeding, in the soybean variety Bolivar, indicates the presence or survival of the Soybean mosaic virus (SMV) or Bean pod mottle virus (BPMV). Treatments were selected based on the percent hilum bleeding in the seed lots including <1% (control), 1, 2, 4, 25 (tr. 2x) and 35% and further subdivided into severity of the hilum bleeding ranging from none to intermediate, or severe hilum bleeding. Stand counts, plant heights, visual disease ratings, and enzyme-linked immunosorbent assay (ELISA) data was collected to compare the treatments. The results from the stand counts, plant heights, and visual disease ratings did not show any association with differences in percent hilum bleeding. When significant differences did occur no trends were noted. Furthermore, only one plant tested positive for SMV and another individual was positive for BPMV with ELISA. Overall, results indicate that hilum bleeding, regardless of percent expression within a seed lot, does not indicate the presence or survival of SMV or BPMV in Bolivar soybean seeds.


A Phomopsis species was isolated from roots, petioles, leaves, and boll tissues of cotton in the southeastern United States. Phomopsis longicolla and P. phaseolina isolated from soybean were used for comparison. Morphological and cultural differences were found between the cotton Phomopsis isolates and the soybean pathogens, P. phaseolina and P. longicolla. Pathogenicity studies determined that all three species produced symptoms on cotton and soybean. Symptoms were more severe for each species on the primary host. Phomopsis isolates from soybean and cotton were distinguished using RFLP analyses of PCR fragments. Primers were used to amplify a 550 bp sequence (Internal Transcribed Spacer 1) of the mitochondrial rRNA gene. PCR fragments from 49 Phomopsis isolates were sequenced. On the basis of host relationship and morphological and molecular data, the Phomopsis isolated from cotton will tentatively be named Phomopsis gossypicola Palmateer, McLean & Morgan-Jones, nomen novum.

Evaluation of ozone gas penetration into commercial packages of orange fruit and control of Penicillium spp. sporulation during cold storage. L. Palou (1), J. L. SMILANICK (2), C. H. Cristosio (1), M. Mansour (2), and P. Plaza (3). (1) Dept. of Pomology, University of California, Davis, CA, USA; (2) USDA-ARS, Parlier, CA, USA; (3) Area de Postcottiola, Centre UdL-IRTA, Lleida, Catalonia, Spain. Phytopathology 93:S69. Publication no. P-2003-0501-AMA.

Penetration of gaseous ozone into citrus packages and its effectiveness in controlling sporulation of Penicillium digitatum and P. italicum were evaluated on artificially inoculated ‘Lane Late’ oranges stored at 12.8°C and exposed to an average ozone concentration of 0.72 ppm (v/v) for 14 days. Oranges were packed naked in standard fiberboard cartons or vented returnable plastic containers (RPCs), or packed in transparent polyethylene bags within RPCs or large fiberboard cartons. Ozone penetration was strongly dependent on the package vent size. As a percentage of the storage room ozone concentration, penetration in RPCs was 82% and relatively uninhibited compared to the poor penetration (9-17%) into other cartons or polyethylene bags. Inhibition of the sporulation of P. digitatum and P. italicum was satisfactory only on oranges packed naked in RPCs.


Listeria monocytogenes is a ubiquitous soil bacterium and an important foodborne human pathogen. Contamination of fresh produce may be an ecological transition between the soil environment and the human host; however, little is known regarding specific Listeria-plant interactions. To identify mechanisms involved in contamination of produce, we are investigating L. monocytogenes gene expression during attachment and growth on cabbage. We performed reverse transcription-PCR with 81 arbitrary primers and used differential display polyacrylamide gels to identify genes that show higher expression in L. monocytogenes cells recovered from cabbage surfaces relative to cells grown in defined medium. Several of these genes encode proteins with no known function. We are assessing the contribution of these proteins to plant colonization by measuring attachment of site-directed mutant L. monocytogenes strains relative to that of wild type L. monocytogenes.


Dahlia is an important ornamental crop grown for its flowers. Several viruses diminish the marketability of dahlias. These include Cucumber mosaic virus, Dahlia mosaic virus (DMV), Impatiens necrotic spot virus, Tobacco streak virus, and Tomato spotted wilt virus. Many of these viruses can be effectively managed or even eliminated using a combination of practices. DMV is considered to be the most economically important viral disease of dahlias. DMV shares many genomic features with members of Caulimoviridae, and is reported to be transmitted by several species of aphids and through infected cuttings and bulbs. Management options for DMV include sanitation, and use of virus-free seed and bulbs for propagation. Tissue culture grown material was effective as an effective means to avoid DMV. The effectiveness of various control options and future prospects for managing DMV will be presented.


Two polygalacturonase (PG) genes from Colletotrichum acutatum were identified by PCR using degenerate primers. These two PG genes were designated as capg1 (Colletotrichum acutatum polygalacturonase 1) and capg2. Full sequences for both genes and their promoters were obtained and analyzed. The open reading frame of capg1 consisted of 1077 base pairs while that of capg2 consisted of 1089 nucleotides. The lengths of the predicted proteins were 358 and 363 amino acids for capg1 and capg2, respectively. The calculated pI of capg1 and capg2 were 8.78 and 9.11, designated as acid proteins and the molecular weights were 35.9 kDa and 37.5 kDa, respectively. The deduced amino acid sequence of capg1 shared 83% identity with C. lindemuthianum capg1, while capg2 showed 85% identity with capg2 of C. gloeosporioides f. sp. malvaceae. Both upstream regions contained CREA and PaC binding consensus sites, indicating that these genes might be controlled by the CREA and pH regulatory mechanism. Using reverse-transcription PCR, it was determined that the expression of the two capg genes depended on the carbon source available as well as the pH of the medium.

Cloning and characterization of the HOG1 homologue CmpK1 from Cryphonectria parasitica. S. M. PARK, E. S. Kim, M. J. Kim, S. M. Yang, and D. H. Kim. Institute for Molecular Biology and Genetics, Basic Science Research Institute, Chonbuk National University, Chonju, Chonbuk, Korea. Phytopathology 93:S69. Publication no. P-2003-0505-AMA.

We examined the biological function of cpmk1, which encodes a MAPK of Cryphonectria parasitica, and its regulation by mycovirus. Sequence comparison revealed that cpmk1 had highest homology with a hog1-like homologue from Magnaporthe grisea. Kinase assays and Northern blot analyses indicated that the CmpK1 pathway was affected specifically in hyphomycosis conditions by the hypovirus CHV1-EP713. Growth defect was observed in the cpmk1-null mutant under hyphomycosis conditions, indicating that cpmk1 functionally belongs to a hog1 subfamily. Moreover, the virus-infected hypovirulent UEP1 strain also exhibited severe osmosensitivity compared with the virus-free isogenic strain EP155/2, thus providing additional evidence for viral regulation of cpmk1. Besides osmosensitivity, disruption of cpmk1 resulted in several, but not all, hypovirulence-associated changes, such as reduced pigmentation, conidiation, laccase production, and cryparin expression. However, the cpmk1-null mutant exhibited an increased accumulation of pheromone gene transcripts. Virulence assays of the cpmk1-null mutant revealed reduced canker area, but not as severe as that of UEP1. These results suggest that mycoviruses modulate the MAPK and thereby provoke the aberrant expression of target genes for viral symptom development.


A 2.8 kb double-stranded (ds) RNA fragment in the fungus that causes black root rot disease on numerous plant species (Chalara elegans) was studied. Partial cDNA clones were obtained by RT-PCR using random primers. One clone (BK183C3) was used for Northern blot hybridization to determine the genetic relatedness among similar-sized dsRNA fragments in 22 C. elegans isolates from different geographic origins. All strains showed a positive hybridization with this probe, indicating a level of homology was present. Random cDNA clones were obtained and aligned with each other to construct a full-length sequence. The linear genome was 2896 bp and
contained one large open reading frame (ORF) in one strand, if mitochondrial codon usage was followed. The putative gene product of this ORF was 705 amino acids in length and showed typical features of RNA-dependent RNA polymerases (RDRPs). Sequence analysis revealed that this dRNA had some homology (59%) to Ophistoma novo-ulmi mitivirus 4-Ld. Mitoviruses are found in the mitochondria of affected fungi; the effects of this virus on the biology of C. elegans is currently under investigation.


Four techniques were evaluated to sample wind-blown splash from canker-infected citrus. Two volumetric samplers (the Cyclone and Burkard Cyclone) and two passive samplers (funnels and panels) were used. The PAS450 sampled no bacteria under controlled laboratory or field conditions. The Burkard Cyclone consistently sampled spray, but even when powered off (8 ml-1 and 5ml-1 of spray sampled when powered on or not, respectively). Both panels and funnels consistently sampled bacteria laden spray (up to 21500 bacteria ml-1 under field conditions. There was a strong positive correlation between panel and funnel catches for total volume sampled (r = 0.9817, P < 0.001) and bacteria ml-1 (r = 0.9780, P < 0.001). Although the number of bacteria ml-1 sampled by the Burkard was correlated with both panel (r = 0.8220, P = 0.007) and funnel (r = 0.7862, P < 0.012) catches, the total volume was not (r = 0.1851, ns and r = 0.1785, ns, respectively). Panels sampled the greatest volume, and effectively collected bacteria-laden wind-blown splash.

Management of sclerotinia blight on peanut with the biocontrol agent Coniothyrium minitans. D. E. PARTRIDGE (1), T. B. Sutton (1), D. L. Jordan (2) and V. L. Curtis (1). (1) Dept. of Plant Pathology; and (2) Dept. of Crop Science, North Carolina State University, Raleigh, NC. Phytopathology 93:S70. Publication no. P-2003-0508-AMA.

Sclerotinia blight of peanut (Sclerotinia minor) is an important peanut disease in North Carolina. Coniothyrium minitans, marketed as Contans WG, is a mycoparasite capable of colonizing sclerotia of Sclerotinia spp. A field experiment was conducted to determine if repeated soil applications of C. minitans would control of sclerotinia blight. C. muntzian was applied in the fall of 1999 and each subsequent fall for 2 years in a field with a history of peanut production and disease. Peanuts were planted in 2001 and 2002. Subplot treatments of fluzinam and the moderate resistant cultivar Perry reduced disease in both years. Applications of fluazinam and the moderate resistant cultivar Perry may provide the best control of sclerotinia blight.

PCR-real time identification of Fusarium oxysporum f. sp. basilici on basil seeds and roots. M. Pasquili, M. L. GULLINO, and A. Garibaldi. Centre for Competence for the Innovation in the Agro-environmental Sector, University of Torino, Via L. da Vinci 44, 10095 Grugliaso (TO), Italy. Phytopathology 93:S70. Publication no. P-2003-0509-AMA.

Fusarium oxysporum f. sp. basilici is the causal agent of wilt and crown rot of sweet basil (Ocimum basilicum L.). In fact this disease is the major problem to this crop. Chemical and biological control are inconsistent means to control the pathogen and few basil ecotypes are resistant. Therefore a prevention strategy is needed. A diagnostic tool able to identify pathogens inside and outside the seed would allow pathogen-free certification of seeds. A previous study developed a PCR assay able to identify the pathogen on the basil seeds and roots. M. Pasquali, M. L. GULLINO, and A. Garibaldi. University of Torino, Via L. da Vinci 44, 10095 Grugliaso (TO), Italy. Phytopathology 93:S70. Publication no. P-2003-0507-AMA.

S. sclerotiorum is required to define the epidemiological roles of ascopores and to establish a transformation system. Fresh apothecia contained primarily immature asci or acia that have released ascopores, with less than 20% undischarged mature ascus. The percent undischarged mature ascus increased up to 70-80% in detached apothecia after incubation at 21°C for 30 h. This was accompanied by a marked increase in ascospore production, up to 5 × 10^5 ascospores per apothecium. Detailed time course studies indicated that percent mature ascus peaked around 36-36 h after incubation. Percent mature ascus and the number of ascospores were similar in closed and open incubations, suggesting that accumulation of volatile substances was not critical to the synchronized maturation. Germination rates of ascospores from naturally matured and the treated apothecia were similar. The post-detachment incubation was effective with all tested isolates of S. sclerotiorum and S. minor.


Infection by Monilinia vaccinii-corymbosi is responsible for mummy berry disease and causes both leaf blight and dry fruit rot in blueberry. In each of 41 crop-year blueberry clones, we selected 10 stems with and 10 stems without symptoms of leaf blight and monitored the proportion of infected leaves and fruit three times during the growing season. Average berry weight and the proportion of flowers that produced healthy fruit were determined for each replicate. Temperature had a significant effect on the rate of LE (P = 0.008). At 25 and 30°C, the rates of LE were linear over time and significantly higher than at 35°C (P = 0.012 and 0.003, respectively). The relationship between temperature and rate of LE was best described by a quadratic polynomial (R² = 0.73).

Postbloom Fruit Drop (PFD), caused by Colletotrichum acutatum, infects petals of citrus flowers and induces the abscission of fruitlets. The PFD model was developed in Florida to schedule fungicide applications based on the amount of inoculum, rainfall and leaf wetness. This model was evaluated for 4 yr in Brazil. A new advisory system (PFD-FAD) was developed to be more widely applicable by incorporating factors such as disease history, varietal susceptibility and the stage of bloom. Applications following the PFD model provided good control of PFD and saved 2 sprays compared to the calendar program in 1999, and 1 to 2 sprays compared to the grower’s choice in 2000, 2001 and 2002. In field tests in 2001 and 2002, PFD-FAD provided good control of the disease with only one spray. The PFD model and the PFD-FAD were equally effective for timely fungicide applications to control PFD in Brazil. PFD-FAD is simpler to use than the PFD model, eliminates unnecessary sprays, and reduces expenses.

Recent outbreaks of hop downy-mildew disease in the South Andean Region of Argentina. B. A. Perez (1), E. Martinez (2), and D. Barreto (1). (1) INTA, YUYA Las Cañadas y De Los Reseros, CC 25 (1712), Castelar; (2) INTA AER El Bolsón, Río Negro, Argentina. Phytopathology 93:S71. Publication no. P-2003-0515-AMA.

Hop disease surveys have been conducted in southwestern Argentina since 2001. The objective of this study was to estimate the prevalence, incidence and severity of downy mildew caused by the fungus, Pseudoperonospora humuli. Early in December 2002, a total of 44 hop crops were monitored in Río Negro and Chubut provinces. The varieties included Buillon, Cascada, CEZ, Haffertau, Nugget, and Trafal. The disease was recorded in all fields surveyed. The incidence of systemically infected plants ranged from 10 to 100% depending on location. The disease severity at each location varied from low to high. The incidence of infected plants during February 2003 reached 40 to 100%. This increase indicates that the disease had spread due to the susceptibility of the current cultivars and weather conditions favorable for the spread of the pathogen.


Pokeweed antiviral protein (PAP) isolated from Phytolacca Americana has been shown to have a broad range antiviral activity. For in vivo experiments, the wild type PAP gene was cloned into a plant transformation vector. We also prepared a DNA construct which produced a deletion of 25 amino acids from the C-terminus of PAP, and two constructs with independent point mutations at the N-terminus (Gly75-Asp), and the active site (Glu76-Ala). These four constructs were then transformed into Citrus paradisi by Agrobacterium-mediated transfer. Nine transgenic plants containing the N-terminal mutant, one containing the active site mutation, and none with the other two constructs were obtained and characterized by molecular methods. The transgenic citrus plants are being graft- and aphid-challenged with CTV and will be analyzed for CTV replication.

Changes in Puccinia graminis populations in Minnesota, 1912 to 2002. P. D. Peterson (1), K. J. Leonard (2), A. P. Roeltls (3), and T. B. Sutton (1). (1) North Carolina State University, Dept. of Plant Pathology, Raleigh, NC 27695; (2) University of Minnesota, Dept. of Plant Pathology, St Paul, MN 55108; (3) formerly Cereal Disease Laboratory, St. Paul, MN. Phytopathology 93:S71. Publication no. P-2003-0517-AMA.

Collection records of Puccinia graminis f. sp. tritici (Pgt) from 1912-2002 were analyzed for changes in race diversity. Aerial collections of P. graminis from barley were analyzed also for changes in forma speciales of P. graminis. After barley eradication in the 1930s, the proportion of Pgt among aerial collections from surviving bushes declined while P. graminis f. sp. secalis increased. Since 1995 the proportion of Pgt collections has increased and that of Pgs has declined, suggesting increased importance of barley to Pgt. The predominant race among both aerial and aerial collections was similar for 6 out of 9 decades. Comparison of normalized Shannon indexes showed that genetic diversity of the aerial populations declined sharply over time, but diversity of the aerial populations remained fairly constant throughout the 90-year study. Factors affecting changes in the pathogen populations and the implications on stem rust epidemics will be discussed.

Spread of viruses in two new cultivars of hop in Australia. S. J. Pethybridge (1) and C. R. Wilson (2). (1) Tasmanian Institute of Agricultural Research (TIAR), University of Tasmania, P.O. Box 447-3523, Burnie, Tas 7320, Australia; (2) TIAR - University of Tasmania, GPO Box 252-54, Hobart, Tas 7001, Australia. Phytopathology 93:S71. Publication no. P-2003-0518-AMA.

Two new cultivars of hop have recently been adopted by the Australian industry, ‘Super Pride’ and ‘Agate’. The objective of this study was to assess reinfestation by Hop mosaic virus (HpMV), Hop latent virus (HpLV), and Apple mosaic virus (ApMV) after planting with virus tested material. Incidence of viruses was monitored in young gardens (<3 years old) of both cultivars at three locations around Tasmania between 1999 and 2001. Incidence of viruses were assessed by either sampling all plants within a garden or systematic selection of 50 plants in a diagonal transect. In the final year of the study, the incidence of HpLV, HpMV, and ApMV ranged from 8-74%, 8-78%, and 0-34% in ‘Agate’ gardens. The incidence of HpLV and HpMV in ‘Super Pride’ gardens was lower and ranged between 5-22%, and 0-22%, respectively. The incidence of ApMV in ‘Super Pride’ gardens was 8% that observed in ‘Agate’ (0-10%). This information will be combined with disease loss data to compile management strategies for these viruses.


Bioterrorism is an ever growing concern, especially since the events surrounding 11 September 2001 attack. If crop pathogens were introduced into this country, like wheat and cotton, were purposefully attacked using phytopathogens, it could cause enormous economic and social disruption. Early recognition of introduced phytopathogens is essential for timely response. We are combining real-time quantitative PCR with terminal restriction fragment length polymorphism methods to detect fungi or bacteria that are pathogenic to unusually high numbers of plant surfaces. These trials numbers on plant surfaces. These trials numbers on plant surfaces. The common goal was set to set a ground work for a crop-protection system in which remote-sensor imagery identifies unusual areas of crop stress. These suspect areas would then be investigated by rapid characterization of microbial genotypes and community structure on the indicated crop plants. We will investigate numerous transsects across pathogen-impacted fields, for comparison, to characterize the patterns typical of natural outbreaks.


Soybean rust has been reported to occur in eastern Asia and Australia for decades. In recent years the disease was introduced and spread rapidly in Africa and South America and become a concern to the US soybean industry. To assess the possible impact of the disease, we use modeling approach to determine potential geographical regions where the fungus could overwinter and serve as a source area for seasonal epidemics. Long-term meteorological averages were used to assess temperature stresses, with CLIMEX, and drought stress. The stresses were integrated to predict survival likelihood of the rust in a given location. Our results suggest many areas in Africa and south Asia could be source regions. In the Western Hemisphere, the rust might persist year-round in Brazil, Paraguay, Central America, the Caribbean, Mexico and Florida. If the rust is introduced into the US during winter it is likely to be restricted to Florida in frost-free areas or areas where the fungus could overcome short periods of freezing temperatures. Occurrence of soybean rust epidemics in the US would depend on uredospores dispersal from south to north.


Arbitrary fragment length polymorphisms (AFLPs) were used to assess genetic diversity among 41 mass and multiple and single basidiospore isolates of Crinipellis perniciosa, cause of witches’ broom of cacao. Nine of 64 primer pairs that were tested produced consistent and informative DNA amplification, and were used to screen all accessions. Fourteen haplotypes were detected. Regional differentiation of C. perniciosa was observed with a mean similarity coefficient of 72%. Cluster analysis and principal component analysis (PCA) grouped isolates from Brazil, Trinidad, and Ecuador together in a major cluster with those from Brazil and Trinidad closer than those from Ecuador. High levels of bootstrap support were found for each grouping and the total variance accounted for in the PCA was 65%. No diversity was observed among basidiospore isolates that were recovered from single basidocarps, reflecting the pathogen’s homocytic (primary homothallic)
reproduction. Minor differences were observed among mass isolates from a broom from Bolivia, indicating that brooms may be infected with more than a single individual. The present results suggest that two populations in Bahia, Brazil may have been introduced from, respectively, the States of Para and Rondonia.


The use of degenerate primers designed to amplify resistance gene analogs has been successful in many crops. The primers are usually based on published resistance gene sequences and, in particular, on conserved regions such as the nucleotide binding site (NBS). In some cases, amplified frag-

ments have been mapped to clusters of known resistance genes. Highbush blueberry (Vaccinium corymbosum L.) cultivars, while not exhibiting absolute resistance to many important fungal diseases such as anthracnose (Colletotrichum acutatum) and mummy berry (Monilinia vaccinii-corymbosi), do have varying degrees of field resistance. Among the more generally resistant cultivars are ‘Elliott’ and ‘Brigitta Blue’. To begin a program aimed at increasing disease resistance, these two cultivars were surveyed for NBS-containing gene fragments. Several potential resistance gene analogs were identified that may have potential both for breeding through marker-assisted-

selection and direct manipulation through biotechnology.


Asexual sporulation by nine species of Bipolaris, Curvularia, Exserohilum, and Drechslera from bermudagrass and ryegrass, three isolates per species, ranged from profuse to nonexistent on potato dextrose agar, cornmeal agar, and Bilay’s medium. Sporulation usually was greatest with species that form relatively small conidia (C. lunata, B. spicifera, B. hawaiiensis) and least with species that form relatively large conidia (B. stenospora, E. rostratum, D. dictyoides). Sporulation by all species on cornmeal agar was enhanced by addition of one or more cellulose substrates that included index cards, filter paper, cotton cloth, and cheesecloth. Growth on index cards and filter paper usually promoted greatest increases in sporulation. Enhancement of sporulation by dematiaceous hyphomycetes, by their growth on cellulose substrates, may assist in production of conidal inoculum and in the identification and quantitative estimation of populations of these fungi from subterranean plant tissues and soil.

Gene expression profiles of the biocontrol agent Pseudomonas fluorescens Pf-5 assessed using oligonucleotide arrays. C. M. PRESS (1), M. Brodhagen (2), and J. E. Loper (1). (1) HCRL USDA-ARS, Corvallis, OR 97330; (2) Dept. Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331. Phytopathology 93:S72. Publication no. P-2003-0524-AMA.

