1995 APS Annual Meeting

ABSTRACTS OF PRESENTATIONS

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The number above an abstract corresponds to its designation in the program of the 1995 APS annual meeting in Pittsburgh, Pennsylvania, August 12–16.

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BACULOVIRUS EXPRESSION OF THE TOMATO SPOTTED WILT VIRUS (TSWV) GLYCOPROTEINS. Tae-Jin Choi[1], Scott T. Adkins[1], Kathryn E. Richmond[1], Barbara A. Israel[2], Kevin T. Schultz[2] and Thomas L. German[1]. Depts. of [1]Plant Pathology and [2]Pathobiological Sciences-School of Veterinary Medicine, Univ. of Wisconsin-Madison, Madison, WI 53706.

G1 and G2 are glycoprotein components of the TSWV membrane. The G1G2 polyprotein leader contains sequence elements directing expression of a toxic signal sequence in *E. coli*. These elements were omitted from a construction of the G1G2 open reading frame in a recombinant baculovirus. Indirect immunofluorescent assays of recombinant baculovirus-infected SF-21 cells with anti-G1 antibody confirmed expression of this glycoprotein. Western blots of infected SF-21 cell extracts with anti-TSWV antibody revealed G1, G2 and their unprocessed precursor. Addition of tunicamycin, a glycosylation inhibitor, to SF-21 cell cultures increased the electrophoretic mobility of G1 and the unprocessed precursor relative to controls.

2

BACKCROSS ANALYSIS OF TOBACCO PLANTS TRANSFORMED WITH CUCUMBER MOSAIC VIRUS REPLICASE GENE SEQUENCES. N. Banerjee, W. M. Wintermantel, and M. Zaitlin, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Transgenic Samsun NN tobacco plants expressing a truncated form of the cucumber mosaic virus 2a replicase gene exhibit a high level of resistance to cucumber mosaic virus disease. A highly resistant line was backcrossed to non-transformed Samsun NN plants. All the F1 progeny were highly resistant. The F2 and F3 progeny showed segregation in the levels of resistance ranging from highly susceptible to highly resistant. The parental lines as well as the progeny were analyzed for transgene copy number as well as for transcript levels. An assessment of the relationship between the degree of resistance, insert copy number, as well as transcript levels will be presented.

3

A SINGLE AMINO ACID IN THE 126 kDa ORF OF TMV M STRAIN PRODUCES A SEVERE SYMPTOM IN TOBACCO. Y. Bao, S.A. Carter, and R.S. Nelson, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

The *Masked* (M) strain of tobacco mosaic virus (TMV) produces non-mosaic symptoms in systemically-infected tobacco leaves compared with the mosaic symptoms caused by the U1 strain. Eight nucleotide changes, resulting in 8 amino acid changes within the 126/183 kDa protein were found to be solely responsible for the symptom difference. Previous results showed that altering 1149 within the 126/183 kDa ORF of the M strain cDNA from an A to a U, as found in the U1 sequence, resulting in a threonine to serine change, yielded progeny virus that produced U1-like symptoms. To determine if the nucleotide or the amino acid change was responsible for the symptom phenotype, two additional site-directed mutations were made. When the codon for threonine was changed from ACA to AGC, which encodes serine as in the U1 protein, the progeny virus produced U1-like symptoms. When ACA was changed to UGC, which encodes cysteine, the progeny virus produced M-like symptoms. These results indicate that it is the amino acid and not the nucleotides at this position that controls the symptom phenotype.

4

ANALYSIS OF SQUASH LEAF CURL GEMINIVIRUS CLONES AND ISOLATES BY COMPONENT-SPECIFIC PCR. J. K. Brown[1], K. Kiesler[1], G. Banks[1], and S. D. Wyatt[2]. [1]Dept. of Plant Sciences, Univ. of Arizona, Tucson, AZ 85721; [2]Dept. of Plant Pathology, Washington State Univ., Pullman, WA, 99164.

A component-specific polymerase chain reaction (CS-PCR) assay was developed to discriminate between the A and B components of two SqLCV strains E and R that have different host ranges, but cause mixed infections in common hosts. CS-PCR primers, designed around unique DNA sequences of cloned SqLCV E and the R, respectively, yielded the expected size fragments from plants biolistically inoculated with the SqLCV clones. Cloned SqLCV, serially transmitted from squash to squash by the whitefly vector, also yielded the expected genomic components by CS-PCR. Field isolates from different host species in the US, Mexico, and Central America contained variable compositions and frequencies of E and R components. The most common genotypes were only E-A and E-B, or various mixtures of E and R components. R-A/R-B were detected only in the presence of E-A or E-B. Six isolates contained only R-A or E-B, suggesting that the type SqLCV E and R genomic components are either absent, in low titer, or that components exist which escaped detection.

5

MUTATIONAL ANALYSIS OF A CONSERVED REGION OF THE TOBACCO MOSAIC TOBAMOVIRUS (TMV) MOVEMENT PROTEIN (MP) AND A SECOND SITE REVERTANT THAT RESTORES FUNCTION. C.M.Deom and X.Z. He. Department of Plant Pathology, The University of Georgia, Athens, Georgia 30602.

Mutations were made in a conserved region of the TMV MP, amino acids 56-96, predicted by computer analysis to be rich in turn structures. A proline to serine change at amino acid 81 resulted in loss of movement function. To avoid recombination with the wild-type MP gene sequence in transgenic MP + tobacco, the mutant TMV was propagated on a transgenic line of tobacco that expresses the sunn-hemp mosaic virus MP. Inoculation of the virus progeny onto tobacco containing the N gene, which responds hypersensitively, indicated the presence of infectious virus at a low frequency. Inoculation of infectious virus onto susceptible tobacco resulted in a systemic infection having symptoms different than wild-type virus, indicating a second-site revertant. This conclusion was confirmed by sequence analysis that revealed conversion of a conserved threonine at amino acid 104 to an isoleucine.

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6

Invasion of minor veins in inoculated *N. tabacum* cv. Xanthi nn leaves by U1-TMV and coat protein mutants of this virus. X.S. Ding, S.A. Carter and R.S. Nelson, Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

We have previously shown that early in infection, TMV accumulates in more vascular parenchymal cells than companion cells in minor veins of inoculated Xanthi nn leaves. Further studies have now shown that two other tobamoviruses and two potyviruses have a similar pattern of vascular invasion in various host plants. To determine the importance of TMV coat protein (CP) and virion in the invasion of minor veins in tobacco leaves, we are now investigating the accumulation of U1-TMV and three CP mutants of TMV in these tissues using *in situ* hybridization. All the mutants are defective in phloem-dependent accumulation. Initial results show that virion is not necessary for the entry of the virus into phloem cells.

7

EXPRESSION OF THE NONSTRUCTURAL PROTEINS OF TOMATO MOTTLE VIRUS IN TRANSGENIC TOBACCO PLANTS. Y. P. Duan, E. Hiebert and C. A. Powell*, Plant Pathology Department, University of Florida, Gainesville, FL 32611; * Ft. Pierce-REC, University of Florida, Ft. Pierce, FL 34945

Transgenic tobacco plants expressing the AC4, BC1 or BV1 open reading frames (ORFs) of tomato mottle virus (TMoV), a bipartite geminivirus, have been generated by *Agrobacterium*-mediated transformation. Transgenic plants expressing AC4 protein, or antisense sequences of either BC1 or BV1 ORFs were phenotypically indistinguishable from wild-type *Nicotiana tabacum* cv. Xanthi. Plants expressing BC1 movement protein (MP) displayed disease symptoms with stunting, mottling and leaf curling on matured leaves. Plants expressing BV1 MP showed slight stunting, distortion and crumpling on newly expanding leaves. The symptom severity varied from plant to plant, and there were no apparent correlations between severity and transgene copy number or transgene protein concentrations. As reported for another bipartite geminivirus, the BC1 protein of TMoV is involved in induction of symptoms. The results also implicate the BV1 protein as a symptom-inducing element.

8

EFFECT OF TEMPERATURE ON RESISTANCE EXPRESSION TO TRIADIMEFON IN *UNCINULA NECATOR*. H.L. Ypema and W.D. Gubler, Department of plant Pathology, University of California, Davis, CA 95616.

Triadimefon has been used in California to control grape powdery mildew since 1982. Resistance to triadimefon in the cooler coastal areas of California is more prevalent than in central and southern CA, where high summer temperatures may have delayed the development of resistance. Single spore isolates of *U. necator*, sensitive (EC_{50} 2.9 µg/ml) and resistant (EC_{50} 45.2 µg/ml) to triadimefon, were maintained in sealed containers and subjected to three applications of 150 µg/ml triadimefon at three week intervals. Conidia production was measured twice during each spray interval. At 25°C or temperatures fluctuating between 15 and 25°C, both isolates continued to grow for more than 53 days. Sporulation of both isolates ceased at 32°C, or at temperatures fluctuating between 22°C and 32°C at 12 hr intervals, irrespective of triadimefon application. At temperatures conducive to sporulation, the first application of triadimefon was lethal to the sensitive isolate, but the resistant isolate sporulated at levels similar to those of the control after three applications. Triadimefon may be effective against *U. necator* in central and southern CA despite the presence of resistance due to temperatures unfavorable for sporulation.

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DIFFERENTIAL REACTIONS OF SOYBEAN CULTIVARS TO *Macrophomina phaseolina*. J. A. Wrather, and S. C. Anand, University of Missouri-Delta Center, P. O. Box 160, Portageville, MO 63873.

Charcoal root rot of soybean, caused by *Macrophomina phaseolina* (Tassi) Goid., causes extensive yield reduction in the United States. Soybean cultivars resistant to this disease have not been identified. We determined the response of five soybean cultivars (Asgrow 3715, Delta Pine 3478, Delsoy 4710, Davis, and Crawford) to *M. phaseolina*. Three inoculation techniques were used on seven-day old seedlings. Inoculated plants were incubated at 28° C for 14 days. Root lesion severity caused by *M. phaseolina* was evaluated on a scale of 1-5 where 1=healthy roots, and 5=necrotic roots. Lesions developed on 60% of the plants inoculated by placing three fungus infested oat seeds next to the seedling root during transplanting. When seedlings were inoculated by transplanting into soil drenched with a mycelium fragment suspension or seedling roots were dipped into a mycelium fragment suspension for three hours just before transplanting, lesions developed on all seedlings of all cultivars. Lesion severity (LS) was significantly greater on Asgrow 3715 (LS=3.6) than Davis (LS=2.5). Lesion severity on Davis and the other cultivars was similar.

10

EVALUATION OF TOMATO BREEDING LINES FOR RESISTANCE TO LATE BLIGHT. Dawn E. Fraser[1], P. B. Shoemaker[1], and R. G. Gardner[2]. Departments of [1]Plant Pathology and [2]Horticultural Science, North Carolina State University, Raleigh, NC 27695.

Six breeding lines of *Lycopersicon esculentum* previously identified as having late blight resistance and cultivar Mountain Supreme were compared to evaluate the efficacy of rate-reducing resistance during naturally occurring epidemics of late blight in unsprayed, replicated field experiments at Fletcher and Waynesville, NC in the summer of 1994. Strains of *Phytophthora infestans* present were A2 mating type and metalaxyl resistant. Disease progressed rapidly on plants of the late blight susceptible/early blight resistant cultivar Mountain Supreme, which at Fletcher had 90% defoliation 22 days after the occurrence of initial symptoms. Mean area under disease progress curves (AUDPC) of combined data from both locations was 1584 and 1608 %-days for 'Mountain Supreme' and WVa 700, respectively. Mean AUDPC's for the other lines tested were less ($P=0.01$) than those above; values were 1141, 1049, 872, 997, and 864 %-days for WVa 63, NC 502-4, (NC 502-4 x WVa 63) F_1 hybrid, NC 24E, and 'Pieraline', respectively.

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DIFFERENTIAL YIELD REACTION AND HOST STATUS OF RESISTANT AND SUSCEPTIBLE SOYBEAN CULTIVARS TO *HETERODERA GLYCINES* RACE 2. S. R. Koenning and K. R. Barker, Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

Experiments conducted in 1992 and 1993 in a field infested with *Heterodera glycines* (SCN) race 2 involved comparison of cv. Hartwig, with multiple SCN race resistance, to the susceptible cv. Deltapine 105. Seed yield of each cv. was suppressed ($P=0.05$) with increasing *H. glycines* population density (Pi). The slope of the regression model relating Pi to yield, however, for the susceptible Deltapine 105 was much steeper than that of resistant Hartwig ($P=0.01$). Final population densities (Pf) of SCN were greatest following culture of the susceptible cv. Reproductive factors (Rf=Pf/Pi) of *H. glycines* on Hartwig and Deltapine 105 were 0.10 and 2.18 respectively, in 1992; and 0.26 and 6.59 in 1993. Number of SCN eggs per cyst were lower ($P=0.01$) for the resistant cv. Hartwig than for the susceptible cv. at soybean harvest. Low numbers of eggs/cyst associated with the resistant cv. may indicate that eggs from plots planted to Hartwig came from the previous crop.

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EVALUATION OF SOFT RED WINTER WHEAT FOR RESISTANCE TO WHEAT SOILBORNE MOSAIC AND WHEAT SPINDLE STREAK MOSAIC VIRUSES. **E. A. Milus, R. C. Gergerich, and S. L. Wickizer. Department of Plant Pathology, University of Arkansas, Fayetteville 72701.**

Both wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV) are known to infest many fields in Arkansas, but the diseases caused by these viruses have been treated as one soilborne virus complex rather than two distinct diseases. Our objective was to evaluate breeding lines and cultivars of soft red winter wheat for resistance to WSBMV and WSSMV. In October 1994, 50 entries were planted at three locations in northeast Arkansas and one location in southeast Missouri known to be infested with one or both viruses. The design was a randomized complete block with four replications. At Feekes growth stage 4 to 6, plots were rated visually for severity of mosaic symptoms, and two plants with the most severe symptoms were selected from each plot. Plants were tested for WSBMV and WSSMV using ELISA, and plants with absorbance values three times greater than the healthy check were considered infected. Entries with high or low frequencies of infected plants were considered susceptible or resistant, respectively. Several entries resistant to both viruses were identified. Screening for resistance based on presence of each virus may be more definitive than screening based on symptoms. The relationship between symptoms and virus presence will be discussed.

13

POTENTIAL USE OF CULTURE FILTRATES AND TOXINS FROM *MACROPHOMINA PHASEOLINA* TO SCREEN GUAYULE GERMPLASM FOR CHARCOAL ROT RESISTANCE. **J.O. Kuti[1], R.L. Schading[1], G.V. Latigo[2] and J.M. Bradford[2]. [1]Dep. Agronomy & Resource Sci. Texas A&M University-Kingsville, Kingsville, TX 78363 and [2]USDA-ARS, CPRS, Weslaco TX, 78596.**

The potential for using cell-free filtrates and toxins (phaseolinone and its derivates) from *Macrophomina phaseolina* for rapid and effective screening procedures for charcoal rot resistance in guayule (*Parthenium argentatum*) germplasm was assessed. The filtrates and toxins were incorporated into Gamborg medium at the rates of 0-50% (v/v) and 0-50 µl ml^{-1} respectively. The medium pH was adjusted to 5.8 before solidifying with 0.8% agar. One-week-old seedlings of ten guayule genotypes were placed in medium, incubated and rated for phytotoxic symptoms 10 days later. Data on growth and phytotoxic responses to the filtrates and toxins were collected. Significant differences were found among the genotypes in response to culture filtrate and toxin inoculations. There was a strong correlation between tolerance to *M. phaseolina* and insensitivities to the filtrates and the toxins among the guayule genotypes.

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FINGERPRINTING MUSKMELON (*CUCUMIS MELO* L.) WITH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) **Yihong Wang, Claude E. Thomas*, Ralph A. Dean**, Department of Plant Pathology & Physiology, Clemson University, Clemson, SC 29634; *USDA Vegetable Laboratory, Charleston, SC 29414

Mapping disease resistance genes will assist current breeding efforts. To find suitable muskmelon (*Cucumis melo* L.) parents for construction of genetic map, twelve muskmelon and four cucumber (*C. sativus* L.) varieties were subject to RAPD analysis. Phylogeny analysis using parsimony (PAUP) was used to analyse the RAPD data. All primers used could distinguish muskmelons from cucumbers and seven were sufficient to differentiate the 16 test varieties. Dissimilarity was greater between than within species. For muskmelon, greatest genetic distances were found between the varieties AY and Perlita, AY and Hymark, AY and Top-mark, and Perlita and Sweet Supreme. Primers produced 5-10 bands with 1-4 of them polymorphic depending on primers. The size of amplified bands were 400-2700 bp, the majority were below 2000 bp. These results show that RAPD can be used to create a genetic map for muskmelon.

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SELECTION OF MICROORGANISMS FOR BIOLOGICAL CONTROL OF SILVER SCURF (*HELMINTHOSPORIUM SOLANI*) IN POTATOES. M.K. Elson and D.A. Schisler. USDA-ARS, NCAUR, Peoria, Illinois 61604.

Silver scurf has become a major storage disease of potatoes primarily because many strains of *Helminthosporium solani* have developed resistance to thiabendazole. Soil and tuber samples were collected from 48 sites throughout the United States. Individual samples of live soil were added to sterilized field soil containing potato periderm (5:93:2 w/w/w, respectively) to produce samples that were chemically, physically and nutritionally similar, but microbiologically dissimilar. After incubation, the samples were assayed for biological suppression of *H. solani* conidiophore production using a whole-tuber/infested soil assay. Conidiophores covered 11 to 75% of the surface area of treated tubers. Over 250 isolates of bacteria, yeasts and actinomycetes were recovered from the 9 most suppressive soil samples. Preliminary results indicate that at least three bacterial isolates suppress silver scurf.

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RISK ANALYSIS FOR ANTAGONISTIC FUSARIUM SPP.. M.L.Gullino, M.Mezzalama and Q. Migheli, DI.VA.P.R.A. , V. Giuria 15, 10126 Torino, Italy.

The risk related to the release in agricultural environments of saprophytic Fusarium spp., active against Fusarium wilts, and of their trasformants, was evaluated. Altered spore pigmentation and fungicide resistance markers, used in combination with molecular tools , such as electrophoretic karyotyping or RAPD, allowed the recognition of selected antagonists several months after their introduction in soil and in the plant rhizosphere. Population dynamics of transformed and non-manipulated Fusarium spp. were reciprocally unaffected when the strains were introduced in soil microcosms . Antagonistic Fusarium spp. actively colonized the rhizosphere of cucumbers grown in non-disinfested soil, but the total number of CFUs of indigenous fungi bacteria recovered in cucumber rhizosphere was not affected by the presence of the antagonists. No evidence for horizontal transfer of benomyl resistance to sensitive strains has been so far observed under natural conditions.

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USE OF RAPD MARKERS FOR IDENTIFICATION OF THE BIOCONTROL AGENT *TRICHODERMA HAMATUM* 382. M. E. Grebus, H. A. J. Hoitink and S. A. Miller. Dept. of Plant Pathology, OARDC/OSU, Wooster, OH 44691.

Trichoderma hamatum isolate 382 (*Th*382) is an effective biocontrol agent against some soilborne plant pathogens in compost-amended substrates. Current detection methods do not provide isolate-specific identification in soil systems. A more effective procedure must be developed. A single primer polymerase chain reaction (PCR) assay was used to generate random amplified polymorphic DNA (RAPD) banding patterns for *Th*382 and 70 isolates of *T. hamatum, T. harzianum, T. koningii, T. viride* and *Gliocladium virens*. Ten primers (Operon H kit) were used to generate the RAPD patterns. Differences in RAPD patterns were observed at isolate, species and genus levels. Ordination procedures including Bray and Curtis polar ordination, and principal components analysis were used to describe and compare the RAPD profiles of the various isolates. This procedure will allow accurate identification of *Th*382 and monitoring of its population in compost-amended substrates. It will also be useful in further classification of the genus *Trichoderma*.

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EVALUATION OF EXOTIC MILD STRAINS OF CITRUS TRISTEZA VIRUS FOR CROSS PROTECTION ON SOUR ORANGE ROOTSTOCK IN BRAZIL. J. Vega[1], G. W. Muller[1], and R. F. Lee[2]. [1] Instituto Agronomico, P.O. Box 28, Campinas, SP, BRAZIL; Research Fellows of CNPq. [2] University of Florida, Lake Alfred, FL 33850

Nine Florida and two Brazilian mild strains of citrus tristeza virus (CTV) were compared in Ponkan tangerine, Galego lime, Marsh grapefruit and Pera sweet orange scions on sour orange rootstock for cross protection in a field trial. Some scion/CTV strain combinations grew normally during the first 3 yr, then declined. Only grapefruit scions with certain mild strains continue to survive in 1995. All plants in the plot were positive with monoclonal antibody MCA-13, specific for most severe CTV strains, in July 1991, but later tests indicated some plants were negative for MCA-13 suggesting the mild strains were suppressing the severe challenge strains. While some of the mild isolates which provided protection up to 3 yr of age may be useful under less severe challenge conditions, none of the isolates evaluated will permit citrus production on sour orange rootstock in Brazil.

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PATTERNS OF VARIATION IN POPULATIONS OF PURPLE AND YELLOW NUTSEDGE-*CYPERUS* spp. C.A.N.Okoli*, D.G. Shilling, R.L. Smith & T.A.Bewick; Dept. of Horticultural Sciences, University of Florida, Gainesville.

Purple and yellow nutsedge, can be readily distinguished by morphological characteristics, but intra-specific variation is not obvious. However, there are both inter- and intra-specific differential response of these weed species to the bioherbicide rust, *Puccinia canaliculata*. RAPDs were used to study variation between and within biotypes of purple and yellow nutsedge populations obtained from different geographical locations. Biotypes within and between purple nutsedge populations showed remarkably limited variation, while yellow nutsedge populations showed extreme variability. Our results suggest that purple nutsedge may be a large regional clone, most likely propagated by asexual means. In contrast, yellow nutsedge, contrary to earlier reports, is spread probably by both viable seeds produced from sexual reproduction and asexually by tubers. These results have implications for the successful biological control of these obnoxious weeds.

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BIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* BY USE OF *PSEUDOMONAS CORRUGATA* AND *BACILLUS SUBTILIS*. G.J. Vandemark, Department of Biotechnology and Biochemistry, CINVESTAV-IPN, Unidad Irapuato, Apdo. Postal 629, Irapuato, Gto., Mexico.

Bacillus subtilis (Bs) and *Pseudomonas corrugata* (Pc) were isolated from the roots of a raspberry plant (*Rubus ideaus* cv. Meeker) and screened for the *in vitro* ability to inhibit the growth of vegetative mycelia of *Botrytis cinerea*. Bs inhibited the growth of *B. cinerea* by 71.3% and 69.6% on Potato Dextrose Agar (PDA) and Tryptic Soy Agar (TSA), respectively. Pc inhibited growth on PDA and TSA by 66.3% and 40.8%. Tests on the ability of these two isolates to inhibit germination of conidia of *B. cinerea* resulted in 0.6%, 14.2%, and 99.2% germination in the Pc, Bs, and control treatments, respectively. Greenhouse tests examining the efficacy of these organisms for the control of grey mold disease of strawberry (*Fragaria x apnanassa* cv. Pajaro) resulted in similar efficacies for Bs and Rovral (Iprodione) in the first two of three harvests, and similar efficacies for Pc and Rovral in the second of three harvests. Rovral demonstrated significantly better efficacy than either Bs or Pc in the last of three harvests.

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EVALUATION OF GENETIC VARIABILITY AMONG *FUSARIUM OXYSPORUM* F. SP. *BETAE* ISOLATES BY VEGETATIVE COMPATIBILITY. **R. M. Harveson, and C. M. Rush. Texas Agric. Exp. Stn., PO Drawer 10, Bushland, TX 79012.**

Over a three-year period (1992-1994), 160 *Fusarium oxysporum* f. sp. *betae* isolates were collected from sugar beet and pigweed plants from seven counties in Texas. They were separated into two groups -- those causing tip rot and those causing only vascular necrosis. Of the 160 isolates, 132 were actually used for vegetative compatibility evaluations. Twenty-eight isolates were chosen as testers, and were paired in all possible combinations to determine the number of vegetative compatibility groups (VCGs) present. Six VCGs have been identified using the 28 testers. The remaining 104 isolates are being screened against one member of each of the 6 established VCGs. To date, 53 isolates have been assigned to VCG 1, with VCGs 2-6 containing 4, 13, 2, 2, and 2 isolates, respectively. No relationship exists between VCG and root rot symptom or host. Results suggest that these populations of *F.* oxysporum are endemic to Texas.

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AN EFFECTIVE AND HOST-SPECIFIC PATHOGEN OF *PAPAVER* SPP. McCarthy, M.K.[1], Pilgeram, A.L.[1], Anderson, T. W.[1], Schultz, M.T.[1], Dolgovskaya, M.[2] and Sands, D.C.[1] Department of Plant Pathology, Montana State University, Bozeman, MT. [2]St. Petersburg Zoological Institute, St. Petersburg, Russia.

Strains of the fungal wilt pathogen, *Fusarium oxysporum* f. sp. *papaver*, were isolated from *Papaver somniferum* plant tissue collected in Thailand, Russia, Colombia, and the United States. The strains were identified as *F. oxysporum* based on morphological characteristics and DNA hybridization with *Fusarium*-specific DNA probes. The pathogenicity of each strain on *P. somniferum* was determined by inoculating a minimum of five plants with $\sim 10^6$ conidia/plant. The identity of strains that were reisolated from diseased plants was confirmed using DNA analysis techniques. Virulence was assessed based on the percentage of plants that showed symptoms of severe vascular wilt and the timing of disease onset. The most virulent strain, CP3A, consistently caused lethal wilt symptoms in 100% of the inoculated plants within six days. Less virulent isolates elicited symptoms on fewer plants or required a longer period of time to cause disease. More than 50 crop species were surveyed for susceptibility to strains of the pathogen, and the observed host range was restricted to species within the Papaveraceae.

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AN EFFECTIVE AND HOST-SPECIFIC PATHOGEN OF *ERYTHROXYLUM* SPP. Sands, D.C.[1], Darlington, L.[2], McCarthy, M.K.[1], Pilgeram, A.L.[1], Ford, E.F.[1] [1]Biological Control of Weeds, Department of Plant Pathology, Montana State University, Bozeman, Montana. [2]Weed Science, United States Department of Agriculture, Beltsville, Maryland.

A fungal pathogen, *Fusarium oxysporum* nova f. sp. *erythroxyli*, was isolated from an *Erythroxylum* plant in Hawaii showing symptoms of vascular wilt and permanent defoliation. The fungus was identified as *F. oxysporum* based on morphological characteristics and DNA hybridization with *Fusarium*-specific DNA probes. Disease symptoms were reproduced in greenhouse pathogenicity tests on *Erythroxylum* and could be observed as early as 7 weeks following inoculation. However, in some instances, wilt symptoms were not apparent for up to eight months. More than 50 crop species were surveyed for susceptibility to the fungus, and the observed host range was limited to *Erythroxylum* spp. Death in field plots consistently exceeded 90% and was observed 7-40 weeks following inoculation. The efficacy of the inoculum was dependent upon the rate of application, formulation, and environmental factors such as moisture and temperature.

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RFLP ANALYSIS OF THE INTERGENIC SPACER REGION (IGS) OF SELECTED ISOLATES OF COLLETOTRICHUM SPP. **T.W. McCormick, J.C. Correll, and D. D. Rhoads[1]. Departments of Plant Pathology and [1]Biological Sciences, University of Arkansas, Fayetteville, AR 72701.**

An extensive collection of isolates of Colletotrichum acutatum, C. gloeosporioides, and Glomerella cingulata (anamorph: C. gloeosporioides), recovered from apple collected from several locations in the southeastern U.S. have previously been characterized for VCG and mtDNA RFLP haplotype. In an attempt to infer the phylogenic relationship within and between these taxa, the IGS region of 20 selected isolates was amplified using PCR (primers IGS2 and CLN12). The fragments recovered ranged in size from 3.0 to 3.5 kb. RFLP analysis of the IGS fragments with four-base restriction enzymes (HhaI and MspI) yielded 4-10 fragments ranging in size from 0.1-1.6 kb. Among isolates of G. cingulata and C. gloeosporioides, the IGS RFLP haplotype generally corresponded to the mtDNA RFLP haplotypes. In contrast, several IGS RFLP haplotypes were identified among isolates of C. acutatum with a single mtDNA RFLP haplotype.

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A PRELIMINARY INTRASPECIFIC PHYLOGENY OF FUSARIUM OXYSPORUM BASED ON THE PARTIAL SEQUENCE OF THE INTERGENIC SPACER (IGS) REGION OF THE RDNA. D.J. Appel and T.R. Gordon. Dept. ESPM, 108 Hilgard Hall, University of California, Berkeley, CA 94720.

Fusarium oxysporum f. sp. *melonis* includes multiple pathogenic races, each of which may be associated with more than one vegetative compatibility group. In order to better understand the relationship between these pathogenic races and between pathogenic and non-pathogenic forms in *F. oxysporum* we have sequenced approximately 1,000 bases of the intergenic spacer (IGS) which separates rDNA repeat units. Fifteen isolates of *F. oxysporum* were examined and a single isolate of *F. subglutinans* was included as an outgroup. The partial IGS sequence of all isolates was readily aligned. Differences between isolates included single and multiple base insertions or deletions and base substitutions. Parsimony analysis identified most parsimonious trees with significant structure. All *F. o. melonis* isolates clustered together, being clearly separated from nonpathogenic strains. Two VCGs, 0131 and 0134, were placed in distinct sub-clusters. In general, sequence similarities did not consistently distinguish any pathogenic races. No nonpathogenic strains were closely related to the *F. o. melonis* isolates, even those which were associated with a pathogen VCG (0131 or 0134). Overall, the results suggest that pathogens arise from within a defined lineage and not from the broader population of nonpathogenic strains.

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CROWN ROT FUNGI OF BANANAS FROM LATIN AMERICA AND THEIR PATHOGENICITY TO GRANDE NAINE AND DISEASE-RESISTANT HYBRIDS. **D. Marin, T. B. Sutton, and S. Blankenship, North Carolina State University, Raleigh, NC 27695.**

Fungi associated with crown rot of bananas were isolated from fruit grown in Mexico, Guatemala, Costa Rica, and Ecuador in Oct and Nov 1993. *Fusarium semitectum* and *Penicillium* sp. were isolated most frequently. *In vitro* growth and *in vivo* growth and pathogenicity of five fungi isolated from Costa Rican (*F. semitectum, F. moniliforme, Penicillium* sp., *Gliocladium roseum* and *Gliocladium* sp.) were determined. The optimum temperatures for growth of *F. moniliforme, F. semitectum, Penicillium* sp., *G. roseum,* and *Gliocladium* sp. were 24.3, >28.0, 21.8, 29.6, and 24.1 C, respectively. All fungi except *Penicillium* sp. grew profusely on the surface of cut crowns; *F. moniliforme* and *F. semitectum* caused the greatest amount of rot. New hybrids released by FHIA, FHIA 1 (Goldfinger) and FHIA 2, were partially resistant to the crown rot fungi. *F. semitectum* and *Penicillium* sp. grew on PDA amended with 10 mg L^{-1} of thiabendazole which may indicate a shift to decreased sensitivity to thiabendazole.

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FUSARIUM WILT OF BASIL UPDATE: DISTRIBUTION, SEEDBORNE NATURE, AND ELIMINATION OF PATHOGEN FROM SEED. S.L. Trueman and R.L. Wick, Department of Plant Pathology, University of Massachusetts, Amherst, MA 01003.

A vascular wilt disease of basil (*Ocimum basilicum* L.), caused by *Fusarium oxysporum* f. sp. *basilicum* (Schlectend.:Fr.) *(Fob),* was first reported in Russia in 1956. The disease was later noted in Italy, France, and more recently in the United States (CA, CO, CT, LA, MD, MA, MI, MN, NM, NY, SC, and OR); Israel; Ontario, Canada; and Australia. The symptoms include longitudinal necrotic stripes on the stem, wilting, stunting, and defoliation. A survey of 27 commercial seed lots from four countries revealed that 59% of the lots exhibited 1 - 27% *Fusarium* contamination. Hot water treatments of 56 - 58°C for 20 minutes decreased *Fusarium* contamination by nearly 100%, without significantly affecting germination rates. Sodium hypochlorite (0.525%) treatments were able to reduce *Fusarium* contamination by approximately 80%. These results strongly support the hypothesis that *Fob* is both a surface contaminant and an internal inhabitant of basil seed. Other treatments currently under review include employing Mycostop and chitosan as seed coatings.

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PHYLOGENETIC RELATIONSHIP OF PYTHIUM WITH OTHER OOMYCETES BASED ON SMALL SUBUNIT RIBOSOMAL DNA SEQUENCES. Weidong Chen, Illinois Natural History Survey, 607 East Peabody Drive, Champaign, IL 61820

Pythium is the largest genus of Oomycetes and is a member of the order Peronosporales. The phylogenetic relationship of Pythium with other genera of Oomycetes was assessed using ribosomal DNA sequences. The nuclear small subunit rDNA of Pythium arrhenomanes was amplified using polymerase chain reaction and cloned into pGEM-3Zf vector. Five clones containing rDNA of P. arrhenomanes were randomly selected and nucleotide sequences were determined on both strands of the rDNA molecule. The rDNA sequence was aligned with those of Phytophthora megasperma, Lagenidium giganteum, Achlya bisexualis, and other eukaryotic organisms. Phylogenetic analyses of the aligned sequences, using criteria of maximum parsimony and neighbor-joining method, showed that Pythium arrhenomanes is more closely related to Lagenidium giganteum (Lagenidiales) than to Phytophthora megasperma (Peronosporales), which is discordant with the current classification system.

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PARASEXUALITY AND HETEROKARYOSIS IN *FUSARIUM OXYSPORUM* F.SP. *CUBENSE* D.N.Kuhn, B.Cortes, T.Pinto, J.Weaver Dept Biological Sciences,Florida International University, Miami, Florida 33199

We have seen evidence of all steps of the parasexual cycle in *Fusarium oxysporum* f.sp. *cubense*, an asexual fungal pathogen of banana. UV generated, single and double auxotrophic mutants formed heterokaryons, the first step of the parasexual cycle. On screening spores from the heterokaryons, we observed diploids that sectored to give either a parental phenotype or a recombinant phenotype, the final stages of parasexuality. After screening 10^4 crosses, we detected strains with altered heterokaryon formation, including inter VCG and inter forma species heterokaryon formation as well as intra-strain incompatibility . For further study of these strains, we have developed a method to force heterokaryon formation between benomyl resistant auxotrophic mutants and wild type strains.

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SOMATIC INTERACTIONS OF *SCLEROTINIA SCLEROTIORUM* STRAINS FROM CABBAGE IN EASTERN NORTH CAROLINA. M.A. Cubeta[1], B.C. Cody[1] and L. M. Kohn[2]. [1]Department of Plant Pathology, North Carolina State University, Plymouth, NC 27962; [2]Department of Botany, Erindale College, University of Toronto, Mississauga, Ontario, Canada L5L IC6.

One hundred and thirty strains of *Sclerotinia sclerotiorum* were isolated from infected cabbage plants from four different fields in eastern North Carolina. Forty randomly selected strains (10 from each field) were paired in all possible combinations on modified Patterson's medium and examined macroscopically for somatic interactions. Excluding the 40 self pairings, 8 of 780 pairings were mycelial compatible with no observed inhibition between paired strains. All self pairings were mycelial compatible. Among eight compatible pairings, eight mycelial compatibility groups (MCG) were identified with two strains represented in each group. Six of eight MCG were obtained after pairing different strains from cabbage fields located 75 km apart. Approximately 99% of the pairings were mycelial incompatible and exhibited somatic interactions manifested by cleared inhibition zones with either small tufts of hyphae or red pigment deposition in the hyphal interaction zone.

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DEVELOPMENTAL ANATOMY OF POLYMYXA GRAMINIS IN ROOTS OF WHEAT. L.J. Littlefield*, R.K. Haddad*, J.L. Sherwood*, and J.H. Whallon**. *Oklahoma State University, Stillwater, OK 74078; **Michigan State University, East Lansing, MI 48824.

The development of Polymyxa graminis, the vector of wheat soilborne mosaic virus, was studied by light, confocal and scanning electron microscopy in roots of hard red winter wheat, cv. Vona, grown at 15 C in naturally infested field soil. Plants, age 12-60 days after planting (DAP), were studied. Intact root segments were used for light and confocal microscopy; three dimensional reconstructions of the fungus were made in the latter. Sections, 2 μm or 8 μm, were cut from roots embedded in resin or paraffin, respectively. The resin or paraffin was then removed chemically; sections were critical-point dried, sputter coated and examined by SEM. Sporangial and cystosoral plasmodia, sporangia and cystosori, which formed by cleavage of large cystosoral plasmodia, were characterized. Plasmodia and young sporangia were first observed ca 12-13 DAP. Sporangia and zoospores were common ca 18-30 DAP; cystosori were common by ca 25-30 DAP, and then remained the predominant stage.

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TAGGING OF QUANTITATIVE LOCI CONTROLLING PATHOGENESIS IN *MAGNAPORTHE GRISEA* BY INSERTIONAL MUTAGENESIS. Y. Shi and H. Leung. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164

We used insertional mutagenesis to tag genes which have quantitative effects on pathogenesis in *M. grisea*. Weeping lovegrass (*Eragrostis curvula*), which is highly susceptible to strain 2539, was used to assay for mutants with defects in pathogenicity. Nine out of 634 (1.4%) integrative transformants exhibited reduced pathogenicity. Co-segregation analysis showed that three mutants (2539T-531, 2539T-144, and 2539T-194) were tagged by plasmid insertion (0.4%). These mutants were apparently normal in vegetative growth, sporulation, and appressorium formation; however, they required a 10-100 fold higher inoculum (10^7 vs 10^5 spores/ml) to achieve wild-type level of pathogenicity. Reduced pathogenicity appeared to be caused by low infection efficiency and reduced colonization of the host tissue. Plasmid DNA carrying the inactivated gene was retrieved from mutant 2539T-531 and the identity of the retrieved sequence was confirmed by gene replacement. Insertional mutagenesis is effective for identifying genes with quantitative expression that are difficult to define by conventional genetic analysis.

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GENETIC ANALYSIS OF EIGHT DEVELOPMENTAL MUTANTS IN *Magnaporthe grisea*. Heng Zhu, Robert D. Gilbert, and Ralph A. Dean. Dept. of Plant Pathology and physiology, Clemson University, Clemson, South Carolina 29634.

To elucidate the mechanisms involved in appressrium formation, several developmental mutants were isolated. Five mutants with reduced appressorium formation on the hydrophobic surface of GelBond were obtained. An additional mutant 17-4/3, which is able to form appressoria on the hydrophilic surface of GelBond, was also isolated. Two further mutants were obtained affected in other developmental processes. One of the mutants, 138-1, can form conidia on complete medium. Another mutant, 62-7, produces conidia which fail to germinate. Genetic analysis was performed to determine the number of affected loci controlling each mutation. Random spore analysis of backcrosses and sibcrosses revealed that 3 of the appressorium mutants contain a single locus mutation. Mutant 17-4/3 and 243-7 were also crossed to study the interaction between the two loci. In order to isolate the genes responsible for the mutations, progeny were obtained from the crosses between the mutants and a mapping strain.

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BACTERIAL CANKER AMONG TOMATO TRANSPLANTS IN THE GREENHOUSE AND IMPACT ON YIELD. M. Hausbeck[1], D. Fulbright[1], F. Louws[2], J. Bell[1], and F. deBruijn[2]. [1]Department of Botany and Plant Pathology and [2]DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Clavibacter michiganensis subsp. *michiganensis* (Cmm), the causal agent of bacterial canker on tomato, was studied among transplants in a greenhouse. Ten-day-old 'Heinz 8704' tomato transplants in 288-cell plug sheets were arranged in four blocks of 40 flats each, surrounded by buffer flats, and placed on overturned flats on an earthen floor of a commercial greenhouse. When 19 days old, 26 seedlings in each of two opposing corners of each block were vacuum-infiltrated or spray-inoculated with a rifampcin- or streptomycin-resistant Cmm strain with a unique Rep-PCR DNA fingerprint. Seven-wk-old transplants were sampled, stomached in buffer, plated onto selective media, and resulting Cmm-like colonies were fingerprinted using Rep-PCR. Cmm spread within blocks from the inoculation site and resulted in significant disease symptoms; it was also detected on asymptomatic transplants. Disease spread was not influenced by inoculation method, although the streptomycin-resistant isolate spread further than the rifampicin-resistant isolate. When 8-wk-old transplants from each block were planted in the field, subsequent fruit yields indicated that symptomatic transplants closest to the inoculation site yielded poorly or not at all. Overall, yields from transplants asymptomatic in the greenhouse were variable but commercially unacceptable. Foliar field samples verified that the Cmm strain was the same one introduced in the greenhouse.

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Optimum CFU Concentrations for Testing Pathogenicity of California Cucurbit Isolates of Monosporascus cannonballus and an Acremonium sp. B. D. Bruton, USDA-ARS, Lane, OK 74555; T.R. Gordon and R.M. Davis, Dept. of Plant Pathology, Univ. of California, Berkeley, 94720, and Davis, 95616.

Monosporascus cannonballus and an Acremonium sp. have been associated with vine decline and/or vine collapse of watermelon and cantaloupe in California. Pathogenicity and virulence evaluations of numerous isolates often have given erratic results. Greenhouse studies were conducted to evaluate seedling disease reaction in response to different CFU concentrations. Three isolates of each fungus were tested using 0, 5, 10, 20, 30, 40, and 50 CFU/g of soil for M. cannonballus and 0, 1,000, 10,000, 20,000, 30,000, 40,000, and 50,000 CFU/g of soil for Acremonium sp. Cucumis melo cv Magnum 45 were evaluated 28 days after planting. Based on root rot ratings, plant stunting, and differential isolate virulence, the optimum CFU/g of soil for evaluating pathogenicity was determined to be 20 for M. cannonballus and 10,000 for Acremonium sp. Some of the less virulent isolates may appear non-pathogenic at these densities but the moderate to highly virulent isolates can be evaluated without overwhelming the plants. Because different isolates of each fungus produce drastically different inoculum densities, it is paramount that CFU's be determined when comparing pathogenicity and virulence between isolates.

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INFLUENCE OF TEMPERATURE ON DISEASE SEVERITY OF *VERTICILLIUM DAHLIAE* IN OKRA ACCESSIONS. **R. S. Potti[1], G. J. Weidemann[1] and E. Lamb[2]. [1]Dept. of Plant Pathology and [2]Dept of Horticulture and Forestry, University of Arkansas, Fayetteville, AR, 72701.**

Verticillium wilt can be devastating to many crop plants including okra *(Abelmoschus esculentus)* at cooler temperatures. Okra accessions that showed resistance to verticillium wilt in greenhouse tests were retested in growth chambers to determine the influence of temperature on identified resistance. Seedlings were root-dip inoculated in replicated experiments and plants were placed into growth chambers at temperatures of 20, 24, 28, and 32 C. Plants were scored for disease severity and mortality after four weeks. Results indicated that disease was severe at 20 C for most accessions. A few accessions showed that severity at 24 and 28 C. All accessions showed the lowest disease severity at 32 C. The susceptible cultivar Jade showed high levels of disease at all temperatures although disease severity was less at 32 C. Results show that the expression of resistance to verticillium wilt is influenced by temperature.

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PHYTOPHTHORA SPP. IMPLICATED IN WARM-SEASON ROOT ROT OF CARROTS IN CALIFORNIA. G.T. Browne, P.J. Wollesen, and A.L. Wolff, University of California Cooperative Extension, Bakersfield 93307

Phytophthora cryptogea (*Pcry*) and *Phytophthora* sp. (*Psp*) were isolated from rotted roots of wilted and dying carrots in Madera and Kern counties during July and September. The affected roots had brown lesions that were typically ≥2 cm diameter when first discovered, but soft rot often ensued and destroyed roots completely. In a growth chamber, full-size carrot tap roots developed severe rot (93-98% of root surface decayed) when transplanted into soil artificially infested with either *Pcry* or *Psp*; significantly less rot developed in carrot roots transplanted in noninfested soil (20%) or soil infested with *Psp* and drenched with 50 ppm metalaxyl (35%). When mid-sized carrot tap roots were inoculated with colonized agar disks, both *Pcry* and *Psp* were pathogenic, but *Pcry* was more virulent than *Psp* was. In 1.5-mo-old potted seedlings with small tap roots, *Pcry* caused severe root rot (73-88%), but *Psp* was nonpathogenic. Therefore, pathogenicity of *Psp* was not established in small carrot tap roots, but both *Pcry* and *Psp* were implicated in rot of full-size carrot tap roots. This is the first report to implicate *Pcry* and *Psp* in rot of carrot tap roots.

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EFFECTS OF DEEP-PLOWING ON DENSITY OF *SCLEROTINIA MINOR* SCLEROTIA AND LETTUCE DROP INCIDENCE. K. V. Subbarao, S. T. Koike, and J. C. Hubbard, Dept. of Plant Path., Univ. of Calif., Davis, c/o U. S. Agric. Res. Stn., Salinas, 93905.

Effects of deep-plowing on the density of *S. minor* sclerotia and disease incidence were evaluated in a field with a history of severe lettuce drop. A 40 x 102 m area was divided into 240 quadrats of 4- x 4-m in four reps. Soil samples were collected and bulked from six random sites within each quadrat before, immediately after, and one lettuce crop after deep-plowing to a depth of 15 cm. Aliquots of 100 g soil from each quadrat were assayed for *S. minor* sclerotia by wet sieving. In two successive lettuce crops following deep-plowing, the total number of plants and the number showing lettuce drop symptoms were counted prior to crop harvest. Significant reductions in the mean number sclerotia and disease incidence occurred on the crop immediately after deep-plowing, however disease incidence was significantly greater in the second crop. Calculated values of Lloyd's index of patchiness showed that the distribution of sclerotia had changed from a highly aggregated pattern prior to deep-plowing to less-aggregated patterns. The altered distribution of sclerotia may have increased the likelihood of a greater number of lettuce plant infections. Deep-plowing is thus unlikely to be a successful management strategy for lettuce drop in high inoculum density fields.

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CHARACTERIZATION OF *PHYTOPHTHORA INFESTANS* ISOLATES FROM FIELD SAMPLES OF POTATO AND TOMATO GROWN IN WISCONSIN AND NORTHERN ILLINOIS. K. D. Marshall and W. R. Stevenson. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706

Field samples of potato foliage, tubers, tomato foliage and tomato fruit exhibiting late blight symptoms were collected in Wisconsin and northern Illinois during the 1994 growing season. *Phytophthora infestans* was isolated in pure culture and isolates were analyzed for mating type, metalaxyl sensitivity and glucose-6-phosphate isomerase (Gpi) genotype (Fry *et. al.*, 1991, Phytopathology 81:1330-1336). Among the 87 isolates (77 from potato, 10 from tomato), 23 isolates were the A1 mating type (16-potato, 7-tomato) and 64 were the A2 mating type (61-potato, 3-tomato). Eighty five isolates were tested for sensitivity to metalaxyl at 5 and 30 µg/ml and of these isolates, 17 were sensitive (17-A1 mating type, 0-A2 mating type); 2 were intermediate/sensitive (2-A1, 0-A2); 33 were intermediate (4-A1, 29-A2); 30 were insensitive/intermediate (0-A1; 30-A2); and 3 were insensitive (0-A1, 3-A2). Two Gpi genotypes were detected among the 85 isolates. All of the A1 mating type isolates were of the 86/100 Gpi genotype and all of the A2 mating type isolates were of the 100/111/122 Gpi genotype.

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EFFECT OF TIME ON LESION LENGTH AND PATHOGEN BUILDUP IN PEA LINES RESISTANT AND SUSCEPTIBLE TO APHANOMYCES ROOT ROT. J.M. Kraft and W.L. Boge, USDA-ARS, RR 2 Box 2953A, Prosser, WA 99350.

Resistance in peas to *Aphanomyces euteiches* has previously been measured by counting oospores in infected root tissue, disease severity ratings, and/or yield in disease nurseries. In a controlled environment, differences in rate of lesion development in resistant and susceptible pea lines is evident and repeatable. Indirect ELISA with a polyclonal antiserum, with increased specificity for oospore antigens, of tissue within the lesion revealed a positive, linear correlation ($r^2 = 0.86$) between lesion length and ELISA readings at 405 nm. Resistance in peas to *A. euteiches* appears associated with slower lesion development and pathogen buildup in pea roots and is inheritable.

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EVALUATION OF PEA *(PISUM SATIVUM)* PLANT INTRODUCTIONS FOR RESISTANCE TO APHANOMYCES ROOT ROT. D. K. Malvick and J. A. Percich. Department of Plant Pathology, University of Minnesota, St. Paul 55108

The objective of this study was to identify pea (*Pisum sativum*) accessions in the USDA plant introduction collection with resistance to *Aphanomyces* root rot. The geographically and morphologically diverse accessions (2,514) were screened once in a greenhouse by inoculating twenty 7-day-old seedlings with zoospores of a single aggressive strain of *Aphanomyces euteiches*. Disease severity index (DSI, 0 = nonsymptomatic and 100 = all plants dead) and percent loss of root and vine biomass due to disease (inoculated vs. noninoculated plants) were determined for each set of 20 plants 12 days after inoculation. Most accessions developed severe root rot. The mean DSI for all accessions was 80 (range: 47-100) and the mean disease rating (DR, sum of percent loss for roots and vines) was 117 (range: 41-185). The DSI and DR for 15 replications of Little Marvel (susceptible control) were 92(±8) and 142(±13), respectively. Only 297 (12%) of the accessions screened had a DR less than 110. These 297 accessions show promise for further evaluation and some may have desirable Aphanomyces root rot resistance traits for use in breeding programs.

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MANAGEMENT OF COMMON ROOT ROT OF PEA *(APHANOMYCES EUTEICHES)* THROUGH CHISEL PLOW INCORPORATION OF AN OAT GREEN MANURE CROP. Jean L. Williams-Woodward, F.L. Pfleger, V.A. Fritz, and R.R. Allmaras, University of Minnesota, St. Paul, MN 55108

Rotation crop residue, oat green manures, and tillage practices were evaluated for management of common root rot of pea (*Pisum sativum*) in a field soil highly infested with *Aphanomyces euteiches*. A late summer oat ('Troy') green manure crop incorporated by chisel plowing increased subsequent dry pea biomass by 165% and pea yield by 245% over fallow controls. Pea biomass and yield were not significantly increased following the incorporation of oat ('Dane') green manure, indicating that the effect of oat on disease reduction may be oat cultivar dependent. Soil incorporation of oat stubble did not reduce subsequent disease severity. Chisel plowing of oat ('Troy') green manure significantly lowered soil inoculum levels in the upper 10 cm of the soil profile compared to inoculum levels from a depth corresponding to 10-25 cm of the soil profile. In contrast, moldboard plowing of the oat green manure resulted in lower inoculum levels at the 10-25 cm depth. Reduction in inoculum levels may correspond to placement of residue associated with tillage practice.

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BENEFITS ASSESSMENT OF THE USE OF FUNGICIDES TO CONTROL FRUIT ROT IN TOMATOES. J.A. Merrick and M.K. Hausbeck, Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing 48824

The effectiveness of the fungicide Bravo 720 (chlorothalonil) at 3.0 pt/A for control of fruit rot on processing tomatoes 'Ohio 7814' was assessed in field plots in Michigan during 1993-1994. Fungicide programs included: 1) 8 sprays at 7-day intervals; 2) 6 sprays at 10-day intervals; 3) 4 sprays at 14-day intervals; 4) 4 and 3 sprays in 1993 and 1994 respectively, scheduled by an early blight forecaster (Tom-Cast) using a threshold of 20 disease severity values; and 5) unsprayed. In both years, all treatments significantly reduced fruit rot compared to the control. The primary fruit rot in 1993 was late blight (*Phytophthora infestans)* resulting in only 8.2% healthy fruit in the unsprayed plot. Chlorothalonil applied every 7 or 10 days resulted in 90.8% and 87% healthy fruit, respectively. Chlorothalonil applied every 14 days or according to Tom-Cast, resulted in 82% healthy fruit. In 1994, the primary fruit rot was anthracnose (*Colletotrichum coccodes*) resulting in 69.3% healthy fruit in the unsprayed plot. Chlorothalonil applied at 7- or 10- day intervals resulted in 97.8% and 93.8% healthy fruit, respectively. The Tom-Cast and 14-day interval treatments both had 90% healthy fruit. An economic assessment revealed that for both years the 7 day application regime provided the greatest benefit per acre.

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STREPTOMYCIN RESISTANCE MANAGEMENT OF PSEUDOMONAS SYRINGAE PV. PAPULANS, THE CAUSE OF APPLE BLISTER SPOT. Tze-chung Huang, Thomas J. Burr, Charles A. Smith, Department of Plant Pathology, Cornell University, NYSAES, Geneva, N.Y. 14456

In 1992, 34% of the *Pseudomonas syringae* pv. *papulans* (Psp) were resistant to streptomycin in a Crispin orchard where streptomycin was regularly sprayed. The resistance determinant in Psp was detected on plasmids of either 94 or 132 kb which transferred among Psp at very low frequencies or not at all. In contrast, prominent resistance plasmids of 83 or 93 kb that were carried by a common foliar and fruit epiphyte (a nonfluorescent yellow *Pseudomonas* sp., closely related to *P. syringae*) were transferred to Psp at very high frequencies ($> 10^{-1}$ per recipient). Applications of streptomycin in the orchard reduced the total number of epiphytic Psp and increased the proportion of resistant strains up to 99.3%. The proportion significantly declined after overwintering in both streptomycin-treated and untreated plots indicating that resistant strains have reduced fitness. Treatments for control of disease were applied three years after the last use of streptomycin (12.8% of Psp resistant). Aliette plus streptomycin and streptomycin plus terramycin showed the best control. Streptomycin alone also provided satisfactory results.

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COMPARISON BETWEEN AGAR PLATING AND BIO-PCR FOR ASSAYING BEAN SEEDS FOR *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA*. N. W. Schaad[1], M. Chang[2], L. Kumagai[3], T. Matsumoto[3], R. L. Forster[4], and E. Hatziloukas[1]. [1]USDA/ARS, FDWSRU, Frederick, MD 21702, [2]Univ. California, Berkeley, CA 94720, [3]California Dept. Food & Agric., Sacramento,CA 94271, and [4]Univ. of Idaho, Kimberly, ID 83341.

BIO-PCR (Schaad, N.W., et al, 1995. Phytopathology 85:243-248) is a highly sensitive technique for detection of *Pseudomonas syringae* pv. *phaseolicola* (PSP) in *Phaseolus vulgaris* seed. However, it has not been tested with naturally contaminated seed lots. Another concern is that BIO-PCR may be too sensitive. To compare BIO-PCR to the MSP agar plating assay (Mohan, S.K., and Schaad, N.W., 1985. Phytopathology 77:1310-1395), a blind test was organized using 52 commercial bean seed lots assayed previously by Idaho or North Dakota (ND) State Certification labs. Samples ranging from 329 to 661 g were washed and aliquots plated onto MSP for viable assay and King et al's medium B (KB) for BIO-PCR. The 45 h washings of the KB plates were stored at -20 C and assayed by BIO-PCR using nested primers derived from a *Tox* gene. Five lots, 95-1, 2, 3, 4, and 5 had been positive in earlier "Dome" (Venette et al, 1987. Plant Dis. 71:984-990) assays in ND. Our MSP plating/pathogenicity tests were positive for lots 95-1, 2, 4, and 6. Six samples were positive by BIO-PCR; ND lots 95-1, 2, 3, 4, and 6 and Idaho lot 92-327. We conclude that BIO-PCR is a rapid, sensitive, and reliable molecular assay which correlates highly with current techniques.

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INVASION AND COLONIZATION OF TOMATO SEEDLINGS BY *PSEUDOMONAS SOLANACEARUM*. E. Saile, and T. P. Denny, Dept. Plant Pathology, Univ. of Georgia, Athens, GA 30602-7274.

We reinvestigated the importance of extracellular polysaccharide (EPS) and endoglucanase (EG), two known virulence factors of *Pseudomonas solanacearum*, for invasion and colonization of tomato. In a growth chamber at 30°C 4-week-old tomato seedlings were inoculated with a wild-type *P. solanacearum* strain and three mutants lacking either EPS, EG, or EPS and EG, by pouring bacterial suspensions onto a fumigated soil mix. Root invasion and movement of the bacteria into the crown region occurred within hours after inoculation with the mutants as well as the wild-type strain. The EPS$^-$ mutants invaded the roots equally well as the wild type but were less proficient in colonizing the stem. Data from petiole inoculations, in which a droplet of inoculum was put on the cut surface of an excised leaf, showed that colonization of the plant stems was impaired in the EPS$^-$ mutants compared with the wild type and the EG$^-$ mutant.

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DETECTION OF *XANTHOMONAS FRAGARIAE* BY AMPLIFICATION OF A REGION RELATED TO THE *HRP* GENES. **P.D. Roberts, J.B. Jones, R.E. Stall, and R.D. Berger. University of Florida, Gainesville, 32611.**

A technique, using polymerase chain reaction (PCR), was developed to detect low numbers of *Xanthomonas fragariae*, causal agent of angular leaf spot of strawberry. Primer selection was determined by sequencing the product that resulted from amplification using the *hrp*-gene-specific primers for *Xanthomonas campestris* pv. *vesicatoria*. Several sets of oligonucleotides with sequences unique to *X. fragariae* were selected. Primer sets amplified a 548 bp and 468 bp region for 49 strains of *X. fragariae* that represented all groups identified by RFLP and fatty acid analysis. No DNA amplification occurred with 17 pathovars of *Xanthomonas campestris* for the 468 bp specific primers. The 548 bp primers amplified a strain of *X. c. pelargonii*. PCR was used to amplify DNA from bacterial cells added to plant extracts at detection levels of 1 x 10^2 cfu/ml and greater. Bacteria were detected on naturally infected plant samples and their presence was confirmed by isolation from plant tissue on agar medium.

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INFECTIVITY TITRATION FOR ASSESSING RESISTANCE TO LEAF SCALD AMONG SUGARCANE CULTIVARS. S. A. Lopes, K. E. Damann, J. W. Hoy, and M. P. Grisham*. Dept. Plant Pathol. and Crop Physiol., La. State Univ. Agric. Ctr., Baton Rouge LA 70803, and *USDA-ARS Sugarcane Res. Unit, Houma LA 70361.

A greenhouse experiment was conducted to determine the potential of infectivity titration to evaluate the resistance of sugarcane to leaf scald disease, caused by Xanthomonas albilineans. Single-bud-cuttings of 14 cultivars were inoculated with suspensions containing 10^1, 10^5 or 10^8 cells/ml of an actively-growing isolate of X. albilineans. Characteristic symptoms of the disease were recorded every 15 days from 45 to 225 days after inoculation. At the final evaluation date, leaf vascular sap was plated on selective medium to detect latent infections. ED_{50} (the bacterial concentration required to infect 50% of the plants) was estimated for each cultivar based on probit analysis of infection percentages. Substantial differences in estimated ED_{50} values were detected among cultivars. These were in agreement with results obtained from experiments conducted under field conditions to evaluate cultivar resistance.

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A COMPARISON OF THREE STRAINS OF *XANTHOMONAS CAMPESTRIS* CAUSING BACTERIAL STREAK OF MILLET. A. Ayub, J.P. Hill, W. M. Brown, Jr., and C. A. Ishimaru. Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Foxtail millet (*Setaria italica*) plants showing red-brown, water soaked leaf streak symptoms were found in eastern Colorado in 1991. *Xanthomonas campestris*, of unknown pathovar affiliation, was isolated, identified, and demonstrated to be the causal agent of this disease. A representative strain was compared with two strains of *X. campestris*, one obtained from pearl millet (*Pennisetum americanum*) in Colorado in 1989 and the other, identified as *X. campestris* pv. *pennamericanum*, isolated from *P. americanum* in Africa. Extensive differential host range studies, including 41 plant species, and diagnostic cellular fatty acid Biolog demonstrated that the two Colorado isolates were very similar to each other, but different from the African strain. The strains of *X. campestris* isolated from foxtail and pearl millets in Colorado are distinct from the pathovar *pennamericanum*.

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SUSCEPTIBILITY OF ANTHURIUM CULTIVARS EVALUATED USING A BIOENGINEERED, BIOLUMINESCENT STRAIN OF *XANTHOMONAS CAMPESTRIS* PV. *DIEFFENBACHIAE*. R. Fukui, H. Muroi, S. C. Nelson and A. M. Alvarez. Dept. of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822-2279.

The infection process in bacterial blight of anthurium was monitored by detecting light produced from a bioluminescent strain of *X. c.* pv. *dieffenbachiae*. The relationship between symptom expression on infected leaves (assessed visually) and the extent of leaf infection (evaluated by bioluminescence emission) varied among cultivars of anthurium. In the early stage of infection (3-4 weeks after inoculation), percentage infected area determined by visual assessment was not significantly different among cultivars regardless of their susceptibility. The subsequent progression of infection was not always accompanied by symptom development, and differences in infection progression were detected by bioluminescence emission. In susceptible cultivars, the severity of leaf infection determined by bioluminescence emission was far greater than the severity determined by visual assessment. In resistant cultivars, infection sites were restricted to areas with visible symptoms. In one susceptible cultivar, infection progressed almost symptomlessly: leaves were severely infected, yet few or no symptoms were seen on infected leaves, which created the illusion that this cultivar is highly resistant.

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INVOLVEMENT OF THAXTOMINS IN PATHOGENICITY OF *STREPTOMYCES SCABIES* ON SEEDLINGS. B.A. Fry, R.H. Leiner, D.E. Carling and R. Loria. Dept. of Plant Pathology, Cornell Univ., Ithaca NY 14853, and Univ. of Alaska Fairbanks, Palmer Research Center, 533 E. Fireweed, Palmer AK 99645

Our long term goal is to identify pathogenicity determinants in plant-infecting *Streptomyces* species. The objectives of this study were to confirm that *S. scabies* is a broad host range seedling pathogen and to investigate the role of the phytotoxin, thaxtomin A, in disease development on seedlings. *S. scabies* significantly ($p<0.05$) reduced shoot and root growth of seedlings of 11 monocot and dicot plants, and caused shoot and root thickening and necrosis. Filter-sterilized cultures of *S. scabies*, which contained thaxtomin A, reproduced disease symptoms. Symptoms on radish seedlings treated with purified thaxtomin A were concentration-dependent: 50-100 µM stopped root and shoot growth and caused tissue necrosis and death, while 10-25 µM caused shoot and root stunting and thickening and inhibited root hair formation. Cross sections of roots and shoots demonstrated that tissue thickening was due to cell hypertrophy. Thaxtomin A is a broad-spectrum phytotoxin and may be responsible for plant pathogenicity in *S. scabies*.

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EFFECT OF BACTERIAL CONCENTRATION ON SURVIVAL UNDER BIOLOGICAL AND ARTIFICIAL OXIDATIVE STRESS. C. Jacyn Baker and Norton Mock, USDA, ARS, Microbiol. Plant Path. Lab., Beltsville, MD

Treatment of plant suspension cells with bacteria results in an immediate burst of active oxygen (AO) production by the plant cells. Our results demonstrate that the amount of hydrogen peroxide (H_2O_2) which accumulates, 5 to 25 µM, is a function of two opposing processes, elicitation and scavenging, both of which are greatly influenced by bacterial concentration. Results demonstrate that H_2O_2 accumulation in plant suspension cells increases with an increase in bacterial inoculum up to 10^8 cfu/ml. At concentrations of 10^9 cfu/ml the H_2O_2 accumulation in plant suspension cells is significantly reduced due to the increased scavenging. Under artificial oxidative stresses involving 1 to 50 mM H_2O_2, bacterial survival is proportional to bacterial concentration. Due to the ability of H_2O_2 to permeate membranes a single bacterial cell is unable to reduce its intracellular H_2O_2 concentration to tolerable levels. Individual cells are dependent on the population as a whole to rapidly reduce the surrounding H_2O_2 concentration to levels which individual cells can tolerate.

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INVERTASE AND CARBOHYDRATE ALTERATIONS ASSOCIATED WITH FOLIAR SYMPTOMS OF BACTERIAL RING ROT. Fang-Ming Lai and Carol Ishimaru, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO, 80523.

Interveinal chlorosis and necrosis are classic foliar symptoms of bacterial ring rot, caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms). Soluble and cell wall invertase activities, and carbohydrate and starch contents were monitored in symptomatic and asymptomatic leaves of potato and eggplant. Soluble and cell wall invertase levels increased and reducing sugars accumulated in chlorotic mesophyll tissues of mature leaves from symptomatic eggplants. In contrast, invertase levels in chlorotic and non-chlorotic mesophyll tissue from symptomatic potato plants and healthy potato plants were similar. Invertase activities in young, newly expanding leaves of potato were significantly lower in inoculated plants than in non-inoculated plants. In both eggplant and potato, starch content was lower in chlorotic mesophyll tissues. Alterations in invertase activity and reducing sugar content associated with foliar symptoms of bacterial ring rot are host and tissue specific.

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DIFFERENTIAL ACCUMULATION OF ANIONIC PEROXIDASE ISOZYMES IN RESISTANT AND SUSCEPTIBLE CABBAGE VARIETIES DURING PATHOGENESIS WITH *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* (XCC). P.A. Gay, K.M. Dodson and S. Tuzun, Department of Plant Pathology, Auburn University, AL 36849-5409 USA.

Resistant [Hancock (HC) and Green Cup (GC)], partially resistant [Cheers (CH)] and susceptible [Strukton (ST) and Perfect Ball (PB)] cabbage varieties were analyzed for anionic peroxidase isozyme accumulation following inoculation with XCC, the causal organism of black rot disease in crucifers. Four-week-old plants were petiole-inoculated with a highly infectious strain of XCC (FD91L) which had been genetically-engineered with the *lux* operon from *Vibrio fischerii* (Shaw and Kado, Bio/Technology 4:560) to allow for bioluminescent determination of bacterial movement *in planta* in a non-destructive manner. Upon symptom development, leaf samples from each variety were taken from three zones (1: non-bioluminescent, non-symptomatic, 2: bioluminescent, non-symptomatic, 3: bioluminescent, symptomatic). Proteins were extracted in a neutral phosphate buffer. Total peroxidase activities were determined spectrophotometrically and individual isozymes were analyzed utilizing an isoelectric focusing gel stained for enzyme activity. Leaf tissues were stained for the presence of lignin with phloroglucinol-HCl. All varieties demonstrated an increase in peroxidase activity upon symptom development, however, this increase was more dramatic in the resistant varieties when compared to susceptible ones. Isoelectric focusing revealed the presence of three anionic peroxidase isozymes in HC and GC (pI 3.6, 3.8 and 4.0) and three in ST and PB (3.6, 3.8 and 4.2). Extracts of the partially resistant variety (CH) contained all four isozymes. The most anionic isozyme (pI 3.6) accumulated during pathogenesis, although earlier and to a greater extent in the resistant than susceptible varieties. Lignin deposition closely correlated with the accumulation of this particular isozyme. In addition, two anionic isozymes (pI 4.0 or 4.2) demonstrated a varietal specificity that was linked to susceptibility or resistance to the pathogen.

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WEATHER AND LEAF WETNESS FORECASTS AS A BASIS FOR LETTUCE DOWNY MILDEW FORECASTS IN COASTAL CALIFORNIA. A.H.C. van Bruggen and G.G.H. Pennings, Dept. of Plant Pathology, University of California, Davis, 95616, and H. Scherm, Dept. of Plant Pathology, Iowa State University, Ames, 50011.

Morning leaf wetness (LW) duration is the most important variable for infection by *Bremia lactucae* (lettuce downy mildew) in coastal California. To predict prolonged morning LW periods, we coupled a dew simulation model to numerical, high-resolution weather forecasts from MESO Inc. (Troy, NY) for June, July, and August. Predicted values at 3-hour intervals for relative humidity (RH), temperature (T), wind speed (WS), and cloud cover were entered into the simulation model to generate forecasts of LW by dew. LW was measured at 1-hour intervals using LW sensing grids. Forecasts at 2-hour intervals for LW, RH, T, WS, rain, and fog drizzle were obtained from Fox Weather (Oxnard, CA). These forecasts were based on several sources, including local weather and LW data from the previous day. Predicted T and RH values from MESO and Fox Weather were regressed against the measured values. The regression slopes did not differ significantly from 1 for T from Fox Weather forecasts, but were significantly low than 1 for T from MESO and RH from Fox Weather and MESO (r^2 =0.23-0.71). Predicted LW events were compared with observed LW. The percentages of hours with correctly predicted LW, missed LW that did occur, or predicted LW that did not occur were 81, 8, and 11% for Fox Weather forecasts, and 67, 28, and 5% for dew simulations with MESO forecasts. Most missed LW events were due to fog drizzle which was not considered in the forecasts using the simulation model.

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SPATIAL PATTERN OF ASTER YELLOWS IN OHIO LETTUCE FIELDS. L. V. Madden[1], L. R. Nault[1,2], D. J. Murrall[2], and M. R. Apelt[2]. Depts. of [1]Plant Pathology and [2]Entomology, Ohio State University, OARDC, Wooster, 44691.

The degree of aggregation of lettuce plants infected by aster yellows phytoplasma (AYP) was investigated in 12 fields. Position of diseased and healthy plants was mapped in a 6-8-x-12-m section of each field; for some analyses, fields were divided into 10-plant quadrats. Mean disease incidence (p) ranged from 0.01 to 0.31. The frequency of diseased plants was described by the beta-binomial distribution, with an index of aggregation (θ) ranging from 0 to 0.17, and positively correlated with p. Distance-class analysis revealed a core-cluster size of only a few plants. However, spatial autocorrelations of p between quadrats were not significant, indicating that the scale of spatial pattern was small compared to field size. Results for this simple-interest disease will be interpreted in relation to the persistent transmission of AYP by its aster leafhopper vector.

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EFFECT OF IRRIGATION ON STEM ROT SEVERITY OF PEANUT AND COMPARISON OF ABOVE-GROUND AND BELOW-GROUND DISEASE RATINGS. Davis, R. F.[1], F. D. Smith[2], T. B. Brenneman[1] and H. S. McLean[3], [1]Dept. of Plant Pathology, Univ. of Georgia, Athens, GA 30602, [2]Rohm & Haas Co., Spring House, PA 19477-0904, [3]Sandoz Agro Inc., Cordele, GA 31015.

Field tests were conducted in 1993 and 1994 in a field with replicated irrigated and non-irrigated sections to evaluate the effect of irrigation on stem rot disease severity and yield loss models, and to examine the relationship between above-ground and below-ground disease ratings. The relationships between the number of cyproconazole applications and stem rot severity and peanut yield also were examined. Rainfall amounts were higher in 1994 than in 1993. Area under disease progress curve (AUDPC) values, below-ground disease ratings, and peanut yield were increased by irrigation in 1993 but not in 1994. Below-ground disease ratings reflected AUDPC in irrigated and non-irrigated plots in 1993 and 1994. Also, for any given AUDPC, the below-ground rating was higher in the non-irrigated plots than in the irrigated plots. Increasing the number of cyproconazole applications decreased both AUDPC and below-ground disease ratings and increased yield. These results indicate that the interrelationships of AUDPC, below-ground stem rot ratings, and yield are strongly influenced by irrigation. Either AUDPC or below-ground stem rot ratings may be used in yield loss models except in non-irrigated fields in dry years when below-ground ratings must be used.

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EFFECTS OF IRRIGATION AND TILLAGE ON SPATIAL DYNAMICS OF *SCLEROTINIA MINOR* SCLEROTIA AND LETTUCE DROP INCIDENCE. K. V. Subbarao, J. C. Hubbard, J. J. Hao, and K. F. Schulbach, Dept. of Plant Path., Univ. of Calif., Davis, c/o U. S. Agric. Res. Stn., Salinas, 93905.

The temporal and spatial dynamics of *S. minor* sclerotia under furrow irrigation with conventional tillage and subsurface-drip irrigation with minimum tillage, and their effects on disease incidence were studied. Grids of 24 (4- x 6-m) contiguous quadrats (1- x 1-m) were established in plots six beds wide and 25 m long under drip and furrow irrigation replicated three times. All lettuce plants were inoculated during the 1993 spring season. Soil samples (100 cm^3) were collected from each quadrat at harvest and after tillage (prior to planting) during 1993 and 1994 spring and fall seasons and assayed for *S. minor* sclerotia by wet sieving. Disease incidence was recorded in each quadrat prior to harvest during 1993 and 1994 fall and 1993 spring seasons. Lloyd's index of patchiness (LIP) was calculated to evaluate the effects of irrigation and tillage methods on aggregation of sclerotia and disease incidence. Plots under furrow irrigation added significantly greater numbers of sclerotia after each lettuce crop and increased significantly over the period of study compared with plots under drip irrigation. The degree of aggregation of sclerotia was decreased by the conventional tillage under furrow irrigation; the distribution patterns and the numbers of sclerotia were changed little by the minimum tillage under drip irrigation. The LIP for disease incidence was always <1 under furrow irrigation and >1 under drip irrigation. Spatial autocorrelograms did not show a consistent autocorrelation for the effects of tillage methods on sclerotial distribution or disease incidence. The combination of lower inoculum added and its aggregated distribution by the minimum tillage make subsurface drip irrigation a valuable cultural practice for lettuce drop management.

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DISTRIBUTION OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIA IN SOIL AND VERTICILLIUM WILT ON CAULIFLOWER. C. L. Xiao and K. V. Subbarao, Dept. of Plant Path., Univ. of California, Davis, c/o U.S. Agric. Res. Station, Salinas, CA 93905.

Distribution of *Verticillium dahliae* microsclerotia in soil and wilt on cauliflower were determined at three sites each in two fields. Each site was a 8 x 8 grid divided into 64 2- x 2-m contiguous quadrats. Soil samples were collected to a depth of 15 cm and bulked from four random sites in each quadrat. The soil was assayed for microsclerotia using the modified Anderson sampler technique. Plants in each quadrat were cut transversely, and the number of plants with or without vascular discoloration were counted. Variance-to-mean ratios (VTM) and Lloyd's index of patchiness (LIP) were used as indicators to evaluate aggregation of both diseased plants and microsclerotia in the field. Spatial correlation analysis was also used to assess the autocorrelation for both diseased plants and microsclerotia among quadrats. Both VTM and LIP for diseased plant counts were less than one at five sites indicating a uniform distribution of the diseased plants. The VTM and LIP for microsclerotia were greater than one indicating aggregation of propagules. The higher disease incidence (77% to 92%) observed in five of the six sites could be explained by the higher inoculum density. Spatial autocorrelograms of propagules showed a significant autocorrelation for the first two lags within rows indicating the influence of row operations on pathogen distribution.

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EFFECT OF DIFFERENT INITIAL SPATIAL PATTERNS OF ZUCCHINI YELLOW MOSAIC VIRUS (ZYMV) ON THE SPATIAL AND TEMPORAL DISEASE SPREAD IN ZUCCHINI SQUASH. P. Rodriguez-de Gonzalez,[1] J. J. Cho,[1] D. Ullman[2] and S. C. Nelson[1]. Plant Pathology. Univ. Hawaii. Honolulu, HI 96826[1]; Univ. California, Davis. CA 95616[2].

Three plots of zucchini squash (cv. "Ambassador"), each with a specific initial spatial pattern of ZYMV-infected plants [random (RP), uniform (UP) or aggregated (AP)], were established in Maui, Hawaii in 1994. Disease incidence was assessed twice per wk until harvest by means of visual symptoms observation and DAS-ELISA. Spatial patterns were mapped and analyzed using Morisita's index, contiguous quadrat variance and two-dimensional and spatiotemporal distance class analyses. Spatial and temporal spread of ZYMV was influenced strongly by initial disease pattern. Disease spread faster for UP than for AP and RP. The different spatial analyses provided complementary spatiotemporal information; however, the distance class analysis supported more complete and quantitative ecological information about the pathosystem. Disease progress curves for the three treatments were described best by the logistic growth model. Rate of disease increase was higher in the UP (r=0.185) than for AP (r=0.153) and RP (r=0.149). Average yield per plant was significantly higher for AP (1.5 kg) than for UP (0.7 Kg).

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OVERWINTERING SITES OF *BIPOLARIS ORYZAE* AND *B. SOROKINIANA* CAUSING FUNGAL BROWN SPOT OF WILD RICE. J.R. Brantner, R.F. Nyvall, and J.A. Percich, Department of Plant Pathology, University of Minnesota, St. Paul 55108

Overwintering sites of *Bipolaris oryzae* and *B. sorokiniana*, causal agents of fungal brown spot (FBS) of cultivated wild rice (*Zizania palustris* L.), have not previously been identified. Pathogen presence was determined in symptomatic (having FBS-like lesions) samples of wild rice residue and grass hosts on dikes surrounding fields in spring 1992-1994. *Bipolaris oryzae* was isolated from 2.4 and 0.6% of residue and dike grass samples, respectively, and *B. sorokiniana* from 0.7 and 2.1% of residue and dike grass samples, respectively. The ability of *B. oryzae* and *B. sorokiniana* to survive over winter in infested wild rice residue and seed and subsequently infect wild rice plants was also determined. Infested residue placed 1.5 m above the soil resulted in significantly increased area under disease progress curves (AUDPC) in 0.25 m^2 plots relative to non-inoculated controls. Infested residue incorporated in and on the soil surface and infested seed sown at a depth of 10-15 cm did not result in increased AUDPC. Non-incorporated wild rice residue and grass hosts on dikes may be important sources of primary inoculum for FBS infection.

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Establishment of a Regional Network for Weather Monitoring and Disease Risk Assessment of Powdery Mildew and Botrytis of Grape Using a Novel Radio Telemetry Technology. W. D. Gubler and C. S. Thomas, Dept. of Plant Pathology, University of California, Davis, CA 95616 and Adcon Telemetry, 11601 Wilshire Blvd., Suite 260, Los Angeles, CA 90025.

Weather stations were established in four counties in California for monitoring weather and disease risk assessment of powdery mildew and Botrytis of grape. Temperature, relative humidity, leaf wetness and precipitation data is transmitted by radio telemetry to a central base station where Windows compatible software instantly plots weather parameters and calculates disease risk. Disease incidence, severity, spray applications and costs were recorded weekly. In 1994 sites with high powdery mildew pressure demonstrated improved crop quality when treated according to the model. Sites with low powdery mildew pressure required 50% fewer sprays than a traditional spray program.

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TWO DIFFERENT GRAM POSITIVE BACTERIA CAUSE A DECAY OF TOMATOES THAT IS SIMILAR TO SOUR ROT. J. A. Bartz, N. C. Hodge, Y. Perez, and D. Concelmo. Plant Pathology Dept., University of Florida, Gainesville, 32611.

Green tomatoes, which had been submerged in non sterile deionized water and then pressure infiltrated, developed a low incidence (< 20%) of lesions typical of sour rot caused by *Geotrichum candidum* within 72 hrs at 25 C. The firm-textured lesions had a dull, greasy color and emitted an odor of lactic acid, but contained no evidence of fungal structures. Instead, high populations of either long rod or short rod-to- coccoid shaped bacteria were observed. These bacteria were isolated in nearly pure cultures on Lactobacilli MRS agar and grew luxuriantly on that medium, whereas growth on nutrient agar was slow and poor. The bacteria were Gram positive and, when inoculated to fresh tomatoes, produced lesions similar in appearance to those observed on the original fruit. The fatty acid profile of the coccoid bacterium was matched most closely with that of *Leuconostoc spp.*, whereas the profile of rod-shaped bacterium was matched to *Lactobacillus spp.* These identifications were consistent with other evidence on the strains' identity including: cell morphology, Gram stain reaction, production of lactic acid in tomatoes, and growth on MRS as compared with nutrient agar.

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INHIBITORY ACTIVITY OF YEAST CELL WALL MATERIALS AGAINST POSTHARVEST FUNGAL PATHOGENS. S. Droby[1], L. Chalupovicz[1], E. Chalutz[1], M.E. Wisniewski[2] and C.L. Wilson[2]. [1] Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, ISRAEL, and [2] USDA-ARS, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430, U.S.A.

Scanning and transmission electron microscopy revealed extensive production of extracellular materials covering the cells of the yeast *Pichia guilliermondii*. In an attempt to study the role of these materials in the antagonistic activity of the yeast against postharvest pathogens, an extracellular material was isolated from cells of the yeast by washing with LiCl followed by 3 days long dialysis to remove the salt. When tested *in vitro*, the isolated material inhibited spore germination and germ tube elongation of several postharvest pathogens. The calculated EC_{50} values for inhibition indicated a diferential response of the various fungi to the material. The EC_{50} for spore germination of these pathogens was obtained at concentrations ranging from 38 µg/ml to 300 µg/ml. Lower concentrations (22-230 µg/ml) of the material were needed to inhibit germ tube elongation. Application of extracellular material to surface wounds of grapefruit resulted in a marked inhibition of infection of *Penicillium digitatum*. Complete inhibition of infection was already evident at a concentration of 500 µg/ml.

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COMPATABILITY OF ECOGEN'S BIOFUNGICIDE ASPIRE™, A YEAST BASED PREPARATION, WITH OTHER FUNGICIDES COMMONLY USED FOR THE CONTROL OF POSTHARVEST DECAY OF CITRUS. H. Katz[1], A. Bercovitz[1], E. Chalutz[2], S. Droby[2], R. Hofstein[3], M. Keren-Tzoor[1]. [1]Ecogen Israel Partnership, P.O.Box 4306, Jerusalem 91042, ISRAEL [2]Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, ISRAEL, and [3]Ecogen Inc, 2005 Cabot Blvd. W., Langhorne, PA. 19047, USA

The yeast biocontrol agent, *Candida oleophila*, recently registered under the name Aspire™ has been tested in semi-commercial and commercial packing house conditions over the past three years. Over 150 experiments have been performed in the pilot packing house located in the ARO, Volcani Institute. Over this period Aspire™ alone consistantly reduced the level of molds caused by *Penicillium digitatum* and *P. italicum* by about 50% when compared to the water control. When applied following a pre-wash of 0.35% SOPP, the molds were reduced by 90% as compared to water control. SOPP alone reduced mold by only 50%. Application of Aspire™ with 50 - 200 ppm of TBZ, or (5-10% of commercial levels) resulted in control levels equivalent to that of the commercial treatment. Aspire™ applied by [illegible]ching, has been shown to be compatable 2-4-D, giberllin, and/or Ridomil subsequently appl[illegible] the wax.

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FACTORS AFFECTING SUSCEPTIBILITY OF ETHYLENE-DEGREENED ORANGES TO DIPLODIA STEM-END ROT. G. Eldon Brown and Jacqueline K. Burns, Florida Dept. of Citrus and Univ. Florida respectively, CREC, Lake Alfred, FL 33850.

Stem-end rot (SER) caused by *Diplodia natalensis* occurs when quiescent mycelia in necrotic tissue on the calyx and disk surface grow into the stem end of the fruit during abscission. The occurrence of SER is significantly enhanced by degreening with high (55 ppm) as opposed to low (2 ppm) levels of ethylene. At high levels of ethylene, activities of the two enzymes involved in fruit abscission, exo-polygalacturonase and cellulase, increased 11 and 13X, respectively. Partially purified abscission enzymes, added to abscission areas of debuttoned fruit before inoculation with mycelia of *D. natalensis*, increased susceptibility of oranges to decay. Conversely, when cycloheximide, 2,4-D, or silver thiosulfate were added to debuttoned fruit before inoculation, the incidence of SER was significantly reduced. Since these compounds interfere with enzyme activity, enhanced SER in response to high ethylene may result from increased enzyme activity that more readily predisposes cells at the fruit base to fungal invasion. Although abscission was more easily stimulated by high ethylene in immature than mature fruit, the immature fruit were less susceptible to SER. This suggests that fruit maturity at the time of ethylene treatment may also impact the ability of the fungus to cause infection.

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PURIFICATION AND CHARACTERIZATION OF AN APPLE POLYGALACTURONASE-INHIBITING PROTEIN. Chenglin Yao[1], William S. Conway[1], and Carl E. Sams[2]. [1]Horticultural Crops Quality Laboratory, USDA-ARS, Beltsville, MD 20705. [2]Department of Plant and Soil Science, The University of Tennessee, Knoxville, TN 37901.

A polygalacturonase-inhibiting protein (PGIP) was purified from mature 'Golden Delicious' apple fruit. The protein had a molecular weight of 45 to 55 kD on SDS-PAGE and an isoelectric point of 4.6. Apple PGIP showed differential inhibitory activity against five polygalacturonase isozymes purifed from *Botrytis cinerea* grown in liquid culture. However, no inhibition was detected against polygalacturonase isolated from apple fruit inoculated with *B. cinerea* or *Penicillium expansum*. Kinetic studies suggested that the inhibition was non-competitive.

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IDENTIFICATION OF CUTINASE PRODUCED BY *ASPERGILLUS FLAVUS* AND ITS ROLE IN AFLATOXIN PRODUCTION IN MAIZE KERNELS. B.Z. Guo[1], J.S. Russin[1], T.E. Cleveland[2], R.L. Brown[2], K.E. Damann[1]. [1]Dept. of Plant Path. & Crop Physio., LSU Ag. Ctr., Baton Rouge, LA 70803; [2]USDA/ARS/SRRC, New Orleans, LA 70179.

Aspergillus flavus can infect developing maize kernels and produce aflatoxin before harvest. Because kernel pericarps contain cutin, experiments were conducted to test for cutinase production by *A. flavus*. In plate assays, cutinase activity was visualized using *p*-nitrophenyl butyrate five days after inoculation of *A. flavus* on medium containing purified apple cutin as the sole carbon source. Cutinase activity was reduced when glucose was provided as a carbon source. pH 8.0 was optimal for the cutinase production. A cutinase from *A. flavus* was isolated after three weeks on liquid medium amended with apple cutin. The molecular weight of this cutinase was about 22-23 kd. Kernels pre-treated with purified cutinase prior to inoculation with *A. flavus* showed levels of aflatoxin production similar to those in wounded kernels. The cutinase activity was strongly inhibited by diisopropyl fluorophosphate (DFP). DFP added to inoculum reduced aflatoxin production in inoculated kernels. These experiments suggest a role for cutinase in pathogenicity of *A. flavus* for infection and aflatoxin production on maize kernels.

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TEXAS NAVEL ORANGE CULTIVARS DIFFER IN RESISTANCE TO POST-HARVEST ROT BY *PENICILLIUM DIGITATUM*. C. G. Eayre, and M. Skaria. USDA ARS, Subtropical Agricultural Research Laboratory, and Texas A&M University-Kingsville Citrus Center, Weslaco, TX 78596.