P. fluorescens Pf-5 is a root-colonizing biocontrol agent that produces a variety of antibiotics including pyoluteorin (Plt). Plt biosynthesis requires PltR, a transcriptional activator of Plt biosynthesis genes. A small-scale array, composed of 81 oligonucleotides representing structural and regulatory genes involved in biological control, was developed to evaluate patterns of gene expression in Pf-5. Arrays were targeted with cDNA transcribed from RNA extracted from cultures of Pf-5 or a pltR mutant. Transcripts of Plt biosynthesis genes were more abundant in Pf-5 than in the pltR mutant, as expected. Five other genes on the array (infC, rank, colI, hupB and mecb) were differentially expressed by Pf-5 and the pltR mutant, and significant differences in transcript levels were confirmed with quantitative PCR. The influence of PltR on transcription of diverse structural and regulatory genes in Pf-5 was demonstrated through analysis of oligonucleotide arrays.

Pathogenesis related volatile metabolites of onion inoculated with fungal and bacterial pathogens: A key to discriminate postharvest diseases. B. Prithiviraj, A. Vikram, and A. C. Kushalappa. Department of Plant Science, McGill University, 21111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada H9X. Phytopathology 93:S72. Publication no. P-2003-0525-AMA.

Erwinia carotovora ssp. carotovora (ECC), Fusarium oxysporum (FOX) and Botrytis allii (BAL) causes significant postharvest losses in onion during storage. The headspace gas of onion cv. Forteleg inoculated with the above pathogens was analyzed using the state of the art portable GC/MS system. The number and relative amounts of volatile metabolites were higher on 3 d after inoculation (dai) than on 6 dai. On 3 dai a total of 213 volatile metabolites were observed as compared to 153 on 6 dai. The pathogen specific metabolites detected were: oxo-4,6-diazacyclooctane-5-thione, Crotonic acid and 4-mercaptobenzene-3-(methylythio)-c-(thio) lactone in FOX, 1-

Prop-1-enyl dicotylpropanoate in BAL. The possible use of disease specific volatile metabolites for detecting and discriminating diseases of onion in storage is discussed. This paper forms the first report of the volatile metabolite profiling of onion inoculated with different pathogens.


A series of trials were conducted in 2002 and 2003 in Yuma, AZ, to evaluate the efficacy of several commercially-available biofungicides in controlling lettuce drop caused by Sclerotinia spp. Trials were established in fields artificially inoculated with either S. sclerotiorum or S. minor. Six products containing one of the following organisms were evaluated: Coniothyrium minitans, Trichoderma harzianum, Glomideum virescens, or Bacillus subtilis. C. minitans significantly reduced the incidence of lettuce drop caused by S. sclerotiorum but was less effective against S. minor. Conversely, the other biocontrol agents were effective against S. minor at varying levels, but had little effect against S. sclerotiorum. Moreover, most biocontrol agents promoted increased head weight and overall yield. In addition, all biocontrol agents were tolerant to standard fungicides used to control lettuce drop including iprodione, vinclozolin, and diconlor. Thus, these agents offer the potential for inclusion into an integrated biological/chemical program for improved management of lettuce drop in Arizona.

Rapid DNA extraction using single field collected sclerotium for the identification of Typhula species and varieties. R. RAGATZ, E. A. Scheef, and G. Jung. Dept. of Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 93:S72. Publication no. P-2003-0527-AMA.

Typhula fungi are a psychrophilic pathogen of grains and cereals found throughout the Northern Hemisphere. Two species, T. incarnata and T. ishikariensis s are highly pathogenic in North America to turf grasses and some cereals during the winter months of the year. Combined they are the second most damaging turf disease in the Great Lakes region and fourth in the United States. PCR primers specific for Typhula incarnata, T. phacorrhiza, and T. ishikariensis var. ishikariensis, var. idahoensis, var. canadensis have been developed but the current molecular identification method takes several months to complete due to slow growth. However, we have developed a rapid, field-based procedure to create PCR amplifiers and an accurate DNA extraction protocol for a single Typhula sclerotium. Coupled with the PCR primers, Typhula varieties and species can be easily identified.


Blackleg, caused by Leptosphaeria maculans, is an economically important disease of canola. Forty-three potential biocontrol agents of the pathogen, with high antifungal activity in both plate and plant assays, were identified. The controls had 100 percent mycelial growth in plates and a maximum disease rating of 9 in plant assays. Bacteria exhibiting inhibition greater than 70 percent on plates and with a disease rating of 0.4 - 3.0, were considered for extraction of the antifungal compounds. The high agar diffusible antifungal activity seems to indicate the role of antibiotics in biocontrol. Thin layer chromatography assays, performed with coated cladosporum spores, showed distinct antifungal zones, indicating the presence of antifungal compounds in the bacterial broth extracts. The antifungal compounds, contained in the zones, once verified for the inhibition of L. maculans, will be isolated, purified and characterized, through flash chromatography, HPLC and NMR, respectively. Also, the ability of the bacteria to inhibit the activity of sirolsmein PL, the necrosis toxin produced by L. maculans is being tested.

Detection of bacterial fruit blotch in watermelon and cantaloupe seeds by seedling PCR. P. S. RANDHAWA (1), H. S. Sohi (1), S. S. Panu (1), N. K. Randhawa (1), and N. W. Schaad (2). (1) California Seed & Plant Lab, 7877 Pleasant Valley Dr., Etiwanda, CA 92562; (2) USDA/ARS, Ft. Detrick, MD 21702. Phytopathology 93:S72. Publication no. P-2003-0529-AMA.

A seedling grow out test is commonly used to detect seed-borne infections of Acidovorax avenae subsp. citrulli, the causal organism of fruit blotch. In this test, seeds are grown in a greenhouse under high temperature and humidity.
After 21 days the seedlings are observed for symptoms. While a grow-out test can be effective in spring and summer, it is less reliable in winter months in California due to sub-optimal seedling growth. Therefore, we have developed an alternative seedling PCR test. In this test, 1,250 seedlings are grown in vermiculite in flats (24" x 12" x 5") with clear plastic covers under 16 hr lights at 25°C. After 12 days, the contents of each box are extracted in 3000 ml of water and 50 ml centrifuged. DNA is extracted from the bacterial pellet and tested by real-time PCR. In a comparison of 100 seed lots tested by the seedling PCR and grow-out methods, 3 lots were found positive by both methods in spring/summer months whereas 4 lots were found positive only by seedling PCR in winter months.


Two M. nivale varieties, majus and nivale, have been reported based on conidial morphology,RAPD markers and ITS region of rDNA. Only vari. nivale has been found on turfgrass. To examine the variation among M. nivale isolates collected from Wisconsin golf courses, RAPD markers were compared among 96 single spore isolates. Two groups were strongly distinguished by the RAPD patterns. A major group (group A) was represented by isolates obtained from northwest (NW) and southwest (SW) regions, while group B was only represented by isolates from NW. Selected isolates from each group were then examined using variety-specific PCR primers and conidial morphology. No differences in shape, conidial length and width were detected among the isolates. Following PCR with primers specific to majus, two groups were observed only with majus isolate obtained from ATCC. DNA from group A amplified only with nivale primers. No amplification product was observed in group B with any of the primers, indicating the possibility of a distinct group. Additional isolates and comparative research are required to validate this hypothesis.


The causal agent of tomato pith necrosis, Pseudomonas corrugata produces antimicrobial compounds and is an effective biological control agent. Limited information on the host range of this bacterium precluded its use as a biological control agent in the field. 2,947 mini-Tn5 insertions in P. corrugata 0782-6 strain were screened for loss of pathogenicity. Four mutants were non-pathogenic to tomato plants but retained their antimicrobial activity. These mutants were screened for their ability to suppress common root rot of peas, caused by Aphanomyces euteiches, in the greenhouse and the field. The non-pathogenic mutants proved superior to the wild-type P. corrugata 0782-6 strain for biological control purposes. A 3541 bp region of chromosomal DNA from 0782-6 was used to complement the mutant with the best antimicrobial activity. Sequence analysis of the 3541 bp region indicated that this fragment may be involved in synthesis of transmembrane proteins, which could regulate the translocation of toxins or enzymes across the bacterial membrane.

Comparison of early and late inoculations of soybean with Bean pod mottle virus, M. G. Redinbaugh (1), J. L. Vacha (1), S. A. Berry (2), and A. E. DORRANCE (2). (1) USDA-Agricultural Research Service; (2) Dept. of Plant Pathology, The Ohio State University, Wooster, OH 44691. Phytopathology 93:S73. Publication no. P-2003-0532-AMA.

In recent years, Bean pod mottle virus (BPMV) has emerged as a new pathogen of soybeans in Ohio along with increased populations of its vector, the bean leaf beetle. In addition, higher incidence of seed coat motting and soybean stems that remain green after pods mature have occurred. Nine northern soybean cultivars and two germplasm lines were evaluated in a field study to determine the effects of early and late virus inoculations. Early inoculation (unifoliate growth stage) with BPMV resulted in foliar symptoms, amplification was observed only with a majus isolate obtained from ATCC. DNA from group A amplified only with nivale primers. No amplification product was observed in group B with any of the primers, indicating the possibility of a distinct group. Additional isolates and comparative research are required to validate this hypothesis.

Phytopathology 93:S73. Publication no. P-2003-0533-AMA.

Lyso bacter enzymogenes strain C3 is a biocontrol agent of several fungal diseases. C3 produces several extracellular enzymes, such as chitinases and beta-1,3-glucanases, which are implicated in biocontrol activity of this bacterium. Recently, a type III protein secretion system (TIII iP) thought to be involved in fungal antagonism has also been identified in strain C3. Although the exact function of the TIII iP system in C3 has yet to be established, RT-PCR data indicates that the pathway is induced in the presence of fungal cell wall constituents. Additional studies indicate the TIII iP system is differentially regulated compared with extracellular enzymes in strain C3, suggesting these traits associated with fungal antagonism are modulated by different mechanisms.


Pecan trees in a five-year-old orchard of 17 cultivars displayed symptoms of an unusual bark canker first noticed in October 2002. Symptoms appeared from ground line up to 3 meters on the central leader and most likely were initiated during the summer of 2002. Cankers developed around buds of the trunk and central leader and were reddish brown, about 2 cm x 4 cm, ovoid and depressed. Chlorophyll degradation, commonly on the southwest side, but some also on the northern side of the tree. Vertical cracking of the cankered bark was increasingly evident as the diameter of affected wood increased. Affected bark, 2 - 3 mm thick, peeled back and dropped leaving a light gray area of under bark which did not appear to be damaged. Fungi associated with twig and limb dieback (Botryosphaeria spp. and Phomopsis sp.) were detected in the necrotic tissue, but not in healthy tissue.


The root knot nematode, Meloidogyne incognita, causes severe crop loss on almost all vegetable crops in the humid tropics. The phase-out of Methyl bromide will require alternative control techniques to improve IPM for sustainable vegetable production in many areas of the world where pesticides are used for the first time. A bacterial antagonistic rhizobacteria (PHPR) have been shown to be effective in biocontrol of root-knot nematodes. The presence of these biological control agents in the root leads to: induced resistance, decreased juvenile penetration, retardation of nematode development or reduced fecundity as well as increased plant growth. Dual inoculation of these two microorganisms may lead to synergistic effects on plant health and thereby sustainable vegetable production. Studies on the interaction between nematode antagonistic PHPR and AMF demonstrated some bacterial isolates are able to enhance mycorrhizal colonization in the root while simultaneously reducing nematode infection. Results on these interrelationships will be presented and future IPM strategies will be outlined.


Plant mitogen-activated protein kinases (MAPKs) are involved in a plethora of signaling pathways associated with stress response and likely act as a converging point for different external stimuli. Up to date, most of the plant MAPKs that have been characterized are from dicotyledonous model species. In this study, we have isolated and characterized a novel jasmonic acid (JA) inducible MAPK gene (OsMAPK6) from rice. The OsMAPK6 DNA is 2384 bp long and encodes a 599-amino acid protein. RNA blot analysis showed that OsMAPK6 was rapidly induced by JA within 30 minutes, peaked at four hours, and decreased six hours after treatment. However, OsMAPK6 was not induced by salicylic acid or benzothiadiazole. Pre-treatment of rice seedlings with JA transiently reduced nematode infection. Results on the interaction between nematode antagonistic PHPR and AMF demonstrated some bacterial isolates are able to enhance mycorrhizal colonization in the root while simultaneously reducing nematode infection. Results on these interrelationships will be presented and future IPM strategies will be outlined.

Assessing the population genetic structure of Cronartium ribicola among multiple hosts and environments. B. A. RICHARDSON (1, 2), N. B. Klopfenstein (1), P. J. Zambino (1), and L. M. Carris (2). (1) USDA Forest Service, RMRS, Moscow, ID; (2) Dept. Plant Pathology, Washington State University, Pullman. Phytopathology 93:S73. Publication no. P-2003-0537-AMA.

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Cronartium ribicola (CR), the causal agent of white pine blister rust (WPBR), has been a major contributor to the decline of five-needle pines in North America. Since its introduction into western North America nearly 100 years ago, CR has spread from Vancouver, B.C. to Wyoming and New Mexico. Phytoplasm, pathogen/ host and evolutionary processes in a pathosystem can be inferred from the genetic structure of extant populations. The interaction of resistance and virulence in WPBR suggests that some CR populations are locally adapted. A population genetics analysis using amplified fragment length polymorphisms (AFLPs) will be conducted on populations from different hosts and environments. AFLP profiles will be analyzed for correlations with host species, canker age, and environmental factors. Knowledge of population genetic structure and host specialization will be useful for host resistance screening, gene deployment, and predicting pathogen adaptation.

Wood stake decomposition on fertilized sites in the northwestern United States. R. C. RIPPY (1,2), N. B. Klopfenstein (1), M.-S. Kim (1), P. J. Zambricki (1), D. S. Page-Dumroese (1), J. A. Moore (2), and P. A. McDaniel (3). (1) USDA Forest Service - RMRS, Moscow, ID 83843; (2) Dept. of Forest Resources; (3) Dept. of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID 83844. Phytopathology 93:S74. Publication no. P-2003-0538-AMA.

Decomposition of soil organic matter is important to the long-term productivity of forest soils. This study is aimed toward evaluating the effects of forest fertilization on wood-decomposing fungi. Decomposition rates of wood stakes from different tree species (lobolly pine, aspen, and Douglas-fir) are being examined at six fertilized sites in Idaho and Washington to compare diversity across different soil and habitat types. Wood stakes were installed in the mineral soil and at the mineral soil/litter interface. Fungi will be isolated from stakes collected at six-month intervals. Molecular techniques (ITS sequencing) and morphological examination will be used to identify fungal isolates. Preliminary results will be presented.

Survey of Colletotrichum spp. isolated from mango in Puerto Rico and Florida. L. RIVERA (1), Y. Lugo (1), R. Mceveen (2), T. Seijo (2), and M. Davis (2). (1) Dept. of Crop Protection, Univ. of Puerto Rico, Mayagüez, P.R. 00681; (2) Dept. of Plant Pathology, Univ. of Florida, Gainesville, FL 32611. Phytopathology 93:S74. Publication no. P-2003-0539-AMA.

A survey of Colletotrichum spp., causing typical anthracnose lesions in mango, was conducted in Puerto Rico and Florida. Morphological, serological and molecular characteristics were examined in 185 isolates. Over 85% of the isolates were identified as C. gloeosporioides (Cg) in Puerto Rico and Florida. Only 5% were C. acutatum (Ca) occurring only in Homestead, Florida. Pathogenicity tests performed on detached leaves and fruits showed that both species were pathogenic to mango. Necrotic lesions produced orange to salmon conidial masses on acerul, 7 days after inoculation. Lesion size ranged from 20-50 mm for Cg, and 7-30 mm for Ca. Colonies showing aerial mycelium were white, gray and/or dark gray, often with conidial masses in PDA. Isolates produced hyaline one-cell, ovoid to oblong, straight or slightly curve conidia, with sizes ranging from 3-20 µm in Cg and 2-12 µm for Ca. ELISA and PCR were used to confirm our findings.


Spartina alterniflora (smooth cordgrass) is the predominant plant species found throughout the vast coastal marshes of Louisiana and the gulf coast region. This species plays a vital role in providing salt marsh habitats and in physically stabilizing coastal wetlands along the northern gulf coast. These habitats are essential for migrant and local bird populations and as nurseries for coastal marine species. It is estimated that 30 percent of US fishery production is derived from Louisiana’s coastal zone. Recently, approximately 158,000 ha of the coastal marsh experienced a rapid dieback, known as the brown marsh syndrome. Not only has this syndrome resulted in an accelerated loss of irreplaceable coastal wetlands, it has also increased concern for the future of restoration efforts in the coastal marshes and barrier islands. Projects related to determining the cause of this dieback and to coastal restoration will be described.


Xanthomonas campestris pv. vitianus, causal agent of bacterial leaf spot on lettuce, has been recognized as a problem in the Florida Everglades. Experiments were conducted to determine the optimum temperature for disease development, host range on agronomic crops (lettuce, endive, mustard, chicory, tomato and pepper) by quantifying internal populations and the effects of wounding on disease development. In temperature studies performed in growth chambers at 15, 20, 25, and 30°C, optimum disease occurred at 25°C. In population experiments in lettuce and endive, populations reached 10^9 and 10^7 CFU/cm2 leaf tissue, respectively. The high populations in endive may indicate that it is a host. Mustard and chicory populations reached maximum levels of only 10^9-10^7 CFU/cm2 and may indicate that these two crops are non-hosts. In wounding experiments where the tissues of chicory, mustard, lettuce, and cress were abraded with sand or carborundum, disease severity compared to non-wounded inoculated plants increased in lettuce only.


Although numerous reports underscore the importance of silicon (Si) in controlling Magnaporthe grisea on rice, no study has associated this effect with biochemical changes commonly involved in host defense responses to this fungal attack. A 2.5-fold increase in the concentration of monolactones A and B was observed in WBC extracts from Si^- plants as compared to leaf extracts from Si+ plants at 96 h after inoculation. Neither two phytoalexins were detectable in leaf extracts from non-inoculated Si^- and Si+ plants. This stimulation of the terpenoid pathway in Si^- plants linked with the increase in the levels of monolactones A and B is possibly a major factor contributing to enhanced blast resistance and helps to explain the smaller lesion size observed on leaves of Si^- plants. This result provides the first evidence of a biochemical basis for Si-mediated blast resistance.


Commercial carrot production systems in Wisconsin rely upon repeated fungicide applications to manage two leaf blights caused by Alternaria dauci and Cercospora carotae. During 2002, five field experiments were conducted that evaluated the efficacy of reduced-risk fungicide programs, field resistance of cultivars and breeding lines, host resistance × fungicide programs and weather-based fungicide programs × host resistance × disease thresholds for initiating fungicide treatment. New fungicide chemistry and increased levels of resistance to foliar pathogens offer effective, reduced-risk alternatives to currently registered products and plant cultivars. Significant differences (P < 0.05) were observed in the disease reaction of 40 cultivars and breeding lines at two locations. Weather-based fungicide programs reduced fungicide inputs while maintaining adequate disease control and high yields. By integrating available management tactics, growers have the necessary tools to sustain carrot production using fewer chemical inputs on disease tolerant cultivars without sacrificing disease control, yield or quality.

Biochemical evidence of bacterial exopolysaccharide production in grapevines infected with Xylella fastidiosa, the causal agent of Pierce’s disease. M. C. ROPER (1), L. C. Greve (2), J. Stevenson (3), J. Labavitch (2), and B. Kirkpatrick (1). (1) Dept. Plant Pathology; (2) Dept. Pomology; (3) Section of Plant Biology, University of California, Davis, CA 95616. Phytopathology 93:S74. Publication no. P-2003-0544-AMA.

Xylella fastidiosa (Xf) colonizes xylem elements of grapevines and during this process the vessels become occluded. Tyloses originate from grapevine xylem parenchyma cells, but the origin of the occluding “gels” is unclear. Bacterial exopolysaccharide (EPS) could be a component of these gels. The Xf genome sequence provides evidence that Xf is capable of producing an EPS similar to xanthan gum produced by Xanthomonas spp. The genome-based EPS structure consists of a glucan backbone substituted with Man-GlcA disaccharide side chains. Gas Chromatography and Mass Spectrometry analysis of polyalcohol and xylem sap from Xf infected grapevines showed a significantly higher content of GlcA and Man as compared to controls. The extracts also showed elevated levels of Gal, GalA, and Ara, constituents of plant.
pectins. These data suggest that the occluding gels in X infected grapevines probably consist of polysaccharides of both host and pathogen origin.


An experimental use permit (EUP) allowed WI potato growers to use azoxystrobin (AZ) fungicide for control of Alternaria solani (early blight) during 1998, AZ received registration in 1999. In 1997-98 field trials, disease progress curves (DPC) for spray programs with AZ treatments resembled flat lines. However, over the past four growing seasons (1999-2002), the DPC’s in field trials treated with AZ (3 sprays) were significantly lower than the baseline 1998 isolates. Variability of foliar, glyphosate application, and US. An IPM program for tomato production with Dactylaria higginsii at Universiti Putra, Selangor, Malaysia. Phytopathology 93:S75. Publication of Plant Pathology, University of Florida, Gainesville, FL 32611; (3) Charudattan (2). (1) USHRL, USDA-ARS, Fort Pierce, FL 34945; (2) Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611; (3) Universiti Putra, Selangor, Malaysia. Phytopathology 93:S75. Publication no. P-2003-0454-AMA.

Dactylaria higginsii is a potential bioherbicide for purple nutseed Cyperus rotundus, one of the most difficult to control weeds in crops in the southern US. An IPM program for tomato production with D. higginsii as one of the components was designed. The system, utilizing fallow season and in-season treatments, was tested in 2001 and 2002. Three fallow treatments (disk fallow, glyphosate application, and D. higginsii application) were combined with in-season treatments of Telone C-33, Telone C-35/Tillam, and Telone C-35/D. higginsii. In the first year, only the in-season treatments had significant effects on purple nutseed density and D. higginsii did not provide a significant level of control. In the second year of study, the fallow season treatments had significant effects on purple nutseed density, with glyphosate-treated plots having significantly fewer nutseed emerging through plastic during the cropping season.


Disease incidence and damage due to Citrus tristeza virus (CTV) in India varies in the different citrus growing regions. Genotype profiles were determined for twenty-one Indian CTV isolates using genotype-specific multiple molecular markers (Hill et al., 1999, Phytopathology 89:336-342) that classify CTV isolates into four genotypes based on amplification of PCR product from four regions of the CTV genome. Fifteen isolates contained only the VT genotype. The other isolates were mixtures of either T30 or T3 with VT or T30, T3 and VT genotypes except isolate BAN-1 which contained a mixture of T36, T30 and T3 genotypes and isolate CTV-B which was classified as a non standard molecular genotype. The T30 genotype-specific primer amplified 696 bp of isolates BAN-1, BAN-2, B194 and B219 from nucleotide 10,722 to 11,467 of the overlapping region of ORF 1b and ORF 2. The blast analysis of the PCR products showed that these four isolates are closely related to the mild T30 isolate. The genotype profiles and sequence analysis revealed that Indian CTV populations contain T30-like genotypes mixed with different T36-, T3- and VT-like severe genotypes.