Citrus growers and shippers reported to us a higher incidence of decay with navel orange cultivar 'N-33' compared to other citrus gift fruit. Tests with wounded fruit showed that 'N-33' had higher incidence of green mold caused by *Penicillium digitatum* than 'Rio Red' grapefruit. To determine if this difference was due to a characteristic unique to the grapefruit, or to a particular susceptibility of 'N-33' or navel oranges in general, tests were conducted with fruit of several citrus cultivars. In two tests with 5 replications with 10 wounded fruit per unit, 'Rio Red', 'Ruby Red', and 'Marsh' grapefruits averaged 7%, 0% and 2% incidence of decay, respectively, while 'Marrs' orange, and 'Everhard' and 'N-33' navel oranges, averaged 20%, 0%, and 24% incidence of decay, respectively. Preliminary bioassays with peel oil of 'N-33' and 'Everhard' navels did not reveal inhibition of *Penicillium digitatum* mycelial growth.

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MYCOTOXIN LEVEL IN 1994 CORN AND SORGHUM IN KANSAS. D. M. Trigo-Stockli, R. Sanchez, and J. R. Pedersen, Dept. of Grain Science & Industry, Kansas State University, Manhattan, KS 66506-2201.

Corn and sorghum grain samples from various locations in Kansas were plated to determine the percentage of kernels invaded with *Aspergillus flavus*, *Fusarium graminearum*, and other fungi. Selected samples were screened for aflatoxin, deoxynivalenol (DON), and zearalenone using Romer Mycotest™ Three Toxin Kit (Thin Layer Chromatography). Fifty percent of corn samples tested indicated aflatoxin and/or DON while 81% of sorghum grain samples indicated either aflatoxin, DON, zearalenone, or combinations of these. The level of aflatoxin ranged from 5 to 60 ppb, and DON and zearalenone levels were 0.5 to 3 ppm.

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EFFECTS OF FORAGE GRASS ROTATIONS ON SOIL MICROBIAL ECOLOGY AND NEMATODE POPULATIONS. N. Kokalis-Burelle, R. Rodríguez-Kábana, D. G. Robertson, W. F. Mahaffee, J. W. Kloepper, and K. L. Bowen. Department of Plant Pathology, Auburn University, AL 36849.

It is hypothesized that the rhizosphere microflora directly affects the infective capabilities of nematodes. Microplot and field experiments were conducted to determine the effects of grass rotations on the ecology of nematodes and microorganisms in soil. Alginate films containing nematode eggs were used in microplot experiments to assess the effects of switchgrass (*Panicum virgatum*) and gammagrass (*Tripsacum dactyloides*) on egg viability, egg parasitism, and emergence of *Meloidogyne incognita* juveniles. Both switchgrass and gammagrass reduced egg viability and juvenile emergence, increased the number of parasitized eggs, and reduced the number of *M. incognita* juveniles in soil compared to peanut or cotton. In field trials, switchgrass and cotton did not support populations of *M. arenaria*. Switchgrass supported higher populations of nonparasitic nematodes than cotton. Peanut with no nematicide following two years of switchgrass provided the same nematode control as continuous peanut plus nematicide. Switchgrass supported lower numbers of rhizosphere fungi than peanut throughout the season, and a distinctly different bacterial microflora compared to continuous peanut and peanut following switchgrass.

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FURTHER CHARACTERIZATION OF MUTANT A-11, A CUCUMBER SPERMOSPHERE PROLIFERATION-IMPAIRED STRAIN OF *ENTEROBACTER CLOACAE*. I. Yucel[1,2] and D. P. Roberts[2]. [1]Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19717 and [2]Biocontrol of Plant Diseases Laboratory, USDA-ARS Beltsville, MD 20705.

Strain A-11 previously identified as a carbohydrate-utilization mutant of the biocontrol bacterium *E. cloacae* did not proliferate in cucumber spermosphere (Roberts *et al.*, 1992, Can. J. Microbiol. 38:1128-1134). Populations of A-11, however, were similar to those of the wildtype *E. cloacae* strain 501R3 in radish, corn, sunflower and pea spermospheres (P=0.05) after 45 h. Therefore, the cucumber spermosphere appears to be unqiue in its inability to support A-11 proliferation. Analyses of amino acid and sugar contents in exudates of the above seeds, revealed that cucumber and radish had comparable levels of L-leucine and glucose equivalents per seed. These results suggest that the quality as well as the quantity of nutrients in a given spermosphere are important for proliferation. Cosmid clones from an *E. cloacae* ECCT501 library that restored A-11's ability to grow to varying degrees on several carbohydrate sources *in vitro* have been isolated and are being subcloned to identify genes important for colonization.

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SEEDLING DISEASE OF WATER-SEEDED RICE: EFFECT OF PLANT AGE ON SUSCEPTIBILITY AND MINIMUM EXPOSURE TIME FOR INFECTION BY *PYTHIUM* SPECIES. S-C. Chun and R.W. Schneider, Dept. of Plant Pathology and Crop Physiology, Louisiana State University Agric. Center, Baton Rouge, LA 70803.

Rice seedling emergence and dry weights were recorded 10 days after plants (0-8 days old) were inoculated with mycelial suspensions of *Pythium arrhenomanes*, *P. myriotylum* and *P. dissotocum*. Susceptibility to all three species was significantly reduced at 2 to 4 days after planting, and seedlings were completely resistant at 8 days after planting. Metalaxyl was applied to the infested flood water at daily intervals beginning at time of planting for up to 10 days. Seedling emergence through the water was determined 10 days after each application. There was a steep decline in emergence following 2 days exposure to inoculum of *P. arrhenomanes* and *P. myriotylum*. In contrast, *P. dissotocum* was much less pathogenic, requiring longer exposure times to cause irreversible seedling damage. These results indicate that rice seedlings become resistant to infection after a very short period of time even though they may still be submerged.

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INTERACTION OF ACEPHATE WITH RHIZOCTONIA SOLANI AG-4 AND SEEDLING DISEASES OF COTTON. Donald R. Sumner, University of Georgia Coastal Plain Experiment Station, Tifton, GA 31793-0748.

Acephate is used as an in-furrow treatment at planting to control thrips (Frankliniella spp.) in cotton. In experiments in field plots on loamy sand soils treatments with acephate increased seedling disease severity and reduced plant stand in cotton. In greenhouse experiments in soil infested with Rhizoctonia solani AG-4 acephate increased post-emergence damping-off in cotton. In laboratory experiments soil was treated with different rates of acephate, placed in glass petri plates, infested or noninfested with R. solani AG-4, planted with cotton, and incubated at 20 or 26 C for 6 days. At both temperatures acephate decreased seedling weight (an average of 15%) and increased root decay, but did not influence the growth rate of R. solani.

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COLONIZATION INTERACTIONS OF *PHYTOPHTHORA CAPSICI* AND *P. NICOTIANAE* IN CO-INFECTED TOMATO PLANTS. J.T. English, T. Ersek, J.E. Schoelz. Department of Plant Pathology, University of Missouri, Columbia 65211.

It is common for the roots of any plant in the field to be infected by more than one fungal pathogen. However, little is known about the impact of co-infection on the behaviors of the pathogenic organisms. In greenhouse experiments, these impacts are being evaluated for *P. capsici* and *P. nicotianae* on tomato. In initial experiments, plants were inoculated on the lower stem with 500 zoospores of either or both species. Each species carried a unique drug resistance marker, permitting easy identification of isolates. The length of stem colonized by *P. capsici* was reduced by less than 9% in plants co-infected with *P. nicotianae*. In contrast, colonization by *P. nicotianae* was reduced by more than 90% in the presence of *P. capsici*. Further evaluations of interspecific interference are being made in other tomato tissues. Similar evaluations are being made when *P. capsici* or *P. nicotianae* co-infect tomato with a new pathogen genotype in the form of a hybrid created from these two species by zoospore fusion.

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SPATIAL AND TEMPORAL DISTRIBUTION OF *PHYTOPHTHORA CACTORUM* IN APPLE ORCHARD SOILS. I.J. Horner[1] and W.F. Wilcox[2]. [1]Horticulture and Food Research Institute of N.Z., Private Bag 92-169, Auckland, New Zealand; [2]Dept. Plant Pathology, Cornell University, N.Y.State Agric. Expt. Stat., Geneva NY 14456.

P.cactorum oospores in New York apple orchard soils were quantified using the SADAMCAP (Soil Air-Dried And Moistened, Chilled And Plated) technique. Relatively high populations were found within 1 m of mature apple trees, with a rapid decline at greater distances and almost nil in the grassed inter-rows. A population gradient was found on hill sites, with high numbers at the bottom and low numbers at the top of slopes. There was a steep decline in population with increasing soil depth: approximately 50% of the total population occurred in the top 6 cm and 85% in the top 15 cm. At depths greater than 20 cm, populations were very low. Studies of colonized fallen fruit and leaves indicate that these tissues contribute to high surface populations. Oospore numbers in soil were highest in early spring, declined steadily throughout the summer, and rose again in late fall. Sporangial/zoospore activity, assessed by baiting techniques, peaked in mid-spring, was sporadic throughout the summer and fall, and ceased in winter.

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EVALUATION OF ISOLATE DIVERSITY OF RHIZOCTONIA SOLANI AG-11. P. Kaufmann and C. S. Rothrock, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701

A new anastomosis group (AG-11) recently has been described from soybean and rice in Arkansas and lupine in Australia (Carling et al. Phytopathology 84:1387-1393). Pathogen diversity among isolates from each geographic region was examined using pathogenicity on seven crops and by isozyme analysis. Pathogenicity was examined in soil artificially infested with 1% potato soil inoculum. Isolates of AG-11 from both regions were pathogenic on cotton, lupine, and wheat. Isolates from Arkansas were generally more pathogenic than Australian isolates, especially on lupine. Some Arkansas isolates of AG-11 also were pathogenic on soybean and radish. Of twenty-four enzymes screened by isozyme analysis; α and ß-acid phosphatase, esterase, glucose dehydrogenase, hexokinase, leucine aminopeptidase, malic enzyme, malate dehydrogenase and superoxide dismutase revealed banding patterns that suggest their use for examining pathogen diversity.

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SENSITIVITY TO BORATE: VARIABILITY AMONG ISOLATES OF *ASPERGILLUS FLAVUS* AND GEOGRAPHICAL DIVERGENCE AMONG *A. FLAVUS* COMMUNITIES. Peter J. Cotty, USDA, ARS, SRRC, P.O. Box 19687, New Orleans, Louisiana 70179

Isolates of *A. flavus* varied in sensitivity to boric acid. Plugs of actively-growing cultures were transferred to media containing boric acid and incubated at 31 C. Radial growth was measured after 3 days. Sensitivity to borate increased with increased alkalinity from pH 6.5 to pH 8.5. Borate-sensitive isolates were obtained from both alkaline (pH > 7.5) and acidic (pH < 6.5) soils. However, highly resistant isolates were only isolated from alkaline soils. Czapek's Dox agar containing 0.5 M boric acid, pH 8.5, completely inhibited growth of most isolates (70% to 95%) from alkaline soils and all isolates (60 isolates were tested) from acidic soils. Variability in sensitivity to borate may reflect variable exposure to borate and demonstrates divergent adaptation of geographically separate fungal communities.

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USE OF NEMATODE COMMUNITY STRUCTURE TO MEASURE DISTURBANCE IN AGRICULTURAL, FOREST AND WETLAND ECOSYSTEMS. Osama Anas, D. A. Neher, S. El-Allaf and M. E. Barbercheck. North Carolina State University, Raleigh, NC 27695.

Maturity and trophic diversity indices of nematode communities (plant-parasitic and free-living) may be useful bioindicators of ecosystem condition in regional monitoring programs. Composite soil samples (20 cores, 2 cm diam., 20 cm deep) were collected from undisturbed (U) and disturbed (D) agricultural, forest and wetland soils in Mountain, Piedmont and Coastal Plain regions of North Carolina. U sites were used as references to calibrate indices in comparison with D sites. U sites were permanent pastures, >20-yr-old forest stands and functioning wetlands. D sites were annually cultivated land, <3-yr-old forest stands and wetlands converted to agriculture. Trophic diversity and maturity indices of nematode communities calculated for spring months 1994 did not distinguish clearly between D and U sites within any ecosystem or ecoregion. Biotic similarity coefficients (B) of nematode community composition (calculated using "BIOSIM1", a FORTRAN IV program) differentiated U and D sites of each ecosystem. Although low B values (0.03 to 0.38) were observed, differences within ecosystems were often greater in March than May. Biotic similarity indices of nematode communities distinguished between D and U sites better than maturity or trophic diversity indices.

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IR-4 REGISTRATION OF MINOR USES OF FUNGICIDES AND NEMATICIDES. D. C. Thompson, W. L. Biehn and R. T. Guest. IR-4 Project, Rutgers University, New Brunswick NJ.

Pesticide uses that are considered minor keep changing, as the costs of reregistration and registration grow larger than the potential profits. EPA describes these uses as having limited potential market volume, irrespective of crop acreage. The changes have resulted in IR-4 working on crops and uses once considered major. Recent expansion of IR-4's budget and staff will aid in this broader group of reregistrations and registrations. Rulings by EPA, now allow IR-4 to accept requests and initiate studies on the new fungicides: tebuconazole, difenoconazole, triflumizole, fenbuconazole, and fludioxonil. IR-4 is also developing data regarding "anticipated residues", that are present following practices, such as washing. This may allow additional uses of older fungicides, such as mancozeb and chlorothalonil.

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COPPER, A KEY ELEMENT FOR DISEASE CONTROL IN WHEAT AND BARLEY CROPS IN ALBERTA. **I.R. Evans, E.D. Solberg, D.C. Penney and D. Maurice, Alberta Agriculture, Food and Rural Development, Agronomy Centre, Edmonton, Alberta, Canada T6H 4P2.**

Until recently, micronutrients were not considered to play a major role in determining disease levels, yield and quality of wheat and barley crops in Alberta. However, data now show conclusively that on deficient soils, copper has a major impact on these factors. Copper-deficient soils are usually sands or light loams with high organic matter (crop residues, manures and peatlands), with high pHs and abundant nitrogen and phosphorus. Amendments of up to 11 kg/ha of copper sulphate (25% Cu) on soil with less than 0.6 ppm available copper more than doubled wheat and barley yields and controlled melanosis, ergot and "take-all". Around 10% of Alberta's cropland (1 million ha) is deficient in available copper.

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SUPPRESSION OF SOUTHERN STEM ROT AND CYLINDROCLADIUM BLACK ROT OF PEANUT WITH FLUAZINAM. T.A. Kucharek, R. Hoover, and J. Atkins. Plant Pathology Dept. (first two authors) and Santa Rosa County Extension Service, University of Florida, Gainesville FL 32611.

Southern stem rot (SSR), caused by *Sclerotium rolfsii*, and Cylindrocladium black rot (CBR), caused by *Cylindrocladium crotalariae*, coexist in peanut fields in Florida. Control of both diseases with one chemical is needed. Two applications of fluazinam, applied in narrow bands of 0.5m or less with one LE 6 80 flat fan nozzle/row, reduced (P=0.5) SSR and increased (P=0.05) yields in three field tests. Three applications of fluazinam applied similarly reduced (P=0.05) CBR and increased (P=0.05) yields in two other field tests. Reduction of wilting associated with SSR and CBR ranged from 40 to 94% and 32 to 61%, respectively. Black pods (CBR) were reduced 65 and 85% in the two tests. Increases in yields ranged from 27 to 51% and 41 to 64% with suppression of SSR and CBR, respectively. Rates of fluazinam ranged from 0.6 to 1.2 kg (a.i.)/ha. All sprays were applied at 207 kPa in 468 L and 234 L of water/ha in the tests for SSR and CBR, respectively.

85

CONTROL OF *PHYTOPHTHORA PARASITICA* USING DIMETHOMORPH (AC 513,873) ON TOBACCO. **T. A. Melton[1] and F. R. Walls[2], [1]Dept. of Plant Pathology, N. C. State University, Raleigh, NC 27695; [2]American Cyanamid, Goldsboro, NC 27530.**

Dimethomorph is a cinnamic-acid derivative that disrupts fungal cell wall formation in oomycete fungi. Six field experiments were conducted during 1994-1995 to evaluate methods and timing of dimethomorph application for control of black shank (*Phytophthora parasitica)* of tobacco (*Nicotiana tabacum).* The chemical was compared to an untreated control and metalaxyl at 1.12 kg a.i./ha broadcast preplant incorporated (bpi) + 0.56 a.i. kg/ha in a band directed at the base of the plant about 5 wk after transplanting (layby). In 1994, dimethomorph applied at 1.0 kg a.i./ha (transplant drench) + 2.0 kg a.i./ha band 1-2 wk later (first cultivation) was as efficacious as the standard metalaxyl treatment. Dimethomorph at 2.0 kg a.i./ha (bpi) + 2.0 kg a.i./ha (first cultivation) + 2.0 kg a.i./ha (layby) was intermediate in efficacy between the above drench treatment and the untreated control. Dimethomorph was toxic to plants when polystyrene flats containing seedlings were floated in a dimethomorph solution (2 g a.i./l) 48 hr before transplanting. Other application methods were efficacious and not phytotoxic.

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EFFICACY OF DIMETHOMORPH FOR THE CONTROL OF TOBACCO BLUE MOLD IN MEXICO. 1990-1995. P. B. Shoemaker[1], C. E. Main[1], J. Jaurez Rangel[2], J. Aburto Garcia[2], V. B. Nikolaeva[2]. [1]Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695, [2]CIICA, Agroindustrias Moderna, Monterrey, Neuvo Leon, Mexico.

Field experiments for the control of tobacco blue mold caused by *Peronospora tabacina* were conducted each winter from 1990 through 1995 near Papantla, Veracruz, Mexico. Treatments common in each of five experiments were a nontreated control and three preventive spray treatments (5-day interval) including 0.28 kg dimethomorph; 1.8 kg mancozeb; and 0.22 kg metalaxyl plus 1.8 kg mancozeb alternating with 1.8 kg mancozeb (metalaxyl/mancozeb) in 467 l/ha . Spray volume increments were 25, 50, 75 and 100% of the max., increasing as plants grew following transplanting. Blue mold occurred each year causing final leaf area damage (% LAD) between 15 and 80% in the nontreated controls. Average % LAD was reduced 93, 21 and 25% by the dimethomorph, mancozeb, and metalaxyl/mancozeb treatments, respectively, compared to the nontreated control in the five experiments. Yields increased an average 578 kg/ha (47%) for the dimethomorph treatment compared to the nontreated control over 4 years (1995 yield data not included). These studies show that dimethomorph is an effective fungicide for the control of blue mold in the presence of metalaxyl resistant strains of *P. tabacina*.

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FIRST REPORT OF BENOMYL-INSENSITIVE *DIDYMELLA BRYONIAE* IN THE UNITED STATES. A. P. Keinath and T. A. Zitter, Clemson Univ. CREC, Charleston, SC 29414 and Cornell Univ., Ithaca, NY 14853.

Benomyl-insensitive isolates of *D. bryoniae* have been identified previously in Europe. Seven isolates were grown on 1/4 PDA amended with 0, 1, 3.2, 10, 31.2 or 100 mg/L benomyl (technical grade, 3 plates/treatment). After 7 days at 21 C, four isolates from FL, NY and SC grew at all concentrations, but the other three did not grow at >1 mg/L. Based on dose response curves, growth of insensitive isolates was reduced 50% at 33.1 mg/L. Of 33 additional isolates tested, 15 isolates from 7 sites in SC were insensitive. In greenhouse tests, 12 watermelon cv. Jubilee II plants were sprayed with 0, 1.5, 15, 150 or 1500 mg/L benomyl (Benlate 50WP) and inoculated 1 day later with 10^5 conidia/ml of either a sensitive or an insensitive isolate. After 3 days at 100% RH, percent leaf area diseased was greater ($P \leq 0.02$) for the insensitive than the sensitive isolate at all concentrations ≥1.5 mg/L. The occurrence of pathogenic, benomyl-insensitive *D. bryoniae* in the eastern US may limit the usefulness of benomyl for gummy stem blight management.

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COMPARISON OF FIVE FUNGICIDES TO METALAXYL WITH RESPECT TO SPORULATION OF *PHYTOPHTHORA CAPSICI* AND DEVELOPMENT OF STEM AND ROOT ROT ON CHILE PEPPER. M.E. Matheron and M. Porchas. Yuma Agricultural Center, University of Arizona, Yuma, 85364.

In vitro studies were conducted to compare sporulation of *Phytophthora capsici* in 1.5% soil extract containing metalaxyl at 1, 10, 100, or 1,000 ppm to sporulation at the same concentrations with dimethomorph, fluazinam, fosetyl-Al, ICIA-5504, or SM-9. Compared to sporulation in the absence of any fungicide, sporangium production by *P. capsici* was reduced at least 90% by 1 ppm dimethomorph, 10 ppm fluazinam or metalaxyl, and 100 ppm fosetyl-Al, ICIA-5504, or SM-9. In a greenhouse trial, root growth on 4-mo-old chile pepper (*Capsicum annum* L.) plants grown in noninfested potting mix was equivalent to that of plants maintained in soil infested with mycelia of *P. capsici* and drenched once with 10 ppm metalaxyl, 50 ppm fluazinam, 100 ppm dimethomorph, and 1,000 ppm fosetyl-Al.

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RESPONSE OF *FUSARIUM* ISOLATES FROM POTATO TUBERS TO BENZIMIDAZOLE FUNGICIDES. L.E. Hanson and R. Loria. Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Fusarium isolates obtained from randomly collected potato tubers with dry rot symptoms were tested in radial growth assays for resistance to three benzimidazole fungicides. Eight species were represented among the 155 isolates tested, including *F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. equiseti, F. oxysporum, F. sambucinum*, and *F. solani*. The response of most isolates to thiabendazole (TBZ) and benomyl, at 5 ppm, was similar and fell into one of two categories: growth less than 30% of the control or 70-100% of the control. However, three *F. sambucinum* isolates were intermediate in their response to TBZ (45-55% of control). One of these isolates did not grow on benomyl, indicating a lack of cross resistance. Growth on thiophanate methyl (25 ppm) did not show discrete categories, and ranged from 0% to 105 % of control. These results suggest that *Fusarium* species respond differently to various benzimidazole fungicides.

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SYNTHETIC PEPTIDE COMBINATORIAL LIBRARIES: AN APPROACH TO IDENTIFYING ANTIMICROBIALS FOR THE CONTROL OF PLANT PATHOGENS. C. F. Gonzalez[1], J.D. Reed[1] and D. L. Edwards[2]. [1]Texas A&M University, College Station, TX 77843 and [2]Ceres Technologies, Inc., San Antonio, TX. 78230.

Peptides are not only key regulators of physiological processes, but also have the potential of acting as antimicrobials to control the growth of phytopathogenic fungi. A synthetic peptide combinational hexapeptide library consisting of four hundred different peptide mixtures, with each peptide mixture consisting of 130,321 individual hexamers, was synthesized. The library was tested for biological activity against selected phytopathogenic fungi. Initial screening of the 400 peptide mixture library identified biologically active peptide mixtures whose first two amino acid residues were designated. An iterative screening has identified a hexapeptide mixture with four designated amino acids (4-6). The 4-6 mixture exhibited an IC_{50} of 136, 273, 50, and 41 µg/ml for *Fusarium oxysporum* f.sp. *lycopersici, Rhizoctonia solani, Pythium ultimum*, and *Ceratocystis fagacearum*, respectively.

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EFFECT OF TWO FUNGICIDES ON FORMATION OF INFECTION CUSHIONS BY SCLEROTINIA MINOR. R.K. Soufi and H.A. Melouk. Department of Plant Pathology and USDA/ARS, Oklahoma State University, Stillwater, OK 74078.

The effect of fluazinam and iprodione, on the number of infection cushions formed by S. minor on cellophane was studied. Root systems of 14-day-old Okrun peanut plants were placed in 10 x 4.9 cm cellophane pouches made of dialysis tubing and individually planted in a soil mix. A suspension of mycelial fragments of S. minor (0.34 g DW in 50 ml water) was incorporated into the top 5 cm of the soil mix. Three days later, 0.23 ml/plant of each of the fungicides was applied at rates equivalent to 0.71 kg ai/ha for fluazinam and 1.136 kg ai/ha for iprodione. Four days after fungicide application, the cellophane was removed, and 10 squares (1 cm^2) were cut from the area of cellophane surrounding the crown of the plant. The squares were stained with cotton blue and the number of infection cushions/cm^2 was counted. One infection cushion/cm^2 was formed in the fluazinam treatment, which was significantly lower ($p = 0.01$) than iprodione or water (4 and 12, respectively).

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ALTERNATIVE FUNGICIDES FOR POSTHARVEST CONTROL OF SILVER SCURF ON POTATO TUBERS. C. Olivier and R. Loria, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Alternative management strategies are needed for silver scurf, a postharvest disease of potato, caused by *Helminthosporium solani*. Our objective was to test carbonate and bicarbonate salts ($KHCO_3$, $NaHCO_3$, K_2CO_3, Na_2CO_3) and propionic and sorbic acids for inhibition of *H. solani*. Radial growth was suppressed with all treatments at concentrations greater than 0.1 M on amended media. Potato tubers were inoculated with spore suspensions, incubated in the dark for 5 days, then dipped in distilled water or 0.2 M solutions of the test compounds. Sporulation was inhibited (<1.4 spores/mm^2) on periderm tissue by $KHCO_3$, $NaHCO_3$, K_2CO_3, Na_2CO_3, propionic acid and sorbic acid, relative to the control (81 spores/mm^2). Percent of the tuber surface with lesions also was significantly ($p<0.01$) reduced . This research has demonstrated that sporulation and disease severity can be reduced with postharvest applications of these compounds, which may provide an alternative to conventional postharvest fungicides for control of silver scurf.

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PHOMOPSIS TWIG BLIGHT OF PEACH: PATHOGENICITY AND INFLUENCE OF TEMPERATURE ON DISEASE DEVELOPMENT. Wakar Uddin and Katherine L. Reynolds, Department of Plant Pathology, University of Georgia, Athens, GA 30602.

A species of *Phomopsis* was consistently isolated from blighted twigs of peach often along with various species of *Botryosphaeria* and *Cytospora*. Pathogenicity tests indicated that *Phomopsis* sp. was the causal agent. One-yr-old peach trees inoculated with *Phomopsis* sp. developed symptoms of the disease and the fungus was consistently reisolated from diseased tissue. Trees inoculated with *Botryosphaeria* spp. and *Cytospora* sp. did not develop symptoms. In another study, the influence of temperature (10, 20, and 30 C) on disease development was investigated. Disease progress at all temperatures was best described by a monomolecular model. Estimates of the rate parameter, r, were not significantly different at 10 and 20 C, but both were significantly greater than that at 30 C. Area under the disease progress curve (AUDPC) at 20 C was significantly greater than that at 10 or 30 C, and the maximum canker length (Y_{max}) at all three temperatures differed significantly from each other, with the highest at 20 C and the lowest at 30 C. Analysis of r, AUDPC, and Y_{max} indicated that 20 C is most favorable for disease development followed by 10 and 30 C.

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EFFICACY OF MIXTURES OF DRY BEAN CULTIVARS IN REDUCING ANTHRACNOSE DEVELOPMENT. Nephtali Ntahimpera, Helene R. Dillard, Ann C. Cobb, and Robert C. Seem, Cornell University, New York State Agricultural Experiment Station, Department of Plant Pathology, Geneva, NY 14456.

Field tests were conducted in 1992, 1993, and 1994 to analyze the effectiveness of cultivar mixtures in reducing bean anthracnose development. The light red kidney bean cultivars, Ruddy, Redkloud, and Sacramento, that are resistant (R), susceptible (S_1), and susceptible (S_2), respectively to the ß race of *Colletotrichum lindemuthianum*, were mixed in different percentages to achieve the following treatments: 100R; $50R+25S_1+25S_2$; $25R+37.5S_1+37.5S_2$; $10R+45S_1+45S_2$; $50S_1+50S_2$; $100S_1$; and $100S_2$. Treatments were planted in May and inoculated by introducing a spreader plant in the center of each square plot of 6.3m. Mixture efficacy of the cultivars was increased as the proportion of the resistant cultivar was increased in the mixtures. Under high disease pressure, susceptible plants grown in mixtures with 10, 25, and 50% of the resistant cultivar Ruddy exhibited 40, 50, and 70% lower disease incidence of anthracnose, respectively as compared to pure stands of the susceptible cultivars. Under low disease pressure, mixture efficacy values in reducing disease incidence were smaller but of similar trend.

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USING FRACTAL GEOMETRY TO COMPARE RHIZOMORPH FORAGING STRATEGIES AMONG SIX ARMILLARIA SPECIES. J.D. Mihail and J.N. Bruhn, Department of Plant Pathology, University of Missouri, Columbia, MO 65211.

Individual genets of *Armillaria* species (*e.g.*, *A. ostoyae* and *A. gallica*) tend to occupy continuous non-overlapping territories. To understand determinants of territory size and shape, the fractal geometry of rhizomorph branching patterns is being used as one metric of foraging strategy. Initial studies of rhizomorph systems of isolates of six *Armillaria* species, developing in a homogeneous, nutrient-rich medium, demonstrated that the species could consistently be grouped based on the magnitude of their fractal dimension (D). In order of decreasing magnitude, they were: *A. tabescens* > *A. mellea* > (*A. gallica* = *A. sinapina*) > (*A. calvescens* = *A. ostoyae*). The range of D in these isolates was 1.481 to 1.897. The rhizomorph systems of species with the most parsimonious rhizomorph foraging strategies (*i.e.*, *A. calvescens*, *A. ostoyae*, and *A. sinapina*) were temporally and spatially invariate with respect to D. The magnitude of D and other metrics of rhizomorph system development, such as growth rate and selective growth toward favored nutrient sources, will be used to simulate the development of genet territories.

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HISTORICAL AND SCIENTIFIC EVIDENCES THAT SUPPORT THE MODERN THEORY OF PERUVIAN ANDES AS THE CENTER OF ORIGIN OF *PHYTOPHTHORA INFESTANS*. **Z. G. Abad[1], J. A. Abad[2] and C. Ochoa[3].** **[1,2]Department of Plant Pathology, NCSU, Raleigh NC 27695-7616. [3]International Potato Center (CIP), P. O. Box 5969. Lima-Peru.**

Although present literature suggests late blight of potato, tomato and pear melon is a new disease (1920's), evidence shows it is much older in South America. Publications described the disease on potatoes in Peru, Bolivia and Colombia prior to 1845. Our research and that of others in Peru (CIP) provide scientific evidence of: 1) resistance to *P. infestans* on wild tuber-bearing *Solanum* species and native potatoes; 2) resistance in wild tomatoes; 3) the pathogen has a wide host range (*Solanaceae* and *Nolanaceae* families) and specialized forms e.g., pear melon, race 1-4-10-11; 5) predominance of tomato and pear melon pathotypes in potato isolates from the Andes; 6) different mitochondrial DNA (type D); 7) old populations with allozymes (Gpi 86/100, pep 92/100) and nuclear DNA fingerprint RG57 (25 loci) similar to European and USA old populations.

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COMPETITIVE DIFFERENCES AMONG ISOLATES OF *SCLEROTINIA SCLEROTIORUM* ON CANOLA. A. D. Maltby and J. D. Mihail, Department of Plant Pathology, University of Missouri, Columbia MO 65211

Four isolates of *S. sclerotiorum*, each belonging to a different vegetative compatibility group, were tested for competitive interactions on canola. When grown on artificial media, there were no significant differences in growth rate or total sclerotial mass among the isolates. Greenhouse studies revealed no significant differences among the isolates in virulence or total mass of sclerotia produced in separate inoculations on the canola cultivar Westar. To study differences in competitive abilities, all possible pairs of isolates were used in dual inoculations. Three different competitive scenarios were examined with inoculations that were spatially and temporally identical, spatially separated but temporally identical, or spatially and temporally separated. These studies revealed significant differences in sclerotial mass among the isolates depending upon the isolate combination. From these results we conclude that *S. sclerotiorum* isolates not different in virulence can differ in ability to compete for the host plant resource.

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AGGRESSIVENESS OF *PHYTOPHTHORA INFESTANS* ISOLATES IN THE PACIFIC NORTHWEST. J. S. Miller, D. A. Johnson, and *P. B. Hamm. Department of Plant Pathology; Washington State University; Pullman WA 99164, and *Hermiston Agricultural Research and Extension Center, Oregon State University, Hermiston OR 97838.

Isolates of *Phytophthora infestans* collected from potatoes in the Columbia Basin from 1992 to 1994 were tested for aggressiveness on detached leaflets of various potato cultivars. Components of aggressiveness include incubation period, latent period, area under the lesion expansion curve (AULEC), and sporangium production. Components of aggressiveness were significantly correlated with each other. Significant differences ($P < 0.05$) were observed among the 30 isolates tested and considerable variability existed among isolates that were metalaxyl resistant (MR) A1 mating type, MR A2 mating type, and metalaxyl sensitive (MS) A1. MR isolates as a group were not consistently more aggressive than the MS isolates. The most aggressive isolate was an A2 mating type. Some A1 isolates, however, were more aggressive than some A2 isolates. AULEC was a good indicator of aggressiveness.

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INFECTION OF TOMATO FRUIT BY *COLLETOTRICHUM COCCODES* UNDER FIELD CONDITIONS. S. Sanogo, S.P. Pennypacker, and R. Stevenson. Department of Plant Pathology, The Pennsylvania State University, PA 16802.

During the 1993 and 1994 growing seasons, 16 potted tomato plants (10-12 wk-old) bearing mature green fruit (4-5 cm diameter) were exposed for 4 days in a field naturally infested with *C. coccodes*. At each of four field sites, four potted plants were placed three feet apart in rows of field-grown tomatoes. In 1993, all rows of field-grown tomatoes had ripening fruit with typical symptoms of*C. coccodes*. During 1994, infected tomato fruit with sporulating lesions were spread at exposure sites to ensure that inoculum was present early in the growing season. Over the two growing seasons, a total of 16 exposures was completed. Preliminary analyses indicated that, of all environmental factors monitored, incidence of anthracnose was correlated ($P<0.05$) to total rainfall and average relative humidity. These results suggest that moisture may have a significant effect on development of tomato anthracnose epidemics.

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A POTATO EARLY BLIGHT FORECASTING MODEL WITH AN INTEGRATED HOST RESISTANCE FACTOR. J.S. Shuman, B.J. Christ and S.P. Pennypacker, Dept. of Plant Pathology, Pennsylvania State University, University Park, PA 16802

The FAST (Forecaster of *Alternaria solani* on Tomato) system, with an integrated host resistance factor, was evaluated for its utility in scheduling fungicide applications to control early blight on potato. Field studies, performed in the summer of 1993 and 1994, utilized a 4x5 factorial in randomized complete block design with 4 replicates. The cultivars Norchip, Norwis, Atlantic, and Katahdin, ranging from susceptible to moderately resistant, were each treated with chlorothalonil using 5 schedules: a 7-day schedule initiated after flowering, a non-sprayed control, and 3 schedules generated by FAST using combinations of critical cumulative severity and rating values. Disease severity and yield components were evaluated. A cost analysis was performed for each treatment using estimated tuber yield, crop value, and spray costs. The cultivars Norchip, Norwis, and Atlantic exhibited best profit margin with the FAST schedule targeted for their respective level of early blight susceptibility. Cost analysis for moderately resistant cv Katahdin, a late maturing variety, indicated that no economic benefits were obtained using any of the spray schedules. Based on cost analysis, disease control, and yield components, we conclude that the FAST system can be successfully used to control early blight and maintain tuber yield while accounting for cultivar resistance.

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NEURAL NETWORKS THAT PREDICT LEAF WETNESS. L.J.Francl, S. Panigrahi, and T. Padhi. Departments of Plant Path. and Agr. Engin., North Dakota State Univ., Fargo ND 58105

Artificial neural network (ANN) models were trained to predict leaf wetness using environmental measurements from a datalogger with output from wetness sensors placed in a wheat crop planted at 5, 10, and 15 g m^{-2}. Three ANN models were developed for different amounts of information. A minimal-input set model (MIS) was based on information commonly available from weather stations. A full-input set model (FIS) used MIS inputs, as well as wetness data from a sensor placed above the crop, maximum wind speed, and temperature and relative humidity from within the canopy. A reduced-input set model (RIS) used MIS inputs and wetness data taken above the crop. Regression of predicted on observed wetness values showed the models explained 42 to 79% of the variation in an independent data set. Some of the error was trivial underprediction of maximum dryness. Error was least between midnight and 0700 and was unbiased with regard to planting rate. Average absolute error for FIS and RIS was 1.2 h per wetness event and models were 90% accurate in predicting wetness. The MIS model erred 1.8 h on average in estimating wetness duration and was 84% accurate. Differences in error rates indicate the value of wetness data as input. These ANN models performed well compared to previously developed models for predicting wetness; moreover, ANN models predicted wetness due to either rain or dew.

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FORECASTING OF INCIDENCE AND TEMPORAL SPREAD OF TWO POTYVIRUSES IN WATERMELON. Mora-Aguilera,G.,[1] Webb,S.E.,[2] Purcifull,D.E.,[1] Zettler,F.W.,[1] Chellemi,D.O.,[3] and Kok-Yokomi,M.L.[2] Plant Pathology Department, University of Florida, Gainesville, FL 32611[1]; CFREC, Leesburg,FL 34748[2]; NFREC, Quincy,FL 32351[3].

Average apparent infection rates of WMV-2/ZYMV epidemics ranging from 0.320-0.409 logit units/day (r^2 >0.93, p≤0.05) were found in four experimental watermelon plots (sown 15 March) at Leesburg, FL in 1994. Time of epidemic duration to 95% incidence was 26 days. WMV-2 was detected with ELISA in 9% of 298 plants in one plot in 26 May, whereas ZYMV was detected in 80%. Fruit yield of single plants or of all plants infected at the same time was directly correlated with the number of days that plants remained healthy. Yield increased at a rate of 0.140 kg day^{-1} (r^2=0.96). Average fruit sugar content of individual melons was correlated with time of infection (r^2=0.818). *Uroleucon pseudambrosiae* (Up), *Myzus persicae* (Mp), *Aphis middletonii* (Am) and *A. spiraecola* (As) were the most commonly trapped (1403,114,94, 49, respectively, of the 2049 aphids caught). Results of arena tests indicate that Up is able to transmit ZYMV; Am, As, and Mp are known vectors of ZYMV. Factor analysis, varimax rotated biplot displays, and lag-time regression of factor scores of aphid vectors with incidence change (Y) were used to develop forecasting models. Disease incidence was best predicted with the model $Y = 0.14 (Up) + 0.12 (Am \times As)$, ($R^2 = 0.94$, Cp-Mallow=1.0).

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NON-TRADITIONAL MEANS OF SELECTING SLASH PINES WITH FUSIFORM RUST RESISTANCE. C.H. Walkinshaw. USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360.

Traditional breeding in selecting disease-resistant trees gathers host materials in high-hazard areas. In this study, rust-free slash pines (Pinus elliottii Engelm. var. elliottii) with good growth and form were selected in low rust areas in Region 8 National Forests. The most resistant of 223 trees were identified by greenhouse tests with Cronartium quercuum (Berk.) Miyabe ex Shirai f.sp. fusiforme. Control-pollinations among resistant trees were made with pollen from each and from trees in forest industry programs. These full-sib progeny had less than 25% infection. Susceptible controls ranged from 85 - 100% infection. This surprising discovery of high resistance from low disease-pressure areas demonstrates that non-traditional procedures can yield valuable results.

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FACTORS LIMITING WESTERN GALL RUST INFECTION ON LODGEPOLE PINE. B. D. Moltzan, P. V. Blenis, and Y. Hiratsuka. Dept. of Agriculture, Food, and Nutritional Sciences, Univ. of Alberta, Edmonton, AB T6G 2P5, Canada.

Western gall rust caused by *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka is very common on lodgepole pine in western Canada. The objective of this study was to determine the relative importance of spore production, spore germination, and candle development in limiting infection. A field study was conducted in 1992 and 1993 near Hinton, Alberta. High levels of spore production occurred during the first and second weeks of June, but by the first week of July, spore counts were minimal. Spore viability remained above 90% from the last week of May through the first week of July. Candle elongation reached 90%, 95%, and 100% of final candle development at the third and forth week of June, and first week of July, respectively. Controlled inoculation of seedlings in different stages of growth showed that infection decreased to 80%, 50%, and 10% of maximum once candles had reached 90%, 95%, and 100% of final development, respectively. These results suggest that a combination of spore availability and host development limit infection in the field. Further histological examination will be conducted to associate periderm and vascular cambium formation to host susceptibility in the field.

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VARIATION IN AGGRESSIVENESS OF *SPHAEROPSIS SAPINEA* MORPHOTYPES ON RED AND JACK PINE. L.T. Blodgett and G.R. Stanosz, Dept. of Plant Path., Univ. of Wisconsin-Madison, 53706.

Sphaeropsis sapinea (syn. *Diplodia pinea*) causes a shoot blight and canker disease of various conifers. Two *S. sapinea* morphotypes ("A" and "B") are recognized and have been suggested to differ in the requirement for wounds to penetrate hosts and in virulence. The aggressiveness of "A" and "B" isolates were compared on wounded and unwounded seedlings of red (*Pinus resinosa*) and jack pine (*Pinus banksiana*) in the greenhouse. Growing shoots tips of two-year-old seedlings were inoculated by placing a colonized agar plug on a wound made by removing a needle fascicle. Unwounded growing shoot tips of one-year-old seedlings were inoculated with 0.4 ml of a conidial suspension (0.5×10^4 spores/ml). Nine "A" and eight "B" isolates were used for wound inoculations and five isolates of each morphotype were used in conidial inoculations. Both methods resulted in some symptom development in red and jack pines inoculated with either "A" or "B" isolates. However, both pine species were more severely affected by "A" isolates than by "B" isolates. This difference was more pronounced for red pines. The large difference in severity of symptoms induced following wound-inoculation of red pine seedlings allows differentiation of isolate morphotype based on host response.

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INCREASED PERMEABILITY OF CONIFER SAPWOOD DURING INCIPIENT BROWN-ROT DECAY. F. GREEN, III, J. Tschernitz, T.A. Kuster and T.L. Highley, Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705-2398.

The permeability of a wood species is primarily a function of the flow rate through pit pores. Pit membranes have been shown to be hydrolyzed by commercial pectinases. Brown-rot fungi produce oxalic acid, and have recently been shown to induce polygalacturonase to pectin. Douglas fir and Southern pine cores were inoculated with P. placenta, G. trabeum, S. incrassata, A. niger or Trichoderma sp. in ASTM soil block tests. Increase to maximum permeability over the first two weeks indicates rapid hydrolysis of pit membranes. Results were confirmed by SEM. We conclude pit hydrolysis is integral to rapid spread of brown-rot decay.

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EFFECT OF FOREST TENT CATERPILLAR AND DISCULA CAMPESTRIS ON SUGAR MAPLE IN PENNSYLVANIA IN 1994. T.J. Hall, Division of Forest Pest Management, Bureau of Forestry, Middletown, PA. 17057-5021.

Severe defoliation caused by *Malacosoma disstria* and other native insect defoliators was observed on *Acer saccharum* in many northern hardwood stands in northern and south-central Pennsylvania in 1994. Subsequent refoliation of trees in affected stands was extremely poor; field examination of felled maples revealed symptoms of leaf anthracnose and blight of leaf, bud, and twig tissue. Microscopic examination revealed conidiomata of *Discula campestris* on leaves, petioles, and twigs. The disease outbreak was associated with unusually moist weather conditions in the affected regions from mid-July through October. Affected stands exhibited extensive crown dieback and potential for significant tree mortality in 1995.

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FUSARIUM PROLIFERATUM IS A COMMON, AGGRESSIVE PATHOGEN OF CONTAINER-GROWN CONIFER SEEDLINGS. R. L. James[1], R. K. Dumroese[2], and D. L. Wenny[1]. [1]USDA Forest Service, Insect and Disease Mgt., Coeur d'Alene, ID 83814. [2]Forest Research Nursery, University of Idaho, Moscow, ID 83844.

Fusarium proliferatum is consistently isolated from roots of diseased and non-diseased container-grown conifer seedlings within nursery greenhouses in the Inland Pacific Northwest. Many conifer species are affected, but Douglas-fir is often severely impacted. F. proliferatum is usually not seed-borne on conifers and increases in abundance on roots as seedlings become older. Inoculum may reside within containers and spread through the air within greenhouses. Pathogenicity tests indicate little variability among isolates with most being very aggressive, causing damping-off and root disease. Control efforts center around reducing inoculum and maintaining high host vigor.

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CHEMICAL TREATMENT OF EASTERN WHITE PINE SEEDS FOR REMOVAL OF *FUSARIUM* PROPAGULES. Cynthia M. Ocamb, USDA Forest Service, North Central Forest Experiment Station, 1992 Folwell Ave., St. Paul, MN 55108

Chemical treatments were examined for disinfestation of eastern white pine (*Pinus strobus*) seeds of *Fusarium* propagules. Unstratified seeds were rinsed in running tap water for 48 hr; agitated in 3 % H_2O_2 for 3 hr, 2 % NaOCl for 10 min, or 0.5 % NaOCl for 10, 20 or 40 min; or left untreated. Three hundred seeds in each treatment were assayed for presence of *Fusarium* species and an additional 150 seeds per treatment were stratified and tested for germinability. Germination levels of chemically-treated seeds were the same as the untreated seeds. Ninety percent of untreated seed were infested with *Fusarium* species. Infestation levels were 4 % for 3 % H_2O_2-treated seed; 14 % for seeds agitated in 2 % NaOCl for 10 min; 50, 41, and 30 % when seeds were agitated in 0.5 % NaOCl for 10, 20, and 40 min, respectively; and 29 % for seeds receiving only a 48-hr rinse. Use of 3 % H_2O_2 for white pine seed disinfestation can significantly reduce pathogen introduction. Disinfesting seed of *Fusarium* spp. may improve performance of seed-applied biological control agents.

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FUSIFORM RUST SPORE GERMINATION BEHAVIOR ON HOST AND NON-HOST SURFACES. P. Spaine, S. Kaneko, J. Kerrigan, B. Richardson, and S. Covert. USDA, Forest Service, Athens, GA 30602; FFPRI, Tsukuba, Japan; Univ. of GA, Athens, GA 30602.

The germination responses of Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme basidiospores (bs) and urediniospores (us) were quantified on host (pine and oak) and non-host surfaces of glass, plastic, 2% water agar and dialysis membrane on agar. Directly-cast bs had 67% direct germination on pine, 56% on membrane, and 25% on the non-host, oak. However, there was a 5-fold increase in indirect germination of bs on oak (50%) compared to pine (10%). Washing the bs increased the %of direct germination on pine and membrane (70% and 93%), but decreased indirect germination on oak (5%). Unwashed us germinated well on membrane and oak surfaces (73% and 48%). Dialysis membrane performed well as an alternative germination surface in all treatments (56-96%).

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FREQUENT AND RAPID EVOLUTION OF HOST RANGE AMONG SPECIES OF *CYTOSPORA*, CANKER PATHOGENS OF TREES. G. C. Adams. Michigan State University, Department of Botany & Plant Pathology, East Lansing, Michigan 48824.

A phylogenetic tree based on DNA sequence provides evidence that individual species of *Cytospora* pathogenic on gymnosperms are most closely related to individual species pathogenic on angiosperms. The relationship reoccurs in terminal taxa in separate clades (groups). Examples of such pairs include: *C. sequoiae/C. magnoliae, C. abietis/C. personata, C. friesii/C. fugax, and C. curreyi/C. cincta.* Many conifer pathogens had been grouped by taxonomists in section Microsporae based on small ascospores. Small ascospores may be a host-influenced character originating with natural selection and convergence.

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USING SUBSURFACE DRIP IRRIGATION TO REDUCE ALTERNARIA LATE BLIGHT OF PISTACHIO CAUSED BY *ALTERNARIA ALTERNATA*. Themis J. Michailides, D. P. Morgan, Dept. of Plant Pathology, and D. A. Goldhamer, Dept. of Water Science and Soils, University of California, Davis, and Kearney Agricultural Center, Parlier 93648.

The effects of subsurface (75 cm deep) drip and flood irrigation on Alternaria late blight of pistachio were compared using a randomized complete-block design with five replications of 12 rows (800 m long) of trees. Subsurface drip irrigation resulted in significantly lower incidence and severity of infected leaves by *A. alternata*. For instance, by commercial harvest time only 11% of the leaves in the drip-irrigated blocks were infected while 54% of those in the flood-irrigated blocks. Furthermore, subsurface drip irrigation reduced the incidence of infected fruit to 22% while 51% of the fruit from trees irrigated by flooding were infected. However, subsurface drip irrigation resulted in ten times more Aspergillus fruit blight (1.3%) than flood irrigation. *A. alternata*, other filamentous fungal, and yeast propagules on leaves and fruits were not affected by the irrigation type. Subsurface drip irrigation resulted in shorter periods of dew, lower relative humidities, and higher temperatures, which can explain the differences in disease levels between the two irrigation systems. In addition, subsurface drip irrigation substantially improved nut quality (more shell splitting and less shell stain and fewer blank nuts) without lowering yield.

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IPM STRATEGY FOR MINIMIZING AFLATOXIN CONTAMINATION IN PEANUTS. K.L. Bowen, T.P. Mack, and S. Wolf, Dept. of Plant Pathology and Dept. of Entomology, Auburn University, AL 36849.

Fungi that produce aflatoxins can be passively vectored by larvae of the lesser cornstalk borer (LCB). Efficient control of LCB can contribute to lower fungal invasion and aflatoxin contamination in peanuts. A system for timing scouting and insecticide applications is called LCB-Days. LCB-Days is the running total of hot dry ($\geq$ 35°C and $<$ 2.54 mm rain) days minus cooler wet days ($<$ 35°C and $\geq$ 2.54 mm rain). When LCB-Days has a positive value, insect scouting is necessary; when LCB-Days $>$ 10, damage is likely. LCB-Days has also been correlated to aflatoxigenic fungal invasion of developing pegs and pods of peanut; therefore, this system could be used to assess the possibility of aflatoxin contamination in peanuts from particular fields or regions.

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Technology Transfer of IPM Information to the Public through a 1-800 Phone Number. David L. Clement, M. K. Malinoski, J. H. Traunfeld, and R. V. Bosmans. Regional Specialists, Cooperative Extension Service, University of Maryland, Home and Garden Information Center, 12005 Homewood Rd. Ellicott City, MD 21042.

The Home and Garden Information Center is a unique program nationally, initiated by the Maryland Cooperative Extension Service, that provides information on Integrated Pest Management (IPM) and Environmental Horticulture topics statewide over a toll free telephone number. Prerecorded information is available 24 hours a day and Horticulture Consultants are available to discuss caller questions 5 days a week. Since 1992, the Center has handled approximately 152,000 caller inquires. Survey data from 1994 indicate 49% of the callers asked questions that required an IPM solution. Of the callers that received IPM solutions to their problems, 58% utilized recommended pesticides in solving their problem and 42% of the callers learned they didn't need a pesticide to solve their problem. In addition to phone consultations the Center staff has produced over 70 fact sheets and 250 prerecorded messages that offer self help diagnostic keys that enable the general public to use the latest IPM approches to pest management. Promotion of IPM solutions includes live broadcasts on TV, and radio as well as newspaper interviews.

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Effect of Nitrogen Rate and Soil pH on Development of Black Root Rot of Burley Tobacco in a Conducive Field Soil. U. J. Harrison and H. D. Shew, Department of Plant Pathology, N. C. State University, Raleigh, 27695.

Field tests were conducted to determine the effects of nitrogen rate and soil pH on populations of *Thielaviopsis basicola* and development of black root rot of burley tobacco in a disease conducive soil. Plots were amended with ammonium nitrate at the recommended rate of 224 kg per ha prior to transplanting or at 28 kg per ha 30 days after transplanting. Half of the plots were limed with calcium hydroxide to raise the pH one unit (5.5 to 6.5). A cultivar with a moderate level of partial resistance to the disease was grown. Populations of the pathogen and soil pH values were determined prior to transplanting, and 30, 60 and 105 days (harvest) after transplanting, and shoot heights were determined on randomly chosen plants 85 days after transplanting. Disease severity was determined by excavating and rating plants after harvest. Populations of *T. basicola* were affected by nitrogen rate, soil pH and initial population density. Final populations of *T. basicola* were lowest and shoot heights greatest in the high N, low pH plots. Disease was most severe in limed plots and was affected by initial inoculum level and soil pH at 30 days after transplanting. Nitrogen rate, pH, and initial inoculum level are significant factors in development of black root rot in a conducive soil.

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RAPID DETERMINATION OF CONIDIAL VIABILITY FOR *BOTRYTIS CINEREA* AND *TRICHODERMA HAMATUM* USING FLUORESCENT MICROSCOPY. R. L. Schading, B. A. Mullin-Schading, and F. D. McElroy. EDEN Bioscience Corporation, 5795 N. E. Minder Rd., Poulsbo, WA 98370.

Conidial viability for two species of deuteromycetous fungi, *Botrytis cinerea* and *Trichoderma hamatum* was determined rapidly using the fluorochrome stain fluorescein diacetate (FDA). FDA fluoresces bright green in viable conidia when viewed using specific fluorescent microscopic techniques. Conidia/deionized water suspensions for two isolates of each species were prepared to be maximum viable, *ca.* 50% viable, and 100% non-viable. The conidial suspensions were tested for viability using FDA and germination counts on water agar. Percentage of viability using FDA was determined by counting all the fluorescing conidia in a microscopic field illuminated by 490 nm UV light and then counting the total conidia in the same field using substage light. No statistical differences were detected between assessment methods using Analysis of Variance ($P=0.05$). Reliable viability data were achieved using FDA in less than one hour compared to 24 hr or more using a standard germination method.

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DETECTION OF AMMONIA IN HAIRY VETCH-AMENDED SOILS AND ITS ROLE IN SOIL SUPPRESSIVENESS TO *THIELAVIOPSIS BASICOLA*. B. L. Candole and C. S. Rothrock, Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701.

Thielaviopsis basicola and black root rot of cotton were reduced in hairy vetch-amended soils. The loss of viability of chlamydospores exposed to the atmosphere of amended soils suggested that a volatile factor, possibly ammonia was responsible for the suppressiveness. A technique using the Aquaquant ammonium test kit (EM Science Co.) was developed to quickly detect and quantify ammonia in atmospheres of hairy vetch-amended soils. This test, based on Nessler's reaction, is specific for the ammonium ion with a lower detection range of 0.025. *In vitro* assays of atmospheres of petri dishes containing 0 to 0.6 μl/ml ammonium hydroxide showed that 0.4 ppm ammonia resulted in complete loss of chlamydospore viability, with an estimated LD_{50} of 0.15 ppm. Experiments using hairy vetch amendment levels of 0%, 0.25%, and 0.75% (w/w), 0.05 ppm ammonia was detected from samples of soil atmospheres trapped in deionized water 24 hr after incorporation. Within 7 days of hairy vetch incorporation, fungicidal concentrations of ammonia ranging from 0.4 to 30 ppm were detected. The results indicate that this technique is useful for detecting and quantifying ammonia in the soil atmosphere.

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EFFECT OF *PSEUDOMONAS SYRINGAE* PV. *TAGETIS* ON COMMON COCKLEBUR (*XANTHIUM STRUMARIUM* L.). H.K. Abbas, D.R. Johnson, B.J. Johnson and D.L. Wyse. USDA-ARS, SWSL, Stoneville, MS; and University of Minnesota, St. Paul, MN.

Ten common cocklebur biotypes, including imazaquin-and MSMA-resistant biotypes were treated with *P. s.* pv. *tagetis*. Bacteria were sprayed at $5x10^8$ cells/ml 2-3 leaf cocklebur plants. Chlorosis of new leaves and petioles, necrosis and apical curl occurred on all biotypes of cocklebur beginning with 3 days after inoculation and progressed with time. Plant height and biomass were reduced 26 to 53% and 55 to 67%, respectively, compared to controls. Tagetitoxin produced by *P. s.* pv. *tagetis* injected into cocklebur plants at 1 μg/plant caused severe chlorosis within 72 h and increased with time up to 14 days. New growth below the injection point also showed chlorosis within 7 days, indicating the toxin is phloem-mobile.

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INFECTION OF THE WEED *SESBANIA EXALTATA* BY SOIL-INCORPORATED MICROSCLEROTIA OF THE FUNGUS *COLLETOTRICHUM TRUNCATUM*. D.A. Schisler and M.A. Jackson, USDA-ARS, NCAUR, Peoria, IL, USA 61604.

Microsclerotial (MS) propagules of *C. truncatum* were produced in submerged culture (Jackson et al., Mycol. Res., in press). When dried MS were encapsulated in formulations of pregelatinized corn flour (PCF), pregelatinized corn starch (PCS) or both, soil-incorporated PCF MS incited the most disease on *S. exaltata* seedlings. Placing formulated MS on Noble water agar resulted in high levels of sporogenic germination for PCF MS (~50-fold increase over MS alone) and secondary MS formation for PCS MS. *C. truncatum* was recovered from seedlings of *S. exaltata* grown in MS-infested pasteurized soil from 3 days after seeding until seedling collapse after 8 days. By day 6, *C. truncatum* was recovered from 24% of seedling samples taken from 7 mm above to 7 mm below the soil line but from only 3% of more distal samples. In total, 65% of the *C. truncatum* isolated was from plant tissues adjacent to the soil line. Light and SEM microscopy studies determined that MS germinated intermittently on *S. exaltata* seedlings *in situ*. Germination was myceliogenic and sometimes sporogenic.

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Establishment of *Cladosporium herbarum* and *Fusarium lateritium* applied to grapevine pruning wounds. P. M. Coleman and J. J. Marois, Department of Plant Pathology, University of California, Davis, CA. 95616.

Establishment of non-pathogenic fungi on the surface of grapevine pruning wounds prior to the arrival of inoculum can prevent infection by the pathogen *Eutypa lata*. Pruning wounds were treated with 10^6/ml conidial suspensions of *Cladosporium herbarum* or *Fusarium lateritium* on 0, 7, or 14 days after pruning, and sampled every 7 days for 28 days. The number of *C. herbarum* recovered from treated or non-treated wounds was 10^5 CFU/wound. The number of *F. lateritium* recovered from wounds treated 0, 7, or 14 days after pruning was 10^5, 10^4, and 10^3 CFU/wound, respectively. *Fusarium lateritium* was not recovered from non-treated wounds.

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STRIGA HERMONTHICA INFESTATION AND HOST PLANT REACTION OF MAIZE(*Zea mays L.*) IN THE SAVANNA ECOSYSTEM. J.M. Fajemisin, S.K. Kim, and J.G Kling, International Institute of Tropical Agriculture, Ibadan, Nigeria

Striga hermonthica (Del.)Benth., a parasitic seed-plant, is a major threat to maize production in the savanna and sahelian zones of sub-Saharan Africa. Two sets of maize -- inbreds and open-pollinated varieties-- were evaluated at Ferkessédougou, Côte d'Ivoire in 1993 and 1994. The plots were artificially infested with *Striga* seed: sand mixture(1:99 ratio by weight) placed 5-8cm deep in each planting hole. There were striking differences among the genotypes in host plant reaction --leaf chlorosis, scorching, plant height reduction, and in reduction in the number and size of ears. Differences among inbreds were greatest for grain yield loss(10-92%) and least for plant height reduction(1-29%). The trend is similar for the open-pollinated varieties. The major determinant of yield loss was reduction in the number of ears; 70% barrenness was observed in the susceptible hybrid. The various forms of expression of host-parasite relationship are being used to investigate genetic variation for resistance in maize, and as part of the strategy to incorporate *Striga* resistance into high yielding genetic backgrounds.

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FREQUENCY OF SEED TRANSMISSION OF ALFALFA BACTERIAL WILT AND DETECTION BY A PCR ASSAY. Deborah A. Samac[1] and Arland E. Oleson[2], [1]USDA/ARS, Dept. of Plant Pathology, Univ. of Minnesota, St. Paul, MN; [2]Biochemistry Department, North Dakota State University, Fargo, ND

Clavibacter michiganense pv. *insidiosum* (*Cmi*), the causal agent of bacterial wilt in alfalfa, is spread primarily through infected plant material although seed transmission occurs at a low frequency. Phytosanitary laws in several countries require alfalfa seed to be certified free of *Cmi*. This study was initiated to determine the frequency of *Cmi* infestation in seed from infected plants, to develop seed lots with known levels of *Cmi*, and to develop rapid tests for detecting the bacterium. Interpollinations were made between highly infected plants of three varieties. Seeds were assayed in batches for *Cmi* by grinding and dilution plating the extract onto a semi-selective medium. *Cmi* was distinguished by colony morphology, KOH reaction, and a specific PCR-based assay. *Cmi* infected seed were produced by 7% of infected plants. Overall, infection occurred in 0.12-1.6% of seed. Seed collected from field-grown plants with severe bacterial wilt symptoms initially had mean *Cmi* levels of 3×10^8 CFU/g, but populations decreased markedly when seed was stored at 24° C.

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EFFECT OF MOSAIC ON YIELD AND QUALITY COMPONENTS OF SUGARCANE. M. P. Grisham and B. L. Legendre, USDA-ARS, Sugarcane Research Unit, Houma, LA 70361.

Shoot-tip culture was used to propagate sugarcane cultivar CP 65-357, a mosaic susceptible commercial cultivar. Approximately 2400 plantlets free of sorghum mosaic virus, the causal agent of mosaic in Louisiana, were transplanted to the field where they were exposed to natural infection by the virus. Stalks from 37 infected and 37 healthy plants were harvested. Stalks from each individual plant were counted, weighted, and measured, then processed to determine juice quality and fiber content. The experiment was repeated over time. Two plant-cane, four first-ratoon, and three second-ratoon crops were analyzed. In most plantings and crops, stalk size and weight were reduced in mosaic affected plants. The most common effect of mosaic on cane quality was an increase in fiber content. Other quality components including juice Brix, sucrose, and purity were not affected by the virus. In past studies, a common cultivar milling factor was used to estimate the yield of recoverable sugar per ton of cane based on a constant fiber content. These results suggest the need to determine fiber content for each sample analyzed to better estimate mosaic effects on yield and quality of sugarcane cultivars.

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DEVELOPMENT OF A MONOCLONAL ANTIBODY SPECIFIC FOR RICE BLAST ISOLATES OF *PYRICULARIA GRISEA*. N. Ramakrishna[1], F.N. Lee[2] and R.C. Gergerich[1]. [1]University of Arkansas, Fayetteville, AR 72701, and [2]University of Arkansas Rice Research and Extension Center, Stuttgart, AR 72160.

Rice blast specific monoclonal antibody has been developed that detected only rice-infecting isolates of *Pyricularia grisea* without any reaction to grass-infecting *P. grisea* or other fungal species associated with rice. The monoclonal antibody detected all races of rice blast including IC-17, IB-33, IB-1, IG-1, IH-1, IB-49, and IB-45. An extracellular antigen concentration of 5 ng/assay for race IC-17 or 0.5 ng/assay for IB-33 was detected by indirect ELISA using the monoclonal antibody. The epitope recognized by the rice blast specific monoclonal antibody was uniformly distributed on the surface of conidia and conidiophores, but not on the germ tubes or appressoria. The monoclonal antibody demonstrated high sensitivity to infected rice blast lesion tissue by indirect ELISA. Stable clones of hybridoma cell line were preserved.

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EXAMINATION OF SIMPLE FACTORS INTERFERING WITH RESULTS IN ELISA. J. Q. Xia, W. Bliss, and C. Sutula. Research Dept., Agdia, Inc., Elkhart, IN 46514.

A sensitive and specific ELISA is mainly based on characteristics of the antibody used in the test. However, an ELISA could be severely affected or ruined by some unexpected factors, especially in detection of pathogens in plant samples. Sample sources in plants, reagents used in routine ELISA, and operator skills were examined in this study. Simple factors that had potential to interfere in ELISA included soil contaminating plant samples, sugars and proteins used in blocking reagents, secondary antibody-enzyme conjugates, plate washings, and artificial contaminations. The effects on test results by the factors depended additionally on the test systems and particular format used in the tests. Results indicat that a reliable ELISA to diagnose plant pathogens requires optimizing its test conditions, as well as using a quality antiserum.

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FITNESS COMPONENTS AND TOLERANCE TO MANCOZEB AMONG GENOTYPES OF PHYTOPHTHORA INFESTANS IN THE UNITED STATES. M. Kato and W. E. Fry. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Three components of fitness on potato, as well as tolerance to mancozeb, were compared between recently immigrated (new) genotypes and a previously existing (old) genotype of Phytophthora infestans in the United States to explore the cause of displacement of old genotypes by new genotypes. To study fitness components, we used two new genotypes (US-7 and US-8) and one old genotype (US-1). Latent period of US-7 was significantly shorter than that of US-1, but lesion area and sporangial production of US-7 were not significantly different from those of US-1. US-8 had a significantly shorter latent period, produced larger lesions and a greater number of sporangia than US-1. We used three new genotypes (US-6, US-7 and US-8) and one old genotype (US-1) to study tolerance to mancozeb, which was measured by minimum inhibitory concentration and EC_{50} on rye B agar media amended with ten concentrations of mancozeb. There were no significant differences in tolerance among genotypes. Increased fitness of the new genotypes rather than tolerance to mancozeb is probably one of the causes of the displacement of the old genotypes in the United States.

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THE PHYLOGENETIC CONSEQUENCES OF ASEXUAL REPRODUCTION IN *FUSARIUM OXYSPORUM* f. sp. *CUBENSE*. R. L. Koenig, H. C. Kistler and R. Ploetz, Department of Plant Pathology, University of Florida, Gainesville.

Single copy RFLP loci were identified in isolates of *F. oxysporum* f. sp. *cubense* representing a diverse geographical distribution, 16 vegetative compatibility groups (VCGs) and three described races. Based on RFLP analysis, a dendrogram was constructed which divided most of the isolates into two genetically distinct groups that aligned with specific VCGs. A small number of isolates representing VCGs that have a narrow geographical distribution comprised discernable, minor groups although, they were allied with one of the two major groups. Similar groupings have been suggested based on RAPD and karotype analyses. Few RFLP haplotypes were observed within each of the major groups. With the exception of isolates belonging to one VCG, no recombinant haplotypes were observed among the isolates comprising the major groups. Generally, however, isolates within a VCG and among VCGs in each major group appear to be clonally derived.

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RECOMBINATION AND GENE FLOW IN FIELD POPULATIONS OF *PHAEOSPHAERIA NODORUM*. S.M. Keller, *J.M. McDermott, *M.S. Wolfe, and B. A. McDonald. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas 77843-2132. *Phytopathology Group, Federal Institute of Technology, CH-8092 Zurich, Switzerland.

Eight nuclear RFLP loci, DNA fingerprints and mitochondrial DNA haplotypes were used to compare the genetic structures of populations of *Phaeosphaeria nodorum* from single wheat fields in Oregon (N=50) and Texas (N=68) and nine fields in Switzerland (N=450). Hierarchical sampling was used for each population. Each population had a low degree of clonality and high levels of gene and genotype diversity distributed on a fine scale. Pairwise comparisons for association among RFLP loci showed that they were in gametic equilibrium, suggesting random-mating within each population. No DNA fingerprints were shared between populations, but all populations shared common alleles at each RFLP locus, often at remarkably similar frequencies. The high degree of similarity among populations suggested that gene flow has operated across long distances.

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CHARACTERIZATION OF A REPETITIVE DNA SEQUENCE FOUND ONLY IN SOME POPULATIONS OF THE BEAN RUST FUNGUS (*UROMYCES APPENDICULATUS*). J. P. Martinez, N. D. Young, and J. V. Groth, Univ. of Minnesota, Dept. of Plant Pathology, St. Paul, MN 55108

The repetitive sequence, UAR-1, has been found in a U.S. population of the bean rust fungus composed mostly of asexual (non-telium producing) isolates. However, UAR-1 is absent from a U.S. population composed primarily of sexually reproducing (telium producing) isolates. We are investigating the genomic organization of UAR-1 and whether UAR-1 represents a transposable element. The genome of *Uromyces appendiculatus* contains at least 40 copies of UAR-1. In DNA-blot analysis of *Eco*RI digested genomic DNA, we observed two classes of UAR-1 fragments. One class consisted of 15-20 single copy fragments ranging in size from approximately 0.2 to 15 Kbp. This pattern suggests that these copies of UAR-1 are dispersed in the genome. The second class contained more than 20 copies of a 600 bp, *Eco*RI fragment. This 600 bp fragment was cloned and its DNA sequence determined. In a search of the EMBL database, no close matches with the DNA sequence of the cloned fragment were found.

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POPULATION ANALYSIS OF *MAGNAPORTHE GRISEA* AT A HIGH DIVERSITY SITE IN THE HIMALAYAS. J.Kumar, R.J.Nelson and R.S.Zeigler. Division of Entomology and Plant Pathology, International Rice Research Institute, 1099 Manila, Philippines.

Traditional rices and millets have been co-cultivated for thousands of years in the central Himalayas, India. We analyzed DNA fingerprints from the rice blast pathogen (*M. grisea*) population from one site over two years. *Eco*R1 digests of total genomic DNA of each isolate were probed using the *M. grisea* repetitive sequence MGR586 and a set of mapped single-copy DNA fragments. Among 26 isolates analyzed for one year, 11 distinct DNA fingerprint groups were identified (each group sharing less than 80% MGR586 band similarity) which were similar to only 2 of the 12 groups identified out a set of 27 isolates from the second year. A high allelic diversity was observed using single copy probes: for the 6 probes analyzed for 49 isolates, an average of 5 band positions were detected . Association between locus pairs was tested using only those loci with intermediate (40-60%) allelic frequencies in all the non-clonal isolates . A high degree of gametic phase equilibrium in the field population was observed . The presence of sexual fertility in the population (reported elsewhere) suggests that the observed MGR fingerprint diversity and gametic phase equilibrium may be the result of sexual recombination in the field.

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EVOLUTION OF DEFENSE MULTIGENE FAMILIES AND ITS CONSEQUENCES FOR PLANT/PATHOGEN COMPATIBILITY. Brian Fristensky, Dept. of Plant Science, Univ. of Manitoba, Winnipeg, MB Canada R3T 2N2 (frist@cc.umanitoba.ca)

Phylogenetic analysis of numerous multigene families for defense proteins (eg. PR10, PR1, β1,3-glucanase) shows that family members within a species are usually more closely-related to each other than to homologues from other species. The tendency of multigene families to undergo evolutionary bottlenecks implies that specializations for different family members must be reassigned frequently as well. That is, differential regulation in response to stimuli such as fungal attack would have to be established *de-novo* in each species. Reassignment of differential regulatory patterns is therefore a potentially important factor in the evolution of plant/pathogen compatibility.

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COMPARISON OF ISOLATES OF *UROMYCES APPENDICULATUS* WITH AND WITHOUT SPECIFIC VIRULENCE TO BEAN LANDRACES OF ANDEAN ORIGIN. C.M. Sandlin, C.M. Araya, J.R. Steadman, and D.P. Coyne, Departments of Plant Pathology and Horticulture, University of Nebraska, Lincoln 68583.

Isolates of the bean-rust fungus exhibited specific virulence on Andean bean landraces. This Andean-specific virulence was demonstrated by rust inoculation onto a set of 30 differential genotypes of *Phaseolus vulgaris*, consisting of five landraces representing six botanical races described for domesticated common beans. Andean-specific isolates were virulent on Andean landraces as well, but not on landraces of Mesoamerican origin. Isolates with virulence to Mesoamerican landraces were virulent on Andean landraces; no Mesoamerican-specific rust isolates were found. RAPD analysis was performed on five geographically distinct pairs of isolates from the Americas. Each of the isolate pairs was from the same country with one isolate Andean-specific, and the other non-specific. The RAPD patterns indicated that the genetic backgrounds of the Andean-specific rust isolates were distinct from those of the non-specific isolates.

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MOTHERHOOD AND THE PRICE OF SEX. John F. Leslie and Keith K. Klein, Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506

Natural populations of heterothallic biological species (= mating populations) in the *Fusarium moniliform* species complex (sexual stage *Gibberella fujikuroi*) consist of males and hermaphrodites. The ratio of males to hermaphrodites varies with the biological species. In the "A" mating population the male:hermaphrodite ratio is 50:50, in the "D" population it is 70:30, and in the "F" population it is 90:10. If P(h) is the proportion of hermaphrodites in the population, then an equilibrium can be found for any P(h) that is dependent on the relative fitness of females and males (Θ), where Θ=(1-P(h)/(1+P(h)). Hermaphrodites always make a larger contribution to the next generation because they contribute all of the female gametes and some of the male gametes. At equilibrium, hermaphroditism costs 1-Θ, otherwise the equilibrium quickly shifts to a population that is all hermaphrodites. In the "A" mating population Θ = 0.33, in the "D" mating population Θ = 0.54, and in the "F" mating population Θ = 0.81. This model also suggests that mating type should be independent of genes for female sterility, and the available data from these species are consistent with this conclusion. The effective population number appears to be much more limited by the availability of hermaphrodites than it is by imbalances in mating type frequencies.

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GENETIC DIVERSITY AND EVOLUTION OF *PHAEOISARIOPSIS GRISEOLA* WITH THE COMMON BEAN IN LATIN AMERICA. M. A.Pastor-Corrales, C. Jara, M. M. Otoya, and M. M. Maya. Plant Pathology section, Bean Program, CIAT, Apartado Aereo 6713, Cali, Colombia.

The domestication of the common bean, *Phaseolus vulgaris*, in two primary centers resulted in two major groups of germplasm: Middle American and Andean South American. The beans from one center differ from those of the other in morphological, biochemical, molecular, and other attributes. Analysis of the genetic diversity of *Phaeoisariopsis griseola* (PG), using virulence and the random amplified polymorphic DNA technique (RAPD), show that Latin American isolates of the angular leaf spot pathogen also fall into two major groups, analogous to the Andean and Middle American bean groups. For the virulence analysis, 55 PG isolates were inoculated on a set of differential cultivars, six Andean and six Middle American. One group of isolates, obtained from large-seeded beans of Andean origin, attacked only the Andean differentials. The other group had a much broader differential range: although isolated from small or medium-seeded beans of Middle American origin, it also attacked some large-seeded differentials. The RAPD technique, which tested DNA from 42 isolates, corroborated the results of the virulence analysis. These findings suggest that PG in Latin America evolved with the common bean: the Andean pathotypes with the large-seeded Andean beans, and the Middle American pathotypes with the small or medium-seeded beans from Middle America.

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CHANGES IN SPECIFIC PATHOGENICITY IN *PHYTOPHTHORA INFESTANS* POPULATIONS IN POLAND. L. S. Sujkowski, S.B. Goodwin, W.E. Fry. First author: Department of Plant Pathology, NCSU, Raleigh, NC 27695-7616; remaining authors: Department of Plant Pathology, Cornell University, Ithaca NY 14953.

Ninety-five isolates of *Phytophthora infestans* from Poland were tested for specific pathogenicity to potato genotypes with major resistance (R) genes using detached leaf assay. Associations between pathotypes and multilocus genotypes (determined from mating type, two allozyme loci and twenty-six RFLP loci) were investigated. Twenty-two isolates collected in 1985-1988 belonged to a single clonal lineage (PO-1) that has probably been present in Europe during the most of the twentieth century. From isolates collected in 1988-1991, thirty belonged to a new clonal lineage (PO-4), and the remaining forty-three represented thirty-eight distinct, new multilocus genotypes. PO-1 had on average slightly less specific pathogenicity factors (5.5) than did the PO-4 (6.6) and the distinct multilocus genotypes (6.7). The old clonal lineage was pathogenically more diverse than the new migrating genotypes. Shannon's diversity index was 0.73 for PO-1 and 0.47 for PO-4.

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NEW VIRULENCE PHENOTYPES OF *PHYTOPHTHORA SOJAE* THROUGH OUTCROSSING PHYSIOLOGIC RACES. R. G. Bhat and A. F. Schmitthenner, Dept. of Plant Pathology, OARDC, The Ohio State University, Wooster, OH 44691.

Physiologic races 7 (FPA^r)and 25 (MEX^r) of *Phytophthora sojae*, virulent on soybean cultivars with *Rps* genes at loci 1a,3a,6,7 and 1a,1b,1c,1k,7, respectively, were crossed using a mixed inoculum technique. Single hybrid-oospore isolates were either selected on a double-drug inhibitor germination medium or screened after colony formation on a germination medium without double drugs. All single-oospore isolates were evaluated for their virulence phenotypes on soybean seedlings by hypocotyl inoculation. Soybean differentials included Williams near-isolines for *rps*, *Rps*1-a, *Rps*1-b, *Rps*1-c, *Rps*1-k, or *Rps*3-a genes, Harosoy near-isolines for *Rps*7 or *Rps*6+7, and P.I. 103.091 for *Rps*1-d. Hybrid oospore isolates exhibited virulence phenotypes of races 1, 3, 4, 5, 20, 21, 22, 25, 30, plus 12 new virulence combinations. Frequencies of race 4, and a new race with complementary virulence of both parents were most common. Virulence of hybrids and preliminary data on F_2 progenies from a few hybrids suggest that there is epistatic interaction among virulence genes.

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COMPARISON OF VIRULENCE OF *TILLETIA INDICA* FROM INDIA, PAKISTAN, AND MEXICO. M. R. Bonde[1], G. L. Peterson[1], G. Fuentes-Davila[2], and J. G. Phillips[3]. [1]USDA/ARS, Foreign Disease-Weed Science Res., Frederick, MD 21702; [2]CIMMYT, Mexico, and [3]USDA/ARS, ERRC, Philadelphia, PA 19118.

Virulence of four *T. indica* field teliospore populations (two from Mexico, one from India, one from Pakistan) were compared on six resistant wheat lines and two susceptible cultivars. Plants (3 reps.) at the boot stage were inoculated by injecting into the boot a water suspension containing 1.0×10^4 sporidia/ml. Plants were incubated 3 days in a mist chamber, then maintained until maturity in a greenhouse. Wheat heads were harvested individually and percentages of seeds infected determined by the presence of sori. Seeds from 10 randomly selected infected heads per treatment were further examined to determine the correlation between percentage of seeds infected and extent of fungal colonization within infected seeds. On the most resistant wheat line (HD-29), percentage of seeds infected varied from 10 to 30%, depending on pathogen aggressiveness. On the most susceptible genotype, infection varied from 55 to 84%. Although there were differences in pathogen aggressiveness, there was no evidence of races. Wheat lines resistant to the Mexican fungal populations were also resistant to those from Asia and vice versa; there was a direct correlation between percentage of seeds infected and extent of fungal colonization with all but one pathogen population.

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POPULATION DYNAMICS OF RACE 1 AND RACE 2 OF COLLETOTRICHUM ORBICULARE ON CUCURBITS. **L. A. Wasilwa, J. C. Correll, and, [1]T. E. Morelock. Depts. of Plant Pathology and [1]Horticulture, Univ. of Arkansas, Fayetteville.**

Race 1 and 2 of C. orbiculare can be distinguished by virulence and vegetative compatibility. Field isolates recovered from cucumber are race 1 whereas as those from watermelon are race 2. However, cross-infection readily occurs under greenhouse inoculation conditions. To examine how host resistance influences the population dynamics of C. orbiculare, race 1 and 2 were co-inoculated onto four differential cultivars under both greenhouse and field conditions. The cucumber cv. Marketer and the watermelon cv. Black Diamond were highly susceptible to race 1 and 2, whereas the cucumber cv. H19 was highly resistant to race 2 and the watermelon cv. Charleston Gray was highly resistant to race 1. To facilitate the monitoring of the frequency of each race, a race 1 wildtype and race 2 nit mutant (and the reciprocal combination) were used. In greenhouse tests, the ratio of race 1 to race 2 isolates recovered from Marketer and Black Diamond were 2:1 and 1:1, respectively. However, the ratio for H19 and Charleston Gray were 4:1 and 1:5, respectively. Similar ratios were observed from field inoculations.

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NICOTIANA TRICHOME EXUDATES AS BIORATIONALS IN PROTECTING CUCUMBERS AGAINST *COLLETOTRICHUM LAGENARIUM* (PASS.) ELL. & HALST DISEASE DEVELOPMENT. B.S. Kennedy, M.T. Nielsen and R.F. Severson, University of Kentucky, Lexington; Deceased, formerly ARS-USDA, Athens, Georgia.

Trichome exudate compounds isolated from leaves of *N. tabacum, N. glutinosa* (accessions 24 and 24a), and 21 other *Nicotiana* species were evaluated for biorational activity against *C. lagenarium* the anthracnose pathogen of cucumber. In dose response experiments test compounds were applied to a water agar surface that was then inoculated with a conidial suspension. Low levels of most test compounds reduced or completely inhibited germination of *C. lagenarium* conidia. Higher levels of the compounds were required to reduce lesion size and number on inoculated cucumber leaves. Over 90% of the inoculated leaves were protected from lesion development when exposed to the highest treatment concentration. Sugar ester mixtures from nine of the species completely inhibited *C. lagenarium* conidium from germinating. These results suggest the potential of *Nicotiana* exudates as biorationals in reducing disease development.

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VIRULENCE OF *RHIZOCTONIA ORYZAE* ISOLATES ON WHEAT AND DETECTION OF THE PATHOGEN IN PLANT TISSUE USING A PCR PROTOCOL. Mark Mazzola, Oi Tak Wong and R. James Cook. USDA-ARS, Washington State University Pullman, WA 99164.

Rhizoctonia solani and *R. oryzae* cause rhizoctonia root rot of wheat in the Pacific Northwest. In a previous study, the isolates of *R. oryzae* examined caused moderate disease at 20 C but mild or no disease at temperatures experienced during wheat stand establishment (10-15 C) (Ogoshi *et al.* Phytopathology 80:784-788). In this study, isolates of *R. oryzae* exhibited a range of virulence on wheat at 12 C. Some isolates induced no disease symptoms on wheat, while other strains reduced emergence up to 90%, delayed root development, caused root rot, and greatly limited wheat biomass production. Primers specific for *R. oryzae* were developed based on sequence analysis of the ITS regions. The primers RO1 and RO2 amplified a 514-bp fragment from purified DNA of *R. oryzae* but not from any of eight anastomosis groups of *R. solani*, binucleate *Rhizoctonia* spp., *Fusarium solani*, *F. sambucinum* or *Pythium graminicola*. *R. oryzae* was detected in plant tissue by boiling infected wheat seeds or root tissue in water and using this plant extract for PCR-amplification.

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SOYBEAN GENOTYPE AFFECTS PRODUCTION OF SCLEROTIA BY *DACTULIOCHAETA GLYCINES*. A.M.C. Schilder, D.A. Florini, and K.E. Dashiell. International Institute of Tropical Agriculture, Oyo Road, Ibadan, Nigeria.

Red leaf blotch, caused by *Dactuliochaeta glycines* was first reported to occur in Nigeria in 1991. To determine whether soybean genotype has an effect on sclerotial inoculum density in the soil after harvest, five soybean genotypes differing in resistance were planted in an infested field. Red leaf blotch severity was assessed as percentage necrotic leaf area three times during the growing season. After harvest, samples were taken from the top 1 cm of soil in each plot. The number of sclerotia was determined by visual counting using a dissecting microscope after elutriation and sieving of a subsample of 300 g soil per plot. Soybean genotype had a significant effect on disease severity ($P<0.05$) and on the number of sclerotia ($P<0.1$) in the soil after harvest. The number of sclerotia averaged 862, 496, 394, 249, and 210 per 300 g dry soil for soybean genotypes TGX 1448-2E, TGX1440-1E, TGX 923-2E, and TGX 1485-1D and TGX 1019-2EN, respectively. The number of sclerotia was not significantly correlated with disease severity. However, early maturing cultivars had the lowest sclerotium production regardless of disease severity. Soybean genotype did not significantly affect sclerotium size. Viability of sclerotia on MEA was over 90%.

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TEMPERATURE INFLUENCE ON PERITHECIAL AND PYCNIDIAL DEVELOPMENT BY *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA*. K.M. Tubajika and J.S. Russin, Dept. Plant Pathol. & Crop Physiol., Louisiana State University Agricultural Center, Baton Rouge 70803

Stem canker, caused by *Diaporthe phaseolorum* var *caulivora* (Dpc), is prevalent in Louisiana soybean fields. Temperature influence on perithecial and pycnidial production and sporulation was determined by inoculating soybean stems of susceptible cultivar 'Northrup King/RA 452' with the pathogen and incubating at 14, 18, 22, 26, and 30 C. The number of perithecia and pycnidia produced and the number sporulating at each temperature were assessed periodically on stems placed on acidified potato dextrose agar. Pycnidia and perithecia were produced from 18 to 26 C. Pycnidia developed 7 to 10 days earlier than perithecia across all temperatures except 14 or 30 C. Maximum perithecial and pycnidial formation occurred on stems incubated at 22 C following 33-40 days incubation. These results indicate that 22 C may be near optimum temperature for perithecial and pycnidial production and sporulation by Dpc on soybean.

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DIFFERENCES IN RATES OF APOTHECIUM DEVELOPMENT BETWEEN POPULATIONS OF *MONILINIA VACCINII-CORYMBOSI*. J.S. Lehman and P.V. Oudemans. Rutgers University, Blueberry and Cranberry Research Center, Chatsworth, NJ 08019.

Incidence of mummy-berry disease depends on coordinated phenological development between blueberry cultivars (*Vaccinium corymbosum*) and populations of *M. vaccinii-corymbosi* (Mvc). To test whether Mvc populations adapt to the phenologies of their host, apothecium development of two fungal populations collected from early and mid-season blueberry cultivars, populations 9420-WT and 9421-WT, respectively, was measured. For population 9420-WT, mean development times for production of above-ground stipes, appearance of an apical dimple on stipes, and production of mature apothecia were 11, 16, and 25 days after exposure to 15-18 C, respectively, and were 6-11 days shorter (30-41%) than those for population 9421-WT. Apical tips of stipes of population 9420-WT expanded at a rate of 0.47 mm/day, 57% faster than stipes of population 9421-WT. Results suggest that Mvc populations can adapt to the phenologies of blueberry cultivars.

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PHYTOPHTHORA ROOT ROT OF CRANBERRIES: IDENTIFICATION OF INOCULUM SOURCES BY LUPIN BAITING. Peter V. Oudemans, Rutgers University, Blueberry and Cranberry Research Center, Lake Oswego Road, Chatsworth, NJ 08019.

Cranberries are cultivated as long-lived perennials. Individual beds may exceed 50 years in age between plantings. Phytophthora root rot can be a major factor in reducing the production life of these beds. The objectives of this study were to identify sources of inoculum and to monitor inoculum levels throughout the year. Use of 5-day-old lupin seedlings as baits in styrofoam boats placed *in-situ* proved to be an effective method for trapping several *Phytophthora* species. Using this approach, three species of *Phytophthora* were regularly trapped from drainage canals surrounding cranberry beds as well as from reservoirs used for irrigation and the streams which feed them. Of the three species isolated, *P. cinnamomi* was the most common and widespread. Weekly samples taken from drainage canals revealed periodic fluctuations in infection rates of the trap plants suggesting this method may be useful in monitoring inoculum levels.

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SYNERGISTIC INTERACTIONS BETWEEN *PRATYLENCHUS PENETRANS* AND VCG 4A ISOLATES OF *VERTICILLIUM DAHLIAE* FROM OHIO AND IDAHO. R. C. Rowe and W. R. Berry, Plant Pathology, Ohio State University, OARDC, Wooster, OH, 44691.

Previous field microplot studies using three Ohio potato-stem isolates each of VCG 4A and 4B of *Verticillium dahliae* (Vd) showed that 4A isolates interacted synergistically with the root-lesion nematode *Pratylenchus penetrans* (Pp) while 4B isolates did not. To confirm this using a more diverse collection of Vd, ten isolates from Ohio (five 4A and five 4B) and nine from Idaho (five 4A and four 4B) were tested, both alone and with Pp. Fumigated soil was infested at 40 Vd microsclerotia/cm^3 soil and 25 Pp vermiforms/100 cm^3 soil, placed into clay-tile microplots and planted with Verticillium-free potato seed pieces (cv. Superior) on 27 May 1994. By late July, foliar chlorosis and necrosis were clearly visible in plants growing in soil infested with both Pp and VCG 4A isolates from both Ohio and Idaho, but not with 4B isolates from either location. Similar symptoms became evident wks later in all plants growing in Vd-infested soil, but in those with 4A plus Pp symptoms were clearly earlier and more severe. Tubers were harvested after plants died naturally. Treatments with VCG 4A isolates alone yielded 67% (Ohio) and 85% (Idaho) of uninfested controls, and those with 4A and Pp, yielded 40 and 49%, respectively. With 4B isolates alone, yields were 82 and 90% of controls, and with 4B and Pp were 69 and 69%.

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VARIATION IN VIRULENCE AMONG CALIFORNIA ISOLATES OF *VERTICILLIUM DAHLIAE* FROM TOMATO AND COTTON. A. C. Sundwall and T. R. Gordon, Department of ESPM, University of California at Berkeley, Berkeley, CA 94720.

A total of 103 isolates of *Verticillium dahliae* were isolated from symptomatic and asymptomatic crop plants in the San Joaquin Valley: 62 from tomato, 35 from cotton, and 6 from other hosts. Single spore cultures were grown on PDA and spore suspensions at a concentration of $8x10^7$ conidia per ml were used to root dip inoculate tomato and cotton seedlings. Inoculated seedlings were then planted in pasteurized potting mix and grown under controlled conditions. Based on symptom development on the differential tomato cultivars Chello, Early Pak 7, Peelmech and LaRossa, isolates exhibited a wide range of virulence, but no clear race differences. Variation in virulence was also apparent on 16 additional commercial cultivars. There was no consistent differential interaction between isolates and hosts. Isolates taken from cotton showed little or no virulence on tomato, but were moderately to highly virulent on cotton.

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RELATIONSHIP OF *APHANOMYCES COCHLIOIDES* POPULATIONS TO SUGAR BEET ROOT ROT SEVERITY IN FIELDS. J.W. Beale[1], C.E. Windels[2], and L.L. Kinkel[1]. [1]Dept. of Plant Pathology, Univ. Minn., St. Paul 55108 and [2]N.W. Exp. Stat., Univ. Minn., Crookston 56716.

To examine the variability of sugar beet root rot severity associated with *Aphanomyces cochlioides* in the field and to assess predictions for field disease based on a greenhouse assay, two 60m^2 plots were marked in each of two fields with a history of disease. In June/July 1994, 99 soil samples were collected within each plot in a randomized block design. Sugar beet seedlings were planted (125 seeds/5 pots/sample) to bait *A. cochlioides* and to determine a root rot index (0-100 scale). In September, field-grown sugar beet roots were rated for rot (0-7 scale) at each sample site. Field symptom severity ratings indicated that the disease was aggregated, and root rot ranged from mild ($\bar{x}$=1.2) to severe ($\bar{x}$=4.8). Potential disease severity predictions from greenhouse assays were positively correlated (P=0.05) with field ratings for all plots; the coefficients ranged from 0.20 to 0.73.

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THE POPULATION OF *FUSARIUM SUBGLUTINANS* F. SP. *PINI*, FOLLOWING RAPID EXPANSION OF PITCH CANKER IN CALIFORNIA. T.R. Gordon, A.J. Storer AND D. Okamoto. DEPT. OF ESPM, 108 HILGARD HALL, UNIVERSITY OF CALIFORNIA, BERKELEY, CA 94720

Pitch canker, caused by *Fusarium subglutinans* f. sp. *pini*, was first identified in California in 1986, where it has caused extensive damage to planted Monterey pines in Santa Cruz and Alameda Counties. Since that time, numerous additional pine species and Douglas fir have become infected under field conditions. The area affected by pitch canker has expanded greatly and now includes all three mainland populations of native Monterey pine. One vegetative compatibility group (VCG) of *F. s. pini*, C1, dominates the Northern California population and is responsible for the expansion of existing disease centers. VCG C3, previously recognized only in Southern California, primarily as a pathogen of nursery trees, is apparently responsible for initiating many new infestations. This may indicate that movement of infected trees has contributed to the long distance spread of pitch canker. The limited diversity of *F. s. pini* in California is consistent with a recent introduction of the pathogen and the absence of sexual reproduction.

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USE OF WASTE CORN AS A NATURAL INOCULUM FOR SCREENING FOR RESISTANCE TO ASPERGILLUS FLAVUS. O.M. OLANYA, D.C. McGEE AND L.H. TIFFANY. Dept. of Plant Pathology, Seed Science Center and Dept. of Botany, Iowa State University, Ames, IA 50011.

Susceptibility of five corn hybrids to natural inoculum of A. flavus was studied at Ames, IA in 1993 and 1994. Waste corn inoculum levels of 0, 1, 5 and 10kg contaminated with A. flavus was applied on the soil surface of plots before silking. Density of A. flavus in the air, incidence on corn husks and silks was determined at four sampling times and found to be correlated with amounts of waste corn applied. Mean kernel infection and number of sporulating kernels at harvest were significantly higher (P=0.05) on Mo17xB73 and LH93xB73 than on LB31xB73, 75R001xB73 and TX6xB73. The same reactions to susceptible and resistant hybrids were obtained in a corresponding artificial inoculum experiment.

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PCR-AMPLIFIED RIBOSOMAL DNA RESTRICTION POLYMORPHISM OF *Puccinia carduorum*. Y. T. Berthier, W. L. Bruckart, P. Chaboudez, and D. G. Luster. USDA/ARS, Foreign Disease-Weed Science Research, Bldg. 1301, Ft. Detrick, Frederick, MD 21702.

To find genetic markers for an isolate of *Puccinia carduorum* being evaluated for biological control of *Carduus thoermeri* (musk thistle), we evaluated polymorphism of internal transcribed spacers (ITS) of ribosomal DNA. A 0.65 Kb region, including the 5.8S gene and the two flanking ITS regions, was amplified by polymerase chain reaction and cut with *Alu* I, *Dra* I, *Eco* RI, *Mse* I, and *Taq* I. The 12 isolates of *P. carduorum* from four hosts showed two different patterns that related to host plant. Restriction patterns of isolates from *P. carduorum* from *C. acanthoides* and *C. thoermeri* were distinct from those of *P. carduorum* from *C. tenuiflorus* and *C. pycnocephalus*. Computerized similarity analysis indicated that patterns within the *C. tenuiflorus-C. pycnocephalus* host group were more homogeneous than patterns within the other group. We concluded that isolates of *P. carduorum* from different hosts can be differentiated whereas those from the same host cannot.

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A REVERSE DOT-BLOT SYSTEM FOR RAPID IDENTIFICATION OF SOME *PYTHIUM* AND *PHYTOPHTHORA* SPECIES. C.A. Lévesque, Agriculture & Agri-Food Canada, Pacific Agriculture Research Centre, 6660 NW Marine Dr, Vancouver, BC, Canada, V6T 1X2.

The main objective of this research was to develop a technology that can identify unknown isolates of *Pythium* or *Phytophthora* species in a single hybridization test. The reverse dot-blot system which was developed was based on species-specific oligonucleotides from different *Pythium* and *Phytophthora* species individually blotted as dots on the surface of a nylon membrane. Oligonucleotides specific to the genus *Phytophthora* and to the Oomycete fungi were also developed. Using DNA from an unknown isolate as template, the region comprising the internal transcribed spacers I and II, the 5.8S gene, and the first 600 bases of the 28S gene of the nuclear ribosomal DNA was amplified and labeled simultaneously by the polymerase chain reaction using universal primers and digoxigenin-dUTP, respectively. A small aliquot of the labeled and amplified product of the unknown isolate was used for hybridization to the reverse dot blot membrane that contained the immobilized species-specific oligonucleotides. Identification was completed by simply identifying the specific oligonucleotide that hybridized with the labeled DNA from the unknown isolate.

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MPG1 HYDROPHOBIN AND SURFACE RECOGNITION. J. L. Beckerman and D. J. Ebbole. Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Upon germination on a hydrophobic substrate, conidia of the rice blast fungus (*Magnaporthe grisea*) form infection structures called appressoria that allow direct penetration of plant cells. We are examining expression of a fungal hydrophobin of *M. grisea* named MPG1 (Magnaporthe Pathogenicity Gene 1). We believe this protein plays a role in recognition of surface hydrophobicity. MPG1 was so named because mutant strains are no longer able to efficiently form appressoria on rice plants and are therefore less pathogenic than wild-type. Upon germination of conidia of MPG1 deletion mutants, the level of appressoria development varies with the degree of hydrophobicity of the substrate, with appressoria formation failing to occur on the more hydrophobic substrates. Conidia germinated under conditions repressive to MPG1 expression resulted in repression of appressoria formation. Taken together, this evidence supports the hypothesis that MPG1 is involved in surface recognition.

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GENETIC ANALYSIS OF THE FUNCTION OF THE FUNGAL HYDROPHOBIN CRYPARIN. D. H. Kim, P. Kazmierczak, L. Zhang, C. Bachmann, and N. K. Van Alfen. Genetic analysis of the function of the fungal hydrophobin cryparin. Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Cryparin is a small cell-surface protein that has characteristics typical of the fungal hydrophobins. This molecule produced by *Cryphonectria parasitica* has significant sequence similarity to the putative phytotoxin cerato-ulmin produced by *Ophiostoma ulmi*. Cryparin is found in aerial hyphae and fruiting bodies of the fungus and is one of the most abundant proteins produced by this fungus in culture. To investigate the function of cryparin, a deletion mutation of the gene was made and the resulting mutant strain analyzed for phenotypic changes. The cryparin null mutation had no visible or quantitative effect on asexual sporulation, pigmentation, extracellular laccase activity, or the ability of the fungus mate. The primary observable phenotype of the mutation was wetability of aerial hyphae, a phenotype which is common to other hydrophobin null mutations.

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RAPD MARKERS AND GENETICS OF RESISTANCE TO *Macrophomina phaseolina* IN BEANS. G. Olaya[1], G. S. Abawi[1], and N. F. Weeden[2]. [1]Dept. of Plant Pathology and [2]Dept. of Horticultural Sciences. Cornell University, Geneva, NY 14456.

The reaction of selected bean (*Phaseolus vulgaris*) accessions to *Macrophomina phaseolina* (Mp) has previously been identified but no information is available regarding the genetic basis of resistance in beans to Mp. In this study, the inheritance of resistance to Mp was characterized using traditional approaches and molecular markers. Inheritance studies were based on a cross between the resistant accession BAT-477 and the susceptible accession A-70. F2 and F3 generations were tested for resistance to Mp by covering the seeds with a layer of soil (150 ml/10-cm pot) infested with sclerotia of Mp (2 g/kg soil) and incubated in growth chambers at 25 C. The ratio of resistant and susceptible plants in the F2 population was close to 9:7. Using bulked segregant analysis, two RAPD markers linked to Mp resistance were identified. Each of the RAPD markers segregated in a 3:1 ratio as expected and the segregation of both markers followed a 9:3:3:1 ratio expected for two unlinked loci. These data are consistant with the hypotheses that in common bean resistance to Mp is controlled by two dominant complementary genes.

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THE CONTRIBUTION OF MEIOSIS AND MITOSIS IN GENERATING KARYOTYPE POLYMORPHISMS IN *PYTHIUM*. Frank N. Martin, Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Significant variation is observed in electrophoretic karyotypes (EK) in the genus Pythium. While isolates of a single species tend to have a similar distribution of chromosome sizes, differences in the number of chromosomal bands and their individual sizes are observed. One mechanism that may contribute to intraspecific polymorphisms of EK is meiotic instability. Depending on the isolate investigated, different types of polymorphisms were generated. For several homothallic species investigated, either no or low levels of polymorphisms were generated by meiosis. In contrast to the results with the homothallic species, significant levels of meiotic instability were detected in the heterothallic species, P. sylvaticum; in total, 80% of the progeny chromosomes were nonparental in size or location of specific coding regions. Mitotic instability did not contribute to polymorphisms of EK in the homothallic species P. oligandrum, as all single zoospore isolates were identical to the parent.

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DNA FINGERPRINTING OF RHYNCHOSPORIUM SECALIS PATHOTYPES. Marcy Johnson, Greg Penner, Andy Tekauz. Winnipeg Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, Manitoba R3T 2M9.

Leaf scald of barley, caused by *Rhynchosporium secalis*, can result in average yield losses of up to 10% in infected fields. A high degree of variability in virulence of the pathogen, as determined by host differential testing, has been observed throughout the world. The virulence variation along with the lack of a clearly defined differential set of cultivars combine to make studies of race distribution and dynamics difficult. Isozyme and rDNA polymorphisms were not useful in distinguishing among or between different pathotypes. Therefore we have used the RAPD technique of PCR amplification to assay a larger proportion of the *R. secalis* genome. Results from RAPD analysis confirmed the expected high degree of molecular variability. The DNA of eight pathotypes was analyzed with 80 10-mer primers resulting in 50% of the amplified bands being polymorphic. The 33 primers with the most distinct amplification were tested again across the original eight as well as four additional pathotypes giving 33% polymorphism. Cluster analysis was performed to compare variation in DNA sequence, as identified by RAPD polymorphisms, to variation in pathogenicity.

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REGULATION OF FUNGAL DEVELOPMENT AND GENE EXPRESSION IN RESPONSE TO PLANT FLAVONOIDS AND ISOFLAVONOID PHYTOALEXINS. David C. Straney and Yijun Ruan. University of Maryland, College Park, MD 20742.

Many soilborne phytopathogenic fungi remain as dormant propagules in soil until the appearance of a potential host stimulates germination. The nature of the plant stimulus is unknown in most cases. We have found that flavonoids, including isoflavonoid phytoalexins, induce spore germination in *Fusarium solani* f.sp. *pisi* (isolates of *N. haematococca* MPVI). Flavonoids are exuded by legume roots and act as important signals for initiation of symbiotic interaction with rhizobial bacteria. The action of specific flavonoids in stimulating germination of this pea pathogen paralleled the specificity of *nod* gene induction in the rhizobia specializing on pea. Flavonoid-responsive germination appears to utilize a signal pathway different than nutrient-responsive germination because the former is prevented by inhibitors of cAMP-dependent protein kinase (PKA), while the latter is not. The signal pathway also appears to be separate than that of isoflavonoid phytoalexin induction of pisatin detoxification activity (*PDA*1 gene) in this fungus. Germination in root exudates was significantly inhibited by the PKA inhibitor, indicating that flavonoids may be as or more important than nutrients as a stimulatory signal in root exudates. We are using genetic and biochemical analysis to study the perception and intracellular signal transduction in this flavonoid response.

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BIOCONTROL OF *PHYTOPHTHORA* WITH BINUCLEATE *RHIZOCTONIA* FUNGI. D. Kelly Cartwright and H. W. Spurr, Jr. Department of Plant Pathology, North Carolina State University, Raleigh.

An inoculation procedure for infecting tobacco seedlings grown in styrofoam float trays in the greenhouse with the black shank pathogen, *Phytophthora parasitica* var. *nicotianae*, was developed. The system proved effective for separating resistant cultivars and evaluating potential biocontrol agents for black shank. Nineteen isolates of binucleate *Rhizoctonia* fungi (BNR) avirulent to tobacco were initially tested for biocontrol of black shank. Three isolates were selected and added to a soilless mix contained in styrofoam trays as BNR-colonized whole rice grains, BNR-colonized pulverized rice particles, or BNR-colonized tobacco seed. The BNR soil treatments resulted in 40-70% control of black shank. More root colonization occurred when BNR isolates were added to the soilless mix on rice substrate than when applied on seed. Disease control was often proportional to colonization by BNR fungi. This float system proved efficient and results were reproducible for selecting these biocontrol agents for black shank.

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ENDOPHYTIC RHIZOBACTERIA AS ANTAGONISTS OF *MELOIDOGYNE INCOGNITA* ON CUCUMBER. J. Hallmann[1,2], J. Kloepper[1], R. Rodríguez-Kábana[1], R.A. Sikora[2], [1]Department of Plant Pathology, Auburn University, AL 36849-5409 USA, [2]Bonn University, Nussallee 9, 53115 Bonn, Germany.

The hypothesis that rhizobacteria can affect plant-parasitic nematodes was tested with endophytic bacteria in greenhouse experiments with *Meloidogyne incognita* on cucumber (*Cucumis sativus*). A total of 72 endophytic bacterial strains were used. The selected bacteria originated from surface disinfested cucumber and cotton (*Gossypium hirsutum*) plants and have previously shown effects against *Rhizoctonia* and *Fusarium*. Cucumber seeds were coated with the bacterial isolates at rates of 10^9 to 10^{10} cfu/seed and planted into sand. After 10 days 1000 second-stage juveniles of *M. incognita* were inoculated per plant. Nematode control was determined 6 weeks after planting by measuring plant growth, gall-index and number of galls and egg masses. Seven bacterial isolates (*Aerococcus viridans, Bacillus megaterium, B. subtilis, Pseudomonas chlororaphis, P. vesicularis, Serratia marcescens, Sphingomonas paucimobilis)* significantly reduced *M. incognita* infection up to 50% compared to nonbacterized controls. Plant growth of the bacteria treated plants was similar to, or greater than, growth of nontreated plants. The antagonism observed in these studies against *M. incognita* suggests that endophytic rhizobacteria should be evaluated in strategies for biological control of nematodes.

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ASSOCIATION OF *BURKHOLDERIA CEPACIA* AND *PSEUDOMONAS* WITH CORN AND SOYBEAN ROOTS AND THEIR ROLE IN SUPPRESSING PRE-EMERGENCE DAMPING-OFF OF SOYBEAN. K.P. Hebbar, J. D. Hackett, D. R. Fravel, R. D. Lumsden, USDA-ARS, Biocontrol of Plant Diseases Laboratory, Bldg. 011A, Beltsville, MD 20705.

Soils from sites designated for a sustainable agriculture project, and under previous alfalfa and corn crop, were evaluated for the presence of *Burkholderia cepacia*. Counts of *B. cepacia* were estimated from 2 week old corn and soybean seedlings by plating dilutions of root macerates on a semi-selective (PCAT) medium. High levels (log 7.8 cfu/g d. wt. root) of *B. cepacia* or a closely related species, *B. gladioli*, were present on corn seedling roots grown in three out of the four soils. However, when root macerates from soybean seedlings grown in the above soils were plated on PCAT medium the predominant bacteria present were *Pseudomonas corrugata* and *P. fluorescens*. This indicates a selective enrichment of *B. cepacia* on corn roots, but not on the soybean roots, and the converse by *P. corrugata* and *P. fluorescens*. In bioassays with soil naturally infested with *Pythium* and *Fusarium* spp., percentage of emergence was higher when soybean seeds were coated with *P. corrugata* strains and *P. fluorescens* (50-60%) than when coated with *B. cepacia* (30%). The stand was only 25% in the pathogen control.

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BNYVV-RELATED INDIGENOUS MILD VIRAL STRAINS FOR BIOCONTROL OF RHIZOMANIA: CHARACTERIZATION OF CANDIDATE ISOLATES AND PRODUCTION OF INOCULUM FOR FIELD TESTING. **B. R. Lovic and C. M. Rush, Texas Agricultural Experiment Station, P.O. Drawer 10, Bushland, TX 79012.**

Rhizomania of sugar beet is caused by beet necrotic yellow vein virus (BNYVV), a soilborne virus vectored by a plasmodiophorous fungus, *Polymyxa betae*. Several soilborne BNYVV-like yet serologically distinct viral strains have been identified in commercial sugar beet fields of Colorado, Texas, and Nebraska from plants exhibiting systemic leaf symptoms and apparently healthy roots. Four such isolates, representing at least two different serotypes, were tested for their pathogenicity to sugar beet and for their ability to protect plants against BNYVV under greenhouse conditions. The biocontrol strategy is based on adaptation of a recently described method (Phytopathology 83:1216-1219) for seed application of BNYVV in the form of viruliferous cystosori of *P. betae*. Growing sugar beet (cv. HH67) from seed in containers concurrently with infected plants exhibiting systemic symptoms proved to be an efficient way of producing inoculum for field testing.

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***BRASSICA NIGRA* LEAF TISSUE REDUCES *FUSARIUM SAMBUCINUM* POPULATIONS IN SOIL AND DECREASES DRY ROT OF POTATO TUBERS.** **H. S. Mayton and R. Loria, Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.**

We are attempting to use *Brassica* species which contain large amounts of allylisothiocyanate (AITC) for control of soilborne fungal pathogens. Macerated leaf tissue (20 g) of either *B. napus* or *B. nigra* was incorporated into 100 g of sterile soil previously infested with *Fusarium sambucinum* (10^3 propagules/g). Incorporation of leaf tissue of *B. nigra* accession 169067 (> 0.15mg AITC/g leaf tissue) significantly reduced ($p<0.05$) the population of *F. sambucinum* compared to an unamended control. The severity of dry rot on the cut surfaces of potato tubers inoculated with the infested soil was also significantly reduced. In contrast, incorporation of *B. napus* cv. Midas (< 0.001mg AITC/g leaf tissue) in soil significantly increased ($p<0.05$) the population of *F. sambucinum* and increased disease severity on inoculated potatoes.

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IMMUNOLOGICAL STUDIES ON THE DETECTION AND LOCALIZATION OF PLANT-ASSOCIATED BACTERIA. A. Quadt-Hallmann*, J.W. Kloepper*, R. Rodríguez-Kábana*, R.A. Sikora**; *Department of Plant Pathology, Auburn University, AL 36849-5409 USA; **Bonn University, Nussallee 9, 53115 Bonn, Germany.

Studies on the microbial ecology of plant-associated bacteria require methods to differentiate an introduced strain from the indigenous bacterial community. Traditionally markers have been based on spontaneous mutations or genetic engineering, both of which may result in pleiotropic mutations. We are developing several immunological systems for detecting plant-associated bacteria, including ELISA, tissue printing, dot blot and immunogold labelling combined with electron microscopic studies. Polyclonal antibodies against *Enterobacter asburiae*, strain JM22 isolated from cotton, were produced in rabbits and tested for their specificity. In addition, monoclonal antibodies were raised in mice. Development and optimisation of the immunological methods revealed a detection limit of 10^4-10^5 cfu/ml using ELISA or dot blot, and 10^3-10^4 cfu/g plant tissue with tissue printing. In electron micrographs, immunogold labeled bacteria were covered with gold particles. Strain JM22 was consistently observed in roots of cucumber, bean and cotton; in cucumber as well as in bean stems up to 20 cm from the soil surface and in leaves of 2-4 wk old cucumber plants. These results de-monstrate that immunological procedures can be used to confirm endophytic colonization of plants by bacteria.

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NONTARGET EFFECTS OF ANTIBIOTIC-PRODUCING PSEUDOMONADS IN WHEAT-SOYBEAN AND WHEAT-FALLOW CROPPING SEQUENCES. R.B. Reeder and B.H. Ownley. Entomology and Plant Pathology Dept., Univ. of Tennessee, Knoxville TN, 37996.

Soil in Conetainers® was planted to wheat (3-4 leaf stage) for 3 cropping cycles. Then, half the Conetainers® were planted to soybean while the remainder were fallowed. At 4 wk, untreated wheat seed or seed treated with *Pseudomonas fluorescens* 2-79 (produces phenazine-1-carboxylate, Phz+), or *P. fluorescens* Q2-87 (produces 2,4-diacetylphloroglucinol, Phl+), or their respective Tn*5* Phz- or Phl- mutants, were planted in the two soils. Bacterial populations of the wheat rhizosphere were assessed at the 3-4 leaf stage. Densities of Actinomycetes, *Arthrobacter*, *Azotobacter*, *Bacillus*, and total bacteria were not affected by seed bacterization with Phl+, Phl-, Phz+, or Phz- strains. Results were similar for bacteria involved in nitrogen transformations in soil. Total fluorescent pseudomonad densities were greater on roots from seed treated with Phl- or Phl+ strains. The introduced pseudomonads (Phl+, Phl-, Phz+, Phz-) accounted for a high proportion of the total pseudomonads recovered. Actinomycetes, *Arthrobacter*, *Bacillus*, and total bacteria from wheat roots were lower in soil previously fallowed than in soil previously planted to soybean. The previous crop (soybean or fallow) did not affect densities of the introduced pseudomonads from wheat roots.

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BIOCONTROL OF FUSARUIM WILT OF COTTON BY *BACILLUS SUBTILIS*, *GLIOCLADIUM VIRENS* AND NONPATHOGENIC *FUSARIUM* SPP. STRAINS. J. X. Zhang[1] and C. R. Howell[2], [1]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843; [2]USDA-ARS, Southern Crops Research Laboratory, College Station, TX 77845.

Two strains of each *Bacillus subtilis* and *Gliocladium virens*, and twenty nonpathogenic strains of *Fusarium oxysporum*, *F. solani*, *F. equiseti*, *F. nygamai* and *F. semitectum* were tested for biocontrol of Fusarium wilt of cotton caused by *F. oxysporum* f. sp. *vasinfectum* (Fov) in the greenhouse. Cotton seeds were treated with the strain preparations, and planted in Fov and root-knot nematode race 3 (*Meloidogyne incognita*) infested field soil in the greenhouse. The experimental results showed that *B. subtilis* and *G. virens* strains significantly ($p \leq 0.05$) reduced disease incidence of Fusarium wilt of cotton compared with the control. Of the nonpathogenic *Fusarium* spp. strains, four strains from *F. solani*, two from *F. oxysporum*, and one each from *F. equiseti* and *F. nygamai*, showed significant ($p \leq 0.05$) reduction of Fusarium wilt of cotton. This suggests that *B. subtilis*, *G. virens* and some strains of *Fusarium* spp. may be potential biocontrol agents of Fusarium wilt of cotton.

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RELATIONSHIP BETWEEN TRAITS OF *BACILLUS MEGATERIUM*, SOYBEAN ROOT AND SEED COLONIZATION, AND SUPPRESSION OF RHIZOCTONIA ROOT ROT. X. Y. Zheng and J. B. Sinclair, Department of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801.

The role of chemotaxis, motility, sporulation, or antagonism of *B. megaterium* B153-2-2 during soybean root and seed colonization, and the correlation between root colonization and suppression of Rhizoctonia root rot were studied in different soil mixtures without or with *Rhizoctonia solani*. Strain B153-2-2 and its mutants with altered trait(s) were applied either as a seed coating or soil application. Results showed that: 1) chemotaxis and antagonism were important for bacterial root and seed colonization; 2) poorly sporulating mutants resulted in low bacterial population after application and less root and seed colonization; 3) motility had no effect on bacterial root and seed colonization; 4) seedlings grown in sandy soil had better bacterial root and seed colonization and lower disease index than those grown in clay soil; and 5) there was a significant ($P = 0.05$) correlation between bacterial root colonization and suppression of root rot.

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THE EFFECTS OF CO-INFECTION OF SELECTED SYSTEMIC VIRUSES ON TITER OF BYDV-PAV AND ITS EFFECT ON THE AGRONOMIC TRAITS IN FOUR HOSTS IN THE GREENHOUSE S. Muratoti[1], I. Latorre[1] and L. L. Domier[1,2], Department of Plant Pathology[1], and USDA-ARS-CPRU[2], University of Illinois, Urbana IL 61801

In the greenhouse the effects of co-infection of selected systemic viruses on PAV strain of BYDV (BYDV-PAV) virus titers were evaluated. Four different hosts, Marshall, Michigan Amber, (wheat cultivars), Clintland 64 (oats) and Hudson Barley were used. The cultivars were planted in pots using a randomized complete block design with three replications. In one set of treatments the plants were inoculated first mechanically with barley stripe mosaic (hordeivirus), brome mosaic virus (bromovirus), wheat streak mosaic virus (potyvirus), wheat spindle streak mosaic virus (potyvirus), BYDV-PAV (luteovirus) and then ten days later with BYDV-PAV. In a second set of treatments the plants were inoculated first with BYDV-PAV and then ten days later with the mechanically transmissible viruses. Ten days after the second inoculations, plants were collected and tested for BYDV-PAV virus titer. The grain yield, kernel weight and tiller number were also analyzed.

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INCIDENCE OF THREE APHID-TRANSMITTED *LILIUM* VIRUSES. T. -C. Deng and F. W. Zettler. Plant Pathology Dept., Univ. Florida, Gainesville, FL. 32611.

Lily symptomless (LSV), tulip break (TBV), or cucumber mosaic (CMV) viruses were detected by indirect ELISA in commercial lily bulbs imported into Florida from Europe (Asiatic and Oriental hybrids) and western USA (*L. longiflorum)* in 1993-1994. TBV and lily mottle virus reference antigens used as controls reacted in ELISA and Western blot tests with the TBV polyclonal antiserum (Derks *et al.* 1982. Neth. J. Pl. Pathol. 88:87-98), but not with PTY 1 (Agdia, Inc.) or ATCC PVAS 766, 767, 769, and 770 monoclonal antisera. Whereas LSV was detected in bulbs of 14/15 Asiatic and 9/9 Oriental cultivars (41% of 380 and 60% of 234 bulbs indexed, respectively), only 6 bulbs were infected with TBV and 1 bulb with CMV. LSV and TBV, but not CMV were detected in all 36 *L. longiflorum* bulbs assayed. LSV was detected in 73% of 86 bulblets derived from infected scale pieces. In contrast with *L. longiflorum*, virus-free commercially available Asiatic and Oriental bulbs representing all cultivars except three were found.

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PARTIAL CHARACTERIZATION AND SEROLOGY OF THE LEAFHOPPER-BORNE MAIZE YELLOW STRIPE TENUI-LIKE VIRUS FROM EGYPT. A.M. Hussein, J.C. Thouvenel*, G.H. Sewify, S.M. Abol-Ela, and E.D. Ammar. Dept. of Economic Entomology, College of Agriculture, Cairo University, Giza; and ORSTOM*, Giza, Egypt.

Capsid and noncapsid proteins of maize yellow stripe virus (MYSV) were purified from maize plants and partially characterized. In SDS-PAGE the capsid protein had an apparent molecular weight of 35.6 kD, whereas the noncapsid protein was 14.7 kD. As with the planthopper-borne tenuiviruses, the capsid protein of MYSV was associated with fine filaments, and the non-capsid protein formed typical needle-shaped crystals. Antisera to both proteins were prepared and used in ELISA and dot-blot tests to detect MYSV in naturally and experimentally infected maize, wheat, barley, oat, and some graminaceous weeds, in addition to detection of MYSV in naturally and experimentally infective Cicadulina chinai leafhoppers.

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A POSSIBLE NEW STRAIN OF POTATO VIRUS M. T.C. Cavileer, R.G. Clarke, D.L. Corsini and P.H. Berger, University of Idaho, Moscow, ID 83844-2339.

Leaf samples from plants of an Idaho potato breeding selection showing mild mosaic symptoms reacted in an inconsistent manner to polyclonal potato virus M (PVM) antisera in ELISA tests. Using reverse transcriptase-polymerase chain reaction and primers based on published PVM sequences, RT-PCR fragments were obtained that appeared to correspond to PVM coat protein in terms of size. The nucleotide sequence was determined, and the predicted amino acid sequence compared to other PVM and carlavirus sequences. While this new isolate is likely a strain of PVM, it is also considerably different than known sequences, particularly the N-terminal 50 residues. These differences may explain poor reactivity to other PVM antisera and indicate that this is a new strain of PVM.

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DETECTION OF PINEAPPLE CLOSTEROVIRUS IN PINEAPPLE PLANTS AND MEALYBUGS USING MONOCLONAL ANTIBODIES J. S. Hu, D. M. Sether, Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, USA; and D. E. Ullman, Department of Entomology, University of California, Davis, CA 95616, USA.

Stable hybridoma cell lines secreting monoclonal antibodies (MAbs) to the pineapple closterovirus (PCV) were produced. PCV was purified from pineapple plants showing typical symptoms of pineapple mealybug wilt. PCV particles were identified by MAbs in ISEM and ELISA in symptomatic and asymtomatic pineapple plants collected from Oahu and Maui, and pineapple collections from USDA-ARS Gemplasm Repository in Hilo, but were not detected from pineapple seedlings. At least two serotypes of PCV were detected. In addition, PCV was detected from mealybugs (*Dysmicoccus brevipes*) collected from wilted pineapple plants, but not from the same species of mealybugs collected from a colony raised on squash. The role of PCV in mealybug wilt of pineapple is being investigated.

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TRANSMISSION OF MAIZE ROUGH DWARF FIJIVIRUS BY VASCULAR PUNCTURE INOCULATION WITH LEAF EXTRACTS AND PARTIALLY PURIFIED PREPARATIONS FROM ROOT EXTRACTS. R. Louie[1,2], O.E. Bradfute[2], and D.T. Gordon[2]. USDA-ARS[1], Dept. of Plant Pathology, OARDC and The Ohio State University[2], Wooster OH 44691.

Virus transmission from extracts of leaves of plants naturally infected with maize rough dwarf fijivirus (MRDV) was achieved by vascular puncture inoculation (VPI) of maize kernels. MRDV was purified from roots of these infected plants by clarifying extracts with carbon tetrachloride or Freon 113 or initially concentrating without clarification using polyethylene glycol. After a low speed centrifugation and then a high speed sucrose density gradient (30, 40, 50, and 60%) centrifugation, the banded virus was collected and dialyzed against the extraction buffer (0.05M Na_2HPO_4, 0.005M sodium EDTA and 0.01M Na_2SO_3, pH 7.8). Best rates of transmission (100 kernels/test) by VPI from leaf extracts and partially purified preparations involving root extracts clarified with carbon tetrachloride or Freon 113 or initially concentrated with polyethylene glycol were 48, 47, 48, and 40%, respectively. Infected plants were stunted and exhibited veinal chlorosis, swollen veins, and enations. Fijivirus-like particles also were observed in leaf and clarified root extract inocula.

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RISK ASSESSMENT OF VIRUS SPREAD AND GENE INTROGRESSION USING TRANSGENIC VEGETABLE CROPS EXPRESSING VIRAL COAT PROTEIN GENES. M. Fuchs and D. Gonsalves. Dept. of Plant Pathology, Cornell University, Geneva, NY 14456.

Some of the potential environmental risks regarding the use and commercialization of virus resistant transgenic plants include the development of modified virions and enhanced weediness of free-living crop relatives due to introgression of viral transgenes. To assess under field conditions the risks of virus transmission via transencapsidation and/or recombination, we evaluated transgenic tomato, squash, and cantaloupe expressing the coat protein (cp) gene of the aphid-borne cucumber mosaic virus (CMV) strain WL to determine their likelihood of mediating spread of the non-aphid transmissible CMV strain C. So far our data does not indicate any spread of CMV-C from inoculated transgenic to healthy nontransformed plants. To analyze the consequences of introgression of viral transgenes, the relative fitness of hybrids of *Cucurbita texana* x transgenic *C. pepo* containing the cp genes of CMV, zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus II (WMV II) was studied under severe disease pressure. Our preliminary data shows that transgenic *C. texana* hybrids were resistant to CMV, ZYMV, and WMV II, and produced more viable seeds than the nontransformed genotypes. Additional field trials designed to assess if risks outweigh the benefits of the use of virus resistant transgenic plants are underway.

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TRANSGENIC *NICOTIANA* SPS. CONTAINING COAT PROTEIN GENE CONSTRUCTS ARE RESISTANT TO TOMATO RINGSPOT NEPOVIRUS. L. M. Yepes, M. Fuchs, J. L. Slightom[1], and D. Gonsalves. Dept. of Plant Pathology, Cornell University, Geneva, NY 14456, [1]Molecular Biology Unit 7242, The Upjohn Company, Kalamazoo, MI 49007.

Tomato ringspot virus (TomRSV) is the most important virus of fruit and berry crops in the Northeastern US and in certain areas of the Pacific Coast where its *Xiphinema* nematode vector species are present. TomRSV causes severe damage to several important perennial crops including apples, peaches, cherries, plums, raspberries, strawberries, and grapes. To engineer resistance to TomRSV, the coat protein (cp) gene and the 3'end untranslated region of a peach isolate were cloned and sequenced. Sense and antisense cp gene constructs were generated by RT-PCR using purified viral RNA, engineered into plant transformation vectors, and transferred to *Nicotiana benthamiana* and *N. tobaccum*, a systemic and a local lesion host of TomRSV, respectively. After challenge inoculation with the virus, several Ro, R1, and R2 resistant transgenic lines containing sense and antisense cp constructs were obtained for *N. benthamiana* and *N. tobaccum* that exhibited variable levels of protection ranging from complete resistance to delay in symptom appearance and reduction in symptom severity. Transformation of several important perennial crops with the cp gene constructs evaluated in *Nicotiana* sps. is underway.

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RESISTANCE TO LMV INFECTION IN LETTUCE DUE TO THE EXPRESSION OF DIFFERENT FORMS OF THE LMV COAT PROTEIN GENE. F.M. Zerbini[1], R.W. Michelmore[2], R.L. Gilbertson[1]. 1. Dep. of Plant Pathology and 2. Dep. of Vegetable Crops, University of California, Davis, CA 95616

Three different forms of the lettuce mosaic potyvirus (LMV) coat protein (CP) gene were cloned into an *Agrobacterium tumefaciens* binary vector and transformed into lettuce cotyledons, cv. 'Cobham Green'. Transgenic plants were generated containing either the full length CP gene, anti-sense (AS) or untranslatable (UT) constructs of the CP gene. Control lines were transformed with the vector alone. R_2 plants were grown in the greenhouse and sap-inoculated with LMV pathotype II. Plants were observed visually for symptoms and tested for viral infection by ELISA eight weeks after inoculation. AS lines did not show any resistance to infection compared to controls. CP and UT lines varied in their degree of resistance, from a slight delay in the onset of symptoms to virtual immunity. Two CP lines and four UT lines were highly resistant, with no symptoms and no viral replication as determined by ELISA. These promising lines will be taken to R_3 and R_4 generations and evaluated in the field.

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DETECTION OF INFECTIOUS TOMATO MOSAIC TOBAMOVIRUS IN FOG AND CLOUDS. Castello, J. D.[1], Lakshman, D. K.[2], Tavantzis, S. M.[2], Rogers, S. O.[1], Bachand, G. D.[1], Jagels, R.[2], Carlisle, J.,[2] and Liu, Y.[1] [1]State University of New York, College of Environ. Sci. & Forestry, Syracuse, 13210; [2]University of Maine, Orono, 04469.

Incidence of tomato mosaic tobamovirus (ToMV) infection of red spruce (*Picea rubens*) in the northeastern US is site related. We hypothesize that infection of spruce is related to exposure of trees to virus-laden clouds or fog. The objective of this study was to determine if infectious ToMV occurs in cloud and/or fog. Twenty-two cloudwater samples from Whiteface Mt. and 22 fog samples from two coastal sites in Maine were collected between June and October 1992, and concentrated by ultracentrifugation. Virus was not transmitted in initial bioassays on *Chenopodium quinoa*. However, ToMV was detected by reverse transcription-polymerase chain reaction in 25 concentrates, and transmitted from a concentrated composite of positive samples to *C. quinoa*. The presence of infectious ToMV in cloud and fog suggests an effective long-distance transport mechanism for this, and possibly other stable viruses.

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AIRBORNE TRANSMISSION OF TOMATO MOSAIC TOBAMOVIRUS TO RED SPRUCE SEEDLINGS ON WHITEFACE MOUNTAIN, NY. R.C. Fillhart, J.D. Castello, G.D. Bachand, State University of New York College of Environmental Science and Forestry, Syracuse 13210

The objective of this study was to investigate the potential for airborne transmission of tomato mosaic tobamovirus (ToMV) to red spruce on Whiteface Mountain, NY (WF). To test the hypothesis that this virus may be transmitted by an airborne mechanism, virus-free seedlings (as determined by enzyme-linked immunosorbent assay (ELISA)), were planted in four raised plywood boxes. The boxes were lined with plastic and filled with a promix-sand (3:1) mix. In previous work, ToMV was not detected in this planting mix. In July, 1993, two boxes were placed at 1015m (site 1) and two at 960m (site 2) on WF. In August 1994, roots were collected from the survivors and tested for ToMV by ELISA. Thirty-two of 40 (site 1) and 33 of 50 seedlings (site 2) tested positive for ToMV. Sixty-nine percent of the infected seedlings from site 1 and 18% from site 2 had a virus concentration greater than 50 ng/g in their roots. We conclude that airborne transmission may be an effective mechanism for the spread of this virus in montane forest ecosystems, and perhaps in other ecosystems, and may be related to cloud exposure.

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A NEW GEMINIVIRUS ASSOCIATED WITH CHLOROSIS OF TOMATOES IN NORTHWESTERN MEXICO. R. L. Gilbertson, Y.-M. Hou, P. Guzman, and M. G. Carvalho. Dept. of Plant Pathology, University of California, Davis, CA 95616.

Tomatoes in the Culiacan Valley of northwestern Mexico are affected by numerous diseases caused by whitefly (WF)-transmitted geminiviruses. Symptoms associated with one of these diseases that has become prevalent during the past two growing seasons include vein yellowing and chlorosis of newly infected leaves, purple discoloration of older leaves, and stunted and distorted growth. Geminivirus infection in tomato plants with these symptoms was established using squash blot hybridization and a general DNA probe for WF-transmitted geminiviruses. Specific DNA probes for tomato yellow leaf curl (Dominican Republic isolate), tomato mottle (Florida), and tomato leaf crumple (Mexico) geminiviruses failed to hybridize with DNA from these plants. A geminivirus DNA-B fragment was amplified from DNA extracted from these plants by the polymerase chain reaction with general geminivirus primers, cloned, and sequenced. DNA sequence comparisons and hybridization results indicate that this virus is distinct from previously characterized WF-transmitted geminiviruses. Putative full-length DNA-A and DNA-B components were cloned from DNA extracted from infected plants. Efforts to establish whether these components comprise the genome of a bipartite geminivirus and whether this virus causes these disease symptoms are ongoing.

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A NEW VIRUS DISEASE IN CHICKPEA IN WASHINGTON STATE CAUSED BY A STRAIN OF RED CLOVER VEIN MOSAIC CARLAVIRUS. R. C. Larsen, USDA-Agricultural Research Service, Route 2 Box 2953A, Prosser, WA 99350, W. J. Kaiser, USDA-ARS, Pullman, WA 99164, and S. D. Wyatt, Dept. of Plant Pathology, Washington State Univ., Pullman, WA 99164.

A strain of red clover vein mosaic virus (RCVMV-ChP) was isolated from chickpea (*Cicer arietinum* L.) grown in the Palouse region of Washington State. Symptoms on chickpea included severe stunting, mosaic, proliferation of axillary buds, and malformation of leaves and branches. Flower and pod formation were severely reduced. The host range was similar to that of the type strain (ATCC RCVMV pv110). However, the viruses could be readily differentiated by mechanical inoculation to *Chenopodium amaranticolor* and *C. quinoa*. RCVMV pv110 produced chlorotic local lesions on the inoculated leaves, while RCMV-ChP produced small (0.5 mm), necrotic local lesions. The RCVMV-ChP virion coat protein was ca. 25,000 Daltons when determined by SDS-PAGE. The virion nucleic acid consisted of a single species ssRNA with a molecular weight of 7.05×10^6. Studies by ELISA indicated no serological relationship between RCVMV-ChP and isolates of pea streak and alfalfa latent carlaviruses. This is the first report of RCVMV occurring in chickpea.

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ADAPTABILITY OF SIDA MOSAIC GEMINIVIRUS DURING INFECTION IN NEW HOSTS. A. M. Abouzid[1], J. E. Polston[2] and E. Hiebert[1]. [1]Plant Pathology Dept.,Univ. of Florida, Gainesville, FL 32611, and [2]GCREC, Bradenton, FL 34203.

Following the establishment of the 'B' biotype whitefly (*Bemisia tabaci*) in Florida many crops such as beans, peppers, tomatoes, and tomatilloes have become naturally infected with geminiviruses. Sequence comparisons of the intergenic region of DNA-A of these new geminiviruses with sida mosaic virus (SiMV) showed sequence similarities ranging from 90-94% while comparisons of the hypervariable region sequences of the DNA-B, showed high divergence ranging from 40-94%. Head-to-tail dimeric clones of both DNA-A and -B of SiMV were infectious when inoculated onto tomato plants by a biolistic procedure. SiMV was also transmitted from *Sida* to tomato by whitefly. Successive whitefly-transmission of SiMV in tomatoes was confirmed by the appearance of symptoms and by PCR amplification. Sequence analyses of the amplified hypervariable region of SiMV DNA-B showed divergence parallel to symptoms alterations from mild symptoms to tomato mottle virus (TMoV)-like symptoms with successive passages. It is proposed that TMoV originated from SiMV in Florida.

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MOLECULAR CHARACTERIZATION OF A DROUGHT-INDUCIBLE PROTEINASE INHIBITOR FROM *ATRIPLEX CANESCENS*. Debbie Villalon,[1] Ronald Newton[2] and John Carney.[1] [1]Institute of Paper Science and Technology, Dept. of Forest Biology, 500 10th str. N.W., Atlanta, GA 30318 and [2]Texas A&M University, Dept. of Forest Science, College Station, TX 77843

This study examines the mechanisms of gene regulation under water-deficit, focusing on a family of Proteinase Inhibitor genes from *Atriplex canescens*. Sequence comparison of several cDNAs indicates a potential for translation and/or stability differences between these transcripts. Quantitative analysis of reverse transcriptase-PCR products shows that different members of this gene family respond in different degrees to various abiotic stresses and hormonal induction. The promoters of two genomic clones include putative transcription factor binding sites such as abscisic acid response elements (ABREs) and additional regulatory motifs. Assay of regulatory sequences is being conducted by reporter gene expression in *Arabidopsis thaliana*. The function of the gene product is being addressed by subjecting the purified protein to a radial gel diffusion assay.

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INFLUENCE OF ELEVATED CO_2 ON DISEASE DEVELOPMENT AND INDUCTION OF PR PROTEINS IN TOMATO ROOTS BY *PHYTOPHTHORA PARASITICA*. N. S. JWA, L. Walling, and P. M. McCool, University of California, Riverside.

Tomato plants grown under high CO_2 level showed possible tolerance against Phytophthora root rot. The total dry weight of tomato plants infected with *Phytophthora parasitica* was higher in plants grown in 700 ppm CO_2 than under ambient CO_2. Both infected and healthy tomato plants which were grown under high CO_2 condition showed larger stem diameters at cotyledon height than those of tomato plants under ambient CO_2. After watering the pot to field capacity, water potentials of diseased and healthy plants were higher in 700 ppm CO_2 than those grown in ambient CO_2. Leaves of tomato inoculated with *Phytophthora parasitica* and grown in hydroponic culture under high CO_2 showed slightly different induction of Pathogenesis Related (PR) proteins. This induction of PR proteins was not related with accumulation of salicylic acid (SA), because HPLC analysis showed no increase in SA concentration after the infection of *Phytophthora parasitica*. A SA treatment through hydroponic culture media induced PR-1a in leaves but no in roots. This may be one possible reason to explain the lack of induced defense resistance against Phytophthora root rot disease in tomato.

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PHENOLICS ASSOCIATED WITH THE RESISTANCE OF COCOA STEMS TO *PHYTOPHTHORA* CANKER. [1]Okey, E.N., [1]Duncan, E.J., [1]Sirju-Charran,G. and [2]Sreenivasan, T.N. [1]Dept. of Plant Science, [2]Cocoa Research Unit, The University of the West Indies, St. Augustine, Trinidad.

Stems of six cocoa clones were inoculated with zoospore suspensions (3×10^5 ml^{-1}) of *Phytophthora palmivora*. Based on their reactions, the clones were categorized into three groups: resistant, IMC 67; moderately resistant, TSH 1188 and ICS 1; susceptible, TSH 1076, SCA 6 and P 18. Ethanol extracts from healthy and inoculated tissues showed the latter to be more inhibitory to *P. palmivora* zoospore germination and growth. Also, extracts from IMC 67 infected tissues were more inhibitory to the pathogen compared to extracts from the other clones. Using TLC, PLC and UV spectral analyses, epicatechin, hydroquinone, chlorogenic and tannic acids were detected in the clones. Salicylic acid was only present in IMC 67 extracts. The amounts of total phenol were higher in IMC 67 and lower in TSH 1076, SCA 6 and P 18. This study indicates the involvement of phenolics in the resistance of cocoa stems to canker.

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GENETICS OF RUST RESISTANCE IN NINE PINTO DRY BEAN GERMPLASM RELEASES. J. R. Stavely, Molecular Plant Pathology Laboratory, USDA, ARS, Beltsville, MD 20705

Type II, erect pinto bean germplasm lines, BelDakMi-Rust Resistant (RR) -1 through -4 and -5 through -9, were released by ARS, USDA, the North Dakota, and Michigan Experiment Stations in 1992 and 1993, respectively. The major source of rust resistance in lines -1 through -4 was plant introduction (PI) 151388, in lines -5 through -7 was PI 181996, and in lines -8 and -9 was PI 190078. All of these PIs and the released lines are resistant to all 65 available races of the bean rust fungus, *Uromyces appendiculatus*. At least three backcrosses with pinto lines or cvs. and field selection in advanced generations were needed in developing and selecting lines to obtain desirable agronomic characteristics for pinto beans. Parental plants of the released lines were crossed with susceptible cvs. Progeny tests indicated all nine lines are homozygous for a single, complex, dominant locus from their respective PI parent that provides resistance to all races. Most of these lines contain a second dominant resistance gene that originated from pinto cv. Olathe and that is effective against many of the races.

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USE OF RECOMBINANT SUBSTITUTION LINES TO MAP AN EYESPOT RESISTANCE GENE ON CHROMOSOME 7A OF WHEAT. R.C. de la Peña[1], T.D. Murray[1] and S.S. Jones[2], [1]Department of Plant Pathology and [2]Department of Crop & Soil Sciences, Washington State University, Pullman, WA 99164.

A gene on wheat chromosome 7A conferring resistance to eyespot disease, caused by *Pseudocercosporella herpotrichoides*, was mapped using chromosome 7A homozygous recombinant substitution lines (RSL). The eyespot reactions of 75 RSL, derived from 'Chinese Spring' (susceptible) hybridized with a disomic substitution line of 'Cappelle Desprez' chromosome 7A in 'Chinese Spring' (resistant), exhibited a 1:1 bimodal distribution for resistance and susceptibility. A wheat chromosome 7A linkage map was constructed and the eyespot resistance gene was mapped by restriction fragment length polymorphism (RFLP) analysis using 28 probes with homologous DNA sequences on the group 7 chromosome of wheat and eight restriction enzymes. Ten polymorphic RFLP markers were identified, and *Xpsr121* was the most closely linked to the resistance gene with 14 % recombination frequency. The greatest polymorphism was observed when genomic DNA was digested with *Eco*RI. Screening of more probes specific for wheat chromosome group 7 is in progress to identify other closely-linked RFLP markers. Identification of markers closely associated with resistance to eyespot will be useful for marker-assisted selection to develop eyespot resistant wheat cultivars.

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GENETIC ANALYSIS OF NEW LEAF RUST RESISTANCE GENES IN BARLEY. Y. Jin[1], G.H. Cui[2], B.J. Steffenson[1], and J.D. Franckowiak[2]. Department of Plant Pathology[1] and Department of Plant Sciences[2], North Dakota State University, Fargo, ND 58105.

Barley accessions PI 531849 and PI 584760 possess resistance to *Puccinia hordei* isolates with wide virulence spectrum. To investigate the inheritance of resistance and allelic relationships to known *Rph* genes conferring leaf rust resistance, crosses were made between these lines and genetic stocks with *Rph*1 to *Rph*12 (except for *Rph*8). The F_2 populations were evaluated for leaf rust reaction at the seedling stage. Results indicated that the resistance genes in PI 531849 and PI 584760 were not allelic to any of the known *Rph* gene loci. Segregation in the F_2 population from the cross between PI 531849 and the *Rph*9 stock showed linkage between resistance genes with an estimated distance of $30.4 \pm 4.5\%$. Linkage was not observed for the resistance gene in PI 584760 and the other *Rph* genes. The segregation pattern in F_2 from PI 584760 cross to the *Rph*5 stock fit better to a pattern of one dominant and one recessive gene ($\chi^2 = 1.05$, $P = 0.31$) than a pattern of two dominant genes ($\chi^2 = 49.12$, $P < 0.01$) although the involved *Rph* genes showed a mode of dominant inheritance in other crosses. The recessiveness for one of the resistance genes may occur if this gene is suppressed in the heterozygous condition.

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IDENTIFICATION OF SEEDLING AND ADULT-PLANT GENES FOR LEAF RUST RESISTANCE IN FOUR SPRING WHEAT CULTIVARS. Jean Q. Liu, and James A. Kolmer. Agriculture and Agri-Food Canada Research Centre, Winnipeg, MB, Canada R3T 2M9.

The hard red spring wheat cultivars recently released in the northern Great Plains of the U.S.A. and Canada are highly resistant to leaf rust (*Puccinia recondita* f.sp. *tritici*). To determine the genetic basis of this resistance, Grandin, AC Domain, CDC Teal, and AC Taber were crossed to the susceptible wheat cultivar Thatcher, F_1 plants were backcrossed to Thatcher, and BCF_2 families were evaluated with a number of different *P.r. tritici* races in both seedling and adult-plant stages. Seedling tests indicated that Grandin has *Lr2a, Lr3, Lr10,* and *Lr16*, AC Domain has *Lr10* and *Lr16*, and AC Teal has *Lr1* plus an unidentified seedling resistance gene. The number and identity of seedling resistance genes in AC Taber is being determined. The BCF_2 families will be evaluated in the adult plant stage to verify the presence of *Lr13* and/or *Lr34*.

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A MAJOR GENE FOR RESISTANCE IN A NATIVE TREE SPECIES, *POPULUS TRICHOCARPA*, AND ITS HYBRIDS. *George Newcombe*[1], Toby Bradshaw[2], Gary Chastagner[1], and Reinii Stettler[3]. [1]Wash. State Univ., Res. & Ext. Ctr., Puyallup, 98371. [2]Ctr. for Urban Hort. GF-15, and [3]Col. For. Res., Univ. of Wash., Seattle, 98195.

Previous attempts to understand the genetics of disease resistance in *Populus*, have been handicapped by nonavailability of progenies beyond F_1s. The development, and use, of a three-generation *P. trichocarpa* x *P. deltoides* (TxD) hybrid pedigree comprising reciprocal backcross progenies and a mapped F_2, have allowed us to begin to characterize genes for disease resistance in *Populus*. We have identified, via an approach combining Mendelian and Mapmaker-QTL analyses, a single dominant gene, Mmd, governing necrotic flecking, which is the major component of resistance in the pedigree to *Melampsora medusae* f.sp. *deltoidae*. The Mmd gene accounted for 83% and 73% of the phenotypic variances in rust severity in field plantings in 1993 and 1994, respectively. Repeated, growth-room inoculation studies confirm the presence of the Mmd gene. The dominant Mmd allele is inherited from the *P. trichocarpa* parent even though it and *M. medusae* f.sp. *deltoidae* have apparently not interacted until the latter was introduced into the Pacific Northwest a few years ago.

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APPARENTLY DISTINCT RESISTANCES IN DURUM WHEATS TO DURUM WHEAT LEAF RUST. A.N. Mishra and A.P. Roelfs. Department of Plant Pathology and USDA-ARS Cereal Rust Lab, University of Minnesota, St. Paul, MN 55108

A total of 22 durum wheats, mostly land races from around the world, were tested as seedlings with 191 durum wheat leaf rust isolates from 11 countries. 'Immer' was resistant to all while 'Arabian durum', 'Local Red', 'RL 6089', and 'Karkov' were susceptible to most isolates. The resistance response of 'Arendeto', 'Mocha de Espiga Branca', and 'Rojal de Almeria' was normally characterized by an infection type (IT) 'Y' (a more compatible response near the leaf tip compared with the leaf base). The low IT was generally a 'Z' (pattern opposite of 'Y' with greater compatibility towards the leaf base) in 'Kubanka' and 'Acme'. Other lines exhibited a range of low ITs from ';1' through 'X' with a fairly common trend for 'Z' or 'Y' patterns. The latter are not common among bread wheat-bread wheat leaf rust interactions. Further, several isolates that were virulent on most of the durum test lines were avirulent on 'Thatcher' and other presumably susceptible bread wheats or on many of the known *Lr* genes. These observations indicate the presence of apparently distinct resistance in the durum wheat and apparently distinct virulence in the durum wheat leaf rust pathogen.

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CHARACTERIZATION OF THE TOBACCO 'VIRGIN A MUTANT' RESISTANCE TO POTATO VIRUS Y INFECTION. R. Acosta-Leal, and Z. Xiong. Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721.

The tobacco breeding line 'Virgin A Mutant' (VAM) is apparently immune to potato virus Y (PVY) NN strain. Therefore, PVY-NN infection at the single cell level was analyzed. Protoplasts from the susceptible tobacco variety 'Burley 21' and the resistant tobacco line VAM were transfected with purified PVY-NN RNA. Infected protoplasts were detected by protoplast printing with capsid protein (CP) antibodies. At 12 hours after transfection (hat), CP was detected in 1-2% of the protoplasts of both tobaccos. The percentage of protoplasts expressing the CP increased with time. At 72 hat, around 20% of the protoplasts of both varieties were positive for the CP. PVY infection was also examined in leaves mechanically inoculated with highly concentrated virus. Protoplasts were subsequently isolated from the inoculated leaf tissue at different time points for protoplast printing. Infected cells were first detected 20 h after inoculation in both varieties but the number of infected cells in VAM tobacco was much lower than in Burley tobacco. These data indicate that PVY-NN can infect single cells of tobacco VAM line. The significance of these findings in relation to the virus resistance mechanism will be discussed.

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INDUCTION OF MUTANTS OF *Capsicum annuum* FOR LOSS OF HYPERSENSITIVITY TO STRAINS OF *Xanthomonas campestris* pv. *vesicatoria* CONTAINING *avrBs2*. R. Stall[1], G. Minsavage[1], A. Cook[1], M. Bassett[1], T. Thai[2], D. Dahlbeck[2], and B. Staskawicz[2]. [1]Univ. of Florida, Gainesville, 32611-0680; and [2]Univ. of California, Berkeley, Berkeley, 94720.

Pollen in flowers from a plant homozygous for the dominant genes, *Bs1*, *Bs2*, and *Bs3*, which confer resistance to different races of *X. campestris* pv. *vesicatoria* (Xcv) was irradiated with gamma rays at 1000, 1500, or 2000 rads. Pollinations of emasculated flowers of susceptible plants with the treated pollen resulted in 63, 48, and 30%, respectively of the number of seeds obtained by similar pollinations with untreated pollen. Only 60.1% of the seeds from pollen treated with 1500 rads germinated. When 14,000 F_1 seedlings from seeds obtained from pollen treated with 1500 rads were screened, 48 lost hypersensitivity to a strain of Xcv that contained *avrBs2*, but retained hypersensitivity to a strain of Xcv that contained *avrBs1*. Some seedlings in progeny of 25 of 35 selfed mutants reverted to hypersensitivity to Xcv-*avrBs2*. Amplification of a DNA sequence located near the *Bs2* gene was used to select homozygotes for the mutated allele from progeny of five plants which had no revertants . The five plants were crossed in all possible combinations and no complementation of hypersensitivity occurred in the progenies inoculated with strains of Xcv containing *avrBs2*.

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IDENTIFICATION OF RAPD MARKERS LINKED TO A ROOT-KNOT NEMATODE RESISTANCE GENE IN TOBACCO USING BULKED SEGREGANT ANALYSIS. Y-H. Yi, R. C. Rufty, and E. A. Wernsman. Dept. of Crop Science, Box 7620, N. C. State University, Raleigh, NC 27695-7620.

Two Burley and two Flue-cured tobacco genotypes differing in their response to root-knot nematode (*Meloidogyne incognita*) were screened with randomly generated 10-mers. Primers producing bands which were polymorphic between nematode resistant and susceptible genotypes were used on a segregating population of maternally-derived doubled haploid lines from the F_1 of the two Burley parents. RAPD markers distinguishing nematode resistant and susceptible bulks were selected and their linkage to the resistance gene was confirmed. It appears likely that screening for root-knot nematode resistance in tobacco can be accomplished using RAPD markers.

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A COMMON SOYBEAN CYST NEMATODE RESISTANCE GENE. V.C. Concibido, R. Denny[1], D. Lange[1], D. Danesh[1], J. Orf[2] and N.D. Young[1]. Dept. of Plant Pathology[1] and Dept. of Agronomy[2], University of Minnesota, St. Paul, MN 55108

To identify genes for soybean cyst nematode resistance (SCN), we analyzed four segregating soybean F_2 populations (Evans x PI 209332, Evans x PI 90763, Evans x PI 88788 and Evans x Peking) and a $F_{5,6}$ nearly-recombinant inbred population derived from the Evans x PI 209332 cross. Among all populations, we identified four independent partial resistance loci significantly associated with SCN resistance. One of these loci, on linkage group G, behaved as a major partial resistance gene and was common in all the populations. This locus explained up to 48.6% of total phenotypic variation in PI 209332, 44.8% in PI 90763, 30% in PI 88788 and 22.5% in Peking populations. Efforts are being directed to further characterize and isolate the region around the SCN resistance gene. Using comparative mapping between *Glycine max*, *Vigna radiata* and *Phaseolus vulgaris*, we have uncovered several *V. radiata* and *P. vulgaris* markers that are tightly linked with SCN resistance in soybean. A high resolution map of the G region is being constructed as a starting point to clone this major partial resistance gene.

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TELONE* SOIL FUMIGANTS REGULATORY AND RESEARCH UPDATE. M. W. Melichar and D. M. Roby, DowElanco, 9330 Zionsville Road, Indianapolis, IN 46268.

The reregistration standard for 1,3-dichloropropene (1,3-D), the active ingredient in Telone soil fumigants, issued September 1986. The last reregistration study due date is August 1995. The Environmental Protection Agency (EPA) placed 1,3-D in Special Review in 1986, which requires EPA to conduct a risk/benefit analysis resulting in issuance of a Federal Register notice of a proposed regulatory decision (PD 2/3) for public comment in early 1995. The EPA will then issue a notice of final determination (PD 4). Current Telone soil fumigant research is focused on methyl bromide replacement and drip applications. Injection trials have tested Telone II or Telone C-17 alone or combined with additional chloropicrin, metam sodium or Tillam as a methyl bromide alternative. Drip application trials of 1,3-D for nematode and disease control also have been evaluated. Results from each set of trials are promising. All 1,3-D containing treatments provided equal nematode control to methyl bromide. The addition of chloropcirin or metam sodium to 1,3-D provided comparable soil-borne disease control to methyl bromide and Tillam furnished very good control of certain weeds, especially nutsedge.

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EFFECT OF FIELD HOST AND TEMPERATURE ON RACE DETERMINATIONS FOR *HETERODERA GLYCINES*. T. L. Niblack, G. S. Smith, J. A. Wrather, H. C. Minor, and R. D. Heinz, Plant Sciences Unit, University of Missouri, Columbia, MO 65211.

Long-term soybean field trials were established in 1991 at three Missouri locations infested with *Heterodera glycines*. Adapted *H. glycines*-resistant or susceptible cultivars were planted in the same plots in 1991 and 1993 or 1992 and 1994, with corn planted in the intervening years, in order to document the effect of host on *H. glycines* populations. Initial race determination tests showed one *H. glycines* population ("Edina") to be a Race 2 and the other two ("Benton City" and "Portageville") were Race 3. After exposure to the same soybean cultivar for two years, isolates from the Edina population (tested as races 2, 4, 5, or 9) showed evidence of "race shift" regardless of the resistance or susceptibility of the soybean cultivar, whereas most of the isolates from the other two populations again tested as Race 3. Greenhouse temperature-tank experiments showed that race determinations on the same nematode isolates were dependent on the soil temperature during the test, with isolates from the Edina population (tested as races 2, 4, 5, 6, 9, 14, or 15) showing more variability than those from the Benton City or Portageville populations.

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SOIL SOLARIZATION REDUCED CITRUS NEMATODE AND PHYTOPHTHORA IN A GRAPEFRUIT ORCHARD IN TEXAS. *Mani Skaria*, Nora Solis-Gracia, and Gregory Panzer, Texas A&M University-Kingsville Citrus Center, Weslaco, TX 78696

A preliminary study in 1992 showed that solarization with plastic mulch reduced citrus nematode, *Tylenchulus semipenetrans* in the soil of an eight-year-old 'Rio Red' grapefruit orchard on sour orange rootstock. Experiments were conducted in 1993 and 1994 with single and double layer plastic, and no plastic as mulch on the east and west sides of six trees/treatment. Soil samples from 4 and 10 inches below the surface were assayed for nematodes in both years and for Phytophthora in 1994. Seasonal drop of nematodes in control samples were 80% and 27.5% in 1993 and 1994, and a combined effect of seasonal drop and mulching reduced the nematode level by 97% and 80% in 1993 and 1994, respectively. Phytophthora was detected in 66% and 100% of 4 and 10 inch respectively of the control soil, and 50% of the mulched soil at 10 inch.

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SOYBEAN CYST NEMATODE STYLET SECRETIONS AND A POTENTIAL SECRETION GENE. Eric L. Davis, Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7616.

Monoclonal antibodies (MAbs) that bind specifically to esophageal gland antigens and stylet secretions of *Heterodera glycines*, the soybean cyst nematode (SCN), have been used to probe Western blots and a SCN cDNA expression library. Two subventral gland MAbs bound to apparently the same broad SCN protein band of approximately 120 kDa, and a third subventral gland MAb bound to a strong band of about 50 kDa. A dorsal gland MAb bound to a strong band of approximately 140 kDa and a weaker band of about 40 kDa. A 585 bp SCN cDNA clone has been isolated with this dorsal gland MAb. Nucleotide sequence analysis of the cDNA predicted an open reading frame that encoded a putative basic protein but provided no significant homologies to reported genes. The cDNA clone hybridized to SCN and *Heterodera schachtii* genomic DNA on preliminary Southern blots, but did not hybridize to genomic DNA from *Globodera tabacum, Meloidogyne incognita,* or *Caenorhabditis elegans*. The initial evidence suggests that this cDNA may represent the first isolated dorsal gland secretory protein gene involved in plant parasitism by a nematode.

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EFFECTS OF TOBACCO CYST NEMATODE CONTROL MEASURES ON TOBACCO LEAF CHEMISTRY. C. S. Johnson, J. Wang, and W. M. Tilson, Virginia Polytechnic Institute & State University, Southern Piedmont Agricultural Research and Extension Center, Route 3, Box 60, Blackstone, VA 23824.

Leaf chemistry is an important indicator of quality for tobacco. Percent total alkaloids and % reducing sugars were evaluated in 1992 and 1993 from cured-leaf samples from each harvest of a flue-cured tobacco cultivar either resistant (NC567) or susceptible (K326) to tobacco cyst nematodes (*Globodera tabacum solanacearum* - TCN). Each cultivar was planted in infested soil either untreated or sprayed with 6.8-7.5 L/ha of fosthiazate. For most harvests, % total alkaloids were higher for NC567 than for K326. Percent reducing sugars were also higher for NC567 in samples from the first harvest. Reducing sugar levels were similar across cultivars at subsequent harvests. Use of fosthiazate also increased % total alkaloids in most harvests, but increased reducing sugar levels only in the first harvest. The ratio of % reducing sugars to % total alkaloids increased in the first and third harvests when fosthiazate was used in both years.

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PHIALOCEPHALA FORTINII, A POTENTIAL FINE ROOT PATHOGEN, ISOLATED FROM RED SPRUCE. S.K. Harney, San Diego State Univ., CA 92182, T.S. Wentworth, P.M. Wargo, USDA Forest Service, Hamden,CT 06514

Nonwoody roots were collected from healthy and declining red spruce (Picea rubens) in ten sites throughout the Northeast and stored at 4C in some of their native soil for 2-3 weeks. Putative dead fine root tips were surface sterilized with 20% Clorox for 10 min., rinsed, and plated out on selective media (PCNB, malt/streptomycin, and rose bengal). Many isolates appeared to belong to Mycelium radicis atrovirens, a broad group of dematiaceous, sterile fungi often associated with conifer roots, and whose ecological role is uncertain. Several isolates were characterized as Phialocephala fortinii-like by RFLP analysis of PCR amplified rDNA. P. fortinii is considered to be a pathogen on stressed conifers, and recently has been shown to be pathogenic on red pine seedlings in greenhouse experiments.

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BLIGHT RESISTANCE IN CHINESE CHESTNUT IS INHERITED FAIRLY SIMPLY. F.V. Hebard, American Chestnut Foundation, Meadowview, VA 24361.

The inheritance of blight resistance was investigated in crosses of Chinese (resistant) and American (susceptible) chestnut. In one orchard, trees were inoculated with *Endothia parasitica* 2 years after planting. Nine of 185 F2 and 105 of 399 B1-F2 (3/4 American) trees survived after two seasons of canker expansion, whereas all the pure American and F1 hybrids had succumbed by early in the second growing season. Five of 12 seedling Chinese chestnut trees, 4 of 5 trees of cv Meiling and 4 of 5 trees of cv Nanking survived through the second growing season. Mean canker size on cv Nanking was significantly smaller than on the other two types of Chnese chestnut. Discriminant analysis after one season classified 8 of the F2 and 22 of the B1-F2 trees as resistant as cv Nanking, which fit a 15:1 ratio, compatible with a model of two incompletely dominant genes controlling blight resistance. The distributions of canker size for the F2 and B1-F2 trees fit two-gene models generated from the mean and standard deviation of canker size in the segregating progeny and controls; under certain assumptions, one and three-gene models also fit. A second orchard was sown with B2 nuts (7/8 American), and trees were inoculated 4 years after planting. Discriminant analysis after one season of canker growth classified 36 progeny as having the resistance of the Chinese, 52 as having that of the F1 hybrid, and 34 as having that of the American controls, which fits a 1:2:1 ratio, again compatible with the two-gene model. The distribution of canker lengths fit the two-gene model and clearly rejected the one-gene model; the three-gene model also did not fit. These data indicate that resistance to blight in Chinese chestnut is fairly simply inherited.

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INTERSTERILITY GROUPS OF *HETEROBASIDION ANNOSUM* IN CANADA. V. L. Raffle and T. Hsiang, Department of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1.

Annosus root rot was first recognized in red pine (*Pinus resinosa*) in southern Ontario in 1955. The incidence of this disease has increased as most of the first rotation of red pine in southern Ontario is now at or approaching the age of first commercial thinning. The intersterility group (S or P) had thus far been determined for only three isolates of *H. annosum* from Ontario and they all belonged to the P group (Commun. Inst. Forest. Fenn. 94(6):1-25). Using crossplating with known tester strains and random amplified polymorphic DNA (RAPD) analyses, we have been investigating which intersterility group or groups of *H. annosum* are present on conifers in southern Ontario and western hemlock (*Tsuga heterophylla*) in British Columbia. All of 36 isolates collected in southern Ontario from various coniferous hosts belonged to the P group. Five isolates from western hemlock in British Columbia belonged to the S group.

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INTERSTERILITY GROUPS OF *HETEROBASIDION ANNOSUM* IN CALIFORNIA: A REAPPRAISAL OF HOST SPECIFICITY, HYBRIDIZATION, AND INTERGROUP GENE FLOW. M Garbelotto, A. Ratcliff, F. Cobb, W. Otrosina, and T. Bruns, Dept. of ESPM, University of California, Berkeley CA, and USFS PSW Research Station, Albany CA.

The reported host specialization of the S and P intersterility groups (ISGs) of *Heterobasidion annosum* is of great importance from ecological and management perspectives and it has never been broadly tested in the field. We devised a PCR method to differentiate the two ISGs called Taxon Specific Competitive Priming (TSCP) PCR . We typed 520 live isolates and dry basidiocarps from 202 trees and 109 stumps in 54 sites over 8 National Forests. All isolates from fir and sequoia stumps or trees were S; isolates from pine stumps were 80% S and 20% P; isolates from pine, incense cedar and western juniper trees were 23% S and 77% P. The recovery of a well-established hybrid genet in a pine center was confirmed by isozyme analysis. The PCR amplification of the mitochondrial ML5-ML6 region was also diagnostic for the two ISGs, but in areas where both fir and pine mortality centers were present, about 10% of S isolates yielded the P-specific fragment. These results indicate the possibility of gene flow in the field between the two ISGs. The presence of S isolates on trees regarded as exclusive P hosts broadens the potential host range of this ISG.

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LEPTOGRAPHIUM PROCERUM IN FIELD-GROWN *PINUS STROBUS* ONE AND TWO YEARS FOLLOWING ARTIFICIAL INOCULATION. J. A. Gray and S. M. Salom. VPI&SU, Blacksburg, VA. 24061-0330.

Procerum root disease of *P. strobus* is most common in trees >6 yrs old, yet *L. procerum* can be isolated from nearly 100% of pales weevil feeding wounds on 2-yr-old seedlings in Christmas tree plantations. This suggests that several years may elapse between inoculation and symptom expression. Field studies were initiated to monitor annual *L. procerum* growth and disease development in *P. strobus*. Trees were inoculated at the base of the stem with artificially infested weevils or with fungal mycelium, and isolations made from the bark and sapwood 1 yr (6- and 8-yr-old trees) and 2 yr (2-, 6-, and 8-yr-old trees) following inoculation. At 1 yr, *L. procerum* was isolated from the bark of 60-73% of weevil-inoculated trees and 93% of mycelial-inoculated trees. It has not been recovered from sapwood, nor from trees control-inoculated with malt extract agar. Second year results will be reported.

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QUANTITATION OF CHITINASE AND ß-1,3-GLUCANASE IN BARK OF AMERICAN AND CHINESE CHESTNUT. L. Shain and R.J. Spalding, Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.

Native protein was extracted from bark of four stems each of American and Chinese chestnut that were either fresh frozen upon collection or incubated for five days in ethylene. Extracts were assayed for chitinase and ß-1,3-glucanase activities by standardized colorimetric techniques. Hydrolase activities were greater in extracts from ethylene-treated Chinese chestnut bark than in comparable extracts from American chestnut bark, although ethylene increased activities in both species as compared to fresh-frozen controls. Chitinase activity increased from means of 9.8 to 183.8 and 8.1 to 479.3 pkat/mg bark, whereas ß-1,3-glucanase activity increased from means of 22.2 to 37.7 and 22.4 to 143.5 fkat in American and Chinese chestnut, respectively. Protein extracts from Chinese chestnut inhibited the chestnut blight fungus more than extracts from similar amounts of bark from American chestnut.

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EFFECTIVENESS OF TRENCHING TO SUPPRESS OAK WILT IN LIVE OAK STANDS OF CENTRAL TEXAS. E. Gehring[1], R. Billings[1], and D. N. Appel[2], [1]Texas Forest Service, P. O. Box 15083, Austin, TX. 78761 and [2]Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

Trenching around expanding oak wilt centers is the cornerstone of the Texas Oak Wilt Suppression Project. This review compares the post suppression evaluations (PSE) of 745 trench barriers installed between 1988 and 1994 to determine their efficacy. The centers were compared by calendar year of trench installation, time after installation to breakout, (oak wilt symptoms found immediately beyond the trench), breakouts per 1,000 feet of trench, mo of treatment, and type of trenching equipment. Overall, only 170 of the 745 trenches had breakouts during a PSE. Fewer centers treated in 1991 (30%) and 1992 (25%) had a breakout as compared to centers treated in 1989 (51%) and 1990 (52%). The majority of the breakouts occurred between 12 and 24 mo after installation. When compared by breakouts per 1,000 ft of trench at 12-mo intervals after installation, fewer breakouts were observed after 1991. Trenches installed during April and May had the lowest percentage of breakouts. The fewest breakouts (16%) were on trenches made with backhoes and rocksaws.

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INOCULUM CONCENTRATION AFFECTS PYCNIAL AND AECIAL SPORULATION ON PINE SEEDLINGS INFECTED BY BASIDIOSPORES OF *CRONARTIUM QUERCUUM F. SP. FUSIFORME*. R. A. Schmidt and T. Miller. University of Florida, Gainesville 32611.

Pine seedlings with fusiform rust galls resulting from an inoculum concentration (IC) study were observed for pycnial and aecial sporulation. A maximum of 10 galled seedlings/pine family/IC of 50, 100, 1000, 5000 basidiospores/seedling were observed on 7 slash and 6 loblolly pine families. Across all families, the percentages of seedlings producing only pycnia was inversely related to the IC in both pine species. Slash pine had 72.4, 61.7, 20.0 and 16.7% of the seedlings with only pycnia for IC's 50, 100, 1000 and 5000, respectively. Results were similar for loblolly pine. Across all families, the percentage of seedlings producing only aecia was directly related to IC's in both pine species. Slash pine had 6.3, 10.0, 33.3 and 50.0% of the seedlings with aecia only for IC's of 50, 100, 1000 and 5000, respectively. Loblolly results were similar. Assuming anastomoses in rust galls of hyphae with 1N nuclei of compatable mating types, these findings may be explained by the differential probability among IC's, of mycelial interactions resulting in dicaryotization of aecial primordia in the absence of pycnial sporulation.

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FIRST REPORT OF PARASITISM OF BALSAM AND FRASER FIR CHRISTMAS TREES BY *PYRULARIA PUBERA*. S. Clark Haynes[1], William L. MacDonald[2] and Lytton J. Musselman[3]. [1]West Virginia Dept. of Agr., Charleston, WV 25305, [2]Plant & Soil Sciences, West Virginia Univ., Morgantown, WV 26506 and [3]Dept. of Biological Sciences, Old Dominion Univ., Norfolk, VA 23529.

Branch dieback and mortality of fir Christmas trees (*Abies balsamea* and *A. fraseri*) have been observed in a Mercer Co., WV tree plantation during the past five years. The 2-6 year-old trees were planted on a site converted from a hardwood forest 12 years earlier. Repeated isolations from symptomatic portions of the trees yielded only saprophytic fungi. Excavation of the fir root systems revealed numerous haustoria and a second associated root network that was traced to oilnut (*Pyrularia pubera*), an endemic phanerogamic root parasite growing throughout the plantation. Although the relationship between the symptoms and parasitism by oilnut has not been established, similar parasitism has been shown to cause comparable damage to Canadian hemlock (*Tsuga canadensis*) in KY.

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A YEAST-DERIVED RNASE THAT CONFERS RESISTANCE TO MULTIPLE PLANT VIRUSES. B. Köhm, D.G. Guerra, T.M. Mowry and P.H. Berger, University of Idaho, Moscow, ID 83844-2339.

Tobacco and potato plants were genetically transformed with a gene originally derived from the yeast, *Schizosaccharomyces pombe*, that encodes an enzyme with activity against dsRNA. Transformed potatoes of cv. Ranger Russet were challenged with either potato leafroll virus (PLRV) or potato virus Y (PVY), and evaluated for current season infection on the basis of symptoms and for virus titer after a winter grow-out test. Several clones tested had no PLRV or PVY symptoms, and the grow-out test confirmed these observations. Tobacco plants, also transformed with this gene, were challenged with cucumber mosaic virus, PVY, potato virus X (PVX) (alone or in combination with PVY), tobacco etch virus, tobacco mosaic virus or tobacco rattle virus. Resistance was observed to all viruses tested. These results, and further characterization of transgenic plants will be discussed in the context of broad-range plant virus resistance.

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A virus-based vector system for evaluating the usefulness of geminivirus gene constructs in conferring pathogen-derived resistance. E.P. Broglio, A.A. Abouzid*, E. Hiebert*, and C.A. Powell

University of Florida A.R.E.C., Fort Pierce, Florida 34945 and *University of Florida, Gainesville, Florida 32611.

The TMV-based vector system pTB2 is being used to express tomato mottle virus (TMoV) genes in order to evaluate their ability to provide pathogen-derived resistance to TMoV. Experimental procedures include cloning of TMoV genes into the TB2 vector, inoculating TB2 transcripts to test plants, TMoV challenge of TB2 infected plants, and measurement of TMoV levels. Infectious TB2 vector constructs under evaluation include the viral replicase (AC1), coat protein (AV1), the AC2 and AC3 genes of TMoV in both plus and minus orientation, as well as frame-shift mutants of the AC1 and AV1 genes. The use of a virus-based vector system allows the rapid screening of a large number of potential resistance-conferring genes. Once identified, resistance-conferring constructs can be used to make virus-resistant transgenic plants by traditional methods.

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SEQUENCE ANALYSIS OF THE 3'-ENDS OF AGROPYRON MOSAIC VIRUS AND HORDEUM MOSAIC VIRUS. R. French and N. L. Robertson. USDA-ARS, Department of Plant Pathology, University of Nebraska, Lincoln NE 68583.

Agropyron mosaic virus (AgMV), hordeum mosaic virus (HoMV), and wheat streak mosaic virus (WSMV) all infect wheat and are members of the eriophyid mite-transmitted *Rymovirus* genus of the *Potyviridae*. To more precisely characterize the relationships among these viruses, 3'-terminal cDNAs of AgMV and HoMV (ca. 2 kb) were generated by RT-PCR, cloned and sequenced. The nucleotide sequence of AgMV was 70% identical to HoMV and the deduced amino acid sequences shared 76% identity but were only ~27% identical to WSMV. Unlike WSMV, AgMV and HoMV showed remarkable sequence similarity to aphid-transmitted potyviruses. Amino acid sequences of AgMV and HoMV corresponding to a portion of the NIb protein were, respectively, 63% and 65% identical to plum pox virus while the last two-thirds of the coat protein regions were 56% identical. Thus, members of the *Rymovirus* genus vary widely in their affinities to other potyviruses.

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SEQUENCE DETERMINATION AND PHYLOGENETIC ANALYSIS OF VIROID-LIKE RNAs ISOLATED FROM SOLANUM CARDIOPHYLLUM IN MEXICO. J.A. Galindo[1], J.P. Martinez-Soriano[1], I. Yucel[2], C.J.M. Maroon[2,3], and T.O. Diener[2,3]. [1]Colegio de Postgraduados, Montecillo, Mex. Mexico; [2]Center Agric. Biotech.; [3]Dept. Plant Biol., University of Maryland, College Park, MD

To identify the possible origin of potato spindle tuber viroid (PSTVd) which was originally isolated from commercial potatoes, wild Solanum cardiophyllum (local names: papita güera and cimantli) were collected from the central part of Mexico and assayed for production of viroid-like symptoms in tomato plants inoculated with leaf extracts. Dot blot hybridization analysis of nucleic acid preparations from six samples of symptomatic tomato tissue, designated OG1 to OG6, indicated the presence of viroid-like RNAs. Sequence determination of cDNA clones of these isolates revealed that they are not identical and that each of the six groups consists of one or more variants. Furthermore, the sequences are not identical to PSTVd or TPMVd. Phylogenetic analysis using maximum parsimony of the OG isolates, together with PSTVd and TPMVd, will be discussed.

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BEET WESTERN YELLOWS VIRUS AND A NEW LUTEOVIRUS ASSOCIATED WITH CHICKPEA STUNT DISEASE IN INDIA. R.A. Naidu, M.A. Mayo[1], S.V. Reddy, C.A. Jolly[1], and L. Torrance[1]. ICRISAT Asia Center, Patancheru, India 502 324; [1]Scottish Crop Research Institute, Dundee DD2 5DA, UK.

Chickpea stunt is an important virus disease of chickpea in the Indian sub-continent. Disease surveys using monoclonal antibodies to potato leafroll and barley yellow dwarf (RPV strain) luteoviruses, showed that more than one virus was present. By using selected oligonucleotide primers and RT-PCR, we have detected sequences encoding luteovirus coat proteins in RNA extract of field-collected diseased plants. Two coat protein sequences were found in the different extracts, suggesting that two viruses were present. One was a strain of beet western yellows virus and the second, which was the more widely distributed, is a new virus as its coat protein sequence was no more than 81% identical to those known for other luteoviruses. In further surveys, a variant of this virus was detected which differed at 14 of 295 nts of the coat protein gene.

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TWO INDEPENDENT MECHANISMS ARE EFFECTIVE IN REPLICASE-MEDIATED RESISTANCE AGAINST CUCUMBER MOSAIC VIRUS
Karl-Heinz Hellwald and Peter Palukaitis, Dept. Plant Pathology, Cornell University, Ithaca, NY 14853

Replicase-mediated resistance is a very effective, though specific strategy to generate resistance against plant viruses. In the case of cucumber mosaic virus, K-CMV has been demonstrated to overcome resistance effective against Fny-CMV. The ability to break resistance was mapped to RNA 2. Chimeric constructs between the full-length cDNA clones of RNA 2 of both strains were generated and inoculated to transgenic plants and protoplasts. Localization of sequence domains needed for resistance breakage and the analysis of protoplast experiments confirmed the existence of two independent mechanisms. The amount of K-CMV sequence in the chimeric construct independent of the location was positively correlated to the ability to accumulate viral RNA in transgenic protoplasts. The ability to infect transgenic plants systemically was mapped to the central polymerase-like domain in RNA 2.

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DIFFERENTIAL TRANSMISSION OF CUCUMBER MOSAIC VIRUS BY TWO APHIDS: MUTATIONS IN THE COAT PROTEIN RESTORE TRANSMISSION BY *APHIS GOSSYPII* BUT NOT BY *MYZUS PERSICAE*
Keith L. Perry, Lee Zhang, and Peter Palukaitis, Cornell University, Ithaca, NY 14853 and Purdue University, West Lafayette, IN 47907

Most strains of cucumber mosaic virus (CMV), such as the Fny strain, are efficiently transmitted by the aphid species *Aphis gossypii* and *Myzus persicae*. The M strain of CMV is transmitted very poorly and this phenotype is a function of the M-CMV coat protein. Mutations were introduced in the coat protein of the transmission deficient M-CMV. Two amino acid changes (Fny-CMV positions 129 and 162) restored partial transmission by *A. gossypii*; three amino acid changes (Fny-CMV positions 129, 162, and 168) restored full transmission (i.e. at the same level as the transmission proficient Fny-CMV). Neither of these modifications to the coat protein rendered the virus transmissible by *M. persicae*. Thus, we have observed a differential transmission of CMV by two species of aphids and we know the specific amino acid determinants in the CMV coat protein which confer this phenotype.