Cabbage leaf curl disease in Jamaica includes a mixed infection with a recombinant geminivirus. M. E. ROYE (1), K. N. Smith (2), I. I. Haye (1), W. M. McLaughlin (2), and D. P. Maxwell (3). (1) Biotechnology Centre; and (2) Department of Basic Medical Sciences, University of the West Indies, Mona; (3) Department of Plant Pathology, University of Wisconsin, Madison, WI. Phytopathology 93:S75. Publication no. P-2003-0548-AMA.

In 1994, cabbage plants exhibited chlorosis, leaf curling and other leaf deformation symptoms. Nucleotide sequence analysis of full-length infectious clones showed that the cabbage plants were infected with cabbage leaf curl virus (CaLCV) from Florida. Nucleotide sequence of a second DNA-A clone (CRFA4) was 84% similar to CaLCV. Closer sequence analysis of CRFA4 showed that it was a recombinant virus. A field survey of cabbage plants showed that 55% of symptomatic plants were infected with CabLCV and 10% with the recombinant virus. One cabbage plant was found infected with macroptilium golden mosaic virus from Jamaica (MaGMV-JM). This suggests that the recombinant virus is transmitted by CabLCV. Although several geminiviruses have been reported from Jamaica this is the first report of a recombinant virus (Roye et al. at 1997, Roye et al 1999). References: Roye et al1997. Plant Dis. 81:1251-1257; Roye et al. 1999. Trop. Agricul. 76:256-262; Zhou et al. 1997. J. Gen. Virol. 78:2101-2111.


Fusarium infested surface debris on soil is considered the primary source of inoculum for Fusarium head blight (FHB) epidemics. Fifty wheat fields in Minnesota were surveyed for soil populations of FHB pathogen. Five surface (0-2 cm depth) soil samples (26 g) were collected 1 m apart along each of two 5 m transects, at least 30 m from the field edge. Samples from each transect were air dried for 4 d at 20-24°C, and sieved through 500 and 250 micron sieves. Aliquots of 1 mg of soil (251-499 micron particle size) were siften onto Komada’s medium, and incubated at 20-24°C for 14 d. Populations of F. graminearum (FG) ranged from 18 CFU/g to 1435 CFU/g. The highest FG populations were found after corn as rotational crop (1159 CFU/g). Populations of FG after soybeans (356 CFU/g) and sunflowers (341 CFU/g) were significantly less than after wheat (642 CFU/g) and dry beans (497 CFU/g). Populations of F. graminearum, F. avenaceum, and F.avenaceum were also high after corn. Data shows that crop rotation, influences the populations of Fusaria present in soils. These findings may assist in the cultural management of Fusarium head blight.


Seventeen pathogenic strains of Pseudomonas cichorii (Pc) were isolated from coffee, Coffea arabica, leaf samples collected from nurseries in eight municipalities of Puerto Rico. Two different inoculation methods were evaluated under in vitro conditions. Inoculation of plant-attached old and young leaves grown under greenhouse conditions and plant-detached young coffee leaves grown under field conditions. Pc was more virulent in older leaves indicating different resistance mechanisms based on leaf age. Both inoculation methods were reliable in identifying resistant genotypes. Three commercial varieties of coffee (Borbón, Pacas and Caturra) were susceptible to bacterial leaf blight while the coffee species Coffea liberica var. Excelsa and Coffea canephora var. Robusta were resistant.

Infection of chile by Phytophthora capsici in relation to soil salinity. S. M. CARREON, New Mexico State University, Dept. of Entomology, Plant Pathology, and Weed Science, Las Cruces, NM 88003. Phytopathology 93:S75. Publication no. P-2003-0551-AMA.

Soil salinity and Phytophthora root rot caused by P. capsici are major concerns in chile production in New Mexico. Soil salinity has been shown to increase plant susceptibility to other species of Phytophthora. It is not known how salinity affects infection by P. capsici. The joint response of chile to salinity and infection by P. capsici was assessed under greenhouse conditions. Additionally, the effect of salinity on mycelial growth and sporangium production by P. capsici was evaluated in the laboratory. Salinity treatments consisted of 5 levels of electrical conductivity (ranging from 1.5 dS/m to 14 dS/m), which were achieved by amending irrigation media with a mixture of sodium chloride and calcium chloride. Disease severity increased with increasing salinity levels. Mycelial growth and sporangium production were not significantly affected by salinity treatments. These results indicate that salinity may exert predispositional effects on chile that promote plant infection by P. capsici.

Identification of the nucleotide substitutions required for Barley stripe mosaic hordeivirus pathogenicity to barley possessing the rsm1 gene. A. Santoso (1) and M. C. EDWARDS (2). (1) North Dakota State University, Dept. of Plant Pathology, Fargo, ND; (2) USDA-ARS Cereal Crops Research Unit, Northern Crop Science Laboratory, Fargo, ND 58105-5677. Phytopathology 93:S75. Publication no. P-2003-0552-AMA.

BSMV strains vary in pathogenicity to hosts such as barley and oat. The barley varieties ‘Morse’ and ‘Modjo-1’ are susceptible to strain CV42. Using recombinant viruses generated from ND18 and CV42, we mapped the
determinants of BSMV pathogenicity to these hosts. Although pathogenicity determinants mapped to the same region previously identified as critical to oat pathogenicity, the specific amino acid substitutions required are not identical. We previously showed that pathogenicity to oat could be conferred to ND18 by as little as a single nt change in the sequence of RNA alpha, resulting in a single amino acid substitution (P-724 to T-724). We now show that pathogenicity to barley is not affected by the PT substitution and that other amino acid substitutions in the same region are essential. The amino acid substitutions may result in conformational changes that influence the functionality of the alpha-a gene.

Response of healthy and diseased peanut pods to calcium nutrition. C. SAUDE (1), H. A. Melouk (1), and M. Payton (2). (1) USDA-ARS and Department of Entomology and Plant Pathology; (2) Department of Statistics, Oklahoma State University, Stillwater, OK 74078. Phytopathology 93:S76. Publication no. P-2003-0553-AMA.

Greenhouse grown peanut plants of ‘Okrun’, a Sclerotium rolfsii-susceptible cultivar, were fertilized with calcium sulfate at two rates (2, 272 Kg/ha and 3,409 Kg/ha) at 75 days after planting (DAP). Calcium content in supplemented soil was ~800 ppm, whereas residual calcium was ~450 ppm. Disease was induced by inoculating plants with S. rolfsii at 100 and 120 DAP. At harvest (150 DAP), mean pod yield on healthy plants was significantly (P = 0.05) higher than those of diseased plants. Calcium content (%) in hulls of pods from healthy and diseased plants receiving calcium supplementation was about 40% higher than those in the controls. Content of calcium in kernels of healthy pods was similar in calcium and non-calcium supplemented plants; however, calcium content in kernels of diseased pods with calcium supplementation was about twice those in the controls. The reason for the increase in calcium content in diseased kernels may be due to chelation by oxalic acid produced by the fungus.

**Xylella fastidiosa taxonomy.** N. W. SCHAAK (1), E. Postnikova (1), M. Fatmi (2), G. H. Lacy (3), and C. J. Chang (4). (1) ARS-USDA, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD; (2) Calif. Dept. Food Agric., Sacramento, CA; (3) USDA/ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD; (4) USDA/APHIS, San Francisco, CA; (5) USDA/APHIS, San Francisco, CA; (4) USDA/APHIS, Los Angeles, CA. Phytopathology 93:S76. Publication no. P-2003-0555-AMA.

**Xylella fastidiosa (Xf) taxonomy.** This is a single species but genetic studies support multiple taxa. To clarify Xf taxonomy, we used DNA homology (S, nucleic procedure) and ITS sequencing to compare 26 strains from 10 hosts. Using stringent conditions (Tm~8 deg C), DNA homology revealed three taxa, with more than 70% homology. Taxon A (grape, alfalfa, maple, and two almond strains), taxon B (peach, elm, plum, wild grape, one almond, and sycamore strains), and taxon C (citrus strains) with mean homologies of 85, 82, and 87%, respectively. Mean reciprocal homologies between taxa A, B, and C were 56, 39, and 46%, respectively. ITS results confirmed the same taxa; A and B, A and C, and B and C had similarities of 98.7, 97.9, and 99.2, respectively. Taxon A strains grow well on PD2 agar but B and C strains do not. Taxon B strains are susceptible to penicillin but resistant to carbencillin and A strains are opposite. We propose taxa A, B, and C be named X. fastidiosa subsp. piercei, agglomeri, and idiorrapusa, respectively.

Evaluation of a on-site, one-hour real-time PCR assay for detecting the citrus canker bacterium in plant samples at port facilities. N. W. SCHAAK (1), D. Oppenorth (2), M. D. Pettitolo (3), M. A. Abdelshife (4), and T. Tedla (4). (1) USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD; (2) Calif. Dept. Food Agric., Sacramento, CA; (3) USDA/APHIS, San Francisco, CA; (4) USDA/APHIS, Los Angeles, CA. Phytopathology 93:S76. Publication no. P-2003-0557-AMA.

The expanding global economy, free trade agreements, and increased air travel. These results show that Xcc is being intercepted at California ports and direct real-time PCR using a Smart Cycler. The total time to assay 14 samples was 45 to 55 min. PCR assays of canker samples collected by inspectors at LAX and SFO ports showed that 7 of 8 and 15 of 28 were positive, respectively. Viable Xcc was recovered from two samples at each port. These results show that Xcc is being intercepted at California ports and that real-time PCR can provide rapid on-site results.


Root knot nematodes (RKN) elicit dramatic morphological changes in susceptible plants and we are interested in the concomitant global changes of host gene expression. We previously used a subtractive cDNA cloning approach to identify approximately 200 tomato genes differentially expressed at nematode larval sites. We routinely scour databases for their homologues and are experimentally analyzing genes with interesting identities. To find additional candidates, we have initiated a cDNA micro-array screen. We identified EST clones defining approximately 4,700 unique genes expressed in tomato roots at TIGR, and to date have arrayed over 1/3 of these genes. In addition, we have assembled a microarray of 768 50-mer oligos defining genes with probable function in giant cell and gall formation. We are currently using our arrays to define temporal changes in tomato root gene expression associated with RKN infection, and also to identify differences in gene expression induced by various species of Meloidogyne, as well as assessing parallel investigations using oligo arrays representing the approximately 16,000 predicted genes of the Medicago genome.


Snow molds, caused by Typhula species, are devastating, psychrophilic fungi that damage turfgrasses and winter cereal crops in the Northern Hemisphere. In Wisconsin, snow mold causes extensive damage to golf courses and sod farms. Over two years, we collected 3,000 samples of snow mold from 100 golf courses across WI, noting coordinates of collection site, temperature zone, duration of snow cover, species of grass, and age of fairway. DNAs of 300 representative isolates were amplified by PCR, and 18S rDNA sequences were determined using the most. GIS programs were used to visualize distribution. Results will be used to help WI turf managers more effectively control Typhula and help researchers gain better understanding about the effects of environment on the fungus.

**Seasonal dynamics of conidial production in the peach scab fungus Cladosporium carpophilum.** H. SCHERM (1), A. T. Savelle (1), R. T. Boozer (2), and W. G. Foshee (2). (1) University of Georgia, Athens, GA 30602; (2) Auburn University, Auburn, AL 36849. Phytopathology 93:S76. Publication no. P-2003-0559-AMA.

Conidia from lesions on 1-year-old twigs constitute the only source of primary inoculum for the peach scab fungus, Cladosporium carpophilum, yet there is little quantitative information about the dynamics of conidial production throughout the season. Starting in late winter, twigs were sampled every 1 to 2 weeks from peach trees untreated with fungicide from a total of 18 site-cultivar-year combinations in the southeastern U.S. Twigs were incubated in a moist chamber, washed, and conidial production potential was determined by microscopic counts in aliquots of the wash water. While the curves summarizing conidial primers differed markedly among sites and years when plotted against day of the year, curves were very similar when numbers were expressed as cumulative totals using date of full bloom as a starting point. Conidial production commenced around bloom and increased logistically to reach 25% and 90% of the cumulative seasonal total by shuck split and 10 weeks after bloom, respectively. Effects of environmental factors on conidial production potential will be discussed.


Eutypa dieback, caused by Eutypa lata, is a threat to longevity of grapevines throughout the world. A project was initiated to describe the progression of Eutypa dieback in a vineyard over time and to develop a reliable method for detection of the pathogen. A ‘Concord’ vineyard in southwest Michigan was visually monitored from 1999 to 2002 for typical Eutypa symptoms (cupped leaves, chlorosis, and stunting of shoots). The incidence of symptomatic vines ranged from 1% in 1999 to 0.6% in 2000, 4% in 2001, and 17% in 2002. None of the vines showed foliar symptoms every year and a few vines died. On average, infected vines had fewer shoots than apparently healthy vines, but shoot number did not decline uniformly. Two morphologically similar fungi were isolated from Michigan vineyards with Eutypa dieback: Eutypella vitis and Eutypella phacorrhiza. Both were genetically identical as determined by sequencing of the ITS region. Both fungi occurred in the same vineyards. The role of E. vitis in disease development is not known. PCR probes were designed for both species and evaluated for detection of these fungi in culture and in infected wood.

Oxalic acid is an important product of fungal metabolism and may play a mechanistic role in fungal degradation of wood. Oxalic acid may mobilize iron and decrease extracellular pH to initiate Fenton-based decay. Also, exchangeable cations may complex with oxalate near the hyphal wall creating a pH gradient. In this study, Melampsora incrassata and Fomitopsis pinicola isolates were grown in basal salts media with modified CaCl2 and MgSO4 concentrations. Oxalate was monitored over four weeks using ion exchange HPLC specifically adapted to resolve oxalate from N-containing compounds. For both fungi, the highest Ca treatment yielded the lowest pH and the highest ratio of soluble/total oxalate. Soluble oxalate was significantly higher in low Ca treatment on yield basis. No concern in the pH gradient between treatments remained constant. Different Mg concentrations did not significantly affect oxalate production. Results also show a clear difference in soluble/insoluble oxalate partitioning between the two species and exhibit significant calcium treatment effects not seen previously with Postia placenta.

Night-time spore deposition of the Fusarium head blight pathogen, Gibberella zeae. D. G. SCHMALZE III (1), E. J. Shields (2), and G. C. Bergstrom (1). (1) Departments of Plant Pathology; and (2) Entomology, USDA-ARS, Parlier, CA 93648; (2) Univ. of California-Davis, Salinas, CA 93905. Phytopathology 93:S77. Publication no. P-2003-0561-AMA.

Temporal patterns of spore deposition of the Fusarium head blight pathogen, Gibberella zeae, were observed over two wheat fields, 0.5 km apart, in Aurora, NY in May/June 2002. More than 88,000 colonies of G. zeae were collected over 20 consecutive dates (40 day/night sampling periods) on a total of 520 wheat spikes. In this selective medium placed 30 cm above wheat spikes. Viable spores were deposited at every sampling period spanning total of 3,840 Petri plates with selective medium. Deposition events (>50 colonies, on average, per plate) were collected at night (sunset to sunrise). Seven major deposition events occurred concurrently of the colonies were collected at night (sunset to sunrise). Seven major deposition events (>50 colonies, on average, per plate) occurred concurrently in the two wheat fields, at all night, and three of these were coincident with rainfall. Cumulative exposure of wheat spikes to viable, airborne spores and the deposition of these spores mainly at night should be considered in the development of model risk models and management strategies for Fusarium head blight.

Alternatives to methyl bromide for vineyard replant – results of ongoing field trials. S. M. SCHNEIDER (1), T. J. Trout (1), G. T. Browne (2), H. Ajwa (3), and J. Sims (4). (1) USDA-ARS, Parlier, CA 93648; (2) USDA-ARS, Davis, CA 95616; (3) Univ. of California-Davis, Salinas, CA 93905; (4) Univ. of California, Riverside, CA 92521. Phytopathology 93:S77. Publication no. P-2003-0562-AMA.

Field evaluation of potential methyl bromide alternatives for vineyard replant must determine not only efficacy of pathogen control at the time of planting the new vineyard, but also the efficacy of pest control and impact on crop growth and yield during the early growth and fruiting years. This paper reports the on-going performance of chemical, cultural, and genetic control measures in field trials planted in 1998 and 2000. Sixty-five year old grapevines were planted in 2001 and 2002 in fields infested with plant parasitic nematodes. Registered and experimental chemicals were applied by shank-injection or drip-irrigation tubing. Soil samples were collected at planting and at harvest to determine nematode populations. Grapevine and berry plants were graded at harvest according to commercial standards. Tree crops will be harvested and graded after the second growing season.

Although there were differences across varieties and rootstocks, iodo-methane, propargyl bromide, 1,3-dichloropropene-chloropicrin, and chloropicrin alone generally controlled nematode populations and resulted in galle-free root systems.


The effect of cropping systems, tillage practices and soil fertility on the occurrence of soil-borne pathogens and nematodes, their interaction and the effect on the yield of cereals is a major concern in the eastern Mediterranean under arid and semi-arid environments. The long-term trials of the International Center for Agricultural Research in the Dry Areas (ICARDA) located in Tel Hadya and Breda, Syria, were used in the present studies. No effect of the fertilizer regime was observed towards the cereal cyst nematode Heterodera latipons or common root rot caused by C. sativus. Barley-vetch rotation is a useful measure for barley yield stability in both agro-ecological zones despite higher levels of H. latipons and C. sativus infestation. However, ten years mono-culture did not result in an inoculum build up of H. latipons and C. sativus with a measurable yield impact. The additional application of compost, demonstrating a H. latipons and C. sativus suppression in barley and wheat, might be an additional control measure for infestation patches in barley and wheat.


The Gram negative soil bacterium, Sinorhizobium meliloti, forms a nitrogen-fixing symbiotic relationship with alfalfa (Medicago sativa) and Medicago trunculata. The DNA sequence of S. meliloti 1021 has been determined and estimated to contain over 6000 open reading frames (ORFs) located on the chromosome and two “megaplasmids” pSymA and pSymB. Using the sequence information, we are carrying out a project involving large-scale genetic manipulation of the S. meliloti genome. Phase one involves cloning of the predicted ORFs into a modified “entry” plasmid for the GATEWAY integrase-mediated recombination cloning system. Phase two of the project involves the transfer of the cloned ORFs into various destination vectors to evaluate the use of these expression vectors for various genetic manipulations with S. meliloti. The resultant constructs will enable expression of predicted proteins, mutation of predicted ORFs and the ability to monitor gene expression using reporter gene fusions. Access to these constructs will be useful in large- and small-scale analysis of S. meliloti.

Impact of root diseases on the health of direct-seeded wheat and barley. K. L. SCHROEDER (1) and T. C. Paulitz (2). (1) Washington State University, Dept. of Plant Pathology, Pullman, WA 99164; (2) USDA-ARS, Pullman, WA 99164. Phytopathology 93:S77. Publication no. P-2003-0566-AMA.

Root diseases of dryland wheat and barley are a primary deterrent to the use of direct seeding in eastern Washington. Previous studies on the impact of tillage on root diseases have reported conflicting results. Two field sites with histories of tillage or direct seeding were selected for study. At each location, plots with and without tillage or inoculated with Rhizoctonia solani AG-8 and Gaeumannomyces graminis var. tritici were initiated. With tillage, there were significantly more tillers and more crown roots than in direct-seeded plots at the same site. Plant height, seminal root number, Rhizoctonia root rot disease incidence and severity, and population density of Pythium spp. did not differ between tillage types, but take-all was more severe in tilled plots although the disease levels were relatively low. There was no difference in yield between tillage types or locations except for an 18% decrease in direct-seeded spring wheat in the first year. These data suggest that differences may be the result of factors other than tillage such as cooler soils or undetected pathogen influences.

Differential susceptibility in cultivars of sweet onion to pink root. K. W. SEEBOULD, Jr. (1), D. B. Langston, Jr. (1), G. E. Boydhan (2), R. L. Torrance (3), and M. J. Cook (3). University of Georgia, (1) Dept. of Plant Pathology, Tifton, GA 31793; (2) Dept. of Crop Science, NC State University; (3) Tattanll Co. CEC, Reidsville, GA 30453. Phytopathology 93:S77. Publication no. P-2003-0567-AMA.

Pink root, caused by Phoma terrestris, is a soilborne pathogen that reduces the yield and quality of sweet onions produced in Georgia annually. In 2002,}

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thirty-one cultivars of sweet onion were evaluated at harvest for symptoms of pink root on root systems to determine the relative susceptibilities of each to the disease. Root systems were evaluated on a 0-10 scale where 0 = no symptoms, 5 = 50% of root system discolored or decayed, and 10 = 100% discoloration or loss. Two of the more widely grown cultivars in GA, ‘Sweet Vidalia’ and ‘Sweet Melissa’, were moderately susceptible to pink root. Highly susceptible cultivars tended to be early maturing types, while the majority of the cultivars least susceptible to pink root matured later. Planting later maturing varieties with resistance to pink root may be useful where disease severity has been high; however, late maturity has been associated with higher incidence of bacterial diseases due to warmer temperatures and should be considered.

Biological variation among wheat streak mosaic virus isolates. D. L. SEIFERS (1), R. French (2), D. Stenger (2), and T. J. Martin (1). (1) Kansas State Univ., ARCH, Hays, KS; (2) USDA-Agricultural Research Service and Department of Plant Pathology, University of Nebraska, Lincoln, NE. Phytopathology 93:587. Publication no. P-2003-0568-AMA.

Biological variation among 52 isolates of Wheat streak mosaic virus (WSMV) was examined by mechanical inoculation of barley (Hordeum vulgare L.), corn (Zea mays L.), oat (Avena sativa L.), pearl millet (Pennisetum glaucum L.), rice (Oryza sativa L.), and wheat (Triticum aestivum L.). All 52 isolates caused systemic infection on “Lodi” oat, “Westford” barley, ‘Borne’ rye, and ‘Tomahawk’ wheat. In contrast, only 6 WSMV isolates infected ‘Spirit’ com, and 11 isolates did not infect pearl millet (94-6667). Nine, 11, and 13 WSMV isolates did not infect sorghum lines SI056, P5560610, and TX626B, respectively, only 2 infected KSS6A. At 18°C, no isolate infected the resistant wheat line KSH96W10-3. However, at 24°C, all but one isolate (EB3) infected this wheat line, indicating that the resistance of KSH96W10-3 wheat was robust at 18°C, yet temperature sensitive with respect to most WSMV isolates.