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THE COMPLETE NUCLEOTIDE SEQUENCE OF TOBACCO RATTLE VIRUS ISOLATE 'ORY'. M.R. Sudarshana and P.H. Berger, University of Idaho, Moscow, ID 83844-2339.

Tobacco rattle virus (TRV) is a bipartite rod-shaped virus that causes corky ringspot, a serious disease in potato. We have determined the complete nucleotide sequence of a Pacific Northwest isolate, Oregon Yellow (ORY). This is the first isolate of TRV to have both of its RNAs sequenced. RNA1, 6790 nucleotides long, while highly conserved with RNA1 sequences of other tobraviruses, contained two unexpected stop codons within the first open reading frame. The presence of these stop codons was confirmed with independently-derived clones. *In vitro* translation indicated that these are functional leaky readthrough codons. In contrast, TRV and tobravirus RNA2s are very heterogenous. RNA2 of TRV-ORY is 3262 nucleotides and contains the coat protein cistron and two other open reading frames. The relationship of these to other tobraviruses and structurally related viruses will be discussed.

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CONSERVATION OF AN ELICITOR REGION WITHIN DIFFERENT TOBAMOVIRUS COAT PROTEINS Z. F. Taraporewala and J. N. Culver, Molecular and Cell Biology Program and Center for Agricultural Biology, University of Maryland, College Park, MD-20742.

The coat protein (CP) of most tobamovirus strains acts as an elicitor of the *N'* gene hypersensitive response (HR) in *Nicotiana sylvestris*. In a preceding study we have mapped a putative "receptor binding site" to the right radial (RR) and right slewed (RS) alpha-helices of the U1 CP. Crystallographic and fiber diffraction studies further indicate that all tobamovirus CPs share a well-preserved 3D fold. This suggests that the RR and RS elicitor region may be structurally and functionally conserved among all tobamovirus CPs. To test this possibility, amino acid substitutions were created within the RR and RS helical region of the ondontoglossum ringspot tobamovirus (ORSV) CP, a non-tobacco strain of TMV. These substitutions resulted in the loss of ORSV CP elicitor function at 29°C but not at 25°C. Interestingly, similar mutations in the U1 CP result in the loss of the HR at both temperatures. These data indicate that the proposed "receptor binding site" is conserved between ORSV and U1 CPs, but that other structural differences between the two CPs also influence host recognition. Additional mutations will be created in the CPs of other tobamovirus strains to further test the conservative nature of this elicitor region.

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A MOLECULAR MARKER FOR CITRUS PARASITISM IN THE BURROWING NEMATODE, *RADOPHOLUS* SPP. D. T. Kaplan, C. M. Vanderspool, USDA, ARS, 2120 Camden Road, Orlando, FL 32803 and C. H. Opperman, North Carolina State University, Raleigh.

Genetic and biological characterization of the burrowing nematode sibling species, *Radopholus citrophilus* and *R. similis* is important to development of practical identification and control methods. Results of 30 day host-indexing studies were used to characterize a collection of 14 burrowing nematode strains for citrus parasitism. A 2.4 kb DNA fragment identified as DK#1 is a sequence characterized amplified region (SCAR) associated with citrus parasitism in burrowing nematode strain DK2. A band of comparable size amplified using SCAR-derived primers and mini-prepped DNA was observed in 7 other burrowing nematode strains (*Radopholus* spp.) but not detected in any of 6 non-parasitic citrus strains. When subjected to restriction analysis involving 17 enzymes, the 2.4 kb fragments that co-migrated with DK#1 yielded identical patterns and size products. Southern blot hybridization experiments confirmed the similarity among co-migrating DNA bands.

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VIRAL REPRESSION OF SEX PHEROMONE GENE EXPRESSION OF *CRYPHONECTRIA PARASITICA*. L. Zhang, A. Carpanelli, and N. K. Van Alfen. Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

The filamentous fungus, *Cryphonectria parasitica*, has a viral disease which acts as a biological control for chestnut blight. The virus affects virulence and sporulation of its host by transcriptionally repressing a number of fungal genes. One set of host genes repressed by the virus are those encoding the sex pheromones of this fungus. Pheromone genes from both mating types have been cloned, and these genes have characteristics common to other known fungal pheromone genes. Expression of the genes are mating-type-specific, but silent copies of the genes are present in the other mating type. The presence of silent copies of these genes indicates that mating type switching may be occurring, and if so, it would explain the observations that this fungus can both outcross and self-fertilize. The viral repression of fungal sex pheromone genes results in perturbation of the fungal sexual cycle, and has implications concerning the ability of the virus to move within host populations.

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PHYSICAL AND CHEMICAL CUES LEADING TO APRESSORIUM FORMATION IN *Magnaporthe grisea*. Robert D. Gilbert and Ralph A. Dean, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634

Infection of rice by *Magnaporthe grisea* begins when the conidium attaches to the leaf surface and germinates. The germ tube grows until the tip attaches to the substratum and differentiates into a highly melanized appressorium. Several environmental cues are involved in triggering appressorium formation. Using a novel bioassay, we have found that specific plant cutin monomers and lipid components induce appressorium formation, even at nanomolar concentrations. Appressorium induction was greatly affected by chain length and the ability of the molecule to form terminal hydrogen bonds. Thigmotrophic effects were also studied and hydrophobicity alone was found to be sufficient to induce appressorium formation in a variety of *M. grisea* strains. A model for infection initiation *in planta* is presented.

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DEGRADATION OF HARPIN BY APOPLASTIC PROTEASE ACTIVITY. R.J. Laby, and S.V. Beer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Apoplastic washing fluids from tobacco, tomato, apple, cotoneaster, and raspberry were each tested for the presence of proteolytic activity. Harpin, a secreted hypersensitive-response (HR) elicitor from *Erwinia amylovora*, was incubated with the fluids, followed by SDS-PAGE analysis to determine whether a 44 kD band, representing full-length harpin, remained. The washing fluids from all species tested contained proteolytic activity, although an activity-stained gel indicated that not all activities share an identical apparent molecular weight. Interestingly, fragments of harpin created in these assays retained their HR-eliciting activity following infiltration into tobacco. However, the pH and temperature optima of the proteolytic activities suggest that they are not highly active under conditions conducive to disease (fire blight) development in *E. amylovora* host plants. Thus the role, in the bacterial-plant interaction, of proteases that might affect harpin is unclear.

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CLONING OF THE CONSERVED REGION OF SOYBEAN 3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE (HMGR) cDNA AND CHARACTERIZATION OF DIFFERENTIAL EXPRESSION OF SOYBEAN *hmg* INDUCED BY SOYBEAN MOSAIC VIRUS (SMV). Xueshu Yu, Chang W. Choi, Heesung Park, Sue A. Tolin, & Carole L. Cramer, Dept. Plant Path., Physiol. & Weed Sci., VPI & SU, Blacksburg, VA 24061-0330

3-Hydroxy-3-methylglutaryl CoA reductase (HMGR) catalyzes the rate-limiting step in biosynthesis of isoprenoids including terpenoid phytoalexins. In soybean, isoflavanoid phytoalexins are considered the predominant form of defense-related antibiotics. Using reverse transcription PCR, we cloned a 494bp cDNA fragment of soybean HMGR, representing the first HMGR sequence from a legume. The soybean *hmg*1 sequence has high homology to all known *hmg* genes. Southern analysis and PCR of genomic DNA indicate that HMGR is encoded by a multigene family in soybean. Northern analysis of mRNA from SMV-inoculated leaves suggest that HMGR gene expression in soybean correlates with *Rsv* resistance to SMV. Soybean *hmg* expression was strongly activated 24h after inoculation with symptomless resistance (R response), had an intermediate level of activation with necrosis (N response), and showed only background levels of expression with systemic mosaic symptoms (S response) and mock-inoculated controls. These results indicate that HMGR plays a role in soybean disease resistance.

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ISOLATION AND CHARACTERIZATION OF cDNA CLONES FOR GENES INDUCED DURING THE DEFENCE RESPONSE OF TOBACCO TO TOBACCO MOSAIC VIRUS. Ailan Guo and Daniel F. Klessig, Waksman Institute, Rutgers University, Piscataway, NJ 08855.

Accumulation of salicylic acid and induction of pathogenesis-related (PR) gene expression are correlated with the hypersensitive response in tobacco plants infected with tobacco mosaic virus (TMV). To gain insights into mechanisms of resistance and to identify potential components of the signal transduction pathway(s) leading to resistance, seven clones have been isolated by differential screening of a cDNA library constructed with RNA from TMV-infected tobacco leaves. Northern analysis showed that all these clones are induced in TMV-infected leaves. Two of these clones encode ethylene-forming enzymes: 1-amino-cyclopropane-1-carboxylate (acc) deaminase and acc oxidase. One encodes 3-hydroxy-3-methylglutaryl(HMG)-coenzyme A reductase. One clone belongs to, but is distinct, from previously identified β -1,3 glucanase (PR-2) genes. Three other clones do not share homology with DNA sequences in the GenBank data base. Sequencing and further characterization of these clones are currently underway.

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OXALIC ACID IS NOT THE SOLE PATHOGENIC DETERMINANT IN Sclerotinia minor ON PEANUT AND LETTUCE. X.Li, H.A.Melouk, J.P.Damicone, and K.E.Jackson. Department of Plant Pathology and USDA-ARS, Oklahoma State University, Stillwater, OK 74075.

Eleven isolates of Sclerotinia minor, including one non-sclerotial forming isolate (9M-N) were used to study the relationship between pathogenicity and oxalic acid production. Oxalate production by all isolates was measured in potato dextrose broth (PDB), in soil, on lettuce leaves and on peanut stems by quantitative enzymatic analysis (Sigma Diagnostics, St.Louis, MO). All isolates produced oxalate, and all were pathogenic to peanut except for 9M-N. All isolates produced oxalate and were pathogenic on detached Romaine lettuce leaves, except 9M-N which was less virulent. The data suggest that oxalic acid is not the sole pathogenicity factor in S.minor on either peanut or lettuce.

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PRODUCTION OF A 24 KDA ELICITOR FROM *FUSARIUM OXYSPORUM* BY FUNGAL ISOLATES AND ITS BIOLOGICAL ACTIVITY AMONG PLANT SPECIES. Bryan A. Bailey, BPDL, USDA, ARS, Beltsville, MD 20705.

A 24 kDa protein which elicits ethylene production and necrosis in leaves of *Erythroxylum coca* was purified from culture filtrates of an isolate of *F. oxysporum* pathogenic to *E. coca*. Polyclonal antisera to native and denatured forms of the 24 kDa protein detected the purified 24 kDa protein on western blots. Among the six *Fusarium* species tested, only *F. oxysporum* isolates produced an antigenically related 24 kDa protein in culture filtrates. The antisera cross-reacted with a 24 kDa protein on western blots of culture filtrates from six of seven *F. oxysporum* formae speciales tested. Several of the breakdown products of the 24 kDa protein produced by heating were recognized by antisera to the denatured 24 kDa protein. The 24 kDa protein induced ethylene production and/or necrosis in a wide variety of plant species. It remains to be determined if the 24 kDa elicitor plays a role in disease development in the *F. oxysporum* interaction with plants.

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DEVELOPMENT OF A TWO-COMPONENT SYSTEM TO ENGINEER NEMATODE RESISTANCE. S Ohl, O Goddijn, F vd Lee, J Klap, J Spiegeler and PC Sijmons. MOGEN International nv, Einsteinweg 97, 2333 CB Leiden, The Netherlands.

Sedentary nematodes have evolved a complex, highly regulated interaction with their plant hosts leading to the formation of nematode feeding structures (NFS) which the nematodes absolutely depend on to complete their life cycles. To engineer nematode resistance by disrupting the NSF we have developed a two-component strategy based on the barnase/barstar system. In this system, a NFS-active promoter confers local expression of the ribonuclease barnase. Leaky expression of barnase in other parts of the plant is neutralized by the concomitant expression of barstar, a highly specific barnase-inhibitor. By analyzing promoter-*gus*A fusions in *Arabidopsis* after infection with *Heterodera schachtii* a number of well known promoters, such as CaMV 35S and RolD, were found to be down-regulated in NFS. Using a gene-tagging strategy, plant promoters were identified which confer fairly specific expression inside the NFS. These regulatory sequences were isolated, recloned in GUS cassettes and transformed back to *Arabidopsis* to confirm their specificity in nematode infection assays. Furthermore, we present first data indicating that the system confers increased resistance in *Arabidopsis* against the beet cyst nematode *Heterodera schachtii*.

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MUTATIONAL ANALYSIS OF GENES INVOLVED IN REPLICATION OF BARLEY YELLOW DWARF VIRUS. B.R.Mohan, S.P.Dinesh-Kumar[1], and W.A.Miller. Plant Pathology Department and MCDB Program, Iowa State University, Ames, Iowa 50011; [1]Present address: USDA Plant Gene Expression Center, Albany, CA 94710.

The roles in replication and viral assembly of different barley yellow dwarf virus gene products and *cis*-acting signals were investigated in oat protoplasts using deleted and mutated transcripts. Of the six open reading frames (ORFs), only ORFs 1 and 2, which encode proteins containing putative replicase domains, were essential for viral replication. Deletion of the coat protein gene reduced the accumulation of genomic RNA but had no effect on subgenomic RNA levels. Nuclease assay established a role for coat protein in protecting the genomic RNA. Deletions and mutations that knocked out expression of ORFs 5 and 6 respectively did not affect viral replication. The replication of the replication-incompetent, deleted RNAs was not supported in *trans* by the coinoculated wild type helper genome, indicating that the replication of BYDV RNA may be *cis*-preferential.

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TOMATO BUSHY STUNT VIRUS MOVEMENT GENES CAN ACTIVATE HOST RESPONSES THAT CONFINE VIRUS SPREAD.
H.B. Scholthof, K.-B. G. Scholthof, and A.O. Jackson*. Dept. of Plant Pathology and Microbiology, Texas A&M Univ., College Station, TX 77843, and *Dept. of Plant Biology, University of California, Berkeley, CA 94720.

In this study we focused on the role of two nested genes at the 3' end of the single stranded RNA genome of tomato bushy stunt virus (TBSV). For this purpose bio-assays were performed with infectious transcripts from wild-type and mutagenized TBSV cDNA plasmids, combined with the expression of individual TBSV genes from a heterologous virus, potato virus X. The results show that p22 is a membrane bound protein that is responsible for cell-to-cell movement whereas the cytosolic p19 promotes long distance spread in an host dependent manner. In addition, p19 elicits local lesion formation on *N. tabacum*, but p22 can induce this response in *N. glutinosa*. Therefore, expression of p19 and p22 can lead to plant responses that effectively counteract the movement function of these proteins.

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THE EFFECT OF DI RNAs ON TOMBUSVIRUS PROTEIN EXPRESSION AND SYMPTOM DEVELOPMENT. K.-B. G. Scholthof, H. B. Scholthof, and A. O. Jackson*. Dept. of Plant Pathology, Texas A&M University, College Station, TX 77843 and *Dept. of Plant Biology, Univ. of California, Berkeley.

Tombusviruses, of which tomato bushy stunt virus (TBSV) is the type member, encode the replicase (p33/p92) proteins from the genomic template and the coat protein (p41) and nested proteins (p19/p22) from two subgenomic (sg) RNAs. The nested proteins are implicated in TBSV movement and host response to infection. Tombusviruses accumulate defective interfering (DI) RNAs which result in amelioration of severe symptoms and reduction of virus accumulation in systemically infected plants. It is unclear if this reduction is due to a general suppression of virus replication, or if other regulatory events play a central role. Time course studies in plants revealed that the DI RNAs suppressed the accumulation of the genomic template and caused an even more substantial reduction of the sgRNAs. Immunoassays revealed a reduced replicase accumulation and almost a complete suppression of the proteins encoded from the sgRNAs. The protective effect of DI RNAs may be due to selective inhibition of p19 expression as well as reduction of the replication of the genomic RNA.

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REPLICASE-MEDIATED RESISTANCE CONFERRED BY THE TOBACCO MOSAIC VIRUS 54-KDA GENE CAN BE OVERCOME BY CHIMERIC VIRUSES. M. E. Sekiya and M. Zaitlin, Dept. of Plant Pathology, Cornell Univ., Ithaca, NY 14853.

Tobacco plants transformed with the 54-kDa putative replicase gene of tobacco mosaic virus are resistant to the U1 strain of tobacco mosaic virus but are susceptible to the L strain of tobacco mosaic virus, also known as tomato mosaic virus. Chimeric viruses were constructed between these two viruses using full-length genomic clones and the resulting chimeras were tested for the ability to break resistance. Resistance breaking mapped to the replicase genes of the L virus, but more than one region of the replicase was found to confer this trait. Chimeras differed in the timing of symptom development on the systemic leaves of transgenic plants and these differences were related to a reduction of viral RNA accumulation in inoculated leaves. Preliminary studies indicate that chimeras differ in their ability to replicate in protoplasts, suggesting that results with the inoculated leaves are due primarily to a suppression of replication rather than cell-to-cell movement. Replicase-mediated resistance conferred by the 54-kDa gene appears to be complex, involving more than one phenomenon, since the ability to break resistance does not map to a single region of the genome. Conclusions about the mechanism of viral resistance will be discussed.

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EXPRESSION OF A POLYCISTRONIC mRNA IN YEAST, *SACCHAROMYCES CEREVISIAE*, MEDIATED BY A PLANT VIRUS TRANSLATIONAL TRANSACTIVATOR Y. Sha, E. P. Brogliot, J. Cannon*, and J. Schoelz, Dept. of Pl. Path. and *Dept. of Mol. Micro. and Immun., Univ. of MO., Columbia MO 65211; †Univ. of Fl, Ft. Pierce, FL 34945

The gene VI product of cauliflower mosaic virus (CaMV) is necessary for the post-transcriptional expression of CaMV genes I - V . As a preliminary step towards identifying host proteins that interact with the gene VI product, we have demonstrated that the gene VI product can transactivate translation of a polycistronic mRNA in the bakers yeast, *Saccharomyces cerevisiae*. A yeast plasmid JS161 was constructed in which the CAT reporter gene was inserted in-frame into gene II of the CaMV genome. Only a very low level of CAT activity was detected in yeast transformed with JS161. To investigate whether the gene VI product would mediate an increase in CAT activity, we cotransformated yeast with JS161 and JS169, which carried gene VI under control of the yeast galactose-inducible promoter *GAL1*. Upon induction with galactose we found that CAT activity in yeast transformed with JS161 & 169 was 19 times higher than the level in the JS161 alone.

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ANALYSIS OF PAPAYA RINGSPOT VIRUS ISOLATES DIFFERING IN PATHOGENICITY ON TRANSGENIC PAPAYA. P. Tennant[1], M. Fitch[2], R. Manshardt[3], J. Slightom[4], and D. Gonsalves[1]. [1]Plant Pathology Dept, Cornell University, Geneva, NY 14456. [2]USDA-ARS, Aeia, HI 96701. [3]Horticulture Dept, University of Hawaii, Honolulu, HI 96822. [4]Upjohn Company, Kalamazoo, MI 49001.

Previous investigations with the transgenic papaya line 55-1 expressing the coat protein gene (CP) of the mild papaya ringspot virus strain from Hawaii (PRV HA 5-1) showed differential reactions to 12 PRV isolates from different geographical regions. The reactions included complete resistance, delay in symptom expression and symptom attenuation, and a shorter delay in symptom expression but no symptom attenuation. Representative PRV isolates of each group from Florida, Jamaica, and Thailand, respectively, were selected for biological and serological analysis. The isolates were serologically similar to PRV HA 5-1 but differed biologically in virulence on papaya. The CP of these isolates have been cloned and the N terminus sequences are being determined. Other transgenic PRV HA 5-1 lines are being tested with these isolates.

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PROBING BROME MOSAIC VIRUS PROTEIN-PROTEIN INTERACTIONS WITH THE YEAST TWO-HYBRID SYSTEM. Z. H. Wang and R. French. Department of Plant Pathology, University of Nebraska, and USDA-ARS, Lincoln NE 68583.

The tripartite RNA genome of brome mosaic virus (BMV), a small isometric virus of cereals, encodes three nonstructural proteins designated 1a, 2a, and 3a. Proteins 1a and 2a are required for viral RNA replication and the 3a protein is required, but not sufficient, for systemic virus movement. As a prelude to examine BMV and host protein-protein interactions, fusion proteins were constructed in yeast two-hybrid vectors. A previously identified *in vitro* interaction between BMV 1a and 2a proteins (Kao *et al.*, 1992, *J. Virology* **66**: 6322-29) was also revealed by *in vivo* two-hybrid assays. In addition, similar assays provided evidence of self-interaction between a central domain of the 1a protein, and of interactions between a C-terminal domain of 2a protein and the 3a protein N-terminus. The latter interaction is of interest in light of the observation that small deletions at the C-terminus of 2a abolish cell to cell movement without affecting RNA replication, and suggest novel targets for control of virus infection. *In vivo* and *in vitro* studies are underway to better map and characterize interacting domains.

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INFECTION OF CUCUMBER MOSAIC VIRUS REPLICASE-RESISTANT TRANSGENIC PLANTS CAN OCCUR VIA THE VASCULAR SYSTEM. W. M. Wintermantel, N. Banerjee, K. L. Perry, and M. Zaitlin. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Transgenic Samsun NN tobacco plants expressing a truncated form of the Fny-CMV 2a replicase gene exhibit a high level of resistance to cucumber mosaic virus (CMV) when inoculated mechanically or by insect vectors. The resistant plants are infectible, however, when in a graft union with an infected plant. The infectious entity can also move quickly through intergrafts of resistant tissue, suggesting that it may move without replicating in the vascular system. This resistance breaking is not a consequence of the selection of virus mutants, since virus isolated from plants in which resistance has broken will not infect seedlings of the resistant plant lines. Progeny of five transgenic plants in which resistance was broken were challenged with CMV; complete resistance was maintained in 4 of the 5 sets of progeny. The results obtained to date are consistent with our earlier observation that in addition to an inhibition of virus replication, replicase mediated resistance to CMV also involves an inhibition of both cell-to-cell and long distance movement. Further experiments involve examining transgenic resistant plants and transgenic plants in which resistance has broken at the DNA and RNA levels.

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EXPRESSION OF BARLEY YELLOW DWARF OR WHEAT STREAK MOSAIC VIRUS COAT PROTEINS IN TRANSGENIC WHEAT. J. L. Hansen[1], P.S. Shiel[1], R.S. Zemetra[1], P.L. McCarthy[1], S.D. Wyatt[2], and P.H. Berger[1], [1]University of Idaho, Moscow, ID 83844-2339 and [2]Washington State University, Pullman, WA 99164.

cDNA clones containing coat protein (CP) genes of either barley yellow dwarf virus (BYDV-PAV) or wheat streak mosaic virus (WSMV) were used for microprojectile bombardment-mediated plant transformation of soft white winter wheat. Two cultivars (Daws and Lambert) were successfully co-transformed, using the *bar* gene as selectable marker. Putative transgenics were assayed by a herbicide resistance bioassay, Southern analysis, PCR assay and western blot analysis. Approximately 50% of the bar+ plants were co-transformed. The selectable marker and CP genes segregated independently in progeny of primary R_0 co-transformants. R_1 progeny have been inoculated with the appropriate virus and results of these tests will be discussed.

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MOLECULAR CHARACTERIZATION AND DETECTION OF SOUR CHERRY GREEN RING MOTTLE VIRUS. Yunping Zhang[1], Jerry K. Uyemoto[2], and Bruce C. Kirkpatrick[1]. [1]Department of Plant Pathology and [2]USDA-ARS, University of California, Davis, CA 95616

Green ring mottle virus (GRMV) causes a serious disease of sour cherry trees which produce deformed, unmarketable fruit. Other susceptible Prunus species include peach, apricot, sweet cherry and flowering cherry. Current detection is by graft indexing on indicator plants which takes several months to complete. Based on cytopathology and virion properties, GRMV has been tentatively classified as a closterovirus, however additional molecular genetic data are needed to confirm this provisional classification. In order to better understand the taxonomic relationship of GRMV to other plant viruses we have cloned and partially sequenced a 10+ kb dsRNA from a GRMV strain originally obtained from a Shirofugen flowering cherry tree in California. Hybridization analysis showed that the California GRMV was related to GRMV isolates from Washington and Canada. Several sets of polymerase chain reaction (PCR) primers were synthesized using sequence information obtained from the GRMV cDNAs. Some of the PCR primer pairs appeared to be universal in that they amplified products from all GRMV strains tested while other PCR primers only amplified specific strains. Results of the sequence analysis of GRMV genome will be presented.

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REASSORTANTS OF TOMATO SPOTTED WILT *TOSPOVIRUS* EXPRESS VARIOUS PHENOTYPES

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Tomato spotted wilt *tospovirus* (TSWV), a member of the Bunyaviridae, has a broad natural host range. In addition, natural isolates are highly variable in symptom type and intensity with the loss of some phenotypic characteristics during mechanical transfers. TSWV's tripartite genomic organization provides the opportunity for phenotypic variations when the RNA segments reassort. To further investigate this phenomenon, reassortants were generated by coinoculation of parental isolates, TSWV-D and TSWV-10, and authenticated by single lesion transfers and with specific genomic segment markers. Reassortants varied from parental isolates in various phenotypic characteristics such as lesion morphology, systemic movement and ability to overcome transgene(N) resistance to TSWV.

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PARTIAL MOLECULAR CHARACTERIZATION OF SEVERAL FUROVIRUSES OF SUGAR BEET FROM THE U.S.A. G. C. Wisler, H.-Y. Liu, and J. E. Duffus. USDA-ARS, Salinas, CA 93905.

Two beet necrotic yellow vein virus (BNYVV) isolates (CA,ID) and four isolates (two each from TX and NE) that are serologically related to beet soil-borne mosaic virus (BSBMV) were compared for size, polyadenylation, cross-hybridization and number of RNA components. RNA-1, -2, and -3 of the two BNYVV isolates were the expected sizes of ca. 6.7, 4.7, and 1.8 kb, respectively. The RNA-1 and-2 from BSBMV of TX were similar to those of BNYVV, but RNA-3 was smaller, at ca. 1.4 kb. An isolate serologically related to BSBMV from NE had RNA-1, -2, and -3 identical in size to BNYVV, but had an additional RNA-4 of ca. 0.9 kb. A new isolate from NE, serologically identical to BSBMV but with a host range wider than that of other sugar beet furoviruses, had an RNA pattern identical to BNYVV. Probes from RNA-1 and -2 of BNYVV were specific to the BNYVV isolates. All RNAs of all isolates examined were polyadenylated, thus are more like BNYVV than the type member of the furovirus group, soil-borne wheat mosaic virus.

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CONTROL OF A GEMINIVIRUS COAT PROTEIN PROMOTER: EVIDENCE FOR A REPRESSOR ELEMENT. G. Sunter and D. M. Bisaro, Plant Biotechnology Center and Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210

Tomato golden mosaic geminivirus (TGMV) has a genome divided between two ~2.5 kb DNA components. Most of the factors required for the replication and expression of this small genome are provided by the host. However, viral AL2 protein (transcriptional activator protein; TrAP) is necessary for expression of the coat protein (CP) gene and the BR1 movement gene in tobacco protoplasts. Recent studies have used promoter/reporter fusions to study expression of TrAP-responsive promoters in transgenic plants. Surprisingly, we have found that plants transgenic for a CP promoter/GUS gene show TrAP-independent expression in vascular tissue. When these plants are inoculated with TGMV, GUS expression is also observed in mesophyll tissue. These results confirm that TrAP is necessary to activate the CP promoter in mesophyll cells, and suggest that a factor present in vascular tissue obviates the requirement for TrAP. Interestingly, if a larger CP promoter fragment is used to construct the transgene, all TrAP-independent expression is repressed. The larger promoter still responds to TrAP, as TGMV infected transgenic plants express GUS in all tissues. Deletion analysis is in progress to delimit the sequence responsible for repression of TrAP-independent expression in vascular tissue, as are experiments to identify host proteins that might interact with TrAP and which might be involved in activation and/or repression of the CP promoter.

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CONSTRUCTION OF TRANSGENIC *PSEUDOMONAS FLUORESCENS* Q69c-80 FOR IMPROVED BIOCONTROL ACTIVITY TO TAKE-ALL.

Dal-Soo Kim, Robert F. Bonsall[2], Linda S. Thomashow[2], and David M. Weller[2]. Washington State University and USDA-ARS, Pullman, WA 99164-6430

Transgenic pseudomonads containing a heterologous 6.5-kb 2,4-diacetylphloroglucinol (Phl) biosynthetic locus on their chromosome were constructed for each of seven biocontrol *Pseudomonas* strains. Transgenic derivatives of one strain, *Pseudomonas fluorescens* Q69c-80 varied greatly in both Phl production as determined by high performance liquid chromatography and ability to inhibit growth of the take-all fungus, *Gaeumannomyces graminis* var. *tritici*. Overproduction of Phl by the transgenic derivatives of Q69c-80 resulted in unstable colony morphology and was lethal to bacterial growth in culture. Some transgenic derivatives of Q69c-80 required lower inoculum doses on seed than the parent to achieve the same level of take-all suppression. However, reducing the inoculum doses was necessary due to their deleterious effect on wheat emergence.

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SUPPRESSION OF PYTHIUM ROOT ROT OF WHEAT BY *PSEUDOMONAS CORRUGATA*. W. Chun and Y. Gao. Plant Pathology Division, PSES, University of Idaho, Moscow, ID 83844-2339.

Susceptibilities of four wheat cultivars (Lambert, Lewjain, Madsen, and Stephens) to *Pythium irregulare*, *P. sylvaticum*, *P. ultimum* var. *sporangiiferum*, and *P. ultimum* var. *ultimum* were determined in field soil artificially infested with oospores. Madsen was the most tolerant while Stephens was the most susceptible to root rot. Further studies used an isolate of *P. u.* var. *ultimum* that was highly virulent and an isolate of *P. irregulare* that was mildly virulent. Madsen and Stephens seed were treated with 1.5% hydroxypropyl methylcellulose suspensions of *Pseudomonas corrugata* (*Pc*) (approximately 10^8 cfu/seed) and planted in non-sterile field soil containing 100 oospores/gm soil of either *P. u.* var. *ultimum* or *P. irregulare*. Results were similar for all host-pathogen combinations. When compared to controls, *Pc* treatment resulted in an increase in seedling emergence (50% to 75%) and plant fresh weight (49% to 75%). A significant reduction in root symptoms and a corresponding increase in plant height were also noted. Thus, *P. corrugata* may be a useful biological control agent for the suppression of root rot caused by *Pythium* spp.

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INFLUENCE OF CULTURAL CONDITIONS ON SPONTANEOUS MUTATIONS IN *PSEUDOMONAS FLUORESCENS* CHA0. Brion K. Duffy, Geneviève Défago, Institute of Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich.

The efficacy of biocontrol agents may be reduced during inoculum production due to mutation in genes important for disease suppression, such as regulators of antibiotic production (eg. *gacA*, *apdA*). Mutations in CHA0wt that confer an orange-enlarged colony phenotype occur at high frequency (to 10^{-2}) in nutrient-yeast broth (NYB) during stationary-phase. All mutants overproduce fluorescent siderophores, >80% are protease$^-$HCN$^-$, 47% gacA$^-$, 40% apdA$^-$, and <5% protease$^-$HCN$^+$. No reversion was observed. Accumulation of mutants was reduced 10^1-10^2 fold or 10^2-10^3 fold by addition of $CuSO_4$ or by 1/10 dilution, respectively. Raising the osmotic pressure of dilute media partially reversed this effect. When grown in dual culture, the ratio mutants:wild-type was 1:9 but was reduced to 1:0.9 in dilute NYB. Addition of Co^{2+}, Zn^{2+}, Cu^{2+}, Mo^{6}, Mn^{2+}, or B^{3+} to full NYB similarly reduced the competitiveness of mutants; Mg^{2+}, Na^{2+}, Fe^{2+}, or Ca^{2+} had little or no effect. The hypothesis that the wt produces a compound in NYB which enhances mutant growth or that mutants produce a compound inhibitory to wt growth is being investigated.

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SUPPRESSION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS* IN POTATO PLANTS BY USING *PSEUDOMONAS CORRUGATA*. K. L. Schroeder, and W. Chun, Plant Pathology Division, PSES, University of Idaho, Moscow, ID 83844-2339.

Pseudomonas corrugata (*Pc*) has both antibacterial and antifungal activities *in vitro* and may have use as a biological control agent. Ten isolates of wild-type *Pc* were examined *in vitro* for inhibition of the ring rot pathogen, *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*). Most isolates of *Pc* inhibited growth of *Cms*. *Pc* isolates 0782-6 and 1090-11 were selected for further testing based on their positive and negative abilities, respectively. Each strain was co-inoculated with *Cms* (strain CIC64S) at two different concentrations (approximately 10^4 and 10^6 cfu/g fresh weight) into stems of Russet Burbank and Shepody potato plants. Bacterial populations were monitored biweekly for eight weeks by dilution plating. At the lower starting concentration, the population of *Cms* decreased significantly when in the presence of 0782-6. Higher starting concentrations of *Cms* increased but were reduced 10- to 50-fold with 0782-6. *Cms* populations were not affected in the control and 1090-11 treatments. *Pc* maintained a population of 10^8 cfu/g fresh weight during the experiment. Thus, *Pc* may have a significant impact on ring rot disease of potatoes.

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BIOLOGICAL CONTROL OF BACTERIAL SOFT ROT USING *ERWINIA HERBICOLA* Eh252. J. L. Vanneste, D. A. Cornish, J. Yu, and L. J. Perry-Meyer, Hort Research, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand.

Erwinia herbicola Eh252, a non-pathogenic epiphytic bacterium originally selected for its ability to control fire blight, was found to inhibit on plate all the isolates that were examined of *Erwinia carotovora* pv. carotovora, *E. carotovora* pv. *atroseptica* and *E. chrysanthemi*. These isolates are mainly responsible for bacterial soft rot on tubers in storage. This inhibition on plate was due to production by Eh252 of a peptide antibiotic which has been shown to play a major role in biological control of fire blight. When potato or carrot slices were treated with a suspension of Eh252 prior to inoculation with *E. carotovora* pv. *carotovora*, development of soft rot was inhibited. The level of inhibition was proportional to the level of Eh252. Transposon induced mutants that did not produce the peptide antibiotic did not control soft rot as well as the wild-type strain. Complementation for antibiotic production completely restored the ability of these mutants to reduce incidence of soft rot.

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SUPPRESSION OF CORN DAMPING-OFF BY *GLIOCLADIUM VIRENS* ISOLATE GL-3. W. Mao, C. McCarthy, P. Hebbar, J. Lewis, R. Lumsden, USDA-ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705.

Fermentor-produced fungal biomass of 2 isolates of *Gliocladium virens* (Gl-3 and Gl-21), 1 isolate of *Trichoderma viride* (Tv-1) and peat-based bacterial slurries of 3 isolates of *Burkholderia (Pseudomonas) cepacia* (Bc-A, B, T) were coated onto corn seeds. Coated seeds were sown in a natural soil, artificially infested with a mixture of *Fusarium roseum* var. *graminearum*, *Pythium ultimum* and *P. arrhenomanes*. Fourteen days after planting at 18C in a greenhouse, seedling emergence in the treatment with isolate GL-3 was 30% to 65% higher than other treatments except with Tv-1 (12%). Isolate Gl-3 significantly decreased the severity of root rot and significantly increased growth rate over the pathogen control. These results indicated that *G. virens* isolate GL-3 has great potential to suppress damping-off of field corn. Although some of the other antagonists tested such as strain Bc-B and Tv-1 reduced the disease serverity, they were not as effective as isolate Gl-3.

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BIOLOGICAL CONTROL OF ASPERGILLUS FLAVUS INFECTION OF COTTON BOLLS. I.J. Misaghi, D.M. Decianne, and P.J. Cotty. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721 and USDA/ARS, Southern Regional Research, P.O.Box 19487, New Orleans, LA 70179.

The efficacy of a bacterial isolate (D1), selected from among several hundred bacterial isolates and identified as Pseudomonas cepacia, to reduce A. flavus infection of cottonseed was evaluated in field trials in Yuma, Arizona in 1993 and 1994. The level of A. flavus-infected cottonseed in plants spray-inoculated with a suspension of D1 was reduced by 90 and 81 percent, compared to that in untreated controls in 1993 and 1994, respectively. The differences between D1-treated and non-treated plants were statistically significant ($p < 0.05$) in both years.

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BIOLOGICAL CONTROL OF GRAPE DOWNY MILDEW BY *FUSARIUM PROLIFERATUM*. S.P. Falk, D.M. Gadoury, and R.C. Pearson, Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456, and A. Sztejnberg, The Hebrew University of Jerusalem, Rehovat 76100, Israel.

Fusarium proliferatum (FP) was evaluated for the control of grape downy mildew (*Plasmopara viticola)* on the cultivars Chancellor and Lakemont from 1992–1994. FP or mancozeb (MZ) were applied at 7- or 14-day intervals. Suspensions of FP were prepared by scraping conidia from a mycelial lawn grown on potato dextrose agar and adjusting to $1–2 \times 10^6$ conidia ml^{-1} in 0.02% Tween 20. FP applied at 7-day intervals reduced severity of cluster infections by 70% on Chancellor in 1992 and by 94% on Lakemont in 1994, compared to the untreated control. On Lakemont, FP reduced incidence of leaf infection by 45% in 1992 and by 49% in 1994, and severity of leaf infection by 66% and 78% in 1992 and 1994 respectively. FP applied every 7 days was as effective as MZ at 14-day intervals on Chancellor clusters in 1992.

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AQUEOUS EXTRACTS OF SPENT MUSHROOM SUBSTRATE REDUCE APPLE SCAB INFECTION . Yohalem, D.S.[1], R.F. Harris[2] and J.H. Andrews[1]. 1. Department of Plant Pathology, University of Wisconsin, Madison; 2. Department of Soils, University of Wisconsin, Madison 53706.

Aqueous extracts of spent mushroom substrate (SMS), anaerobically fermented for seven days and amended with spreader-sticker, were applied at weekly intervals to apple trees (cv MacIntosh) from green tip until petal fall and at biweekly intervals through the remainder of the 1994 growing season at two locations in Wisconsin. Extracts prepared from two sources of SMS reduced the leaf area affected by *Venturia inaequalis* (Cke)Winter relative to water and spreader-sticker controls when evaluated on the Horsfall-Barrat scale. Disease incidence was also significantly decreased. The extracts were not as effective in inhibiting disease as captan sprayed at the same intervals. No difference was detected between extracts with and without spreader-sticker. Significantly higher, persistent populations of bacteria, but not of fungi, were detected on leaves treated with the various compost formulations relative to untreated and captan treated leaves.

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SUPPRESSION OF DOLLAR SPOT BY HYPOVIRULENT ISOLATES OF *SCLEROTINIA HOMOEOCARPA*. T. Zhou[1] and G. J. Boland[2]. [1]Agriculture & Agri-Food Canada, Pest Management Research Centre, Vineland Station, ON Canada L0R 2E0. [2]Departmet of Environmental Biology, University of Guelph, Guelph, ON Canada N1G 2W1.

The potential of using hypovirulent isolates of *Sclerotinia homoeocarpa* for suppression of dollar spot disease on creeping bentgrass (*Agrostis palustris*) was evaluated in growth room and field conditions. In growth room, application of sodium alginate pellets containing hyphae of hypovirulent isolate Sh12B to grasses inoculated with virulent isolates Sh48B or Sh14D resulted in significantly ($p=0.05$) lower disease ratings (32.1 or 17.9% reduction, respectively) compared to the virulent control treatments 10 days after application. On swards of creeping bentgrass, dollar spot was significantly ($p=0.05$) suppressed 79.9, 60.0 and 36.1% by applications of isolate Sh12B formulated as mycelial suspension, colonized oatmeal:sand medium, and sodium alginate pellets with the fungal hyphae, respectively, compared to control plots inoculated only with virulent isolate Sh48B. The field experiment was repeated once and similar results were obtained.

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A *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* MUTANT NEGATIVE FOR PRODUCTION OF A DIFFUSIBLE SIGNAL IS ALSO IMPAIRED IN EPIPHYTIC DEVELOPMENT. A. R. Poplawsky and W. Chun. Plant Pathology Division, Dept. P.S.E.S., University of Idaho, Moscow ID 83844-2339.

Strains of *X. c. campestris* with insertion mutations in the *pig*B operon are reduced 80-90% in xanthomonadin and extracellular polysaccharide (EPS) production, and also do not produce the diffusible signal, DEF. Since extracellular DEF restores xanthomonadin and EPS production to *pig*B mutants, it is apparently necessary for full expression of these traits. The *pig*B mutants inoculated into cauliflower showed normal black rot symptomatology. However, epiphytic populations of a *pig*B mutant increased from 5.0×10^4 cfu/g fresh weight to only 3.5×10^6 cfu/g, whereas the parent strain increased to 4.0×10^7 cfu/g over a two week period at 25° C and 85% RH. Under less favorable conditions (25°C and 60% RH), epiphytic populations decreased to 1.8×10^4 cfu/g for the *pig*B mutant, but increased to 2.7×10^6 cfu/g for the parent strain after three weeks. Thus, DEF may be important for epiphytic development of the pathogen. The *pig*B mutant has been restored by gene exchange, and the epiphytic fitness of this strain is currently being tested.

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CHARACTERIZATION OF *ERWINIA AMYLOVORA* STRAINS FROM DIFFERENT HOSTS AND GEOGRAPHIC AREAS. J.-H. KIM, C. H. ZUMOFF, A. TANII*, R. J. LABY and S. V. BEER. Department of Plant Pathology, Cornell University, Ithaca, NY 14853 USA; *Donan Agricultural Experiment Station, Hokkaido 041-12, JAPAN

Eighty-four strains of *E. amylovora* isolated from different hosts in diverse geographical areas were compared for microbiological, molecular biological and plant pathological properties. All strains were tested with the BIOLOG™ system to determinine metabolic capabilities. DNA of several representative strains was hybridized with the cloned *hrp* gene cluster of strain Ea321, and it was tested for ability to direct synthesis of a PCR product from primers derived from the sequence of the reportedly ubiquitous plasmid of *E. amylovora*, pEA29. The strains formed five groups based on all assay results. Strains isolated from pomaceous hosts in North America, Europe, the Middle East and New Zealand formed the largest group. Strains from *Rubus* species from North America formed two distinct groups, as did strains isolated from Asian pear. These results suggest that division of *E. amylovora* at the infrasubspecific level may be justified. The host-specificity of the strains is under investigation.

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CHARACTERIZATION OF ANTAGONISM OF TOMATO RACE 3 STRAINS OF *Xanthomonas campestris* pv. *vesicatoria* TO OTHER STRAINS OF THE SAME BACTERIUM. S. M. Tudor-Nelson[1], J. B. Jones[2], G. V. Minsavage[1], and R. E. Stall[1]. University of Florida, [1]Gainesville, 32611-0680, and [2]Bradenton, 34203-9324.

Zones of inhibition around colonies of the tomato race 3 (T3) strain of *X. campestris* pv. *vesicatoria* (Xcv) oversprayed with strains of tomato race 1 occur on nutrient agar, trypticase soy broth agar, and a minimal medium, but not on King's medium B. Glucose levels in the minimal medium did not affect the size of the zones. On nutrient agar strains of T3 were antagonistic to all of 25 strains of the A group and two of six strains of the B group of Xcv. All of 10 strains of T3 produced antagonism to strains of the A group. Antagonism occurred to five of 14 other pathovars of *X. campestris*. Antagonism did not occur to four other genera of plant pathogenic bacteria. Cell-free extracts of T3 strains were inhibitory and the activity was destroyed at 80 C. Three clones which conferred inhibitory activity similar to a wild type strain were identified from a genomic library of T3 DNA. These clones, when conjugated into a sensitive strain, caused the strain to be antagonistic to other sensitive strains. The results of Southern hybridization and marker exchange experiments with the clones support that more than one antagonistic agent is produced by T3 strains.

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CHARACTERIZATION OF EXOPOLYSACCHARIDE SYNTHESIS BY *ERWINIA AMYLOVORA*. K. Geider, P. Aldridge, S. Bereswill & P. Bugert, Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-69120 Heidelberg, Germany

The causative agent for fire blight on pome fruit trees and other Rosaceae, *Erwinia amylovora*, produces capsules of the acidic exopolysaccharide (EPS) amylovoran. They can be specifically stained with an FITC-labeled lectin. Conditions and assays for formation of amylovoran were established. It is synthesized at all growth stages of the cells well into the stationary phase. Its synthesis is regulated by several *rcs*-genes and factors of the environment such as salt or the presence of sorbitol, typical for rosaceous plants. EPS-synthesis is induced by $CuSO_4$, which can be toxic for the bacteria in some conditions. Amylovoran-deficient mutants were non-pathogenic. A large chromosomal region with genes involved in synthesis of amylovoran was characterized by sequence analysis. Possible gene functions were deduced from homology to protein sequences in data libraries that include assembly, transport, and polymerization of the repeating units.

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HOMOLOGY GROUPS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* (*Xcv*) BASED ON DNA HYBRIDIZATION IN MICROTITER PLATES. H. Bouzar, J. B. Jones, R. E. Stall & J. J. Sudberry. Univ. of Florida, 5007 60th St. E., Bradenton 34203.

Genetic relatedness among representative *Xcv* strains was determined using nonradioisotope-labelled DNA with hybridization performed in a 96-well microtiter plate (Ezaki et al., Int. J. Syst. Bacteriol. 39: 224). Following hybridization of a biotinylated DNA probe to the target DNA, streptavidin-conjugated β-D-galactosidase and a fluorogenic substrate were added, and DNA reassociation was measured using a fluorometric plate-reader. The data confirmed that the nonamylolytic (Amy^-), race T1 group A strains have less than 50% homology with the Amy^+, race T2 group B strains and most likely belong to a different species. The group A strains had high homology (>70%) with both the Amy^+, race T3 strains and a group of Amy^+, race T1 strains that were serologically similar to group A strains. Also, there were apparently at least two more species; these were phenotypically similar to group B strains; one of them included the type strain of *X. c. gardneri*. Thus, it appears that at least four *Xanthomonas* species are pathogenic to tomato and/or pepper.

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ANALYSIS OF PATHOGENICITY OF A *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* B728A Δ*HRPZ*::*NPTII* MUTANT ON BEAN. Amy O. Loniello, James R. Alfano, David W. Bauer, and Alan Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908

Pseudomonas syringae pv *syringae* has been shown to secrete HrpZ via the Hrp secretion (type III) pathway, and the isolated protein is sufficient to cause an HR on tobacco plants. Marker-exchange mutagenesis, using cloned *P. s. syringae* 61 *hrp* genes, was used to construct mutations in the highly virulent bean pathogen B728a. A *hrpK*::ΩSp mutant was unable to secrete HrpZ and was drastically reduced in ability to cause the HR in tobacco. A Δ*hrpZ*::*nptII* mutant was still able to cause HR on tobacco and appeared as virulent as the wild type when infiltrated into bean leaves. However, preliminary results indicate that this mutant is reduced in virulence when inoculated onto the surface of bean leaves. These results indicate that HrpZ is not necessary for pathogenicity on bean and suggest that other proteins involved in pathogenicity are secreted through the Hrp pathway.

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PARTIAL PURIFICATION OF A PUTATIVE PHEROMONE FROM *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*. J. Cui, J. K. Fellman, A. R. Poplawsky, and W. Chun. Plant Pathology Division, PSES, University of Idaho, Moscow, ID 83844-2339.

Xanthomonas campestris pv. *campestris* (*Xcc*) strains with induced or naturally occurring mutations in the *pig*B operon are deficient in the production of xanthomonadin pigments, extracellular polysaccharide (EPS), and a diffusible extracellular factor (DEF). A DEF purification assay was developed based on the ability of the DEF, at low concentrations, to restore pigment and EPS production to *pig*B mutants in a minimal medium. The DEF activity was not affected by heat (100°C for 1 hr), but was sensitive to alkali conditions. Based on ultrafiltration, the molecular weight of the active principle is less than 500. It is soluble in polar solvents but can be partitioned into non-polar solvents when acidified. Ability of the DEF to induce specific responses in other like individuals at low concentrations is typical of bacterial pheromones. Thus, the *Xcc* DEF may play an important role in life cycle processes of this pathogen. Further studies will be focused on final purification and structural analysis of the DEF.

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FUNCTIONAL ANALYSIS OF THE SYRINGOMYCIN BIOSYNTHESIS ENZYME, SYRC, OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. J.-H. Zhang, I. Deligiovas*, I. Grgurina*, and D.C. Gross. Dept. Plant Pathology, Washington State University, Pullman 99164, and *Dipartimento di Scienze Biochemiche, Università 'La Sapienza' di Roma, Italy.

Sequence analysis of *syrC* and other genes in the *syr* cluster required for syringomycin production indicated that the phytotoxin is synthesized by the thiotemplate mechanism of peptide synthesis. The *syrC* ORF was predicted to encode a ~48-kDa protein, SyrC, containing a motif characteristic of thioesterases and a zinc-binding motif characteristic of carboxypeptidases. To determine the function of SyrC in syringomycin synthesis, the *syrC* gene was subcloned into the expression vector, pTrcHis-C, and overexpressed in *Escherichia coli*. The overexpressed SyrC protein was used in studies of enzymatic activity and to generate a polyclonal antiserum for Western analysis. In vitro assays showed that SyrC catalyzed the release of CoA from a 3-hydroxydodecanoyl-CoA substrate, suggesting that SyrC functions in the formation of 3-hydroxydodecanoyl-L-serine. The enzymatic activities of wild-type SyrC and mutant derivatives, obtained by site-directed mutagenesis or deletion of a putative motif, will be compared to identify the active sites of SyrC.

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SENSITIVITY OF *CLADOSPORIUM CARYIGENUM* TO PROPICONAZOLE AND FENBUCONAZOLE. [1]K. L. Reynolds, [2]T. B. Brenneman, [3]P. F. Bertrand, and [2]A. K. Culbreath, Department of Plant Pathology, University of Georgia, [1]Athens, GA, 30602, [2]Coastal Plain Experiment Station, and [3]Rural Development Center, Tifton, GA, 31793.

One hundred monoconidial isolates of the pecan scab fungus, *Cladosporium caryigenum*, were obtained from each of two pecan orchards in Georgia during May 1994. The orchards, located in Troup and Jeff Davis Counties, had never been exposed to sterol-inhibiting fungicides and were isolated from commercially sprayed orchards. Isolates were grown on PDA amended with propiconazole or fenbuconazole at 0, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, or 5.0 μg ml^{-1}. After 4-5 wk at 25 C, the diameter of each colony was measured. Relative growth was expressed as the colony diameter on each fungicide concentration as a percentage of the diameter on unamended PDA. ED_{50}-values for fenbuconazole were lognormally distributed with a mean of 0.19 and 0.25 μg ml^{-1} for Jeff Davis and Troup County, respectively. ED_{50}-values for propiconazole were lognormally distributed with a mean of 0.12 and 0.17 μg ml^{-1} for Jeff Davis and Troup County, respectively. There was a significant positive correlation between propiconazole and fenbuconazole sensitivity at both locations indicating potential for development of cross-resistance to these compounds.

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RESIDUAL EFFECTS OF TREATMENT ON CONTROL OF OLIVE LEAF SPOT CAUSED BY *SPILOCEA OLEAGINEA*. B.L. Teviotdale and G.S. Sibbett. University of California, Kearney Ag. Center, Parlier, CA 93648.

Olive trees treated annually in two consecutive winters with one or two applications of copper fungicide were left untreated the immediate following winter, then treated the next winter with one application of Bordeaux mixture. Percent healthy and diseased leaves among ten adjacent leaf pairs were evaluated on 20 randomly selected shoots each May. In a second experiment, cupric hydroxide was applied once in November or January or in both November and January on annual or biennial schedules for three years. All trees were treated the following two winters with cupric hydroxide. In May of the first three years, percent healthy and diseased leaves were determined among ten adjacent pairs of leaves on ten shoots which were selected on each tree before treatments were made. Similar measurements were made for ten pairs of leaves on 20 randomly selected shoots on each tree the last two years. In both orchards, following similar treatment after experiments ended, treatment effects were measurable.

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DEVELOPMENT OF ONTOGENIC RESISTANCE TO POWDERY MILDEW (*UNCINULA NECATOR*) IN FRUIT OF CONCORD GRAPEVINES. David M. Gadoury and Robert C. Seem. Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, 14456.

Vitis labruscana 'Concord' has been reported to be more resistant to powdery mildew than cultivars of *V. vinifera*, because foliar infection is inconspicuous before late summer. Thus, fungicide applications are delayed on Concord, and continue until veraison when fruit are thought to become resistant. We inoculated Concord clusters at prebloom, fruit set, 6 mm fruit, and 12 mm fruit. Ontogenic resistance developed up to 6 weeks before veraison. Concord fruit were nearly immune by the time they reached 6 mm dia. (2 wk after fruit set). Inoculation at prebloom or at fruit set resulted in severely diseased berries. The rachis remained susceptible to infection throughout the season. A single application of myclobutanil at a critical phenological stage (prebloom) provided season-long control of fruit infection. Thus, severe fruit infection apparently is a consequence of early events in the epidemic cycle. Control of powdery mildew on Concord fruit requires early-, not late-season protection.

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EFFECTS OF PARTIAL HOST RESISTANCE AND DURATION OF LEAF WETNESS ON DEVELOPMENT OF PECAN SCAB. W. W. Turechek and K. L. Reynolds, Department of Plant Pathology, University of Georgia, Athens, GA. 30602.

The effects of partial host resistance and leaf wetness on the development of pecan scab were evaluated. Seedlings of cultivars Wichita (susceptible), Cape Fear (moderately susceptible) and Elliott (moderately resistant) were inoculated with conidia of *Cladosporium caryigenum*, enclosed in plastic bags to maintain leaf wetness and placed in a growth chamber at 20 C. Trees were removed from the bags after 3, 6, 12, 24, 36 or 48 hr of leaf wetness and placed in a greenhouse to allow development of disease. After 2 or 3 wk, disease severity, mean lesion area and spores/lesion on leaves exposed to 36 or 48 hr of wetness were determined and found to be significantly greater for Wichita than the less susceptible cultivars. Disease severity on leaves exposed to 48 hr of leaf wetness was significantly greater in Cape Fear than Elliott. After 36 hr of leaf wetness, lesions on leaves of Wichita produced approximately 100 X more spores than on leaves of Elliott or Cape Fear. Ninety to 100% of the lesions from Wichita and Elliott sporulated, whereas only 70-84% of the lesions sporulated in Cape Fear. Results show that reduced spore production and lesion area may be responsible for partial resistance in Cape Fear and Elliott.

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DIFFERENTIATION OF SPECIES AND PATHOTYPES OF *ELSINOE* CAUSING SCAB ON CITRUS. L.W. Timmer, Univ. Florida, CREC, Lake Alfred 33850; Mui-Keng Tan, M. Priest and P. Broadbent, Biol. & Chem. Res. Inst., Rydalmere NSW 2116, Australia

Three types of scab have been described: 1) the widespread citrus scab caused by *Elsinoe fawcettii* (*Ef*) with 2 pathotypes in Florida, 2) sweet orange scab caused by *E. australis* (*Ea*); 3) Tryon's scab in Australia caused by *Sphaceloma fawcettii* var. *scabiosa* (*Efs*). Hyaline conidia of the 3 types did not differ in size or shape. Colonies of *Ea* were vinaceous to black whereas those of *Ef* and *Efs* were beige to brown. In detached leaf assays on rough lemon (RL), sour orange (SO) and grapefruit (GF), one Florida biotype affected RL, SO and GF, the other only RL and GF. *Efs* affected only RL and *Ea* did not affect any of the 3 hosts. In greenhouse inoculations, *Efs* was pathogenic on leaves of RL, Rangpur lime, Eureka lemon, Cleopatra mandarin, but not on SO, GF, trifoliate orange, citranges or Key lime. Restriction analysis of the ITS region of ribosomal DNA indicated clear differences between *Ea* and *Efs* or *Ef*, but no differences between *Efs* and *Ef*. RAPD analyses differentiated isolates within species.

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IN VITRO SUPPRESSION OF *ENTOMOSPORIUM MESPILI* AND EFFICACY OF VARIOUS FUNGICIDES FOR CONTROL OF ENTOMOSPORIUM LEAF AND BERRY SPOT OF SASKATOON. P.S. BAINS, and R.M. LANGE, Crop Diversification Centre - North, RR6, Edmonton, Alberta, Canada T5B 4K3 and R.J. Howard, Crop Diversification Centre - South, SS 4, Brooks, Alberta, Canada T1R 1E6

Entomosporium mespili (DC. ex Duby) Sacc. has become an important pathogen of saskatoon (*Amelanchier alnifolia* Nutt.). Fourteen fungicides were tested *in vitro* for their ability to restrict radial growth and conidiospore germination of a monoconidial *E. mespili* isolate. Benomyl, imazalil, myclobutanil, propiconazole, thiabendazole and thiophanate-methyl completely restricted growth and, with the exception of myclobutanil, totally inhibited germination at 1 ppm a.i., the lowest concentration tested. Benomyl, sulfur and thiophanate-methyl strongly reduced the incidence of the disease in greenhouse experiments. Thiophanate-methyl, however, was strongly phytotoxic. In a field experiment, one application of chlorothalonil, myclobutanil, triforine, thiophanate-methyl or propiconazole at the white-tip plant growth stage failed to control the disease on fruit, although propiconazole reduced disease severity on lower leaves early in the growing season. Isolation of the pathogen from infected fruit and pedicels collected during winter suggests that these structures may be important over-wintering sites for *E. mespili*.

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DIFFERENCES IN SUSCEPTIBILITY OF PEACH VARIETIES TO PHOMOPSIS FRUIT ROT. P. Fenn and H. Barczynska. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

A Phomopsis sp. has caused an important pre- and postharvest fruit rot of processing clingstone peaches in Arkansas. The purpose of this research was to screen peach varieties for differences in susceptibility to Phomopsis fruit rot. Fruit from a variety trial at Clarksville, AR were picked at the firm mature stage, washed in water with 0.02% Tween 20, rinsed and air dried. The fruit were inoculated with one 20 μl drop containing 5 X 10^4 pycnidiospores of the fungus cultured on PDA. Fruit were incubated at 21° C in covered flats to maintain high humidity. Incubation period (time to lesion appearance) and rate of lesion growth were recorded. Significant differences were found for both average incubation period (range 2.1-10.4 days) and lesion growth rate (0.81-1.5 cm/day) among the 75 varieties screened. The average incubation period was not significantly different between 30 clingstone (5.7 days) and 45 freestone (6.2 days) varieties, but lesion growth was about 7% faster on clingstones than freestones. Screening could identify useful sources of genetic resistance to fruit rot.

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GLOMERELLA CINGULATA AND *COLLETOTRICHUM* SPP. FROM CRANBERRY IN NEW JERSEY. C.M. Stiles and P.V. Oudemans. Rutgers University, Blueberry and Cranberry Research Center, Chatsworth, NJ 08019.

A survey of cranberry fruit rot (CFR) was conducted across 36 cranberry beds in NJ during harvest in 1994 to determine the frequency and distribution of the major fruit-rotting fungi. Of 15 fungal species implicated in the CFR complex, *Physalospora vaccinii* (found in 36 beds) and *Glomerella cingulata* (*Colletotrichum gloeosporioides*) (in 33 beds) were the most commonly isolated species, comprising 13 to 98% and 1 to 86% of rotted fruit/sampling site, respectively. Isolates of *G. cingulata* varied in colony morphology, including clumped-perithecial types, scattered-perithecial types, and conidial types. Chromogenic isolates of a distinct species, similar to *C. acutatum*, were recovered infrequently from only 6 bogs. Isolates were tested for sensitivity to chlorothalonil; EC_{50} values ranged from 0.08 to >80 µg/ml, and were higher for chromogenic isolates than for *G. cingulata* isolates.

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THE RELATIONSHIP OF SHELL DISCOLORATION TO FUNGAL DECAY FOR PISTACHIO NUTS. M. A. Doster and T. J. Michailides, Dept. of Plant Pathology, Univ. of California, Davis/Kearney Ag. Center, Parlier 93648.

Although only a small percentage of pistachio nuts in commercial orchards have both hull and shell split, these nuts ("early splits") are the major source of fungal-decayed kernels. Fortunately, such nuts frequently have discolored shells which could assist in removal during processing. Nuts that split their hulls earlier in the season had more shell discoloration than nuts that split later. For example, 67% of the nuts that had hulls split before 12 August had extensive shell discoloration (>10% shell surface) compared to only 13% of the nuts that split after 26 August. Discoloration along the suture where the shell splits was a common characteristic for early splits. Pistachio nuts were obtained from two processors, separated according to the shell appearance, and evaluated for kernel quality. Nuts in the following four categories frequently had fungal-decayed kernels: oily shell, crinkled shell, extensive dark brown shell discoloration, and limited (<11%) dark brown shell discoloration along the suture. However, nuts with limited dark brown discoloration (none along the suture) or with yellow shell discoloration almost never had fungal-decayed kernels. Therefore, the processors need to remove only those nuts with certain types of shell discoloration.

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IDENTITY AND DISTRIBUTION OF *PHYTOPHTHORA* SPP. CAUSING ROOT ROT OF RASPBERRY IN CHILE. W. F. Wilcox and B. A. Latorre, Dept. Plant Pathology, Cornell University, NY State Agr. Expt. Sta., Geneva 14456 and Facultad de Agronomía, P. Universidad Católica de Chile, Santiago

From Oct.-Dec. 1993, *Phytophthora* spp. were isolated from diseased raspberries collected from 18 plantations in Chile. Plantations were located along a 1,000-km north-south axis from Nogales (semi-arid, 32.6°S) to Osorno (maritime, 40.8°S). The *Phytophthora* spp. and their distribution (northern, central, and/or southern zones) were: *P. fragariae* var. *rubi* (8 sites, central and southern only); *P. megasperma* (8 sites, southern only); *P. cryptogea* "type A", similar to tree fruit and kiwifruit isolates from Calif. and Chile (8 sites, northern and central only); *P. cryptogea* "type B", similar to tree fruit isolates from the Great Lakes region (5 sites, central and southern only); *P. citricola* (4 sites, all zones); and an unidentified homothallic and unidentified heterothallic species (1 southern and 1 northern site each, respectively). All species except *P. megasperma* were highly virulent on cv. 'Heritage' in greenhouse tests. This is the first known record of *P. fragariae* var. *rubi* in the southern hemisphere.

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FUNGI ASSOCIATED WITH MANGO DECLINE IN FLORIDA. Randy C. Ploetz, David Benscher, and Aimé Vázquez. University of Florida, TREC, 18905 SW 280th Street, Homestead, FL 33031.

Mango, *Mangifera indica*, suffers from a decline syndrome in south Florida which resembles disorders in other producing regions; symptoms include tip dieback, gummosis, defoliation and vascular discoloration. The etiology of these diseases is confused. To date, only *Botryosphaeria ribis* and *Diplodia* sp. have been reported to be important causal agents in Florida. During a re-examination of the disease in Florida, fungi in affected stem tissue were assessed. Samples were taken primarily from cvs. Keitt and Tommy Atkins in commercial orchards and an experimental planting at the University of Florida in Homestead (TREC). Pieces of cambium, 1 cm^2, were removed from the interface of symptomatic and healthy tissue, surface-disinfested in 70% ethanol (10 sec) and 10% household bleach (2 min), and submerged in molten PDA (45C) amended with 100 mg streptomycin sulfate/L. In alphabetical order, the following fungi were recovered: *Alternaria alternata*, *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Dothiorella dominicana*, *Fusarium* spp., *Lasiodiplodia theobromae*, *Pestalotiopsis mangiferae*(?) and *Phomopsis anacardii*(?). The relative abundance of the fungi that were recovered varied according to season. In preliminary pathogenicity trials, *L. theobromae*, *C. gloeosporioides*, *P. anacardii*(?), and two species of *Fusarium* systemically colonized wound-inoculated stems, and caused vascular discoloration and, with the exception of the *Fusarium* spp., gummosis. Results from this and ongoing experiments will be discussed with regard to the previous reports on *B. ribis* and *Diplodia* sp. Mango decline in Florida is probably a disease complex involving several different fungi.

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REDUCING POSTHARVEST DECLINE OF CUT FLOWERS. C.A. Greer and J.J. Marois, Department of Plant Pathology, University of California, Davis, CA 95616.

The main focus of procedures to extend vase life of cut flowers has been on finding vase solution additives that will eliminate bacteria from the vase solution system. In this study, an exclusionary approach utilizing a stem-end filter to bar bacteria from xylem elements was tested. Rose (cv. Samantha) stems were surface disinfested with sodium hypochlorite, ethanol or hydrogen peroxide; re-cut under sterile water and fitted into syringe filter units. The junction of the stem and filter unit was sealed with laboratory film or rubber tubing. Roses were then placed in individual 1% sucrose vase solutions. Roses with filters had a significantly longer (3-5 days) vase life than control roses. This is the first direct evidence, without the use of biocides, implicating physical plugging of the xylem elements by bacterial populations in the vase solution as the cause of postharvest decline of cut flowers. Results of these experiments also indicate that an exclusionary approach might be useful in extending vase life of cut flowers in commercial applications.

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REDUCTION OF PHYTOPHTHORA BLIGHT OF MADAGASCAR PERIWINKLE BY SOIL SOLARIZATION. R.J. McGovern, J.P. Begeman and J.C. Capece. University of Florida-IFAS, Southwest Florida Research and Education Center, Immokalee, 33934

Two experiments were conducted in southwest Florida to evaluate the effectiveness of soil solarization to reduce Phytophthora blight (*Phytophthora nicotianae*) of Madagascar periwinkle (*Catharanthus roseus* 'Cooler Peppermint'). In 1993, 0.6 kg (dry weight) of hard red, winter wheat kernels infested with the fungus were incorporated to a depth of 15.2 cm in 3.6x3.6 m plots and in 1994, 1.6 kg of inoculum were used. Half of the plots were solarized using clear polyethylene mulch from 27 Sep to 18 Oct in 1993 and 30 Sep to 2 Nov in 1994. Following solarization the mulch was removed and 58-64 two-month-old periwinkle plants were planted in each plot using a 30.5 cm spacing. In 1993 plants were watered by overhead irrigation and in 1994 through subirrigation. A randomized complete block design with three or five replicates was used. Phytophthora blight was monitored for 42 and 30 days, respectively, in 1993 and 1994. Solarization significantly (LSD, $p=0.05$) reduced both the relative area under the disease progress curve [AUDPC (-87.8%)] and final blight incidence (-78.7%) in 1993, and AUDPC (-28.1%) in 1994. Failure of solarization to reduce final blight incidence in 1994 may be attributed to flooding conditions following an unseasonably late tropical storm.

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BICARBONATES AND BOTRYTIS: V. CONTROL OF GRAY MOLD ON GREENHOUSE GROWN GERANIUMS. Palmer, C. L., R. W. Langhans, R. K. Horst, and H. W. Israel. Departments of Floriculture and Ornamental Horticulture and of Plant Pathology, Cornell University, Ithaca, NY, USA 14853.

Bicarbonates inhibit *Botrytis cinerea* (Pers.) *in vitro* colony growth and conidial germination, as well as altering viability of ungerminated conidia (Phytopathology 84:546,1065). Bicarbonate effects on gray mold incidence *in planta* have not yet been investigated. Therefore, infected seedling geranium cultivars Red Elite and Scarlet Elite were sprayed weekly with 0, 25, and 50 mM NH_4HCO_3 or $KHCO_3$. Data were collected biweekly on foliar and floral disease ratings and sporulation. Foliar and floral ratings were significantly lower for 25 mM $KHCO_3$, 25 mM NH_4HCO_3, and 50 mM NH_4HCO_3 than for 0 mM and 50 mM $KHCO_3$. No differences in sporulation occurred among treatments. Some phytotoxicity was observed with 50 mM $KHCO_3$; none with NH_4HCO_3. Phytotoxicity accounted for higher ratings with 50 mM $KHCO_3$ by providing additional infection sites for *B. cinerea*. At appropriate concentrations, bicarbonates effectively manage gray mold of geraniums. (Supported by H&I Agritech Inc., Ithaca, NY 14850.)

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BICARBONATE - BASED FUNGICIDES CONTROL SCLEROTINIA AND PYTHIUM ON TURF. M.S. Szyndel[1], R. K. Horst[1,2], H.W. Israel[1,2], M. S. Lajoie[3]. [1]H & I Agritech ,Inc., Ithaca NY, 14850, [2]Dept. Plant Pathology, Cornell University, Ithaca, NY, 14850, and [3]Church & Dwight Co., Inc., Princeton, NJ, 08540.

The efficacies of bicarbonate-based experimental formulations, alone and in combinations with commercial fungicides, to control dollar spot and Pythium blight diseases on turf grasses were tested in greenhouse research. Bent grass, *Agrostis palustris*, infected with *Sclerotinia homeocarpa* or *Pythium aphanidermatum* were treated with sodium, potassium or ammonium bicarbonate containing formulations: 2126-17A, 2311-136, 2311-65, 2311-67. Efficacy in combinations with recommended rates of Terraclor (PCNB) for *Sclerotinia* and Truban (Etridiazole) for *Pythium* was examined. Only formulations 2311-65 and 2311-67 were efficacious in control of both pathogens although severe phytotoxicity occurred at 0.59% active ingredient (A.I.). When formulation and fungicide were applied in combination and compared with either product alone, marked additive effects and enhanced growth were observed. Dollar spot disease was reduced c. 90% by combinations of 1/4 x to 1/2 x both formulations (0.15% and 0.3% A.I.) and 1/4 x to 1/2 x Terraclor (recommended label rate), and 1/2 x 2311-65 (0.3% A.I.) combined with 1/2 x Truban reduced Pythium blight by c. 93%. We thus infer that it is possible to reduce to half or more the amounts of traditional pesticides to control turf diseases by supplementing with environmentally compatible formulations.

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REACTION OF CULTIVARS OF FLOWERING, KOUSA, AND HYBIRD DOGWOODS TO POWDERY MILDEW. A. K. Hagan, C. H. Gilliam, G. J. Keever, and D. Williams, Auburn University, AL 36849.

Severity of powdery mildew (Oidium spp.) was assessed in a 2 yr-old planting of 36 cultivars of flowering (Cornus florida), kousa (C. kousa), and hybrid (C. florida x kousa and C. nuttallii x florida) dogwood. Powdery mildew severity was visually rated on 4 Aug 94. Generally, the flowering dogwood cultivars were more susceptible to powdery mildew than the kousa or hybrid dogwood selections. Of the 24 flowering dogwood cultivars screened, only the cv. 'Cherokee Brave' and 'Dwarf White', were free of symptoms of this disease. Leaf discoloration and distortion along with some light premature leaf shed were seen on most flowering dogwood cultivars. Although discrete colonies of Oidium spp. were noted on the leaves of all eight hybrid dogwood selections, no leaf distortion or premature leaf shed occurred. Little if any sign of powdery mildew fungus was noted on the leaves of the 4 kousa dogwood cultivars.

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SPATIAL DYNAMICS OF PHYTOPHTHORA ROOT ROT OF HEMLOCK. V. L. Smith and J. S. Ward. Depts. of Plant Pathology & Ecology and Forestry & Horticulture, Connecticut Agricultural Experiment Station, New Haven 06504

A naturally-occurring epidemic of root rot in a commercial nursery planting (1 ha) of hemlock (*Tsuga canadensis*), caused primarily by *Phytophthora cactorum* and *P. cinnamomi*, was monitored August through October 1994 and April through June 1995. The source of inoculum for the epidemic is unknown. Onset of disease followed 3 days of heavy rain after several months of severe drought. Trees (4-5 yr old) in a section of field measuring approximately 0.2 ha were rated every 2 wk for foliar symptoms of root rot (wilting, redness of needles, and defoliation). A nearest-neighbor probability analysis, based on chi-square ratio analysis, was conducted on ratings of 1,440 trees planted at a 1.2-m spacing, to determine the likelihood of adjacent plants having the same rating. Initially, healthy trees and dead trees were randomly arranged within the field ($P=0.146$). The number of trees rated as healthy declined from 492 to 474, and dead trees increased from 67 to 124 in six wk. Spatial clustering of dead and dying trees developed ($P<0.001$), indicating that spread of disease from declining trees to neighboring healthy trees was occurring.

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PHYTOPHTHORA LEAF BLIGHT, CAUSED BY *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANA*, ON *AECHMEA FASCIATA* R. T. McMillan, Jr. and W. Graves. University of Florida, Tropical Research and Education Center, 18905 S. W. 280th Street Homestead, FL 33031

During the summer of 1994, in a commercial greenhouse 25 to 30% of 1000, 1-year-old bromeliads, *Aechmea fasciata* (Lindl.) Bak., showed large blackened necrotic lesions on the leaves. *Phytophthora parasitica* Dast. var. *nicotiana* (B. de H.) Tucker was recovered from the infected leaves. The fungus was isolated on a vegetable oil, nitrate agar. Koch's postulates were completed. Typical black, water-soaked, lesions developed in 4 to 7 days on all six healthy 6-month-old plants of *Aechmea fasciata* that had been inoculated with hyphae of the fungus on 4-mm agar blocks and maintained in a dew chamber for seven days. The pathogen was consistently reisolated from the diseased leaves. The identity of the isolate was confirmed by the mycology laboratory of the Fla. Dept. of Agr., Div. of Plant Ind., Gainesville, FL.

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THE EFFECTS OF PRIMARY CULTURAL PRACTICES ON DOLLAR SPOT INTENSITY ON WARM-SEASON TURFGRASSES. W.P. Moss, A.K. Hagan, K.L. Bowen, R. Dickens, Dept. of Plant Pathology and Dept. of Agronomy, Auburn University, AL 36849.

The field effects of mowing height, N rate, and irrigation rate on the intensity of dollar spot disease (*Sclerotinia homoeocarpa*) on four inoculated warm-season turfgrasses was evaluated. St. Augustinegrass was asymptomatic. Interactions of factors were significant for the symptomatic turfgrasses. Disease intensity was inversely related to irrigation rate and mowing height for centipedegrass, inversely related to N rate or to irrigation rate at a low mowing height for zoysiagrass, and inversely related to N rate for bermudagrass. On high cut zoysiagrass, disease intensity increased as irrigation rate increased. Primary cultural factors interacted to produce turfgrass-specific responses to *S. homoeocarpa*.

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MANAGEMENT OF TEMPORAL VARIATIONS IN STRESS INDUCING FACTORS AT SOUTHERN CALIFORNIA GOLF COURSES. Larry J. Stowell, PACE Turfgrass Research Institute, 1267 Diamond Street, San Diego, CA 92109.

Southern California experiences drought conditions between May and September annually. The limited rainfall results in exclusive use of irrigation water to meet turfgrass evapotranspirational demands. Unfortunately, even the purest well waters (256 mg/l total dissolved salts) contain sufficient dissolved salts to induce dramatic changes in soil chemistry. For example, soil sodium expressed as a percentage of the total extractable cations, can fluctuate from 9% following summer irrigation to 2% following winter rainfall periods. Likewise, soil potassium can fluctuate between 200 mg/kg following summer irrigation to 90 mg/kg following winter rainfall. Soil sulfates can range between 330 mg/kg following the summer irrigation and 74 mg/kg following winter rainfall. Although management programs have been implemented to limit accumulation of dissolved salts in the soil, attempts to control the chemical composition of the soil solution have been difficult. The best management strategies currently in place will be discussed.

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TRANSCAPSIDATION IN TRANSGENIC PLANTS EXPRESSING POTYVIRUS COAT PROTEINS. J. Hammond[1], H. Puringer[2], H. Steinkellner[2], A. da Câmara Machado[2], and M. Laimer da Câmara Machado[2]. [1]USDA-ARS, FNPRU, BARC, Beltsville MD 20705 and [2]Univ. of Agriculture, Vienna, Austria.

Transgenic plants expressing the coat protein (CP) of either bean yellow mosaic virus strain GDD (BYMV-GDD) or plum pox virus strain NAT (PPV-NAT) were inoculated with BYMV Ideal A, BYMV PV0287, PPV-NAT, or other potyviruses. Virus preparations from infected plants were analyzed by ELISA, Western blotting, and immuno-specific electron microscopy (ISEM). Transgene CP was readily detected in preparations from BYMV CP plants with a BYMV-GDD-specific monoclonal antibody. ISEM with gold-labelled BYMV antibodies showed significant incorporation of BYMV-GDD CP into PPV particles.

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DNA REPLICATION AND HOST RANGE PROPERTIES OF TWO PSEUDORECOMBINANT BIPARTITE GEMINIVIRUSES. Y. M. Hou and R. L. Gilbertson, Dept. of Plant Pathology, University of California, Davis, CA 95616.

Infectious pseudorecombinant (PR) bipartite geminiviruses were made by exchanging DNA components of bean dwarf mosaic (BDMV) and tomato mottle (ToMoV) geminiviruses. The PRs (PR1 = BDMV DNA-A and ToMoV DNA-B; and PR2 = ToMoV DNA-A and BDMV DNA-B) induced mild symptoms in systemically infected *Nicotiana benthamiana* plants. PCR analysis indicated that the DNA-B titers of both PRs were reduced compared to those of wild type BDMV and ToMoV. DNA components of BDMV, ToMoV, PR1, and PR2 were electroporated into *N. tabacum* protoplasts and DNA-A and DNA-B replication was analyzed by Southern hybridization. De novo replication of all DNA-A components and BDMV DNA-B was detected as early as 48 hours after electroporation, whereas replication of the DNA-B components of both PRs was delayed and reduced. ToMoV DNA-B replicated poorly, even when combined with ToMoV DNA-A. These results suggest that host factors may play an important role in the efficient replication of DNA-B. Host range is being investigated by agroinoculation of these viruses into *N. benthamiana*, bean, and tomato.

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THE EASTERN AND WESTERN STRAINS OF PEANUT STUNT VIRUS (PSV) REPRESENT TWO DISTINCT SUBGROUPS BASED ON SEROLOGY AND SEQUENCE HOMOLOGY. C.-C. Hu and S. A. Ghabrial, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The complete nucleotide sequences of the genomes of two strains of PSV, an eastern strain (ER) and a western strain (W), have been determined and compared to the previously published sequences of the Japanese strain, PSV-J. The RNAs of PSV-ER had significantly higher percentages of nucleotide sequence identity with those of PSV-J than with those of PSV-W. Whereas the percentages of identity between PSV-J and PSV-ER were 91, 94 and 89%, respectively, for RNA 1, 2 and 3, the corresponding values between PSV-J and PSV-W were 78, 74 and 76%. ELISA and western blot analysis, using antisera to PSV-ER and PSV-W, clearly distinguished between strains ER and W. Strain J was serologically closely related to PSV-ER. PSV strains, like those of cucumber mosaic virus, the type member of the genus *Cucumovirus*, appear to fall into two distinct subgroups distinguishable serologically and based on the percentage of nucleotide sequence identity.

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GENOME ORGANIZATION AND EXPRESSION OF A DSRNA VIRUS INFECTING A PLANT PATHOGENIC FUNGUS. S. Huang and S. A. Ghabrial, Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

The mycovirus Helminthosporium victoriae 190S virus (Hv190SV), a member of the family *Totiviridae*, contains a monopartite dsRNA genome of 5,178 bp. Although the capsid is encoded by a single gene, it contains three closely related polypeptides, p78, p83 and p88. Whereas p83 and p88 are phosphoproteins, p78 is nonphosphorylated. A full length cDNA clone of the viral dsRNA was constructed and its sequence revealed the presence of two overlapping large open reading frames (ORFs). The 5' proximal ORF (ORF 1) codes for the capsid protein and the 3' ORF (ORF 2) contains all the consensus RNA-dependent RNA polymerase (RDRP) sequence motifs and is in the -1 frame relative to ORF 1. The product of ORF 2 was identified as a separate virion-associated minor polypeptide with an *Mr* value of 92,000. Hv190SV thus differs from other totiviruses which express RDRP by fusing ORFs 1 and 2 via a translational frameshift. The mechanisms of translation of ORFs 1 and 2 will be discussed.

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CHARACTERIZATION OF DEFECTIVE FORMS OF CUCUMBER MOSAIC VIRUS RNA3 GENERATED IN CMV 3A-TRANSGENIC PLANTS Igor B. Kaplan and Peter Palukaitis,
Dept. Plant Pathology, Cornell University, Ithaca, NY 14853

Movement protein-deficient mutants of CMV that are able to accumulate only in CMV 3a-expressing transgenic plants were shown to undergo deletion of the 3a gene. The deletion mutants were selected for at different rates and all evolved to stable forms of RNA 3. Sequence analysis of the stable deletion mutants showed that they contained either in-frame deletions within the 3a gene, or a complete deletion of the entire 3a gene. The nature of the deletion mutant was determined by the nature of the initial mutation rendering the 3a gene dysfunctional. Several intermediate forms of one deletion mutant were also detected. The characterization of these intermediates will be discussed.

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THE INTERACTION OF A SINGLE, RECESSIVE GENE IN *NICOTIANA CLEVELANDII* AND GENE VI OF CAULIFLOWER MOSAIC VIRUS RESULTS IN SYSTEMIC NECROSIS. **Lorant Kiraly and James E. Schoelz, Department of Plant Pathology, University of Missouri, Columbia MO**

Cauliflower mosaic virus (CaMV) strains D4 and W260 can be distinguished by the type of systemic symptoms they induce in *Nicotiana clevelandii*; D4 induces a systemic mosaic while W260 induces systemic necrosis in addition to the mosaic symptom. To determine which W260 genes are responsible for the systemic necrosis symptom, we constructed chimeric viruses between D4 and W260 and found that W260 gene VI elicited systemic necrosis. We also present evidence that the systemic necrosis trait in *N. clevelandii* is governed by a single, recessive gene. To investigate the inheritance of systemic necrosis, we crossed *N. clevelandii* with *Nicotiana bigelovii*, a host that reacts with a systemic mosaic symptom upon infection with W260. All F1 plants developed a systemic mosaic after inoculation with W260 and the F2 generation segregated 3:1 for systemic mosaic vs. necrosis. These results indicate that the systemic necrosis symptom in *N. clevelandii* is induced by the interaction of a single, recessive host gene and gene VI of CaMV.

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CLONING AND SEQUENCING OF PEACH ROSETTE MOSAIC VIRUS RNA 1. **A.H. Lammers, R.F. Allison, and D.C. Ramsdell. Dept of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.**

The nepovirus peach rosette mosaic virus (PRMV) infects peach, grapevine, and highbush blueberry. The bipartite virus consists of two single stranded RNA molecules that are separately encapsidated and estimated to be 8 and 7 kilobases, respectively. The virus is transmitted by nematode and by seed. A grapevine isolate of PRMV was propagated on *Chenopodium quinoa*. RNA was extracted from purified virions and oligo d(T) primed cDNA was synthesized. Additional cDNA clones were primed by oligonucleotides designed to complement the desired upstream sequence. All cDNA clones were sequenced from a series of nested deletion mutants. The complete nucleotide sequence and analysis of RNA 1 will be reported. The genomic organization and translational strategy will be compared with that of other plant viruses.

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FUNCTIONAL ANALYSIS OF THE MOVEMENT PROTEIN OF CUCUMBER MOSAIC VIRUS. Qiubo Li and Peter Palukaitis, Dept. of Plant Pathology, Cornell University, Ithaca, NY14853

The movement protein (3a) of cucumber mosaic virus has been expressed in *E. coli* and the isolated protein was found to have single-stranded (ss) RNA binding activity. Nine site-directed mutants of the 3a gene were made and analysed for their biological function and for the ability of the altered 3a proteins to bind ss RNA. Although all the mutants except one can systemically infect the 3a transgenic tobacco, five of them can also infect non-transformed tobacco, producing delayed or milder systemic symptoms compared to wild type cucumber mosaic virus. There is no quantitative relationship between the infectivity and the ability to bind ss RNA of the mutants. However, minimal RNA binding activity of the 3a protein is essential for its biological function. Sequence motifs in the 3a protein that affect host range and RNA-binding were assigned and will be described.

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PARTIAL GENOME ORGANIZATION OF GRAPEVINE LEAFROLL-ASSOCIATED CLOSTEROVIRUS 3. Kai-Shu Ling, Roger F. Drong*, Jerry L. Slightom* and Dennis Gonsalves. Department of Plant Pathology, Cornell University, Geneva, NY 14456. *Molecular Biology Unit 7242, The Upjohn Company, Kalamazoo, MI 49007

About 70% of grapevine leafroll-associated closterovirus 3 (GLRaV 3) RNA genome has been sequenced thus far. At least nine potential open reading frames (ORFs) were revealed. Putative helicase domains were identified on the 5' terminal region. A second ORF encoded a protein which is similar to the Supergroup 3 RNA-dependent RNA polymerase. Similar heat shock 70 and heat shock 90 proteins that have been identified in other closteroviruses were also present in GLRaV 3. A tentative coat protein gene was identified after sequencing three immunopositive clones. A possible divergent coat protein gene was also identified that shares similarity to the tentative coat protein gene. Analysis of the GLRaV 3 genome organization reveals a close similarity to two other closteroviruses that have been sequenced: beet yellows and citrus tristeza. A phylogenetic analysis placed GLRaV 3 into the closterovirus group.

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BACTERIAL EXPRESSION OF SINGLE CHAIN ANTIBODIES SPECIFIC TO CITRUS TRISTEZA CLOSTEROVIRUS. K. L. Manjunath[1], M. Hooker[2], H. R. Pappu[3], S. S. Pappu[3], C. A. Powell[2], M. Bar-Joseph[4], C. L. Niblett[3] and R. F. Lee[1], [1]University of Florida, CREC, Lake Alfred, FL 33850, [2]University of Florida, Plant Pathology Dept., Gainesville, FL 32611, [3]University of Florida, AREC, Ft. Pierce, FL 34945, and [4]The Volcani Center, Bet Dagan, Israel.

A monoclonal antibody (Mab), 17G11, having a broad spectrum recognition of citrus tristeza virus (CTV) isolates was selected for construction of single chain antibody gene (SCAB). Total RNA was isolated from hybridoma cells expressing 17G11 Mab, and the variable regions of heavy chain (V_H) and light chain (V_L) of the immunoglobulin G gene were amplified separately and cloned into the SmaI site of pUC 118 vector. The V_H and V_L genes were linked with a 14 amino acid peptide. This SCAB gene was subcloned under the pEL B leader sequence of pET 22b (+). The protein was expressed in a T7 expression system using *E. coli*, strain BL21 (DE3). The binding affinity of the expressed protein purified from the periplasmic spaces with the antigen was studied.

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MECHANICAL TRANSMISSION OF GENOMIC CLONES OF MAIZE STREAK GEMINIVIRUS. P. Ngwira[1], R. Louie[1,2], J. Njuguna[1], D. M. Bisaro[3], and D. T. Gordon[1]. Dept. of Plant Path, OARDC, The Ohio State University (OSU)[1], USDA-ARS[2], Wooster, 44691 and Plant Biotech. Center and Dept. of Molecular Genetics, OSU[3], Columbus, 43210.

Viral dsDNAs (RF) were isolated from maize leaves infected with Nigerian (MSV-N) and Kenyan (MSV-K) isolates of maize streak virus, respectively. The DNAs were restricted with Bam HI and cloned into plasmid pGEM7 as a 1.5-mer containing two copies of the plus strand origin of replication (MSV-N) or as a tandem duplication (MSV-K). Infectivity of MSV DNA in the recombinant plasmids was tested by vascular puncture inoculation (VPI) of Seneca Chief sweet corn seeds. In the first test, MSV-N and MSV-K were transmitted to 0 of 50 and 23 of 49 plants from VPI seeds, respectively, and in the second to 3 of 96 and 24 of 98 plants, respectively. MSV-N infected plants exhibited mild symptoms which first appeared 10 days after inoculation (dai), whereas MSV-K infected plants exhibited severe symptoms beginning 7 dai. Mostly dsDNA RF was recovered from MSV-N infected plants, whereas all genomic DNA forms, including ssDNA, were recovered from MSV-K infected plants. This is the first report of mechanical transmission of cloned MSV DNA without the aid of biolistic delivery or agroinoculation. We expect that this technique will greatly facilitate the genetic analysis of MSV.

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A PROBABLE EVOLUTIONARY LINK BETWEEN THE MARAFIVIRUSES AND THE TYMOVIRUSES. Michael C. Edwards and Zhijun Zhang. Dept. of Plant Pathology, North Dakota State Univ. & USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105-5677.

The Marafiviruses are a small group of monopartite, (+) sense, RNA viruses reported to replicate in their leafhopper vectors as well as in their plant hosts. Since very little is known about these viruses, we began an investigation of the molecular biology of oat blue dwarf virus (OBDV), a Marafivirus which can be found annually in oat, barley, and flax in the upper Midwest. Clones representing more than half of the OBDV genome were obtained using oligo-dT primed cDNA synthesis. The remaining 5'-end sequence was cloned after ligation-anchored PCR. Extensive similarities between OBDV and the Tymoviruses were found in genome sequence and organization. Similarities in nonstructural gene organization and encoded amino acid sequences were particularly strong in putative protease, helicase, and polymerase domains. Cytidine content was comparable to that typical of Tymoviruses. A strong similarity to the 'Tymobox' also was found, both in nucleotide sequence (81%) and relative genome position. The observed levels of similarity were surprising considering the biological distinctions between these two virus groups. Our results have significant implications for the evolution of the Marafi- and Tymoviruses.

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M.B. Fiely, J.C. Correll, and T.E. Morelock. Comparison of seedling damping-off of spinach in naturally and artificially infested Arkansas and Texas field soils. University of Arkansas, Fayetteville, AR.

COMPARISON OF SEEDLING DAMPING-OFF OF SPINACH IN NATURALLY AND ARTIFICIALLY INFESTED ARKANSAS AND TEXAS FIELD SOILS. M.B. Fiely, J.C. Correll, and T.E. Morelock[1]. Dept. of Plant Pathology and [1]Dept. of Horticulture, University of Arkansas, Fayetteville, AR 72701.

Seedling damping-off and Fusarium wilt of spinach, caused by Fusarium oxysporum f.sp. spinaciae (Fos) are important disease problems in most spinach production areas in the U.S., except Texas. The objective of this study was to document the putative suppressiveness of Texas soils to damping-off of spinach caused by Fos. Soil was collected from spinach disease nurseries in Arkansas and Texas. The experimental treatments for each soil consisted of autoclaved, autoclaved + Fos (0.5 g barley straw inoculum/kg soil), native, and native + Fos soil. Post emergence damping-off in the autoclaved, autoclaved + Fos, native, and native + Fos treatments was 3, 42, 26, and 27% in Texas soil, and 9, 80, 68, and 80% in Arkansas soil, respectively. The pH of the Texas soil was >8.0 whereas the Arkansas soil was <7.0. Our hypothesis is that soil pH and/or calcium levels may be involved in the suppressiveness of the Texas soil.

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EVALUATION OF *TRICHODERMA ATROVIRIDE* IN CONTROLLING *RHIZOCTONIA SOLANI* OF POTATO UNDER POTATO FIELD CONDITIONS IN MONTANA. J. H. McBeath, E. Carpenter* and M. Sun*. Agricultural and Forestry Experiment Station, University of Alaska Fairbanks, AK 99775, and *Potato Laboratory, Montana State University, Bozeman, MT 84322.

The efficacy of controlling *Rhizoctonia solani* with four isolates of *Trichoderma atroviride*, a cold tolerant, wide-spectrum mycoparasite isolated from Alaska, was evaluated under potato field conditions in Montana. Eight treatments were tested in a randomized complete block design with five replications. The infestation of *R. solani* in 1994 was generally low. Russet Burbank potatoes treated with biotype 453 displayed significantly fewer lesions at the lower stems of the potato plants. In addition, potato plants treated with *T. atroviride* isolates CHS 861, CHS 901, biotype 453 and biotype 603 seemed to produce fewer malformed, knobby potato tubers. Results from Montana field trials were consistent with three year's field observations in Alaska.

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EVALUATION OF MARKING SYSTEMS FOR MONITORING ENVIRONMENTAL RELEASES OF RHIZOBACTERIA. W.F. Mahaffee[1], J.W.L. van Vuurde[2], J.M. van der Wolf[2], M. van den Brink[2], E.M. Bauske[1], and J.W. Kloepper[1], [1]Dept. Plant Pathology, Biological Control Institute, Auburn University, AL; [2]IPO-DLO Wageningen, the Netherlands.

The use of genetic markers for monitoring environmental releases of rhizobacteria has been hypothesized to reduce their ecological fitness; however, the testing of this hypothesis has been limited because of difficulties in monitoring the unaltered wild-type strain. The technique of Immunofluorescent Colony Staining (IFC) allows for detection and enumeration of the unaltered wild-type strain. A field release on cucumber was established with seed treatments of a wild-type Plant Growth-Promoting Rhizobacterial strain *Pseudomonas fluorescens* 89B-27, its bioluminescent derivative (lux; 89B27::tn*4431*), a spontaneous rifampicin resistant mutant (rif) and a noninoculated control. Seed and root samples were taken 0, 7, 14, 21, 35, and 80 days after planting and processed for enumeration by spiral plating on 5% or 50% TSA amended with antibiotics as needed. The wild-type and all other treatments were processed for IFC by pour plating samples in 5% TSA, incubating, air drying, staining with a FITC labeled polyclonal antiserum and counting fluorescent colonies. Bacterial populations measured by IFC were greater for all samples throughout the growing season but did not differ from each other. Populations of rif and lux, as measured by spiral plating, were lower than the wild type and their IFC determined populations. Introduced rhizobacteria were still detected 80 days after planting using IFC but not with traditional plating. These data indicate that traditional recovery methods underestimate the populations and survival of genetically marked bacteria.

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SEWAGE AND SPENT MUSHROOM COMPOST SOIL AMENDMENTS TO SUPPRESS THE SOILBORNE FUNGAL PLANT PATHOGEN *CYLINDROCLADIUM SCOPARIUM*. Hunter, B. B., L. R. Bulluck, J. R. Newhouse, Department of Biological and Environmental Sciences, California University of Pennsylvania, California, PA 15419 and Thomas Hall, PA Bureau of Forestry.

Soil compost amendments are known to suppress fungal pathogenesis of economically-important plants. These amended soils are suppressive soils. Fungal pathogen numbers often decrease because suppressive soils support increased numbers of competing microbes and/or produce chemicals which alter the pathogen's ability to infect plants. Selected composts must be of high quality and commercially produced so that chemical compositions and concentrations are similar from application to application. Our research objective was to determine whether sewage and mushroom composts, as soil amendments, singly and in various concentrations, could reduce soilborne numbers of the imperfect fungal plant pathogen, *Cylindrocladium scoparium*. Soil from Penn Nursery, Spring Mills, PA, known to be infested with *C. scoparium*, was amended with the two composts. The geranium baiting soil bioassay, a differential selective medium, and a soil chemical procedure were used to determine the presence of *C. scoparium* in test and control soils. Our data demonstrated that *C. scoparium* soilborne populations were reduced and that the reduction was maintained for at least 10 months. Experimental and control data were significantly different according to a Two-Way ANOVA. Studies are ongoing to ascertain how the suppression is reducing *C. scoparium* numbers in nursery soils.

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MECHANISMS OF INHIBITION OR KILLING OF *Pythium dimorphum* BY *Trichoderma* spp. **Xiaoan Sun and J. P. Jones, Dept. of Plant Pathology and Crop Physiology, La. Expt Stn., LA State University Ag. Center, Baton Rouge, LA 70803**

Four *Trichoderma hamatum*, six *T. harzianum*, five *T. piluliferum*, two *T. koningii*, one *T. pseudokoningii*, one *T. viride* and one *Gliocladium* sp. isolates were all able to kill or inhibit to some degree *P. dimorphum* in vitro. They all produced some antibiotic substances which permeated through a dialysis membrane into underlying corn meal agar (CMA). Four *T. hamatum*, two *T. harzianum*, two *T. koningii*, one *T. viride*, one *T. pseudokoningii*, and *G.* sp. made the underlying medium lethal to *Pythium dimorphum* while the others only inhibited growth. The isolates of *Trichoderma* which physically penetrated or curled around *Pythium* hyphae usually had the least actual effect on *Pythium*. All ten of the *Trichoderma* isolates that allowed some, reduced, *Pythium* growth physically penetrated or curled around *Pythium* hyphae. Two of the isolates which totally inhibited *Pythium* also penetrated or curled; only one of the lethal isolates penetrated or curled around *Pythium* species. These results indicate that there is a range of mechanisms employed by *Trichoderma* spp. which result in biocontrol, and that procedures for selection biocontrol candidates should take all these mechanisms into account.

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EFFECTS OF SOLID MATRIX PRIMING ON BIOCONTROL FUNGAL GROWTH AND SEED GERMINATION. WANG, Zheng-guang and Kenneth Conway, Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74048.

Integration of biological control fungi into solid matrix priming (SMP) process serves a dual purpose. The objective of this project is to investigate the compatibility of SMP with biocontrol agents and SMP effects on seed germination. Solid matrix priming was carried out by mixing a variety of seeds with 6 different priming materials at a 1:3 ratio (w/w), water content of 30%. The biocontrol fungi *Laetisaria arvalis* 206 and *Trichoderma harzianum* 110 formulated previously were added to priming matrix at 1, 2, 3% (w/w). Fungal population growth was recorded at day 0, 3, 7, and 14 post priming. Both fungi were highly compatible with materials tested and both successfully colonized seed coats. However, fungal population began to decline after 7 days of priming. Seed germination test revealed that solid matrix priming significantly accelerated germination.

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PGPR-MEDIATED INDUCED SYSTEMIC RESISTANCE AGAINST ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON CUCUMBER. N. Martínez-Ochoa, J. W. Kloepper, and R. Rodríguez-Kábana, Department of Plant Pathology, Biological Control Institute, Auburn University, AL 36849-5409.

Strains of Plant growth-promoting rhizobacteria (PGPR), which previously demonstrated induced systemic resistance (ISR) against several cucumber diseases, were tested for capacity to induce systemic resistance against root-knot nematode. A split-root system was used to allow spatial separation of the bacterial inducer and the nematodes. One half of two-week-old cucumber 'SMR-58' roots were inoculated with a bacterial drench (log 7 cfu/ml in 50 ml 0.85% NaCl) and the other half with nematodes (about 4,000 eggs embedded in sodium alginate films). Experiments were conducted in both growth chamber (50% RH, 12 hr photoperiod at 26 C) and greenhouse environments. Total number of root-knot galls, number of egg masses, and root-knot severity index were recorded five weeks later. Treatment with some PGPR strains resulted in significant root-knot reduction compared to non-treated disease controls in all parameters. Results indicate that PGPR-mediated ISR may result in protection against pathogenic nematodes.

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INDUCED SYSTEMIC RESISTANCE BY SELECT PLANT GROWTH-PROMOTING RHIZOBACTERIA AGAINST BACTERIAL WILT OF CUCUMBER AND THE BEETLE VECTORS. G. Wei, C. Yao, G.W. Zehnder, S. Tuzun, and J.W. Kloepper, Department of Plant Pathology, Biological Control Institute, Alabama Agricultural Experiment Station, Auburn University, AL 36849.

In our previous work, select strains of plant growth-promoting rhizobacteria (PGPR) induced systemic resistance (ISR) of cucumber against several plant pathogens in the greenhouse. When field trials were conducted, control was seen against indigenous bacterial wilt, caused by *Erwinia tracheiphila* which is vectored by the spotted and striped cucumber beetles. The objective of this study was to determine if protection with PGPR-ISR was activated against the pathogen, the vector, or both. Continued field and greenhouse trials indicated that PGPR treatments reduced beetle numbers, beetle feeding activity, and incidence of the beetle-transmitting wilt. Levels of the triterpenoid cucurbitacin, a feeding stimulant for cucumber beetles, were reduced significantly compared to nontreated plants. When the pathogen was mechanically inoculated into PGPR-treated and nontreated plants, the disease progress was reduced by PGPR. These results demonstrate that PGPR-ISR leads to protection against both the bacterial wilt pathogen and beetle vectors.

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INDUCED SYSTEMIC RESISTANCE (ISR) OF CUCUMBER BY STEM-INJECTION AND SEED TREATMENT WITH PGPR. K. Jetiyanon, G. Wei, S. Tuzun, and J.W. Kloepper, Department of Plant Pathology, Alabama Agricultural Experiment Station, Auburn University, AL 36849-5409.

Previous studies demonstrated that strains of plant growth-promoting rhizobacteria (PGPR) could induce systemic resistance against cucumber pathogens when applied as seed treatment. To conduct detailed studies of mechanisms of PGPR-mediated ISR, such as assessing the role of bacterial metabolites, application methods other than seed treatment need to be developed. The objective of this work was to determine if stem-injection of PGPR could lead to ISR at levels similar to seed treatment. Eight PGPR strains were inoculated onto cucumber seeds at planting time. Three weeks later plants were challenge-inoculated with 30 10 μl drops of *Colletotrichum orbiculare* (10^5 spores/ml). In a second experiment, suspensions of the same PGPR were injected 14 days after planting into the base of the stem. One week later plants were inoculated with *C. orbiculare*. Necrotic lesions caused by *C. orbiculare* were measured 7 days after challenge. Five PGPR strains caused significant reductions in mean lesion diameter using both stem-injection and seed treatment.

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COMMUNITY-LEVEL CHANGES IN BACTERIAL ENDOPHYTES OF CUCUMBER CAUSED BY PLANT GROWTH-PROMOTING RHIZOBACTERIA WHICH INDUCE SYSTEMIC DISEASE RESISTANCE. C.M. Press, W.F. Mahaffee, and J.W. Kloepper, Dept. of Plant Pathology, Biological Control Institute, Auburn University, AL 36849-5409 USA.

Several plant growth-promoting rhizobacteria (ISR-PGPR) were isolated which induced systemic resistance in cucumber to *Colletotrichum orbiculare*, *P. syringae* pv. *lachrymans*, *Erwinia tracheiphila*, and cucumber mosaic virus. Experiments were conducted to evaluate the hypothesis that ISR-PGPR alter the community structure of bacterial endophytes in inoculated plants compared to noninduced or pathogen-induced control plants. Two strains, 90-166 (*Serratia marcescens*) and INR7 (*Bacillus pumilus*) were inoculated onto cucumber seed and plants were monitored for changes in populations of bacterial endophytes. Plants were induced either by seed treatment with PGPR or by cotyledon injection of *C. orbiculare* (IR control). All plants were challenged with *C. orbiculare* at 21 days after planting (DAP), and disease was scored at 28 DAP. Stem segments were surface-disinfested, triturated, and plated on 5% TSA. Total bacterial endophyte populations were determined, and 45 colonies were picked at random for identification by fatty acid analysis. Identifications were used to examine the bacterial endophyte community structure. Endophyte community structure of plants treated with 90-166 differed significantly (P=0.05) from the disease control, whereas the IR control was not significantly different from the disease control (P=0.8). However total populations of endophytic bacteria from PGPR-treated plants did not significantly differ from either induced resistance or disease controls. These results demonstrate that treatment with ISR-PGPR alters the endophyte community structure of cucumber which may play a role in PGPR-mediated induced resistance.

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SALICYLATE PRODUCTION BY PLANT GROWTH-PROMOTING RHIZOBACTERIA WHICH INDUCE SYSTEMIC DISEASE RESISTANCE IN CUCUMBER. C.M. Press, M. Wilson, J.W. Kloepper, and S. Tuzun, Department of Plant Pathology, Biological Control Institute, Auburn University, AL 36849-5409 USA.

Salicylate (SA) has been demonstrated to induce systemic resistance to several pathogens in cucumber as well as other crops. Strains of plant growth-promoting rhizobacteria (ISR-PGPR) have been isolated which induce systemic resistance to *C. orbiculare*, *P. syringae* pv. *lachrymans*, *Erwinia tracheiphila*, and cucumber mosaic virus. Experiments were designed to evaluate the hypothesis that some ISR-PGPR produce salicylate which induces resistance in cucumber. A bioassay was developed using *Pseudomonas fluorescens* strain HK44 containing the reporter plasmid pUTK21. The reporter plasmid contains a transcriptional fusion between the *luxCDABE* genes from *Vibrio fischeri* and the *nahG* (salicylate hydroxylase) gene from the salicylate catabolism operon of *P. putida* (pNAH7). Exposure of pUTK21 to salicylate results in increased bioluminescence. HK44 was used as a reporter strain to screen PGPR strains for the production of SA. Approximately 64% of tested ISR-PGPR produced SA including *Serratia marcescens* strain 90-166, which has demonstrated repeated control of disease caused by a variety of pathogens in greenhouse and field experiments. Salicylate is a precursor in the synthesis of pyochelin (a siderophore), and this led to the hypothesis that SA production by 90-166 was iron (Fe^{+++}) regulated. SA production by 90-166 decreased *in vitro* with increasing Fe^{+++} levels. SA production was also regulated by phenylalanine (PHE) and tryptophan (TRP). Further experiments were conducted to evaluate the effects of altering Fe^{+++}, PHE or TRP concentrations *in planta*. The results of these experiments will be discussed.

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SPATIAL DISTRIBUTION AND ASCOSPORE RELEASE BY *GIBBERELLA ZEAE* IN ARTIFICIALLY INOCULATED FIELD PLOTS OF CORN.
W.G.D. Fernando, B. Vigier, L.M. Reid and J.D. Miller, Agriculture and Agri-Food Canada, Plant Research Centre, Ottawa, ON K1A 0C6, Canada.

An early and late maturing variety of corn planted in 15 x 15 m plots were artificially infested with perithecial inoculum of *G.zeae*. Corn kernals colonized by *Fusarium graminearum* were spread in the centre of each plot in a 1 x 1 m area. An early and late inoculation two weeks apart were carried out before silking occured in either variety. Spore houses placed 1.5 m away from the inoculum source. Spores were collected on Fusarium selective media (PCNB) and on slides coated with vaseline and hexane. Spore release was nocturnal. There was a minimum lag period of 24h between rainfall and spore release. Disease incidence over distance from inoculum source showed a dispersal gradient, mostly in the windward direction. There was no difference in disease incidence due to early or late inoculation on either variety.

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INFLUENCE OF WHEAT STRAW AGE ON SPORE PRODUCTION OF *PYRENOPHORA TRITICI-REPENTIS* IN THE NORTHERN GREAT PLAINS. J.M. Krupinsky. USDA-ARS, Northern Great Plains Research Laboratory, P.O. Box 459, Mandan, ND 58554-0459.

Rotorod spore samplers were used to detect the spore production of *P. tritici-repentis* from naturally infected wheat straw and wheat straw artificially inoculated with *P. tritici-repentis* from one through four growing seasons, 1989-1994. Support frames were used to hold infested wheat straw above the spore samplers. Conidia, *Drechslera tritici-repentis*, production was greatest during the first year the straw was monitored and declined each following year. With one exception during an unusually wet year, ascospore production was very low compared to conidia production during the first year. Generally, ascospore production was greatest during the second year the straw samples were monitored.

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EFFECT OF CULTIVAR AND CULTURAL PRACTICES ON THE EPIDEMIOLOGY AND SEVERITY OF RICE BLAST DISEASE. D.H. Long, D.O. TeBeest and F.N. Lee, University of Arkansas, Fayetteville.

Field experiments were conducted to measure rice blast development and the effect of N fertilization on the incidence and severity of blast on eight selected rice cultivars in small plots in Arkansas. Our results indicate that leaf blast does not develop continuously throughout the season and that the application of nitrogen at preflood above the recommended rates significantly increased the severity and incidence of leaf blast on some cultivars. Differential cultivar responces to nitrogen effects were detected using AUDPC values for disease severity, disease incidence, number of lesions per plant and total lesion area. The incidence of collar rot was significantly correlated to neck blast incidence in small plots for all cultivars. The incidence of leaf blast and and collar rot may have important implications in predicting the occurrence and severity of neck blast and potential yield losses.

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EFFECTS OF PLANTING DATE AND SEED SIZE ON COMMON ROOT ROT SEVERITY IN HARD RED WINTER WHEAT IN THE TEXAS PANHANDLE. **J. M. Shriver, C. M. Rush, and K. M. Vaughn. Texas Agric. Exp. Stn., PO Drawer 10, Bushland, TX 79012.**

A dryland field study was begun in the fall of 1994 to determine if planting date and/or seed size affected severity of common root rot caused by *Bipolaris sorokiniana*. Seed of five cultivars were separated into three size categories using no. 6 and no. 10 size sieves and were planted on Sept. 6 and Oct. 7, 1994, into a field naturally infested with *B. sorokiniana*. Stands planted with small seed were significantly greater at both planting dates. In March, plants were harvested and examined for disease severity. Disease severity was quantified by rating subcrown internodes on a 0-4 scale, and a disease index (D.I.) was calculated. For the September planting date, TAM200 had a significantly higher D.I. (2.43) than all cultivars tested, and there were no differences among seed sizes. In the October planting, D.I.'s for Siouxland 89 (1.63) and TAM200 (1.53) were significantly higher than that of Hawk (0.94), and plants grown from large seed had a significantly lower D.I. (1.11) than unsieved or small seed. Disease indices for September were higher than those for October.

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POPULATION DENSITIES OF *PHYTOPHTHORA SOJAE* AS AFFECTED BY TILLAGE PRACTICES IN THE NORTH CENTRAL U.S. F. Workneh, X.B. Yang, and G.L. Tylka, Department of Plant Pathology, Iowa State University, Ames 50011

Sixty-one locations, each with adjacent tilled and non tilled soybean fields, were identified in Minnesota, Iowa, Illinois, and Indiana in June 1994. In each field soil was randomly sampled at 0-7.5 and 7.5-15 cm depths, and composite samples from each depth were kept separate. Population densities of *P. sojae* were determined in samples from each field and each depth with a leaf-disc bioassay method. Twenty leaf discs were used per two subsamples of each sample. The percentage of leaf discs colonized was determined by plating the leaf discs onto a selective medium containing hymexazol. Paired t-test analysis of the percentage of discs colonized revealed that samples from no-till fields in Illinois and Indiana had significantly greater densities of *P. sojae* than those from the respective adjacent tilled fields ($P<0.02$ and $P<0.01$, respectively). Samples from Iowa and Minnesota showed similar trends, but the differences were not significant. In samples from all states, no-till fields had greater densities of the pathogen in 0-7.5 cm depth than in 7.5-15 cm. Conversely, samples from conventionally tilled fields had greater densities in 7.5-15 cm than in 0-7.5 cm, indicating the importance of debris placement.

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USE OF RAPD AND SPECIES-SPECIFIC PCR PRIMERS AS AIDES IN IDENTIFYING SDS ISOLATES OF *FUSARIUM SOLANI*. L. E. Gray[1] and L. Achenbach[2]. [1]USDA/ARS, Department of Plant Pathology, University of Illinois, Urbana, IL 61801, [2]Department of Microbiology, Southern Illinois University, Carbondale, IL 62901.

Randomly amplified polymorphic DNA (RAPD) markers were used to characterize different isolates of *Fusarium solani* that cause soybean sudden death syndrome. Twenty isolates representing collections from various locations in Illinois and other states were evaluated along with eight isolates of *F. solani* from other plant hosts. The SDS isolates were readily distinguished from other non-SDS isolates based on banding patterns generated with five 10-base primers. Repeatability of banding patterns with different primers has been compared between laboratories using different sources of *Taq* DNA polymerase, DNA samples, and thermocyclers. A species-specific PCR primer pair was used to separate SDS isolates from non-SDS isolates of *F. solani*. This primer pair specifically amplifies DNA from only SDS isolates and can be used to identify newly collected isolates. Pathogenicity tests of all fungal isolates are being conducted in the greenhouse to determine the severity of root rot on inoculated soybean plants as well as the severity of foliar SDS symptoms.

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DIALLEL ANALYSIS OF EIGHT ELITE MAIZE LINES FOR RESISTANCE TO DIPLODIA EAR ROT. A.E. Dorrance, E.L. Stromberg and H.L. Warren. Dept. of Plant Pathology, Physiology and Weed Science, VPI & SU Blacksburg, VA 24061-0331.

An F1 generation from a diallel cross with eight parents was evaluated for resistance to diplodia ear rot (DER), caused by the fungus *Stenocarpella maydis*. F1's, and reciprocals were planted in a randomized complete block with 4 replications. Germplasm was inoculated with 5mls of a pycnidiospore suspension (5,000 spores/ml) placed in the whorl at 10 and 17 days prior to tasseling. Ear rot ratings were taken at harvest. A nonorthogonal analysis was conducted and no reciprocal effects were found. Ear rot ratings of reciprocal crosses were pooled to determine general (GCA) and specific combining effects (SCA). GCA effects were significant but SCA effects were not. These results indicate additive genetic factors are involved in resistance to DER. This F1 germplasm will be re-tested in crop year 1995.

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OVERWINTERING OF SOILBORNE FUNGI ON DEAD COTTON ROOTS. R.E. Baird[1] and W. E. Batson[2], [1]RDC, Box 1209, The University of Georgia, Tifton, GA 31794; [2]P.O. Drawer PG, Ent. and Plant Path., Mississippi State, MS 37962.

Following the 1994 growing season, cotton (*Gossypium hirsutum L.*) roots of cv. Deltapine 90 were left in the soil after mowing at Miss. State, MS and three locations in GA. including Calhoun, Midville, and Tifton. During the first week of each month from December, 1994 through April, 1995, five randomly selected root systems were removed at each site and sent to the Tifton laboratory for assaying. Ten pieces (1 cm long) of each tap and feeder roots per root system were assayed in the lab. Results from the first year showed that isolation frequencies for all fungal species varied per root sample location and between sampling dates. Pathogens which cause boll rot and seedling diseases were commonly isolated, but they varied per location and sampling date. Common boll rot fungi were *Lasiodiplodia theobromae, Alternaria* spp., several *Fusarium* spp., and important seedling disease pathogens including primarily *Fusarium oxysporum*, but only low percentages of *Rhizoctonia* spp. and *Pythium* spp. were observed. Other commonly isolated fungi were *Melanospora* sp. and *Phoma* spp.

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RANDOM AMPLIFIED POLYMORPHIC DNA ANALYSIS OF *Xylella fastidiosa* PIERCE'S DISEASE AND OAK LEAF SCORCH PATHOTYPES. **J. Chen[1], O. Lamikanra[1], C. J. Chang[2], & D. L. Hopkins[3], [1]Florida A&M University, Tallahassee, FL 32307, [2]University of Georgia, Griffin, GA 30223, [3]University of Florida, Leesburg, FL 34784.**

Random amplified polymorphic DNA (RAPD) analysis was conducted with 14 primers to 17 strains of *Xylella fastidiosa*. There was a high degree of similarity among the seven Pierce's disease (PD) strains (Sxy > 0.93) and the seven oak leaf scorch (OLS) strains (Sxy > 0.96). The two groups were different with a similarity index of 0.67, confirming the presence of a PD DNA cluster and suggesting a new OLS cluster; whereas, the control plum leaf scald (two strains) together with the periwinkle wilt strain have much smaller similarity value (0.44) to PD and OLS clusters.

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EVIDENCE FOR CLONING OF A PATHOGENICITY DETERMINANT FROM *STREPTOMYCES SCABIES*. R. A. Bukhalid and R. Loria, Department of Plant Pathology, Cornell University, Ithaca NY 14853.

Our goal is to identify pathogenicity factors from *Streptomyces scabies*, a cause of potato scab. We have cloned a 9.4-kb DNA fragment (RB101) from *S. scabies*, which allows the non-pathogen *S. lividans 66* TK24 to colonize and sporulate on potato tuber slices. Mutation analyses demonstrated that activity was conferred by a 1.6-kb DNA fragment from RB101. When used as a probe in high stringency Southern analysis, this 1.6-kb fragment hybridized to 13 *Streptomyces* strains which are pathogenic on potato, and which produce the phytotoxin, thaxtomin A. These include *S. scabies*, *S. acidiscabies*, and other unidentified pathogenic *Streptomyces* spp. The probe did not hybridize to the 13 nonpathogenic strains which do not produce thaxtomins, with the exception of one strain which had previously been shown to be pathogenic. Extracellular compounds produced by *S. lividans* TK24 (pRB101) produced lenticel browning and collapse, and darkening of the periderm of immature potato tubers. Additionally, culture extracts of pRB101 completely inhibited seedling growth in a radish seedling assay.

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ISOLATION AND CHARACTERIZATION OF THE GENE IN *PANTOEA CITREA* RESPONSIBLE FOR THE PINK DISEASE OF PINEAPPLE. J.-S. Cha[1], C. Pujol and C. I. Kado, Dept. of Plant Pathology, University of California, Davis, CA 95616; [1]Dept. of Agricultural Biology, Chungbuk National University, Chungbuk, Korea 360-763.

Pink disease of pineapple is caused by *Pantoea citrea*, an opportunistic pathogen that invades through pineapple florets and affects interior tissues of the fruit. The symptoms are subtle and usually remain undetectable visually in the field. The effects of infection become apparent after the cored fruit is canned. All infected sites are stained with a reddish-brown to pink pigment, a result that is unmarketable. The biochemical basis of this staining reaction has remained a mystery for many years. We have examined *P. citrea* using molecular approaches and have identified and isolated a gene responsible for causing the post canning pigmentation. This pink disease gene (*pnk*) has been sequenced and its predicted protein product analyzed. The results indicate that the *pnk* gene encodes the enzyme D-glucose dehydrogenase (GDH). A mutation in *pnk* abolishes the pink disease phenotype and this mutation can be complemented with the wild-type *pnk* gene. These results indicate that GDH is a key enzyme contributing to the pink disease.

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PLASMID-BORNE PATHOGENICITY GENES OF *XANTHOMONAS CAMPESTRIS* PV. MALVACEARUM. P.K. Chakrabarty and D.W. Gabriel. Plant Pathology Dept. University of Florida, Gainesville, FL 32611.

Three plasmids of size 55, 31.2 and 7.4 kb were isolated from a race 18 isolate of *X. campestris* pv. malvacearum. The bacterium was cured of all three plasmids in high frequency (3.1%) with heat (42° C). Virulence of the cured strains was drastically reduced. Transformation of a cured strain using plasmid DNA resulted in colonies carrying all seven possible combinations of the three plasmids. Each of the plasmids contributed additively to virulence. An African strain (proposed race 19) of the pathogen, XcmN, with no known avirulence (*avr*) genes, was similarly cured of one of its two plasmids and exhibited drastically reduced virulence. Virulence of this cured strain was restored by *avrb6*, a member of an avirulence/pathogenicity gene family cloned from a race 2 isolate of the the pathogen. The genes on the XcmN plasmid responsible for pathogenicity but not encoding avirulence are being analyzed.

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ISOLATION OF TWO *PSEUDOMONAS SOLANACEARUM* MUTANTS DEFECTIVE IN PRODUCTION OF HOMOSERINE LACTONE-LIKE COMPOUNDS. A.B. Flavier and T.P. Denny. Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Homoserine lactone (HSL) derivatives act as intercellular signal molecules to regulate diverse processes in numerous bacteria, including phytopathogens. *Pseudomonas solanacearum* produces HSL-like compounds that induce expression of HSL-inducible reporter genes in other bacteria, including *Agrobacterium tumefaciens*. As an initial approach to investigate the role of HSL in *P. solanacearum*, random Tn*5* mutants were screened for failure to induce expression of the *A. tumefaciens tra::lacZ* reporter. One strain, AI-1, made no detectable HSL-like compounds, while another, AI-2, produced reduced levels. Extracellular polysaccharide production (EPS) in both mutants was reduced on minimal but not on rich media at 30°C, but for AI-2, EPS production was normal at 25°C. Cosmid clones were identified in a *P. solanacearum* genomic library that restored both HSL and EPS production to each of the mutants. Clones that restored HSL production to AI-2 did not complement AI-1, suggesting the inactivation of different and probably unlinked genes in the two mutants.

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CLONING OF A NOVEL *PEL* GENE FROM A *PELABCEX PEHX ERWINIA CHRYSANTHEMI* EC16 MUTANT USING TRANSPOSON TN*5*TAC1. Jong H. Ham, James R. Alfano, and Alan Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853-4203.

E. chrysanthemi mutant CUCPB5047, Δ(*pelA pelE*) Δ(*pelB pelC*)::28bp Δ(*pelX*)Δ4bp *pehX*::ΩCm^r, was constructed. CUCPB5047 is still able to macerate plant tissue, albeit with reduced virulence, and produces a second set of pectate lyase (Pel) isozymes *in planta*. We mutated CUCPB5047 with Tn*5*tac1 and screened for IPTG-dependent Pel production. All hyperexpressing Pel$^+$ mutants produced a single Pel isozyme with a very alkaline pI, as determined by activity-stained isoelectric focusing gels. A Km^r *Sac*I fragment from Pel$^+$ Tn*5*tac1 mutant CUCPB5066 was cloned into *Escherichia coli* NM522 and sequenced. *pelK* encodes a novel, asparagine-rich protein, with an N-terminal signal peptide and 29% identity with the C-terminal 500 amino acids of PelX. PelK shows no sequence similarity with PelE, but the two isozymes have similar enzymological properties and macerate plant tissues equivalently. We are now constructing *pelK* mutations in CUCPB5047 with the further goal of cloning the genes encoding additional Pel isozymes.

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IDENTIFICATION OF ACHOLEPLASMA STRAINS FOR THE HETEROLOGOUS EXPRESSION OF PHYTOPLASMA GENES. Christine D. Smart and Bruce C. Kirkpatrick. Department of Plant Pathology, University of California, Davis CA 95616.

Because phytoplasmas have not been cultured *in vitro*, nothing is known about phytoplasmas genes that mediate insect transmission or plant pathogenicity and host specificity. One strategy for identifying such phytoplasma genes is to identify a culturable mollicute that could express phytoplasma genes. We used a comparatively variable phylogenetic marker, the 16/23S rDNA spacer region (SR), to evaluate similarities between phytoplasmas and several Acholeplasma species. We sequenced the SRs of more than 60 distinct phytoplasma strains and all contained only a single $tRNA^{Ile}$ within the SR. *Acholeplasma laidlawii*, *A. axanthum*, *A. oculi*, and *A. sp. J233* all contained two tRNA genes, $tRNA^{Ile}$ and $tRNA^{Ala}$, within their SRs. However, *A. modicum* and *A. brassicae* only contained a single $tRNA^{Ile}$ in their SR, an organization that was previously found only in the phytoplasmas. This similarity, together with 16S rDNA sequence data, suggests that *A. modicum* and *A. brassicae* may be the best potential hosts for replicating and expressing phytoplasma genes. We are now evaluating whether these *Acholeplasma* species can replicate and express phytoplasma plasmid genes.

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CHARACTERIZATION OF THE *AGROBACTERIUM VITIS* POLYGALACTURONASE GENE *PEHA* AND ITS PROTEIN PRODUCT. Thomas C. Herlache[1], Xinan Pu[2], Thomas J. Burr[2], and Alan Collmer[1]. [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853. [2]Department of Plant Pathology, New York State Agricultural Experiment Station, Geneva, NY 14456.

Agrobacterium vitis causes crown gall on grape and is unique among *Agrobacteria* spp. in its ability to cause grape-specific root decay and systemic infections. The decay observed on juvenile grape roots is associated with production of a single polygalacturonase (PG) isozyme with an acidic pI. Derivatives of *A. vitis* strain CG49 carrying mutations in the PG-encoding gene *pehA* do not cause root decay and are reduced in tumorigenicity on woody grape stems. Sequencing of the *pehA* gene revealed a predicted 532 amino-acid protein with homology to PGs from *Erwinia carotovora* and *Pseudomonas solanacearum*. The PGs have similar pH and temperature optima, but time-point analysis of enzymatic reaction products revealed differences in their catalytic properties. These three PGs also differ in several properties potentially important in pathogenesis, including tissue maceration and cell killing.

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SHOOT TIP CULTURE OF PERIWINKLE INFECTED BY *Spiroplasma citri* OR ASTER YELLOWS PHYTOPLASMA. C. J. Chang and Ming Chen. Dept. of Plant Pathology, Univ. of Georgia, Griffin, GA 30223.

Shoot tips (1 cm) of periwinkle (*Catharanthus roseus* L.) infected by *S. citri* (SC) or aster yellows phytoplasma (AYP) as well as those of healthy periwinkle were grown in Murashige and Skoog medium containing 1 mg/l of α-naphthalenacetic acid (MS1N). Shoot tips of AYP-infected as well as healthy plants had normal shoot and root growth, whereas those of SC-infected plants had growth of calluses and shoot proliferations with limited growth of root primordia after 11 weeks in MS1N medium. The stem and leaf dry weight:fresh weight ratios were 0.12, 0.15, and 0.24 for healthy, AYP-, and SC-infected plants, respectively. The dry weight:fresh weight ratios were 0.06, 0.11, and 0.18 for healthy, AYP-, and SC-infected plants, however, the latter ratio represented more root primordia and calluses than actual roots.

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DEVELOPMENT OF SPECIFIC PROBES FOR IDENTIFICATION OF THE SUGARCANE PATHOGENS, *XANTHOMONAS CAMPESTRIS* PV. *VASCULORUM* AND *X. ALBILINEANS*. E. Hatziloukas[1], N. J. Panopoulos[2], Z. K. Wang[3], J. C. Comstock[3], and N. W. Schaad[1]. [1]USDA/ARS, FDWSRU, Frederick, MD 21702, [2]Inst. Molec. Biol. & Biotech., Crete, Greece, [3]USDA/ARS, Canal Point, FL 33438.

Several DNA probes and a pair of PCR primers were tested for identification of *Xanthomonas campestris* pv. *vasculorum* and *X. albilineans*, the causal agents of gumming and leaf scald of sugarcane, respectively. One probe, a 9.5-kb fragment from the xanthomonadin biosynthetic gene cluster of *X. campestris* pv. *campestris*, hybridized to all xanthomonads tested (*X. oryzae* pv.'s *oryzae* and *oryzicola*; *X. c.* pv.'s *campestris, vesicatoria,* and *vasculorum*) including *X. albilineans* but not to several non-xanthomonads. A pair of degenerate primers designed to amplify a short segment of *rcsA*, the regulatory gene of the capsular polysaccharides biosynthesis, amplified (with varying efficiency) discrete bands from either purified DNA or intact cells from several Gram-negative bacteria including *Escherichia coli, Erwinia* spp., and *Xanthomonas* spp. The amplification products from both sugarcane pathogens were cloned and tested by Southern hybridization. Preliminary results indicate that the clones are organism-specific and they will be used to identify their genomic counterparts from which specific primers will be selected.

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EVIDENCE FOR A BACTERIAL ETIOLOGY OF PAPAYA BUNCHY TOP DISEASE. M. J. Davis[1], J. B. Kramer[1], F. H. Ferwerda[2], and B. R. Brunner[3]. [1]University of Florida, TREC, 18905 SW 280 Street, Homestead, FL 33031; Department of Horticulture, University of Puerto Rico, [2]Mayaguez, PR 00680, and [3]Agricultural Experiment Station, HC 01 Box 11656, Lajas, PR 00667.

A total of 95 papaya trees with symptoms of papaya bunchy top (PBT) from 12 countries throughout the American Tropics were assayed by polymerase chain reaction for the presence of 16S rRNA genes of mycoplasma-like organisms, but none were found. No differences were detectable between healthy and diseased papaya DNA samples. Abnormal fluorescence was consistently observed by epifluorescence microscopy in the region between the xylem and phloem in transverse sections of PBT-affected petioles when stained by DAPI or acridine orange. Bacteria were found in the region by transmission electron microscopy and observed in expressed sap by light microscopy. The bacteria were associated with PBT-affected, but not with healthy, papaya trees. The bacteria measured 0.25-0.35 µm in width and 0.8-1.6 µm in length and had a Gram-negative type cell envelope. All attempts to isolate the bacterium in axenic culture have failed.

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SPECIFIC IDENTIFICATION OF STRAINS OF *XYLELLA FASTIDIOSA* CAUSING CITRUS VARIEGATED CHLOROSIS DISEASE USING SEQUENCE CHARACTERIZED AMPLIFIED REGIONS Margaret R. Pooler and John S. Hartung, USDA ARS Fruit Lab, Room 111 Bldg 004 BARC-West, Beltsville, MD 20705

RAPD-PCR assays with twenty-two 10 nucleotide primers produced 77 discrete polymorphic bands which were used to create a similarity matrix. This matrix was consistent with other systematic data. The CVC strains of *X. fastidiosa* (S. American origin) were well separated from the strains from other hosts (N. American origin). Several of the RAPD-PCR products were cloned and sequenced to create Sequence Characterized Amplified Regions. PCR reaction conditions are described which produce amplified products with all strains of *X. fastidiosa* as template (species-specific) and only with *X. fastidiosa* from CVC as template (disease-specific). The latter were derived from polymorphic RAPD products rather than from bands amplified only from CVC templates.

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A COMPUTER-CONTROLLED ENVIRONMENTAL CHAMBER FOR THE STUDY OF AERIAL FUNGAL SPORE RELEASE. T. R. Gottwald and T. M. Trocine, USDA-ARS, Horticultural Research Lab, Orlando, FL 32803.

An environmental chamber was designed to study aerial release of spores of ascomycetes and hyphomycetes, based on a previous device (Phytopathology 79:189-191). Relative humidity (RH), temperature, red (660 nM) and infrared (880 nM) light, leaf wetness, wind speed, vibration, and rain events are controlled and monitored within the chamber via a RTC-HC11 real-time controller and data acquisition system. A BASIC computer program is uploaded to and controls the system by requesting a profile of environmental conditions that change through time according to user specifications. The controller interacts with a stepper motor, solenoids and relay switches via a feedback system based on data received from solid state RH, temperature and leaf wetness sensors. The data acquisition system records environmental data from the chamber in RAM memory that can be downloaded to a PC for correlation with spore release data. Spores released from fungal specimens on plant tissues and cultures that are placed into the chamber and subjected to the desired interactions of environmental conditions are collected on a continuous volumetric spore trap at an exhaust port from the chamber. The device is presently being used to study *Mycosphaerella citri* and *Alternaria citri* spore release.

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DEVELOPMENT OF A SPRAY FORECAST MODEL FOR TOMATO POWDERY MILDEW. R.A. Guzmán-Plazola; J.J. Marois; R. M. Davis. University of California. Davis, CA. 95616.

The effect of temperature, relative humidity and leaf wetness on spore germination, latent period and disease development was studied in controlled environments and in fresh market tomato fields. *Leveillula taurica* induced disease at 20 or 25 C but not at 30 C. At 25 C, the latent period was 9-10 days, with visual symptoms evident 3-5 days later. Lower temperatures delayed symptom development. Conidia were formed at relative humidities (RH) of 20-90%. Optimum disease development (lesion size and percentage of diseased area) occurred at 60-70% RH. Temperatures of 20-25 C and high RH favored fungal growth and sporulation; diurnal shifting to higher temperature and lower RH allowed spore release and favored yellowing and darkening of lesions. Free water on the leaf surface was not required for spore germination or fungal development. An experimental forecast model is being developed based on data from controlled environment and field conditions; its usefulness to predict when fungicide sprays are required to control powdery in tomato fields will be investigated.

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LONG-TERM DYNAMICS OF WHEAT RUSTS IN CHINA AND THE U.S. IN RELATION TO THE EL NIÑO / SOUTHERN OSCILLATION. H. Scherm and X. B. Yang, Department of Plant Pathology, Iowa State University, Ames 50011

The El Niño / Southern Oscillation (ENSO) is one of the strongest mechanisms of interannual climatic variation. Because it influences global temperature and precipitation patterns, long-term plant disease records may contain an ENSO-related signal. We used cross-spectral analysis to establish coherence and phase relationships between the Southern Oscillation Index (SOI), which is a measure of the ENSO, and long-term (>40 yr) dynamics of wheat stripe rust in five regions of northern China and wheat stem rust in four regions of the midwestern U.S. The coherence relationships revealed consistent and significant ($0.01 < P < 0.10$) co-oscillations between the rust series and the SOI series at temporal scales characteristic of ENSO events. The five stripe rust series were coherent with the SOI at periodicities of 2.0-3.0 and 8.0-10.0 yr, and three of the four stem rust series were coherent with the SOI at a periodicity of 6.8-8.2 yr. The phase relationships showed that, in most cases, the rust series and the SOI series co-oscillated out of phase, suggesting that the associations between them are indirect.

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Seasonal Development and Release of Venturia inaequalis Ascospores. Donald E. Aylor, The Connecticut Agricultural Experiment Station, New Haven 06504

To manage apple scab (caused by Venturia inaequalis) it is important to know the start and the duration of the ascospore release season. Diseased leaves were collected twice a week from the field for three seasons, and the time course of release of V. inaequalis ascospores from these leaves was determined in a standardized test. In addition, ascospores in the air above the source in the field were monitored throughout the season. The cumulative numbers of ascospores either released from leaves or in the air above the source were described well by a logistic function of time. The cumulative release of ascospores from leaves was found to lag behind the cumulative number of airborne ascospores. This lag suggests a late-season decrease in the dispersal potential of ascospores which could have important implications for management.

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MICROGEOGRAPHIC VARIATION OF *XANTHOMONAS ORYZAE* PV.*ORYZAE*. C.M. Vera Cruz[1], E. Ardales[2], J. Talag[2], R. Sridhar[2], R.J. Nelson[2], D.Z. Skinner[1], T.W. Mew[2] and J.E. Leach[1]. [1]Kansas State University, Manhattan, KS 66506-5502; [2]International Rice Research Institute, Manila, Philippines.

Genetic variation was measured in a collection of 162 *Xanthomonas oryzae* pv. *oryzae* strains that were systematically sampled from a farmer's field where the pathogen was endemic. The field was planted to an improved rice cultivar Sinandomeng with no known bacterial blight resistance genes. The amount and distribution of variation were measured by restriction fragment length polymorphism (RFLP) analysis with the repetitive transposable element probe IS*1113* and rep-PCR, which is based on DNA amplification using short bacterial repetitive elements (specifically, ERIC and REP primers). The strains were consistently grouped into two distinct lineages by both fingerprinting techniques. Rep-PCR revealed higher haplotypic diversity (H_t = 0.415) than RFLP (H_t = 0.338). Both techniques showed that the spatial distribution of haplotypes varied across the field. The most frequently observed haplotype was distributed in all 25 blocks. Two blocks showed significantly higher haplotypic diversity (H_s=0.833 and 0.733) than the total haplotypic diversity for the population (H_t = 0.415).

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A MECHANISTIC MODEL OF THE RESPONSE OF A FOLIAR PARASITE TO COMBINED EFFECTS OF TEMPERATURE AND DURATION OF LEAF WETNESS. Duthie, J.A. and Damicone, J.P., Department of Plant Pathology, Oklahoma State University, Lane, OK 74555.

In order to forecast outbreaks of disease, combined effects on a foliar parasite of temperature (T) and duration of leaf wetness (W) often are evaluated in controlled environments. Typically, the response surface (Y) is characterized empirically by an intrinsically linear, polynomial equation in which the estimated parameters are numerous and have no clear biological meaning. Alternatively, Y may be described more parsimoniously by the product of two intrinsically nonlinear equations (Y_1 and Y_2) that are derived from prior knowledge of the constituent processes. If $Y_1=\{\exp[(T-a)^2/b]\}^{-1}$, then Y decreases at an intrinsic rate of *a* and approaches a lower limit of Y=0 as T increases or decreases from an optimum at T=*b*. If $Y_2=c\{1-\exp[-(W/d)^e]\}$, then Y increases monotonically from a lower limit of Y=0 at W=0 to an upper limit of *c* when W is large, at an intrinsic rate of *d*, with a shape of *e*. This mechanistic model suggests a basis for an ecological classification of foliar parasites. Mechanistic models and polynomial models will be compared for distinguishing the response of *Cercospora* sp. among three cultivars of peanut.

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USING MATRIX MODELS TO EVALUATE THE INFLUENCE OF *RHIZOCTONIA* SPP. ON POPULATION DYNAMICS IN BIRDSFOOT TREFOIL. Emery, K.M. and English, J.T. Dept. of Plant Pathology, Univ. of Missouri, Columbia, MO 65211.

Failure of the perennial forage legume, birdsfoot trefoil, to persist under disease pressure is a major limitation to its productivity and utilization. Infection by *Rhizoctonia* spp., in particular, affects all stages in the life cycle of birdsfoot trefoil including seed germination, survival, growth and reproduction. Matrix population models are being used to investigate the relationship of epidemics incited by *Rhizoctonia* spp. to the dynamics of reproduction, recruitment, and survival. Rates of transition related to these processes are being compared between host populations in open or closed canopies in which disease levels differ. Matrix models provide insights into the relationship of disease in a particular growth stage with population survival. Analyses to date have shown that disease affects recruitment strongly and thus, influences stand persistence. These models should aid in making management decisions that improve stand longevity and productivity.

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RELATIONSHIPS BETWEEN DISEASE INCIDENCE MEASURED AT DIFFERENT SPATIAL SCALES. G. Hughes[1], N. McRoberts[2], L. V. Madden[3] and T. R. Gottwald[4]. [1]Institute of Ecology and Resource Management, University of Edinburgh, Edinburgh EH9 3JG, Scotland, UK; [2]Department of Plant Science, Scottish Agricultural College, Ayr KA6 5HW, Scotland, UK. [3]Department of Plant Pathology, Ohio State University, OARDC, Wooster, OH 44691; and [4]Horticultural Research Laboratory, USDA-ARS, Orlando, FL 32803.

Using theory (based on the properties of the binomial and beta-binomial distributions, and on an empirical 'power-law' relationship between the observed and binomial variances of disease incidence) and data (based on observations of grape downy mildew, caused by *Plasmopara viticola*, and of citrus scab, caused by *Elsinoe fawcettii*), we describe the development of relationships between disease incidence measured at two levels within a spatial hierarchy, and discuss the possible applications of such relationships in plant disease epidemiology.

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ENVIRONMENTAL AND FUNGICIDE EFFECTS ON EPIDEMICS OF GRAPE POWDERY MILDEW. Robert C. Seem and David M. Gadoury, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, 14456.

Weather and disease data from sprayed and unsprayed vines were analyzed for a 10-yr period. Initial foliar *incidence* was lower on sprayed than unsprayed vines, but the rates of increase (*R*) were equal. In contrast, *R* of foliar *severity* was reduced by fungicide use. Foliar incidence after 1.0 infected leaf/shoot was more accurately predicted by time (days) than by environmental factors. Severity of fruit infection at harvest was correlated with number and intensity of rain events before bloom. Our results suggest that fungicides, as presently used, provide spatially and/or temporally discontinuous protection, and suppress powdery mildew by limiting local spread upon infected tissue; *i.e.*, *R* of severity rather than incidence is reduced. Furthermore, typical temperatures in New York vineyards may not limit, nor determine rates of secondary development of powdery mildew. Finally, severe fruit infection may be a consequence of severe ascosporic (primary) infection resulting from numerous rain events before bloom.

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GENETIC FINGERPRINTING AND RELATEDNESS DETERMINATION OF *AGROBACTERIUM VITIS* STRAINS BY RESTRICTION FRAGMENT ANALYSIS OF rRNA GENES. E. A. Momol[1], W. F. Lamboy[2], C. L. Reid[1], T. J. Burr[1]. Depart. of Plant Path., and Hort. Sci. and USDA-ARS, PGRU[2], Cornell University, Geneva, NY.

Restriction fragment patterns from rRNA genes were used to genetically fingerprint fifteen different strains of *Agrobacterium vitis* and to evaluate relationships among them. Strains were obtained from *Vitis riparia* roots growing in several different geographic regions. Four of the strains were tumorigenic, four others had biological control properties against tumorigenic *A. vitis* and the remaining seven had neither of these characteristics. Using locus-specific primers, the regions of DNA coding for the 16S rRNA and the intergenic spacer region between 16S and 23S were amplified by PCR. Restriction digests of the amplification products yielded 127 scorable fragments. For every pair of strains, Nei-Li similarity coefficients were computed from restriction fragment presence / absence data. UPGMA cluster analysis placed the four biocontrol strains into a single group based on their Nei-Li similarities. In addition, sets of restriction fragments were found that distinguished all of the 15 strains from one another. Based on these results, we conclude that ribo-fingerprinting is a valuable tool for measuring the genetic diversity in *A.vitis*.

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GENETIC DIVERSITY OF *ERWINIA AMYLOVORA* STRAINS AS DETERMINED BY RAPD FRAGMENTS. M. T. Momol[1], E. A. Momol[1], W. F. Lamboy[2], J. L. Norelli[1], H. S. Aldwinckle[1], and S. V. Beer[3]. Departments of Plant Pathology, Geneva[1] and Ithaca[3], and Hort. Sci. and USDA-ARS, PGRU[2], Cornell University, Geneva, NY.

RAPD fragments were used to assess genetic diversity among 16 strains of *Erwinia amylovora* (Ea). Strains were chosen to represent different geographic regions and host plant origins. One strain each of *Erwinia herbicola* and *Agrobacterium vitis* were used as outgroups. Six ten-mer primers resulted in the detection of 98 different RAPD fragments. Nei-Li similarity coefficients were computed for every pair of strains from data on fragment presence / absence. Relatedness between strains was determined by UPGMA clustering. With the exception of two strains isolated from Asian pear, strains of Ea isolated from *Pomoideae* formed a single group. Two strains isolated from *Rubus* formed a third group. RAPD fragments were found that enabled each of the three groups to be unambigously distinguished from each other and from the outgroups. RAPD banding profiles were found that enabled all 16 of the Ea strains to be distinguished from one another. More strains must be studied to determine whether the differences reported here among Ea strains reflect a continuum of diversity or isolated, disjunct subgroups.

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MUTATIONAL ANALYSIS OF THE ROLE OF THE *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* HRPZ IN ELICITATION OF THE HYPERSENSITIVE RESPONSE IN TOBACCO. James R. Alfano, David W. Bauer, Timothy M. Milos, and Alan Collmer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853-4203.

P. s. syringae 61 *hrpZ* encodes a 341 amino acid protein, HrpZ, that has been shown previously to elicit the hypersensitive response (HR) on plants. *Escherichia coli* cells carrying pHIR11 with several internal *hrpZ* deletions retained their ability to elicit an HR on tobacco. However, a complete deletion of hrpZ from pHIR11 resulted in loss of HR elicitation activity. When this mutation was marker exchanged into the chromosome of *P. s. syringae* the mutant, CUCPB5057, was still capable of eliciting an HR on tobacco. Several partially purified, histidine-tagged, truncated HrpZ forms retained their HR-inducing activity. Paradoxically, an amino-terminal portion that contains the first 151 amino acids of HrpZ and a carboxyl-terminal portion that contains amino acids 126 to 280 of HrpZ are stable and capable of eliciting the HR on tobacco. Taken together, these results suggest that elicitor activity is not confined to one region of *hrpZ* and that *P. s. syringae* 61 contains multiple elicitor proteins that travel the type III secretion pathway.

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CLONING AND CHARACTERIZATION OF A PECTATE LYASE GENE FROM *PSEUDOMONAS SYRINGAE* PV. *LACHRYMANS*. David W. Bauer and Alan Collmer, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The ability to produce pectolytic enzymes has been described for several pathovars of *Pseudomonas syringae*, but the role these enzymes play in disease has not been investigated. We cloned a gene for a pectate lyase (designated *pelP*) from *P. syringae* pv. *lachrymans* strain 859. Sequencing of the gene revealed that mature PelP is 350 aa in length and about 37.4 kD. The protein sequence has high similarity to pectate lyases from *P. fluorescens* and *P. viridiflava*, and moderate similarity to PelE from *Erwinia chrysanthemi* and a pectate lyase from *Bacillus subtilis*. PelP and PelE were found to be very similar in several of their enzymatic properties, including pH optimum, substrate utilization, calcium dependence, and macerating ability. The presence of homologs of *pelP* in several other *P. syringae* pathovars was shown by probing genomic DNA blots with *pelP* DNA. The *pelP* gene was mutated by insertion of an ΩKm fragment and marker exchanged into the highly virulent strain of *P. syringae* pv. *lachrymans*, Pla5. The mutants no longer produced pectate lyase in King's B medium. Pathogenicity assays on cucumber cotyledons revealed that the mutants were as virulent as the wild-type parent. Thus, *pelP* does not appear to play a role in pathogenicity.

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HARPIN IS EXPORTED VIA THE TYPE III SECRETION PATHWAY. Adam J. Bogdanove, Zhong-Min Wei, Li Ping Zhao, and Steven V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Complementation groups VI, VII, and VIII of the *hrp* gene cluster of *Erwinia amylovora* were shown to be required for harpin secretion. Complementation group VI contains the previously characterized *hrpI* gene,encoding HrpI, a member of the LcrD family of secretory proteins. The remaining portions of the roughly 11 kb region encompassing complementation groups VI, VII, and VIII were sequenced. The entire region was found to contain, including *hrpI*, eleven contiguous or overlapping open reading frames (ORFs). All but two of these ORFs are similar to and for the most part colinear with genes in *Yersinia* previously shown to be involved in the secretion and regulation of Yops, including a conserved cluster of genes thought to encode inner membrane and cytoplasmic components of the Type III secretion pathway. This pathway is used by a variety of bacteria for export of diverse proteins including virulence factors and flagellar components. Analysis of a nonpolar mutant confirmed that the Type III pathway functions in *E. amylovora* in the export of harpin.

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INVOLVEMENT OF A TWO-COMPONENT SYSTEM AND 3-HYDROXYPALMITIC ACID METHYL ESTER (3-OH PAME) IN THE REGULATION OF VIRULENCE IN *PSEUDOMONAS SOLANACEARUM*. S. J. Clough, M. A. Schell, and T. P. Denny, Dept. Plant Pathology, Univ. of Georgia, Athens, GA 30602-7274.

Three genes *phcB, S* and *R* were identified in an operon. *phcB* is required for production of 3-OH PAME. PhcS and PhcR have amino acid sequence similarities to sensors and response regulators of two-component systems for signal transduction, respectively. We hypothesize that PhcS and PhcR act together to repress *phcA*, a global regulator of virulence, and this repression is relieved by 3-OH PAME because: (1) nonpolar mutations affecting only *phcB* phenotypically resemble *phcA* mutants (ie: 20 to 50 fold reduction in extracellular polysaccharide production, endoglucanase and pectin-methyl-esterase activities and an increase in motility), (2) *phcB* polar mutations, *phcS* or *phcR* mutations, and *phcB/S* or *phcB/R* double mutants all show near wild-type levels of PhcA-regulated virulence factors, and (3) 3-OH PAME induces nonpolar *phcB* mutants to a wild-type phenotype.

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THE HRPZ PROTEINS OF *PSEUDOMONAS SYRINGAE* PVS. *SYRINGAE, GLYCINEA* AND *TOMATO* ARE ENCODED BY OPERONS CONTAINING *YERSINIA YSC* HOMOLOGS AND EXHIBIT SIMILAR ELICITOR ACTIVITY. Gail Preston[1], Hsiou-Chen Huang[2], Alan Collmer[1]. [1]Department of Plant Pathology, Cornell University, Ithaca, NY 14853-4203. [2]Agricultural Biotechnology Laboratories, National Chung-Hsing University, Taichung, Taiwan 40227 R.O.C.

Pseudomonas syringae pvs. *syringae, glycinea* and *tomato* are host-specific plant pathogens that elicit the defense-associated hypersensitive response (HR) in non-host plants. DNA sequence analysis of the *hrpZ* operons from *P.s.* pv. *syringae* and *P.s.* pv. *tomato* shows that *hrpZ* is the second of six ORFs in a polycistronic operon. The four ORFs downstream of *hrpZ* display varying levels of similarity to four colinearly arranged *ysc* genes that are involved in a Type III protein secretion pathway in *Yersinia* spp., indicating that the *hrpZ* operon contains genes required for HrpZ secretion. Purified HrpZ proteins from *P.s.* pvs. *syringae, glycinea* and *tomato* elicited a visible HR in tobacco and tomato (but not soybean). This reaction could be blocked by coinfiltration of metabolic inhibitors, indicating that the necrosis observed is an active plant response and that HrpZ does not appear to directly determine host range.

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AUTOINDUCTION CONTROLS EXTRACELLULAR POLYSACCHARIDE PRODUCTION AND WATERSOAKING ABILITY IN *ERWINIA STEWARTII*. S. Beck von Bodman, D. R. Majerczak, S. K. Farrand, and D. L. Coplin.

Erwinia stewartii , the etiological agent of Stewart's wilt on sweet corn, produces an autoinducer signal, *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL). The gene product of *esaI* is required for OHHL synthesis. Esal is a LuxI homologue. A linked gene, *easR*, is a *luxR* homologue. An *esaI* mutant strain of *E. stewartii* is nonpathogenic on sweet corn and does not produce extracellular polysaccharides (EPS). These mutant phenotypes were restored by complementation with a functional copy of *esaI* and also by exogenous addition of OHHL. Reporter gene fusions in *cpsA*, *cpsB*, and *cpsD* express at lower levels in the *esaI* mutant background. Similarly, some reporter fusions located in the *wtsB* operon, which is required for watersoaking, show decreased activity in the *esaI* mutant background. Since the *wts* gene cluster serves to secrete harpin-Es, this is the first demonstration that autoinduction can play a role in controlling host-pathogen interactions mediated by regulating both EPS synthesis and Hrp functions.

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NUCLEOTIDE SEQUENCE ANALYSIS OF THE *ERWINIA STEWARTII CPS* GENE CLUSTER FOR SYNTHESIS OF STEWARTAN. D.L. Coplin[1], D.R. Majerczak[1], P. Bugert[2], and K. Geider[2]. [1]Dept.of Plant Pathology, The Ohio State University, Columbus OH 43210. [2]Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-69120 Heidelberg, Germany.

Erwinia amylovova and *Erwinia stewartii* form related exopolysaccharide (EPS) capsules, which are the products of the *ams* and *cps* gene clusters, respectively. The nucleotide sequence of 18.5 kb from the *cps* cluster was determined. Thirteen ORFs were arranged in the order *cpsA, B, I, C, D, E, F, G, H, J, K, L* and *M*. The *cpsA, B, I, C, H, J, K, L* and *M* ORFs were homologous to *ams* genes mapping at the same position. Data base searches suggested functions for several genes. CpsA and AmsG may add galactose to the lipid carrier; CpsB and AmsH and CpsC and AmsA are membrane proteins that may be involved in export and polymerization of the repeating unit; CpsI and AmsI have homologies to acid phosphatases; and the CpsD, CpsE, CpsF, and CpsG ORFs have features of glycosyl-transferases.

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STRUCTURE OF A POLYGALACTURONASE REGULATORY OPERON IN *PSEUDOMONAS SOLANACEARUM*. Caitilyn Allen and Jaqueline Gay. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

An operon, *pehSR*, is required for wildtype expression of the virulence factor polygalacturonase (PG) in *P. solanacearum*, incitant of bacterial wilt disease. *pehSR* mutants produce reduced levels of three extracellular PGs; when *pehSR* is present in multiple copies, bacteria overproduce PG and acquire increased motility. The operon contains 3 ORFs; the first two appear to encode a typical prokaryotic two-component regulator while the third has 60% amino acid homology to an enterobacterial amidase involved in regulation of ampicillin resistance (AmpD). Reporter gene experiments indicated that expression of *pehSR* is itself regulated by growth conditions and by PhcA, a global regulator of virulence functions, suggesting that a complex regulatory cascade controls virulence genes in this pathogen.

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· Semi-Automated analysis of complex rep-PCR genomic fingerprints of pathogenic bacteria. F.J. Louws, U. Rossbach, S. Rossbach, M. Schneider and F.J. de Bruijn. Michigan State University, East Lansing, MI 48824.

Uniform PCR conditions and primers corresponding to repetitive sequences (rep-PCR) can be used to rapidly identify pathogenic bacteria, differentiate closely related strains, and assess genetic diversity of pathogenic populations. The utility of rep-PCR would be enhanced through semi-automated analysis of the complex genomic fingerprint profiles generated. Conventional agarose gel images, containing up to 27 strain-specific fingerprint profiles, were scanned, standardized and combined to perform pattern-matching and perform cluster analysis employing computer assisted statistical software packages. Alternatively, primers were labeled with fluorescent dyes and resultant PCR products were automatically size called employing an ABI 372 DNA sequenator coupled to GENESCAN software. "Bar-code" like rep-PCR fingerprints could be manipulated for strain identification, diagnosis and analysis of population diversity. In addition, differential color labeling of gene-specific primers and rep primers enabled the simultaneous sampling of particular genes and their genomic background. Semi-automated technologies to analyze rep-PCR fingerprint patterns combined with concurrent detection of specific genes should prove useful for devising disease management recommendations and conducting ecology and epidemiology studies.

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ISOLATION OF PLASMID-BORN PATHOGENICITY GENES IN *ERWINIA HERBICOLA* PV. *GYPSOPHILAE*. S. Manulis[1], L. Valinsky[1], R. Nitazan[2], A. Lichter[2], and I. Barash[2]. [1]Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan, 50250 and [2]Dept. of Botany, Tel Aviv University, Israel.

Erwinia herbicola pv. *gypsophilae* (*Ehg*) induces gall formation in gypsophila. Our previous work has demonstrated that the pathogenicity of *Ehg* is associated with a plasmid (pPATH). Insertional inactivation of either IAA or cytokinin biosynthethic genes located on pPATH caused substantial reduction in gall size but did not eliminate gall initiation. Two clusters of non-pathogenic mutants were obtained on a cosmid clone of pPATH using the transposon-reporter Tn3-Spice. One cluster was located near the phytohormones biosynthetic genes and did not show homology to any known genes. The other cluster was located 12 kb downstream to the first one. DNA sequencing of 2 ORFs in this region showed a significant homology to genes involved in protein secretion system (type III) which was identified in animal pathogenic bacteria.

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GENETIC FINGERPRINTING OF *ERWINIA AMYLOVORA* BY REPETITIVE SEQUENCE-PCR AND PCR-RIBOTYPING. Patricia S. McManus and Alan L. Jones. Department of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing, 48824-1312.

Genetic diversity of *Erwinia amylovora* isolated from apple, pear, and *Rubus* spp. from different geographic locations was assessed by PCR-ribotyping and repetitive element-PCR (rep-PCR). For PCR-ribotyping, DNA primers were used to amplify sequences located between 16S and 23S ribosomal RNA genes. Electrophoresis of PCR-ribotyping products revealed banding patterns (=fingerprints) that distinguished tree-fruit isolates from *Rubus* isolates, but that were similar among isolates within the tree-fruit group or within the *Rubus* group. For rep-PCR, outwardly-directed primers corresponding to conserved repetitive (REP, ERIC, and BOX) elements in bacteria were used to amplify sequences located between the elements. Electrophoresis of rep-PCR products revealed a predominant fingerprint for each of the REP, ERIC, and BOX primer sets characteristic of 62, 59, and 51 of 67 tree-fruit isolates, respectively. Among 14 *Rubus* isolates, no single REP, ERIC, or BOX fingerprint was predominant. We conclude that tree-fruit isolates of *E. amylovora* from widely separated geographic regions are genetically homogenous.

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STABLE TRANSFORMATION OF THE GRAM-POSITIVE PHYTOPATHOGENIC BACTERIUM *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS* WITH SEVERAL CLONING VECTORS. M. Laine[1], H. Nakhei[2], J. Dreier[2], D. Meletzus[2], R. Eichenlaub[2], and M. Metzler[1]. [1]Dept. of Biology, Univ. of Turku, BioCity 6A, 20520 Turku, Finland. [2]Fakultät für Biologie, Gentechnologie/Mikrobiologie, Universität Bielefeld, 33501 Bielefeld, Germany.

This is the first report of transformation of *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*), the potato ring rot bacterium, with plasmid vectors. Three of these plasmids, pDM100, pDM302 and pDM306, contain the origin of replication from pCM1, a native plasmid of *C. m.* subsp. *michiganensis* (*Cmm*). We constructed two new cloning vectors, pHN205 and pHN216, using the origin of replication of pCM2, another native plasmid of *Cmm*. Plasmids pDM302, pHN205 and pHN216 were stably maintained without antibiotic selection in various strains of *Cms*. We observed that for a single plasmid, different strains of *Cms* showed significantly different transformation efficiencies. We examined the effect of a number of factors on transformation efficiency. The best transformation efficiencies were obtained when *Cms* cells were grown on DM-agar plates, harvested during early exponential growth phase and used fresh (without freezing) for electroporation. Optimal electrical conditions were two pulses, 20 sec apart, at a field strength of 15 kV/cm, giving time constants of 11.2 and 10.0 msec and transformation efficiencies of up to 4.6×10^4 CFU/µg of pHN216 plasmid DNA.

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A COMPARISON OF SALICYLIC ACID AND TOBACCO MOSAIC VIRUS AS INDUCERS OF DEFENSE COMPOUNDS AND RESISTANCE IN TOBACCO TO TMV AND PERONOSPORA TABACINA. Chengsong Xie, Joseph Kuc. Department of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Tobacco mosaic virus (TMV) and salicylic acid(SA) induce resistance to some viral, bacterial and fungal diseases in plants. Inoculation of the lower leaves of N-gene containing tobacco with TMV induced an increase in SA locally and systemically. SA has been suggested to be required for induced systemic resistance even though it is not a translocated signal. Inoculating 2-3 lower leaves of tobacco with TMV induced systemic resistance to TMV and Peronospora tabacina, whereas SA induced resistance to TMV, but not P. tabacina. Both TMV and SA increase the activities of chitinase and ß-1,3-glucanase locally and systemically, but the chitinase activity induced by TMV locally and systemically was higher than the activity induced by SA. TMV induced high peroxidase activity locally and systemically by 4 days after induction, but SA induced low peroxidase activity in inoculated leaves and a delayed increase in systemic leaves. Both appeared to induce the same patterns of basic and acidic PR proteins (PR 1-5, ß-1,3-glucanase and chitinase) in tobacco. TMV induced low molecular weight compounds which have antifungal activities, but SA did not. From the data, it appears that TMV and SA induced some resistance mechanisms in common. SA is not likely to be the only factor involved in the signal transduction of TMV-induced systemic resistance to P. tabacina in tobacco plants. The translocated signal(s) for induced systemic resistance and mechanism(s) for activation of multiple putative defense compounds remain unknown.

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PURIFICATION AND CHARACTERIZATION OF AN ACIDIC ß-1,3-GLUCANASE FROM CUCUMBER INDUCED BY *COLLETOTRICHUM LAGENARIUM* AND TOBACCO NECROSIS VIRUS Cheng Ji and Joseph Kuc.
Department of Plant Pathology, University of Kentucky, Lexington, KY 40546

An acidic ß-1,3-glucanase was induced in cucumber leaves inoculated with *Colletotrichum lagenarium* or tobacco necrosis virus as well as in the leaves above those inoculated with the pathogens. The enzyme is extracellular and migrates together in native-PAGE with a putative bifunctional chitinase, suggesting a similar three dimensional structure of the two hydrolases and a possible compartmental juxtaposition of them in the plant. The enzyme was purified to apparent homogeneity. Only one isozyme was detected (molecular weight, ca. 38 kD as estimated by SDS-PAGE; isoelectric point [pI], pH 3.6; specific activity, 26 µmol glucose equivalents liberated from laminarin min^{-1} mg^{-1} protein). Partial amino acid (64 AA) sequencing of the ß-1,3-glucanase revealed similarities (from 49% to 72%) to sequences of published ß-1,3-glucanases. A time course study indicated that the increase of the ß-1,3-glucanase activity was associated with induced resistance against *C. lagenarium*.

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REGENERATION OF TOXIN RESISTANT SOYBEAN EMBRYOGENIC SUSPENSION CULTURES AND TESTING OF REGENERANTS FOR RESISTANCE TO *FUSARIUM SOLANI*. H. Jin[1], G. L. Hartman[1,2] and J. M. Widholm[3]. [1]Dept. of Plant Pathology, [2]USDA/ARS, [3]Dept. of Agronomy, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801

Soybean embryogenic suspension cultures established from cv. Jack immature cotyledons were selected for their insensitivity to toxic culture filtrates of *Fusarium solani*, the causal agent of soybean sudden death syndrome (SDS). The embryogenic cultures were challenged with a sublethal concentration of fungal culture filtrates for 1 to 2 months. Those surviving embryogenic cultures were transferred to regeneration medium. Out of 74 plants regenerated, 69 set seeds. The R_1 (first-selfed generation) and R_2 plants of regenerants were inoculated with a *F. solani* SDS isolate. The foliar severity was rated on a 1 to 5 scale: 1 = no foliar symptoms to 5 = interveinal necrosis and drying of both unifoliate and trifoliate leaves. Of the regenerants, 1% of each of the R_1 and R_2 plants had a foliar severity of 1; 29% of the R_1 and 26% of the R_2 plants were rated 2 or 3; and 70% of the R_1 and 72% of the R_2 plants were rated 4 or 5. Of the nonregenerated control plants, 11% were rated 2 or 3 and 89% were rated 4 or 5. Plants in the R_1 and R_2 generations were more resistant than nonregenerated control plants to *F. solani*, indicating that the resistance may be heritable.

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CLONING AND MAPPING OF CHALCONE SYNTHASE AND PHENYLALANINE AMMONIA-LYASE GENES IN SORGHUM. Yunxing Cui, Clint Magill, Jane Magill, and Richard Frederiksen. Texas A&M University, College Station, TX 77843-2132.

Two gene segments, 535bp PAL1-1 and 620bp CHS2G were cloned from sorghum by PCR and by shown to have >70% base sequence identity with phenylalanine ammonia-lyase and chalcone synthase genes from other species. The clones probed a locus for each gene on a sorghum RFLP linkage map. A Northern analysis showed that PAL and CHS mRNAs accumulated more rapidly and to higher levels in seedlings after inoculation with *Bipolaris maydis* than that in controls. No significant increase of either PAL or CHS mRNA was seen in seedlings of BTx635 after inoculation with *Sporisorium reilianum*. The accumulation of mRNA in resistant lines after inoculation with *Peronosclerospora sorghi* was higher and lasted longer than that in susceptible lines.

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EXPRESSION OF A BACTERIAL AVIRULENCE GENE IN TRANSGENIC TOBACCO INDUCES THE HYPERSENSITIVE CELL DEATH. Y. Huang and J. H. McBeath. Dept. of Plant and Animal Science, UAF, AK 99775

AvrRxv, a bacterial avirulence gene cloned from *Xanthomonas campestris* pv. *vesicatoria*, confers the hypersensitive reaction on several non-host plants. A chimeric gene cassette consisting of *avrRxv* placed between promoter and terminator of proteinase inhibitor II was constructed and introduced into tobacco to investigate the molecular mechanisms underlying *avrRxv* expression in tobacco. At two weeks after transformation, the majority (over 90%) of the *avrRxv* transformed calli showed signs of necrosis, whereas the control tissue transformed with 35S-GUS did not. By six weeks after transformation, calli transformed with *avrRxv* showed strong necrotic reactions. In addition, *avrRxv* transformed calli grew slower than the control tissues. These results suggest that expression of the *avrRxv* gene in tobacco is lethal to tobacco cells.

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EFFECTS OF THE NECROSIS TOXIN PRODUCED BY *PYRENOPHORA TRITICI-REPENTIS* ON THE MEMBRANES OF WHEAT. JB Rasmussen[1], LJ Francl[1], S Meinhart[2], C Kwon[1], and TP Freeman[1]. Depts of Plant Pathology[1] and Biochemistry[2], North Dakota State University, Fargo, ND.

Certain isolates of *Pyrenophora tritici-repentis*, the causal agent of tan spot on wheat, produce a toxin (Ptr toxin) that affects only certain cultivars of wheat. Ptr toxin induces necrosis in sensitive cultivars within 24 to 48 hr of application, but little is known of its mode-of-action. Partially-purified preparations of Ptr toxin were infiltrated into the cultivars ND495 (toxin sensitive) and Erik (insensitive). Transmission electron microscopy revealed that the plasmalemma and the chloroplast membrane had lost integrity in ND495 within 4 hr of toxin treatment. Massive cell death was evident by 18 hr post-infiltration. Similar events were found in toxin-treated Erik but fewer cells were involved. Other experiments demonstrated enhanced electrolyte leakage in ND495 but not in Erik 4 hr after toxin treatment. The loss of membrane integrity is the most rapid effect of Ptr toxin observed to date.

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IN VITRO ANTIFUNGAL ACTIVITY OF TOBACCO CLASS I CHITINASE AND CLASS I ß-1,3-GLUCANASE RELIES ON SYNERGY. Marianne B. Sela-Buurlage, Anne S. Ponstein, Els J.P. Van Deventer-Troost, Saskia Kroon-Swart, Peter J.M. van den Elzen, Leo S. Melchers. MOGEN, Leiden, The Netherlands

The vacuolar class I chitinase (Chi-I) and class I ß-1,3-glucanase (Glu-I), from TMV inoculated tobacco cv. Samsun NN leaves have been shown previously to possess potent antifungal activity *in vitro* towards *Fusarium solani* both individually and in synergy (Sela-Buurlage *et al*,, 1993, Pl Phys 101: 857-63). Routinely, purity of these proteins was ensured with SDS-PAGE, Western analysis and enzymatic assays. Genes corresponding to these two proteins had been identified. Transgenic tobacco plants had been generated overexpressing modified single genes. Through introduction of additional stop codons, proteins, Chi-I* or Glu-I*, were retargeted extracellularly (Melchers *et al*, 1993, PMB 21: 583-93). Upon purification from the intercellular washing fluids, enzymatic activities on artificial substrates remained unchanged, indicating that targeting had not influenced the nature of these proteins. However, *in vitro* assays showed a dramatic decrease in activity for both hydrolases when applied separately on *F. solani*. Antifungal activity could be restored by amending with 1% of the opposite hydrolase, indicating the enormous synergy of both proteins.

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PHOSPHOLIPASE D ACTIVITY AND LOCATION IN RICE DURING INTERACTIONS WITH *XANTHOMONAS ORYZAE* PV. *ORYZAE*. Scott A. Young[1], Xuemin Wang[2], and Jan E. Leach[1]. Department of Plant Pathology[1] and Department of Biochemistry[2], Kansas State University, Manhattan, KS 66506-5502

Phospholipase D (PLD) activity has been shown to increase during freeze injury, water stress, pathogen infection, senescence, and seed germination. Polyclonal anti-PLD antibodies, raised against PLD from castor bean, cross-reacted with a 92 kDa protein from leaves of rice. PLD was monitored in rice during compatible and incompatible interactions with *Xanthomonas oryzae* pv. *oryzae* over time (0-120 h) by using an activity assay and immunoblots. Three forms of PLD (type 1, 2, 3) were identified based on their mobility during nondenaturing polyacrylamide gel electrophoresis. Type 1 was only observed in tissues that had been infiltrated. Type 2 and 3 were detected up to 48 h in all interactions. After 72 h, only type 2 was detected in incompatible interactions. PLD was located in the membrane of mesophyll cells in both incompatible and compatible interactions at 3 and 24 h after infection by biochemical fractionation and immunoelectronmicroscopy. Northern analysis on transcript accumulation of both PLD and PLC will be reported.

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CHARACTERIZATION OF POTATO SESQUITERPENE CYCLASE CDNA CLONES. M. Zook. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

The first biosynthetic step specific for the potato sesquiterpene phytoalexins is catalyzed by a sesquiterpene cyclase. Three different PCR products were obtained from potato genomic DNA using primers derived from a genomic clone of the tobacco sesquiterpene cyclase. A comparison of the putative coding sequence of the potato PCR products with coding sequence of a tobacco sesquiterpene cyclase gene revealed a high level of homology at the nucleotide and amino acid level. One of the potato PCR products was used to screen a cDNA library made from arachidonic acid-treated potato tuber tissue. Several full-length cDNA clones were isolated which also have a high level of homology (79%) at the nucleotide and amino acid level with the N-terminal coding region of a tobacco sesquiterpene cyclase gene. The isolation of cDNA clones will facilitate further analysis of the potato sesquiterpene cyclase gene(s).

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ENHANCED OXALATE PRODUCTION BY THE BROWN-ROT FUNGUS *GLOEOPHYLLUM TRABEUM* UPON EXPOSURE TO TCA CYCLE ACIDS. Jon H. Connolly and Jody Jellison, Department of Plant Biology and Pathology, University of Maine, 04469-5722.

Wood decay fungi are consistent producers of oxalate and oxalate salts, and these metabolites may have an important role in mechanisms of wood decay. Oxalate production by *Gloeophyllum trabeum*, an aggressive brown rot fungus, was monitored by microscopic examination of crystal production and by HPLC. Oxalate production was augmented significantly above glucose controls by 10 mM acetate, succinate, malate, citrate, fumarate, glyoxylate, and malonate at pH ca. 4.6. In sodium acetate and sodium citrate cultures at pH levels of ca. 6.5 and 7.5 respectively, oxalate upregulation occurred before it did in the free acid cultures at pH 4.6. These results suggest that in *G. trabeum*, oxalate is normally derived from organic acids in the TCA cycle, and that this metabolic path can be influenced by environmental pH.

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USE OF NITRATE NONUTILIZING MUTANTS OF *FUSARIUM MONILIFORME* TO TRACK ITS SYSTEMIC INFECTION IN MAIZE. Dunhua Zhang and Richard A. Shelby, Department of Plant Pathology, Auburn University, AL 36849 USA.

Nitrate nonutilizing mutants (*Nit* mutants) of *F. moniliforme* were generated on 2.5% chlorate medium and used as inoculum to coat surface-sterilized corn seeds. These infected seeds were planted in methyl bromide fumigated soil in the greenhouse. Plants grown from these seeds were apparently asymptomatic; however, the inoculated *Nit* mutants were consistently recovered from every part of the plants from seedling to maturity. Asymptomatic grains had an infection rate of 36% for *Nit* mutants and 42% for wild types of *F. moniliforme* when surface-sterilized and plated on sterile agar. *Nit* mutants had almost the same infection capability as the wild types of *F. moniliforme*; however, they were much more easily identified than the wild types. The advantages of using *Nit* mutants as an inoculum source could be extended to study plant resistance to or biological control of this fungus.

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ANALYSIS OF GENES INVOLVED IN COPPER RESISTANCE AND CYTOCHROME *C* BIOGENESIS IN *PSEUDOMONAS FLUORESCENS*. C. H. Yang, H. R. Azad, and D. A. Cooksey. Dept. of Plant Pathology, Univ. of California, Riverside CA 92521.

A chromosomal locus essential for copper resistance and competitive survival was previously identified in the biocontrol strain 09906 of *Pseudomonas fluorescens*. Further analysis of this locus revealed five open reading frames with homology to genes involved in cytochrome *c* biogenesis, *cycJ, cycK, tipB, cycL*, and *cycH*. Tn*3-gus* mutagenesis showed that expression of the genes was constitutive but enhanced by copper, and that the genes function both in copper resistance and biogenesis of active cytochrome *c*. However, one mutant in *cycH* was copper-sensitive and oxidase-positive, suggesting that the products of these genes, rather than cytochrome *c* activity, confer resistance.

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MOLECULAR CLONING OF A β-1,4-ENDOGLUCANASE GENE FROM *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS*. M.J. Laine and M.C. Metzler. Dept. of Biology, Univ. of Turku, BioCity 6A, 20520 Turku, Finland.

We have found that cellulase is important pathogenicity factor for *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*), the potato ring rot bacteria. To characterize this enzyme, we isolated a full length cellulase-encoding phage clone from a *Cms* genomic library. This clone showed a high degree of similarity to *Bacillus polymyxa* β-1,4-endoglucanase gene. At the amino acid level the degree of identity was over 50% in putative coding region, suggesting that the gene encoding for cellulase in *Cms* is β-1,4-endoglucanase. The corresponding gene in *B. polymyxa* is a cell-associated endoglucanase, of which very little is secreted outside the bacterial cell. We are determining whether the *Cms* cellulase gene is also cell wall associated and studying the regulation of the gene.

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DETECTION OF THE WATERMELON FRUIT BLOTCH PATHOGEN ON SEEDS WITH THE POLYMERASE CHAIN REACTION. G. V. Minsavage, R. J. Hoover, T. A. Kucharek, and R. E. Stall. University of Florida, Gainesville, 32611.

Oligonucleotide primers were identified for specific amplification of DNA of *Acidovorax avenae* subsp. *citrulli* (Aac) by the polymerase chain reaction (PCR). Amplification of DNA from six other plant pathogenic bacteria in the genus *Acidovorax* occurred, but the amplified fragments could be distinguished from Aac by restriction enzyme analysis. No amplification of DNA of 60 other bacteria occurred. The watermelon fruit blotch pathogen was detected routinely on naturally contaminated watermelon seeds by a method involving a phosphate buffer wash of seeds to which polyvinylpolypyrrolidone, sodium ascorbate, and potassium bisulfite were added for inactivation of PCR inhibitors, followed by concentration of bacterial cells, a CTAB DNA extraction, and PCR amplification of DNA. Detection by PCR amplication with one round of 30 cycles correlated better with results of seed transmission tests than did detection with nested primers in two rounds of amplification. The nested-primer PCR protocol increased detection sensitivity to the genomic equivalent of a single bacterial cell.