Experiments were conducted in 2001 and 2002 to compare development of early leaf spot (ELS) (Cercosporidac aracodicola Hori), peanut pod yield, and market grade characteristics under overhead sprinkler irrigation (OSI) and subsurface drip irrigation (SDI). Fungicides were applied bi-weekly, based on weather advisories, or not at all. With fungicides applied, no difference was found between irrigation systems in ELS control and peanut defoliation. Fungicides optimized ELS control, and when based on weather advisories, some fungicides had a significant affect on ELS. Without fungicides, ELS incidence was lower under SDI than under OSI. Pod yield was higher without fungicides in 2001 under SDI compared to OSI, and was similar in 2002. No difference in yield was found when fungicides were applied. Percentage of total sound mature kernels, extra large kernels, and fancy pods were higher under both OSI and SDI than non-irrigated peanut, with no difference perceived of these parameters between irrigation systems or fungicide programs. Potential for hyperspectral and multispectral techniques will be evaluated in the future using similar disease management strategies and irrigation systems.

Population dynamics of the lance nematode on a creeping bentgrass putting green in Kansas. D. SETTLE (1), J. Fry (2), N. Tisserat (1), and T. Todd (1). (1) Kansas State University, Dept. Plant Pathology; and (2) Division of Horticulture, Manhattan, KS 66506. Phytopathology 93:578. Publication no. P-2003-0570-AMA.

The effects of cultural practices on lance nematode (Hoplolaimus galeatus) population dynamics and their relationship with turf damage on a sand-based putting green were studied. Treatments consisted of 4 bentgrass cultivars, 2 clipping heights, and 2 irrigation regimes arranged in a strip-split plot design. Total nematode populations increased through June and July and peaked in August. Populations fell 50% by September but increased again in November. No differences in host preference were detected among cultivars in either year. In 2001, daily irrigation increased juvenile nematode densities by 40-50% compared to alternate day irrigation. The effect of clipping height varied with cultivar. Our study demonstrates that peak lance nematode densities on bentgrass putting greens occur in late summer, a period that coincides with root mortality, and that cultural practices can influence nematode population dynamics.


Viruses were implicated in reducing New York snap bean yields in recent years. We sampled 12 bean fields at bloom (20 quadrats per field, five plants per quadrat) in 2002, and assayed individual plants for Alfalfa mosaic virus (AMV), Cucumber mosaic virus (CMV), Bean common mosaic virus (BCMV), and Bean yellow mosaic virus (BYMV) by ELISA. Fields were either early- (EP) or late-season planted (LP), and were adjacent to alfalfa (a putative source of these viruses) or >1.5 km from alfalfa. Mean incidences of virus-infected plants were 26.0% for AMV, 40.7% for CMV, 33.6% for BCMV, and 16.4% for BYMV. Incidences of AMV or CMV-infected plants were significantly lower (P < 0.05) in EP fields. Proximity of beans to alfalfa did not affect virus infection incidences. Aggregated patterns of virus-infected plants were more frequent in fields adjacent to alfalfa (41% of the time) compared with fields remote from alfalfa (17% of the time).


Tanos™, a mixture of Famoxadone™ (famoxadone) and cymoxanil, is a new fungicide for U. S. potato growers. Tanos™ controls both potato early blight (Alternaria solani) and late blight (Phytophthora infestans). It effectively controls strains of A. solani with reduced sensitivity to chlorothalonil and strains of P. infestans resistant to phenylamide fungicides. Tanos™ has long-lasting preventive activity with outstanding washoff resistance. Tanos™ also provides post-infection, local systemic control of late blight and excellent control of both foliar and stem blight. The wetttable granule formulation is convenient and effective when applied by granular or air- or chemigation equipment. Tanos™ in combination or alternation with contact fungicides (EBDC’s or chlorothalonil) increases the range of useful fungicide attributes and reduces the risk of resistance development.


Several isolates of Potato virus Y (PVY) with characteristics of strains which cause tobacco vein necrosis (PVYV) or potato tuber necrosis (PVYN) were obtained from potatoes (Solanum tuberosum L.) grown in the United States. Several isolates have been partially characterized at the biological and molecular levels. These isolates have been associated with symptoms in tubers resembling those reported for PVYN. Inoculation of cv. Ranger Russet produced symptoms atypical for PVYV infection of this cultivar in greenhouse tests. RFLP and sequence analyses of the P1 and CP cistrons indicated nucleotide sequence similarities to European tobacco and potato necrosis strains, as well as isolates that appear to be novel. Indications of potential sites of recombination between PVYN and PVYV were obtained. Full-length RT-PCR products have been generated for further analysis.


The G7 isolate of Soybean mosaic virus (SMV) was analyzed for nucleotide changes after infecting soybeans with an infectious plasmid-derived cDNA clone. The virus was passaged for 5 generations in a permissive soybean host. From each generation, populations of virus cDNA clones were generated after RT-PCR using minimal cycling conditions with a high fidelity DNA polymerase. The portions of the genome analyzed included the 5'-UTR, P1 and CP cistrons, and the 3'-UTR, and their mutation frequencies were determined. For the protein coding regions, the overall mutation frequency was similar to that reported for other RNA viruses, with few deletions or insertions. The frequency of transversion mutations was similar to transition mutation frequency. The 3'-UTR was observed to have a lower frequency of mutation, but there were more base insertions than in the protein coding regions.


C. zeae-maydis causes grey leaf spot of maize and produces the phytotoxin cercosporin. Little is known about the biosynthetic pathway or factors that
regulate cercosporin production. Analysis of a cDNA subtraction library representing genes up-regulated during toxin biosynthesis revealed a sequence highly similar to MAP kinases in other fungi. Sequence analysis indicated that the gene, designated CZK3, contains a 4119-bp ORF and encodes a polypeptide highly similar to Wip1, a MAP KKK in S. pombe. Gene disruption of CZK3 suppressed expression of genes predicted to participate in cercosporin synthesis and abolished cercosporin production. The disrupted mutant grew faster than wild type but was deficient in conidiation and elicited only small chlorotic spots on inoculated maize leaves. Complementation restored wild-type levels of conidiation, growth rate, and virulence as well as cercosporin production. The results suggest that cercosporin is a virulence factor in maize pathogenesis, but the pleiotropic effects of CZK3 disruption precluded definitive conclusions.

Control of apple powdery mildew with products different from traditional fungicides. P. L. SHOLBERG, J. Boule, T. Beveridge, and T. Li. Agriculture & Agri-food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada V0H 1Z0. Phytopathology 93:S79. Publication no. P-2003-0576-AMA.

Powdery mildew is an important disease of several prominent apple cultivars in North America. Many interesting products have been identified for its control; however, they are not linked with decreased thrips transmission efficiency. Eight glycoproteins (G1/G2) and accumulation of defective interfering RNAs (DI RNAs) are exclusively transmitted by thrips in nature. Deletions in the envelope region of the DI RNAs mapped to the M RNA. The mutation frequency of DI RNAs was determined in apple leaves. P. L. SHOLBERG, J. Boule, T. Beveridge, and T. Li. Agriculture & Agri-food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia, Canada V0H 1Z0. Phytopathology 93:S79. Publication no. P-2003-0576-AMA.

Lack of technologies to produce and deliver effective biological control agents (BCAs) is a major barrier to their commercialization. A myriad of variables associated with BCA cultivation, formulation, drying, storage, and reconstitution processes complicates agent yield maximization. An exploratory approach was developed towards a well-organized and integrated approach to optimizing these process variables. The assays involve growing the BCA of interest in flasks or fermentors, formulating cells harvested from growth cultures, delivering microdroplets of formulated cells to microplate wells, air- or freeze-drying droplets, storing plates, reconstituting dried cells, and monitoring the rate of cell growth to a specified yield using a plate-reading spectrophotometer. Relevant variables (ingredients, temperature, etc.) are tested at each step of the assay process to view their individual and combined impact on resultant microbial activity. The utility of this method to evaluate many treatments is demonstrated on strains of Pseudomonas fluorescens and Enterobacter cloacae known to suppress fungal plant diseases.


High populations of root-lesion nematode (RLN) damage wheat in some annual field crop rotations in eastern Oregon and Washington. RLN populations have been inversely correlated with yield of locally adapted cultivars. Australian cultivars were evaluated in replicated plots at three nonirrigated Oregon locations. Cultivars were selected for resistance (R), susceptibility (S), tolerance (T), or intolerance (I) to RLN. Pratylenchus species were enumerated in roots in each plot before harvest in 2001, and in soil in each plot before planting in 2002. Grain was harvested with a plot combine. ‘Machte’ (S/I) and ‘Sunvalle’ (R/T) yields declined steadily as RLN increased from zero to 2,500 per gram root (fresh weight) in 2001, or increased from zero to 11,000 per kilogram soil in 2002. ‘Kichaufi’ (R/T) yields declined when populations in mature roots exceeded 1,500 per gram but did not decline with pre-plant populations up to 11,000 per kilogram soil. Genetic tolerance plus resistance in ‘Kichaufi’ maintained yield of wheat in plots containing high populations of P. neglectus plus P. thornei.


Fusarium pseudograminearum (Fp) is among the complex of pathogens causing Fusarium crown rot of wheat in eastern Oregon and Washington. Wheat varieties and lines (70 to 100 entries per year) were evaluated over three years in inoculated or non-inoculated paired plots. Five Fp isolates on millet-seed substrate were mixed and dispensed 1-inch above wheat seed at planting. Crown rot incidence and severity were measures for genetic resistance. Grain yield differential among paired plots was the measure for susceptibility. Ten entries were resistant. Disease was moderate (10 to 12 percent [%] mean yield reduction due to inoculation; 0 to 28% range among entries) during two years, and was intense (57% mean; 32 to 85% range) during the third year. Wheat entries ranked as most tolerant (less than 10% reduction during both “moderate” years) were also most tolerant but failed to adequately protect wheat (up to 70% yield reduction) when disease was intense. We conclude that genetic resistance will be required when cultural and climatic conditions are highly favorable for expression of Fusarium crown rot.


Anthracnose diseases of strawberry are caused by several Colletotrichum species. A subset (“supercore”) of the Fragaria core collection maintained at the USDA National Clonal Repository, Corvallis, OR, contains an elite group of 15 native F. virginiana clones and 12 F. chiloensis clones of widely differing genotypes. We evaluated this supercore collection for anthracnose resistance by inoculating plants with conidial suspensions of isolates of C. acutatum, C. fragariae and C. gloeosporioides. No Fragaria sub-grouping was more resistant than any other. Individual accessions within groups were identified as sources of resistance. The virulence of Colletotrichum isolates varied depending on the Fragaria accession challenged. Apparent resistance to anthracnose in accessions from different geographic origins, Peru, British Columbia, California, Montana and Mississippi, and in two Fragaria species.
species, suggests there is a wealth of genetic resources in native octoploid germplasm that can be used to develop resistant cultivars.

**Susceptibility of detached peanut (Arachis hypogaea) plant parts to Sclerotinia minor.** D. L. SMITH and B. B. Shew. Department of Plant Pathology, North Carolina State University, Raleigh, NC. Phytopathology 93:S80. Publication no. P-2003-0583-AMA.

A method for evaluating resistance of peanut to *S. minor* using detached leaflet inoculation was modified to compare susceptibility of peanut, petiole, main stem, and branches of three peanut lines: NC 12C (highly susceptible), NC 7 (moderately susceptible), and N96076L (resistant). Main stems, primary lateral branches, petals, and single leaflets still attached to their petioles were detached, placed into plastic boxes and inoculated by affixing a plug of an actively growing culture of *S. minor* to the part. The lateral branchlets and leaflets inoculated were either at a vegetative or flowering node. Plant parts were incubated (20°C and 100% humidity) and lesion lengths were measured each day for seven days after inoculation. Responses of all lines were consistent across plant tissues with N96076L exhibiting the highest levels of resistance. Main stems and branches of all lines were more resistant than leaves and petals. Petal tissue susceptibility to *S. minor* suggests that disease management and breeding programs should focus on protecting petals from infection.


A highly susceptible (NC 12C) and a moderately resistant peanut cultivar (Perry) were planted in field plots in Perlquimans Co, NC and treated with fungicide (fluzinam) at three rates to establish different disease severities amongst plots. Fungicide and cultivar effects on yield were additive. Modelled weather data were obtained from Skybit Inc and related to weekly changes in incidence of Sclerotinia blight. Growth chamber studies were conducted to examine the effects of temperature and moisture on sclerotial germination, mycelial growth, and colonization. Soil was adjusted to water potentials of -7.2, -10, and -100 kPa and placed in glass jars. Sclerotia or mycelial plugs were placed on soil in glass jars and incubated at 18, 22, 26, and 30°C. Leaflets of three peanut lines were inoculated with mycelial plugs and incubated in jars under the same conditions. Sclerotial germination was greatest at 26-30°C and -7.2 kPa (saturated), whereas mycelial growth was greatest at 18-22°C at all water potentials. A combination of soil temperature between 18-22°C and soil moisture near -10 kPa (field capacity) was optimal for infection of leaves by *S. minor*.


The coelomycete fungus *Sirococcus conigenus* causes shoot blight in many conifer species in Europe and North America. Some previous investigations of this fungus hinted at host-related variation. To investigate this possibility, 47 isolates from *Pinus, Picea, Tsuga, Cedrus* and *Larix* species from North America and Europe were examined by inter-simple sequence repeat (ISSR) polymerase chain reaction. Cluster analysis of the data revealed two main groups: the P group containing most *Picea* and *Pinus* isolates, all *Larix* isolates and one *Cedrus* isolate; and the T group containing all *Tsuga* isolates and one *Cedrus* isolate. Phylogenetic analysis of the internal transcribed spacer (ITS) and 5.8S nuclear rDNA sequences performed on a subset of isolates supported this distinction. Phylogenetic analysis of the 18S nuclear rDNA sequence from a *Tsuga* sp. isolate, a *Pinus* sp. isolate, and *S. clavigignenti-juglandacearum* places *Sirococcus* in the Ascomycete class Sordaromycetes.


The sugar beet nematode (SBN) causes serious damage to sugar beet crops in the U.S. SBN-resistant trap crop radish cultivars reduce soil populations of *H. schachtii* when second-cropped following harvest of malting barley in Wyoming. Five SBN-resistant oil radish cultivars were evaluated in the field for SBN control for two years. Radishes were seeded in August of 2000 and 2001 at a rate of 25 kg/ha. Soil samples were taken and SBN densities were determined at seeding and seven (2001) to ten (2000) weeks after seeding. Reproductive factors (Rf) decreased with increasing initial SBN population densities (Pi). In the first year, Rf values ranged from 0.02 for ‘Colonel’ to 0.44 for ‘Rimbo’ at Pi > 2.5 eggs/J2/ml soil. ‘Colonel’ reduced nematode populations more than ‘Adagio’ and ‘Rimbo’ at Pi < 2.5 eggs/J2/ml. In the second year, Rf values varied between 0.23 for ‘Adagio’ to 1.55 for ‘Rimbo’ for all Pi, and no differences in Rf values were detected between radish cultivars. ‘Colonel’ is currently being used in sugar beet production areas in several western states as a component of an integrated management program for control of the SBN.

**Postharvest biological control of grey and blue mould on apple.** D. Spadaro, A. Garibaldi, and M. L. Gullino. Centre of Competence for the Innovation in the Agro-environmental Sector, University of Turino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Phytopathology 93:S80. Publication no. P-2003-0587-AMA.

**Botrytis cinerea and Penicillium expansum**, among the most dangerous postharvest pathogens in pome fruits, are primarily controlled by synthetic fungicides. Biological control with micorobial antagonists has emerged as a promising alternative. Several strains belonging to the species *Metschnikowia pulcherrima* proved to be highly effective in the control of grey and blue mould, in controlled and semi-commercial conditions. The mechanism of action was investigated: living cells of the yeasts are necessary for biocontrol, while culture filtrates are ineffective, in *in vivo* and *in vitro* experiments. Competition for nutrients was evaluated through an *in vitro* system of cultivation of the antagonist separated from the pathogen. The cell suspension of one antagonistic strain, applied alone or associated to heat treatment and sodium bicarbonate, acibenzolar-s-methyl, or ethanol application, offered a high control of both pathogens.


Infection of tobacco stems and leaves by pathogenic *Erwinia* spp. prior to harvest can result in barn rot, i.e., dissolved pith and dark brown to black stems and lamina. Curing tobacco with high moisture and low air movement favors these pathogens, facultative anaerobes that use nitrates for respiration and form nitric oxide. Nitric oxide reacts with alkaloids (nicotine) to form nitrosamines. Chemical analyses were made of flue-cured tobacco samples (50 stems) and healthy stems and laminas were compared to diseased ones. Nitrosamines increased 2-30 times in diseased tissues with a corresponding decrease in total alkaloids and sugars. Barn rot control during curing can decrease nitric oxide and nitrosamines.


Disomic substitution lines (LDN-DIC) were prepared by replacing chromosomes of *Langdon durum* with those from *Triticum dicoccoides* (TDIC). These lines show significant differences in response to Fusarium head blight (FHB) when inoculated with *Fusarium graminearum* by the single spikelet method. Several lines showed increased resistance to FHB. In contrast, the line LDN(DIC-2A) was highly susceptible to FHB, just as is the TDIC accession that contributed the chromosomes to the substitution lines. In F-1 hybrids with other substitution lines, the gene(s) on 2A behaves as a “susceptibility gene” that acts in an additive manner. In most F-1 hybrids involving the LDN(DIC-2A), the FHB severity was intermediate between the parents. However, in F-2 populations derived from crosses to FHB resistant lines, the distribution suggests that the gene(s) on chromosome 2A not only confers increased FHB susceptibility, but suppresses the effect of any resistance genes.

**Combining ability of genes conditioning Fusarium head blight resistance in the Langdon durum - dicoccoides disomic substitution lines.** R. W. STACK (1), F. D. Miller (2), and L. R. Lopma (2). (1) Plant Pathology Dept., N. D. St. Univ., Fargo, ND 58105; (2) USDA-ARS, NCSL, Fargo, ND 58105. Phytopathology 93:S80. Publication no. P-2003-0590-AMA.

Disomic substitution lines (LDN-DIC) prepared by replacing chromosomes of *Langdon durum* with the corresponding ones from *T. dicoccoides* show significant differences in response to Fusarium head blight (FHB). The disomic lines with the lowest FHB severity (LDN-DIC-3A, -6B) and with the highest FHB severity (-1B, -2A, -6A) were intercrossed in a diallele series. The F-1 hybrid plants were grown in replicated trials in the greenhouse and inoculated at anthesis with *Fusarium graminearum* by the single spikelet method. FHB response was determined visually 3.5 weeks after inoculation; at maturity inoculated heads were harvested and % scabby kernels.
determined. All five lines showed significant general combining ability (GCA) for FHB severity (positive or negative) and three of the five showed GCA for scabby grain as well. Only a few of the individual crosses showed specific combining ability (SCA). The results support the view that individual genes for FHB resistance may be combined.

Precision of visual and radiometric disease evaluations. K. STEDDOM (1), M. Bredehoef (2), M. Kahn (3), and C. M. Rush (1). (1) Texas Ag. Exp. Sta. Bushland, TX 79012; (2) SMSCB, Renville, MN 56284; (3) NDSU, Fargo, ND 58105. Phytopathology 93:8S1. Publication no. P-2003-0591-AMA.

We investigated the precision of a hand held radiometer and visual estimates of disease severity for Cercospora leaf spot of sugar beet. Two field trials, Willmar, MN and Drayton, ND were evaluated. Two raters gave visual ratings of disease severity for a radiometer and visual readings for each trial. Both radiometer readings and visual estimates were repeated a second time immediately following the first repeat. The renormalized difference vegetative index (RDVI) was calculated from the radiometric data. RDVI and visual estimates from the first repeat were regressed against the second repeat with R² for visual of 0.92 and 0.93 for Drayton and 0.75 and 0.90 at Willmar, while RDVI gave 0.78 and 0.79 for Drayton and Willmar respectively. Regression between visual and RDVI gave R² values of 0.74 and 0.73 for Drayton and 0.69 and 0.25 for Willmar. Averaging RDVI from each repeat had a larger effect on precision than averaging visual estimates. This data suggests that readings with a radiometer can be used to replace visual estimates of disease, but on crops with a highly variable canopy many readings will be needed.


Field plots were solarized in 1998 using Thermofil-IR single layer (SL) and double layers (DL) soil salitization (SS) amended with furlfur (F) or urea (U), and the control (O) for two periods (31 days for SL and 28 days for DL; and 76 days for SL and 64 days for DL). The objectives were to evaluate weed control (WC) one year after SS and damping-off (DO) (Rhizoctonia solani) and WC two years following SS in soils amended with or without F plus allyl isothiocyanate (AI) or a reduced rate of U on ‘Kentucky Wonder’ snapbeans. Results in 1999 and 2000 showed complete WC following SS of <93% in 1999 and ≤63% in 2000. Percent DO were 35, 24, 25, 26, and 41% in NSO, SLO1, SLO2, DLO1, and DLO2, soils, respectively. Combining F plus AI to all treatments was most effective in significantly reducing DO. Percent DO for NSF, SFL1, SFL2, DFL1, and DFL2 were 11.11, 10, 14.0%, respectively.


A Penn State survey conducted during 2001-2 evaluated grapevine decline across Pennsylvania and in the Finger Lakes and Lake Erie regions of New York due to fungi, viruses, and nematodes. Symptoms of Petri and Esca disease were found at most of the 27 locations surveyed. Declining vines were collected from survey sites for laboratory analysis, Phaeomoniella chlorotica, Phaeoacremonium aleophilum, and Phaeoacremonium angustius, and an undescribed Phaeoacremonium sp. were recovered from symptomatic vines from 16/18 sites sampled and from 18 different cultivars including Vitis vinifera, Vitis labrusca, and French American hybrids ranging from 3-45 years old. Isolates were identified based on previous descriptions and by internal transcribed spacer (ITS1–5.8S–ITS2) rDNA sequences. Phaeomoniella and Cylindrocarpon spp. were isolated from Petri and esca disease fungi in Cylindrocarpon sp., Phomopsis spp., Verticillium sp., Gliocladium sp., and Fusarium graminearum. P. proliferator, F. cinctum, and F. oxyporum were also frequently isolated and identified based on morphology.

Pythium spp. isolated from roots, stems and leaves of Bermudagrass in non-fumigated and fumigated plots in Florida. C. M. STILES (1), P. A. Ayers (2), C. M. Stiles (3), and A. D. Child (4). (1) Univ. of Florida-IFAS, (2) Dept. of Plant Pathology, Gainesville, FL 32611-0680; (3) Dept. of Plant Pathology, Everglades Research and Education Center, Belle Glade, FL 33430-8033. Phytopathology 93:8S1. Publication no. P-2003-0594-AMA.

In summer 2001, field plots near Gainesville, FL, were cultivated, methyl iodide fumigated, and spriggled with Bermuda grass (Cynodon sp., ‘Tifdwarf’). One strip of the field, previously sampled (2000-2001) for Pythium spp., was left non-fumigated. Pythium spp. were isolated monthly from the roots, stems and leaves (SL) tissue in both treatments beginning in October. The frequency of Pythium spp. recovered from field plots ranged up to 15% in March and April, and up to 22% from SL in February. In the previous year, P. graminicola and P. irregular are had dominated, but after fumigation and re-planting, P. graminicola comprised only 11% of all isolates. Pythium irregular was comprised 30% of all isolates, but was only found in non-fumigated plots. A different species, P. ultimum var. ultimum, not isolated in the previous year, was recovered (34%) from the re-planted field in February and March 2002, and was more common in fumigated than in non-fumigated plots.