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FINGERPRINTING GENOMES OF POTATO SCAB-CAUSING *STREPTOMYCES SPECIES* WITH REPETITIVE EXTRAGENIC SEQUENCES AND THE POLYMERASE CHAIN REACTION TECHNIQUE. F. Spooner, C. Medina, and D. Fulbright. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Genomic fingerprints were generated for various *Streptomyces* species pathogenic toward potato by the polymerase chain reaction technique utilizing repetitive extragenic sequences REP, ERIC, and BOX (Louws, et al, 1994). Genomic fingerprints of *S. scabies* (common scab pathogen) and deep-pitting *Streptomyces* isolates from United States and Canadian culture collections were compared to the deep-pitting isolate D.P. (Spooner et al., 1994), to determine whether fingerprints similar to D.P. occurred in other geographic localities. The D.P. isolate was phenotypically similar to four Canadian deep-pitting isolates (Faucher et al., 1992) lacking melanin production in culture and bearing spores in flexuous chains; however, genomic fingerprints of the Canadian isolates differed from D.P. The D.P. genomic fingerprints also differed from five deep-pitting species reported by Archuleta and Easton (1981). Genomic fingerprints for the Loria *S. scabies* isolate RL 232 (Spooner et al., 1994) and D.P. were very similar; however, the isolates caused different types of scab lesion and exhibited different cultural morphology and physiology. The genomic fingerprint for RL 232 differed from *S. scabies* isolates from Washington, North Dakota, Idaho, Montana, Connecticut, West Virginia, Iowa, and Maine. As a group, potato scab-causing *Streptomyces* appear to lack uniform genomic organization. This may account for the difficulty in developing useful criteria for species identification by traditional approaches.

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Erwinia carotovora rsmA functions as a global regulator in enterobacterial species. Y. Cui, A. Chatterjee, and A. K. Chatterjee, Dept. Plant Pathol, Univ. of Missouri, Columbia MO 65211

The *rsmA* gene of *Erwinia carotovora* subsp. *carotovora* (Ecc) has extensive homology with the *csrA* gene of *Escherichia coli*. Southern hybridizations under stringent conditions revealed the presence of *rsmA* homologs in soft-rotting and non-soft-rotting *Erwinia* as well as in *Enterobacter aerogenes, E. coli, Salmonella typhimurium, Serratia marscesens, Shigella flexneri* and *Yersinia pseudotuberculosis*. The Ecc71 *rsmA* suppresses the expression of genes for glycogen synthesis, production of extracellular enzymes and an antibacterial antibiotic, motility, flagella, and carotenoid, indigoidine and prodigiosin synthesis. Thus, *rsmA* homologs are predicted to function as global regulators of secondary metabolic systems in various enterobacteria.

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A MODIFIED TWO-COMPONENT REGULATORY SYSTEM IS REQUIRED FOR CORONATINE PRODUCTION. A. Peñaloza-Vázquez[1], M. Ullrich[1], A.M. Bailey[2], and C. Bender[1], 110 NRC, Oklahoma St. Univ., Stillwater, OK 74078[1], and CINVESTAV-IPN, Irapuato, Gto. 36500 MEXICO.[2]

Coronatine (COR), a phytotoxin produced by *P. syringae* pv. *glycinea* PG4180, consists of coronafacic acid (CFA) linked by an amide bond to coronamic acid (CMA). In PG4180, the genes encoding COR synthesis are clustered within a 32-kb contiguous region of a 90-kb plasmid designated p4180A. Two Tn*5* mutants, D4 and F7, failed to produce COR unless both CFA and CMA were added to the culture medium. Sequence analysis of a 3.4-kb *Hin*dIII fragment which complemented D4 and F7 revealed three ORFs designated *corR1, corR2*, and *corS*. When translated, *corR1* and *corR2* showed similarity to response regulators, and *corS* showed relatedness to environmental sensors. Transcriptional fusions of the *corR1* and *corR2* promoter regions to a promoterless glucuronidase (GUS) gene indicated these genes are constitutively expressed at 18 and 28°C. In contrast, GUS fusions showed the *corS* promoter was significantly more active at 18°C than 28°C, a trait also observed for the CFA and CMA biosynthetic promoters.

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CHARACTERIZATION OF GENES FROM *PSEUDOMONAS FLUORESCENS* INVOLVED IN THE SYNTHESIS OF PYRROLNITRIN. Philip E. Hammer, Steve Hill and James Ligon. CIBA Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, North Carolina 27709-2257.

Pseudomonas fluorescens strain BL915, an effective biocontrol agent against damping off diseases caused by *Rhizoctonia solani*, produces several antifungal compounds including chitinase, cyanide and pyrrolnitrin (Prn) under coordinate regulation by *gafA*. In a poster at this meeting Hill et. al describe the isolation of a 6 kb genetic region involved in Prn production. Here we describe the characterization of the Prn gene region. The region was sequenced and four putative open reading frames (ORFs) were identified. Deletions were created in each ORF and crossed back into BL915 by double homologous recombination. A deletion in any one of the ORFs abolished pyrrolnitrin production but did not affect production of the other antifungal compounds. For each ORF the translation start site was identified by sequence analysis and complementation. Each coding region was cloned behind a P_{tac} promoter and used to complement the respective ORF deletion mutant.

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A GENETIC APPROACH TO IDENTIFY PYRROLNITRIN BIOSYNTHESIS AND OTHER GLOBALLY REGULATED GENES IN *PSEUDOMONAS FLUORESCENS*. Stephen T. Lam, R. Allen Frazelle, Nancy Torkewitz, and Thomas D. Gaffney. CIBA Agricultural Biotechnology, P. O. Box 12257, Research Triangle Park, NC 27709-2257

The production of antifungal compounds, including pyrrolnitrin, in the biocontrol *Pseudomonas fluorescens* strain BL915 are coordinately regulated by the *gacA/lemA* global regulatory system. Mutants defective in either *gacA* or *lemA* are no longer effective in disease control. To identify *gacA/lemA* regulated genes, we tagged them with a transposon (TnCIB*116*) containing a promoterless *lacZ* gene. Starting with a collection of random transposon mutants in a GacA$^-$ strain, we introduced the *gacA* gene on a plasmid into each mutant. Mutants which showed differential *lacZ* expression in a GacA$^+$ vs. a GacA$^-$ background contained transposon insertions in genes which were regulated by *gacA*. Such mutants were screened for pyrrolnitrin (Prn) production and biocontrol activities (see poster by Torkewitz et al). The insertion junction from a Prn- mutant was used to isolate the wildtype sequence by hybridization (for characterization of the Prn gene region see poster by Hill et al).

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GENETICS AND COMPLEMENTATION OF DNA REGIONS INVOLVED IN AMYLOVORAN SYNTHESIS OF *ERWINIA AMYLOVORA* AND STEWARTAN SYNTHESIS OF *ERWINIA STEWARTII*. Frank Bernhard[1], Peter Bugert[1], Klaus Geider[1], Doris Majerczak[2], and David Coplin[2]. [1]Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-69120 Heidelberg, Germany. [2]Dept. of Plant Pathology, The Ohio State University, Columbus OH 43210 USA.

Erwinia amylovora and *Erwinia stewartii* form similar exopolysaccharide (EPS) capsules, which are the products of the *ams* and *cps* gene clusters, respectively. Nucleotide sequence analysis indicated that these DNA regions share many common functions. Mutants in these regions are nonpathogenic, and can be complemented by clones of the heterologous gene cluster. Depending on the chromosomal mutation, this complementation required a set of EPS genes. Degradation analysis with *Erwinia* EPS-phage depolymerases and lectin binding assays indicated changes in the structure of EPS from many merodiploids. This suggests a conversion of the EPS type by expression of the foreign EPS genes.

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Identification of a DNA region from the native plasmid, pCXC100, of Clavibacter xyli subsp. cynodontis (CXC) and its stabilizing function in other plasmids in transformed Escherichia coli and CXC. T.Y. Li and T.A. Chen, Department of Plant Pathology, Rutgers University, New Brunswick, N.J. 08903.

A DNA library of the native plasmid, pCXC100, of the Gram+ bacterium, CXC, was constructed in the plasmid vector PBR325. One fragment contained a plasmid-stabilizing region (SR) which, when subcloned into several different plasmids, increased the stability of the plasmid in transformed bacteria hosts over time. For example, SR subcloned into the cosmid cloning vector pLAFR3 was maintained at 100% in a host cultivated over 100 generations on non-selective media compared to pLAFR3 without SR, which lost over 1% per generation. In addition, the SR rendered the stabilization of the plasmids in both Gram+ (CXC) and Gram- (E. coli) hosts, suggesting its function was independent of the plasmid replicon and the host bacterium. Restriction map and deletion analyses indicated the SR was located on a 0.6 Kb region near the replicative origin of pCXC100. DNA sequence of this region was determined, and a search within Genebank revealed no homology with other DNA sequences. This is the first report that a SR of a plasmid from a Gram+ bacterium that functions on plasmids originating from Gram- bacteria.

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COMPLEMENTATION GROUPS II AND III OF THE *ERWINIA AMYLOVORA HRP* GENE CLUSTER ARE REQUIRED FOR SECRETION OF HARPIN. Ji-Hyun Kim, Zhong-Min Wei, and Steven V. Beer. Department of Plant Pathology, Cornell University, Ithaca, NY 14853 USA

Insertional mutagenesis of complementation groups II and III of the *hrp* gene cluster of *E. amylovora* resulted in *hrp*-minus strains. Lysis of the strains revealed harpin present inside the cells but not extracellularly. The 6.2-kb region corresponding to transcriptional units II and III was sequenced. It contains ten putative genes in two operons. Four genes are homologous to *Yersinia ysc*, *Shigella flexneri mxi*, *Burkholderia solanacearum hrp*, *Xanthomonas campestris hrp*, and *Rhizobium fredii nol* genes, the products of which are involved in the interaction between bacteria and their hosts. The products of two genes of operon III also are similar to proteins involved in the early stages of flagellar biogenesis. A gene of operon II encodes a protein highly similar to members of the PulD superfamily, which are required for both the Type II and Type III secretion pathways. The protein products were visualized by the T7 polymerase/promoter expression system. Tn*phoA* translational fusion suggested that two of the gene products are envelope-associated.

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NUCLEOTIDE SEQUENCE ANALYSIS OF *Pel* GENES FROM *Xanthomonas campestris* pv. *malvacearum* and *Pseudomonas viridiflava*. C.-H. Liao and L.-J. Wong, ERRC, USDA-ARS, Philadelphia, PA 19118.

X. c. pv. *malvacearum* produces an extracellular pectate lyase (PL) with an estimated Mr of 40 KDa and pI of 9.5. The *pelX* gene coding for this enzyme was located in a 1.8-kb *Pst I* genomic fragment. Sequence analysis of this fragment revealed the presence of an ORF of 1,131 bp. Nucleotide sequence of the *pelV* gene from *P. viridiflava* was also determined. The PelX and PelV proteins exhibited 80 % identity in a.a. sequences. Only 43-44 % identity in a.a. was observed between the PelE from *Erwinia chrysanthemi* and PLs from *P. viridiflava*, *P. fluorescens* and *X. c.* pv. *malvacearum*.

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ASSESSMENT OF GENOMIC VARIABILITY IN *XANTHOMONAS CAMPESTRIS* PV. MANGIFERAEINDICAE. Lionel Gagnevin and Olivier Pruvost, CIRAD-FLHOR, 97455 St. Pierre, Reunion, France.

Xanthomonas campestris pv. mangiferacindicae (*Xcm*) causes mango black spot disease. We assessed the relationships among strains within pathovar mangiferacindicae by using restriction fragment length polymorphism (RFLP) analysis. The probes were a 1.9 kb DNA fragment, containing a repetitive element cloned from *Xcm*, and two clones, which contain an avirulence gene and the *hrp* gene cluster, respectively, from *X. oryzae* pv. oryzae. One hundred strains of *Xcm* were clustered after analysis with each of the three different probes into groups that were consistent with culture phenotypes or host origin. The most typical strains, found in the largest cluster, are those with white colonies and which were isolated from mango or pepper tree (in the same family as mango). Less typical strains were either yellow pigmented or were isolated from ambarella, an other host of the same family as mango.

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CHARACTERISTICS OF PEPPER BACTERIAL SPOT PATHOGEN RACES THAT OVERCOME THE *BS2* GENE FOR RESISTANCE. C.S. Kousik & D.F. Ritchie. Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

A major limiting disease in bell pepper production is bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria*. Gene *Bs2* confers resistance to the three most common races 1,2,3 and to race 0. Strains which overcome *Bs2* were isolated from diseased pepper. One strain was compatible on ECW-10R (contains *Bs1*) and ECW-20R (*Bs2*) but incompatible on ECW-30R (*Bs3*). Another strain was compatible on ECW-20R and ECW-30R but incompatible on ECW-10R. These strains were designated races 4 and 5 respectively. A third strain (designated race 6) compatible on ECW-10R, 20R, and 30R was selected by passing race 4 through ECW-30R. Strains of races 4,5, and 6 were copper resistant and streptomycin sensitive. Growth curves for races 1-6 did not significantly differ in 0.8% nutrient broth (NB). However, strains of races 4,5 and 6 multiplied slower in 0.4 and 0.2% NB than races 1,2, and 3 after 32h. Growth curves for races 4-6 did not differ from curves for races 1-3 in susceptible ECW and most were within 0.5 log units. Growth of races 5 and 6 was similar to races 2 and 3 in ECW-30R. Races 4 and 6 multiplied to similar levels as race 1 in ECW-10R. Chlorosis caused by race 6 developed at a slower rate compared to the other races.

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IDENTIFICATION OF TWO BACTERIAL BLIGHTS AND THEIR SUPPRESSION BY *PANTOEA AGGLOMERANS*. Braun A. and D.C. Sands. Dept. Plant Pathology, Montana State University, Bozeman, MT 59717.

It has been reported in Montana and Idaho that two major types of kernel blight of barley occur in wet years or in irrigated areas. One type of blight affects the embryo of the barley kernel (basal blight), and the other type affects the lemma of the barley kernel (spot blight). The two blights are visible as dark brown tissue discoloration and reduce the germination rate 30-60%. Barley cultivars seem to be most susceptible to spot blight when heads are in the early milk stage and to basal blight in the soft dough stage. Two different groups of *Pseudomonas syringae* have been found as causal agents. In our studies we report an in-depth identification of the two syringae groups using molecular biological approaches to detect differences in their genomic structure in addition to biochemical identification systems, including MIDI and Biolog. RFLPs and Southern hybridization with 3 DNA probes (*syrB, hrp10, and hrp14* genes) demonstrated differences in the genetic relatedness of 30 *P.syringae* pathovars and strains. Pulsed Field Gel Electrophoresis using a CHEF-GE was done for the detection of macrorestriction fragment patterns and evaluated for its ability to demonstrate taxonomic differences on the pathovar and strain level. In addition, Epiphytic population studies in greenhouse experiments revealed that an application of *P.agglmerans* bacteria 3 days prior to an application of *P. syringae* bacteria strains Ps 552 (basal) and Ps 418 (spot) reduced the percentage of infection of the two blight diseases between 50-100% depending on the *P. agglomerans* strain used and the blight disease screened. Similar results were found in field studies, suggesting that *P. agglomerans* bacteria can be used effectively to control the two blight diseases.

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DEVELOPMENT OF A PCR-BASED METHOD FOR DETECTING CORONATINE-PRODUCING STRAINS OF PSEUDOMONAS SYRINGAE ON PLANT MATERIAL. D.A. Cuppels and T. Ainsworth. Agriculture & Agri-Food Canada, London, Ontario, N5V 4T3, Canada.

P. syringae pv. tomato DC3000, which causes bacterial speck of tomato, produces the phytotoxin coronatine. A nonradioactively-labeled 4.6-kb DNA probe originating from the gene cluster that controls toxin production, in combination with the semiselective growth medium VB-tar, is the basis of a quantitative assay we developed for monitoring this pathogen on transplant seedlings. In the present work, a 1.48-kb *Pst*I fragment from this probe was sequenced and the information used to design PCR primers. These primers directed the synthesis of a 640-bp DNA fragment in PCR assays containing template DNA from coronatine-producing P. syringae strains; the fragment did not appear in assays employing DNA from non-toxin producers. The primers work well in direct PCR screens of P. syringae pv. tomato colonies grown on VBtar and in PCR screens of eluate from bacterial speck lesions.

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INFLUENCE OF ROOT EXUDATES ON THE PHOSPHOLIPID COMPOSITION OF THE ROOT COLONIZING BACTERIUM, *PSEUDOMONAS AUREOFACIENS*. D. Jurkonie[1], P. Bakker[2], J. Lawrence[1], D. Kluepfel[1]. Dept. of Plant Pathology & Physiology, Clemson University, Clemson, SC[1] and University of Utrecht, Utrecht, The Netherlands[2].

The plant rhizosphere is directly influenced by the root and its exudates. Presumably, different plant types produce exudates of various qualities and quantities. Experiments were performed to test the effect the root exudates of different plant types have on the fatty acid composition of *Pseudomonas aureofaciens*. Magenta boxes containing 100 g of sterile white sand were inoculated with 20 ml of *P. aureofaciens* suspended in sterile liquid fertilizer. Wheat, tomato, corn or soybean seedlings, grown on sterile germination paper, were transferred to the magenta boxes. The fatty acids of root washings were extracted and analyzed with gas chromatography, which resulted in the generation of fatty acid retention time profiles. These profiles were then compared by cluster analysis, which revealed that root exudates of different plant types influence the phospholipid composition of *P. aureofaciens*.

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CHARACTERISTICS OF STRAINS OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA* (XCV) FROM PROCESSING TOMATOES IN OHIO. F. Sahin and S. A. Miller, Dept. Plant Pathology, Ohio State University-OARDC, Wooster, OH 44691

Approximately 50% of the 57 pathogenic strains of Xcv isolated from processing tomatoes in NW Ohio in 1994 were also pathogenic on peppers. All strains were identified to species as *X. campestris* but usually not to pathovar *vesicatoria* by fatty acid methyl ester (FAME) analysis. Slightly more than half of the strains were tomato race 1 (T1), while most of the remaining strains were T2. None of the strains were resistant to 30 μg/ml copper sulfate, and only a few (< 1%) were resistant to 20 μg/ml streptomycin sulfate. The vast majority of the strains (95%) were amylolytic. There was no correlation between physiologic race of these strains and amylolytic activity. Strains reacting postively in indirect enzyme-linked immuno-sorbent assay (ELISA) with one or more of eight Xcv-specific monoclonal antibodies were grouped into three serovars.

395 Withdrawn

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EFFECTS OF TEMPERATURE AND NITROGEN FERTILIZATION ON INFECTION PROCESS IN LEAVES OF ANTHURIUM INOCULATED WITH A BIOENGINEERED, BIOLUMINESCENT STRAIN OF *XANTHOMONAS CAMPESTRIS* PV. *DIEFFENBACHIAE*. R. Fukui, H. Muroi, S. C. Nelson, and A. M. Alvarez. Dept. of Plant Pathology, University of Hawaii at Manoa, Honolulu, HI 96822-2279.

The process of leaf infection in anthurium plants was monitored by detecting light emission from a bioluminescent strain of *X. c.* pv. *dieffenbachiae*. The severity of leaf infection was greater when plants were grown under a higher temperature and was a direct function of degree-day in susceptible, intermediate and resistant cultivars of anthurium. Each function had a x-intercept, representing length of latent infection, and the slope value indicated the rate of daily increase in disease severity. The slope value for the susceptible variety was approximately three times higher than that for the resistant variety, but the value of x-intercept was not considerably different among three varieties. When both susceptible and resistant plants were fertilized with 0, 70, 140 and 210 μg/ml of nitrogen (in the form of ammonium nitrate), the severity of leaf infection was greater at 70 or 140 μg/ml than at 0 μg/ml, but 210 μg/ml of nitrogen did not always result in the highest severity of leaf infection. The use of the bioluminescent strain is expected to resolve the early controversy on the effects of temperature and nitrogen on susceptibility to bacterial blight.

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SURVIVAL OF *PSEUDOMONAS ANDROPOGONIS* IN GRAIN SORGHUM DEBRIS. L. B. Muriithi, L. E. Claflin, and B. A. Ramundo, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506

Seventy grain sorghum (*Sorghum bicolor L.*) accessions were inoculated with *Pseudomonas andropogonis* at the 8-leaf stage of growth. Leaf, stem, and seed samples were collected from each of two resistant, intermediate, and susceptible accessions at six-week intervals beginning in October. Bacterial colonies from the samples were identified by biochemical and physiological tests. Recovery of *P. andropogonis* from leaf tissue declined from 1.65 X 10^7 cfu/g in October to 4.0 X 10^4 cfu/g in March. Stalk tissue yielded *P. andropogonis* from each sampling period. Recovery from seed was sporadic and only from susceptible genotypes.

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ANALYSIS OF ANIONIC PEROXIDASE ISOZYMES IN HYDATHODE FLUID OF RESISTANT AND SUSCEPTIBLE CABBAGE VARIETIES DURING PATHOGENESIS WITH *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* (XCC). P. A. Gay and S. Tuzun, Dept. of Plant Pathology, Auburn University, AL 36849 USA.

Hydathode fluid from resistant (R) [Hancock (HC) and Green Cup (GC)], partially resistant [Cheers (CH)] and susceptible (S) [Strukton (ST) and Perfect Ball (PB)] cabbage varieties were analyzed for anionic peroxidase isozymes during pathogenesis with XCC. Four-week-old plants were petiole-inoculated with XCC (FD91L) and hydathodal fluids were collected from both non-infected and infected plants from each variety. Total peroxidase activities were determined spectrophotometrically and individual isozymes were analyzed utilizing an iso-electric focusing gel stained for peroxidases. Hydathode tissue was stained for the presence of lignin using phloroglucinol-HCl. All varieties contained at least 50- to 100-fold greater peroxidase activities in hydathode fluid when compared to other tissues sampled (root, stem and leaf). Total peroxidase activity was greater in hydathode fluid from inoculated plants when compared to non-inoculated ones. Isoelectric focusing revealed that non-inoculated HC and GC (R) contained predominantly one anionic peroxidase isozyme (pI 4.0), whereas ST and PB (S) contained another one (pI 4.2). Partially resistant variety (CH) had lower levels of both isozymes. Inoculated resistant plants (HC, GC and CH) showed an increase in several peroxidase isozymes, including one (pI 3.6) which appears to be correlated with increased lignin deposition. Preliminary studies indicate that hydathodal fluids have direct antibacterial properties against XCC.

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THE PRESENCE AND PHYTOTOXICITY OF FUMONISINS AND AAL-TOXIN IN *ALTERNARIA ALTERNATA*. H.K. Abbas and R.T. Riley, USDA-ARS, SWSL, Stoneville, MS; and USDA-ARS, RRC, Athens, GA.

An isolate of *A. alternata* produced 3.1 ppm fumonisin B1 (FB_1) when grown on PDA as determined by CD-ELISA. The presence of FB_2, FB_3, and AAL-toxin were confirmed in spores and mycelia of *A. alternata* by continuous flow fast atom bombardment/mass spectroscopy. This is the first report of AAL-toxin, FB_2, and FB_3 in spores and mycelia and confirms the presence of FB_1 in *A. alternata*. Spores and mycelia of *A. alternata*, FB_1 (70 μM), and AAL-toxin (5 μM) caused stem canker symptoms and accumulation of free sphingoid bases (phytosphingosine and sphinganine) in tomato plants (*asc/asc*). AAL-toxin appears to be the major factor in pathogenesis of *A. alternata*. The fumonisins and AAL-toxin both are demonstrated in the same fungus, showing that biosynthetic pathways coexist in *A. alternata*.

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FUSARIUM SOLANI DNASE IS A SIGNAL FOR INCREASING EXPRESSION OF NON-HOST DISEASE RESISTANCE RESPONSE GENES, HYPERSENSITIVITY, AND PISATIN PRODUCTION. Lee A. Hadwiger, Dept. of Plant Pathology, Wash. State Univ., Pullman, WA and Ming-Mei Chang, Dept. of Biology, SUNY, Geneseo, NY

Pea endocarp tissue suppresses growth of the bean pathogen, *Fusarium solani* f. sp. *phaseoli* (Fsp) in 6 h. The major components of this response are also induced by a DNase released from Fsp. These components including phytoalexin production and the accumulation of RNA homologous with the PR genes DRR49, DRR206, and DRR230 are induced by Fsp DNase within time frames and up to levels comparable to those induced by Fsp. Fsp DNase also induces resistance against *F. solani* f. sp. *pisi*, hypersensitive discoloration, and nuclear distortion in the pea cells. 680 Fsp DNase units representing only 1 μg protein induces resistance in 1 g pods against f. sp. *pisi*. We propose that nuclear changes caused by the Fsp DNase DNA nicking action enhance the transcription of pea defense genes.

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ANALYSIS OF THE PRODUCTION OF A PUTATIVE CALCIUM-BINDING PROTEIN DURING A HYPERSENSITIVE REACTION. A. J. Baldwin, J. L. Jakobek, and P. B. Lindgren, Department of Plant Pathology, North Carolina State University, Raleigh, N.C., 27695-7616.

We have identified a bean cDNA, designated Hra32, corresponding to a transcript which accumulates during the expression of a hypersensitive reaction (HR) in response to Pseudomonas syringae pv. tabaci. This transcript also accumulates after infiltration with a P. s. pv. tabaci Hrp⁻ mutant and other treatments which do not induce a HR. Significantly, the pattern of Hra32 transcript accumulation during these latter interactions was very distinct when compared to that observed after infiltration with wildtype P. s. pv. tabaci. Sequence analysis predicted the Hra32 transcript to encode a 17 kDa protein of 161 amino acids. The predicted protein shares no significant homology with other putative defense proteins, however, it contains 4 putative calcium binding domains. To facilitate the isolation of Hra32-antibody, a gene fusion between between the entire Hra32 coding region and the carboxy-terminus of the glutathione S-transferase gene has been generated. The resulting fusion protein has been purified for antibody production. Analyses of the accumulation of the Hra32 product during plant-bacterial interactions will be discussed.

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ANTIMICROBIAL PEPTIDES: DESIGN AND APPLICATION
C. M. Catranis, C. A. Maynard, and W. A. Powell. State University of New York - College of Environmental Science and Forestry, Syracuse, NY 13210

The objective of this study was to synthesize antimicrobial peptides and transform *Populus* spp. with the peptide encoding genes. One 18-amino acid and five 20-amino acid amphipathic peptides were designed, synthesized, and tested with bioassays *in vitro*. Antimicrobial activity was solely dependent upon the presence of positive charges on the hydrophilic side of the peptide. Four peptides (ESF1, 5, 6, 12) were inhibitory to conidial germination for three fungal pathogens: *Cryphonectria parasitica, Fusarium oxysporum*, and *Septoria musiva*. ESF 12 (18 amino acids) was also inhibitory to growth of three bacterial pathogens: *Erwinia amylovora, Pseudomonas syringae,* and *Agrobacterium tumefaciens*. Inhibition to germination of host plant pollen was not detected. Minimal inhibitory concentrations were similar to those of naturally occurring magainin and cecropin. A fast growth poplar clone, the 'OGY' hybrid was transformed with gene constructs encoding ESF12.

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DIFFERENTIAL RESPONSE OF PROTOPLASTS, ISOLATED CELLS, AND TISSUES OF RESISTANT AND SUSCEPTIBLE CRUCIFERS TO DESTRUXIN B PRODUCED BY *ALTERNARIA BRASSICAE*. T. R. Sharma and J. P. Tewari, Dept. of Agric., Food, and Nut. Sci., Univ. of Alberta, Edmonton, AB., Canada T6G 2P5.

Destruxin B (DB), a pathotoxin produced by *Alternaria brassicae* is a major determinant of Alternaria blight of crucifers. A comparative study on the effects of DB on protoplasts, isolated cells, and intact tissues of susceptible (*Brassica juncea*) and resistant (*Camelina sativa, Capsella bursa-pastoris*) crucifers was carried out. Isolated cells and intact tissues of all the species were sensitive to DB depending upon time of exposure to the toxic stress. Freshly isolated mesophyll protoplasts of *C. sativa* and *C. bursa -pastoris* were more sensitive to DB than the *B. juncea* protoplasts. Comparative response of hypocotyl derived protoplasts and isolated cells of *B. juncea* to DB indicated that protoplasts were insensitive to DB at 30 µg/ml for up to 4 days of incubation while the isolated cells were sensitive under similar conditions. Differential response of protoplasts, cells and intact tissues indicate the possibility of absence/modifications of DB receptor sites on the freshly isolated protoplasts of *B. juncea* due to enzymatic removal of cell wall.

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INDUCTION OF PATHOGENESIS-RELATED PROTEINS BY CYST NEMATODE AND SALICYLIC ACID IN SOYBEAN. Jeng-Sheng Huang[1] and James A. Knopp[2], Departments of Plant Pathology[1] and Biochemistry[2], North Carolina State University, Raleigh, NC 27695

Salicylic acid (SA) is a plant signal molecule that is involved in both local and systemic resistance to viral, bacterial, and fungal infections. During the resistance reaction, SA activates the expression of genes encoding pathogenesis-related (PR) proteins. This study is designed to investigate the role of SA in resistance to the cyst nematode (*Heterodera glycines* Ichinohe) in soybeans. Our results indicate that Lee 60, a soybean cultivar susceptible to race 3 of the nematode, when treated with SA, had reduced nematode populations, suggesting that SA may be involved in nematode resistance. Intercellular fluids removed from roots of control and nematode-inoculated Lee 68 and analyzed by SDS-PAGE had similar protein banding patterns. Samples prepared from nematode-infected, SA-treated Lee 68 and nematode-infected Peking, a resistant cultivar, had several new protein bands that were absent in their untreated controls. One protein band with an apparent molecular weight of 28 kD appears in response to both nematode infection and SA treatment. The biological function of this putative PR protein is being studied.

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CHARACTERIZATION OF p30 OF WHEAT SOILBORNE MOSAIC FUROVIRUS (WSBMW). X. M. Zhu AND J. L. Sherwood. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078

The 3' end of RNA1 of the bipartite genome of WSBMV contains an ORF that codes for p37 that is proposed to modulate virus movement. In western blots, polyclonal antibody made to p37 expressed in *Escherichia coli* detected a 37kD protein in the cell wall fraction of WSBMV infected-wheat 15 days post inoculation. In a gel retardation assay, p37 was found to bind to single stranded RNA. The ORF for p37 was cloned into plasmid pTMV3'NAS provided by C.M.Deom (U of GA) and substituded for the p30 gene of tobacco mosaic virus (TMV). The recombinant TMV 3'fragment was then substituted for the 3'half of the TMV clone L19 provided by R.S.Nelson (S.R.Noble Foundation) to produce pTMVWS1. When RNA transcripts from pTMVSW1 were inoculated to *Nicotiana tabacum* L. cv. Xanthi-nc, symptoms did not develop. However, localized lesions developed when transcripts from pTMVWS1 were inoculated to TMV p30 transgenic *N. tabacum* cv. Xanthi-nn. The 37kD protein of WSBMV has characteristics associated with plant virus movement proteins, but did not complement movement of TMV in tobacco.

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PSEUDOMONAS SYRINGAE PV. *TAGETIS*, A BIOCONTROL FOR CANADA THISTLE (*CIRCIUM ARVENSE*). K.J. Jones, M.G. Goering, S. Savage. Mycogen Corporation, 5501 Oberlin Dr., San Diego, CA 92121 and D.J. Johnson, Dept. Agron. and Plant Genetics, Univ of Minnesota, St. Paul, MN 55108.

Pseudomonas syringae pv. *tagetis* (Pst) when sprayed onto Canada Thistle with the surfactant Silwet L-77 infects rapidly and produces a toxin that causes a persistent apical chlorosis. This effect is selective for certain members of the Asteraceae, and a few other hosts. Most agricultural species are not affected. Growth of Canada thistle is severely inhibited (number of stems reduced, biomass reduced) in the season of application. Overwinter survival of treated plants is much reduced. Artificial inoculation requires surfactant. Movement of disease from inoculated to non-inoculated plants does not occur. Effective control of Canada thistle was demonstrated in MN in 1993-4. In 1994-5 field trials were conducted in MN, CO, SD, and MD to test the effect of biotype and environment. Wider scale testing in 15 states across the mid-west were initiated in the spring of 1995.

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INTERACTIONS BETWEEN THE BIOCONTROL AGENT *GLIOCLADIUM ROSEUM* AND THE PATHOGEN *BOTRYTIS CINEREA* ON RASPBERRY. H.Yu and J.C.Sutton. Dept. of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Leaf disks, stem segments, and flowers of raspberry were dip-inoculated with *G. roseum* (10^7 conidia/mL), with *B. cinerea* (10^6 conidia/mL), or with both fungi, and incubated in >95% relative humidity at 21-23°C. At various times after inoculation, leaf disks, epidermis of the stem segments, and stamens of inoculated flowers were cleared and stained in lactophenol containing 0.05% trypan blue, and examined by light microscopy and laser scanning confocal microscopy. *G. roseum* suppressed conidial germination of *B. cinerea* by >85% on the leaves, but by <5% and <3% on the stem segments and stamens, respectively. Parasitism of germinated conidia and germ tubes of *B. cinerea* by *G. roseum* was observed frequently on the stems, infrequently on the leaves, and not at all on the stamens. Abundant mycelium of the antagonist was present on the stamens. *G. roseum* markedly suppressed penetration frequency of *B. cinerea* in the stem segments and stamens. We conclude that hyperparasitism was a major mechanism of biocontrol and that nutrient competition was probably involved on the leaves.

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NATURAL SPREAD OF BACTERIAL ANTAGONISTS OF *ERWINIA AMYLOVORA* AMONG PEAR BLOSSOMS AND THEIR EFFECT ON FIRE BLIGHT CONTROL. R. Nuclo, K.B. Johnson, D. Sugar and V.O. Stockwell, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR, 97331-2902.

Dispersal of both *Pseudomonas fluorescens* strain A506 and *Erwinia herbicola* strain C9-1 from treated to non-treated pear blossoms, and the effect of their dispersal on fire blight were investigated. An orchard block, 10 rows by 4 trees per row, was used. At 30% bloom, the center two rows were sprayed with a mixture of A506 and C9-1. Immediately after spraying, antagonists were detected only on treated blossoms. As bloom progressed, both A506 and C9-1 spread to non-treated blossoms located up to 4 rows distal to the treated blossoms. At 80% bloom, *Erwinia amylovora* was introduced to all trees by honey bees that had been forced to walk through powdered, freeze-dried cells of the pathogen as they exited their hive. After full bloom, detection of blossoms with *E. amylovora* populations > 10^5 was highest in the outermost rows, and decreased significantly ($P < 0.05$) with proximity to treated rows. Diseased blossom clusters decreased linearly from 4.5% in the outermost rows to 2.9% in the treated rows. These results suggest that both A506 and C9-1 spread naturally after application, and that this spread is an important factor in obtaining disease control.

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CHITINOLYTIC ENZYMES AND (β 1→3) GLUCANASES PRODUCED BY SOYBEAN ROOTS, RHIZOCTONIA SOLANI AND TRICHODERMA HARZIANUM IN SOIL. F. K. Dal Soglio, B. L. Bertagnolli, J. B. Sinclair and D. M Eastburn. Dept. of Plant Pathology, University of Illinois at Urbana-Champaign, Urbana, IL 61801-4709.

A model system of pathogenicity/biocontrol was used to investigate the origin of chitinolytic enzymes and (β 1→3) glucanase in soybean rhizosphere during resistance/pathogenicity/antagonism process. Culture filtrate assays showed that T. harzianum Th008 and soybean produced β-N-acetylhexosaminidase, chitobiosidase, endochitinase and (β 1→3) glucanase, and R. solani 2B-12 produced all these enzymes but endochitinase. Under greenhouse conditions, cv. Williams 82 seeds, noninoculated or inoculated with Th008, were planted in soil noninfested or infested with 2B-12. At day 15 after emergence, the rhizosphere (0 to 2 cm) was assayed for enzyme activity. Th008 produced endochitinase. β-N-acetylhexosaminidase and (β 1→3) glucanase production was related to root infection by 2B-12, probably associated with plant resistance mechanism.

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BIOLOGICAL CONTROL OF COTTON SEEDLING ROOT DISEASE IN TWO SOILS BY *PSEUDOMONAS AUREOFACIENS*. M.A. Mulesky, G.H. Lacy, and C. Hagedorn. Dept. of Plant Pathology, Physiology, and Weed Science, VPI & SU, Blacksburg, VA 24061.

Greenhouse trials were conducted to evaluate the efficacy of *P. aureofaciens* L-850 in suppressing *Pythium ultimum*- and *Rhizoctonia solani*-induced cotton seedling disease. By varying soil inoculum densities of *P.u.* and *R.s.*, cotton seedling stands were established ranging from 2.1 to 85.4% in two soil types (pH 5.7 and 8.0) to assess the effectiveness of L-850 in controlling the pre- and postemergence damping-off and seedling root decay phases at different disease intensity levels. Percent emergence, disease severity, foliar fwt and dwt, root fwt and total root area/plant were dependent on soil type and pathogen level. L-850 efficacy differed with respect to disease phase, equalling that of the standard fungicide in some cases. The results indicate the importance of determining disease pressure in the soil prior to selecting a control strategy (cultural, chemical, biological).

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CONTROL OF DOLLAR SPOT OF TURF WITH BIOCONTROL AGENT-FORTIFIED COMPOST TOPDRESSINGS. M. E. Grebus[1], J. W. Rimelspach[2] and H. A. J. Hoitink[1]. [1]Dept. of Plant Pathology, OARDC/OSU, Wooster, OH 44691. [2]The Ohio State University Dept. of Plant Pathology, Columbus, OH 43210.

In a two-year study, compost topdressings fortified with the biocontrol agents *Trichoderma hamatum* 382 and *Flavobacterium balustinum* 299r_2 were evaluated for efficacy in control of dollar spot (*Sclerotinia homoeocarpa*) on creeping bentgrass pv. penncross. During the first year, biocontrol agent-fortified composted yard waste, leaf humus and municipal biosolids topdressings were applied once (0.6 cm depth) and compared with a commercial fungicide (chlorothalonil) and untreated turf. The turf plots were infested with rye grain inoculum of *S. homoeocarpa* and significant ($p=0.05$) disease control was observed in the fortified composted municipal biosolids topdressing and chlorothalonil treatments only. In the second year, plots were topdressed monthly with biocontrol agent-fortified composted municipal biosolids and compared with chlorothalonil and untreated turf. The compost topdressing yielded significantly ($p=0.05$) less disease than the untreated control but significantly ($p=0.05$) more disease than chlorothalonil.

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Effects of various dew and incubation temperatures on infection components of wild type and mutant isolates of Colletotrichum gloeosporioides f. sp. aeschynomene on northern jointvetch. Y. Luo and D.O. TeBeest, Department of Plant Pathology, University of Arkansas, 217 Plant Sci. Fayetteville, AR 72701.

Four infection components, infection efficiency (lesion number), latent period, lesion expansion rate and sporulation, were measured at different dew and incubation temperatures for infection of northern jointvetch by three isolates (wild type, benomyl resistant [B21], and nitrate reductase deficient [Nit A]) of C. gloeosporioides f. sp. aeschynomene in growth chamber experiments. Components were measured for each dew temperature X incubation temperature interaction with each isolate. The number of lesions produced by B21 and Nit A was more sensitive to dew and incubation temperature interactions than was infection by the wild type isolate. Incubation temperatures caused significant differences in the latent period between isolates. Incubation at 32C reduced lesion expansion rates for B21 when compared with expansion at 20C and 28C. Incubation temperatures did not significantly affect expansion rates for the wild type or Nit A. In-vivo sporulation was inconsistent for mutant isolates.

414 Withdrawn

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EFFECT OF GROWTH MEDIUM AND PATHOGEN INTRODUCTION TIME ON BIOCONTROL BY *BACILLUS* SP. BA55 AGAINST *T. CUCUMERIS* ON TOBACCO. J.P. McMahan and B.H. Ownley. Ent. & Plant Pathology Dept., Univ. of Tenn., Knoxville 37996.

Thanatephorus cucumeris causes target spot and soreshin of tobacco. *Bacillus* sp. strain BA55 can provide significant control of target spot on tobacco seedlings. However, variability in performance of BA55 has been observed. In this study, the effects of bacterial culture medium and time of pathogen introduction on biocontrol by BA55 on Burley tobacco (TN86) were tested in the greenhouse. The experiment was a 3×2 factorial in a RCB with 3 control treatments, 2 pathogen introduction times, and 4 replicates. The control treatments were: untreated check, BA55 cultured in Minimal C Broth (Min C), or BA55 grown in Nutrient Broth Yeast Extract (NBY). Min C promotes spore production while NBY promotes vegetative growth. The pathogen introduction times were 1 day or 6 days after application of the control treatments. After 4 wk, disease severity and shoot fresh weight were determined. The control × time interaction and the main effect of time were not significant. The main effect of control was significant ($P=0.01$) for disease rating. Compared to the untreated check, BA55-Min C reduced disease severity due to target spot and soreshin by 46%. BA55-Min C also increased shoot fresh weight by 36%, but the difference was not significant.

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EFFECT OF INVERT EMULSION ON VIRULENCE AND HOST RANGE OF *MYROTHECIUM VERRUCARIA*, A PATHOGEN OF *EUPHORBIA ESULA*. S.M.Yang and S.C.Jong. USDA/ARS, Frederick,MD 21702, and ATCC, Rockville,MD 20852.

Nine isolates of *Myrothecium verrucaria*, including five non-pathogens, were used to inoculate 14 plant species in the presence and absence of dew. Two isolates from *Euphorbia esula* in China and ATCC isolates 22798 and 24571 were pathogenic, whereas ATCC isolates 26265,20540,18398,26146, and 9095 were non-pathogenic. Virulence was determined by atomizing 4- to 6-wk-old plants with a 2% sucrose solution or an invert emulsion (IE) containing 10^8 conidia/ml. Plants inoculated with sucrose solution were incubated in a dew chamber at 30 C for 18 hr then moved to greenhouse at 20-25 C, 50-60% RH. Disease severity was recorded after 2 wk. All isolates atomized with IE severely infected or killed *Chenopodium album, E. esula, Glycine max, Helianthus annuus,* and *Physalis ixocarpa*. Only certain isolates affected *Amaranthus retroflexus, Carduus pycnocephalus, Convolvulus arvensis, Gossypium hirsutum, Medicago lupina, M. sativa, Rumex crispus, Solanum tuberosum,* and *Trifolium pratense*. Except for *E. esula*, which required several sprays, 6-wk-old plants were normally killed by two sprays. Sucrose or IE alone either slightly injured or had no effect. Most isolates had no effect when treated without dew and without IE. *M. verrucaria* was reisolated from infected plants but not the control plants. These preliminary results suggest that IE can increase the virulence and widen the host range of *M. verrucaria*.

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STORAGE WAXES THAT SUPPORT GROWTH OF *CANDIDA OLEOPHILA* FOR BIOCONTROL OF *PENICILLIUM DIGITATUM* ON CITRUS. R. G. McGuire and R. D. Hagenmaier. USDA - ARS, 13601 Old Cutler Rd., Miami, FL 33158.

One candidate for biocontrol of green mold on citrus is the epiphytic yeast, *C. oleophila*. The incorporation of this microorganism in wax coatings can reduce decay over periods of cold storage that may last six months. As liquids, hospitable coatings should have a pH below 8.25, less than 8% ethanol, and concentrations of morpholine and ammonia below 1.5% and 0.3%, respectively. From these criteria, four formulations (two based upon candelilla wax, one composed of a blend of candelilla and carnauba waxes, and one comprising carnauba and polyethylene waxes) have been developed. As dilutions containing 5% total solids, these coatings maintain yeast populations above 10^5 cfu/ml as long as 2 hr. In the dried film on grapefruits, surface populations of the yeast climb to levels between 10^5 and 10^6 cfu/cm^2.

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PREDICTABLY SUPPRESSIVE BIOCONTROL AGENT-FORTIFIED COMPOST-AMENDED POTTING MIXES. M. E. Grebus[1], K. A. Feldman[2], C. A. Musselman[1] and H. A. J. Hoitink[1]. [1]Dept. of Plant Pathology, OARDC/OSU, Wooster, OH. [2]Earthgro Inc., Lebanon, CT.

Commercial scale development of composted yard waste and softwood bark amended potting mixes fortified with the biocontrol agents *Trichoderma hamatum* 382 and *Flavobacterium balustinum* 299r_2 was continued. Rhizoctonia and Pythium damping-off of radish and cucumber, respectively, and Fusarium wilt of radish were controlled consistently in the biocontrol agent-fortified, but not in the natural compost mixes. Furthermore, Pythium and Thielaviopsis root rots of poinsettia were controlled significantly better ($p=0.05$) in the fortified mixes than in either the natural compost-amended or peat mixes. These suppressive effects lasted through six months of storage of the mix followed by production of a poinsettia crop. The rate of hydrolysis of fluorescein diacetate (FDA) in the biocontrol agent-fortified mixes was significantly ($p=0.05$) higher than in either natural compost-amended or peat mixes.

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BIOLOGICAL CONTROL OF FUSARIUM WILT OF WATERMELON USING NON-PATHOGENIC ISOLATES OF *FUSARIUM OXYSPORUM*. R. P. Larkin[1], D. L. Hopkins[2], and F. N. Martin[3], [1]USDA-ARS, Biocontrol of Plant Diseases Laboratory, Beltsville, MD 20705; University of Florida, [2]CFREC, Leesburg 34748 and [3]Gainesville 32611.

Nonpathogenic strains of *F. oxysporum* isolated from watermelon roots growing in a soil suppressive to Fusarium wilt of watermelon were tested for their ability to control disease in greenhouse and field tests. Several isolates significantly reduced disease incidence (35-75% reduction) in greenhouse tests compared to infested field soil controls. Individual isolates produced comparable levels of control as the suppressive soil. Antagonists were added to field soil as chlamydospores and achieved control at inoculum levels of 50-100 cfu/g soil when pathogen inoculum levels averaged 100-200 cfu/g soil. Evidence of an induced systemic resistance to disease was observed as a result of colonization of roots by some antagonistic isolates in split-root tests. Reduced disease incidence and increased yield was also observed in preliminary field tests when seedlings were colonized by selected antagonists prior to transplanting.

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PRODUCTION OF ANTIBODY TO GLUCOSE OXIDASE FROM TALAROMYCES FLAVUS AND ROLE OF THE ENZYME IN BIOCONTROL OF VERTICILLIUM DAHLIAE. S. K. Stosz and D. R. Fravel. Biocontrol of Plant Diseases Laboratory, USDA, ARS, Beltsville, MD 20705.

Glucose oxidase from Talaromyces flavus was purified by HPLC and used to raise polyclonal antisera in rabbits. The antibody was tested against culture filtrates of several common soil microbes and was found to be highly specific to T. flavus. Culture filtrates of T. flavus grown in media that either repressed or induced glucose oxidase were collected. In the presence of glucose, only filtrates from inducing media were able to kill microsclerotia of Verticillium dahliae. Immunoprecipitation studies using this antibody were successful in removing the glucose oxidase from filtrates and reducing biocontrol expression in a plate assay. Amending antibody-treated filtrates with glucose oxidase restored biocontrol activity.

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BIOLOGICAL CONTROL OF CUCUMBER MOSAIC CUCUMOVIRUS IN *CUCUMIS SATIVUS* L. BY PGPR-MEDIATED INDUCED SYSTEMIC RESISTANCE. Georg S. Raupach, John F. Murphy, and Joseph W. Kloepper, Department of Plant Pathology, Biological Control Institute, Auburn University, AL 36849-5409 USA.

Plant growth-promoting rhizobacteria (PGPR) strains, previously demonstrating induced systemic resistance (ISR) against several cucumber diseases, were tested for ISR activity against cucumber mosaic cucumovirus (CMV) in greenhouse trials. Using cucumber cultivar 'Straight 8', PGPR application was performed either as a seed treatment or soil-drench at planting. Virus was inoculated mechanically onto cotyledons of plants at the first true leaf stage, or onto the first true leaf of plants that were at 2-3 true leaf stage. Viral antigen was detected using ELISA. PGPR treatment protected against CMV disease incidence or reduced disease severity, depending on the inoculation system. Following cotyledon inoculation, protected cucumber plants were nonsymptomatic and no viral antigen was detectable in noninoculated leaves. In contrast, with CMV inoculation of 1st true leaves, PGPR treatment decreased disease severity, the development of severe mosaic symptoms caused by CMV, although virus accumulated to similar levels to the nontreated controls. These results indicate that PGPR-mediated ISR may produce a form of biological control of CMV in cucumber.

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EFFICACY OF SOIL DRENCHES WITH ERADICATIVE FUNGICIDES AND SEED TREATMENTS WITH AERATED STEAM FOR CONTROL OF CLAVICIPITACEOUS ENDOPHYTES IN GRASS GERM PLASM. A. D. Wilson, D. G. Lester, USDA Forest Service, Southern Hardwoods Lab, P.O. Box 227, Stoneville, MS, 38776, and W. J. Kaiser, USDA Agricultural Research Service, Western Regional Plant Introduction Station, Washington State University, Pullman, WA, 99164.

The effectiveness of soil drench treatments with systemic and eradicative fungicides (benomyl, fenarimol, imazalil, prochloraz, and propiconazole) in controlling *Acremonium* endophytes in *Hordeum brevisubulatum* ssp. *violaceum* (PI 440420), *Festuca arundinacea* 'Tribute', and *Lolium perenne* 'Ellett' (PI 462339) was tested with potted plants in the greenhouse at 2-5 PPT fungicide concentrations. Significant control of endophyte in *H. brevisubulatum* was observed only with propiconazole, but phytotoxicity caused growth distortion and reduced height and dry weight. Fenarimol and prochloraz also significantly reduced total chlorophyll content. Similar results occurred in *F. arundinacea* and *L. perenne*. Seed treatments with aerated steam for 10 min at 58 C and 3-5 min at 60 C significantly reduced endophyte viability 26.8-83.3% in *L. perenne*, however, seed germination was reduced 48.0-87.0% and height of surviving seedlings was reduced 15.6-22.1%.

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EFFECT OF CARBOXIN, METHYL CELLULOSE AND SEED PELLETING ON ONION SMUT (*UROCYSTIS CEPULAE* FROST) INCIDENCE AND ONION YIELDS.
M.R. McDonald and S. Janse. Muck Research Station, Kettleby, On. L0G 1J0 Canada.

The efficacy of different rates of PRO GRO (30% carboxin and 50% thiram) was evaluated alone or with a 1% methyl cellulose sticker on raw onion seed or incorporated into seed pellets. The effect of various types of pellets on smut incidence was also investigated. Onions, cultivars Taurus and Fortress, were seeded in organic soil with a history of onion smut in 1993 and 1994; cv. Capable was also seeded in 1994. Application of PRO GRO to onion seed reduced the incidence of smut and increased yields of Taurus and Fortress. A low rate (3.9 g ai/kg seed) of carboxin was less effective than the recommended rate (7.8 g ai/kg seed) in reducing smut. Increasing the rate to 22.5 g ai did not improve efficacy. Onions grown from seed treated with PRO GRO and methyl cellulose had less smut early in the season than those treated with PRO GRO alone (52% and 67% infection, respectively on June 16, 1994) and also had a higher yield when seed was treated with 15 g ai/kg PRO GRO (442 and 223 bu/acre respectively). Capable onions grown from seed with a Springkote pellet had less smut and higher yields than seed with a Splitkote Radiant Red pellet.

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EFFICACY OF SURFACTANTS IN THE CONTROL OF ZOOSPORIC ROOT-INFECTING FUNGI. S.L. Rasmussen, M.E. Stanghellini, D.H. Kim, and P. Rorabaugh. Department of Plant Pathology, University of Arizona, Tucson, 85721.

Zoosporic fungi are among the most destructive root pathogens in recirculating hydroponic cultural systems (Plant Disease 78:1129-1138) and zoospores have been implicated as the primary, if not sole, infectious propagule responsible for spread of the fungus via the recirculation system. Previous in vitro studies (Phytopathology 77:112-114) demonstrated that zoospores are rapidly lysed and killed when exposed to a surfactant. Current in vitro experiments, employing cucumbers as the susceptible host and *Pythium aphanidermatum* as the pathogen, demonstrated the efficacy of surfactants in the control of root disease caused by this fungus. Amending the nutrient solution with a nonionic surfactant (final concentration, 20 ug/ml) resulted in complete control of the spread of the fungus via zoospores. In the absence of a surfactant, all plants (8) within a recirculating system, which consisted of two rockwool slabs (each containing 4 cucumber plants) connected to a common 50 L reservoir, were killed within 4-5 weeks following hypocotyl-inoculation of a single plant.

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EVALUATION OF CHLORPYRIFOS AGAINST *ASPERGILLUS FLAVUS*. K.L. Bowen and G.R. Garner, Dept. of Plant Pathology, Auburn University, AL 36849.

Aspergillus flavus-type fungi produce aflatoxins in agricultural commodities and can be passively vectored into peanuts by larvae of the lesser cornstalk borer (LCB). Control of LCB can contribute to lower fungal invasion and aflatoxin contamination in peanuts with chlorpyrifos the insecticide of choice. Chlorpyrifos technical, 4EC, and 15G were evaluated *in vitro* for reducing radial growth of an *A. flavus* field isolate. Reduction of relative populations of *A. flavus* in soil by 4EC and 15G formulations was also evaluated. *In vitro*, 4EC was most effective in reducing radial growth. Fungal growth *in vitro* was 100% inhibited by 4EC at 100 mg a.i./ml of substrate; while 15G provided 10% inhibition at 100 mg a.i./ml of substate and technical was ineffective.

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Comparison of different bioassays for evaluating fungicides. A.L. O'Leary[1], R.K. Jansson[2], J.C. Davis[1], R.L. Shea[1], and R.A. Dybas[3]. [1]Ricerca, Inc., Painesville, OH 44077, [2]Merck Research Laboratories, Three Bridges NJ 07065 and [3]Merck Research Laboratories, Rahway, NJ 07065.

A method was developed to screen antifungal compounds which are available in limited quantities. The screen was comprised of seven diseases. Fourteen plants were sprayed with a volume of ca. 8.5% of that needed in the standard screen. The major difference between the screens was the use of an airbrush applicator to deliver the test materials. Plants were effectively covered with the reduced volume in the miniature screen with little or no run-off using this apparatus. The standard screen utilized a higher-volume spray nozzle which resulted in a considerable amount of run-off. In addition, plants were tested at a younger growth stage that allowed for consistent disease development. When the two methods were compared, standard protectant fungicides had very similar ED50 values against the diseases. The systemic fungicides had ED50 values which were from five to eight times greater using the miniaturized method.

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ICIA5504- A NOVEL, BROAD SPECTRUM, SYSTEMIC FUNGICIDE FOR WHEAT. J. R. Godwin, Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, England RG126EY.

ICIA5504 (BSI proposed common name azoxystrobin) is a highly active, very broad spectrum, systemic fungicide for use on a wide range of crops. It is an analogue of the naturally occurring strobilurins and has a novel mode of action. Field trials over the last four years in both Europe and the USA have shown ICIA5504 to be an excellent wheat fungicide. It is particularly effective against the rusts (*Puccinia* spp.), *Septoria tritici, Stagonospora nodorum,* tan spot (*Helminthosporium tritici-repentis*), *Helminthosporium sativum,* sooty moulds (*Cladosporium* spp.) and sharp eyespot (*Rhizoctonia cerealis*). Moderate to good control can be achieved if applied preventatively. The breadth of spectrum and persistence of effect of ICIA5504 means that the green leaf area is maintained until very late in the season. This feature, together with excellent crop safety and very good protection of the ears, results in outstanding increases in yield and grain quality following treatment with ICIA5504. Field data will be presented to highlight these properties in comparison to existing commercial standards including propiconazole.

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METHYL IODIDE, A DIRECT REPLACEMENT FOR METHYL BROMIDE AS A SOIL FUMIGANT. Howard D. Ohr[1], James J. Sims[1], Nigel M. Grech[1], and J. Ole Becker[2], Departments of Plant Pathology[1] and Nematology[2], University of California, Riverside, CA 92521

Methyl bromide is being phased out of production and use by 2001 in the United States because of its role in stratospheric ozone depletion. Methyl iodide is a logical replacement for methyl bromide for several reasons. Its spectrum of activity and efficacy as a soil fumigant appears to equal that of methyl bromide; methyl iodide is a liquid with a boiling point of 42°C making it easier to handle and safer for workers to use than methyl bromide; and compared to the ozone depletion potential (ODP) of the chlorofluorocarbon CFC-11, which is 1, methyl bromide has an ODP of 0.6 whereas that of methyl iodide is estimated to be 0.02. In accordance with the Montreal protocol all ozone depleters with a rating of 0.2 or higher are to be removed from production and use. In both laboratory and field trials, when compared at molar equivalent rates, methyl iodide has proven to be equal to or better than methyl bromide in controlling tested soilborne fungal plant pathogens, nematodes and weeds.

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EFFECTS OF FOLIAR FUNGICIDES ON DISEASE SEVERITY, YIELD AND TEST WEIGHT IN OATS. D. Gallenberg, D. Reeves, M. Thompson and L. Hall, Dept. of Plant Science, South Dakota State University, Brookings, SD, 57007.

Foliar fungicide trials were conducted in eastern South Dakota at two sites in 1992 and 1993, and at three sites in 1994, to evaluate the response of oats to various foliar fungicide treatments. Overall disease pressure varied each season, however crown rust was the predominant foliar disease problem observed in all three years. Crown rust susceptible varieties were used in all tests. Despite very high yield potentials in 1992, there was no response to fungicide application at either test site. Under heavy crown rust pressure in 1993, most of the foliar fungicide treatments resulted in reduced disease severity and increased yield and test weight at both locations. With light to moderate crown rust pressure in 1994, all treatments significantly reduced disease severity and increased yield at all three locations, and increased test weight at two locations. Foliar fungicides appear to be an economical treatment on oats in some seasons.

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EFFECT OF FOLIAR FUNGICIDES ON REDUCING SCAB (FUSARIUM HEAD BLIGHT) IN HARD RED SPRING WHEAT. D. Gallenberg, J. Rudd, M. Thompson and B. Farber, Dept. of Plant Science, South Dakota State University, Brookings, SD, 57007.

Evaluations were made on selected foliar fungicide treatments in 1993 and 1994 for their effect in reducing scab (Fusarium head blight) in hard red spring wheat. Under heavy scab pressure in 1993 on a single variety, mancozeb treatments applied at boot and boot plus 10 days and Tilt applied at boot significantly reduced scab incidence and severity and increased yield and test weight. However, overall scab levels were still very high and yields and test weights very low even in the best fungicide treatments. In 1994, under moderate scab pressure on 3 varieties varying in scab response, disease ratings were generally not reduced by fungicide applications. However, several fungicide treatments applied during and after heading, including mancozeb, Folicur and Govern, resulted in increased yield and test weight. Overall scab levels were lower and yields/test weights higher than in 1993. Although high levels of scab control by foliar fungicides are not possible, they may represent a realistic option for reducing scab and increasing yield and test weight, particularly in more tolerant varieties.

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EFFECTS OF SILICON AND FUNGICIDES ON LEAF AND NECK BLAST DEVELOPMENT IN RICE. K. Seebold, L. Datnoff, F. Correa-V. and G. Snyder, University of Florida, Belle Glade and CIAT, Colombia.

Rice was treated with silicon alone applied at 400 kg/ha or with the fungicides tricyclazole, 300 g/ha, and edifenfos, 1 L/ha, to evaluate their effects on the development of leaf and neck blast (*Pyricularia grisea*) at two locations in eastern Colombia. Disease levels were highest at Santa Rosa, where silicon alone significantly reduced leaf blast by 10% to 20%. Leaf blast also was reduced with silicon plus fungicides by 46% to 52%. Neck blast was not significantly affected by silicon alone; however, silicon plus fungicides reduced the incidence by 31% to 96%. At Altillinura where disease levels were lower, silicon alone significantly reduced leaf blast by 46% to 56% while silicon plus fungicides reduced blast by 74% to 78%. Neck blast was significantly reduced 40% by silicon alone, while silicon plus fungicides reduced neck blast by 75% to 98%. Under conditions of low disease intensity, low rates of silicon may provide sufficient blast control but high disease pressure will require fungicide inputs. This information suggests that the number of fungicide applications also might be reduced.

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EFFECT OF USING INFECTED WHEAT SEED FOR SOWING, ON THE NATURAL INCIDENCE OF KARNAL BUNT (*Tilletia indica*). Guillermo Fuentes-Davila. International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F.

Wheat seed production in Karnal bunt quarantined areas from northwest Mexico must comply with the norm of 0% infected seeds/kg. In that region, high relative humidity, cloudiness and rainfall have been associated with disease incidence, however, no influence by infected seed has been detected. Experiments were initiated in the Yaqui valley to evaluate the effect of using 5, 10, 100, 250 and 500 infected seed/kg and were repeated in the same land during subsequent wheat cycles. In 1989-90 the infected seed range per plot was 2-22; the greatest number of infected seeds was obtained with the treatment of 100, while the treatment with 500 had less than the untreated check plot. In 1990-91 the range was 973-1559, the check had the greatest number of infected seeds. During 1991-92 disease incidence was even higher with a range of 21242 to 42298. While the treatment with 5 showed the highest number of infected seeds, the treatment with 500 had 29194. Karnal bunt incidence in south Sonora was low in 1989-90, moderate during 1990-91 and high during 1991-92, which was reflected in the results obtained in the experiments. These results indicate that using 5 to 500 infected seeds per kg for sowing, does not influence a greater incidence of Karnal bunt since the soil in those areas is already infested.

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THE ROLE OF THE USDA-ARS CEREAL RUST LABORATORY VIRULENCE SURVEYS. D. L. Long[1], J. J. Roberts[2], K. J. Leonard[1] and D. V. McVey[1]. [1]USDA-ARS, Cereal Rust Laboratory, St. Paul, MN 55108 and [2]USDA-ARS, Georgia Agricultural Experiment Station, Griffin, GA 30223

Cereal rust surveys coordinated by the Cereal Rust Laboratory (CRL) contribute to timely identification of pathogen virulence shifts in United States rust populations. New virulences often force major wheat cultivars to be abandoned, e.g. ProBrand 812 in the Southern Plains in 1986. Rust-screening programs at the CRL and other institutions identify new sources of rust resistance. Breeders' advanced lines are screened using regionally selected virulence populations based on survey rust collections. Virulence data from surveys and resistance genes identified through screening enable breeders to develop cultivars with essential new rust resistances. Protection provided by the prompt utilization of rust resistance can be estimated from yield loss data and cultivar acreages.

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OCCURRENCE OF *MONOGRAPHELLA NIVALIS* VAR. *MAYOR* AND *FUSARIUM CULMORUM* ON BARLEY IN GERMANY IN 1992. A. Westphal[1], A. v. Tiedemann[2] and H. Fehrmann. Institut für Pflanzenpathologie und Pflanzenschutz, Universität Göttingen, Germany. Current addresses: [1] Dept. Nematology, University of California, Riverside, CA 92521; [2] Boyce Thompson Institute , Cornell University, Ithaca NY 14853.

Monographella nivalis var. *mayor* and *Fusarium culmorum* were isolated from 3-5 mm long, grayish-green, to later black leaf spots on the upper leaves of barley (winter and spring varieties). These symptoms have been known on barley for several years, but could not be clearly related to any pathogenic organism. Leaves with these leaf spots were collected from field and greenhouse trials at the University of Göttingen. The symptoms were reproduced by inoculating secondary leaves of greenhouse grown barley with conidial suspensions of each of the two pathogens. The pathogens were later reisolated. Cytological examination showed cell necrosis due to the infection with these two fungi. The spots first appeared on lower leaves early in the growing season. Spots did not occur on internediate leaves but they did appear on leaves in late tillering stages. Since both pathogens could be isolated from different plant parts during development, endophytic growth as a latent phase of the fungi was hypothesized. Seed testing with German barley seeds revealed a high abundance of these pathogens, indicating seed contamination as a potential source for the observed leaf disease.

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OPTIMIZING INOCULUM PRODUCTION FOR *RAMULISPORA SORGHI*. Xiude Xu, L. E. Claflin, B. A. Ramundo and D. J. Jardine. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506.

Ramulispora sorghi is the causal agent of sooty stripe disease of sorghum. An effective screening protocol was desired due to the increased incidence of the disease in Kansas for the past two years. Various media were evaluated to maximize conidial production. Sorghum leaf extract, sorghum grain extract and Bilai media were found to produce abundant quantities of conidia. The optimum pH for conidial production was between 5.0 - 6.0 with an optimum temperature of 24-28 C. Sclerotia were observed on sterilized sorghum grain and sorghum leaf tissue 25-30 days after inoculation and matured in about 50 days.

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SOURCES OF RESISTANCE TO *Stagonospora nodorum* IN SOFT RED WINTER WHEAT. Barry M. Cunfer and Jerry W. Johnson. Department of Plant Pathology and Department of Crops and Soils, University of Georgia, Georgia Station, Griffin, GA 30223.

Seven soft red winter wheat breeding lines with resistance to *Stagonospora nodorum* were selected in field trials during at least three seasons and in several greenhouse trials. Several experimental lines have a level of leaf colonization by *S. nodorum* which is 5-10 times less than susceptible lines but similar to the resistant cultivar Oasis at decimal growth stage 47 in the field. All are resistant to leaf rust and all but one are resistant to current field populations of powdery mildew. All lines have good agronomic characteristics and have been included among the elite lines of the Georgia breeding program during the past four years.

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QUANTIFICATION AND CLASSIFICATION OF RESISTANCE TO TAKE-ALL AMONG WINTER WHEAT VARIETIES: IN VIVO EVALUATION. E. Ismail, H.T. Wilkinson, W.L. Pedersen and H. Fouly, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Avenue, Urbana, IL 61801.

Wheat take-all, caused by *Gaeumannomyces graminis* var. *tritici*, is both common and potentially destructive wherever wheat is produced. An initial population of over 200 winter wheat lines were evaluated for take-all severity using a 28-day, in vivo assay. The initial evaluations resulted in the identification of a sub-set of 60 entries, including both commercial cultivars and experimental lines, with disease reaction ranging from severely rotted to significantly reduced rot. Further quantification of disease using disease severity, root dry weight, and shoot dry weight measurements revealed that variation existed among these 60 entries. The disease severity reactions were divided into three groups: high disease; intermediate disease; and low disease; however, most of the entries were placed in the first two categories. One entry, IL-83-1698-1, consistently displayed a low mean disease severity score of 1.9 (less than 40% of the roots displaying symptoms). The in vivo assay system provided repeatable and simple means to rate large numbers of individual plants.

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YIELD LOSS-SOUTHERN LEAF BLIGHT SEVERITY RELATIONSHIPS IN HYBRID MAIZE INFECTED WITH 19 ISOLATES OF COCHLIOBOLUS HETEROSTROPHUS RACE O IN NORTH CAROLINA. M. L. Carson, USDA-ARS, Raleigh, NC, 27695-7616.

There is surprisingly little published information on the ability of *Cochliobolus heterostrophus* race O to cause yield losses on maize in the southeastern U.S.. The relationship between various measures of southern leaf blight (SLB) severity and maize grain yield and yield components was examined using data from a 2 yr trial at Plymouth NC, in which the relative aggressiveness of isolates of *C. heterostrophus* race O was being tested. Estimated yield losses of 0.47 and 0.33 % for each percent SLB severity at 2 wks post-silk, were calculated for 1992 and 1993, respectively. A predicted 0.01% loss for each area under the disease progress curve(AUDPC) unit was calculated in both years. These yield loss-SLB severity relationships were consistent with those reported from the Midwest. Losses were attributed mainly to reduced kernel weight, but ear length and kernel numbers were also significantly reduced, possibly indicating the importance of SLB occurring prior to anthesis.

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INCIDENCE OF *FUSARIUM GRAMINEARUM* AND VOMITOXIN LEVELS IN BARLEY GENOTYPES IN NORTH DAKOTA. B. Salas[1], B.J. Steffenson[1], and H.H. Casper[2]. Dept. of Plant Pathology[1] and Vet Science/Microbiology[2], North Dakota State University, Fargo, ND 58105.

Severe epidemics of Fusarium head blight (scab) occurred on barley in North Dakota in 1993 and 1994. Ten (1993) and sixteen (1994) barley genotypes grown at Fargo, ND were assayed for *Fusarium* infection and deoxynivalenol (vomitoxin) levels in kernels after harvest. In both years, *Fusarium graminearum* was the primary causal organism of the epidemic. *Fusarium poae*, *F. avenaceum*, and *F. sporotrichioides* were isolated also, but frequencies were low (<5%). Significant differences ($P<0.01$) were observed among the barley genotypes for infection by *F. graminearum* and vomitoxin levels. Chevron was confirmed to be resistant to *F. graminearum*; in 1993 it had the lowest infection incidence and vomitoxin level (5.1%, 0.6 ppm), and in 1994 it ranked among the lowest (9%, 0.7 ppm). Vomitoxin levels were highly correlated ($r=0.68$ to 0.92) with the incidence of *F. graminearum*. Thus, assays of vomitoxin may be useful in screening for resistance to *F. graminearum* in barley.

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IDENTIFICATION AND PATHOGENICITY OF *PYTHIUM* SPECIES ASSOCIATED WITH WINTER RYE IN GEORGIA M.J. BLACK and B.M. Cunfer, Department of Plant Pathology, Unversity of Georgia, Athens, GA 30602, and Georgia Station, Griffin, GA 30223.

Nearly 600 isolates of *Pythium* were recovered from 22 field soils supporting small grains. *P. irregulare* was recovered from 95% of all locations surveyed and was the most commonly encountered species, accounting for 23% of all isolates. *P. echinulatum*, *P. ultimum*, *P. vanterpoolii*, *P. sylvaticum* , *P. torulosum*, *P. aphanidermatum*, *P. myriotylum*, and *P. spinosum* were recovered less frequently and are listed in descending order of frequency. Selected isolates, grown on a cornmeal-sand mixture, were mixed with steam-treated field soil (1:10) for pathogenicity tests. Day/night temperatures of 25 and 20 C were used for this experiment. Emergence of rye seedlings from soils infested with *P. myriotylum* and *P. aphanidermatum* was reduced 60-100% compared to controls. *P. irregulare* and *P. ultimum* reduced seedling emergence 20-30% and caused a noticeable stunting of surviving plants. All other species failed to cause significant reductions in emergence or plant size. These results suggest a possible link between *P. irregulare* and poor stand establishment of winter rye in Georgia.

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REDUCTION IN YIELD AS AN INDICATOR OF RELATIVE RESISTANCE TO SCLEROTINIA TRIFOLIORUM IN ALFALFA R.G. Pratt and D.E. Rowe, USDA, ARS, FRU, P.O. Box 5367, Mississippi State, MS 39762.

Three cultivars of alfalfa and a new germplasm with enhanced resistance (MSR) were grown in soils naturally infested with S. trifoliorum. Half of plots were sprayed repeatedly with vinclozolin when apothecia appeared in late autumn, and only trace disease developed by early spring. Half of plots were not sprayed and natural disease was moderate to severe. Mean dry-matter yields in unsprayed plots were 38-49% of yields in sprayed plots for the three cultivars and 89% for MSR in one year. Results indicate that differences in yield reduction may be used to compare resistance of alfalfa entries to S. trifoliorum in the field.

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PREVALENCE OF *APHANOMYCES EUTEICHES* AND *PHYTOPHTHORA MEDICAGINIS* IN IOWA ALFALFA IN 1994. G.P. Munkvold and W.M. Carlton. Dept. of Plant Pathology, Iowa State University, Ames, 50011.

Soil and/or root samples from 118 alfalfa stands in 44 of 99 Iowa counties were collected to determine the presence of *Aphanomyces euteiches* and *Phytophthora medicaginis*. Collections were made from sites believed to have a high probability of root disease problems. Presence of these fungi was assayed by baiting soil and root samples with healthy alfalfa seedlings (cv. Vernal) in water and 5 μg/ml metalaxyl in petri plates. *A. euteiches* and *P. medicaginis* were recovered from 75.0% and 35.6% of soil samples and 16.9% and 7.7% of root samples, respectively. Of the soil samples infested with *P. medicaginis*, 81.1% also were infested with *A. euteiches*. *A. euteiches* and *P. medicaginis* were detected in 32 and 27 counties, respectively. *A. euteiches* was recovered from 89.3% of sites in NE Iowa (the area with the greatest alfalfa production). Virulence of *A. euteiches* isolates was tested on several alfalfa lines by zoospore inoculation of seedlings; Iowa isolates varied considerably in virulence and several were found to be virulent on lines with *A. euteiches* resistance. *A. euteiches* may be more prevalent in Iowa than *P. medicaginis*, and currently available resistant cultivars are susceptible to some Iowa isolates.

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DNA ANALYSIS OF A *STAGONOSPORA*-LIKE SPECIES CAUSING EASTERN GAMAGRASS LEAF SPOT DISEASE. L. Wang[1], R.L. Bowden[2], W. Chen[3], K. Subramaniam[1] and P.P. Ueng[1] [1] Plant Mol. Biol. Lab. USDA-ARS, BARC-West, Beltsville, MD 20705 [2]Dept. of Plant Pathology, Kansas State Univ., Manhattan, KS, and [3]Illinois Natural History Survey, Univ. of Illinois, Champaign, IL,

Sixteen isolates of a fungal pathogen causing leaf spot disease in eastern gamagrass in Kansas were compared with *Stagonospora* species from cereals and other grasses. Twenty probes from *S. nodorum* and *S. avenae* were used in RFLP analysis. Only 4 - 6 of the 20 probes detected DNA bands in this pathogen, whereas most of the probes detected DNA bands in *Stagonospora* spp. Even though this pathogen is morphologically similar to *Stagonospora*, the genetic similarity between this pathogen and *Stagonospora* spp.is low.

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EFFECTS OF LUPIN/WHEAT ROTATION AND TILLAGE ON BROWN SPOT OF LUPIN. D.J. Collins[1], D.W. Reeves[2,3], and E.V. Van Santen[3], [1]Dept. of Plant Pathology, Auburn University, AL 36849, [2]USDA-ARS National Soils Dynamics Lab, Auburn, AL 36831, and [3]Dept. of Agronomy and Soils, Auburn University, AL 36849.

A lupin/wheat rotation was established in the fall of 1992 to evaluate agronomic performance and disease severity of the white lupin cultivar 'Lunoble' (*Lupinus albus* L.) under conventional and conservation tillage systems. The lupin rotations fell in three groups: 1) wheat-pearl millet-lupin, 2) wheat-soybean-lupin, and 3) lupin-summer fallow-lupin. Brown leaf spot, casued by *Pleiochaeta setosa* (Kirchn.) Huges was rated in the second year of the rotation. Brown spot was most severe in the lupin/fallow conservation tillage rotation. In the conventional lupin/fallow rotation brown spot was reduced. Some wheat-soybean-lupin rotations had higher levels of brown spot under both tillage systems. Lupins planted after pearl millet had the lowest brown spot ratings.

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STABLE TRANSFORMATION OF *COLLETOTRICHUM TRIFOLII* BY ELECTROPORATION. Nichole R. O'Neill[1], Nancy Brooker[2], James A. Saunders[1], and John Lydon[2], [1]Soybean and Alfalfa Research Laboratory, [2]Weed Science Laboratory, USDA, A RS, Beltsville, MD 20705.

Electrotransformation was evaluated as a procedure for transforming *Colleotrichum trifolii*, the causal agent of alfalfa anthracnose. Whole fungal spores and spores generated from protoplasts were subjected to a range of electroporation conditions. Transformation frequency and fungal viability was optimized using an exponential pulse wave generator and varying the number of pulses, field strength of the pulse (kV/cm), and internal resistance to control pulse duration. Transformants were obtained using a fungal expression vector that contained a bacterial gene encoding hygromycin B phosphotransferase. Isolates transformed by PEG or electroporation were evaluated for virulence, race specificity, mitotic stability, and gene integration. Restriction digests of transformants provided evidence for single copy, tandem repeat integration, and multiple random integration events in the fungal genome. Transformation by both procedures resulted in isolation of mitotically stable transformants which varied in cultural characteristics and virulence but retained wild-type race specificity. Electroporation and PEG DNA uptake resulted in similar transformation frequencies. The introduction of stable, dominant, selectable, antibiotic resistance genes in this race will facilitate disease resistance studies by enabling rapid detection of the fungal race in host tissues.

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EFFECTS OF VIRUS, NEMATODES, AND DROUGHT ON GROWTH AND PERSISTENCE OF WHITE CLOVER. M. R. McLaughlin, and G. L. Windham, USDA-ARS, Forage Unit, Mississippi State, MS 39762.

Field plots of half-sib lines of white clover (*Trifolium repens* L.) with and without hypersensitive resistance to peanut stunt virus (PSV) were established in Oct. 1991 using factorial treatments in a split-plot design where half of the plots were irrigated to allow measurement of natural drought effects. Our objective was to determine effects and interactions of PSV, root-knot nematodes (RKN) (*Meloidogyne incognita*), and drought on growth and persistence. PSV was inoculated in April and RKN in May 1992. Irrigation was from Jul.-Oct. 1992 and 1993. PSV reduced herbage yield (HY). In the presence of RKN, irrigation increased HY, while drought reduced HY. Persistence (stolon density) was reduced by PSV, RKN, and drought, but no interactions occurred. Persistence was reduced most by RKN, resulting in nearly complete loss by Nov. 1993. PSV reduced leaf area and petiole length more in irrigated plots. RKN reduced leaf area and petiole length, but not as much as PSV. Seed yields were reduced by RKN, but increased slightly by drought and PSV.

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VARIATION AMONG CREEPING BENTGRASS VARIETIES IN RECOVERY FROM EPIDEMICS OF DOLLAR SPOT. P. Vincelli, J. C. Doney, Jr, and A. J. Powell, Depts. of Plant Pathology and Agronomy, University of Kentucky, Lexington, 40546-0091

Twenty varieties of four *Agrostis* spp. were maintained under fairway conditions in a RCB design with 3 reps. In 1991-93, dollar spot was allowed to develop from natural inoculum until epidemics were well-established; at that time, initial disease intensity was assessed and one half of each plot was treated with 305 g cyproconazole/ha. Ten to 14 days later, disease intensities in both treated and untreated subplots were compared to each other and to the initial disease intensity separately for each variety. Three general patterns of recovery were observed among creeping bentgrass (CBG) varieties: (A) significant recovery only with fungicide treatment; (B) some recovery without treatment but greater recovery with treatment; and (C) equal recovery with or without treatment. Most CBG varieties did not exhibit the same recovery pattern in all three years. In 1991 and 1993, most CBG varieties exhibited pattern A. In contrast, nearly half of the varieties tested exhibited pattern C in 1992, recovering equally with or without a fungicide application. Differences among years are likely due to different weather conditions.

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HOST RANGE OF *PYRICULARIA* FROM RICE, CEREALS AND AMENITY GRASSES. Angela Purchio-Muchovej[1] and J. J. Muchovej[2], [1]A&J Agronomic Diagnostics, PO Box 25, Lloyd, FL 32337, [2]Ornamental Horticulture, Florida A&M University, Tallahassee, FL 32307.

Host range of 23 isolates of *Pyricularia* from various localities and host species was tested by inoculating 32 species of greenhouse grown grasses with conidial suspensions. All isolates were pathogenic to their original hosts. The virulence of isolates from wheat and rice, inoculated on their respective hosts, varied. Susceptible rice cvs presented a greater disease index than resistant or intermediate cvs. Greater severity was observed on wheat cvs. All wheat isolates used for cross-inoculations were from the same cv, but different localities. Rice 'IAC-4440', sorghum 'BR-300' and *Paspalum plicatulum* were resistant to the majority of isolates tested. Isolates from *Digitaria sanguinalis* caused disease on various ornamental, forage and weedy grasses tested, including cereals, except blast resistant rice cvs. Generally, all isolates were pathogenic to the majority of host species tested. Fifteen grass species not yet reported as hosts were susceptible to various isolates.

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Epidemiology of Powdery Mildew on Poinsettia. B. Shaw and M. Hausbeck. Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

Poinsettia is susceptible to powdery mildew (PM) (*Oidium* sp.) with symptoms first appearing as small circular colonies on the abaxial and adaxial surface of bracts and foliage, coalescing to colonize large areas of plant tissue, and in some cases defoliating and eventually killing the plants. During 5 Dec 1994 to 31 Mar 1995, atmospheric concentrations of PM conidia were monitored with Burkard volummetric spore traps in two research greenhouses containing PM-infected, multiple-stem poinsettias with mature bracts. To determine viability of atmospheric conidia, healthy poinsettias were placed in each greenhouse weekly, removed after seven days, and incubated in a greenhouse environment conducive to PM development. Weather-recording instruments in each greenhouse provided hourly records of temperature (temp.) and relative humidity (RH). Conidia in the atmosphere were common from 0900 to 1800 hours and not associated with routine grower activity. Peak conidial concentrations often were associated with a rapid change in temp., RH, or both. Average temp. in the two greenhouses was similar (20 C), although average RH was higher in one greenhouse (64%) than the other (53%). Average daily conidial concentrations in the atmosphere were higher (1,221 conidia/m^3 air/hr) and disease more severe (defoliation and plant death) in the high RH greenhouse compared to the low RH greenhouse (317 conidia/m^3 air/hr). Plants exposed to high atmospheric conidial concentrations showed PM colonies within an average of 9.4 d; plants exposed to lower conidial concentrations showed PM colonies within an average of 11.4 d.

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INFLUENCE OF PH, POTASSIUM, PHOSPHORUS, AND MAGNESIUM ON ROOT ROT OF *VIOLA WITTROCKIANA* CAUSED BY *THIELAVIOPSIS BASICOLA*. Copes, W. E., and Hendrix, F. F. University of Georgia, Athens, GA 30602.

Fertility treatments were applied every 2 days in three factorial experiments of pH (4.7 and 6.2) X NO_3:NH_4 ratio (100:0, 67:32, 25:75); NO_3:NH_4 ratio (75:25, 25:75) X K (133, 233, 333 ppm); and Mg (5, 25, 45 ppm) X P (5, 15, 25 ppm) to pansy plants both noninoculated and inoculated with *T. basicola* in sand medium. Disease incidence was lowest with high NH_4-N (25:75 NO_3:NH_4 ratio), a low level of K (133 ppm) in conjunction with the 25:75 NO_3:NH_4 ratio, and a low level of P (5 ppm). The NO_3:NH_4 ratio was the dominant nutritional factor which reduced disease incidence. Increasing potassium and phosphorus levels resulted in an increase in disease incidence. The influence of pH had no effect on disease incidence in sand medium (CEC=0.225) presumably because element availability from sand surfaces into substrate solution was low.

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RECURRENCE OF POWDERY MILDEW ON OVER-SUMMERED ASYMPTOMATIC POINSETTIAS. *S. H. Kim*, T. N. Olson, and C. E. Portmann. Plant Disease Diagnostic Lab., PA Dept. of Agriculture, Harrisburg 17110.

Powdery mildew (PM) on poinsettia (*Euphorbia pulcherrima*), has recurred in PA since 1990, when the disease was first encountered as a new disease to PA and the US. The infected plants became asymptomatic during each summer in greenhouses in PA. To investigate the recurrence of *Oidium* sp. on the asymptomatic poinsettias, % foliage infection on 40 PM-infected Dark Red Hegg poinsettias was recorded at weekly intervals in a greenhouse during June '94 - May '95. The foliage >5cm in length, occurring on a stem 20cm from the apex was monitored for PM sign development. Prior to initial PM sign development on Sep 21, the greenhouse was isolated to avoid an inoculum source other than from the initial 40 infected plants. All the foliage of each of 40 symptomatic plants became asymptomatic during Aug 10 - Sep 14, '94. The PM reappeared on Sep 21, '94 (21-26C), and the 40 individual plants reached 100% foliar infection by March 1, '95 (17-23C). This suggested that *Oidium* sp. over-summered with the asymptomatic poinsettias, and that the asymptomatic plants could be the source of the inoculum.

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COMPARISON OF dsRNA IN WESTERN AND EASTERN ISOLATES OF *Discula destructiva*. S. D. McElreath, J.-M. Yao, and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Twenty-eight isolates of *Discula destructiva* Red., the dogwood anthracnose fungus, from the northwestern United States and 1 from British Columbia, Canada were examined for double-stranded RNA (dsRNA). Seven (24%) were positive, including 6/23 isolates from Oregon and 1/1 from Washington. The isolate from British Columbia and 4 isolates from Idaho were negative. Estimated segment sizes by agarose gel electrophoresis ranged from *ca.* <0.6 to 5.0-6.0 kbp. No two banding profiles were the same. DsRNA was not detected in 22/29 western isolates even when doubling the amount of mycelium usually extracted and overloading the gels. Previously, we found dsRNA in 80/80 *D. destructiva* isolates from the eastern United States (Current Microbiology 29:57-60, 1994). Twenty-three isolates (29%) had one or more large segments (8-12 kbp). Doublets of 3-4 kbp were common in eastern isolates but were not present in western isolates. Banding profiles of all of the western isolates were different from those of the eastern isolates.

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SUSCEPTIBILITY OF POINSETTIA CULTIVARS TO POWDERY MILDEW. S. H. Kim[1], *T. N. Olson*[1], and A. H. Michael[2]. [1]Plant Disease Diagnostic Lab., PA Dept of Agriculture, Harrisburg 17110, and [2]Penn State Univ, University Park 16802.

Powdery mildew (PM) of poinsettia (*Euphorbia pulcherrima*), has recurred in PA since 1990, when the disease was first encountered as a new disease to PA and the US. To investigate susceptibility of popular cultivars in PA, 11 cvs. with bracts were placed next to a PM-infected cv. Dark Red Hegg. Each cultivar was replicated 6 times in a greenhouse, 16-23C; and % foliage with PM signs was analyzed at 3, 4, and 5 wks. after the exposure to PM (Duncan's multiple range test, P=.05). At 5 wk, % leaf infection of Dark Red Hegg, V-17 Angelika White, Pink Peppermint, Hot Pink Hegg, Topwhite Hegg, V-17 Angelika Marble, Lilo Red, Supjibi Red, Red Sails, Jingle Bells 3, and Freedom Red was 29, 50, 50, 53, 60, 63, 77, 83, 96, 100, & 100%, respectively (LSD=24); whereas % bract infection was 58, 62, 80, 77, 83, 86, 92, 97, 86, 90, & 99%, respectively (LSD=27). Freedom Red was significantly more susceptible than Dark Red Hegg and V-17 Angelika White; % foliage infection of Freedom Red increased from 79% to 100%, V-17 Angelika White 2% to 56%, and Dark Red Hegg 14% to 43%, at 3rd and 5th wk, respectively.

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INFLUENCE OF DAY/ NIGHT TEMPERATURES ON THE SUSCEPTIBILITY OF POINSETTIAS TO *BOTRYTIS CINEREA*. P.M. Pritchard[1], M.K. Hausbeck[1], and R.D. Heins[2]. [1]Dept. of Botany and Plant Pathology and [2]Dept. of Horticulture, Michigan State University, East Lansing, MI 48824.

The susceptibility of poinsettias with maturing bracts to *Botrytis cinerea* when grown under day/night temperatures of 16/16, 19/19, 22/22, 16/19, 19/22, 16/22, 19/16, 22/19, and 22/16 C for three (Expt. 1) or six weeks (Expt. 2) prior to inoculation was investigated. Plants were inoculated with a suspension of 2.7 x 10^5 conidia/ml and incubated at 20 C. After 8 days in Expt. 1, 16/16, 16/19, and 19/16 C treatments had 72-75% bract and 9-18% leaf infection with sporulation occurring on ≤25% of the bracts and ≤8% of the leaves. All other treatment combinations had greater bract (≥96%) and leaf (33-62%) infection; sporulation was also greater on bracts (55-94%) and on leaves (14-37%). In Expt. 2, plants were three weeks more mature at time of inoculation, and ≥ 99% of the bracts became infected regardless of treatment. Leaf infection was 17-24% for treatments 22/22, 19/22, and 22/19 and ≤13% for all other treatments. Sporulation on bracts was 18% for the 16/16 C treatment and 51-79% for all other treatments. Sporulation on leaves was ≤13% regardless of treatment. Overall, mature bracts were more susceptible than leaves or immature bracts. Plants grown at higher average temperatures were more susceptible to *B. cinerea*, apparently due to increased maturity.

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TOXIC METABOLITE DETECTION SYSTEM DEMONSTRATES DIFFERENCES BETWEEN *Discula destructiva* AND *Discula*, TYPE II. D. E. Wedge and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

A seedling root bioassay, an allelopathic screening method commonly used in herbicide research, was used to study toxic metabolites of fungi associated with dogwood anthracnose. Three isolates of *Discula destructiva* Red. and 1 isolate of *Discula* sp. (Type II) were grown under identical conditions. Culture filtrates from 6 replicates of each isolate were pooled, filtered and screened for biological activity using seeds of *Cornus* spp. and radish (*Raphanus sativus*). Seedling root measurements after exposure to the toxic metabolites demonstrated that the culture filtrates of *D. destructiva* isolates were significantly more toxic than those of the Type II *Discula* sp. Excellent correlation between the *Cornus* spp. and *R. sativus* was established.

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SEPARATION OF *Discula destructiva* TOXIC METABOLITES FROM CULTURE FILTRATE. D. E. Wedge[1] M. B. Riley[2] and F. H. Tainter[1] Departments of Forest Resources[1] and Plant Pathology and Physiology[2], Clemson University, Clemson, SC 29634-1003.

Toxic metabolites of *Discula destructiva* Red., the causative agent of dogwood anthracnose were studied. A procedure combining ultrafiltration, lyophilization and rotary evaporation was used to produce consistent isolation of low-molecular-weight toxic metabolites in a partially purified culture filtrate (PPCF). Reverse phase high performance liquid chromatography (HPLC) was used to further separate this PPCF. Samples were collected at five minute intervals and toxicity was tested using *Cornus* spp. and radish (*Raphanus sativus*) seedling bioassays. Bioassays demonstrated highly significant differences in activity between HPLC samples. Cultures grown on potato-dextrose broth (PDB) contained several active fractions, one fraction was noted in PDB without *D. destructiva* growth. Biologically active fractions were confirmed using a chemically defined media that had no toxic characteristics. Isolation and identification of these active components will be used to develop a quantitative method for the comparison of *D. destructiva* isolates and those associated with Type II *Discula* sp.

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DETECTION OF A VIRUS INFECTING PORTULACA HYBRIDS IN KENTUCKY AND KANSAS GREENHOUSES. B.C. Eshenaur[1], U.E. Jarlfors[1], K.A.Kelly[2], and J. O'Mara[3]. Univ of KY[1], Lexington, 40546 AGDIA INC[2]. Elkhart IN 46514 and KS State Univ[3]. Manhattan, KS 66506.

Portulaca hybrids grown in the ornamental greenhouse trade in Kentucky and Kansas were exhibiting stunting and irregular leaf margins. Using the electron microscope, leaf dips of infected plant tissue revealed numerous negatively stained flexous rods. Thin sections showed banded aggregates of the virus particle as well as crystalline inclusions. The virus was transmitted by mechanical inoculation to *Nicotiana benthamina* (systemic infection) and *Chenopodium amaranticolor* (local lesions). An ELISA screen run on the infected plant tissue and indicator plants gave a positive reaction to Papaya Mosaic Virus antiserum.

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INFLUENCE OF AMBIENT OZONE CONCENTRATIONS AND PLANT AGE ON FOLIAR INJURY IN PUMPKIN. M. T. McGrath, Dept. of Plant Pathology, Long Island Hort. Res. Lab, Cornell Univ., Riverhead, NY 11901.

'Spirit' pumpkin plants were transplanted on six dates between 25 May and 2 Aug in 1993 and in 1994. Ozone was monitored with a TECO Model 49 UV Photometric Ozone Analyzer. In 1993, ozone-induced foliar injury was first observed in each of the six plantings 18-36 days after transplanting. Hourly ozone concentrations were $\geq$ 60 ppb prior to symptoms for 48, 37, 86, 99, 72, and 67 hrs for the six successive plantings, respectively. Cumulative exposures to ozone $\geq$ 60 ppb were 3387, 2642, 7220, 7768, 5352, and 5325 ppb•hrs, respectively. In 1994, severe injury was seen 2 days after the year's highest daily ozone concentrations on 13 July (23 hrs $\geq$ 30 ppb, 14 hrs $\geq$ 60 ppb, 4 hrs $\geq$ 100 ppb) in pumpkins transplanted 26 and 43 days before exposure. After the second highest daily ozone concentrations on 2 July (24 hrs $\geq$ 30 ppb, 11 hrs $\geq$ 60 ppb, 0 hrs $\geq$ 90 ppb), only one plant (transplanted 32 days before) developed symptoms. Plants were exposed to higher ozone levels during the week before 13 July (56 hrs $\geq$ 60 ppb) than before 2 July (22 hrs $\geq$ 60 ppb).

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YIELD AND BIOMASS OF CROP PLANTS IRRIGATED WITH TNT AND RDX CONTAMINATED WATER. M. Simini, Geo-Centers, Inc., Aberdeen Proving Ground, MD 21010-0068; and R.T. Checkai, U.S. Army, ERDEC, Aberdeen Proving Ground, MD 21010-5423.

Crops grown in site-collected soil were irrigated with water containing 2,4,6-trinitrotoluene (TNT) and cyclotrimethylenetrinitramine (RDX) to simulate field conditions. Pots were watered to field capacity throughout the life-cycle of the crop with 2, 20, and 100ppb RDX; 2, 100, and 800ppb TNT; 100ppb RDX + 800ppb TNT; or uncontaminated water. Yield and biomass of tomato fruit, bush bean fruit, corn stover, and soybean seeds were significantly ($p=0.05$) less when irrigated with the RDX+TNT treatment compared to controls. Lettuce leaves and radish root yield and biomass were unaffected by treatment level. Loading of RDX and TNT in response to evapotranspiration was greatest for tomato/corn/soybean/bush bean and least for radish/lettuce. Plant tissue contaminant concentrations will be presented and discussed.

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SIMULATION OF THE EFFECTS OF WINTER INJURY ON THE GROWTH OF RED SPRUCE. J. A. Laurence, D. A. Weinstein, R. J. Kohut, and R. G. Amundson. Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, NY 14853-1801.

Winter injury has been implicated as a factor in the decline of some high elevation red spruce stands in northeast North America. We used a physiologically based simulation model, TREGRO, to investigate the potential impact of winter injury on the growth of sapling-size trees. Winter injury in the field causes loss of the most recently produced foliage. In our simulations, removal of up to 100 percent of the youngest needle cohort resulted in significant reductions in growth. Growth reductions were not distributed equally in the simulated tree; stem, fine root, and coarse root growth were affected more than branch or new needle growth. Simulations indicated that effects of increasing defoliation were not linear. By the end of the second year after simulated defoliation, relative growth rates of defoliated trees matched those of the controls, but 50 percent defoliation resulted in a 20 percent reduction in total tree weight after four simulated years. Similar reductions in growth have been measured in the field.

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Ozone-Induced Accelerated Senescence in Four Tree Species: A Function of Growth Habit. E.J. Pell, B.W. Brendley, J.P. Sinn, Department of Plant Pathology, the Pennsylvania State University, 211 Buckhout Laboratory, University Park, PA 16802

Two-year-old seedlings of black cherry (*Prunus serotina*), northern red oak (*Quercus rubra*), and sugar maple (*Acer saccharum*), and ramets of hybrid poplar (*Populus maximowizii x trichocarpa* clone 245) were grown in a set of eight charcoal-filtered open-top chambers. Half of the chambers received 0.08 $\mu l\ l^{-1}$ ozone (O_3) from 1000 to 1800 h each day of the growing season. At regular intervals from emergence to senescence, foliage was subjected to gas exchange analysis and quantification of Rubisco protein. Ozone-induced an acceleration of foliar senescence and loss of Rubisco in older foliage of the free-growing genotypes, hybrid poplar and black cherry. In association with this response net photosynthesis declined; this response, could not be attributed entirely to reduction in stomatal conductance. Northern red oak exhibited a reduction in net photosynthesis in concert with a reduction in stomatal conductance. Sugar maple foliage did not exhibit O_3-induced reductions in either parameter. Neither of the latter two fixed-growing genotypes exhibited O_3-induced accelerated senescence or a reduction in Rubisco content. Explanations for differential responses will be explored with attention to growth habit among the four genotypes.

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A NEURAL NETWORK MODEL TO PREDICT SOYBEAN RUST. X.B. Yang, W.D. Batchelor, and A.T. Tschanz. Dept. of Pl. Path., Dept. of Agri. & Bio. Sys. Engi., Iowa State Univ., Ames, 50011, USDA-APHIS-PPQ, Beltsville, MD 20705.

A neural network was developed to predict disease severity of soybean rust. The multiple layer feed forward network was developed with inputs of planting date, days to maturity, first day that disease was observed, crop age, number of days that relative humidity exceeded 90%, and degree days for soybean and rust development. Historical data collected from Asian Vegetable Research and Development Center in Taiwan was used for network training and testing. The data set consisted of observations from 72 sequential planting experiments over 2 years for two soybean varieties, with 576 scenarios (observations) per variety. A separate network was developed for each variety. The training set consisted of 80% of the available scenarios and the test set consisted of the remaining 20% of the scenarios. A sensitivity analysis on the number of hidden nodes was used to obtain the network architecture that gave the highest correlation between predicted and measured disease severity for the test cases, $r = 0.96$ for $n = 115$. The maximum error in predicting disease severity for the test cases was < 20%. This was much more accurate than previous attempts to predict disease severity using process-oriented models.

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EPIDEMIOLOGY OF *XANTHOMONAS FRAGARIAE* ON STRAWBERRY: YIELD LOSS AND DISEASE MANAGEMENT. P.D. Roberts[1], R.D. Berger[1], J.B. Jones[2], and C.K. Chandler[2]. [1]University of Florida, Gainesville, 32611.[2] Bradenton, 34203.

In field plots of strawberry 'Sweet Charlie', the increase of angular leaf spot was studied in two seasons. After inoculation, disease severity increased to 25% by 13 weeks in 1994, and to 10% by 21 weeks in 1995. Yield was reduced in inoculated plots by 12% in 1994 and 5% in 1995 compared to healthy plants. Sprays at label rates (X) of a combination of cupric hydroxide and mancozeb applied at 7 to 14-day intervals provided considerable disease control, but phytotoxicity reduced plant size and yield. Sprays at 0.05X of the combination or cupric hydroxide alone applied 2-3 times per week were not phytotoxic and disease was reduced ca. 50%. The spread of the bacterium from inoculated plants was minimal; thus, yield losses to angular leaf spot might be avoided if disease-free transplants are used.

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MANAGEMENT OF EPIDEMICS OF OAT CROWN RUST THROUGH THE CONTROL OF THE INITIAL WAVES OF DISEASE BY REDUCED RATES OF FUNGICIDE. C. A. Forcelini and R. D. Berger, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

The fungicide mancozeb (75 DF) was applied to oat plants at 100% (1.8kg a.i./ha, 1 × wk) or 25% (0.45kg a.i./ha, 3 × wk) of label rates to control crown rust (*Puccinia coronata*). In the greenhouse, the AUDPCs that resulted from 15 daily inoculations were 0.632 (non-sprayed), 0.001 (25% rate), and 0.004 (100% rate). In the field, the AUDPCs after three latent periods ($p=9$) were 1.515 (non-sprayed), 0.057 (25% rate), and 0.494 (100% rate). The better control of rust by the lower rate was credited to the more frequent spray applications which maintained an adequate level of fungicide on the leaf surface and protected new plant tissue that emerged between applications of fungicide. When fungicide sprays were initiated at several thresholds of disease severity (0.0001, 0.001, 0.01, and 0.05), the AUDPCs were 0.89, 1.181, 1.334, and 1.236 (25% rate) and 0.823, 0.935, 0.991, and 1.116 (100% rate), respectively. Decisions to manage epidemics based on thresholds were unsuccessful. Preventive control of initial disease waves with reduced rates of fungicide may have applicability to manage epidemics of a broad range of pathosystems.

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RICE BLAST FORECASTING SYSTEM BASED ON NEAR REAL-TIME MICROCLIMATIC DATA. E.W. Park and K.R. Kim, Dept. of Agricultural Biology, Seoul National University, Suwon Korea, 441-744.

A rice blast forecasting system was developed by integrating a weather monitoring system and a simulation model for rice blast development. An automated weather station (AWS) was installed in a rice paddy field to monitor air temperature, relative humidity, leaf wetness, solar radiation, wind speed, and water temperature. Near real-time weather data were transmitted directly from the AWS to a PC in the lab via the public telephone line. The rice blast model being driven by hourly weather data consisted of several submodels simulating spore dispersal, spore deposition, infection, infection rate, latent period, symptom appearence on leaves and panicles, lesion expansion, host growth and initial heading date. The whole system was programmed in C for OS/2 which provides GUI, multitasking, and profile management subsystems. The rice blast model was tested by comparing the predicted and the observed data from 2 year field experiments. The result of validation experiment suggested possible use of the system for rice blast management although over-estimation of disease severity was noted in the late season.

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USE OF GEOGRAPHIC INFORMATION SYSTEMS TO GENERATE DISEASE PREVALENCE, INCIDENCE, AND SEVERITY MAPS FOR SEED CORN PRODUCTION IN 1992. F. W. Nutter, Jr., S. N. Wegulo, and C.A. Martinson, Dept. of Plant Pathology, Iowa State University, Ames, 50011

Geographic information systems (GIS) are systems used to facilitate the input, storage, retrieval, analysis, and output of geographical information. In excess of 500 seed corn production fields in Iowa were inspected during August of 1992 for phytosanitary purposes. Field location (township), disease prevalence, incidence, and severity data were recorded for each disease. Common rust was the most prevalent disease in 96% of fields inspected followed by common smut (88%), Northern corn leaf blight (73%), Stewart's wilt (38%), Helminthosporium leaf spot (33%), gray leaf spot (12%), and eyespot (10%). Incidence and severity maps were generated to display the geographical distribution of disease intensity assessments. Although the 1992 disease database has a number of assumptions and potential sources of bias, the application of GIS offers a new tool to document and analyze year to year variation in the geographic distribution of seed corn diseases.

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COMPARISON OF EPIDEMICS CAUSED BY PHYMATOTRICHUM OMNIVORUM IN TRELLISED APPLE ORCHARDS. C. Kenerley[1], K. Ivors[1], D. Appel[1], and S. Nelson[2]. [1]Dept. Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843 and [2]Dept. Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Root rot epidemics caused by *Phymatotrichum omnivorum* were monitored in five, commercial apple orchards in Kerrville, Tx during two, consecutive growing seasons (1993-1994). Plant mortality at the beginning of disease assessments (1993) ranged from 8 to 16%. At leaf fall (1994), mortality among orchards ranged from 35 to 46%. Regression diagnostics for data from both years and all orchards indicated that temporal changes in disease incidence were described adequately by simple, linear models ($r = 0.95$-0.99). Relative Area Under the Disease Progress Curve (RAUDPC) values were significantly different among orchards for 1993 and 1994 and for orchards between years. From 1993 to 1994, a decline in RAUDPCs was observed in three orchards, whereas an increase in RAUDPCs was recorded for the other two orchards. The pathogen survived between growing seasons as mycelial strands on asymptomatic trees and on trees that had been killed by the fungus. Sclerotia of *P. omnivorum* were recovered from 62% of the areas sampled within orchards. Spatiotemporal distance class analysis detected significantly nonrandom patterns of disease increase during 1993-1994 for all orchards. Patterns of disease increase occurred both within and across trellis rows. Significant edge effects were detected in 4 of the 5 orchards.

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GERMINATION OF *SEPTORIA LYCOPERSICI* SPORES DURING INTERRUPTED WET PERIODS

T.E. Engle, S.K. Parker, M.L. Gleason, E.J. Braun, and F.W. Nutter, Jr., Departent of Plant Pathology, Iowa State University, Ames IA 50011.

Pycnidiospores of *Septoria lycopersici*, obtained from fresh lesions on greenhouse-grown tomato leaves, were exposed to alternating periods of presence or absence of free water at 25± 0.03° C. Ten-$\mu\ell$ droplets of a spore suspension (1-3 x 10^5 spores/ml) on glass cover slips were exposed to 12 hr in free water at 100% RH, then 12 hr at 100, 95, or 50% RH above glycerol/water solutions following air drying of the 95 and 50% RH treatments, then 12 hr in free water at 100% RH. In each trial, fifty spores from each of 6 cover slips were examined for germination under a microscope after each 12-hr period. The experiment was repeated six times. Spores exposed to 95% or 50% RH from 12 to 24 hr exhibited significantly lower percent germination after 36 hr than those which remained at 100% RH for 24 or 36 hr. The results are preliminary evidence that interruptions in wet periods inhibit the germination of *S. lycopersici* spores.

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HOST RANGE OF *PHYTOPHTHORA INFESTANS* IN CENTRAL PERU AND LIST IN THE WORLD SINCE 1840'S. RESISTANCE ON WILD TOMATOES.
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Late blight is an important disease on potato, tomato and pear melon in Peru the center of origin of the crops. In surveys to determine other hosts in the Coastal area during the winter season (14-18C, 90-95% humidity) natural infection was found in "Las Lomas" on: *Solanum medians, S. montanum, S. senecioides* and *S. tuberiferum;* and *Nolana gayana* and *N. humifusa* of the family *Nolanaceae;* in the highlands on *S. laxissimum;* in Lima on ornamentals *S. sitiens* (from Chile) and *S. bukasovii* (from Peru). Wild relatives of tomato: *Lycopersicon chilense, L. peruvianum,* and *L. pimpinelifolium,* as well as *S. lycopersicoides, S. ochranthum (L. pennelli)* and *S. sitiens,* showed partial resistance or resistance; the commercial cultivar "Huando" was highly susceptible. In laboratory inoculation with potato isolates races 0tm and 1-3-7tm (tomato and pear melon patothypes), and pear melon specialized form race 1-4-10-11 pt (potato and tomato patothypes) 24 *Solanaceous* and *Nolanaceous* (11 not previously reported) were susceptibles with fungal sporulation. The host range of *P. infestans* was expanded from 63 to 113 hosts.

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VERTICILLIUM DAHLIAE RACE 2 IN TOMATO FIELDS IN SOUTHERN ONTARIO
G.K. Tenuta, G. Lazarovits, K.F. Dobinson, Agriculture and Agri-Food Canada, Pest Management Research Centre, London ON N5V 4T3.

Ten fields in Essex County were surveyed in 1993 and 1994 to assess the incidence of *Verticillium* infection in processing tomato plants and to determine how prevalent race 2 strains are in the tomato fields. Soil samples were collected from each field prior to planting, and a non-destructive sampling technique, in which leaf petioles were taken from the lower portion of randomly selected tomato plants, was used to assess infection by *V. dahliae*. Race types of *V. dahliae* isolates collected from soil, trap plants and leaf petioles of field-grown tomatoes were determined by a root dip inoculation method, with Bonny Best (race 1- and race 2- susceptible) and H1350 (race 1-resistant) as differential cultivars. *V. dahliae* was present in the soil of all fields, 31 to 41% of plants were infected by mid-August, and 98% of isolates typed were race 2. Our data indicate that Verticillium wilt is no longer being adequately controlled by planting race 1-resistant tomato varieties, and the prevalence of race 2 *V. dahliae* in the fields suggests that verticillium wilt has the potential to again have an economic impact on tomato production in southern Ontario.

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RELATION OF LIGHT INTENSITY AND TEMPERATURE TO SCLEROTIAL GROWTH OF *COLLETOTRICHUM COCCODES*. S. Sanogo, S.P. Pennypacker, and R. Stevenson. Department of Plant Pathology, The Pennsylvania State University, PA 16802.

A solar simulator, operated in a temperature-controlled chamber, was used to investigate the effects of light intensity and temperature on sclerotial growth of *Colletotrichum coccodes*. Two sclerotia were placed equidistant (5mm) from the center of 3.5-cm petri plates containing 2% water agar medium. The plates were subjected to five light intensities (33, 105, 209, 416, and 602 $w.m^{-2}$) of electromagnetic radiation (>300 nm) and four temperatures (16, 22, 28, and 34 C). Actual temperature of sclerotia within each plate was estimated by a thermocouple placed on the agar surface. At each light intensity one plate was irradiated daily for 14 hrs followed by 10 hrs darkness for a period of 4 days. Preliminary regression analysis indicated that the mean radial growth of sclerotia was dependent upon both temperature and light intensity ($Ra^2=0.79$, $P<0.0001$).

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INVASION OF PATHOGEN POPULATIONS BY CYTOPLASMIC ELEMENTS THAT CAUSE HYPOVIRULENCE: A MODEL.
D.R. Taylor[1], A.M. Jarosz, R.E. Lenski & D.W. Fulbright, Michigan State University, East Lansing 48824-14312. [1]Present address: University of Virginia, Charlottesville 22903.

A deterministic model was constructed to explore factors controlling the invasion of cytoplasmic elements (e.g. double-stranded RNAs or mitochondrial mutants) into populations of plant pathogenic fungi. Reductions in pathogen fitness due to the presence of the cytoplasmic element, ability of the element to spread contagiously through the pathogen population, and plant population size strongly influenced the ability to invade. Elements that caused only small reductions in pathogen fitness could easily invade large pathogen populations, as long as contagious spread was greater than zero. In contrast, high contagious transmission rates were needed for invasion when pathogen fitness was severely reduced or plant populations were small.

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VCG AND DNA FINGERPRINT DATA SUGGEST THAT FUSARIUM OXYSPORUM F. SP. MELONIS RACE 1 HAS RECENTLY BEEN INTRODUCED INTO NEW YORK STATE. D. T. Schroeder, T. R. Gordon, and D. Okamoto. Dept. of ESPM, Univ. of Ca, Berkeley, 94720, and T. L. Zuniga, and T. A. Zitter. Dept. of Plant Pathology, Cornell Univ., Ithaca, NY, 14853.

The recent occurrence of Fusarium wilt on race 2 resistant varieties of muskmelon and the identification of race 1 isolates in New York has raised questions concerning the introduction of race 1 populations into the state. Did race 1 emerge from native race 2 populations or was it introduced? A collection of 46 Fusarium oxysporum f. sp. melonis isolates from New York State was subjected to VCG and DNA fingerprint analyses to determine a likely origin. Without exception the race 1 isolates fell into the same VCG as race 1 isolates from Maryland (the only previously reported case of race 1 in the United States). In addition, DNA fingerprinting supported the strong affinity between the New York and Maryland race 1 isolates. Both methods of analysis discounted the hypothesis that race 1 emerged from indigenous race 2 populations. Our conclusion based on this data is that the partial failure of muskmelon race 2 resistance in New York is due to recent introductions of race 1 into the state.

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TRACKING ANCIENT EPIDEMICS: SURVEY OF PLANT PATHOGENS OF PRECERAMIC PERU. J. B. Ristaino[1], Z. G. Abad[1], and D. Ugent[2]. [1]Dept. of Plant Pathology, North Carolina State University, Raleigh, NC, and [2]Dept. Plant Biology, Southern illinois University, Carbondale, IL.

Ten ancient potato tubers (*Solanum tuberosum* L., sensu lato) unearthed from the middens of four archaeological sites located near Casma, Peru were examined. These ancient tubers represent some of the oldest known mummified specimens of potato in the world (2000 BC-1200 BC). We will address the following questions: 1) Do these ancient specimens contain morphological evidence of plant pathogenic fungi or actinomycetes; and 2) Can DNA be amplified from the organisms observed and the identity of the organisms be confirmed utilizing PCR technology? Specimens were handled aseptically in a laboratory without a history of work with potato pathogens. One of the tubers showed visible symptoms of scab. We have observed evidence of fungal mycelium on two of the specimens by scanning electron microscopy (SEM). Fungal hyphae were observed adjacent to a lenticel on one tuber. Ribosomal DNA will be amplified and sequenced to identify pathogens of epidemiological significance.

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Chen, J., and G.W. Bird. Simulation model of early-season ontogeny of ***Solanum tuberosum*** in the presence of ***Pratylenchus penetrans*** and ***Verticillum dahliae***. Department of Entomology, Michigan State University, East Lansing, MI 48824.

A computer model simulating the early-season ontogeny of ***Solanum tuberosum*** cv. Superior in the presence and absence of ***Pratylenchus penetrans*** and ***Verticillium dahliae*** was developed using C++ computer language. The model simulates the eight components of the below-ground architecture of ***S. tuberosum***. Data for the model were obtained from a greenhouse experiment using soil from a potato field with known infestations of ***V. dahliae*** and ***P. penetrans*** and high incidence of potato early-die symptoms during the previous 15 years. The best-fit linear, polynomial, logarithmic, or exponential function was tested and linked in the model. Average r^2 value of the simulated linear, polynomial, logarithmic, and exponential growth of ***S. tuberosum*** system components was 0.739, 0.922, 0.669, and 0.708 in the presence of the two pathogens, respectively. The model is designed to be linked to SUBSTOR, a potato simulation that is part of the CERES crop model system.

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COMPARISON OF rDNA SEQUENCES OF LOW TEMPERATURE BASIDIOMYCETES. Claudia Jasalavich and Bruce Gossen, Agriculture and Agri-Food Canada, Research Station, Saskatoon, Sask., Canada.

The low temperature basidiomycetes (LTBs) are a group of psychrophilic plant pathogens which cause diseases such as cottony snow mold of turf grass and fruit rot of apples in cold storage. Only the form found on wheat produces sclerotia. Most isolates are sterile. Currently LTBs are called *Coprinus psychromorbidus* based on a basidiocarp found on alfalfa. In order to compare the different forms of LTBs, we amplified and direct sequenced by PCR the nuclear 5.8s rDNA, the internal transcribed spacers, and a variable portion of the mitochondrial small rDNA from 8 LTBs and from 4 other species of *Coprinus*. Based on phylogenetic analyses of the aligned DNA sequences, the LTBs are comprised of at least three taxa, probably three different species in more than one genus.

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COMPARISONS OF THE INTERNAL TRANSCRIBED SPACER REGIONS OF rDNA IN *OPHIOSPHAERELLA KORRAE*, *O. HERPOTRICHA* AND *LEPTOSPHAERIA NARMARI*. N. A. Tisserat and S. H. Hulbert. Kansas State University Manhattan, KS 66506.

The internal transcribed spacer (ITS) regions of the rDNA of *Ophiosphaerella korrae*, *O. herpotricha*, and *Leptosphaeria narmari*, three fungi that cause spring dead spot on bermudagrass, were amplified with the universal primers ITS4 and ITS5. Amplification of DNA from isolates of each fungus resulted in either a 590- or 1,019-bp fragment. The ITS1 and ITS2 sequences of the 590-bp fragment varied by less than 5% among the three fungal species, indicating a close phylogenetic relationship and a need for taxonomic revision of *L. narmari*. The larger 1,019-bp fragment, found in certain isolates of each species, included the 590-bp sequence plus a 429-bp insert located 2 bp downstream from the ITS5 primer sequence. Preliminary sequence data indicates the insert is similar among these species. The presence of ITS length polymorphisms should be considered during selection of sequence regions for species-specific primers.

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CHARACTERIZATION OF THE **A**-PHEROMONE GENE OF *USTILAGO HORDEI*. C.M. Anderson and J.E. Sherwood, Dept of Plant Pathology, Montana State University, Bozeman, MT 59717

The secretion of chemical mating factors is the first step in the formation of conjugation tubes and the subsequent conversion of *Ustilago hordei* haploid sporidia to pathogenic dikaryotic mycelia. A region of the *U. hordei* mating-type **A** genome having homology to the *U. maydis* mfa1 gene was cloned and transformed into *U. hordei* mating-type **a** cells. The resulting transformants constituitively produced conjugation tube-like structures. Nested deletions of the cloned fragment were constructed and transformed into mating-type **a** cells to determine which to use for sequencing. Sequence information revealed an open reading frame of 126 bases that would encode a 42 amino acid pheromone precursor. The last four amino acids of this precursor, CVVA, represent the CAAX motif common to farnesylated, carboxymethylated fungal pheromone precursors. Synthetic peptides representative of possible mature pheromone peptide were evaluated for their ability to induce conjugation tube formation in **a** cells. Expression of the pheromone gene under different environmental conditions was analyzed. A more complete understanding of the early stages of the sexual processes in this fungus will allow the development of strategies to prevent this cycle in the field, thereby providing an efficient, biologically specific means of controlling cereal diseases.

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MOLECULAR CHARACTERIZATION OF *FUSARIUM* PATHOGENS OF PAPAVER. Pilgeram, A.P., Weaver, M.B., Morgan, C.T., and Sands, D.C. Biological Control of Weeds, Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

Strains of *Fusarium* pathogenic to poppy were characterized using several molecular techniques. Pathogens from Russia, Colombia, Thailand, and the United States were evaluated using rDNA and RAPD analysis. The strains from Thailand, isolated from within a single region of poppy cultivation, were nearly clonal and could be readily distinguished from other foreign isolates using RAPD analysis. The greatest genetic diversity and pathogenicity were observed in the Colombian strains which were also isolated from plants within a single region. In addition, the relationship of the genotypes of individual strains relative to their pathogenicity was investigated.

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IDENTIFICATION OF DNA AMPLIFICATION FINGERPRINTING (DAF) MARKERS FOR RACE-SPECIFIC DETECTION OF *FUSARIUM OXYSPORUM* F.SP. *MELONIS*. K. Yagiz[1], J. Qiu[1], E.A. Momol[2], and S. Tuzun[1], [1]Dept. of Plant Pathology, Auburn University, AL 36849-5409; and [2]Dept. of Plant Pathology, Cornell University, Geneva, NY 14456-0462.

Wilt and yellowing diseases of muskmelon, caused by *Fusarium oxysporum* f.sp. *melonis*, are serious problems of muskmelon production worldwide. Although four different races of the pathogen (0, 1, 2, and 1.2) have been identified based on their disease reactions to the host, molecular markers that permit reliable distinction of the races are not currently available. The objectives of this research were to identify polymerase chain reaction (PCR) based DAF markers for race-specific detection and to determine the genetic relatedness among the races. Genomic DNA extracted from single spore cultures of two sets of races (one from France and the other from the USA) was PCR-amplified using a single 8-mer or 10-mer primer of arbitrary sequences. Partial amplification products were also subjected to restriction digest analyses. DAF banding patterns were studied by polyacrylamide gel electrophoresis followed by silver staining. Of 40 primers tested, 14 revealed race-specific DAF profiles. Jaccard's similarity coefficients (r) between paired races ranged from 0.38 (Race 2 - Race 1.2) to 0.64 (Race 0 - Race 1). Cluster analysis using a total of 246 bands generated from 14 selective primers indicated presence of distinct clusters (Race 2 vs. others) in each set of races. Our results demonstrated that DAF procedure is a simple, rapid, and reliable technique that can be used to distinguish different races of *Fusarium oxysporum* f.sp. *melonis* as well as to determine genetic relationship among the races.

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MAPPING AVIRULENCE GENES IN THE RUST FUNGUS, *Puccinia graminis*. Les J. Szabo, Paul J. Zambino and Anne R. Kubelik, Cereal Rust Laboratory, USDA-ARS, and Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108

Extensive genetic and physiological studies of cereal rust diseases have provided the foundation for many of our current concepts about host-parasite interactions. However, we know very little about the molecular biology of rust fungi, in part due to the obligate biotrophic nature of these pathogens. A genetic mapping population has been generated by crossing two North American isolates of *Puccinia graminis* f.sp. *tritici* and selfing a single F_1 progeny. In this F_2 mapping population ten avirulence/virulence phenotypes are segregating. Eight of these segregate as single dominant genes (*Avr*6, *Avr*8a, *Avr*9a, *Avr*10, *Avr*21, *Avr*28, *Avr*30, and *Avr*U), while *Avr*9d and *Avr*flk are segregating with ratios indicating that two genes are involved (3:13 and 15:1 respectively for avirulence:virulence). Linkage analysis showed that Avr10 and AvrU are approximately 6 cM apart. RAPDs and bulked segregant analysis have been used to identify three RAPD markers linked to *Avr*8a or *Avr*28. We are continuing to look for additional RAPD markers closely linked to these avirulence genes in order to isolate them by map-based cloning.

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GENE RESCUE OF PROTEIN KINASE A (*cpk*A) MUTANTS OF *Magnaporthe grisea*, USING THE *bar* SELECTABLE MARKER SYSTEM, RESTORES APPRESSORIUM FORMATION
Julius C. Brooks and Ralph A. Dean, Dept. of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634

The *bar* gene, which encodes phosphinothricin acetyltransferase, has been successfully used to develop an efficient transformation system for several filamentous fungi. Here, the *bar* gene was used as a selectable marker in *Magnaporthe grisea*. A plasmid containing the *cpk*A gene and the *bar* gene under the control of the *Aspergillus nidulans trp* C promoter was constructed and used to restore cAMP dependent protein kinase to strains in which the *cpk*A gene had been deleted. Transformants, resistant to the herbicide Basta, were able to form appressoria at levels indistinguishable from wild-type and were able to successfully infect rice. This is further molecular genetic evidence that *cpk*A is directly involved in pathogenicity and appressorium formation.

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IDENTIFICATION OF G PROTEIN α SUBUNITS FROM *Magnaporthe grisea*. **Shaohua Liu and Ralph A. Dean,** Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

The polymerase chain reaction was performed on *Magnaporthe grisea* genomic DNA using degenerate oligonucleotide primers complimentary to a conserved portion of G protein α subunits. PCR products were cloned into pBluescript II KS+, and the relationship among the clones was determined by Southern hybridization. Representative clones were sequenced and at least three G protein α subunits have been identified. pSL 1-1 is very similar (85% amino acid identity in the cloned region) to *GPA1* of the animal pathogenic yeast *Cryptococcus neoformans*. pSL 2-3 and pSL 8 are virtually identical to *gna1* (97%) and *gna2* (98%), respectively, of the fungus *Neurospora crassa*. RNA blot analysis showed that these three G protein α subunits are strongly expressed during *M. grisea* sporulation but weakly or not expressed during vegetative growth. Our results suggest that these G protein α subunits are involved in fungal sporulation and/or pathogenesis.

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PATHOGENIC VARIATION AMONG *Albugo candida* ISOLATES FROM WESTERN CANADA. S. Mathur, C.R. Wu, S.R. Rimmer, Department of Plant Science, University of Manitoba, Winnipeg, Canada R3T 2N2

Thirty seven isolates of *Albugo candida*, collected from different geographic locations in western Canada, were tested for virulence on a total of 14 accessions from four *Brassica* species to determine variability and distribution of races. Isolates were either mass isolations or single-pustule isolates (SPI) derived from mass isolates. Most isolates were virulent only on *B. rapa* and were classified as race 7. These isolates could be subdivided into two groups (named race 7a and 7v) on the basis of their virulence on cv. Reward. Isolates 28-7 and 29-1 were avirulent to all the differentials except the rapid cycling *B. rapa* CrGC 1-18. Two isolates, 11-16 and 41-4, which could infect cultivars of both *B. rapa* and *B. juncea*, could be hybrids between race 2 and race 7.

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USE OF RANDOM AMPLIFICATION OF POLYMORPHIC DNA TO DIFFERENTIATE RACES AND ISOLATES OF *Albugo candida*. C. Wu, S. Mathur, S.R. Rimmer, Department of Plant Science, The University of Manitoba, Winnipeg, Canada R3T 2N2

Thirty seven isolates of *Albugo candida* from different geographic locations in western Canada had previously been characterized for virulence on 14 *Brassica* accessions. These isolates were examined for genetic variation using RAPD. Using five random primers, fingerprint patterns were generated for each isolate. The classification of *A. candida* isolates based on RAPD analysis was similar to the virulence classification on *Brassica* differentials. A higher level of polymorphism was found between different races than subgroups within races. Most isolates were grouped as race 7 and could be subdivided into two subgroups (7a and 7v). Evidence that isolate 11-16 could be hybrid between race 2 and 7 was supported by the RAPD data. One avirulent isolate, 28-7, had entirely different banding patterns to other isolates. Primer 760 could distinguish among races 7a, 7v, 2a, 2v, 1 and 9.

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DETECTION OF DOUBLE-STRANDED RNA IN *VENTURIA INAEQUALIS*. T. Zhou and R. DeYoung. Agriculture and Agri-Food Canada, Pest Management Research Centre, Vineland Station, ON Canada L0R 2E0.

Single-conidium isolates of *Venturia inaequalis*, the causal agent of apple scab, were obtained from apple leaves and fruit collected from 67 different commercial or abandoned orchards, and wild apple trees in all of the Ontario apple growing areas. Nucleic acids were extracted from fungal mycelia using a simplified microwave method and analyzed via agarose gel electrophoresis. Twenty-six of the 279 isolates screened had nucleic acid band(s) additional to genomic DNA and single-stranded RNA. The additional band(s) were confirmed as double-stranded RNA (dsRNA) fragments by a specific extraction procedure and by digestion with DNase, and RNase at high and low salt concentrations. The number of dsRNA fragments from different isolates varied from one to five, with most isolates containing four fragments. The sizes of the dsRNA fragments ranged from approximately 2.5 to 6.2 kb.

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EVALUATION OF SPECIFICITY AND SENSITIVITY OF PCR PRIMERS FOR IDENTIFICATION OF *TILLETIA INDICA*. M.A.S.V. Ferreira[1], P.W. Tooley[1], E. Hatziloukas[1], M.R. Bonde[1], C.Castro[2], and N.W. Schaad[1]. [1]USDA/ARS, FDWSRU, Frederick, MD 21702, [2]CENARGEN/EMBRAPA, Brasilia, D.F., Brazil 70849-970.

A 2.3 kb mitochondrial DNA fragment isolated from an Indian isolate of *Tilletia indica*, the causal agent of Karnal bunt of wheat, was cloned and partially sequenced. A pair of external primers was used to amplify the DNA from mycelial cultures of 17 *T. indica* isolates from India, Mexico, and Pakistan. The amplification products were detected on ethidium bromide stained agarose gels and specificity confirmed by Southern hybridization. No PCR products were detected with DNA from 19 isolates of *T. barclayana*, a morphologically similar smut species or seven isolates of four other *Tilletia* species affecting wheat and other grasses. The detection threshold of the assay was 500 pg of total DNA as determined on ethidium bromide stained agarose gels. To further increase sensitivity, heminested PCR was employed, using one internal and one external primer in a second round of amplification. This lowered the detection limit to 1 pg of template DNA. The results show that these primers can be used to differentiate *T. indica* from other *Tilletia* species. A PCR method to detect *T. indica* teliospores in contaminated seed lots by using our primers is currently being developed.

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Use of a competitive polymerase chain reaction to quantify DNA of *Leptosphaeria maculans* during blackleg development in oilseed rape.
G.S. Mahuku, P.H. Goodwin and R. Hall, Dept. of Environmental Biology, University of Guelph, Guelph, ON, N1G 2W1.

An assay based on competitive PCR was developed to quantify *Leptosphaeria maculans* during blackleg development in oilseed rape leaves using primers specific to the highly virulent type. Coamplification of *L. maculans* with a heterologous internal control template resulted in accurate quantification of 1 to 10^9 copies of target DNA. The quantity of DNA per lesion in resistant and susceptible cultivars increased during the first 12 days and then declined. The trend was most evident in the susceptible cultivar Westar. The decline in detectable fungal DNA coincided with abundant sporulation, necrosis and onset of leaf senescence. *L. maculans* DNA could be detected after inoculation of the cultivar Glacier even though the lesion did not expand from the inoculated site. The assay is rapid, accurate and very sensitive, and can be incorporated into conventional disease screening programs.

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PCR AMPLIFICATION OF RIBOSOMAL DNA FOR SPECIES IDENTIFICATION OF *PHYTOPHTHORA*. J. B. Ristaino, G. Parra, M. Madritch, R. French, and D. Fraser. Dept. of Plant Pathology, North Carolina State University, Raleigh.

We have developed a PCR procedure to amplify *Phytophthora* DNA from infected tissue for quick species identification. This procedure involves amplification of the 5.8s ribosomal DNA gene (rDNA) and internal transcribed spacers (ITS) with ITS primers 5 and 4 and yields an amplified product approximately 946 bp in length. PCR amplification with primers ITS5 and ITS2, and ITS3 and ITS4 yield smaller amplified products approximately 363 bp and 612 bp in length, respectively. Restriction digest of the larger 946 bp product with Rsa I cut *P. infestans* rDNA into three smaller fragments. All tomato and potato isolates of *P. infestans* from North Carolina fields yielded the same unique PCR restriction pattern which was distinctive from *P. erythroseptica*. Restriction patterns from Rsa I digests of *P. nicotianae* and *P. megasperma* were unique, while Hae III digests separated *P. citrophthora* from *P. cryptogea*, and *P. cinnamomi* from *P. fragariae*. *P. citricola* and *P. capsici* patterns were similar with digests from both enzymes. This technique will provide a powerful tool for species identification in *Phytophthora*.

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MOST VIRULENT MYCOPARASITE OF LICHENS IS ALSO A POST HARVEST PATHOGEN OF CARROTS. **G. C. Adams and B. R. Kropp. Michigan State University, Department of Botany & Plant Pathology, East Lansing, Michigan 48824. Utah State University, Biology Department, Logan, Utah 84322.**

Homology of DNA sequence has provided genetic proof that the basidiomycete *Athelia arachnoidea* is the teleomorph of *Rhizoctonia carotae*. *A. arachnoidea* is a well known lichen parasite and *R. carotae* is a common cold-storage pathogen of carrot in the northern hemisphere. Connecting the two disparate literatures on the natural history of the fungus has provided new insights into the epidemiology of the carrot disease. Basidia and sclerotia are formed abundantly on senescent leaves of several common deciduous trees in autumn. These propagules may provide the inoculum on late harvested carrots.

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COLONY MORPHOLOGY DIVERSITY AMONG MGR586 DNA FINGERPRINT GROUPS OF THE RICE BLAST PATHOGEN, PYRICULARIA GRISEA. T. L. Harp and J. C. Correll. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

DNA fingerprinting has been used to characterize regional genetic diversity of the rice blast pathogen, Pyricularia grisea. Four distinct MGR586 DNA fingerprint groups (A, B, C and D) have been found in the contemporary (after 1992) blast pathogen population with multiple haplotypes (isolates with 1-20% fingerprint variation) within each of the four groups. The colony morphology and growth rate of 10-20 monoconidial isolates from each of the four groups were examined. Colony morphology and growth rates were determined on water agar after 12-21 days. The colony morphology was classified based upon the appearence of the mycelial pattern at the colony perimeter. Four colony morphologies generally have been observed: round-dense (RD), starred-feathery (SF), round-sparse (RS), and round-irregular (RI). There was a correspondence between colony morphology and DNA fingerprint group. Isolates in fingerprint groups A and C shared an RD morphology. Isolates in group B had an SF morphology while isolates in group D had either an RS or an RI morphology. Isolates in group A and C could be distinguished based on growth rate where isolates in group C had a faster growth rate on water agar.

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METHOD OF SEED SELECTION AND ASEPTIC CULTIVATION OF SEEDS FOR HYDROPONIC SYSTEMS. Oluwasanmi Areola, Fraline Castillo, Roy I. Konyeaso, Olufisayo A. Jejelowo,Department of Biology, Texas Southern University, Houston, Texas 77004 & Dan Barta, Johnson Space Center, Houston, Texas

Seeds of Peanut (*Arachis hypogea*) and spinach (*Spinacia oleracea*) were surface sterilized with sodium hypoochlorite and sown on potato dextrose agar. Seedlings which showed no contamination were selected and transferred into sterile plant growth containers. The plant containers were specially designed to enable the study of host-parasite interactions within an aseptic hydroponic environment. This poster describes the set up for plant cultivation and the sterilization techniques employed. The results of the experiments comparing growth under aseptic conditions with growth in the open are also discussed.

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A QUEST FOR THE BINDING SITE OF A BACTERIAL HR ELICITOR, HARPIN$_{Pss}$. M. E. Hoyos, C. M. Stanley, * S. Y. He, S. Pike , and A. Novacky, University of Missouri, Columbia, MO, * University of Kentucky, North Lexington, KY.

Harpin$_{Pss}$, the elicitor of bacteria-induced HR isolated from *P. s.* pv. *syringae*, causes K^+ efflux and extracellular alkalinization in tobacco suspension cultured cells (SCC). Living, fixed and permeabilized tobacco SCC and protoplasts were treated with harpin$_{Pss}$, anti harpin antibody, and Cy 5-tagged secondary antibody in a confocal laser microscopy search for the harpin binding site. Control SCC and protoplasts treated with primary and secondary antibodies, but not harpin, did not fluoresce. Only the outer part of the cells fluoresced when tobacco SCC were treated with harpin and both antibodies. No signal was observed in the interior of fixed and permeabilized cells indicating that the harpin molecule was not internalized. Protoplasts treated with harpin and antibodies did not fluoresce. Tobacco leaves were inoculated with *P. s.* pv. *syringae*, the HR-inducing bacterium from which harpin$_{Pss}$ was isolated. Sections were cut, treated with harpin$_{Pss}$ antibody and gold conjugated secondary antibody, and observed with transmission electron microscopy. Gold labeling was localized mostly in the apoplast 8 h after inoculation. These results suggest that harpin$_{Pss}$ interacts with the plant cell wall. (Supported by USDA grant)

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THE ROLE OF THE CELL WALL IN BACTERIAL HR INDUCTION. M. E. Hoyos, S. Pike, and A. Novacky, University of Missouri, Columbia, MO.

HR induction is associated with pH changes in the extracellular medium, K^+ efflux, and cell death. Alkalinization of the extracellular medium is often used as an assay for HR in suspension cultured cells (SCC). The role of the plant cell wall (CW) in HR induction has not been yet determined. We used tobacco SCC and protoplasts isolated from tobacco SCC to address this question. Both SCC and protoplasts were treated with *P. s.* pv. *syringe* (Pss) and harpin$_{Pss}$, the HR elicitor isolated from Pss. Changes in pH in the extracellular medium were measured hourly. Different media were tested and one was selected that satisfied two basic requirements: 1) protoplast viability for 3 days and 2) alkalinization in the external medium of tobacco SCC in response to both bacteria (by 4 h after treatment) and harpin$_{Pss}$ (by 1 h after treatment). Alkalinization was first observed 1h after protoplasts were inoculated with Pss, and it continuously increased for the remainder of the experiment (6 h). No pH change was detected in the medium of protoplasts treated with harpin$_{Pss}$ during this time. These results suggest that bacterial products other than harpin contribute to the pH changes in protoplasts treated with Pss and that receptors or factors necessary for the action of harpin$_{Pss}$ are removed with the CW. (Supported by USDA grant)

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DOUBLE-STRANDED RNAS IN THREE POPULATIONS OF *CRYPHONECTRIA PARASITICA* AND THEIR RELATIONSHIP WITH BRANCH RECOVERY. Anita L. Davelos, Jennifer K. Schaupp, Dennis W. Fulbright and Andrew M. Jarosz. Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824-1312.

Recovery of American chestnuts from chestnut blight is thought to be associated with the presence of double-stranded (ds) RNAs in the pathogen, *Cryphonectria parasitica*. We sampled 150 trees at each of three sites in Michigan (County Line, Grand Haven, and Roscommon) to determine if the proportion of recovering trees differed among sites and if recovery was associated with the presence of dsRNAs. A significantly smaller proportion of recovering trees was found at Roscommon (45% versus 69% for Grand Haven and 79% for County Line). The Roscommon site also had a significantly smaller proportion of cankers containing dsRNA in the isolates of the fungal pathogen. However, dsRNA was found in cankers of both recovering and dying branches with equal frequency at all sites. The diversity of dsRNA types based on banding patterns differed across sites with Grand Haven having the greatest diversity and Roscommon having the least. The mere presence of dsRNA does not seem to be sufficient for recovery of the American chestnut.

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PROTEASE ACTIVITY IN THE VASCULAR WILT FUNGUS VERTICILLIUM DAHLIAE. K.F. Dobinson, N. Lecomte, G. Lazarovits, Agriculture and Agri-Food Canada, Pest Management Research Centre, London ON N5V 4T3.

When grown in liquid cultures containing skim milk, strains of *Verticillium dahliae* and *V. nigrescens* produced protease activity that hydrolyzed the chromogenic substrate azocasein. Both race 1 and race 2 *V. dahliae* tomato isolates tested produced the protease, as did isolates from potato, strawberry and Japanese Maple, as well as a *V. nigrescens* strain. All isolates were pathogenic on eggplant and tomato, with the exception of the Japanese Maple isolate, which did not cause disease on tomato, and the *V. nigrescens* strain, which was not pathogenic on either host. Production of protease is therefore not a determinant of host range. Nonpathogenic isolates colonized the roots of both eggplant and tomato to some extent. A protease-deficient mutant, generated by transformation of a race 1 *V. dahliae* strain, was no longer pathogenic on tomato and eggplant. To determine if loss of pathogenicity and protease activity cosegregate, parasexual genetic crosses are being carried out between complementary nitrate non-utilizing derivatives of the wild-type strain and protease deficient mutant. Purification and characterization of the protease activity are also in progress.

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VIRULENCE AND POLYMORPHIC DNA RELATIONSHIPS OF *PUCCINIA STRIIFORMIS* F. SP. *HORDEI* TO RUSTS OF BARLEY, WHEAT, AND BLUEGRASS IN THE USA. X. M. Chen, R. F. Line, and H. Leung. Dept. of Plant Pathol. and USDA-ARS, WSU, Pullman, WA 99164.

Relationships of *Puccinia striiformis* f. sp. *hordei* to *P. s. tritici*, *P. s. poae*, *P. hordei*, *P. recondita* f. sp. *tritici*, and *P. graminis* f. sp. *tritici* in the USA were determined by virulence and random amplified polymorphic DNA (RAPD) analyses. Isolates of *P. s. hordei* were virulent on some wheat cultivars and of *P. s. tritici* were virulent on some barley cultivars; *P. s. hordei* was avirulent on most wheat cultivars and *P. s. tritici* was avirulent on most barley cultivars. *Puccinia s. hordei* and *P. s. tritici* did not infect bluegrass and *P. s. poae* did not infect barley or wheat. Fourteen races of *P. s. hordei* were detected using 11 barley differential cultivars. RAPD analyses separated the isolates of *P. s. hordei*, *P. s. tritici* and *P. s. poae*. *Puccinia s. hordei* and *P. s. tritici* were more closely related to each other than they were to *P. s. poae*. The formae speciales of *P. striiformis* were not closely related to *P. hordei*, *P. r. tritici*, or *P. g. tritici*.

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TRANSIENT INDUCTION OF SORGHUM PATHOGENICITY-RELATED PROTEINS AND A LEUCINE-RICH PROTEIN AS A HOST RESPONSE TO FUNGAL PENETRATION

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[1]Department of Botany and Plant Pathology, and [2]Department of Horticulture, Purdue University, West Lafayette, IN 47907

Through differential screening, three nearly full-length cDNAs from a sorghum library synthesized from mesocotyl tissues 24h after infection with *Bipolaris maydis* race O were identified. Sequence data of two of these cDNAs revealed homology to Pr-1 and Pr-5 (thaumatin-like) anti-microbial proteins, respectively. The sequence of a third cDNA (designated S-LRR) was found to have no significant homology to any previously identified gene. However, translation to a putative peptide revealed homology to protein kinase receptor proteins and the leucine-rich repeat domain of the *Cf 9* and *RPS 2* resistance genes. Leucine-rich repeats are believed to be the site of protein-protein interactions. Results from RNA gel-blot analyses show that mRNA from each of these genes begins to accumulate in both mesocotyls and juvenile leaves at 6h post-inoculation. This accumulation is coincident with the formation of appressoria and attempted penetration of the fungus. Therefore, in addition to anti-microbial phytoalexins, juvenile sorghum seedlings rapidly accumulate putative anti-microbial proteins. Further, these data suggest that the synthesis of anti-microbial proteins and phytoalexins share common elements in transcriptional regulation through the signal transduction pathway.

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EFFECTS OF CAMALEXIN ON GERMINATION AND GERM TUBE GROWTH OF *ALTERNARIA SOLANI*. Olufisayo Jejelowo, Department of Biology, Texas Southern University, Houston, Texas 77004 & Raymond Hammerschmidt, Dept. of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Camalexin (the *Arabidopsis* phytoalexin) inhibited germ tube growth of *Alternaria solani*. EC_{50} at 8 micrograms per ml and the minimum inhibitory concentration (MIC) at 80 micrograms per ml. In presence of the phytoalexin, conidia often formed more germ tubes than in control and the germ tubes formed in presence of the phytoalexin appeared distorted. Addition of 50 micrograms per ml or higher concentration of phytoalexin resutled in swelling and bursting of hyphal tips. Leaves of *A. thaliana* (columbia) were highly resistant to *A. solani* and produced camalexin in response to infection by conidia of *A. solani*. Comparison of the rate of phytoalexin accumulation with the inhibitory concentration suggests that phytoalexins are involved in the resistantce of *Arabidopsis thaliana* to *Alternaria solani*.

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EFFECTIVENESS OF SODIUM HYPOCHLORITE AS A SURFACE STERILIZING AGENT. Fraline J. Castillo, Oluwasanmi Areola, Roy I. Konyeaso & Olufisayo Jejelowo. Department of Biology, Texas Southern University, Houston, Texas 77004

In this study, spinach seeds, *Spinacia Oleracea*, pretreated with *Pythium spp.*, *Alternaria spp.* and *Aspergillus spp.* were exposed to different concentrations of sodium hypochlorite (0.0071-0.71) M for various time intervals and then planted on petri dishes containing potato dextrose agar medium (PDA). After 6 days at 25/18 C day/night temp., about 100% of all seeds sterilized for 1 min. at concentrations lower than 0.142 M were still contaminated. There was a steady decrease in contamination with time of exposure to surface sterilization agent and for all concentration increases. The observed time for total elimination of contamination for various NaClO concentrations were; 4 min. for 0.71M: 5 min. for 0.568 M: 6 min. for 0.426 M: 7 min. for 0.355 M: 6 min for 0.248 M: 6 min for 0.142 M: 6 min for 0.071 M. For a concentration of 0.0071 M, contamination was still observed after 18 min. in about 30% of the seeds.

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HETEROGENEITY IN B COMPONENT SEQUENCES FROM GEMINI-VIRUS(ES) INFECTING PEPPER IN EGYPT. A. M. Abouzid[1], H. H. Ahmed[2], J. E. Polston[3] and E. Hiebert[3]. [3]Plant Pathology Dept.,Univ. of Florida, Gainesville, FL 32611, [3]GCREC, Bradenton, FL 34203 , and [2]Botany Dept.,University of Suez Canal, Ismaillia, Egypt.

A new disease of pepper (*Capsicum annuum* L) was observed in northeastern Egypt during 1988. Pepper cultivars were severely affected by systemic leaf curl and distortion symptoms, and the disease was associated with high populations of the whitefly, *Bemisia tabaci*. Total DNA extracted from infected plants hybridized weakly in dot blots with a TYLCV DNA probe. Segments of DNA-A (PALiv1978-PAR1c496, ~1.1 kb) and of DNA-B (PBL1v 2040-PCRc1, ~ 0.4-0.6 kb) were amplified by PCR and cloned from infected pepper. The nucleotide sequences of both DNA-A and DNA- B segments were compared with sequences from similar regions for the previously sequenced geminiviruses. The intergenic region of DNA-A, which is unique for distinct geminiviruses, showed the highest sequence similarity (74%) to the squash leaf curl virus. Surprisingly, three different DNA-Bs (~ 0.4, 0.46 and 0.6 kb) were amplified by PCR. The hypervariable region sequences of three B component clones showed a sequence similarity ranging from 35-60% to other geminiviruses. The variability in B component sequences of the pepper geminivirus may provide information regarding the adaptability of geminiviruses during infection in a new host.

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ANALYSIS OF RESISTANCE TO EASTERN FILBERT BLIGHT IN SELECTED HAZELNUT CULTIVARS. N.K. Osterbauer[1], T.L. Sawyer[1], S.A. Mehlenbacher[2], and K.B. Johnson[1]. [1]Dept. of Botany & Plant Pathology and [2]Dept. of Horticulture, Oregon State University, Corvallis 97331.

Inheritance of resistance to eastern filbert blight, caused by *Anisogramma anomala*, was studied in *Corylus avellana* seedlings. Seven parent cultivars were crossed in 12 combinations. In the field, new shoots of 1-yr-old trees were inoculated with ascospores in the spring of 1992. After a cold dormancy, the proportion of wood diseased was measured. Offspring of the parent VR6-28, which is heterozygous for a single dominant resistance gene, segregated 1:1 for resistance. Offspring of the remaining parents were analyzed for general (GCA) and specific (SCA) combining ability. Based on GCA values, `Gem' and `Tonda di Giffoni' were superior pollen parents whereas `Willamette' and `Casina' were superior nut parents for transmission of resistance. `Tonda Gentile delle Langhe' and `Ennis' were poor pollen and nut parents, respectively. Both GCA and SCA were significant in this experiment. This is in contrast to results from a previous study (Phytopath. 84:1117).

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RESISTANCE OF SOME WILD AND CULTIVATED ACCESSIONS OF ERUCA AGAINST RACES 2 AND 7 of ALBUGO CANDIDA. V. K. Bansal[1], J. P. Tewari[1], I. Tewari[1], C. Gómez-Campo[2], and G. R. Stringam[1], [1]Dept. of Agric., Food, and Nut. Sci., Univ. of Alberta, Edmonton, AB., Canada, T6G 2P5, [2]Dep. Biologia Vegetal, ETS de Ingenieros Agrónomos, Universidad Politecnica, 28040 Madrid, Spain.

Eleven accessions of Eruca were screened against Albugo candida (Pers. ex Lev.) Ktze. race 2 (from Brassica juncea L.) and race 7 (from B. rapa L.) using the cotyledon inoculation method. All accessions were resistant to race 2. All wild and one cultivated genotype were found to be resistant to race 7. The remaining five cultivated genotypes had 8% to 50% disease incidence (DI) but the disease severity was low in all cases. To estimate DI, only plants with white rust pustules were counted and plants with necrotic or chlorotic flecks were considered resistant. These results suggest that race 7 has some virulence against the cultivated genotypes. Therefore, Eruca genotypes may be useful sources of resistance to race 2 and have only limited value for resistance to race 7. Since Eruca and Brassica are close phylogenetically and are known to produce somatic and sexual hybrids, introgression of white rust resistance from Eruca to Brassica may be possible.

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GREENHOUSE TECHNIQUE FOR DETECTION OF PHYSIOLOGICAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN SOYBEAN. B. W. Pennypacker and O.E. Hatley, Agronomy Dept., Penn State, University Park ,16802.

Physiological resistance to *Sclerotinia sclerotiorum* is difficult to assess in the field due to confounding effects of phenology and plant architecture. Limited-term inoculation (Plt. Dis. 67:784-786) was modified to allow rapid greenhouse screening of germplasm. Plants grown in pots on a bench with a solenoid-controlled mist system were inoculated at growth stage V2-V3 by placing a pathogen-infested 5 mm wedge of carrot root in the axil of the first trifoliolate leaf. Inoculum was removed after 48 hr. Plants were misted 1 min every 20 min for the first 24 hr, and then 1 min every 30 min . Physiological resistance was assessed 5 da after inoculation. Ambient light was supplemented with metal-halide lamps and daylength was 12 hr. Resistant plants showed a small, reddish brown, hypersensitive lesion at the inoculation site; the stems of all other plants were collapsed. The screening method required a minimum of plant handling and clearly differentiated plants possessing physiological resistance to *Sclerotinia sclerotiorum.*

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GERMPLASM OF *OCIMUM BASILICUM* RESISTANT TO *FUSARIUM OXYSPORUM.*
R. REUVENI, N. DUDAI and E. PUTIEVSKY, *ARO,* Newe Ya'ar Research Center, P.O.Box 90000, Haifa 31900, Israel

Fusarium oxysporum in sweet basil (*Ocimum basilicum* L.) causes stunting of the plants, browning of vascular tissue, dark longitudinal streaks on stems, severe wilting and defoliation. Two stem-originated isolates of *F. oxysporum* were pathogenic to basil but not to 9 species of *Labiatae, Cucurbitaceae, Solanaceae* and *Compositae*, indicating that the causal organism is *F. oxysporum* f.sp. *basilicum*. These isolates were used for additional resistance tests. Resistant germplasm was identified in several basil plants of a local cultivar which was introduced from USA and adjusted at Newe Ya'ar for local environmental and agronomic needs. Seeds were planted in the greenhouse in highly naturally-infested soil. Symptomless plants were selected for self breeding as a source for seeds of resistant germplasm which was identified by artificial inoculations with both isolates of the pathogen. Further selection tests to improve resistance were conducted in the greenhouse on infested soil up to F4. All individuals of the present genetic line were symptomless while all individual plants of the susceptible cultivar defoliated three weeks after planting in the infested soil. As this genetic line demonstrates resistance against *F. oxysporum*, and has a similar composition of essential oil, further crosses will be made to introduce this resistance into other germplasms of sweet basil of high commercial and agronomic value.

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PROBABLE SOURCE OF SEEDLING RESISTANCE TO WHEAT LEAF RUST IN 'THATCHER'. A.N. Mishra and A.P. Roelfs. Department of Plant Pathology and USDA-ARS Cereal Rust Lab, University of Minnesota, St. Paul, MN 55108

Bread wheat cultivar Thatcher has been documented to carry *Lr22b* alone, expressed only in the adult plants. Thatcher is considered "universal suscept" in the seedling stage to leaf rust of bread wheat. However, Thatcher was recently shown to be seedling resistant to many Ethiopian durum wheat leaf rust isolates. Thatcher was developed from an interspecific double cross involving an F1 from two bread wheats Marquis and Kanred, and the one from Marquis and Iumillo durum wheat. It was not clear if seedling resistance in Thatcher was derived from its durum parent or from one of the bread wheats. Our seedling tests of Thatcher and its parental lines with 20 Ethiopian durum leaf rust isolates indicate that the source of seedling resistance in Thatcher is probably Kanred. The low infection types (ITs) on Thatcher and Kanred were identical whereas significantly higher ITs were produced on Marquis and Iumillo when tested with the same isolates. Conversely, isolates giving high ITs on Thatcher and Kanred produced lower ITs on Marquis and Iumillo. Tests with more number of isolates and the genetic studies are in progress to confirm these observations.

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STRESS INDUCED NEGATION OF DURABLE SINGLE-GENE RESISTANCE TO BLACK ROOT ROT OF TOBACCO. M.E. Hood and H.D. Shew, Department of Plant Pathology, North Carolina State University, Raleigh 27695.

Single-gene resistance to black root rot (*Thielaviopsis basicola*) derived from *Nicotiana debneyi* is considered complete and has been durable since its introduction in the 1960's. Effects of stress factors on expression of resistance and disease development were examined in two burley tobacco cultivars of *Nicotiana tabacum* with the *N. debneyi* resistance gene (Tennessee 90; Kentucky 15). Under normal growing conditions, few or no lesions develop on the roots of these cultivars, lesion expansion is acutely limited, and viable secondary inoculum is not produced. The ability of *T. basicola* to colonize and reproduce on root tissue of resistant cultivars increased significantly when the host was nutrient deficient or when foliar damage occurred. Other root pathogens also were examined as stress factors that could affect pathogenesis of *T. basicola* on resistant cultivars.

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FOLIAR SPRAYS OF PHOSPHATES INHIBIT POWDERY MILDEW DEVELOPMENT IN FIELD-GROWN NECTARINE AND APPLE TREES.
M. Reuveni, V. Agapov, and R. Reuveni, Golan Res. Inst., Univ. of Haifa, P.O.Box 97, Kazrine 12900, ARO, Div. of Plant Pathology, Newe Ya'ar Res. Cent., Haifa 31900, Israel

Foliar sprays of 0.4-1.0% solutions of KH_2PO_4 and commercial systemic fungicides inhibited development of powdery mildew fungi on fruits and leaves of field-grown nectarine (*Sphaerotheca pannosa*) and apple (*Podosphaera leucotricha*) trees. The fungicides-based treatments were more effective in controlling the disease than the phosphate. Alternating treatments of phosphate salt with an appropiate systemic fungicide in both crops, however, enhanced the inhibitory effect against the mildew and was similar to that of the commercial treatment with systemic fungicides. Phosphate solutions were not phytotoxic to plant tissue. Fruits harvested from nectarine trees sprayed with phosphate alone or in alternation with fungicides were similar in their size distribution to those harvested from the commercial fungicides-based treatment and larger than those obtained from control non-treated trees. A significant increase in β-1,3-glucanase and peroxidase activity was detected in infected leaves as compared with non-infected ones. The inhibitory effectiveness of phosphate salts makes them useful "biocompatible" fungicides for application in the field for disease control.

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REDUCTION OF TOMATO EARLY BLIGHT BY COMBINING SOIL SOLARIZATION AND BIOLOGICAL CONTROL STRATEGIES
C. Stevens[1], V. A. Khan[1], L. D. Ploper[2], P. Backman[2], R. Rodriguez-Kabana[2], D. J. Collins[2], J. E Brown[2], and M. A. Wilson[4]. [1]Tuskegee University, Tuskegee Inst. AL. 36088, [2] Dept. Plant Pathology, Auburn University AL 36849, [3]Dept. of Hort. Auburn University, AL. 36849, [4]Dept. of Agri. Cape Girardeau Southeast Missouri Univ. MO 63701

Combinations of solarized soil (SBS), bare soil control (BS), black plastic mulched soil (BM), row cover (RC), fungicide (chlorothalonil) and biological treatments (Bacillus cereus) were evaluated. SBS vs BS treatments were main plots, mulch and row covers splitplots and foliage treatments split-splitplots. Application of either foliar treatment was superior to BS. Using a 1/2 rate of fungicide on plants from solarized soil treatments showed equal or comparable reduction of the disease when compared to tomatoes grown in BS with high rates of the fungicide. Combined treatments of solarized + BM, BM with or without RC and low rate of fungicide or biological agent, were the most effective when compared to BS + fungicide, indicating that integration of plasticulture and biological strategies can reduce early blight below the levels of commercial fungicide applied to tomatoes grown on BS.

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THE PHYTOTOXICITY OF VARIOUS FUSARIUM SPECIES TO WEED AND CROP SPECIES. H.K. Abbas, C.M. Ocamb and B.J. Johnson. USDA-ARS, SWSL, Stoneville, MS; and USDA, Forest Service, St. Paul, MN.

Fifty-two Fusarium cultures: *F. oxysporum* (9), *F. oxysporum* var. *redolens* (7), *F. moniliforme* (4), *F. polyphialidicium* (20), *F. proliferatum* (6), *F. solani* (3), *F. tricinctum* (2) and *F. equiseti* (1), were isolated from soil, crops, white pine, and weeds. A homogenate of each isolate was sprayed on 12 varieties of 1-2 wk old plant species. Apical chlorosis on corn, sunflower, wheat, black nightshade, jimsonweed, and morningglory was associated with some isolates of *F. polyphialidicum*. Necrosis and stunting were caused by several taxa: *F. proliferatum* (83% of the isolates tested), *F. oxysporum* var. *redolens* (75%), *F. solani* (33%), and *F. tricinctum* on cotton, sunflower, black nightshade, common cocklebur, hemp sesbania, jimsonweed, prickly sida, sicklepod, and velvetleaf. Mortality was asociated with *F. moniliforme, F. proliferatum* (67%), *F. oxysporum* var *redolens* (43%) and *F. solani* (33%) on black nightshade, jimsonweed, prickly sida and sicklepod. *F. polyphialidicum* inoculations resulted in unique apical chlorosis. This is the first report of *F. oxysporum* var. *redolens* causing necrosis and mortality of these weed and crop species.

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USE OF *ACREMONIUM DYOSPYRI* TO CONTROL PERSIMMON TREES IN PASTURES. N. A. Tisserat. Kansas State University, Manhattan 66506-5502.

Persimmon trees(*Diospyros virginiana*) are invasive weeds in southeastern Kansas pastures. The trees sucker from roots to form large (30 m-diameter), clonal groves that are not easily eradicated by conventional herbicides. The vascular pathogen *Acremonium dyospyri* was tested for use as a mycoherbicide. A total of 60 trees in three groves was inoculated in June 1994 by wounding trunks near the soil line with a hatchet, then applying 5-10 ml of a 10^6 spores/ml suspension of *A. dyospyri*. By August, 47 trees had wilt symptoms and of these, 10 had died. To determine the rate of disease progression, four trees, equally spaced around the perimeter of each of three groves (50 to 129 stems), were inoculated in June. All inoculated trees and four adjacent, non-inoculated trees developed wilt symptoms by August. Preliminary results indicate that *A. dyospyri* is a relatively fast-acting, effective silvicide. Wilting apparently progresses from tree stem to stem within the grove by systemic fungal movement through a common root system. Therefore, it does not appear that all stems in the grove need to be inoculated for effective control.

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THE DIFFERENTIATION OF *CHONDROSTEREUM PURPUREUM* ISOLATES BY CYCLOHEXIMIDE SENSITIVITY AND L-DOPA ACTIVITY. S.F. Shamoun[1], E.T. Sela[1], T.D. Ramsfield[1], and J.A. Micales[2], [1]Canadian Forest Service, Pacific Forestry Centre, 506 W. Burnside Rd, Victoria, B.C. V8Z 1M5, Canada and [2]Forest Products Lab., 1 Gifford Pinchot Dr, Madison, WI 53705, USA.

Chondrostereum purpureum is a wound pathogen of hardwood trees, and is also under evaluation as a mycoherbicide for unwanted brush species in forestry and utility rights-of-way. A biochemical test (L-DOPA staining) was used to determine the genetic identity. There was no formation of a zone of antagonism (or black line) between the confronting margins of heterokaryon Canadian isolates in the presence of L-DOPA. European and New Zealand isolates when paired with each other or with Canadian isolates produced a black line after similar treatment. Cycloheximide sensitivity was determined by radial growth on cycloheximide-amended malt agar. There was little intraspecific variation in sensitivity among Canadian, European and New Zealand isolates (LD_{50} 0.85-1.50). These biochemical data indicate that *C. purpureum* isolates share common metabolic pathways. Such genetic uniformity is crucial for the registration of *C. purpureum* as a mycoherbicide.

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EVALUATION OF PATHOGENICITY OF SELECTED GLYPHOSATE TOLERANT STRAINS (*FUSARIUM OXYSPORUM).* Tiourabaev, K.S.[1], Schultz, M.T.[2], McCarthy, M.K.[2], Anderson, T.W.[2], Weaver, M.B.[2], Morgan, C.T.[2], and Sands, D.C.[2] [1]Institute of Zoology, Academy of Science of Kazakhstan, Almaty, Kazakhstan. [2]Biological Control of Weeds, Department of Plant Pathology, Montana State University, Bozeman, MT.

The virulence of glyphosate-tolerant mutants of *F. oxysporum* was compared with the virulence of the wild-type culture on host and non-host plants in the presence and in the absence of glyphosate. The growth of wild-type *F. oxysporum* was restricted in media supplemented with as little as 5 mM glyphosphate. Pathogenic strains of *F. oxysporum* were exposed to increasing concentrations of glyphosphate in minimal media (5 mM, 10 mM, 15 mM, 20 mM, 25 mM). Single colonies were considered tolerant if they grew on media with $\geq$ 10 mM. Plants were inoculated with wild-type or tolerant strains with and without glyphosate cotreatment.

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COMPARISON OF *FUSARIUM* SPP. FOR THEIR ABILITY TO PRODUCE INHIBITORY SUBSTANCES ON GROWTH OF *LEMNA MINOR* L. Ronald F. Vesonder, Mycotoxin Research, National Center for Agricultural Utilization Research, USDA/ARS, 1815 N. University Street, Peoria, IL 61604.

Fusarium spp. from the sections *Liseola* [*F. moniliforme* (*FM*), *F. subglutinans* (*FS*), *F. proliferatum* (*FP*) from the host corn], Martiella and Ventricosum [4-*F. solani* (*FSo*) from the hosts soybeans and potatoes] and Elegans (*F. oxysporium* (*FO*) host unknown] were each grown on rice and examined for production of substances inhibitory (IS) to the duckweed *Lemna minor* L (*LM*). Aqueous methanol extracts of rice culture material (RCM) obtained from each *Fusarium* sp. contained IS to LM. The RCM extracts were chromatographed by reverse-phase C_{18} column chromatography. LM bioassay directed-fractionation led to the identification of fumonisins in *FM* and *FP* RCM, moniliformin in *FS*, *FP*, and *FSo* RCM, and fusaric acid in *FO* RCM. Several fractions from each of these species also contained IS other than the ones identified. IS were studied by spectroscopic methods, and evidence will be presented to show that one such metabolite is an isomer of fusaric acid and another has a molecular formula of $C_{30}H_{28}O_5$ and one is a substituted tetrahydrofuran. It is not known how these latter metabolites inhibit plant functions.

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USE OF THE POLYMERASE CHAIN REACTION TO INVESTIGATE THE COLONIZATION OF DYERS WOAD BY A SYSTEMIC RUST. Bradley R. Kropp, Karen Flint and Sherman V. Thomson, Department of Biology, Utah State University, Logan, Utah 84322

A systemic rust tentatively identified as *Puccinia thlaspeos* attacks the weed dyers woad (*Isatis tinctoria*). Dyers woad is normally biennial and overwinters after the first season as a rosette which, in the case of infected plants, is often asymptomatic. The distribution of the pathogen in an asymptomatic rosette was studied using PCR and detected in most of the leaves and in the roots of the plant. Infected plants which had bolted during the second season, were also sampled at different developmental stages. As expected, symptomatic parts of these plants were found to be colonized by the fungus. Rust was also consistently detected in asymptomatic branches, leaves and roots of the plants. The study is directed at the eventual develoment of *Puccinia thlaspeos* as a biocontrol system for dyers woad.

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EXPRESSION OF SINGLE-CHAIN ANTIBODY FRAGMENTS (ScFv) SPECIFIC FOR CORN STUNT SPIROPLASMA (CSS) IN *E. COLI*. Y.-D. Chen and T.-A. Chen. Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

Corn Stunt disease, caused by *Spiroplasma kunkelii*, is one of the important limiting factors for maize production in several countries. To date, no effective control measures are available. We have attempted to develop transgenic maize plants resistant to *S. kunkelii* by expressing anti-CSS antibody genes. A panel of monoclonal antibodies (MAbs) against CSS were produced. Total mRNA isolated from hybridoma cell lines producing MAbs with strong deformation ability to CSS was used for the first strand cDNA syntheses. The Variable heavy (V_H) and light chain (V_L) genes of the antibodies were amplified in two separate PCR reactions and joined together by a neutral linker DNA. These assembled single-chain DNA fragments were then ligated to a phagemid and expressed in *E. coli*. Expressed ScFv antibodies were screened by ELISA and their specificity was confirmed by western blot analyses. The genes encoding these antibodies were sequenced. Several positive ScFv antibodies were also selected to confirm their deformation ability to CSS, and at least one of them was shown to be effective. This particular gene has since been cloned into a plant expression vector and used for maize transformation.

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UNIVERSAL AMPLIFICATION AND ANALYSIS OF 16S rDNA FOR CLASSIFICATION AND IDENTIFICATION OF *CLAVIBACTER* SPP. AND SUBSP. IN *C. MICHIGANENSIS*. I.-M. Lee[1], D.E. Gundersen[1], B. Mogen[2], and R.E. Davis[1]. Molecular Plant Pathology Laboratory[1], USDA ARS, Beltsville, MD 20705 and Dept of Biology[2], University of Wisconsin, River Falls, WI 54022-5001.

Oligonucleotide primers for PCR were designed from a *Clavibacter* spp. 16S rRNA gene sequence and were employed for specific amplification of 16S rDNA from representative spp. and subsp. in the genus *Clavibacter*, gram-positive bacteria causing numerous plant diseases, including potato ring rot. A 16S rDNA fragment, approximately 1.5 kb, was amplified from each representative strain. The amplified 16S rDNA sequences were analyzed after digestion with restriction enzymes. Based on cluster analysis of the RFLP profiles members of the genus *Clavibacter* were differentiated into groups and subgroups consistent with established species and subspecies comprising the genus. Five subgroups corresponding to five subspecies, *C. michiganensis* subsp. *michiganensis*, *C. michiganensis* subsp. *nebraskensis*, *C. michiganensis* subsp. *sepedonicus*, *C. michiganensis* subsp. *tessellarius*, and *C. michiganensis* subsp. *insidiosus* clustered together and formed a major group. 16S rDNA represents a potential taxonomic tool that allows rapid and sensitive means for simultaneous detection and differentiation among members of *Clavibacter*.

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PHYTOPLASMA INFECTION: A BENEFICIAL FACTOR FOR PRODUCTION OF COMMERCIAL BRANCHING POINSETTIA CULTIVARS? I.-M. Lee[1], M. Tiffany[2], D.E. Gundersen[1], and M. Klopmeyer[2]. Molecular Plant Pathology Laboratory[1], USDA ARS, Beltsville, MD 20705 and Ball FloraPlant[2], West Chicago, IL 60185-2698.

A graft-transmissible branching agent has been known to be associated with commercial free-branching (self-branching) poinsettia cultivars. Thus far, attempts to identify the agent have failed. Because excessive branching is one of the characteristic symptoms induced by phytoplasma infections, phytoplasma etiology has been suspected. In the present study, direct- and nested-PCR using universal primer pairs designed for amplification of phytoplasma 16S rDNA were employed for detection of putative phytoplasma(s) that might be associated with branching poinsettia plants. Rooted cuttings of 21 commercial poinsettia cultivars, including non-branching ones, were tested for the presence of phytoplasma(s). Phytoplasma-specific 16S rDNA was amplified from each of all free-branching cultivars by direct- or nested-PCR. No PCR products were obtained from non-branching cultivars, e.g. Regal Velvet. Preliminary characterization of RFLP profiles of the phytoplasma 16S rDNA PCR products indicated that the phytoplasmas associated with all branching cultivars are very similar and are most closely related to 16S rRNA group III (peach X-disease and related phytoplasmas), subgroup III-E (type strain SP1, spirea stunt).

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DETECTION AND IDENTIFICATION OF A NEW PHYTOPLASMA ASSOCIATED WITH CHERRY LETHAL YELLOWS IN CHINA. I.-M. Lee[1], S. Zhu[2], D.E. Gundersen[1], C. Zhang[3], and A. Hadidi[2]. Molecular Plant Pathology Laboratory[1] and National Germplasm Resources Laboratory[2], USDA ARS, Beltsville, MD 20705 and Plant Quarantine Institute[3], Ministry of Agriculture, Beijing, P.R. China.

An outbreak of a lethal yellows disease on Chinese cherry (*Prunus pseudocerasus* Lindl.) was reported in 1989 in Sichuan province, China. The diseased cherry trees developed a diffused yellow discoloration of foliage in late spring, defoliated prematurely, and produced little or no fruit. Infected trees die in 3-4 years. Phytoplasma etiology was suspected on the basis of electron microscopy observation of diseased tissues and on the basis of the symptom remission resulting from tetracycline, but not penicillin, treatment. In the present study, a nested-PCR assay using two universal primer pairs designed for specific detection of all phytoplasmas was employed for detection of putative phytoplasma(s) present in these diseased cherry trees. Phytoplasma-specific PCR products (16S rDNA) were obtained and analyzed with restriction enzymes. Based on RFLP profiles of 16S rDNA the phytoplasma associated with diseased cherry was identified as a new subgroup of phytoplasma 16S rRNA group V (elm yellows and related phytoplasmas).

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EUROPEAN CORN BORER AS A VECTOR OF *FUSARIUM MONILIFORME* IN SYMPTOMATIC AND ASYMPTOMATIC INFECTION OF CORN KERNELS. E. A. Sobek and G. P. Munkvold. Dept. of Plant Pathology, Iowa State University, Ames, 50011.

Field and greenhouse experiments were conducted to assess the ability of the European corn borer (ECB) to vector *F. moniliforme* from corn plant surfaces to kernels. In field and greenhouse experiments, plant surfaces were sprayed with a spore suspension of *F. moniliforme* in the dough stage; treatments were ear wounding, ECB's placed in ear shoot axil, ECB's infested with *F. moniliforme*, and a non-treated control. Ears were evaluated at maturity for ECB damage, *F. moniliforme* symptoms, and asymptomatic infection. In the greenhouse, *F. moniliforme* strain EA-2 was used to spray plant surfaces and recovered strains was tested for vegetative compatibility with EA-2 using nitrate-nonutilizing mutants. ECB's significantly increased ear rot symptoms and asymptomatic infection compared to the wounded treatment. Strain EA-2 was detected on ECB's and in 28-38% and 4-8% of kernels in the ECB and wounded treatments, respectively. In the field, ECB's did not increase infection over the wounded treatment, except when ECBs were previously infested with *F. moniliforme*. Results indicated that ECBs can act as vectors, but ECB wounding may be just as important in increasing *F. moniliforme* infection.

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SALIVARY GLAND ULTRASTRUCTURE OF TWO SPIROPLASMA VECTORS, *CIRCULIFER TENELLUS* AND *DALBULUS MAIDIS*. Astri C. Wayadande, Ginger R. Baker* and Jacqueline Fletcher, Dept. of Plant Pathology and Dept. of Physiological Sciences,* Oklahoma State University, Stillwater, OK 74078.

The salivary glands of leafhoppers *C. tenellus* and *D. maidis* consist of symmetrically paired principle glands and smaller accessory glands. The principle glands of the two species differ in cell arrangement and cytology. *C. tenellus* principle glands consist of seven acinar cell types, all of which are binucleate with the most ventral cells arranged in a rosette. Six *D. maidis* principle gland cell types, also binucleate, are loosely arranged around the salivary duct. Each cell type is easily distinguished by unique staining and ultrastructure. Large canaliculi, salivary bodies, and endoplasmic reticulum ramify through the cytoplasm of most principle gland cells. Individual cells are surrounded by a featureless basal lamina. Extensive basal infolding of the apical plasmalemma is similar in all cells of both species. Paired accessory glands lie anterior to a cuticular process in *C. tenellus*, ventrally in *D. maidis*, and consist of a distal multi-celled lobe and proximal tubular section. Currently, our work is directed at locating the site of spiroplasma invasion of the salivary glands.

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NITROGEN AS AN INFUSION AND POLYMERIZATION MEDIUM FOR LR WHITE EMBEDDING OF WHOLE SMALL INSECTS. [1]K.F. Harris, [1]Z. Pesic-Van Esbroeck, and [2]J.E. Duffus. [1]Virus-Vector Research Laboratory, Texas A&M University, College Station, TX 77843-2475 and [2]USDA-ARS, Sugarbeet Production Research Unit, 1636 E. Alisal St., Salinas, CA 93905.

Oxygen-free embedding of whole whitefly virus vectors in LR White at moderate temperature preserves viral antigenicity while yielding high-quality specimens which can be serially sectioned for light and electron microscopy and immunocytochemistry. The protocol includes "infusing" the liquid resin with N_2, evacuating the N_2-infused resin, covering embeddings with film, and polymerizing in a nitrogen atmosphere. The technique ought to be equally adaptable to studying plant viruses in whole specimens of other arthropod vectors such as aphids, leafhoppers, planthoppers, thrips and mites.

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MORPHOLOGY OF THE WHITEFLY FEEDING APPARATUS RELATIVE TO NONCIRCULATIVE PLANT VIRUS TRANSMISSION. [1]Z. Pesic-Van Esbroeck, [1]K.F. Harris, and [2]J.E. Duffus. [1]Virus-Vector Research Laboratory, Texas A&M University, College Station, TX 77843-2475 and [2]USDA-ARS, Sugarbeet Production Research Unit, 1636 E. Alisal St., Salinas, CA 93905.

The comparative morphology of *Bemisia*'s feeding apparatus was studied by light microscopy and analyzed relative to known noncirculative virus-vector interactions. Similarities among whitefly, aphid and leafhopper feeding apparatuses suggest that whiteflies too are capable of ingestion-egestion behavior and that noncirculative whitefly-transmitted viruses are cuticula-borne: carried at specific sites on the cuticula lining the lumina of the maxillary food canal and cibarium (antecibarium, cibarial valve and postcibarium) of the feeding apparatus. It appears that virus carried at sites beyond the feeding apparatus in the pharynx of the foregut of homopteran vectors would not be available for inoculation by egestion.

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IMMUNOCYTOCHEMICAL LOCALIZATION OF SQUASH LEAF CURL VIRUS (SLCV) IN SQUASH AND THE SWEET POTATO WHITEFLY. [1]Z. Pesic-Van Esbroeck, [1]K.F. Harris, and [2]J.E. Duffus. [1]Virus-Vector Research Laboratory, Texas A&M University, College Station, TX 77843-2475 and [2]USDA-ARS, Sugarbeet Production Research Unit, 1636 E. Alisal St., Salinas, CA 93905.