Root diseases of wheat and barley in southeastern Idaho have not been extensively studied. Therefore, surveys were conducted in June/July 2001 and 2002 to assess root disease severity and identify soil-borne pathogens present in 81 wheat and 52 barley fields in 13 southeastern Idaho counties. Pathogenic fungi isolated from root lesions included Fusarium spp., Bipolaris sorokiniana, Rhizoctonia solani, and Gaeumannomyces graminis var. tritici. Based on root lesions associated with these fungi, mean root disease severity index values ranged from 4 to 18 and 2 to 34 in 2001 and 2002, respectively. Greenhouse pathogenicity tests conducted on soft winter wheat indicated Fusarium culmorum and B. sorokiniana isolates to be the most virulent of the species tested. Nematode soil assays revealed that 96% of the fields had lesion nematodes (mostly Pratylenchus neglectus), and 78% had stunt nematodes (Tylenchorhynchus spp.). Other nematode species were detected in less than 18% of the fields. The cereal cyst nematode (Heterodera avenae) was found only in 2 fields.

Genetic characterization of differential reactions among Host Group 3 common bean cultivars to NL-3 K strain of Bean common mosaic necrosis virus. C. A. STRAUSBAUGH (1), P. N. Miklas (2), S. P. Singh (1), and R. L. Forster (1). (1) Univ. of Idaho, Res. and Ext. Center, Kimberly, ID 83341; (2) USDA/ARS, Prosser, WA 99350; (3) Oregon State Univ., Corvallis, OR 97331. Phytopathology 93:8S1. Publication no. P-2003-0596-AMA.

A previously unrecognized recessive resistance gene (or allele) was identified in three Host Group (HG) 3 common bean cultivars Olathe, Victor, and UI 37, based on genetic analysis of plants from five populations screened with the NL-3 K strain of Bean common mosaic necrosis virus (BCMVN). The gene (or allele) was associated with resistance to leaf stunting and deformity and reduction in plant height. The gene (or allele) provides similar, but slightly better resistance characteristics than the bc-1 P gene that is characteristic of HG 3 cultivars. Traditional HG 3 cultivars like Redlands Greenleaf B with bc-1P are susceptible to NL-3 K, whereas this newly identified gene (or allele) conditions resistance to NL-3 K. To gauge the full breeding value of this newly identified gene (or allele), allelism tests with existing genes namely bc-1e, and further characterization of responses to all Bean common mosaic virus (BCMV) and BCMVN strains need to be conducted.


The root-knot nematode, M. incognita is an obligate plant endoparasite, which has evolved a complex feeding relationship with its host plant. Previous studies showed that an Arabidopsis thaliana endoglaculase (AtCell) promoter was activated in the feeding cells of root-knot nematodes in transgenic tobacco and Arabidopsis plants. Gus activity was also visible early in the lateral root primordia. Different Δ deletions of the AtCell promoter deletion constructs showed that the region between -1639 and -1171 was essential to provide specificity of GUS expression in nematode feeding cells. Studies are under way to examine the development of nematode feeding cells in A. thaliana antisein Cell plants that express reduced levels of the CEL1 protein.
Potential host range of *Phytophthora* *inflata*. B. M. Sundick, J. K. Schneider, S. L. Jensen-Tracy, and G. W. HUDLER. Dept. of Plant Pathology, Cornell University, Ithaca, NY. Phytopathology 93:S82. Publication no. P-2003-0598-AMA.

*Phytophthora* *inflata* Caroselli and Tucker has been tentatively identified as a primary pathogen associated with European beech (*Fagus sylvatica*) decline in the northeast U.S. because it has been found consistently in and isolated from diseased bark to the exclusion of any other fungus or Oomycete. Here we report results of experiments to test the ability of *P.* *inflata* to cause visible symptoms on detached leaves and/or logs from local plants. Results of inoculations of detached leaves or small logs suggest that species of *Acer*, *Ulmus*, *Syringa*, *Liriodendron* and *Tilia* are potential hosts in the field. No species in the Fagaceae, either as detached leaves or as small logs, showed no evidence of colonization by the pathogen in our tests.


Dispersal of conidia of *P. viticola*, a common pathogen of grape, was studied for 2 seasons in vineyards of the Finger Lakes and Lake Erie grape regions of NY. Spores were collected in rainwater beneath 1-m sections of top-wire conduits. In 2001, a relatively dry season, peak spore release coincided with bloom, with low levels of release before and after. A peak of spore release near bloom was observed again in 2002, although earlier peaks of comparable magnitude also were detected during the wet prebloom period. Virtually no spores were collected >4 wk postbloom. Although recent studies have shown grape berries and rachises to be susceptible to infection throughout the growing season (PLANT DIS. 85: 517-520), we obtained control of all rachis infections with fungicides applied between bud break and bloom; no fruit infection occurred. Seasonal inoculum patterns should be an important determinant of disease management programs.


Historically, stem and leaf rusts have been among the most devastating diseases of wheat, with written accounts of epidemics dating back to ancient Greek and Roman times. In North America, major epidemics have occurred from the late 1800’s through the 1970’s. During the cold war, the United States and the Soviet Union studied rusts of wheat, with written accounts of epidemics dating back to ancient times. The rusts were studied in the Soviet Union from the late 1800's through the 1970's. During the cold war, the United States and the Soviet Union studied rusts of wheat, with written accounts of epidemics dating back to ancient times. The rusts were studied in the Soviet Union from the late 1800's through the 1970's. During the cold war, the United States and the Soviet Union studied rusts of wheat, with written accounts of epidemics dating back to ancient times. The rusts were studied in the Soviet Union from the late 1800's through the 1970's. During the cold war, the United States and the Soviet Union studied rusts of wheat, with written accounts of epidemics dating back to ancient times.


*Pseudomonas fluorescens* strain A506 produces an antibiotic toxic to *Erwinia amylovora* in defined culture media containing at least 0.1mM FeCl3. To estimate the relative abundance of iron on blossoms, we used an iron biosensor [iron-regulated promoter (pvd) fused to an ice nucleation reporter gene (inaZ)] in A506. In four experiments on pear and apple trees, A506 pvd-inaZ expressed high ice nucleation activity (INA) on blossoms indicating limited iron bioavailability. A506 pvd-inaZ expressed less INA on blossoms sprayed with an iron chelate (0.1mM FeEDDHA) than on blossoms sprayed with water, indicating that 0.1 mM FeEDDHA increased the levels of iron available to A506 pvd-inaZ in this habitat. Lower concentrations (0.01mM) of FeEDDHA did not significantly increase iron available to A506 on blossoms. These results indicate that apple and pear blossoms represent an iron-limited environment, which is unlikely to support antibiosis by A506, and adding at least 0.1mM FeEDDHA is required to significantly increase the level of bioavailable iron to A506 on blossoms.


The root-knot nematode (*RKN*) resistant ‘Charleston Belle’ (*C. pepo*) was similarly iden****alyzed but susceptible to ‘Keystone Resistant Sistant’ (*RKN*) were compared as spring crops for managing *RKN* (*Meloidogyne incognita*) in fall-cropped cucumber (*Cucumis sativus*) and squash (*Cucurbita pepo*) at Blackville, SC and Tifton, GA. CB was resistant and *KRG* was susceptible at both sites. Cucumber plants grown after CB ha****d lower (*P < 0.001) root gall severity indices (GI) than plants grown after *KRG* (4.2 vs. 4.9 on a 1 to 5 scale). Cucumber yields were 87% heavier (*P < 0.001) following CB than *KRG*. Squash plants grown after CB had higher gi (*P < 0.001) than plants grown after *KRG* (4.0 vs. 4.8). Squash yields were 55% heavier (*P < 0.01) following CB than *KRG*. These results demonstrate that *RKN* resistant bell pepper cultivars such as *CB* will be useful tools for managing *M. incognita* in double cropping systems with cucurbits.

The IR-4 Project with the U.S. EPA, California's Department of Pesticide Regulation and Canada's Pest Management Regulatory Agency obtained 531 new food-use clearances, 482 new ornamental-use labels and 91 new biopesticide registrations. Coordinated IR-4 data supported many Section 18 uses. More than 70% of these clearances are chemicals and uses identified as “reduced risk” by EPA. The U.S. EPA is actively reviewing triazole fungicides to develop a reduced residue program for iodomethane on all crops. Fluodioxin post-harvest use on stone fruit finally made it to a national label. Efficacy work continues to be evaluated for development of symptoms for 21 days after inoculation. As a result, a filamentous fungus and the causal agent of rice blast on bentgrass, creeping bentgrass by species from the respective plant families. We inoculated 17 plant species harvested from seedlings, and sampled for avenacin. Avenacin was not detected in any root samples from bentgrass or wheat but was detected in oats. It is unknown whether avenacin activity is also required for infection of bentgrass by Gs. This study was initiated to assess the ability of bentgrass to produce avenacin and subsequently to determine the pathogenicity of Gs on bentgrass. Bentgrass, wheat, and oat root tissues were harvested from seedlings, and sampled for avenacin. Avenacin was not detected in any root samples from bentgrass or wheat but was detected in oats. Isolates showed strong host specialization to the five exotic hosts and to all three native species of Moraceae. Thus, excluding those from Gnelina, isolates host-specialized to exotic hosts were not host-specialized to their respective Brazilian relatives.

The five hosts and measured length of xylem discoloration after 23 days. Native Moraceae were not available. Isolates showed strong host-specialization to the five exotic hosts and to all three native species of Verbenaceae. In the other native hosts, the three isolates from a given host behaved differently, and the isolates were not specialized to the respective host families. Thus, excluding those from Gnelina, isolates host-specialized to exotic hosts were not host-specialized to their respective Brazilian relatives.

Chemical characterization of Fusarium solani f. sp. glycinis by image analysis of HPLC chromatograms. U. Thrané (1), S. Li (2), and G. L. Harwood (3). (1) Technical University of Denmark, BioCentrum-DTU, DK-2800 Kgs. Lyngby, Denmark; (2) University of Illinois, Dept. Crop Sciences, Urbana, IL 61801; (3) USDA-ARS, Urbana, IL 61801. Phytopathology 93:883. Publication no. P-2003-0610-AMA.

Fusarium solani f. sp. glycinis, the cause of soybean sudden death syndrome (SDS), was classified with chromographic image analysis on full chromatographic matrices obtained by high performance liquid chromatography with UV detection of culture extracts. F. solani non-SDS causing strains were used for comparison. Three chromatograms of each SDS and non-SDS F. solani were selected for calculation of reference chromatograms for identification by chromatographic image analysis. The strains are identified by the cophenetic magnitudes between each chromatogram and each of the two calculated reference chromatograms, according to the highest similarity. In all cases the chemical identification of the SDS-causing isolates were consistent with the culture morphology and pathogenic identification. Secondary metabolite profiles contained sufficient information for classification and species identification indicating that F. solani f. sp. glycinis is a distinct taxon.


Total number of R-genes in genome seems to be insufficient to confer resistance to the multitude of pathogens that a plant species is likely to encounter. Here, we report the diverse features of polymorphism at five R-gene loci in A. thaliana. Some of these loci appear to harbor thousands of alleles, revealing an impressive ability to recognize its natural enemies. For each of these R-genes, we sequenced 23-28 A. thaliana ecotypes and 1-6 alleles of the congener A. lyrata. The R-genes that we studied revealed very different evolutionary dynamics. Rps5 and its homolog, Rfl1, showed a slow rate of evolutionary change characterized by very low diversity (0.08%) or 0.38% Ka/Ks (0.39–0.38 between species), and no sequence exchange between homologs. There was basically a single haplotype at Rps5 and two haplotypes at Rfl1, apparently existing for millions of years. In contrast, rapid evolution was observed at Rpp8 and its homolog locus K, evident by frequent sequence exchange between homologs, high Ka/Ks (2.23–4.40 between homologs or species), and moderate diversity (2.9–7.0%). There was also extensive allelic diversity: 44 distinct alleles were found in a survey of 50 sequenced alleles in this gene family. Alleles at each of these loci were intermixed in a gene tree, and differ at 3.4% one from another. Interestingly, Rpp13 has both high diversity (6.9%) and high Ka/Ks ratio (1.99), but has less distinct alleles. Within 25 ecotypes, there were only 11 different alleles grouping into 5 subgroups. Therefore extensive variation in the dynamics of the R-genes that we studied existed. The diversity and the number of diverse alleles are dissimilar, as is the frequency of recombination. The diverse feature of polymorphism in R-genes provides a key to understanding patterns of variation, and may inform strategies of achieving efficient plant resistance breeding and preservation of R-gene resources.


Phytophthora capsici was first described in 1922 as the agent of blight on pepper (Capsicum annuum) in New Mexico. This pathogen was subsequently reported on several other plants species. Phytophthora blight, caused by P. capsici, is the most serious threat to the production of cucurbits, eggplants, peppers and tomatoes in Illinois. To develop an effective method to manage Phytophthora blight of vegetables, this study was conducted to determine the host range of P. capsici in Illinois. Seedlings of the plants were grown in a greenhouse and inoculated by adding 2 ml of zoospore suspension of P. capsici (2 x 10^5 spores/ml) onto soil around each seedling. A combination of six isolates of P. capsici was used to inoculate plants. Seedlings were evaluated for development of symptoms for 21 days after inoculation. As a...
result, in addition to the known hosts, green bean, beet, carrot, chard, lima beans, onion, radish, Swiss pea, tobacco, and two weed species, nightshade (Solanum sp.) and Vineet leaf (Abutilon theophrasti) were found as experimental hosts of P. capsici. No detectable infection occurred on basil, broccoli, cabbage, cauliflower, celery, chive, dill, kohlrabi, mustard, parsley, popcorn, and soybean.


Genetic variation among 23 isolates of Phytophthora capsici from Illinois was assessed by comparing Internal Transcribed Spacer (ITS) regions of rDNA and using Inter Simple Sequence Repeat (ISSR) and Amplified Fragment Length Polymorphism (AFLP) markers. ITS and ISSR markers were used to amplify the ITS1, 5.8S and ITS2 regions. Fifteen restriction enzymes were used to digest the PCR product of ITS regions. No differences were detected among the isolates. Thirteen ISSR primers were used to amplify genomic DNA of the isolates. No polymorphic bands were detected among the isolates from the same field. However, several polymorphic bands among isolates from different locations and different hosts were observed. Our preliminary data showed that there are close relationships among the isolates from the same location, while isolates from different locations are more distant. Similar polymorphism was detected using 24 AFLP primers. Results of this study suggested that there is no or non-detectable genetic variation among the isolates from the same field, but genetic variation can easily be detected among the isolates from different locations.

Susceptibility of some eastern oak species to sudden oak death caused by Phytophthora ramorum. P. W. TOOLEY (1) and K. L. Kyde (2). (1) USDA-ARS, Ft. Detrick, MD 21702; (2) University of Rhode Island, Kingston, RI 02881. Phytopathology 93:S84. Publication no. P-2003-0614-AMA.

To evaluate susceptibility of eastern oak species to P. ramorum, 2- to 3-year-old seedlings of white oak (Quercus alba), Northern red oak (Q. rubra), chestnut oak (Q. prinus), cherrybark oak (Q. falcata var pagodifolia), and coast live oak (Q. agrifolia) were inoculated with P. ramorum. Agar plugs from 10-14 day old colonies on 2% V8-juice agar were placed in stem wounds 5 cm above the soil line. Trees were incubated at 20°C in a greenhouse cubic for 9 weeks, then debarked and lesion areas were traced and measured. P. ramorum caused significantly more (P < 0.01) disease than the controls on all five oak species in two replicated experiments (25 trees/species/experiment). White and chestnut oak sustained the largest lesions, followed by oak, then cherrybark oak, and coast live oak. The results suggest that, following wound-inoculation, P. ramorum can infect seedlings of eastern oak species under greenhouse conditions. Whether these species would serve as hosts of P. ramorum in nature is unknown.

Genome sequence and host range of maize fine streak rhadovirus (MFSV). C.-W. Tsai (1), J. C. Todd (1,3), M. G. REDINBAUGH (2,3), K. TUBAJIKA (1), E. L. Civerolo (1), G. Purteka (2), L. Willie (2), E. D. Ammar (1), W. Styer (1), and S. A. Hogenhout (1). (1) NCSU, Dept. of Plant Sciences; and (2) Plant Pathology, Ohio State University; (4) UCCE, Bakersfield, CA. Phytopathology 93:S84. Publication no. P-2003-0617-AMA.

Gene expression in Catharanthus roseus infected with vinca virensce. E. TUMBAN (1), R. Richins (2), and M. Shaw (1). (1) Natural Sciences, NMHU, Las Vegas, NM; (2) NMSU, Las Cruces, NM. Phytopathology 93:S84. Publication no. P-2003-0618-AMA.

Phytoplasmas are wall-less prokaryotes which cause hundreds of plant diseases such as maize bushy stunt, cabbage witches’ broom, onion yellows, etc. Vinca virensce is a phytoplasma that causes symptoms of virensce, phylloxy and witches’ broom. The mechanism(s) by which these symptoms are caused is still unknown, although it has been postulated that they may be due to physiological disturbances such as hormonal imbalance. If hormone imbalances are involved, some genes of the plant may have a role to play at an early stage of protein synthesis. My interest is to investigate which plant genes are involved. RNA isolated from infected C. roseus was transcribed to cDNA and cloned in E. coli. The cDNA was probed with radioactive probes synthesized from mRNA of both infected and healthy plants. Plaques which were expressed differentially were selected and their cDNAs have been sequenced. Preliminary results from BLAST search have identified cDNA clones with homology to enzymes from different plants. Some enzymes of interest cysteine protease, Dna J and CYFP77 are currently being analyzed to find out what role they may be playing in the disease symptoms.


Approaches for quantifying net photosynthesis (PN) on strawberry leaves infected with strawberry leaf scorch were investigated on greenhouse-grown Jewel and Kent plants. Plants were inoculated with a 1 x 10^6 conidial suspension of D. earlinae and placed under various conditions to generate a range of disease severities. PN was measured on individual leaflets using both a narrow leaf (small) and conifer (large) cuvette in a biotron set to constant, near-saturating light levels. Percent infected leaflet area was determined digitally using the program ASSESS. Disease severity ranged from 0 to 100%. There was a strong negative linear correlation between PN and percent infected leaf area. PN response curves for the two cuvettes were similar, with leaflet readings being typically higher for the small cuvette. Leaflets between 15 and 80 days old show no significant differences in PN. This information will be coupled with return yield, biomass and disease intensity data from field grown strawberries and used to develop economic thresholds for disease management decisions.
Intracellular processing and secretion of the fungal hydrophobin cryparin. M. Turina (1), P. KAZMIERCZAK (2), and N. Van Alfen (2). (1) IVV-CNR, Str delle Cacce 73, 1-10135 Torino, Italy; (2) UC-Davis, Plant Pathology, Davis, CA 95616. Phytopathology 93:S85. Publication no. P-2003-0620-AMA.

Cryparin is an abundant class II fungal hydrophobin found in the cell walls of fruiting bodies of the fungus Cystocercis parasitica. This protein is necessary for the eruption of the fungal fruiting body through the bark of infected trees. Large amounts of cryparin are secreted in liquid culture allowing its use in the study of vesicular protein secretion. The precryparin is processed by cleavage of the signal peptide and then the propeptide is cleaved by a Kex2-type endoprotease. The role of the Kex2-type protein processing on secretion of this protein was studied by site-specific mutagenesis of the Kex2 recognition site. Antibodies were raised against a His-tagged cryparin protein and used to detect the presence of the cryparin in the cell wall and culture fluid of C. parasitica. GFP fusion was also used to study the localization of cryparin. Results indicate that Kex2 processing is not necessary for secretion and that cryparin localizes within discrete bodies in the hyphae.

Possible role of structural components of the secretory pathway in the replication cycle of Cystocercis parasitica. M. Turina (1), D. Wilk (2), and N. VAN ALFEN (2). (1) IVV-CNR, Str delle Cacce 73, 1-10135 Torino, Italy; (2) UC-Davis, Plant Pathology, Davis, CA 95616. Phytopathology 93:S85. Publication no. P-2003-0621-AMA.

CHV1 infection of the filamentous ascomycete Cystocercis parasitica, the causal agent of chestnut blight, results in hypovirulent phenotype. Previous studies showed a consistent proliferation of host vesicles where virus replication and dsRNA accumulation occur. Heavy water-ficoll gradients were used to separate subsets of vesicles from the microsomal fraction of virus infected and uninfected strains was maintained over time whereas in healthy mycelia the amount of vesicles dramatically decreases after an early accumulation. A subset of vesicles shown to contain viral dsRNA and proteins reacting to CHV1 infected C. parasitica contain an enriched protein band reacting with anti-clathrine heavy chain antibodies and anti-middle component of its adapter complex 1 in Western blot analysis. The finding that clathrin coat associated vesicles accumulate in hypovirulent strains of C. parasitica prompted us to clone the C. parasitica clathrin heavy chain gene and the middle component of its adapter complex 1 in order to investigate their role in CHV1-C. parasitica interaction.


Cucumis melo L., cultivars were evaluated for relative susceptibility to vine decline caused by Monosporascus cannonballus Pollack & Uekker in Imperial County in 2002. Seeds of 14 cantaloupe cultivars (Caravelle, Cruiser, Don Carlos, Esteem, Goldmine, Gold Rush, Hymark, Impac, Laredo, Mission, Oro Rico, Primo, Sol Real, Valley Pac) and 10 mixed melon cultivars (Emerald, Golden Beauty, Golden Casaba, Honey Ace, Mega Brew, Morning Ice, Santa Fe, Saturno, Silver World, Sun Canary) were sown and irrigated on 10 April. On 27 June, withering, foliar necrotic lesions, and a dry rot of the roots with M. cannonballus were observed. Vine collapse was rated in each plot on 1 July. On 11 July, root symptom severity of three plants per plot was rated. Mixed melon types had lower vine decline and root symptom severity than cantaloupe cultivars tested as determined by orthogonal contrasts (P < 0.001). Esteem had the lowest vine decline severity of the cantaloupe cultivars tested (P ≤ 0.05).


Silver scurf, caused by Helminthosporium solani, is a blemish disease of the potato tubers. No effective method is currently available to control this postharvest disease. Chlorine presents an interesting chemical option to help manage this disease which causes important economic losses. The objective of this study was to evaluate the effect of chlorine atmospheres on the development of potato silver scurf. Potato tubers were infected with H. solani and stored at 15°C in hermetically sealed chambers containing 0% (control), 2, 20 and 201 mg available chlorine / L moist air for different periods. The exposure of tubers for 40 days to a chlorine concentration of 2 mg/L moist air markedly reduced silver scurf. A two-day exposure to 20 mg/L was also found to be effective in reducing silver scurf. The results suggest that there is a potential for controlling silver scurf in stored potato tubers with optimal chlorine concentration in the atmosphere and exposure time.

Beet pseudoyellow virus, a new virus in strawberry. I. E. TZANETAKIS (1) and R. R. Martin (1,2). (1) Dep. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331; (2) USDA-ARS, Corvallis, OR 97330. Phytopathology 93:S85. Publication no. P-2003-0624-AMA.

In our effort to determine the causal agent of strawberry pallidosis disease we identified a single plant that was not infected with the strawberry pallidosis associated virus (SPaV). Granting from this plant produced the typical symptoms of the pallidosis disease in Fragaria virginiana and was asymptomatic in Fragaria vesca plants. The dsRNA extracted from this isolate was similar but not identical to that of SPaV indicating that another crinivirus may be associated with the disease. We developed degenerate primers for the polymerase gene of criniviruses and used them in the PCR of the virus with these primers and with random primers. Clones from the heat shock protein 70 homologue gene (HSP70h) indicated that the virus was Beet pseudoyellow virus (BPYV). We were unable to identify dsRNA species larger than ~9000 bp and suggest that BPYV is a typical crinivirus with a bipartite rather than a monopartite genome as reported previously. Phylogenetic analysis of the HSP70h, the coat protein and the minor coat protein of the virus indicate that BPYV is most closely related to SPaV.


Blackberry plants that exhibited a chlorotic line pattern symptom were tested for known viruses of Rubus by ELISA. DsRNA extracted from symptomatic plants that tested negative in all ELISA tests had multiple bands with the largest approximately 9000 bp. Cloning with random primers, sequencing and a BLAST search revealed sequence similarity to the minor coat protein (CPm) of criniviruses. Generic crinivirus polymerase primers were designed and used to amplify a fragment of the polymerase gene that had homology to criniviruses. Specific primers were designed for the CPm and the polymerase genes of the new virus and used in RT-PCR to test for the virus. The virus was detected, with both primer sets in symptomatic blackberry plants from Arkansas, North Carolina and South Carolina. No amplification products were detected when these primers were used in RT-PCR tests with healthy blackberry plants or with two other criniviruses.


Tomato streak virus (TSV) has many serotypes. Attempts to use RT-PCR for detection of TSV in strawberry using primers based on published sequences were unsuccessful. We cloned and sequenced a portion of an isolate of TSV from strawberry, and when the sequence was aligned with published sequences there was approximately a 70% identity. We then sequenced the coat protein gene of ten TSV strawberry isolates from Japan and the United States. Phylogenetic analysis showed that strawberry isolates of TSV form a distinct cluster compared to isolates from other herbaceous hosts and the published TSV sequences. The differences are significant enough to assign a new name to the strawberry isolates of TSV. We suggest “Strawberry necrotic shock virus” from the name Frazier first used to describe the disease caused by this virus in strawberry in 1962.

Molecular characterization and epidemiology of the strawberry pallidosis virus. I. E. TZANETAKIS (1,2), A. B. Halgren (2), K. E. Keller (3), W. M. Wintemantel (4), and R. R. Martin (1,2,3). (1) Molecular and Cellular Biology Program; and (2) Dept. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331; (3) USDA-ARS, Corvallis, OR 97330; (4) USDA-ARS, Salinas, CA 93905. Phytopathology 93:S85. Publication no. P-2003-0627-AMA.

A Closterovirus belonging to the Crinivirus genus has been identified as a potential causal agent of strawberry pallidosis and was designated as Strawberry pallidosis associated virus (SPaV). The virus is present in California, Eastern U.S., and may be present in other strawberry production regions as well. The complete heat shock protein 70 homologue and coat protein genes of the virus have been sequenced. Phylogenetic analysis indicates that SPaV is most closely related to Curticbit yellow stunting disorder virus with which is shares 34% amino acid identity in the coat protein gene. Transmission studies have identified Tridraulinae vaporariorum.
the greenhouse whitely as a potential vector of the SPaV. Transmission studies involving other whitely species are in progress. We have developed antibodies against the recombinant coat protein of the virus and we are currently producing monoclonal antibodies.

Comparative analysis of Pyricularia grisea causing simultaneous outbreaks of gray leaf spot of perennial ryegrass in U.S. and Japan. W. UDDIN (1), Y. Tosa (2), and G. Vijji (1). (1) Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (2) Faculty of Agriculture, Kobe University, Kobe 657-8501, Japan. Phytopathology 93:886. Publication no. P-2003-0628-AMA.

Isolates of Pyricularia grisea causing gray leaf spot of perennial ryegrass (Lolium perenne) in golf course fairways in U.S. and Japan were characterized using the repetitive DNA elements, MGR586, Pot2, and MAG. Two groups of grisea isolates from Japan, two groups of TALF (anamorph G. graminicola) were identified. The major group (TALF) was similar to the isolates from U.S. with the exception of an isolate from Maryland. There were three sub-populations among TALF isolates from Japan, of which one was identical to several U.S. isolates collected from Pennsylvania. Geographically, the isolates from U.S. were more diverse than those from Japan. The isolates collected in U.S. in 1995 and 1996 were more diverse than those collected from 1998 to 2001; however, the diversity decreased over time, and consequently a single group became predominant. The results suggest that the predominant group of the P. grisea isolates associated with the outbreaks of gray leaf spot in Japan and those from U.S., belong to the same lineage.


The effects of cultural management practices on the development of anthracnose basal rot in mixed annual bluegrass and creeping bentgrass putting greens were evaluated. The experiment was set up as split-plot design that included two factors: mowing height and verticutting (110, 77, and 51 mm for putting greens were evaluated. The experiment was set up as split-plot design. The turf was inoculated with Colletotrichum graminicola (4 x 10^4 conidia/ml of water) 6 h after the treatment applications. There were significant effects (P = 0.05) of mowing heights and verticutting on severity of anthracnose basal rot. Disease severity was significantly higher in turf that received deep verticutting than the treatments that received moderate or no verticutting, regardless of the mowing height. Similarly, disease severity was significantly higher in close-cut turf than in tall-cut turf, regardless of the verticutting depth. This study indicates that certain turfgrass management practices contribute to development of anthracnose basal rot in putting greens.

Transcriptional profiling of tomato tissue treated with coronatine. S. R. UPPALAPATI (1), P. Ayoubi (2), and C. L. Bender (1). (1) Dept. of Entomology and Plant Pathology; and (2) Dept. of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078; uppalap@okstate.edu. Phytopathology 93:886. Publication no. P-2003-0630-AMA.

Coronatine (COR), a phytoxoto produced by Pseudomonas syringae, has structural and functional similarity to jasmonic acid. In the present study, a cDNA microarray was utilized to identify COR-regulated genes in tomato. Approximately 1.3% of 7,714 unique genes showed a significant change in expression (>2-log fold increase or decrease over untreated control) within 12 h after treatment with exogenously applied, purified COR (0.2 mM). Some of the COR-regulated genes were represented in pathways previously associated with COR-induced biological processes. However, a much larger number of genes were not previously associated with COR, and some of these were novel. The coronacid acid (CFA) portion of the COR was active and regulated ~60% of the COR-regulated genes. The results indicate that COR regulates tomato via multiple phytohormone pathways. The COR-regulated genes identified in this study will also be useful in identifying the multiple targets for COR in planta.

Detection and identification of Didymella bryoniae in plant samples and greenhouse air samples by polymerase chain reaction. R. S. UTKHEDE and C. A. Koch. Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1000, Agassiz, BC V0M 1A0. Phytopathology 93:886. Publication no. P-2003-0631-AMA.

Two species specific pairs of primers were evaluated for detecting and identifying Didymella bryoniae (anamorph Phoma cucurbitae-caracorum), causal agent of gummy stem blight of greenhouse cucumbers, in tissue and air samples, by the polymerase chain reaction. One primer in each pair was a universal primer that has been used for amplification of septate fungi. The other primer in each pair was an oligonucleotide designed from sequence information in the GenBank database to be specific to D. bryoniae. These two oligonucleotides were selected as species-specific probes in hybridization reactions. Both primer pairs were able to distinguish D. bryoniae from other fungal genera, and from most other species of Didymella and Phoma. Both primer pairs gave positive results for cucumber tissue infected with D. bryoniae, and negative results for uninfected tissue and for tissue infected with Botrytis cinerea. Both pairs of primers were able to detect D. bryoniae from air samples collected using a rotation impaction sampler.


Gibberella pellisalis is the causative agent of dry rot in stored potato tubers. Enterobacter cloacae S11:T:07 (NRRL 21050) is a proven antagonist against G. pellisalis. One concern in using S11:T:07 is shelf-life after biomass production. Freeze-drying biomass in the presence of osmolytes has been shown to extend shelf-life. Two CLAs were identified that were shown to extend the shelf-life of freeze-dried cell populations over time. In the present study 100Ml. turanose or arginine was added to a semidefined medium that was inoculated with S11:T:07, harvested after 96h and freeze-dried. Dried cells were stored at room temperature over 21 days. Efficacy testing and cell survival was evaluated periodically; S11:T:07 cells survived freeze-drying better when grown with turanose than with arginine (-1.6 log units) or no osmolyte (-1.6 log units). Over a 21 day testing period, cells grown in the turanose medium showed superior survival of freeze-drying and reduced rot by 34% (<0.05).


Radial growth and sporulation were compared among newly discovered members of the sotoot botch complex, which colonize the cuticle of apple fruit. Isolates from six clades were grown on potato dextrose agar (PDA), malt extract agar (MEA), and carnation leaf agar (CLA). Colony diameter was measured after 2 weeks of incubation at 25°C, and conidia were counted after 4, 11, and 18 days of growth at 25°C. Overall, there were significant effects of isolate and media on radial growth, as well as a significant isolate x media interaction. Radial growth was significantly greater on MEA than with turanose (0.7 log units) than with arginine (-1.6 log units) or no osmolyte (-1.6 log units). Over a 21 day testing period, cells grown in the turanose medium showed superior survival of freeze-drying and reduced rot by 34% (<0.05).


Aphanomyces euteiches causes severe root rot of peas. Resistance is limited in commercial pea cultivars. Lack of progress in breeding for resistance may in part be due to limited discriminatory power of typical screening procedures, which rate disease severity using an integer scale. We developed a real time fluorescent PCR assay specific for A. euteiches. The susceptible cultivar Genie and the moderately resistant breeding line 180693 were inoculated with two different pathogen isolates. Plants were rated for disease severity using an index of 0 (healthy) – 5 (extreme root necrosis) and the amount of pathogen DNA in roots was determined using the PCR assay. For the two different inoculum levels for both pathogen isolates, significantly more pathogen DNA was detected in the susceptible cultivar than in the moderately resistant population. In seven of eight experiments the Spearman correlation between pathogen DNA quantity and disease severity index was positive and significant (P < 0.05). This real time PCR assay may be a useful selection tool to discriminate among plants that are indistinguishable based on visual assessment of disease severity.

Citrus tristeza virus (CTV) is worldwide in distribution. A continuous supply of reliable antibodies is needed for management of CTV in budwood certification, cross protection, eradication and other CTV management programs. The coat protein gene of CTV isolate, T36 was cloned in PET 22b vector (Novagen) and expressed in Escherichia coli, BL21 DE3. The expressed protein was purified using a nickel column. A naïve human single chain antibody (ScFv) library, provided by the Centre for Protein Engineering, MRC Centre, Cambridge, England, was screened against the expressed protein. After three rounds of biopanning, several clones producing ScFvs specific to CTV CP were identified. ScFvs produced from selected clones were screened against the expressed protein and the infected tissue. Five clones were further tested against diverse CTV isolates from different geographic regions.


Bacteria isolated from cranberry stem galls were screened for indole acetic acid (IAA) production. A subset (ca. 16%) of isolates that produced the highest levels of IAA were identified by 16S rRNA gene sequencing. Those similar (>96%) to members of the Enterobacteriaceae caused galls on micropropagated cranberry plants. Potent IAA producers that did not cause galls in our assay included some species of Pseudomonas, Agrobacterium rubi and Xanthomonas campestris. Genes from the indole-3-acetamide pathway for IAA biosynthesis, normally found in gall-forming bacteria, were not detected in representative Pantoea agglomerans strains. Bacteria were also isolated from 17 galls of cranberry where symptoms had not been seen. Comparison of IAA production among bacteria from the different sites will be reported. Thin sections of field galls and galls from micropropagated plants were observed microscopically. Gall tissue proliferated from the vascular cambium and broke through the outer bark or epidermal tissues. Xylem from the stem could be seen leading directly into the heavily vascularized gall tissue.


Cephalosporium stripe of wheat caused by Cephalosporium gramineum can be seed transmitted. Little is known about the extent to which this occurs in commercial seed lots; therefore, the objective of this study was to develop PCR primers for detection of the fungus in wheat seed. Twenty isolates from 37 locations in WA were analyzed using AFLP to identify genotypes. Five isolates with distinct AFLP patterns were chosen and the nuclear ribosomal ITS 1, 5.8s and ITS 2 were amplified with universal primers ITS 1 and 4. The PCR products were sequenced and aligned with Phyllostropha hurelata and Rhynchosporum secalis sequences to identify potential primers specific for C. gramineum. One set of primers amplified a 527 bp product from the genomes of 105 isolates of C. gramineum from WA, MT, KS and Japan, but not from 27 isolates of wheat seed mycdata. The same amplicon was produced from wheat seed infected with C. gramineum, thus demonstrating the potential of these primers to specifically detect the fungus in seed.

Basal levels of expression of defense-related genes in soybean cultivars varying in partial resistance to Phytophthora sojae. M. E. VEGA-SANCHEZ (1), M. G. Redinbaugh (2), and A. E. Dorrance (1). (1) The Ohio State University, Dept. of Plant Pathology; (2) USDA-ARS, Wooster, OH 44691. Phytopathology 93:S87. Publication no. P-2003-0638-AMA.

The genetic mechanisms of partial resistance to P. sojae in soybean are poorly understood. To explore the hypothesis that higher levels of basal expression of defense-related genes correlate with high levels of partial resistance, a Northern blot analysis was carried out. Total RNA was extracted from roots and cotyledons of fourteen soybean cultivars having low, moderate and high levels of partial resistance to P. sojae. Basal levels of transcripts for the seven defense-related genes tested were detected in all cultivars. Most mRNAs had higher levels in roots than in cotyledons, especially transcripts for PR-1a and basic peroxidase. Variation in basal defense gene expression levels and partial resistance to P. sojae exists in soybean roots or cotyledons.


Mannitol, a known quencher of reactive oxygen species (ROS), is thought to be involved in plant-pathogen interactions by allowing fungi to counteract the ROS-mediated plant defenses. We are studying Alternaria alternata, a causal agent of brown spot in tobacco. To assess the role of mannitol in A. alternata interactions with soybean, we are crossing fungal strain unable to produce mannitol dehydrogenase (Mdh), the enzyme responsible for mannitol synthesis in this fungus. A 2Kb fragment containing a gene with strong homology to published MtDHs was cloned, and a disruption construct was made using hygromycin as a selectable maker. A. alternata protoplasts were transformed using the construct, and hygromycin-resistant transformants were recovered. Enzyme assays for four putative transformants showed no activity for MtDH. These putative MtDH disruptants are being further characterized by Southern analysis and for mannitol production. Mannitol knock-out strains will be assayed for pathogenicity on tobacco.


Colletotrichum graminicola causes anthracnose leaf blight, stalk rot, and vascular wilt (‘top dieback’) of corn. C. graminicola produces two, developmentally distinct, types of conidia. One is falcate and produced from conidiogenous cells in acervuli that also contain setae, whereas the other is ovoid and produced directly from submerged hyphae not contained within a fruiting structure. Our goal is to compare the development of ovoid and falcate conidia in vitro in response to various environmental and chemical factors. We developed an in vitro bioassay that involves growth of the fungus from sporulated mycelia in a nutrient-poor agarose medium. Surprisingly, falcate as well as ovoid spores were produced from hyphae that were submerged beneath the surface of the medium. Individual hyphal branches appeared to be competent to produce only one of the two types of spores. Studies are underway to examine the effect of various nutrient sources and pharmacological inhibitors on these developmental processes. A better understanding of the stimuli that trigger production of one or the other type of spore may shed light on their comparative functions in vivo.

Characterization of Pyricularia grisea from various geographic regions of the United States and Japan using vegetative compatibility and pathogenicity assays. G. VII (1), W. Uldin (1), and J. C. Correll (2). (1) Dept of Plant Pathology, The Pennsylvania State University, University Park, PA 16802; (2) Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701. Phytopathology 93:S87. Publication no. P-2003-0641-AMA.

Isolates of Pyricularia grisea causing gray leaf spot of perennial ryegrass (Lolium perenne), from various geographical regions of the U.S. and Japan, collected between 1995 and 2000, were characterized for vegetative compatibility and pathogenicity. Nit mutants were used to assess the vegetative compatibility. All of the perennial ryegrass isolates of P. grisea from throughout the U.S. (except one from Maryland), and 5 of the 6 isolates from Japan, belonged to a single vegetative compatibility group (VCG), representing a widely distributed clonal population. Pathogenicity tests indicated that there were significant differences (P = 0.05) in virulence among the isolates of P. grisea from perennial ryegrass within this single VCG. Isolates of P. grisea from eight other gramineous hosts were vegetatively incompatible with the isolates from perennial ryegrass. In addition, there was a strong correspondence between VCG and molecular haplotypes.


Fungicide runoff following application for turf disease control was modeled using TurfPQ, a simulation model developed for turfgrass (J. Environ. Qual., 2001, 30:1033). Spray programs suitable for Kentucky golf course fairways and lawns were tested using a 21-yr period of weather data for Lexington, KY. Half-life and Koc values were obtained from published sources; other input parameters were selected to simulate conditions typical in local swards. Predicted fungicide concentrations in runoff (mg/L) were compared to LC50 values for Daphnia magna (“water flea”) and rainbow trout. All simulated chlorothalonil applications to fairways produced runoff with concentrations exceeding LC50’s for both indicator species. For some applications, predicted concentrations of azoxyystrobin, iprodione, and PCNB in runoff exceeded LC50 values of at least one indicator species. Under the conditions simulated, predicted concentrations of DMI fungicides, thiophanate methyl, and metalaxyl were well below LC50’s for indicator species. Tactics for reducing the risk of fungicide runoff will be discussed.
Genetic analysis and QTL identification of soybean resistance to Sclerotinia stem rot. T. D. Vuong (1) and G. L. Hartman (2). (1) Dept. of Crop Sciences; (2) Dept. of Crop Sciences, University of Illinois and USDA-ARS, Urbana, IL 61801. Phytopathology 93:S88. Publication no. P-2003-0643-AMA.

Sclerotinia stem rot (Sclerotinia sclerotiorum) is a major disease of soybean in the north-central states of the US. Partial resistance to this pathogen has been reported; yet there is limited understanding of inheritance of the resistance. The objectives were to determine the genetics of the resistance and identify marker genes associated with the resistance. Six crosses were made among susceptible and partially resistant soybean genotypes. Segregating F2 and F3 populations were used for genetic analysis indicating that resistance to S. sclerotiorum was quantitatively inherited. Simple sequence repeat (SSR) assay was utilized for the identification of markers and analysis of QTL in F4:5 recombinant inbred lines. Of over 500 SSR primers screened, 51% showed polymorphisms. SSR marker analysis will provide the basis for constructing populations for QTL resistance genes.


Bacterial fruit blotch (BFB), caused by Acidovorax avenae subsp. citrulli (Aac), is a serious threat to cucurbit production. Using 65 strains recovered from 5 hosts in 8 countries, we determined that Aac clustered into two groups, A and B. Strains from group A were more aggressive on watermelon (average severity = 3.16) than on cantaloupe fruit (average severity = 3.06). Hence, while there appears to be host specificity, this study did not distinguish between members of groups A and B.


Eyespot (Tapesia yallundae, TY and T. acutiformis, TA) of winter wheat causes yield loss in some locations and years in the UK. Disease progress for each species is not fully understood. The development of TY and TA on leaf sheaths was related to thermal time (accumulated degree-days) in five field experiments at Rothamsted, England (1985/86, 1986/87, 1987/88 and two in 2000/01). Depending on cultivar, season and sample the mean number of living leaves infected was 1.0 - 2.0 and 1.0 - 2.7 leaves per plant for TY and TA, respectively. The cumulative number of leaf sheaths penetrated or infected was consistently and positively correlated to thermal time from sowing date, with no significant differences in infection or penetration rates between TY and TA. Inoculation of wheat plants under controlled environment conditions with TY and TA showed no difference in number of leaf sheaths infected (3.43 and 2.99 leaf sheaths infected, respectively, at 10°C 200 m³). Although differences in progress of disease may exist between TY and TA, it was not discernable under the experimental conditions here.


Two experiments were conducted in north central Florida to examine the effects of winter cover crops on plant-parasitic nematode population dynamics. In the first experiment, six winter cover crops (wheat, rye, oat, lupine, hairy vetch, and crimson clover) were inoculated in a rotation with a summer corn crop. At the end of the corn crop in year one, population densities of root-knot nematodes were lower on corn following rye or oat, but not in year two. Wheat was a good host to stubby-root nematodes, whereas vetch was a poor host. In year two, stubby-root nematodes were not influenced by the treatments, but in 2004, plots after corn was grown. The second experiment was in a split-plot design in which rye or lupine was planted into field plots with histories of five summer cover crops: soybean, cowpea, sudax, sunn hemp, and corn. Population densities of root-knot and spiral nematodes were affected by previous summer crops, especially sunn hemp, but not by the winter cover crops present at the time of sampling. These data suggested that winter cover crops were not effective in suppressing plant-parasitic nematodes, especially root-knot nematodes, compared to summer cover crops.

Analysis of genetic and environmental factors that regulate production of syringopeptin by Pseudomonas syringae pv. syringae. N. Wang, S. E. Lu, and D. C. Gross. Texas A&M University, Dept. of Plant Pathology & Microbiology, College Station, TX. Phytopathology 93:S88. Publication no. P-2003-0649-AMA.

Syringopeptin and syringomycin are necrosis-inducing lipopeptides produced by Pseudomonas syringae pv. syringae. The syringopeptin (syp) and syringomycin (syr) gene clusters are located adjacent to one another on a large genomic island approximately 145 kb in size. Previously, it was demonstrated that specific plant signal molecules activate syringomycin biosynthesis genes, such as the sybB1 synthetase gene. This study examined the effect of phenolic plant signal molecules on induction of the sypA reporter gene associated with syringomycin biosynthesis. The effect of mutation of potential regulatory genes located at the left border of the syp cluster on expression of a sypA:uidA reporter also was examined. Results will be presented that contrast the similarities and differences in the regulation of syringopeptin and syringomycin biosynthesis for strain B301D of P. syringae pv. syringae.


Secretory proteins synthesized in the esophageal glands of plant-parasitic nematodes play important roles in parasitism. A parasitism gene (SVY46) expressed exclusively in the dorsal gland of parasitic stages of the soybean cyst nematode (SCN), Heterodera glycines, encodes a protein with a signal peptide and has similarity with CLAVATA3 of Arabidopsis thaliana. Factor(s) in nematode stylet secretions induce novel gene regulatory cascades causing the paralyzed root cells to differentiate into unique feeding cells. The similarity of SVY46 with CLAVATA3 may suggest a signaling function of this nematode gland secretion that mimics a natural plant peptide. Antiserum raised against the SVY46 gene product localized the expressed protein within the nematode dorsal gland cell and its extension, indicating a movement of this protein towards the nematode stylet. SVY46 has been
cloned into a binary vector and expressed in soybean hairy roots to observe any phenotype changes in plant cells caused by in planta expression SYV46 from SCN.


The Arabidopsis RPP7 gene activates race-specific resistance to the downy mildew pathogen through a signaling mechanism that does not require accumulation of salicylic acid and is not suppressed by mutations in a variety of defense signal transducers (e.g., pad4-1, ndr1-1, npr1-1, pbs2-1). We have constructed a series of double mutants to test for additive or functionally redundant contributions by known defense signaling components. Most of these combinations display a slightly enhanced level of asexual sporulation, with the ndr1/pad4 combination having the strongest effect. All of the double mutants are capable of inducing the HR, but this response is delayed to varying degrees. These observations suggest that RPP7 activates resistance through multiple signaling pathways that collectively regulate the kinetics of the HR. The RPP7 gene belongs to a cluster of eight highly related CC-NBS-LRR genes on Chr.1. The RPP7 gene is 18.6 Kbp long and is comprised of long introns within short 5’ and 3’ UTRs. The RPP7 transcript is alternatively spliced.


Tilletia fusca is a smut fungus infecting two species of annual fescues, V. microstachys and V. octoflora, in North America. Microsatellite markers were developed to investigate the mating system and genetic differentiation of T. fusca infecting sympatric host populations at two sites in central Washington. T. fusca populations from V. microstachys showed greater genetic diversity than populations from V. octoflora. Mean number of alleles/locus was 1.4 for T. fusca from V. octoflora and 3.9 from V. microstachys. Pairwise genetic distances between populations indicated that T. fusca is differentiated by host rather than by geographic distance. All populations showed significant heterozygote deficiency with inbreeding coefficient FIS = 1, indicating that T. fusca is completely inbreeding. The result is concordant with observations of T. fusca and related species that the majority of primary basidiospores conjugate while attached to the basidium. This result suggests that T. fusca populations have become genetically isolated by host specialization and inbreeding.

Molecular and pathological variation in streptomyces causing common scab. L. A. WANNER. USDA-ARS Vegetable Lab, 10300 Baltimore Ave, Beltsville, MD 20705. Phytopathology 93:S89. Publication no. P-2003-0653-AMA.

Common scab is the fourth most important potato disease, and affects root and tuber crops world-wide. Scab is caused by streptomycetes, phylogenetically diverse soil-inhabiting gram-positive bacteria. Most are not plant pathogens. To better understand pathogenesis and variability in symptoms, we are investigating pathogenicity determinants and virulence factors in plant pathogenic streptomycetes. Pathogenicity is associated with production of the toxin thaxtomin and with a proposed pathogenicity island (PAI) containing a gene for a pathogenicity factor (nec1) within a conserved region that may be horizontally transferred into distantly related streptomycetes to produce plant pathogenic strains. We isolated streptomyces from scabby potatoes from several US states. Disease varied in severity and appearance in potato and radish. All pathogenic isolates contain the txtA gene, but distinct from PRSV-W was also found in the same area. These results provide a strong fluorescent signal whereas soybean agglutinin gave a weak to moderate signal. X. WANG, K. W. Kim, L. A. WANNER. USDA-ARS Vegetable Lab, 10300 Baltimore Ave, Beltsville, MD 20705. Phytopathology 93:S89. Publication no. P-2003-0654-AMA.

Black root rot is a serious disease of pantry, and infected plugs are a common method for introduction of this disease into a greenhouse operation. Re-used greenhouse plug trays were assayed for the presence of T. basicola spores, and commonly used greenhouse disinfectants were evaluated for efficacy in reducing spore viability. It was confirmed that T. basicola spores can survive on recycled plug trays and are capable of infecting new crops seeded into the re-used trays. Some disinfectants were not effective at label rates for complete disinfestation of Thielaviopsis spores. Residual organic matter (potting mix and roots) significantly reduced the efficacy of certain disinfectants. The production practice of using recycled plug trays carries the risk of infecting new plug crops with black root rot.

Lectin binding to salivary glands of two Spiroplasma vectors, Circulifer tenellus and Excitius exiiusius. A. WAYADANDE (1), S. Palermo (2), and J. M. McDowell (1). (1) Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK; (2) Dept. of Entomology, University of Torino, Torino, Italy. Phytopathology 93:S89. Publication no. P-2003-0655-AMA.

Spiroplasma citri and S ikancellui traverse the gut and salivary glands of female vector leafhoppers during transmision, likely adhering to specific cellular components at the salivary gland interface. Seven FITC-conjugated lectins were used to characterize the salivary gland surface of two Spiroplasma vector leafhoppers, Circulifer tenellus and Excitius exiiusius. Salivary gland pairs were excised, frozen, incubated with individual lectins and examined for fluorescent signal. Lectins showed different binding capacity to salivary glands of the two leafhoppers. Concanavalin A and wheat germ agglutinin provided a strongly fluorescent signal, whereas soybean agglutinin gave a moderately strong signal. Peanut agglutinin and Ricinus agglutinin gave a weak to moderate signal. Ulex europaea agglutinin and Dolichos biflorus agglutinin provided no signal to weak signal in both species. Based on the known binding specificities of these lectins, results suggest the presence of glucosyl residues and lack of galactosyl or fucose residues on the salivary gland basal lamina.


Because Florida experiences frequent introductions of pathogens and insect vectors, we conducted a statewide survey of field-grown cucurbits to determine if any new viruses were present. In 2002, 931 samples, mostly from melons and squash, showing mosaic symptoms, were tested by ELISA for nine viruses. Samples with symptoms that did not react in any test were used to mechanically inoculate squash. Papaya ringspot virus-type W (PRSV-W) was detected in 65% of south Florida samples, and watermelon mosaic virus-2 was found in 85% of samples from north Florida. Zucchini yellow mosaic virus was more common in south Florida than in the past. Other viruses were found, including watermelon leaf mottle virus, tomato spotted wilt virus, and cucumber mosaic virus. An ilavivirus (a strain of tobacco streak virus) was detected in 45% of 119 plants sampled from a field in south Florida. A potyvirus that appears from preliminary sequence information to be related to but distinct from PRSV-W was also found in the same area. These results show the need for ongoing surveys.


An epidemic of canker disease was observed in a plantation of hybrid poplar clone NM6 (Populus nigra x P. maximowiczii) in northern Wisconsin in the summer of 2000. This is noteworthy because NM6 has been considered for large-scale short rotation, intensive culture plantations due to its fast growth and putative canker disease resistance. The DNA fingerprint obtained from samples of 931 samples, mostly from melons and squash, showing mosaic symptoms, were tested by ELISA for nine viruses. Samples with symptoms that did not react in any test were used to mechanically inoculate squash. Papaya ringspot virus-type W (PRSV-W) was detected in 65% of south Florida samples, and watermelon mosaic virus-2 was found in 85% of samples from north Florida. Zucchini yellow mosaic virus was more common in south Florida than in the past. Other viruses were found, including watermelon leaf mottle virus, tomato spotted wilt virus, and cucumber mosaic virus. An ilavivirus (a strain of tobacco streak virus) was detected in 45% of 119 plants sampled from a field in south Florida. A potyvirus that appears from preliminary sequence information to be related to but distinct from PRSV-W was also found in the same area. These results show the need for ongoing surveys.


Novozymes newly registered Bacillus SB3086 based biofungicide EcoGuard was tested at six university turf centers in the United States during the 2002
inhibits energy production in plant pathogenic fungi and is highly active against spore germination and mycelial growth. Tanos™ controls a wide range of tomato diseases such as early blight (Alternaria solani), late blight (Phytophthora infestans), anthracnose (Colletotrichum cereoides), target spot (Corynespora cassicola), and leaf spot (Septoria lycopersici). Tanos™ also improves control of bacterial spot (Xanthomonas campestris pv. vesicatoria) and bacterial speck (Pseudomonas pv. tomato) when combined or alternated with copper or EBDC fungicides. Disease management programs that combine or alternate Tanos™ with other appropriate fungicides are highly effective and reduce the risk of resistance development.


Woodwasps vector wood decay fungi (WDF) to many eastern hardwoods resulting in substantial decay damage in addition to the galleries made by tunneling larvae. WDF isolated from the mycangia of four xiphydriid and two tremicine woodwasps were evaluated for their potential to decay sapwood of eight eastern hardwood species over a two-year period. The wood decay potential of these fungi was assessed in vitro using weight loss as a measure of decay. Xiphydriid WDF from xiphydriid woodwasps (as a group) caused mean weight losses comparable to losses caused by xiphydriid woodwasps from both primary and secondary xiphydriid woodwasps. Wood decay potential of tremicine woodwasps caused significantly more decay the second year than the first year, but there was no significant difference in weight loss caused by WDF from tremicine woodwasps between years. The decay type in all cases was endophytic white wood decay, and less than 1% of the wood was removed. Among all fungi tested, individual strains caused weight losses up to 17% the first year, and up to 26% the second year. The extent of decay by mycosymbionts of individual woodwasps was host-specific.


Resistance to Striga hermonthica was evaluated in 274 Pennisetum glaucum subsp. monodii entries in four trials in Mali and Niger from 1997 to 1998. Seventy five selected entries were evaluated in three additional trials in Niger and Nigeria in 2000. Mean emerged striga per plant ranged from 0.2 to 14.9 across the seven trials, and from 0.8 to 8.8 for the 75 entries. Nine entries were resistant to both methods, and are likely to be sources of stable striga resistance for cultivated pearl millet in West Africa.


Oospores of A. cochlioides form in diseased sugar beet roots and are assumed to survive in soil for years. Field trials were done for 2 yr to assess oospore survival in solarized (S) and non-solarized (NS) plots. Sugar beet hypotyls (2-cm long) containing 7-9 wk-old oospores (mean = 8,000 and 15,000 in 2001 and 2002, respectively) were put in nylon mesh bags (10 micron pore size, 1 hypocytol/bag) and buried at 8, 15 and 23 cm in mid July. A polyethylene tarp covered S plots from mid July to early September; NS plots were fallow (3 x 9 m, 6 x 9 m plots in 2001 and 2002, respectively). Then, mesh bags were retrieved; hypotyls were rated for decomposition; and number of viable oospores were counted. No significant differences in oospore viability occurred in S (23%) and NS (44%) plots and in 2002, numbers of living oospores were the same in S (1.745) and NS (1.166) plots. Thus, 1 solatization was ineffective in reducing viability of A. cochlioides oospores and 2 oospore survival depended upon integrity of infected sugar beet roots.

The glume and leaf blotch, caused by *Phaeosphaeria nodorum* (anam. *Stagonospora nodorum*), is a common disease on small grains. For a better understanding of the pathogenesis a series of cross inoculation experiments were conducted under greenhouse and field conditions in which two winter varieties of wheat and barley were inoculated with five isolates of *P. nodorum* derived from these three host species. Disease severity of seedlings and adult plants was evaluated on leaves and ears, respectively, using necrotic area as disease parameter. In addition, a DAS-ELISA and xylanase assay, respectively, were used for the quantitative determination of fungal infection. Triticale and wheat were usually more infected than rye at all growth stages. Likewise, the symptoms on triticale and wheat were generally more obvious than on rye. *P. nodorum* infected rye seedlings, but did not cause noticeable symptoms. This result suggests that susceptibility of rye is not equally expressed at all growth stages of development.

**Baseline sensitivity distribution of *Colletotrichum graminicola* (turfgrass anthracnose) to thiophanate-methyl and detection of resistance in California populations.** F. P. WONG. Dept. of Plant Pathology, Univ. of Calif., Riverside, CA 92521. Phytopathology 93:S91. Publication no. P-2003-0667-AMA.

Thiophanate-methyl [TM] is a benzimidazole fungicide frequently used for the control of turfgrass anthracnose. In 2002, 280 isolates were collected from four golf courses: three greens (exposed sites) and a fairway where fungicides had not been previously applied (baseline site). Sixty isolates from the baseline site were tested using a mycelial expansion assay on 1/4-PDA amended with 0 to 30 mg/l TM at half-log serial dilutions. ED₅₀ values ranged from 0.14 to 2.3 mg/l with a mean value of 0.75 mg/l. Twenty isolates from 26 NY sites exposed to TM for a discriminatory dose of 10 mg/l. The remaining isolates were tested at the discriminatory dose of 10 mg/l TM. For the three exposed sites, 95, 93 and 92% of the isolates had more than 80% growth relative to the check plate (RG); 86% of baseline site isolates had 0 to 10% RG, while the remaining 14% had 10 to 30% RG at this discriminatory dose. This is the first report of TM resistance in California for this pathogen and suggests that the viability of future TM use for the control of this pathogen be reexamined.


Bosalid (BAS 510 F) and pyraclostrobin are carboxin and QoI fungicides, respectively, in development for grapevine disease control. In 1999 and 2001, boscalid and pyraclostrobin provided 99% control of cluster mildew, pyraclostrobin 99% control of powdery mildew, 99% control of downy mildew, 97% control of black spot, 93% control of gray mold, 92% of the isolates had more than 80% growth relative to the check plate (RG); 86% of baseline site isolates had 0 to 10% RG, while the remaining 14% had 10 to 30% RG at this discriminatory dose. This is the first report of TM resistance in California for this pathogen and suggests that the viability of future TM use for the control of this pathogen be reexamined.

**A new method for detecting spatial patterns in disease survey data, including data from both airborne and soilborne diseases.** F. P. WONG (1), T. R. MAIER (3), S. R. Rodermel (1,2), and T. J. Baum (1,3). (1) Interdepartmental Genetics Program; (2) Dept. of Botany; (3) Dept. of Plant Pathology, Iowa State University, Ames, IA 50014. Phytopathology 93:S91. Publication no. P-2003-0672-AMA.

The new method detected aggregation that was not detected by V/M and LIP, and in many cases, the results were consistent with the results from variogram and spatial autocorrelation analyses. The new method can be applied to many types of data, including binomial diseased or healthy plant counts, incidence, and number of diseased plants or pathogen propagules although directional and edge effects may limit its application.

**The leaf as a key determinant of ephiphytic microbial population dynamics.** S. T. WOODY (1), R. N. Spear (1), E. V. Nordheim (2), A. R. Ives (3), and J. H. Andrews (1). (1) University of Wisconsin, Dept. of Plant Pathology; (2) Dept. of Statistics; (3) Dept. of Zoology, Madison, WI 53706. Phytopathology 93:S91. Publication no. P-2003-0670-AMA.

The abundance of phylloplane microorganisms typically varies over several orders of magnitude among leaves sampled concurrently. The reason for the variation is unclear because heretofore sampling was destructive. We developed a method to repeat the same sample at different times by removing progressively more proximal 1-cm-wide transverse segments. *Aureobasidium pullulans* (Ap) densities were determined as cfu/cm² of segment by a grind-plate procedure. Variability in Ap population densities among subsamples of a given leaf was 1/3 to 1/9 the variability among whole leaves harvested concurrently. Sequential harvest of leaf segments did not introduce unstable changes in density on a daily time scale. Among-leaf effects were the major source of variance. Changes in Ap density tended to be synchronous among leaves, with the rank-order Ap density of leaves largely being maintained through time. Persistent differences in habitat (leaf) quality likely are responsible for variation in Ap density among leaves.


A new method for detecting spatial patterns was designed based on the variance of moving window averages. Data sets were derived by averaging the original values within a moving window, and indices were calculated based on their variances at different window sizes. Different types of analyses were used to test the method in conjunction with commonly used methods. Regardless of data types, the new method correctly determined the spatial patterns except when directional and edge effects were strong. This method was also employed to assess spatial patterns in disease survey data, including data from both airborne and soilborne diseases. The new method detected aggregation that was not detected by V/m and LIP, and in many cases, the results were consistent with the results from variogram and spatial autocorrelation analyses. The new method can be applied to many types of data, including binomial diseased or healthy plant counts, incidence, and number of diseased plants or pathogen propagules although directional and edge effects may limit its application.

**Nitric oxide synthase in higher plants: An attempt to clone the gene.** J. E. Wubben (1), T. R. MAIER (3), S. R. Rodermel (1,2), and T. J. Baum (1,3). (1) Interdepartmental Genetics Program; (2) Dept. of Botany; (3) Dept. of Plant Pathology, Iowa State University, Ames, IA 50014. Phytopathology 93:S91. Publication no. P-2003-0669-AMA.

Nitric oxide (NO) is a signal molecule involved in intercellular communication and immune responses in mammalian cells, produced during the conversion of L-arginine to L-citrulline as catalyzed by nitric oxide synthase (NOS). There is evidence for the presence of both NO and NOS in higher plants, including the involvement of NO in disease resistance. Hitherto, NOS has not been isolated from higher plants nor the gene cloned. We have attempted to clone the NOS gene through RT-PCR amplification using wild type of *Arabidopsis thaliana* and pea tissue undergoing a hypersensitive reaction as templates and primers designed to target: 1) functional domains and consensus sequences in NOSs from animal species, 2) NOS gene of *Physarum polycephalum* and 3) hypothetical genes in the Arabidopsis genome. RT-PCR was not able to amplify any of the target fragments except one 400bp fragment amplified from a gene sequence in Arabidopsis with similarity to *Helix pomatia* br-1 (NOS) protein. The gene encoding NOS (or its functional equivalent) in higher plants may be quite different from NOS genes in other species.
on spring-sown oat (123 Kg/ha), 6.1 on bare soil and 3.8 to 4.7 on annual medics (45 Kg/ha). Percent ground cover at harvest and % fruit with FFR were 89% and 5% in rye (101 Kg/ha), 88% and 10% in rye (56 Kg/ha), 85% and 5% in rye + hairy vetch (56 Kg/ha ea), 19% and 30% in fall-sown hairy vetch (56 Kg/ha), 23% and 23% in spring-sown oat (123 Kg/ha), 1% and 25-39% in spring-sown annual medic and 0% and 46% in bare soil.


Winter pears in the U.S. are primarily produced in the Pacific Northwest (PNW). Phacidiopycnis pyri, the anamorph of the discomycete Pochthienia pyri, is the causal agent of Phacidiopycnis rot, a newly recognized postharvest disease in the U.S. The fungus was found on freeze-treated and cankers on twigs. Little information is available on pathogenicity of this fungus to pear tree. To determine the distribution of the fungus, dying and dead bark were sampled from pear orchards in PNW, and examined for presence of pycnidia of the fungus. Decayed fruit from storage were also collected from some locations for isolation of the fungus. In the orchard, 2-year-old twigs were wounded with or without freezing at the wound sites, and then inoculated with the fungus. The fungus was found to be widespread in PNW. Incidence of trees infected by the fungus in pear orchards ranged from 0 to 100 percent. Monthly tree inoculations in the orchard indicated that the fungus did not cause canker on wound-inoculated twigs but it established on freeze-treated twigs. Thus the fungus appeared to be a weak canker-causing pathogen on pear trees.

A new postharvest fruit rot in d’Anjou pears caused by Sphaeropsis pyriputrescens sp. nov. C. L. XIAO (1) and J. D. Rogers (2). Washington State Univ., (1) TFREC, Wenatchee, WA 98801; (2) Dept. of Plant Pathology, Pullman, WA 99164. Phytopathology 93:S92. Publication no. P-2003-0675-AMA.

A new postharvest fruit rot in d’Anjou pears in Washington State was discovered during a disease survey in 2001-02. Symptoms were stem-end rot, calyx-end rot, and wound-associated rot, which originated from infections of stem, calyx, and wounds on fruit surfaces, respectively. The decayed area was first water-soaked then appeared brown. During the late storage period from March to May 2002, fruit from 19 of 39 lots had this disease, accounting for 2 to 21% of the decayed fruit. The causal agent, Sphaeropsis pyriputrescens sp. nov., was consistently recovered from decayed fruit with symptoms described above. In the laboratory, fruit were inoculated with spore suspensions of the fungus at stem, calyx, and wounds on fruit surfaces, and then stored at 0°C. Decay symptoms were observed within 30 days. In the field, inoculated fruit were isolated and then re-isolated from the same host. The fungus, S. pyriputrescens sp. nov., has been characterized and described as a new species. The fungus grows at the pear storage temperature -1 to 0°C, with optimum temperature between 15 to 20°C, little or no growth at 30°C but no growth at 35°C. The fungus appears to be a low temperature species.


The potato mop-top virus (PMTV), recently discovered to be present in Canada and the United States, has potential for causing significant quality defects in processing potatoes. The coat protein gene of six North American isolates was sequenced and found to have 99-100% homology; they had 97-99% homology with a European isolate. The potato mop-top virus coat protein gene in RNA 3 and the triple-gene-block in RNA 2, were designed for detection of PMTV by RT-PCR. PMTV RNA was detected by RT-PCR in parenchymous tissue from the stem and bud ends of potato tubers and in peridermal tissue immediately after harvest and after 2-6 mo of cold storage. Identity of RT-PCR amplicons was confirmed by restriction analysis using HindIII or BstEII. The presence of PMTV in RT-PCR positive samples was verified by ELISA and in some cases by bioassay on Nicotiana debneyi. Efficiency of screening seed lots for PMTV was improved by combining tissue from up to 100 tubers into a single composite sample. Preliminary tests indicated that a single positive tuber could be detected at this composite level using RT-PCR but not ELISA.

Use of SADE statistics to study spatial dynamics of plant disease epidemics. X. M. Xu (1) and L. V. Madden (2). (1) Horticulture Research International - East Malling, West Malling, Kent ME19 6BJ, UK; (2) Department of Plant Pathology, Ohio State University, Wooster, OH 44691-4096. Phytopathology 93:S92. Publication no. P-2003-0677-AMA.

Using a stochastic simulation model, relationship of the SADE $I_s$ statistic with initial epidemic conditions, spore dispersal distance, sampling quadrat size and other spatial statistics was investigated. Most variation in $I_s$ was attributable to the initial spatial pattern of infected plants and quadrat size. Increasing quadrat size led to decreased values of $I_s$. Variability in $I_s$ between replicate runs also depended on spatial patterns: lowest and greatest for the clumped and random patterns, respectively. $I_s$ increased initially and then decreased with increasing incidence. Overall, $I_s$ was not sensitive to the change in median spore dispersal distance. Using artificially generated data sets, we found that SADE statistics were dependent on number and absolute positions of patches, and not just on their relative positions, and were less sensitive to changes in spatial gradients than spatial autocorrelograms. Results indicate potential challenges in interpreting SADE statistics in relation to the underlying physical and biological processes.


Host resistance is the major method to control rusts. The occurrence of yellow rust (Puccinia striiformis f. sp. triticci), leaf rust (Puccinia triticina), and stem rust (Puccinia graminis f. sp. tritici) in wheat in Africa, Middle East, West and Central Asia has been documented. The long-term co-evolution of wheat wild relatives, such as Aegilops and Triticum sp. with rust in most of these regions may have resulted in accumulation of efficient resistance genes in natural populations. These can be exploited as sources of resistance. Over 550 Aegilops accesions from 40 taxa in 20 species and 200 Triticum accesions from 13 taxa have been evaluated for single gene and combined resistance under artificial inoculation of seedlings and adult plants. Of Aegilops accesions 10.9% showed resistance to three rusts. Resistance to individual rust varied from 12.8 to 41.3%. About 65% of Aegilops triuncialis acessions had combined resistance to yellow and leaf rust. Triticum acessions showed relatively low resistance levels with 8.3% resistance to stem rust and 4% combined resistance to all three rusts. AFLP analysis showed high diversity among and within the Aegilops and Triticum sp.


The suitability of a bioherbicide as a component of an IPM program relies on its field efficacy and also on compatibility with other pest control measures employed during the cropping season. The effects of selected pesticides on the biocontrol agent Dactylaria higginsii were determined using mycelial growth and spore germination as indicators of pesticide sensitivity. Oxyfluorfen, fosetyl-Al, thiophanate-methyl, and dicofol completely inhibited the growth of D. higginsii. Diuron, glyphosate, metamitron, and copper hydroxide reduced mycelial growth while the growth on cyromazine- and imazapyr-amended media was comparable to growth on PDA (control). Germination of spores treated with cyromazine, imazapyr, copper hydroxide, mfenoxam, and diuron were higher or equal to those treated with water (control). Germination of spores treated with thiophanate, glyphosate, fosetyl-Al, dicofol, fosetyl-Al, and oxyfluorfen were significantly lower than the control.


Current resistance screening methods for soybean sudden death syndrome caused by Fusarium solani f. sp. glycines (FGS) are inconsistent in foliar symptom expression. Studies were conducted to determine the cause of inconsistent symptoms. Inoculation of soybean seeds with 1-cm radicle were dip-inoculated in spore suspension before transplanted. Three weeks later plants were assessed and grouped by presence of foliar symptoms. Discoloration in phloem or xylem tissues of tap roots were assessed and isolations were made to confirm FSG colonization in each tissue. All inoculated plants had discolored phloem tissue in taproot. However, discoloration was limited to phloem cylinders in plants without foliar symptoms. Discoloration occurred in both phloem and xylem tissues in plants with foliar symptoms and isolations confirmed colonization of FSG in xylem tissue. Our data suggest that 1) fungal colonization in xylem is critical to foliar symptom expression; 2) radicle infection increases likelihood of fungal colonization in xylem.

REATEINING EFFECTS OF THOROUGH-HARDNESS ON MYCOTOXIN PROFILES AND INTRAGENIC VARIATION IN GIBBERELLA ZEAE ITS PRIMARY USES IN MAIZE. D. M. ZAPATA. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR. Phytopathology 93:S93. Publication no. P-2003-0685-AMA.

Phytophthora ramorum, the causal agent in sudden oak death and related diseases, is now actively expanding from southwestern Oregon to many tanoak communities where the pathogen has been found in Oregon are substantially different from infested areas in California, with a new suite of potential host species present. We have screened more than 30 species of native shrubs and herbs for susceptibility to foliar infection by the pathogen using detached leaf assays. Most species screened appear susceptible to some degree in these tests when compared to known hosts, though evidence from field-collected material or whole-plant inoculations are needed to confirm the disease phenotype.

Characterization of pathogenic races of Xanthomonas campestris pv. phaseoli (Xcp) isolated from infected bean leaves in Nicaragua. C. Jochum (1), L. Parker (2), L. J. W. ZANZOTTO (1), K. N. ZADOK (1), and T. G. YUEN (1). (1) USDA-ARS Nematology Laboratory, Beltsville, MD 20705; (2) Department of Soil Science, University of P.R., Dept. of Crop Protection, P.O. Box 9030, Mayaguez, P.R. 00680-9030. Phytopathology 93:S93. Publication no. P-2003-0686-AMA.

Xanthomonas campestris pv. phaseoli (Xcp) isolated from infected bean leaves in Nicaragua, Costa Rica and Puerto Rico were compared using rRNA patterns and pathogenicity on 69 Phaseolus vulgaris genotypes. Strains were characterized by rRNA ribotyping using the restriction enzymes EcoRI, PstI and PvuII. One ribopattern was observed with EcoRI, two with Psti and three patterns with PvuII. Diversity among strains in rRNA at the infrasubspecific (pathovar) level was detected with the enzymes PvuII and PvuII. Differences in rRNA showed the presence of variable regions at the pathovar level. Twelve genotypes were identified with resistance to at least one bacterial strain and were selected for their specific reaction to the strains. Because of the specificity of the reactions, the strains from Nicaragua, Costa Rica and Puerto Rico are considered to be pathogenic races of Xcp. These races are not distinguishable by phenotype but they can be recognized by the variable regions detected in rRNA with PstI and PvuII.

Chemical-mediated lethal effects of N-Viro soil against Heterodera glycines. I. A. ZASADA (1) and M. Tenuta (2). (1) USDA-ARS Nematology Laboratory, Beltsville, MD 20705; (2) Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2. Phytopathology 93:S93. Publication no. P-2003-0687-AMA.

N-Viro Soil (NVS) is an alkaline stabilized municipal biosolid used as a soil amendment. Previous research demonstrated lower populations and reduced egg hatch of Heterodera glycines after NVS application. The mechanism(s) of nematode suppression by NVS and amendment are unknown. This study evaluated NVS-mediated changes in soil chemical properties and their impact upon survival of second-stage juveniles of H. glycines. In soil microcosms, NVS was applied at rates corresponding to 0, 12, 34 and 68 t/ha. There was a reduction in nematode survival at all NVS application rates. Increasing rates of NVS were strongly correlated with higher soil pH levels. Cations, anions, and NH4 concentrations in soil solution were also correlated with nematode survival in amended soil. This study is part of a comprehensive research program initially identifying abiotic and biotic mechanisms responsible for the reduction of plant-parasitic nematodes in NVS-amended soil. Thenceafter the use of NVS will be optimized in a strategy to control plant disease.


The ability to distinguish many fungal species depends upon the context within which species are defined. Morphological, biological, and phylogenetic species recognition criteria have different foci, often utilize different data types, and require different types of analyses. Each type of species recognition criterion has unique uses and drawbacks when applied to plant pathogenic fungi. For example, based on morphology Gibberella zeae is a single homogeneous species. Biological criteria (interfertility) suggest that G. zeae contains several subspecies with less than 2% interfertility between subspecies less than within subspecies. Phylogenetic criteria applied to molecular data may be interpreted to mean that G. zeae is composed of relatively distinct phylogenetic species. Similar problems exist within other Fusicladium, Gibberella fujikuroi and G. intermedia (biological species C and D of the G. fujikuroi complex) are morphologically indistinguishable, but can be distinguished by mycotoxin profiles, host associations, limited interfertility, and DNA sequence differences. However, some isolates can
complete meiosis to produce viable progeny. Similar problems are associated with putative species clusters that include *F. andiavaz* and *G. konza*.


We have previously reported the occurrence in nature of two distinct subgroups (I and II) of BPMV strains as well as reaissantants between the two subgroups. Now we report on the isolation and molecular characterization of an interstrain recombinant RNA1 from the severe reaissant strain CB-1. Two types of local lesion isolates, mild and severe, were derived from strain CB-1 after two passages on Pinto bean, with the majority of the isolates being severe. Whereas CB-1 and all local lesion isolates examined contained only one type of RNA1 (subgroup I), the RNA1 present in the mild isolates belonged to either subgroup I or II. Interestingly, the severe local lesion isolates contained at least two distinct types of RNA1 including an interstrain RNA1 (I-II) recombinant molecule. The same recombinant RNA1 (I-II) molecule was isolated from the original CB-1 strain using RT-PCR cloning and sequencing. Structurally similar recombinant RNA1 molecules were also generated after four passages in soybean using a mixed infection with infectious RNA1 transfectant from two distinct strains, suggesting the presence of recombination hot spots.


A collection of 267 isolates was established from soybean plants with characteristic stem canker lesions and symptoms in South Dakota during 1999-2001. RFLP analysis of the ITS5 region of nuclear ribosomal DNA, was carried out to aid in identification of individual isolates. PCR amplified DNA fragments defined by the ITS4 and ITS5 primers were digested with restriction enzymes Alu I, Hha I and Rsa I. Analysis of resultant RFLPs enabled classification of isolates among different groups within the *Diaporthe/Phomopsis* complex. Two hundred-six isolates (77%) were identified as *D. phaseolorum var. caelovora* (DPC), the causal pathogen of northern stem canker, and included both known subgroups: 163 DPC A (79%) and 43 DPC B (21%); 40 isolates (15%) belonged to *Phomopsis longicolla*, causal pathogen of Phomopsis seed decay; a third group of 21 isolates (8%) could not be identified with certainty to a previously characterized group but most closely resembled *D. phaseolorum var. meridionalis* (DPM), the causal pathogen of southern stem canker. RAPD and very high variability studies were also conducted and indicate variability among isolates within sub groups.


Fludioxonil manufactured by Syngenta Inc. is in process for EPA registration for the postharvest disease control of fruits including citrus. The efficacy of Fludioxonil for the control of stem-end rot and green mold caused by *Penicillium digitatum* (DPM), the causal pathogen of *C. ZHANG and S. A. Ghabrial. University of Kentucky, Dept. of Plant Pathology, Lexington, KY 40546. Phytopathology 93:S94. Publication no. P-2003-0692-AMA.

Fusarium head blight, caused by *Gibberella zeae*, is a devastating disease of wheat worldwide. *Cryptococcus nostaenisi* OH 182.9 has been evaluated as an effective biocontrol agent for this disease. Development of a dried product of OH 182.99 would have potential advantages of ease of handling, favorable economics and acceptance by end users. OH 182.9 was grown for 48 and 72 h in amended complete liquid media (SDLC) with C/N ratios of 6.5, 9, 11, 15 and 30. Total biomass production and cell survival for 15 d after freeze-drying were evaluated. Biomass production of OH 182.9 was similar for all cell age by medium C/N combinations. In general, cells harvested at 48 h survived freeze-drying better than those harvested at 72 h. Survival of freeze-dried cells was greatest for cells grown for 48 h in SDLC C/N 30 medium. Cells produced in C/N 6.5 medium generally exhibited the poorest survival. The difference in freeze-dried cell populations between superior and inferior treatments was more than 3 log units. Results of biocontrol efficacy will be reported.


Predation by soil mesofauna may impact the population dynamics and activities of soilborne phyto-pathogenic fungi. However, direct experimental evidence illustrating faunal controls over soilborne pathogens is limited. We examined the effects of the addition of a fungivorous nematode (*Apheleschenes avenue*) and two collembolan species (*Hygopestraxurpera* and *Sinella curviseta*) to an organic soil on disease incidence in tomato caused by *Pythium ultimum*. Compared to a defaunated control, introduction of both species or complete lack of fauna (*H. perplexa* and *S. curviseta*) resulted in suppression comparable to their no- pathogen control. These results indicate that faunal grazing on pathogens can significantly contribute to disease suppression, suggesting that effective management of soil fauna may facilitate control of some root pathogens in organic soils.

Biological control of *Sclerotinia sclerotiorum* infection in canola by *Bacillus* sp. Y. Zhang, W. G. D. Fernandez, and F. Daayf. Dept. of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada. Phytopathology 93:S94. Publication no. P-2003-0695-AMA.

*Sclerotinia* stem rot caused by *Sclerotinia sclerotiorum* (SS) is found worldwide on canola and rapeseed. Eight *Bacillus* strains isolated from canola and wheat plants showed antifungal activity against SS on both PDA and LBA plates. Four *Bacillus* strains were tested in whole plant assays. Canola petals were pre-treated with bacteria, then inoculated with SS ascospores 24 h later. After 17 d, plants pre-treated with bacteria had only 0.0-1.0 disease severity compared to 4.5 in control plants. Disease incidence (DI) on stems was 0.0-0.125 and 0.75 for pre-treated and control, respectively. DI on leaves was 0.0-0.5 and 1.0 for pre-treated and control plants, respectively. Disease progress over time was significantly reduced with bacterial treatments, indicating either curtailment or inhibition of ascospore germination. The antifungal area of inhibition on the plate seems to indicate that antibiotics play a role in biocontrol. Antibiotic agents will be isolated, purified and characterized, using TLC, HPLC and NMR methods, respectively. The mechanism of ascospore inhibition will be studied microscopically.

A single dominant gene designated Rsol that conditions the hypersensitive response (HR) to Xanthomonas oryzae pv. oryzae (X. o. pv. oryzae), the casual agent of rice bacterial leaf streak, was identified from maize inbred line B73. All X. o. pv. oryzae strains tested carried a non-host avirulence gene designated avrRsol. An NBS-LRR gene family that co-segregates with the Rsol gene was recently identified in gene mapping populations. Silencing this NBS-LRR gene family by RNAi knocked out the ability of maize to respond to X. o. pv. oryzae, suggesting that at least one gene of this family conditions the Rsol function. Expression studies demonstrated three out of five members of the NBS-LRR family are transcribed and only one, Rsol-1, accounts for the most of the transcript. An HR-like cell death occurs after transient expression of the Rsol-1 and the avrRsol genes in the maize line Mo17 (xol/xol). This suggests the Rsol-1 gene is the family member that confers the Rsol defense reaction. Stable expression of the Rsol gene in maize and rice to test its ability to confer resistance are on going.


We previously reported the isolation and molecular characterization of the multifunctional cellular protein Hv-p68, which is induced by virus infection in the plant pathogenic fungus Helminthosporium victoriae. Hv-p68 was proposed to play a role in viral RNA replication and pathogenesis. Hv-p68 genomic DNA cloned downstream of promoter Hv-p68 overexpression vectors were constructed and transformed into virus-free and virus-infected isolates of H. victoriae. Transformants overexpressing Hv-p68 were confirmed by northern hybridization and western blot analyses. Surprisingly, overexpression of Hv-p68 enhanced fungal growth in all transformants and had no effect on the diseased phenotype in the virus-infected isolates. On the other hand, a significant increase in the concentration of Hv-p68 transcript was observed in Hv-p68 transformed isolates compared to the original isolate. These findings suggest that the Hv-p68 overexpression affected the virus infection process.


ATP-binding cassette (ABC) is a highly conserved protein domain of approximately 215 amino acids; it plays a pivotal role in transducing energy from ATP to diverse biological processes. S. kunkelli is a helical, wall-less bacterium that causes corn stunt disease. The macrofusor has a compact genome with a gene set approaching the minimal complement for cellular life. A total of 22 ABC domains were identified during a preliminary annotation of a draft S. kunkelli genome sequence. 15 ABC transporters were present in 18 proteins and are components of 16 functional systems. Of the 16 systems, 15 are ABC transporters and the remaining one is an enzyme complex involved with DNA repair. Assembly and analysis of the 15 ABC transporters permitted predictions of the functions for the S. kunkelli ABC transporter systems. 7 importers, 5 exporters, and 3 with unclear pumping directions. These transporters handle a wide variety of substrates and are critical for nutrient uptake, multidrug resistance, and possible virulence.

Evaluation of Glycine species for resistance to Bean pod mottle virus. C. Zheng (1), R. GERGERICH (1), and P. Chen (2). (1) Dept. of Plant Pathology, Univ. of Arkansas; (2) Dept. of Crop, Soil and Environmental Sciences, Univ. of Arkansas, Fayetteville, AR 72701. Phytopathology 93:95. Publication no. P-2003-0699-AMA.

Bean pod mottle virus (BPMV) is widespread in soybeans in the U.S. No resistance has been identified in extensive evaluations of commercial cultivars and Plant Introductions (PIs) of Glycine max. Four BPMV isolates representing the diversity of this virus were used to study the reaction of diverse collections of two Glycine species to BPMV infection. Seedlings were mechanically inoculated twice with virus, and the inoculated plants were evaluated for symptoms and tested for virus by ELISA four weeks after the first inoculation. A mixture of 284 S. kunkelli transformants were resistant to all BPMV isolates and contained no virus. Fourteen of 118 G. soja PIs showed tolerance to BPMV, however, virus was detected in these plants. Thirty-seven of 118 G. soja PIs exhibited necrosis following BPMV inoculation. The resistance to BPMV expressed in G. tomentella and the necrotic reaction expressed in G. soja may be useful for the development of resistant soybean cultivars if this resistance can be incorporated into G. max by interspecific crosses between these species.


Tomato bushy stunt virus (TBSV) encodes a 22 kDa protein (P22) that is required for cell-to-cell movement. The aim of this project is to characterize the interaction of P22 with host components. P22 is an RNA-binding phosphorylated protein, which in overlay assays binds to a 45 kDa host protein, yeasts-two-hybrid studies showed that P22 interacts with a newly identified host homeodomain protein (HF22). Peptides were synthesized to a carboxyl region (246-260) unique to HF22 and used to produce antiserum in rabbits. Western blots probed with this antiserum revealed the presence of a 45 kDa protein in leaf extracts of healthy and infected plants. It has yet to be determined directly whether this antiserum-reactive protein is P22. However, co-sedimentation of different plant species showed that the signal for the 45 kDa band was most distinct for hosts of TBSV supporting a putative role of this protein during TBSV infections.

Generation of ethylene-insensitive transgenic rice via dsRNAi-mediated suppression of the EIN2 ortholog. X. Zhou and Y. YANG. University of Delaware, (1) Dept. of Plant Science, (2) Dept. of Plant Pathology, Texas A&M University, College Station, TX 77843. Phytopathology 93:595. Publication no. P-2003-0701-AMA.

Increasing evidence has shown that ethylene signaling is involved in the activation of host defense response in addition to its important role in plant growth and development. In Arabidopsis, EIN2 is a key component of ethylene signal transduction and is the only gene known whose recessive mutations lead to complete ethylene insensitivity. To determine the role of ethylene signaling in rice defense response, we have isolated the EIN2 ortholog from rice by screening a cDNA library. The full-length EIN2 cDNA in rice is 1,196 bp in length and encodes an estimated molecular mass of 126.3 kD and an isoelectric point of 5.61. RNA blot analysis suggested that EIN2 was constitutively expressed in rice leaves and was not inducible by the treatment of Magnaporthe grisea or the treatment of ethylene, jasmonic acid and salicylic acid. Using the double-stranded RNA interference (dsRNAi) approach, we have generated a total of 32 transgenic rice plants whose expression of endogenous EIN2 transcripts were efficiently suppressed. Potential insensitivity of these EIN2 dsRNAi transgenic mutants to ethylene treatment is currently being evaluated. Results from physiological and pathological tests will be presented.

Watermelon colonization by Fusarium oxysporum f. sp. niveum and quantification of Fusarium wilt resistance. X. G. ZHOU (1) and K. L. EVENTS (2). (1) University of Delaware, Salisbury, 21801; (2) University of Delaware, Georgetown, DE 19947. Phytopathology 93:595. Publication on no. P-2003-0702-AMA.

A more detailed understanding of the quantitative colonization of watermelon plants by Fusarium oxysporum f. sp. niveum (FON) during Fusarium wilt development may help develop a quick, innovative method to identify Fusarium wilt resistance. Six days after greenhouse root-dip inoculation with a chlorate-resistant marked race 1 isolate of FON, watermelon seedlings of 18 cultivars had lower-stem densities of 10^5, 10^6, and 10^7 CFU/g tissue for highly resistant, moderately resistant, and susceptible cultivars, respectively. Percent wilt was highly and positively correlated with CFU/g in roots, crowns, or lower stems, and with the ratio of the FON density in lower stem to root. Two years of field studies confirmed these relationships and a negative correlation (r^2 > 0.58) between tissue colonization and fruit yield occurred. Wilt resistance was related to limited internal-plant movement of FON during a season. FON density in seedling stems is a good indicator of resistance and can be utilized to quantify wilt resistance in watermelon.

Effect of foliar insecticide timing on incidence of Bean pod mottle virus. A. D. ZIEMS (1), L. J. Giesler (1), and T. E. Hunt (2). (1) Dept. of Plant Pathology; and (2) Dept. of Entomology, University of Nebraska, Lincoln, NE 68583-0772. Phytopathology 93:95. Publication no. P-2003-0703-AMA.

Bean pod mottle virus (BPMV) is an important virus effecting soybeans in the north central region of the United States. The primary vector for BPMV is the Cerotoma trifurcata (Forster) bean leaf beetle (BLB). A study was conducted to determine the optimal application time of a foliar insecticide lambda-cyhalothrin (Bayer AgriScience) to control BLB and to manage incidence of BPMV. The foliar insecticide was applied at growth stage VC, V2, and targeting F1 BLB population. BLB population counts were taken every five days from mid-April to the end of September. Plant tissue samples were taken at growth stage V2, V5, V9, R1, and R3 to determine BPMV incidence. The application targeting F1 BLB was the only treatment that gave a significant reduction in beetle population (alpha = 0.05) from the non-treated plots. This treatment had a 6% lower BPMV incidence (P < 0.01)
from the non-treated plots at growth stage R3. The V2 foliar application had a 17% lower BPMV incidence ($P = 0.01$) than the non-treated plots at growth stage R1. There was no reduction in BPMV incidence in the vegetative growth stages from any insecticide treatment. Further research is needed to develop management recommendations based on insecticide application time.

**Importance of mutualistic endophytic fungi in banana growing in soils suppressive to endoparasitic nematodes.** A. zum Felde (1), L. Pocasangre (2), and R. A. SIKORA (1). (1) Soil Ecosystem Phytopathology, Institute for Plant Diseases, University of Bonn, Nussallee 9, 53115 Bonn, Germany; (2) INIBAP-LAC, c/o CATIE, 7170 Turrialba, Costa Rica. Phytopathology 93:S96. Publication no. P-2003-d704-AMA.

The purpose was to investigate claims of nematode suppressive soils in banana plantations in the Motagua Valley of Guatemala. Root samples were collected from four farms where nematode suppression had been reported. Nematodes were extracted, counted and identified. *Radopholus similis*, *Helicotylenchus* spp. and *Meloidogyne* spp. were recovered from all farms. The number and species per 100 g root were significantly different between fields. The extremely low density of the most damaging nematode, *R. similis*, from two of the four farms sampled support the designation of these areas as nematode-suppressive. However, it is questionable whether the soils of these areas should be called suppressive, as the nematodes are endoparasitic. Further investigations clearly showed that fungal endophytes in the root tissue contributed significantly to nematode population suppression.