Studies were conducted to localize SLCV, a circulative whitefly-borne geminivirus, in squash (*Cucurbita* sp.) and its vector *Bemisia tabaci* (Genn.) using light and transmission electron microscopic (TEM) immunolabeling procedures. Light microscopic examination of immunogold-silver stained squash leaf sections showed that SLCV is phloem-restricted. The latter was confirmed by TEM and immunogold labeling of virions in ultrathin leaf sections. Fibrillar rings, characteristic of geminiviruses, were observed in the nuclei of infected phloem parenchyma cells. Immunogold-TEM of viruliferous whiteflies indicates unique interactions between SLCV and *Bemisia*'s digestive and salivary systems.

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PCR-BASED DETECTION OF POTATO VIRUS Y (PVY) IN VIRULIFEROUS APHIDS. S.H. Hidayat[1], L.R. Kabrick[1], J.A. Wyman[1], and T.L. German[2]. Depts. [1]Entomology, and [2]Plant Pathology, University of Wisconsin, Madison, WI 53706.

A sensitive reverse transcription-polymerase chain reaction (RT-PCR) method was developed to detect PVY in aphids which transmit it in a non-persistent manner. DNA primers specific for PVY were constructed based on the nucleotide sequence of the coat protein. These primers were utilized for synthesis of cDNA and PCR amplification of a 500 bp fragment from extracts of viruliferous aphids. The amplification products were further identified by Southern hybridization with a DNA probe of the cloned PVY coat protein gene. Two aphid species, *Myzus persicae* and *Macrosiphum euphorbiae* , were examined for their ability to acquire the virus from infected plants. RT-PCR was able to amplify a specific viral fragment from groups as well as individual viruliferous aphids. Simultaneously, PVY transmission efficiency of *M. persicae* and *M. euphorbiae* was tested, to correlate the ability of each aphid species to acquire the virus with their ability to transmit it. The RT-PCR assay allows for sensitive detection of PVY in viruliferous aphids and provides a valuable tool to study the epidemiology of PVY as well as to study virus-vector relationships.

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RELATIONSHIPS AMONG THREE POTYVIRUSES AS DETERMINED BY IMMUNOGOLD LABELLING. R.Y. Wang, D.W. Thornbury and T.P. Pirone. Dept. of Plant Pathology, University of Kentucky, Lexington, KY 40546.

Relationships among tobacco vein mottling virus (TVMV), potato virus Y (PVY) and tobacco etch virus (TEV) were evaluated by immunogold labelling of virus particles using polyclonal antisera and leaf dip preparations from infected plants. Close immunological relationships, evidenced by uniform labelling of gold particles along the filamentous virus particles, were found between TVMV and PVY (using PVY antiserum against TVMV or TVMV antiserum against PVY) and between TVMV and TEV (using TVMV antiserum against TEV or TEV antiserum against TVMV). There was no difference in labelling by homologous or heterologous antisera. However, when PVY antiserum was tested against TEV, or TEV antiserum against PVY, only one end of the virus particles was labelled, demonstrating the presence of an epitope common to both and probably all three viruses. Treatment of purified TEV with PVY antiserum prevented aphid transmission of TEV, whereas treatment with PVY antiserum did not.

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WHITEFLY MORPHOLOGY (HOMOPTERA: ALEYRODIDAE) AND TOMATO MOTTLE GEMINIVIRUS. W.B. Hunter, E. Hiebert, S. Webb, J. Polston, and J. Tsai. University Florida, Gainesville.

Internal anatomy and morphology of the whitefly, Bemisia tabaci (Gennadius), was examined using light- and electron-microscopy. Whiteflies that successfully transmitted virus were frozen in buffer and subsequently fixed in 2% Paraformaldehyde, 1% glutaraldehyde, and embedded in LR White resin, for examination of virus. Location of geminivirus in tissues was elucidated by immuno-gold labeling and immuno-fluorescence techniques.

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DETECTION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS* BY PCR AND *IN SITU* HYBRIDIZATION. X. Li and S. H. De Boer, Agriculture Canada, Pacific Agriculture Research Centre, 6660 N.W. Marine Dr., Vancouver, B.C., Canada V6T 1X2.

Genomic DNA of *Clavibacter michiganensis* subsp. *sepedonicus*, the causal agent of bacterial ring rot of potato, was used to develop specific primers for the polymerase chain reaction (PCR) and an oligonucleotide probe for *in situ* hybridization. The 16S rRNA gene and adjacent intergenic spacer region of *C. m. sepedonicus*, *C. m. michiganensis*, *C. m. insidiosus*, *C. m. nebraskensis* and *C. m. tesselarius* were amplified and sequenced. An oligonucleotide probe and a pair of PCR primers specific for *C. m. sepedonicus* were selected on the basis of the sequence data. The probe, labelled with rhodamine dye, specifically hybridized to the rRNA of *C. m. sepedonicus* cells fixed on microscopic slides in *in situ* hybridization, but did not hybridize to strains of the other subspecies of *C. michiganensis*, *Rathayibacter* spp. and other bacteria. *C. m. sepedonicus* was identified simultaneously by *in situ* hybridization and immunofluorescence, using rhodamine and fluorescein, respectively. In PCR, the primers specifically amplified a 215 base pair fragment when *C. m. sepedonicus* genomic DNA was used as template. The sensitivity of PCR was 20-200 times greater than ELISA and immunofluorescence. Amplification products were obtained for all potato tuber samples that were positive by ELISA and immunofluorescence, while tubers from healthy plants were all negative by both serological tests and PCR.

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IDENTIFICATION OF *ERWINIA AMYLOVORA* BY PCR-ASSAYS. S. Bereswill & K. Geider. *Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-69120 Heidelberg*, Germany

Molecular identification of the fire blight pathogen by DNA hybridization was replaced with PCR-analysis. Primers were created from a 0.9 kb *Pst*I-fragment of the common plasmid pEA29. After cell lysis in the assay with Tween 20, down to 50 cells could be detected. The 0.9 kb band was obtained from strains isolated in various geographical regions and was also seen in the presence of other plant-associated bacteria. Independently from the plasmid pEA29, a chromosomal fragment from the *ams*-region (for *am*ylovoran *s*ynthesis) was used for amplification which produced a specific signal of 1.6 kb and was found for all *Erwinia amylovora* strains assayed, but not for other plant-associated bacteria. Additional information to identify *E. amylovora* was obtained by PCR based on the bacterial 16S rDNA and subsequent digestion of the amplification products with restriction enzyme *Hae*III. Finally, RP-PCR (Random Primer, RAPD PCR) produced a pattern of bands which was unique for *E. amylovora*.

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A QUANTITATIVE COLORMETRIC PCR ASSAY FOR THE DETECTION OF *STAGONOSPORA NODORUM* AND ITS COMPARISON TO ELISA. James Beck[1], Laurent Etienne[2], Andres Binder[2] and James Ligon[1], [1]CIBA Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, NC 27709, [2]Ciba-Geigy Limited, CH-4002 Basel, Switzerland.

We have previously reported on the development of a polymerase chain reaction (PCR)-based assay which detects the causal agent of glume blotch in wheat, *Stagonospora nodorum* (*Phytopathology*, in press). This assay is based on primers designed to the internal transcribed spacer (ITS) regions of *S. nodorum*. These *S.* nodorum-specific primers were integrated into the quantitative colormetric format described by Nikiforov *et al.* (PCR Methods and Applications 3:285-291). Results are presented here that compare the quantitative colormetric PCR assay to our exisiting ELISA for *S. nodorum*.

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ANTIBODIES RECOGNIZE A KARNAL BUNT (*Tilletia indica*) TELIOSPORE WALL GLYCOPROTEIN. D. G. Luster[1], M. R. Bonde[1], G. L. Peterson[1], M. A. Hack[1], and A. J. Russo[2]. [1]USDA-Agricultural Research Service, Bldg. 1301, Ft. Detrick, Frederick, MD 21702-5023; [2]Mount St. Mary's College, Emmitsburg, MD 21727.

We are developing immunofluorescence assays to identify teliospores of *Tilletia indica*, the causative agent of Karnal bunt of wheat. Proteins were phenol-extracted and precipitated from a saline-washed, 3000 *g* spore wall pellet of pulverized *T. indica* teliospores. Spore wall extracts were used to immunize two rabbits every two weeks for six weeks, and the IgG fraction from serum drawn at eight weeks post-immunization was purified by protein A/G affinity chromatography. Western blots of spore wall proteins probed with anti-*T. indica* spore wall IgG indicate that the antibodies recognize a 37-43 kDa protein(s) present in the *T. indica* 3000 *g* spore wall fraction that is absent or undetectable in the spore wall of *T. barclayana*, a closely related rice kernel smut. The mannose-specific lectin, concanavalin A, also binds to the 37-43 kDa protein(s) on Western blots. Cleavage of N-linked mannose from *T. indica* spore wall proteins with endoglycosidase H produced a new, immunoreactive 28 kDa deglycosylated protein not detected in endoglycosidase H-digested *T. barclayana* teliospore wall proteins. These results suggest that the *T. indica* 37-43 kDa spore wall protein(s) is a glycoconjugate with varying amounts of asparagine-linked mannose. Experiments are in progress to determine the chemical identity and specificity of the epitopes recognized by the antibodies.

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DEVELOPMENT OF MONOCLONAL ANTIBODIES RECOGNIZING DIFFERENT EPITOPES OF THE RICE BLAST PATHOGEN, *PYRICULARIA GRISEA*. N. Ramakrishna[1], F.N. Lee[2] and R.C. Gergerich[1]. [1]University of Arkansas, Fayetteville, AR 72701, and [2]University of Arkansas Rice Research and Extension Center, Stuttgart, AR 72160.

Hybridoma cell lines producing monoclonal antibodies recognizing different epitopes of *Pyricularia grisea* have been developed by four fusions of myeloma cell line NS-1 with spleen cells from Balb/c female mice immunized with cell wall, extracellular or intracellular fungal antigens. Extensive screening of over 1600 hybridomas simultaneously against seven different antigens of *P. grisea* led to the development of 145 monoclonal antibodies of varying affinities, specificities and sensitivities that were characterized as: (a) recognizing only rice blast isolates of *P. grisea*, (b) recognizing both rice-infecting and grass-infecting isolates of *P. grisea* only, and (c) recognizing both rice- and grass-infecting *P. grisea* as well as a few other fungal species associated with rice. Monoclonal antibodies recognizing either protein or carbohydrate epitopes were identified.

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SENSITIVITY OF THE PARTICLE CAPTURE IMMUNOASSAY IN EARLY DETECTION OF BROWN-ROT FUNGI. C.A.Clausen, L.Ferge, and T.L.Highley, Forest Products Laboratory, One Gifford Pinchot Dr., Madison,WI 53705-2398.

Sensitivity of the particle capture immunoassay (PCI) was evaluated using a scaled-up modification of the soil block test. Southern pine 2X4's, 34" long were inoculated on one end with the brown-rot fungus, Postia placenta, and tested for brown-rot decay using PCI. Hyphal growth was visible 5-7" from the site of inoculation, but fungal antigens were detected by the PCI over the entire length of the test unit. The PCI uses antibody-coated latex particles to capture extracellular hemicellulases which are secreted by brown-rot fungi. High moisture content apparently enhances movement of fungal enzymes ahead of the hyphal growth, enabling the PCI to detect the presence of brown-rot antigens in culture-negative samples. PCI is a sensitive method for a detection of incipient decay.

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DETECTION OF POTATO SPINDLE VIROID FROM INFECTED TISSUE USING POLYMERASE CHAIN REACTION TECHNOLOGY. A.M. Shamloul, A. Hadidi and [1] R.P. Singh. National Germplasm Resources Laboratory, ARS-USDA, Beltsville, MD 20705;[1] Agriculture and Agri-Food Canada Research Center, Fredericton, NB, Canada E3B YZ7

DNA primers specific for potato spindle tuber viroid (PSTVd) were constructed based on the nucleotide sequence of the upper central conserved region and its adjacent segment. The antisense primer was complementary to (nt 69-88) and the sense primer was homologous to (nt 89-113). primers were utilized for RT-PCR amplification of a full length PSTVd cDNA (359 bp) from GeneReleaser™ treated clarified sap or total nucleic acids obtained from PSTVd-infected potato tubers (eyes, periderm, cortical zone or pith), true seeds, pollen and leaves. PSTVd cDNA was amplified from as little as 10 pg of infected total nucleic acids. No amplification was obtained from uninfected tissue. PSTVd cDNA from the severe strain of PSTVd had a slower electrophoretic mobility than that of the mild strain. PSTVd cDNA hybridized to a PSTVd cRNA probe. Our RT-PCR assays indicate that PSTVd can be directly detected from infected tubers or seeds without the need of their germination as required by other known detection methods.

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AN ON-SITE ELISA FOR DETECTION OF TOMATO SPOTTED WILT VIRUS AND IMPATIENS NECROTIC SPOT VIRUS. J. Q. Xia, C. Sutula, and D. Marti. Research Dept., Agdia, Inc., Elkhart, IN 46514.

A simple, sensitive test based on double-antibody sandwich (DAS) ELISA has been developed to simultaneously detect two important tospoviruses, tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV). The test can be performed by persons without special training or laboratory equipment. Up to six plant samples to be tested are placed in plastic pouches containing premeasured sample extract buffer, then macerated by rubbing each pouch with a pen. Samples are transferred into wells of an ELISA strip with straws, antibody-enzyme conjugate is added, and the strip is incubated for two hours. After washing with tap water, substrate is added to develop color. A positive control and a buffer well are provided. The test can be completed in three hours and has the same sensitivity as regular DAS ELISA.

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THE 3'-TERMINAL NUCLEOTIDE SEQUENCE OF A CALADIUM ISOLATE OF DASHEEN MOSAIC VIRUS (DSMV-CH). R. H. Li, F. W. Zettler and E. Hiebert, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

The sequence of the 3'-terminal 3159 nucleotides of the DsMV-Ch genome (Li et al., 1994, Phytopathol. 84:1105) was determined. The region contains the nucleotide sequence which encodes the carboxyl terminus of the NIa protease , the NIb RNA polymerase, and the coat protein. The genomic organization of this region is similar to those of other potyviruses. The large ORF encoding the polyprotein is followed by a 249 nucleotide non-coding region and a poly(A) tail. Two consensus NIa cleavage sequences [V-x-x-Q/G(A)] were found in the polyprotein. The overall nucleotide sequence homology of the coding region (the 3'-terminus of the NIa, the NIb, and the CP) of DsMV-Ch compared with those of other sequenced potyviruses is between 57-68%, and the amino acid sequence homology is between 70-82%. Phylogenetic alignment of the genomic sequences indicates that DsMV is a member of the bean common mosaic virus subgroup in the Potyviridae.

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EFFECT OF LEAF WETNESS AND SHADE ON SURVIVAL OF *Xanthomonas campestris* pv. *citrumelo* ON CITRUS LEAVES. J.H. Graham, Citrus Research and Education Center, Univ. Florida, Lake Alfred 33850; T.R. Gottwald and T.D. Riley, USDA-ARS, Orlando, FL 32803

Survival of splash-dispersed *X.c.* pv. *citrumelo* (Xcc strain F1) was evaluated on leaves of Swingle citrumelo trap plants placed within a grid and below the canopy of citrus bacterial spot-diseased plants. Following evening rain or overhead irrigation, diseased plants were removed and Xcc recovered from trap plants by leaf washing from 24 to 96 hr after wetting and prior to disease development. Plants were subjected to shading, to full sun, or to rewetting and periodic misting to maintain leaf wetness (LW). Shading and misting prolonged Xcc survival by extending the duration of LW, whereas Xcc declined to low or nondetectable levels within hours after leaf surfaces dried in full sun or with no misting. Rewetting of leaves with irrigation following a dry period of several hours did not cause a resurgence in Xcc but maintained populations at low levels as long as LW was sustained. These findings confirm the role of rain, dew, and overhead irrigation for survival of Xcc.

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EFFECTS OF SHADING ON SHORT TERM COLONIZATION BY BACTERIAL BIOLOGICAL CONTROL AGENTS IN TURFGRASS. L. J. Giesler, and G. Y. Yuen, Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

Shading of the turfgrass canopy is assumed to support increased microorganism activity (e.g. pathogen infection). The goal of this research was to determine how shading affects microenvironmental conditions, and how these conditions affect phylloplane colonization by applied bacterial strains. Shade cloth was suspended over tall fescue turf plots and environmental parameters were measured in shaded and unshaded plots. Shading increased leaf wetness duration and levels of relative humidity and decreased canopy air and foliage temperatures. Bacterial strains (*Enterobacter cloacae*, *Bacillus megaterium*, and *Pseudomonas* sp.) were applied to all plots. Population numbers either increased or decreased in both shaded and unshaded plots, depending on the strain. 8 days after application, *E. cloacae* populations were higher in unshaded plots compared to shaded plots, whereas *B. megaterium* populations were higher in shaded plots. Numbers of *B. megaterium* colonization sites, as determined by shoot imprinting on agar media, were greater in shaded plots than in unshaded plots. It is concluded that measurable differences in the microenvironment occur as a result of shading, and bacterial response to these changes is strain-specific.

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DEPOSITION PATTERNS OF AEROSOLIZED BACTERIA APPLIED TO CROP FOLIAGE. L. M. Dandurand and G. R. Knudsen, Plant Pathology Division, University of Idaho, Moscow.

Aerosolized bacterial suspensions (*Erwinia herbicola* 112Y) were applied at different concentrations and volumes to foliage of 2-mo-old bean plants. Plants were arranged in rectangular grids, with selective 'gravity' plates and water-sensitive cards suspended at upper canopy level. Wind speed and direction were monitored at 1-s intervals. Deposition of bacteria on upper and lower canopy leaves was quantified by washing and dilution plating, colonies on gravity plates were counted, and deposition on individual cards was quantified with video image analysis. All deposition patterns were mapped by spatial coordinates. Each monitoring method yielded qualitatively and quantitatively different information. At low wind speeds (ca. 1 m/s) and higher bacterial concentrations, cards provided more spatial resolution than gravity plates and more accurately predicted recovery from leaves. At higher wind speeds (ca. 5 m/s, turbulent), gravity plates provided more spatial resolution as the mean particle size landing within the grid increased. The experimental results were used to test and improve an aerosol dispersal simulation model.

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EPIPHYTIC YEASTS OF CITRUS FRUIT TOLERANT TO EXTREME CONDITIONS ARE EFFECTIVE ANTAGONISTS OF GREEN MOLD DECAY. S. Droby[1], S. Lischinsky[1], L. Cohen[1], S. Manulis[2], R.K. Mehra[3] and J.W. Eckert[4]. [1]Dept. of Postharvest Science of Fresh Produce, and [2]Dept. of Plant Pathology, ARO, The Volcani Center, P.O.Box 6, Bet Dagan 50250, ISRAEL, [3]Dept. of, Entomology/Environmental Toxicology and [4]Dept. Plant Pathology, University of California, Riverside, CA 92521, U.S.A.

This study was aimed at isolation and characterization of epiphytic yeast population of citrus fruit antagonistic to green mold decay caused by *Penicillium digitatum*. Ability to grow on elevated concentrations of NaCl, glucose and wide range of temperatures was used as a critirium for the initial selection. Of 202 yeast isolates, randomly selected following plating of surfaces washings and isolations from wounds, 69 grew at temperatures ranging from 5 to 40°C. Of 69 isolates, about 60 found to grow on PDA amended with either 10% NaCl or 50% glucose. Following an assay performed on wounded grapefruit to test biocontrol activity, 46 isolates exhibited 70% or more inhibition of infection. Among these antagonist, 4 yeast isolates were highly effective against *P. digitatum* on grapefruit. RAPD-PCR analysis showed that population of the 69 yeasts tested consisted of only three groups of isolates, suggesting the presence of only three different yeast species.

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MODIFIED XTS AGAR FOR ISOLATION OF XANTHOMONAS CAMPESTRIS PV. TRANSLUCENS FROM WHEAT AND BARLEY SEED. R.L. Forster[1], C.A. Strausbaugh[1], and N.W. Schaad[2]. [1]Univ. of Idaho Res. and Ext. Center, Kimberly, 83341; [2]USDA/ARS Foreign Disease-Weed Research Unit, Frederick, MD 21702.

The gentamicin concentration (8 mg gentamicin sulfate/L = ca. 4800 µg gentamicin/L) in XTS agar (Phytopathology 75:260-263) may be toxic to some strains of Xanthomonas campestris pv. translucens (Xct). To test further the effect of gentamicin in XTS, we determined the recovery of five different strains and assayed eight naturally contaminated wheat and barley seed lots using XTS prepared with 2900, 2600, 1450, and 0 µg gentamicin/L. Results showed that recovery of Xct was reduced 10 to 1,000 times when gentamicin was increased from 1,450 to 2,900 µg for one strain and five seedlots, whereas the others were affected only slightly. Elimination of gentamicin in the medium had little effect on increased recovery of Xct past that at the 1450 level but permitted saprophytic growth of bacteria in some seed assays making those plates uncountable. Thus, if gentamicin is required, 1450 µg/L is recommended for maximum effective recovery of Xct.

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EFFECTS OF INOCULATION METHOD AND CAPTAN ON SEED TRANSMISSION AND SYSTEMIC INFECTION OF MAIZE BY *FUSARIUM MONILIFORME*. G.P. Munkvold and W.M. Carlton. Dept. of Plant Pathology, Iowa State University, Ames 50011

In 1994, we conducted field experiments to assess the frequency of *Fusarium moniliforme* seed transmission and systemic infection in maize grown from seed inoculated in the field or in the laboratory. Seed produced in 1993 was inoculated with *F. moniliforme* strain EA-1 by spraying a spore suspension on the silks at growth stage R1-R2. Infection of mature seed and plants by strain EA-1 was confirmed by vegetative compatibility of recovered strains with EA-1 using nitrate-nonutilizing (*nit*) mutants. Following laboratory inoculation by soaking hybrid seed in a spore suspension, EA-1 was detected in 100% of seed and in ≤68% of seedlings, but in only ≤20% of mature ears and ≤22% of mature infected kernels. Following field inoculation, EA-1 was detected in ≤72% of seed, ≤78% of mature ears and ≤48% of mature infected kernels. Strains compatible with EA-1 were detected in <2% of plants from noninoculated seed. Seed inoculation in the laboratory, a method used in prior research, may underestimate the importance of systemic infection and seed transmission in maize. Post-inoculation captan treatment (0.625 g a.i./kg seed) had no effect on *F. moniliforme* seed transmission from either laboratory- or field-inoculated seed planted in the greenhouse.

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ANTIFUNGAL ACTIVITY IN GERMINATING TALL FESCUE SEED. Batzer, J.C. and Gwinn, K.D. University of Tennessee, Dept. Entomology and Plant Pathology, Knoxville, TN 37910-1071.

Endophyte infection of many grasses results in enhanced resistance to pre-emergence damping-off pathogens. To determine if antifungal compounds are produced in germinating seed, tall fescue (*Festuca arundinacea* cv Pixie) seed lots with endophyte (*Acremonium coenophialum)* infestation levels of 79 % (EH) and 29% (EL) were compared. Seeds (5g) were scarified, surface sterilized then allowed to imbibe water (50 ml). Water was decanted and mixed 2:1 with 3% agar (imbibition agar). Seeds were ground in buffer, then mixed with 2% water agar (macerate agar). Culture plates (6-well) were used to pair EH and EL treatments. Cores (5 mm) of *Rhizoctonia solani* and *Pythium aphanidermatum* were transferred to media. Diameter of colonies was measured. The experiment was performed three times. Data were analyzed with a paired t-test. Growth of *R. solani* was significantly greater on EL macerate agar than on EH macerate agar at 72 h but not at 48 h or 96 h. Growth was greater on EL imbibition agar only at 48 h. Growth of *P. aphanidermatum* was significantly greater on EL imbibition agar only at 48 h. Growth of *P. aphanidermatum* on macerate agar was not affected by endophyte status.

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ISOLATION OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* FROM DETACHED TOMATO SEED FIBERS. M. D. Ricker, Heinz U.S.A., 13737 Middleton Pike, Bowling Green, OH 43402.

Seeds were extracted from tomato fruit infected with *C. m.* subsp. *michiganensis* (*Cmm*) by crushing the fruit and dissolving the pulp with pectinase. Seventy-five g of air-dried seed were shaken for 30 min in an inverted 1.89 L juice bottle on a wrist action shaker. Fibers (seed hairs) that were dislodged by shaking fell through a nylon mesh screen into a polyethylene bag. Fiber samples were soaked overnight at 2 C in phosphate buffer (0.1 M; pH 7.4) with Tween. A series of dilutions were plated on semi-selective agar. Isolation of *Cmm* was confirmed with pathogenicity tests. Shaking fibrous or smooth seed for up to 1 hr did not reduce germination. The bacterium remained viable on detached fibers for at least 13 mo. This is the first report of isolation of *Cmm* from detached tomato seed fibers. Development of a non-destructive assay, as a supplement to traditional methods, may facilitate the testing of larger percentages of seedlots.

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DEVELOPMENT OF A MULTIPLEX PCR SEED HEALTH TEST TO DETECT AND IDENTIFY THREE FUNGAL SEED-BORNE PATHOGENS OF BARLEY. J Alderson, E J A Blakemore and J C Reeves, National Institute of Agricultural Botany, Molecular Biology and Diagnostics Section, Huntingdon Rd, Cambridge, CB3 OLE, UK.

Diseases of barley caused by Pyrenophora graminea (leaf stripe) and the two forms of P. teres (f. teres and f. maculata, causing net blotch and a spot form of net blotch respectively) are capable of producing economically significant yield losses. Two strategies have been used to obtain primers for identifying and detecting Pyrenophora spp. The first, random amplified polymorphic DNAs (RAPDs), has been successful in identifying a DNA amplification product specific for each of the Pyrenophora spp. mentioned above. An alternative strategy used ribosomal RNA gene sequences, which have been useful in taxonomic studies because they have variable internal transcribed spacer (ITS) regions, useful for identification at an intraspecific level. Sequence data was obtained for the ITS region from different isolates of Pyrenophora spp. Screening a large number of geographically diverse Pyrenophora spp. isolates with primers obtained from both strategies will be carried out before developing a multiplex PCR seed health test.

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CRYOPRESERVATION OF HEALTHY AND DISEASED COTTONSEED M.H. Wheeler, USDA, ARS, SCRL, Cotton Pathology Research Unit, 2765 F&B Road, College Station, Texas 77845.

Liquid nitrogen (-196°C) was used to preserve healthy and deteriorated cottonseed over various time periods from 72 hr to 90 days. The seeds were then returned to 25°C for several days and evaluated for quality, using the Cool-Warm Vigor Index test. Storage at -196°C had no significant effect on the seed germination and vigor of different varieties of seed, but it often caused minor to severe cracks in the cotyledonary tissues. Typical seedlings, 10 to 25 mm in length, were planted in peat pellets and allowed to grow for 2 to 3 wk. They were then transferred to potted soil and allowed to develop into mature plants. The cotyledonary changes often slowed growth of the plants but only occasionally prevented seed from developing into adult plants capable of producing cotton. This suggests that the cryogenic method may be suitable for long term preservation of cottonseed germplasm.

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SENSITIVITY OF SELECTED MAIZE INBREDS AND THEIR HYBRIDS TO BARLEY YELLOW DWARF VIRUS RMV-IL. R. L. Tinyre, C. J. D'Arcy, W. L. Pedersen, and A. D. Hewings, Department of Plant Pathology, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801 and USDA-ARS MWA Peoria, IL 61604.

Ten dent maize inbreds, A619, A632, A634, B68, B73, B84, Cm105, Mo17, Pa91, and Va22, were tested under field conditions during two growing seasons (1992 and 1993) for their sensitivity to BYDV (RMV). Forty-five hybrids were created from the ten inbreds and tested under field conditions in 1993. Plants were inoculated at the six leaf stage with *Rhopalosiphum padi* reared on Hudson barley infected with RMV. Leaf tissue was collected from the inoculated and control plants a month after flowering and assayed for RMV using double antibody sandwich enzyme-linked immunosorbent assay. In 1992, infection ranged from 20-80% in inoculated inbreds, with eight of the inbreds having more than 50% of the plants infected. In 1993, infection ranged from 11-74% with eight inbreds having more than 35% of the plants infected. For the hybrids, infection ranged from 7% to 87%. Fourteen hybrids had less than 25% of the plants infected, 16 hybrids had between 25-50% of plants infected, and 14 hybrids had between 50-75% of the plants infected. All of the inbreds and hybrids tested were susceptible to RMV infection.

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A VIRUS-LIKE DISEASE OF CORN AND WHEAT IN THE HIGH PLAINS : ULTRASTRUCTURAL ASPECTS. K.-K. Ahn[1], S.G. Jensen[2], E.J. Anderson[1], R.C. Gergerich[1] and K.S. Kim[1]. [1]Dept. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701, [2]USDA-ARS, Univ. of Nebraska, Lincoln, NE 68583

A new disease affecting corn and wheat has been epidemic in the high plains region of the central US and viral etiology has been suggested based on symptomatology, eriophyid mite-transmissibility and detection of a 32 kd protein apparently associated with this disease (Phytopathology 84:1158). Thin section electron microscopy of symptomatic leaves consistently showed the presence of quasi-spherical double membrane-bound particles (DMPs) of 100-150 nm in diameter which were structurally indistinguishable from those associated with fig mosaic, rose rosette, yellow ringspot of redbud, and thistle mosaic diseases, a group of eriophyid mite-borne diseases of unknown etiology (Phytopathology 83:1402). When DMPs occurred as aggregates, they were associated with electron-dense amorphous inclusions. In many cells, DMPs occurred together with pinwheel inclusions indicating co-infection with wheat streak mosaic potyvirus which was serologically detected in symptomatic plants. Neither DMPs nor pinwheel inclusions were observed in healthy control leaves. The number of DMPs in cells that contained pinwheel inclusions was many times greater than in cells without pinwheel inclusions. Immunogold labelling using antiserum produced against the 32 kd protein exhibited specific labelling of DMPs and associated amorphous inclusions. This suggests that the 32 kd protein represents a viral protein originating from DMPs and associated inclusions.

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POTATO VIRUSES IN THE OJOS NEGROS VALLEY , BAJA CALIFORNIA, MEXICO. J. GUEVARA. INIFAP-CECOEN. APDO. POSTAL 2777. ENSENADA, B. C. MEXICO.

Viral diseases are the major phytopathological problem in the potato crop, for this reason was done a survey to detect the incidence of viruses in the potato, in the Ojos Negros Valley. 250 plant samples were collected from 5 potatoes fields during October 1994, and were tested against eleven plant virus antisera using the DAS-ELISA test. The viruses identified were PVY°, PVS,PLRV and PVX with incidences of 64, 48, 34, and 17%, respectively, of the potatoes samples, whereas PVYn, BWYV, TSV,PVA, PVM, AMV and TRV were not detected. This is the first report of viruses that infect potatoes in the Ojos Negros Valley, Baja California, México.

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PCR-BASED DETECTION OF GEMINIVIRUSES IN TWO WHITEFLY VECTORS. A. M. Idris and J. K. Brown. Department of Plant sciences, University of Arizona, Tucson, AZ 85721.

Virus-vector transmission parameters were evaluated and compared for chino del tomate (CdTV) and tomato yellow leaf curl-Thailand (TYLCV-Th) geminiviruses using *Bemisia tabaci* and *B. argentifolii* (formally A and B biotypes). Adults whiteflies were allowed acquisition access periods ranging from 30 min to 72 hrs, prior to analysis by PCR. Ingestion of geminiviruses by whiteflies was confirmed by PCR amplification of a 550 bp coat protein gene fragment. In addition, PCR was used successfully to detect CdTV and TYLCV-Th in viruliferous whiteflies that ingested from single and/or mixed geminiviral infections. Virus-specific degenerate PCR primers, designed to amplify the pre-coat protein region of the A-components, resulted in the detection of viral DNA fragments of the expected sizes, 400 bp and 500 bp, for CdTV and TYLCV-Th, respectively.

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PARTIAL CHARACTERIZATION AND MOLECULAR CLONING OF TOMATO INFECTIOUS CHLOROSIS VIRUS (TICV). Hsing-Yeh Liu, G. C. Wisler, and J. E. Duffus. USDA-ARS, 1636 East Alisal Street, Salinas, CA 93905.

A new yellowing disease of tomato was found in California in 1993. The inciting closterо-like virus, TICV, was transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*) in a semipersistent manner, but was not mechanically transmissible. Flexuous filamentous particles with a modal length of 850-900 nm were consistently observed in leaf dip preparations of TICV-infected plants. Complementary DNA synthesis and cloning used the virion RNA (ca. 8.0 kb) isolated from TICV-infected *Nicotiana clevelandii* as a template. These clones specifically reacted with purified TICV-RNA, dsRNA, as well as with RNA extracted from TICV-infected plants in dot blot analyses. No reactions were observed in dot blots against other whitefly transmitted closteroviruses including beet pseudo yellows, lettuce infectious yellows, lettuce chlorosis, or cucurbit yellow stunting disorder.

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CHARACTERIZATION OF GRAPEVINE ASSOCIATED CLOSTEROVIRUSES BY WESTERN BLOTTING. Iudit Monis and Rick Bestwick. Agritope, Inc., Research and Development Department. 8505 SW Creekside Place. Beaverton, OR 97008.

Various closterovirus-like particles have been described in association with different types of leafroll and corky bark diseases. Because Koch's postulates have not been completed with the viruses causing these diseases, the causal agents are referred to as grapevine leafroll associated (GLRaV) and grapevine corky bark associated (GCBaV) viruses. We have isolated viruses from several grapevine cultivars determined to be ELISA positive for different GLRaV serotypes (*e.g.*, GLRaV I, II, IIb, III, and IV) and GCBaV. The virus extracts were analyzed by Western blot immunoassay using different monoclonal and polyclonal antibodies. The results show that grapevine cultivars often carry mixed infections of serologically unrelated GLRaV and GCBaV. Our work has demonstrated that a polyclonal antibody assumed to only react to GLRaV II also reacts to GLRaV I, IIb, and IV viral strains. A simple and sensitive method for the detection and identification of different GLRaVs and GCBaV by Western blot immunoassay was developed.

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MOLECULAR CLONING AND SEQUENCE ANALYSIS OF CYMBIDIUM MOSAIC AND ODONTOGLOSSUM RINGSPOT VIRUSES. K. Barry, J. S. Hu, A. Kuehnle, and N. Sugii. Depts. of Plant Pathology and Horticulture, Univ. of Hawaii, Honolulu, HI 96822.

A Hawaiian strain of Cymbidium mosaic virus (CyMV) was isolated from *Dendrobium* orchid, and a cDNA library was constructed. Clones containing the coat protein (CP) gene and movement protein (MP) gene were identified by colony hybridization and polymerase chain reaction (PCR). DNA sequence comparisons indicate that the CP and MP genes of the Hawaiian CyMV shares >95% sequence identity with the respective genes of the Singapore CyMV isolate. The Hawaiian strain of Odontoglossum ringspot virus (ORSV) was isolated from *Cattleya* orchid. The CP and 54 kDa putative replicase genes were cloned by RT-PCR. Sequence comparisons of the CP region reveal over 99% homology to that of the Japanese ORSV isolate. The genes of CyMV and ORSV were introduced by bombardment into protocorms and protocorm-like bodies of *Dendrobium* for production of virus-resistant transgenic plants.

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ULTRASTRUCTURAL STUDIES ON SEED TRANSMISSION OF CUCUMBER MOSAIC VIRUS IN SPINACH. Y. Yang, K.S. Kim and E.J. Anderson, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Cucumber mosaic virus (CMV) was recently reported to be seed-transmitted in spinach, as determined by seed grow-out tests and ELISA. To understand the nature of CMV seed transmission, cytological localizations of the virus in spinach seed and reproductive organs were investigated by thin-section electron microscopy and immunogold labeling. Analysis of ovary, anther and endosperm tissues from CMV infected spinach demonstrated that virus particles were present in these tissues. In ovary, virus particles appeared as discrete spheres of approximately 25 nm in diameter. They occurred exclusively in the cytoplasm and were often associated with amorphorous inclusion bodies. Also, fine fibril-containing vesicles, characteristic of single-strand RNA viruses, occurred in the central vacuole along the tonoplast of virus-containing cells. In endosperm cells, virus particles appeared to accumulate near the tonoplast and protrude or invaginate into the central vacuole as membrane bound bodies of various sizes. Immunogold labeling revealed gold particles specifically associated with the areas in the cytoplasm where virus particles occurred. These results indicate that CMV can replicate in spinach reproductive organs and seed tissues. These studies provide preliminary information regarding the mechanism of CMV seed transmission in spinach.

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TOBACCO MOSAIC VIRUS ENCODING GREEN FLUORESCENT PROTEIN. Yue Lin Zhang and Ulrich Melcher, Dept. Biochem. & Molec. Biol., OAES & Oklahoma State University, Stillwater OK 74078.

The green fluorescent protein (GFP) of *Aequorea victoria* is a revolutionary *in vivo* marker of gene expression and protein localization. To test whether GFP can be used as marker for tobamovirus replication and movement, the GFP gene was cloned in a tobacco mosaic virus (TMV)-based cDNA plasmid vector. In preliminary experiments, transcripts of this DNA (TMV:GFP) infected *Nicotiana tabacum*. GFP was expressed in infected leaves as evidenced by green fluorescent spots visible on the infected leaves under long wavelength UV irradiation. The positions of the spots correlated with the location of TMV in the leaf. Wild type TMV-infected leaves did not fluoresce green. The successful construction of TMV:GFP and epifluorescence microscopy should assist our investigation of local TMV infection of the non-host turnip. As a positive control for replication and movement in turnip we will use turnip vein-clearing tobamovirus (TVCV) expressing GFP. A TVCV-based vector analogous to that used in the construction of TMV:GFP was constructed, using an infectious TVCV cDNA clone recently assembled in our laboratory (Lartey, Voss, Zhang and Melcher, unpublished). Expression of GFP from the TVCV-based vector will be attempted. Supported by OAES and NSF-EPSCoR.

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TIME COURSE STUDIES OF TWO ISOLATES OF IMPATIENS NECROTIC SPOT VIRUS. Dienelt, M. M., Lawson, R. H., and Hsu, H. T. U.S. National Arboretum, USDA, Beltsville, MD 20705

Infection cycles of two morphologically and serologically distinct isolates of Impatiens necrotic spot virus, INSV B and HT-1, were compared in Nicotiana benthamiana. In early infections of both isolates, rough endoplasmic reticulum (rER) formed single or concentric circular formations. Regions enclosed by rER developed extensive networks of smooth endoplasmic reticulum (sER), Golgi bodies, vesicles, and paired parallel membranes. Nucleocapsid protein (N protein) was identified at these internal membranes by immunogold labelling with antisera to N proteins of both isolates. Both isolates produced nucleocapsid aggregates (NCAs) within this area; in HT-1 infections, amorphous inclusions occurred as well. Early to mid infections displayed double enveloped virions in cytoplasm, rows of particles within sER, and extensive budding and/or fusing of membranes and virions. In older INSV B infections, rER with clustered particles were predominant. Older HT-1 infections displayed NCAs, paired parallel membranes, and particles in sER representative of mid-infection. Cytopathological features that may relate to a maturation defect in HT-1 will be discussed.

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TWO DISTINCT TOSPOVIRUS NUCLEOCAPSID PROTEINS IN THE SAME INCLUSION BODIES. Lawson, R. H., Dienelt, M. M., and Hsu, H. T. U.S. National Arboretum, USDA, Beltsville, MD 20705

A unique variant of impatiens necrotic spot virus, INSV HT-1, was recovered in 1991 when INSV Igg, was serially passaged in Nicotiana benthamiana at high temperatures (HT) (27/24 C,day/night). Unlike Igg, HT-1 forms virions and does not react with antiserum (AS) to INSV N protein. AS to HT-1 N protein did not react with our Igg cultures in 1993/1994. Immunogold labelling was used to test for HT-1 in embedded tissue of 1991 Igg cultures, including: our original culture, HT passages that yielded HT-1, and passages at 21/18C day/night, comprising low temperature (LT) controls. Antisera to INSV and HT-1 N proteins labelled nucleocapsid aggregates (NCAs) in the original culture, first three HT passages and all LT passages. Double label tests of the original culture confirmed that INSV and HT-1 N proteins were present in the same NCAs. By the 4th HT passage, virions appeared and by the 10th passage NCAs were strongly labelled by HT-1 AS but not by INSV AS. HT-1 was, therefore, present in our original Igg culture, with HT-1 separated in 1991 and Igg separated later. This is the first report of two distinct tospovirus N proteins in the same inclusion body.

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SEQUENCE ANALYSIS OF THE COAT PROTEIN REGION OF WHEAT STREAK MOSAIC VIRUS (WSMV) ISOLATES. K.D. Chenault, R.M. Hunger, and J.L. Sherwood. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Wheat streak mosaic (WSM), caused by wheat streak mosaic virus (WSMV), is an important disease in hard red winter wheat (*Triticum aestivum* L.). Analysis by serology with monoclonal antibodies and polyclonal antiserum (Phytopathology 83:1371), and by PCR and RFLP mapping (Phytopathology 83:1356) has indicated variation in the coat protein region of isolates of WSMV. The coat protein region of nine isolates of WSMV was cloned by RT-PCR using primers that were inclusive of nts 398-1825 (J. Gen. Virol. 72:499) that resulted in PCR fragments of approximately 1427 bps. The PCR products were sequenced, and restriction analysis results were consistant with those attained by serology and RFLP mapping. The variability in the coat protein region may be useful for evaluation of isolate-cultivar interaction and the epidemiology of WSM.

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GENETIC ANALYSIS OF CALIFORNIA BEET YELLOWS CLOSTEROVIRUS FIELD ISOLATES. R. Creamer and C-.H. Yang, Department of Plant Pathology, University of California, Riverside, CA 92521.

Beet yellows closterovirus (BYV) is the type member of the closterovirus group and an important pathogen of sugarbeets in California. Nucleic acid sequence of isolates of BYV have been published from Europe and Russia, but no information is available on the genetics of the virus isolates found in the U.S. Five isolates of BYV were collected from sugarbeets from different growing regions in California in 1994, a sixth in 1992, and a seventh isolate in 1960. The gene encoding the virus coat protein from each isolate was amplified using PCR and the sequence determined. Sequence similarity was very high between all California isolates, suggesting that there is very little diversity in the virus within the state, and that there has been little divergence in this gene over the last 30 years. Sequence comparison of the gene from California isolates with those from Great Britain, Germany, and Russia also yielded high similarity.

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TESTING FOR EXPRESSION OF TWO CITRUS TRISTEZA VIRUS GENES IN INFECTED CITRUS TISSUE. V.J. Febres[1], H.R. Pappu[1], S.S. Pappu[1], R.F Lee[2] and C.L. Niblett[1]. [1]University of Florida, Plant Pathology Dept., Gainesville, FL 32611-0680. [2]CREC. Lake Alfred, FL 33850.

The genes for p18 and p20 are located toward the 3' end of the citrus tristeza virus genome. These two genes potentially encode proteins of 18.3 kDa and 20.5 kDa, respectively. Amino acid sequence comparison indicates statistically significant similarities between p20 and p21, a putative protein encoded by the beet yellows closterovirus genome. No significant homologies with p18 were found in current protein databases. These two genes were expressed in *E. coli* as fusion proteins for the production of polyclonal antibodies. The antibodies were used to test for the expression of these genes in infected citrus tissue extracts using Western blot. The p20 was detected and cell fractionation studies indicate that it accumulates in the soluble protein fraction. The antibody for p18 was reactive to the protein used as antigen but did not reveal the presence of p18 in infected tissue. It is possible that p18 is produced in very small quantities or at a very specific stage during viral replication. No function in the virus replication cycle is yet assigned to either protein.

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SEROLOGICAL ASSAYS FOR THE DETECTION OF WHEAT STREAK MOSAIC VIRUS IN A SINGLE WHEAT CURL MITE. T. MAHMOOD, G. L. Hein, and R. C. French. Department of Plant Pathology and Entomology, University of Nebraska, Lincoln, NE 68583

Wheat streak mosaic virus (WSMV) is transmitted by the wheat curl mite, *Aceria tosichella* Keifer. A major difficulty in working with wheat curl mite is its very small size (250 *u*), making it extremely difficult to detect the virus in individual mites. Immunofluorescent and dot immunobinding assays were developed to detect the presence of WSMV in single mites. Virus-specific immunofluorescent microscopy detected the presence of WSMV near the anterior end of viruliferous mites. With dot immunobinding assay, WSMV was detected in mites fed on tissue infected with the virus but not in mites maintained on healthy plants. Both immunofluorescent and dot immunobinding assays were sufficiently sensitive to detect virus in individual mites and can provide practical means to determine percentage of viruliferous mites in field collections.

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ULTRASTRUCTURAL CHANGES IN THE FLAG LEAVES OF BARLEY YELLOW DWARF INFECTED OAT LINES. Petra H. Nass and Cleora J. D'Arcy, University of Illinois, Department of Plant Pathology, 1102 South Goodwin Avenue, Urbana, IL 61801.

Similar cytopathological changes can be found in phloem cells of the primary leaves of barley yellow dwarf virus (BYDV) infected tolerant (T) and sensitive (S) oat lines. A correlation between the extent of cytopathological damage and tolerance, however, does not seem apparent. In this study changes of the ultrastructure in the flag leaves of infected T and S lines were analyzed to determine whether there is a relationship between cytopathological changes in the flag leaf phloem and tolerance to BYDV infection. Ten-day-old seedlings of Coast Black oats (S), IL 86-5262 (T), IL 86-1150 (T), IL 602T (T), and IL 602S (S) were inoculated with BYDV PAV-IL by approximately 30 *Rhopalosiphum padi* L. Two to three months after inoculation samples were taken from the midrib of fully extended flag leaves and prepared for electron microscopy. Phloem obliteration was absent from BYDV infected T lines and cytopathological alterations were rarely observed. BYDV infected S oat lines showed occasional necrosis of phloem cells. The phloem cells of infected CBO flag leaves were all obliterated or contained large aggregates of viral particles.

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ANALYSIS OF THE SYSTEMIC MOVEMENT OF PEA ENATION MOSAIC VIRUS. J.S. Skaf, D.G. Rucker, S.A. Demler, and G.A. de Zoeten. Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824. In the symbiotic association of the two genomic components of pea enation mosaic virus (PEMV), the *Luteovirus*-like component (RNA1) provides the structural and aphid transmission functions but has not been shown to be capable of establishing a systemic infection on its own. The functions essential for the systemic movement of the virus are furnished by the *Umbravirus*-like component (RNA2). Stop and frame shift mutations introduced into the 26/27 kDa ORFs of RNA2 abolished the systemic movement of both viral RNAs as assayed by symptom expression and northern blot analysis. Deletion of the coat protein ORF from RNA1 did not affect the movement of either RNA but resulted in stunting, necrosis, and wilting symptoms more severe than those produced by the wild-type infection.

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DETECTION OF WHITEFLY-TRANSMITTED GEMINIVIRUSES IN AQUEOUS EXTRACTS USING DEGENERATE PRIMERS AND POLYMERASE CHAIN REACTION. S.D. Wyatt[1] and J.K. Brown[2]. [1]Dept. Plant Pathology, Washington State University, Pullman, WA 99164; [2]Dept. Plant Sciences, University of Arizona, Tucson, AZ 85721.

The DNA of monopartite and bipartite whitefly-transmitted (WFT) geminiviruses was amplified following direct addition of aqueous leaf extracts to a polymerase chain reaction (PCR) mix, thus eliminating the need to prepare purified nucleic acid extracts prior to PCR amplification. Several degenerate primer pairs were designed and successfully applied to the targeting, amplification and detection of a suite of geminivirus isolates, representative of a broad biogeographic range. This approach allows for rapid, sensitive and accurate detection of a diverse array of WFT geminiviruses in cultivated and weed hosts with minimal sample preparation. It is also effective with more highly purified DNA or partially purified virion preparations. The versatility of the method makes this the simplest detection system available to date for group-specific detection of WFT geminiviruses.

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Cherry Virus A: cDNA Cloning of dsRNA, Nucleotide Sequence Analysis and Serology Reveal a New Plant Capillovirus in Sweet Cherry. Dr. Wilhelm Jelkmann, Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Fruit Crops, Schwabenheimer Str. 101, D-69221 Dossenheim, Federal Republic of Germany.

The nucleotide sequence (7383 nucleotides) of a newly identified member of the genus *Capillovirus*, cherry virus A (CVA), was obtained from cDNA clones. The cDNA was generated from double-stranded RNA extracted from plant tissue infected with little cherry virus (LCV). Low amounts of LCV dsRNA served as template nucleic acid and enabled the construction of a library of which unexpectedly 7.5% of the recombinant plasmids were specific for CVA. The genome organization of CVA resembles that of apple stem grooving virus (ASGV), the type member of the genus *Capillovirus* and is composed of a 266 kDa polyprotein (ORF1), a 52 kDa ORF2 located within ORF1 and a poly(A) tail. The coat protein (CP) is located in the C-terminal region of ORF1 and was identified in immunoblot analysis and estimated to be of 24 kDa. Antiserum was obtained by expression of antigens as fusion proteins in *Escherichia coli*. No serological cross reaction was obtained in immunoblot analysis with ASGV, apple chlorotic leafspot trichovirus (ACLSV), apple stem pitting virus (ASPV), and cherry mottle leaf virus (CMLV) antisera. Flexuous filamentous CVA virions were identified in extracts of sweet cherry by immunosorbent electron microscopy (ISEM) and decorated with the antiserum to the fusion protein. CVA was identified in three cherry sources of different disease status by ISEM, immunoblot analysis, and hybridization to dsRNA. CVA is not closely related to any of the currently described diseases in cherry but is has all the properties of a capillovirus. It is suggested that CVA should be classified as a new member of the genus *Capillovirus*.

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ISOLATION, CHARACTERIZATION AND UTILIZATION OF STRONG PROMOTERS FROM *CLAVIBACTER XYLI* SUBSP. *CYNODONTIS*. M. Haapalainen[1], M. Karp[2], N. Kobets[3], E. Piruzian[3] and M.C. Metzler[1]. [1]Dept. of Biology and [2]Dept. of Biotechnology, [1,2]University of Turku, Biocity, 20520 Turku, Finland; [3]Institute of Molecular Genetics of the Russ. Acad. Sci., 46 Kurchatov Sq. 123182 Moscow, Russia.

Clavibacter xyli subsp. *cynodontis* (*C.x.c.*) is a gram-positive coryneform bacterium occuring as an endophytic parasite of bermudagrass. It is able to colonize the xylem of other plants, and it causes no disease symptoms under non-stress conditions. Engineering *C.x.c.* to produce proteinaceous insecticides and fungicides in high quantities inside a host plant could make it useful for plant protection. To find strong promoters for *C.x.c.* for high expression of heterologous genes, we developed a sensitive promoter-probe plasmid method utilizing the broad host range vector pRK415 and a click beetle luciferase gene. Small fragments of chromosomal DNA were inserted in front of the promoterless luciferase gene to create a promoter library. We screened the library transformants using a CCD camera. The strongest promoters thus found, which give over 2500 times more light than the promoterless control, were characterized. These promoter sequences proved to be highly GC-rich, as is the whole chromosomal DNA of *C.x.* subsp. *cynodontis*. The regions probably recognized by a *C.x.c.* σ-factor are very different from the consensus sequences of the strong promoters of *E. coli* and *Bacillus*. Instead, they most closely resemble the promoters of streptomycetes. We are using these promoters to express anti-fungal enzymes in transformed *C.x.c.*

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THE *tolC*-COMPLEMENTING GENE OF *ERWINIA CHRYSANTHEMI*. O. L. Johnson[1], J. Fralick[2] and M. San Francisco[1], [1]Texas Tech University, [2]Texas Tech University Health Science Center, Lubbock, TX 79404.

TolC is a minor outer membrane protein of *Escherichia coli* involved in a number of functions including resistance to detergents and tolerance to Colicin EI. This protein also plays a role in *E. coli* as an element in the alpha hemolysin secretion pathway. Studies were carried out to determine whether a functional homologue of the *E. coli* TolC exists in *E. chrysanthemi*. Using an *E. chrysanthemi* cosmid library a TolC-deficient *E. coli* strain was screened for recovery of at least one TolC function, *viz.*, deoxycholate resistance. Tn*PhoA* mutagenesis of the complementing cosmid was carried out to identify *E. chrysanthemi* nucleotide sequences required for DOC resistance. Low stringency Southern blot analysis using the *E. coli* tolC gene as a probe identified a 2 kb Pst I fragment of the *E. chrysanthemi*-tolC-complementing cosmid.

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EVALUATION OF PCR PRIMERS FOR DETECTION OF *XANTHOMONAS ALBILINEANS*, THE CAUSAL AGENT OF LEAF SCALD DISEASE. Yong-Bao Pan, Michael P. Grisham, and David M. Burner, USDA/ARS, Sugarcane Research Unit, Houma, Louisiana 70361

Genomic DNA was extracted from seven Louisiana isolates of *Xanthomonas albilineans* (*Xa*), nine strains of other *Xanthomonas* species, and five non-*Xa* bacteria strains isolated from the sugarcane tissue. Three sets of PCR primers, L1/G1 (Jensen et al., 1993, Appl. Environ. Microbiol. 59:945-952), T3A/T5B (Welsh and McClelland, 1991, Nucleic Acid Res. 19:861-866), and A1a4/Ile2 (Honeycutt et al., 1995, *unpublished*) were tested on the genomic DNA. Only A1a4/Ile2 was found to be *Xa*-specific and amplified an approximate 70 bp product. Four combinations among A1a4, Ile2, L1, and G1 were also tested. Under an optimized PCR program, the primer set A1a4/L1 specifically amplified an approximate 360 bp product from all *Xa* isolates that was detectable by conventional agarose gel electrophoresis and ethidium bromide staining. This product was also amplified from *Xa*-infected sugarcane and sweet corn tissues but not from healthy ones. In addition, our PCR protocol is simple, sensitive, rapid, and inexpensive. The potential of this PCR protocol in diagnosis of leaf scald disease in sugarcane production and breeding will be discussed.

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Characterization of a global regulatory gene, *rsmA*, of *Erwinia carotovora* subsp. *carotovora*. Y. Cui, A. Chatterjee, Y. Liu, C. K. Dumenyo, and A. K. Chatterjee, Dept. Plant Pathol., Univ. of Missouri, Columbia, MO 65211

We have discovered a novel regulatory gene, *rsmA* (*rsm* = *r*epressor of *s*econdary *m*etabolism) in *E. carotovora* subsp. *carotovora* strain 71. An RsmA$^+$ plasmid suppresses pectate lyase, polygalacturonase, cellulase and protease production, *N*-(3-oxohexanoyl)-L-homoserine lactone (HSL) synthesis, and plant pathogenicity in *E. c.* subsp. *atroseptica*, *E. c.* subsp. *carotovora* (Ecc), *E. c.* subsp. *betavasculorum*, and *E. chrysanthemi*. Nucleotide sequencing, transcript assays, and protein analysis established that a 183 base pair open reading frame encodes RsmA. In Ecc strain 71, *rsmA* inhibits transcription of *hslI*, a *luxI* homolog required for HSL biosynthesis. These observations suggest that RsmA controls gene expression in soft rotting *Erwinia* by modulating the levels of HSL.

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ISOLATION OF GENES REQUIRED FOR ALGINATE BIOSYNTHESIS IN *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. S.P. Kidambi and C.L. Bender. 110 NRC, Oklahoma St. Univ., Stillwater, OK 74078.

Pseudomonas syringae pv. *syringae* FF5(pPSR12) is a stably mucoid strain which produces large amounts of the exopolysaccharide alginate in vitro. Alginate-defective (Alg$^-$) mutants of FF5(pPSR12) were generated by EMS and Tn*5* mutagenesis. A genomic library of FF5(pPSR12) was constructed in cosmid pRK7813 and mobilized into several Alg$^-$ mutants. Cosmid pSK2 restored alginate production to two Tn*5* mutants, and Southern hybridization analysis revealed that this cosmid contained homologs of *algD*, *alg8*, *algG*, *algL*, and *algA*, which are genes required for alginate biosynthesis in *Pseudomonas aeruginosa*. The organization of the alginate biosynthetic operon in *P. syringae* pv. *syringae* FF5 is similar but not identical to that previously described for *P. aeruginosa* 8830.

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PHENAZINE GENE EXPRESSION IN *PSEUDOMONAS AUREOFACIENS* 30-84 IS REGULATED IN PART BY A *GACA* HOMOLOGUE. Chancey, S.T., Pierson, L.S. III. Department Of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Two new mutants of *Pseudomonas aureofaciens* 30-84 deficient in phenazine antibiotic production were isolated. Mutant 30-84W arose spontaneously while mutant 30-84.A2 was isolated by transposon Tn*5* mutagenesis. Both mutants are Phz$^-$ and are unable to produce HCN. Both overproduce a fluorescent siderophore and siderophore production is no longer repressed by the presence of iron in the medium. Neither mutant is complemented by previously identified phenazine genes. Introduction of a cosmid from a genomic library of wild type 30-84 into both mutants resulted in the production of a novel brown pigment and restoration of siderophore regulation by iron. Brown pigment production was dependent on a functional phenazine biosynthetic region. This cosmid also restored 30-84W to Phz$^+$ but not 30-84.A2. The mutation in 30-84W is complemented by a cloned *gacA* gene from *P. fluorescence* CHA0. In other pseudomonads, *gacA* is involved in the regulation of antibiotic and cyanide production. This cosmid had no effect on 30-84.A2. These results suggest that phenazine gene expression is regulated, at least in part, by a *gacA* homologue and that phenazine antibiotic synthesis may be linked to siderophore biosynthesis.

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CLONING, CHARACTERIZATION, AND HETEROLOGOUS EXPRESSION OF GENES FROM *PSEUDOMONAS FLUORESCENS* INVOLVED IN THE SYNTHESIS OF PYRROLNITRIN. Steve Hill, Stephen T. Lam, Philip E. Hammer, and James Ligon. CIBA Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, North Carolina 27709-2257.

Pseudomonas fluorescens strain BL915 is an effective biocontrol agent against *Rhizoctonia solani*-induced damping off. This bacterium produces chitinase, cyanide, and pyrrolnitrin (Prn) (a secondary metabolite that inhibits *R. solani* and other fungi) under coordinate regulation by *gafA*. A mutant deficient in Prn production was created in a related strain BL914 (see Lam *et al.* poster). This mutant was used to isolate a cosmid clone of 30 kB from BL915 which complemented the Tn5 mutation in BL914. This clone was subjected to site-directed mutagenesis and individual Tn5 mutations were mapped and crossed back into the wild-type BL915 chromosome via double homologous recombination. Each mutant was assayed for Prn production and a genetic region of 6 kb was found to be involved in Prn production. This genetic region was sequenced and four open reading frames were identified. When expressed in *E. coli* and *Pseudomonas* the genes conferred the ability to produce Prn which was confirmed by HPLC, GC-MS, and NMR analysis.

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IDENTIFYING *GacA*-REGULATED GENES IN *PSEUDOMONAS FLUORESCENS* THAT PLAY A ROLE IN BIOLOGICAL DISEASE CONTROL. Nancy R. Torkewitz, M. Ellen Thompson, R. Allen Frazelle, Krista Gates, and Stephen T. Lam. CIBA Agricultural Biotechnology, P.O. Box 12257, Research Triangle Park, North Carolina 27709-2257

Transposon (TnCIB *116*) mutants of Pseudomonas fluorescens strain BL914 which showed differential expression in a GacA+ vs. a GacA- background (see poster by Lam et al) were screened for in-vitro production of known anti-fungal compounds and biocontrol efficacy in cotton / *Rhizoctonia solani* greenhouse assays. Many of the mutants produced all of the known antifungal compounds and provided good disease control. A pyrrolnitrin- mutant was identified; it provided reduced disease control. However, other mutants which produced all of the known antifungal compounds of the parent strain also provided reduced disease control. This method may lead to identification of *gac*A-regulated genes that are important in disease control.

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MOLECULAR ANALYSIS OF THE *ams*-REGION INVOLVED IN EXO-POLYSACCHARIDE SYNTHESIS OF *ERWINIA AMYLOVORA*. P. Bugert, S. Bereswill & K. Geider, Max-Planck-Institut für medizinische Forschung, Jahnstr. 29, D-69120 Heidelberg, Germany

Erwinia amylovora causes fire blight on apple and pear trees and other rosaceous plants. A capsule of the complex acidic exopolysaccharide (EPS) amylovoran protects the cells against recognition by plant defense mechanisms. The repeating unit of amylovoran consists of one glucuronic acid and four galactose residues. Its synthesis is regulated by *rcs*A and *rcs*B, which have been recently characterized. Most of the structural genes are located in the *ams*-operon, which is transcribed as a 16 kb mRNA. In addition to an RcsA-dependent promoter in front of *ams*G, individual genes within the operon are apparently preceded by sequences with promoter activities. The twelve genes of the *ams*-operon are followed by two genes involved in the synthesis of the EPS-precursors UDP-glucose and UDP-galactose. The *ams*-region corresponds to the *cps*-region of *Escherichia coli* at 44 min on the chromosomal map. Adjacent genes in *E. amylovora* are homologous to *udk-dcd* of *E. coli* and the *rfb*-region of *Salmonella thyphimurium*.

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CLONING AND CHARACTERIZATION OF AN *EXO*-POLYGALACTURONASE GENE FROM *PSEUDOMONAS SOLANACEARUM*. Qi Huang, Yaowei Kang, and Caitilyn Allen. Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706.

The bacterial wilt pathogen *P. solanacearum* produces one *endo*- and two *exo*-extracellular polygalacturonases (PG). The previously-described *endo*-PG PehA is a minor virulence factor, while an *exo*-PG, PehB, plays a more important role in disease development as determined by eggplant seedling bioassays. By complementation of a *pehB*::Tn*phoA* mutant. we cloned the Strain K60 *pehB*, which encodes a 75,000 kD MW *exo*-PG with an isoelectric point of 7.2. The cloned gene is being used for DNA sequence analysis, further enzyme characterization, and construction of multiple-PG deficient mutants. These mutants will allow us to test the individual and collective roles of *endo*- and *exo*-PG in bacterial wilt pathogenesis.

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LOSS OF GENES DUE TO CHROMOSOMAL REARRANGEMENTS IN AN INSECT NON-TRANSMISSIBLE LINE OF *SPIROPLASMA CITRI* BR3. Fengchun Ye, Ulrich Melcher and Jacqueline Fletcher. Departments of Plant Pathology and Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, OK 74078.

Spiroplasma citri, the causal agent of several important plant diseases, is transmitted from plant to plant by leafhoppers. Interaction between this wall-less prokaryote (Mollicute) and the insect involves passage of the spiroplasma through several physical barriers within the insect. An insect non-transmissible line, BR3-G, resulted from maintainance of the original insect transmissible line, BR3-3X, in plants by grafting over ten years. Physical genome mapping showed a chromosomal inversion and a 10-20 kb deletion at each of the two recombination sites in the genome of BR3-G. A 5.6 kb segment mapped to one of the deletion regions has been cloned and sequenced. It has at least seven ORFs, including a gene closely related to the transposase of spiroplasma virus SpV1-R8A2, which is possibly involved in the genetic rearrangements in this line. Another ORF encodes a membrane protein that shares weak similarity with the *Mycoplasma hominis* adhesin protein, a key element in mycoplasma attachment to host cells. Thus, it may be involved in the insect-spiroplasma interaction. Further study of this protein and its possible role in insect transmission are in progress.

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A BACTERIUM RELATED TO *Acidovorax facilis* OCCURS ON WATERMELON SEEDLINGS. N.C. Hodge[1], S. M. Baird[3], R. G. Gitaitis[3], D. L. Hopkins[2], and R. E. Stall[1]. University of Florida, [1]Gainesville, 32611-0680, and [2]Leesburg, 34748-8232; [3]University of Georgia, Tifton, 31793-0748.

Watermelon fruit blotch(WFB), caused by *A. avenae* subsp. *citrulli*, substantially affected watermelon production in continental USA in 1989, and every year thereafter. Another bacterium which can be confused with the WFB pathogen has been isolated each year since 1992 from watermelon seedlings. This bacterium causes restricted lesions on watermelon cotyledons with little or no watersoaking, and produces a hypersensitive reaction in tobacco. The organism rots watermelon fruit when wound-inoculated, but does not cause the fruit blotch symptom when spray inoculated onto fruit surfaces. Although the two bacteria have similar cultural characteristics, they differ in fatty acid profiles, malonate utilization, and colony morphology on a semi-selective medium, WFB 44. Fatty acid profiles of the non-aggressive bacterium matched most closely to those of *A. facilis* entries in the MIDI library (TSBA 3.80).

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VIRULENCE AND HYPERSENSITIVITY OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *SEPEDONICUS*. R. Nissinen[1], M.J. Laine[1], S.H. De Boer[2], P. Bauer[3], C. Ishimaru[3] and M.C. Metzler[1]. [1]Department of Biology, University of Turku, Biocity 6A, Turku, FIN-20520; [2]Agriculture Canada Research Station, 6660 NW Marine Dr., Vancouver, BC V6T 1X2, Canada; [3]Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523.

Pathogenicity and virulence of *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*), causal agent of bacterial ring rot, is strain and host dependent. Potato is the only natural host, although eggplant is used routinely as an indicator species. Fully expanded leaves of 8-9 week-old tobacco plants (cv. Xanthi) were infiltrated with about 10^8 cfu/ml of *Cms*. After 24-72 h at 20 C, infiltrated tissues were generally chlorotic and sometimes collapsed and necrotic. With the exception of one strain, all strains highly virulent in potato produced an HR-like reaction in tobacco. The strain that was highly virulent in potato but did not give an HR was markedly less virulent in eggplant. Strains that were not virulent in either eggplant or potato produced no reaction or only a slight chlorosis in tobacco. Thus, virulence of *Cms* is correlated in most cases with production of an HR-like reaction in a nonhost.

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FUNGAL INHIBITION AND SUPPRESSION OF ROOT DISEASE USING CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA*. Brion K. Duffy, Geneviève Défago, Institute of Plant Sciences, Swiss Federal Institute of Technology, Zürich CH-8092.

Pseudomonas aeruginosa is a cosmopolitan sp. with indistinguishable forms including human pathogenic isolates which raises questions for risk assessment when strains are used for biocontrol of plant diseases or bioremediation. From a collection of 19 clinical isolates screened *in vitro* for inhibition of *Gäumannomyces graminis*, *Rhizoctonia solani*, *Pythium ultimum*, and *Phomopsis sclerotioides* 8-15 were as effective or better than standard biocontrol strains of *P. fluorescens* (2-79, CHA0) and *P. aeruginosa*. (7NSK2, Lec1) depending on the pathogen. In natural soil, take-all suppression on wheat by 2 of the most inhibitory strains was comparable to 2-79 and 7NSK2. A clinical isolate from Australia, PAO1, controlled *Rhizoctonia* and *Pythium* on corn, *Pythium* on cucumber, and take-all to a level comparable with CHA0. A pyoverdine-pyochelin-salicylate-negative mutant was less competetive in the rhizosphere and was not disease suppressive while a *lasR*-pyocyanin-HCN-negative siderophore-overproducing mutant performed equal to or better than the wt. This suggests that siderophore production, a virulence factor in human pathogenesis, is also a primary mechanism of plant-disease suppression by PAO1. Avoiding species of clinical importance such as *P. aeruginosa* is the surest approach to avoid potential risks.

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SPREAD OF *PSEUDOMONAS SYRINGAE* IN THE PHYLLOSPHERE OF SNAP BEAN SEEDLINGS. S.S. Hirano, K.D. Fourrier, B.K. Riely, L.S. Baker, and C.D. Upper*, Dept. of Plant Pathology, USDA-ARS*, Univ. of Wisconsin, Madison, WI.

Spread of two genotypes of *Pseudomonas syringae* (Ps) across three different surfaces was examined in the field. Each plot consisted of three concentric squares. The sink or innermost square (6 m x 6 m) was surrounded by a 6 m wide barrier zone (snap bean, soy bean or bare ground). The barrier zone was surrounded by a 6 m wide source area. Source areas were planted with seeds inoculated with Ps B728a (wild type, causal agent of bacterial brown spot) or NPS3136 (*lemA*::Tn*5* non-lesion forming mutant of B728a). The first leaf samples were taken within 2 days after emergence of the bean seedlings in the sources. At this time, B728a and NPS3136 were detected in the sink areas at an overall frequency of 4.4% (n = 450 samples from 18 sinks). One week later, the frequency of detection in the sinks had increased to 51.8% (n = 450). There was no significant effect of bacterial genotype on the extent of spread at either sampling time. However, the nature of the barrier zone may have had an effect. Population sizes of the Ps strains were 3.80, 3.47, and 2.72 (as mean log cfu/leaf) in sinks surrounded by bare ground, soy bean, and snap bean, respectively. Thus, substantial spread of Ps occurred very early in the development of the bean seedlings and surfaces that were not favorable for growth of Ps did not impede and may have facilitated spread of Ps for distances > 6 m.

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INSECT-MEDIATED DISPERSAL OF *PSEUDOMONAS SYRINGAE* IN A BEAN FIELD. E.M. Groth, S.S. Hirano, L.S. Baker, and C.D. Upper*, Department of Plant Pathology and USDA-ARS*, University of Wisconsin, Madison, WI 53706.

Field experiments were conducted to examine the role of immigration in the development of populations of *P. syringae* (Ps) on snap bean leaves. Immigration was monitored continuously for 14 days by exposing petri dishes containing a medium selective for Ps. Deposition plates were exposed for 2 hr (day) or 4 hr (night) at each of 52 sites in a 90 m x 200 m bean field. When summed over the 14 days, the number of Ps detected on the deposition plates when the leaves were wet with dew (0200 to 0800) was roughly twice that found when the leaves were dry (0800 to 1600) and aerosol deposition was expected (Lindemann & Upper, Appl. Environ. Microbiol. 50:1229-1232, 1985). It was not uncommon to find 10 to 100 colonies of Ps distributed nonrandomly on the deposition plates exposed during the early morning hours. The patterns were suggestive of insects tracking across the plates. Of 47 insects trapped in petri plates shortly after sunrise, 67.5% were found to harbor Ps on their surfaces. The majority were flies of the genus *Delia* and potato leafhoppers (*Empoasca fabae*). Thus, insects may play an important role in dispersal of Ps when leaves are wet with dew.

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CLASSIFICATION OF PATHOGENIC STRAINS OF *STREPTOMYCES* SPP. USING FATTY ACID ANALYSIS. J.H. Bowers, L. L. Kinkel, and R. K. Jones. Dept. of Plant Pathology, University of Minnesota, St. Paul 55108.

Scab-inducing strains of *Streptomyces* isolated in Minnesota were distinguished from saprophytic field isolates and disease suppressive strains using cluster analysis of fatty acid profiles. Associations were observed for field isolates that closely matched the pathogenic fatty acid profile. All pathogenic, thaxtomin-producing isolates were identified by fatty acid analysis. In addition, all isolates that were not related to the pathogen library were non-pathogenic and non-thaxtomin producing. However, research in Canada using the same analysis, though slightly different methodology, could not differentiate pathogenic and non-pathogenic strains (Int. J. Syst. Bacteriol. 44:561-564). To address this inconsistency, we collected pathogenic *Streptomyces* isolates from the United States, Canada, and other regions of the world. Fatty acid profiles of these isolates are compared against our pathogen library to determine whether the variance among strains is geographic or otherwise. These results provide crucial insight into the potential utility of using fatty acid analysis to help resolve this complex taxonomic group and to determine whether pathogens are geographically localized or can be represented by a generalized fatty acid library.

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Worldwide Genetic Variation in *Xanthomonas albilineans*. M. J. Davis[1], C. J. Warmuth[1], P. Rott[2], M. Chatenet[2], and P. Baudin[2]. [1]University. of Florida, TREC, 18905 SW 280 Street, Homestead, FL 33031; [2]Centre de Coopération Internationale en Recherche Agronomique pour le Développement, CIRAD-CA, BP 5035, 34032 Montpellier Cedex, France

Leaf scald disease of sugarcane is caused by *X. albilineans*. Restriction fragment length polymorphisms (RFLP) of high molecular weight DNA fragments produced with *Spe*I and separated by pulse-field gel electrophoresis were used to examine the genetic variability of 217 strains of the pathogen from throughout the world. Most strains (203) could be assigned to one of four groups. The remaining 14 strains were not assigned to these groups due to their unusual RFLP patterns. Recent outbreaks of leaf scald in Florida, Louisiana, and Texas apparently followed the introduction of a genetic variant of the pathogen into the southeastern United States. Strains of only two genetic groups were identified among the 85 strains examined from the United States. The existence of genetic variants of the pathogen and their limited geographic distribution are important considerations in the management of leaf scald disease.

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ANALYSIS OF THE *ExuT* DNA IN *ERWINIA CHRYSANTHEMI*. M. Melkus, B. Haseloff, T. Freeman and M. San Francisco, Texas Tech University, Lubbock, TX 79409.

Products of pectin degradation are taken up into *E. chrysanthemi* for utilization as carbon sources and pectinase induction. The uptake of these compounds must contribute to the virulence phenotype of the bacterium. EC16 and CUCPB0873 GA transport mutants were generated using *nptI/sacB/sacR* marker exchange mutagenesis, and assessed for their relevance to the virulence phenotype. *exuT* mutants compared to wild type *Erwinia* grown on GA-supplemented minimal media had retarded growth. The infection rate of the mutant stains compared to the wild type *Erwinia* revealed delayed infection in tobacco leaves and witloof chicory. Localization of the gene products encoded by the 3.4 kb *EcoRV* fragment was carried out using the T-7 RNA polymerase expression system.

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MOLECULAR CLONING OF THE BLUE-PIGMENT RELATED GENES FROM *ERWINIA CHRYSANTHEMI* (ECH) STRAIN RA3B AND USE OF THE GENES FOR IDENTIFICATION OF ECH BY PCR TECHNOLOGY. Hsiou-Chen Huang[1], M.-K. Chu[1,2], R.-H. Lin[1], K.-C. Tzeng[2] and S.-T. Hsu[2]. [1]Agricultural Biotechnology Labs. and [2]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan.

Some strains of Ech form an extracellular insoluble blue pigment, indigoidine, in several growth media. In this study, a 6-kb *Eco*RI fragment cloned in pCPP30 was isolated from Ech RA3B chromosome and could restore pigmentation to three transposon-induced nonpigmented mutants of this strain. The nucleotide sequence analysis, Tn*phoA* mutagenesis, and complementation assay revealed that this DNA fragment contained one partial open reading frame (orf) and at least five complete orfs, designated as *argG*, *pecS*, *pecM*, *idgA*, *idgB* and *idgC*, respectively. Except for the argG gene, these genes controlled the blue pigment synthesis. Also, an ERIC sequence was found to be located downstream of *idgB* gene. Two pairs of primers complementary to *pecS* and *idgC* genes, respectively, could be used to identify 93% of Ech strains by PCR. The PCR results indicate that most strains of Ech should have blue-pigment related genes which may not express in some strains.

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GALLIC ACID PRODUCTION IN *XANTHOMONAS CAMPESTRIS* pv. *PRUNI*. S. J. Kent and D. F. Ritchie. Dept. of Plant Pathology, Box 7616, North Carolina State University, Raleigh, 27695.

Gallic acid is considered an antimicrobial compound. Its production by bacteria is uncommon except for degradation of gallotannins. Strains of *X. c.* pv. *pruni* (*Xcp*), the causal agent of bacterial spot in stone fruit, differ in both timing and quantity of gallic acid produced in defined medium containing quinic acid. Gallic acid was measured spectrophotometrically using a rhodanine assay. Gallic acid production ranged from 2 ug/mL to 70 ug/mL for 73 strains inoculated at levels of 10^6 - 10^7 cfu/mL into defined medium containing 5 mg/mL quinic acid. Bacterial populations of high gallic acid producing strains increased two log units over three days and then declined. Populations of low gallic acid producing strains remained the same or decreased less than one log unit over six days. Gallic acid production in medium containing 100 - 500 ug/mL quinic acid was detected in some strains at less than 2 ug/mL. Since quinic acid esters occur widely in stone fruit, the possibility that gallic acid may act as a pathogenicity/virulence factor in *Xcp* is being investigated.

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PROTOCOLS FOR SAMPLING EPIPHYTIC BACTERIA: HOW LONG DO I SHAKE, WHAT DO I SHAKE IT IN, ETC.? Ken Pernezny, Janice Collins, and Myrine Hewitt. Everglades Research and Education Center, University of Florida, Belle Glade, FL 33430

Several factors were tested for their effect on recovery rates and population estimates of epiphytic, plant-pathogenic bacteria on leaves of homologous hosts. When a 10^8 cfu/ml suspension of *Xanthomonas campestris* pv. *vesicatoria* was sprayed on shoots of pepper plants and allowed to dry and detached leaves shaken for time spans of 5 min to 2 hr, no significant differences in log cfu/g leaf tissue were found (5 min, 7.14; 20 min, 7.23; 1 hr, 7.45; 2 hr, 7.50). Within the same time frame, no differences were evident when phosphate-buffered saline was compared to a washing buffer containing 0.1% peptone, or with sterile, distilled water. No differences were found when three rotary shaker speeds were compared (100 rpm, 7.29; 250 rpm, 7.72; 350 rpm, 6.99). Similar results were recorded for *Pseudomonas cichorii* on celery and *Erwinia carotovora* subsp. *carotovora* on lettuce. Similar results were found when bacterial suspensions were allowed to dry for 24 hr. Therefore, researchers have considerable latitude to choose a protocol on the basis of labor, time, and monetary constraints.

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EFFECT OF RATOON STUNTING DISEASE RESISTANCE ON DISEASE SPREAD IN SUGARCANE IN FLORIDA. **J. C. Comstock[1], J. M. Shine, Jr.[2], M. J. Davis[3], and J. L. Dean[1] (Retired). [1]USDA-ARS, and [2]Florida Sugar Cane League, Canal Point; and [3]U of FL, TREC, Homestead.**

Spread of ratoon stunting disease (RSD) by harvesting was influenced by cultivar resistance. RSD spread was in the direction of harvest down the row to uninoculated plants. RSD increased in uninoculated plants through the croping cycle as did the number of hand harvests. The annual rate of spread was correlated (r=0.93) to the number of colonized vascular bundles (CVB). The % RSD stalk infection was correlated (r=0.8) to CVB in commercial fields that had no history of phytosanitary or heat-therapy control practices. Two cultivars in commercial fields had <3% infected stalks without any control measures.

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SPREAD OF *XANTHOMONAS CAMPESTRIS* PV. *TRANSLUCENS*. IN WHEAT STUDIED WITH A RIFAMPICIN-RESISTANT STRAIN. K.M. Tubajika[1]., J.S.Russin[1]., C.A. Clark[1] and S.A. Harrison[2]. [1]Dept. Plant Path. & Crop Phys. and [2] Dept. Agron. Louisiana State University Agricultural Center. Baton Rouge 70803

Xanthomonas campestris pv. *translucens* (Xct) causes bacterial leaf streak and black chaff in Louisiana wheat fields.Two experiments, using strain 88-14^{rif} of Xct, were conducted to study the temporal and spatial spread of BLS from line and point inoculum sources in susceptible (Florida 304) and resistant (Terral 101) wheat cultivars. Spread of Xct was determined bi-weekly beginning 29 Jan. Five leaves were randomly-sampled at 2 m interval, bulked, and assayed for the presence of Xct 88-14^{rif} on Wilbrink's medium amended with cycloheximide, rifampicin and cephalexin, all at 100 mg/l. Xct was detected at 0, 2, 4, and 6 m distance intervals in Florida 304 and at 0, 2, and 4 m in Terral 101, 40 days after inoculation. Bacterial population decreased with increasing distance from point and line sources. Bacterial populations were higher at each distance in Florida 304 than in Terral 101. Disease gradients will be compared to pathogen detection gradients in the different varieties.

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SUDDEN DEATH SYNDROME OF SOYBEAN: FURTHER CHARACTERIZATION AND PURIFICATION OF THE TOXIN PRODUCED BY *FUSARIUM SOLANI*. H. Jin[1], G. L. Hartman[1, 2] and J. M. Widholm[3]. [1]Dept. of Plant Pathology, [2]USDA/ARS, [3]Dept. of Agronomy, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Ave., Urbana, IL 61801

A 17 kD phytotoxic polypeptide was identified from culture filtrates of *Fusarium solani* the causal agent of soybean sudden death syndrome. The phytotoxoin was heat unstable, negatively charged, absorbed by 10% charcoal and destroyed by proteinase K. Purification of the phytotoxin was achieved by gel filtration of Sephadex G-50 chromatography followed by ion exchange chromatography on a DE-52 column. The purified protein migrated as a single band on SDS polyacrylamide gels. The N-terminal 15 amino acids were sequenced. Samples containing this single protein caused browning on soybean calli, necrosis on detached soybean cotyledons, and caused yellowing, curling, and drying on intact cotyledons and leaves of seedlings.

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REVERSAL OF EDDHA-MEDIATED GROWTH INHIBITION BY ADDITION OF IRON-BINDING COMPOUNDS PRODUCED BY *POSTIA PLACENTA* AND *GLOEOPHYLLUM TRABEUM*. Brian Doyle and Jody Jellison, Dept. of Plant Biology and Pathology, University of Maine Orono, ME 04469

In the wood decaying basidiomycetous fungus, *Postia placenta*, growth inhibition by EDDHA-induced iron limitation was reversed by the addition of ferrated iron-binding compounds (putative siderophores) isolated previously from *P. placenta*. A diffuse zone of growth characterized by large diameter colonies displaying aerial hyphae surrounded the delivery tube which was partially embedded in the solid plating medium. Similar results were also observed in *G. trabeum* which received iron-binding compounds previously isolated from *G. trabeum*. These iron nutrition bioassays suggest that the iron-binding compounds isolated from *P. placenta* and *G. trabeum* might function as siderophores for these wood-decay fungi.

600

DISSECTION OF THE BIOCHEMICAL AND REGULATORY EVENTS IN THE BIOSYNTHESIS OF CERCOSPORIN. Maura J. Meade and R. G. Upchurch. Department of Plant Pathology, Box 7616, North Carolina State University, Raleigh, NC 27695.

The phytopathogenic fungus, *Cercospora kikuchii*, produces a pathogenicity factor of polyketide origin. This toxin, cercosporin, has a biosynthetic pathway which is largely uncharacterized. We have been using various biochemical and molecular biological techniques in an attempt to elucidate the cercosporin biosynthetic pathway. Newly developed organic extraction procedures and subsequent biochemical analyses have led to the investigation of three fluorescent compounds as potential pathway intermediates. Cercosporin biosynthesis in this organism is thought to be similar, if not identical, to toxin synthesis in other *Cercospora* species. Because of the broad range of plants infected by cercosporin producing fungi, an understanding of this biosynthetic pathway may have far-reaching implications. Regulatory events are being investigated by genetic complementation of a toxin regulatory *C. kikuchii* mutant.

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DIFFERENTIAL EXPRESSION OF PATHOGENESIS-RELATED PR10 MULTIGENE FAMILY PARALLELS EVOLUTION OF PLANT/ FUNGAL COMPATIBILITY. S. Tewari and B. W. Fristensky, Dept. of Plant Science, Univ. of Manitoba, Winnipeg, MB, Canada R3T 2N2.

RT-PCR was used to analyze transcript accumulation of individual PR10 multigene family members in domestic and wild species of pea in response to inoculation with *Fusarium solani* f. sp. *phaseoli* and f. sp. *pisi*. In domestic pea, PR10a transcript accumulated rapidly while PR10b accumulation was not only delayed but also lower in abundance in response to salicylic acid, abscissic acid and the incompatible pathogen, *F. s.* f. sp. *phaseoli*. In response to *F. s.* f. sp. *pisi*, both PR10a and PR10b expression was delayed. PR10c was not induced with any treatment. Wild peas, in contrast, responded with higher accumulation of PR10b than PR10a in response to both pathogens.

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ELICITATION OF PHYTOALEXINS IN CAPSELLA BURSA-PASTORIS IN RESPONSE TO ALTERNARIA BRASSICAE. L.D. Jimenez[1], W.A. Ayer[1], and J.P. Tewari[2], [1]Department of Chemistry, and [2]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

The common weed, Capsella bursa pastoris (L.) Medic. (shepherd's purse) is known to be resistant to Alternaria brassicae (Berk.) Sacc. and elicits phytoalexins when challenged by this pathogen. Three phytoalexins were isolated and identified as camalexin ($C_{11}H_8N_2S$), 6-methoxycamalexin ($C_{12}H_{10}N_2SO$), and N-methyl-camalexin ($C_{12}H_{10}N_2S$). Camalexin and methoxycamalexin were originally described from Camelina sativa (L.) Crantz. N-methyl-camalexin is a new phytoalexin, though this compound has been known through chemical synthesis. Resistance to disease and flea beetles, cold tolerance, and short life-cycle are reported traits of C. bursa-pastoris that make it a promising candidate for use in canola and mustard improvement programs.

603

SUPEROXIDE DISMUTASE: A DIFFERENTIATION PROTEIN EXPRESSED IN *UROMYCES* GERMLINGS DURING EARLY APPRESSORIUM DEVELOPMENT. Jana S. Lamboy, Harvey C. Hoch, and Richard C. Staples. Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.

Germlings of the bean rust fungus, *Uromyces appendiculatus*, detect penetration sites on the surface of the host leaf by thigmosensing topographical features. Within 2-4 min after the apex of a urediospore germ tube encounters the cuticular lip of a stomate, the germling ceases polarized growth and begins to swell over the aperture. The mechanism by which the cells detect topographical signals is not understood, however, previous experiments indicated that the initiation process does not involve de novo gene expression. In order to detect post-translational modifications, the protein profiles of induced and non-induced germlings were compared at the earliest stages of appressorium formation, and a 21 kDa differentiation protein was identified by a shift in isoelectric point. The N-terminal amino acid sequence exhibited homology with superoxide dismutase (SOD), and antibodies to a synthetic peptide fragment with that sequence recognize copper/zinc isozymes of SOD in native gels. Electroelution of the active enzyme bands and separation with SDS PAGE suggest that the enzyme in protein extracts of topographically induced germlings is an 85 kDa tetramer. We will investigate the possible roles of reactive oxygen species in intracellular signaling and adhesion processes.

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TRANSGENIC STRATEGIES FOR NEMATODE RESISTANCE IN TOBACCO. Andrea M. Skantar, Christopher G. Taylor, David M. Saravitz, Mark A. Conkling, and Charles H. Opperman, Depts. of Plant Pathology and Genetics, N. C. State University, Raleigh, NC.

Root-knot nematodes are obligate plant parasites that induce development of an elaborate feeding site during root infection. Feeding site formation results from a complex interaction between the pathogen and the host plant in which the nematode alters patterns of plant gene expression within the "giant cells" destined to become the feeding site. Our goal is to interfere with development of the feeding site via the specific expression of toxin genes within the giant cells. The nematode's inability to establish a proper feeding site would lead to death of the pathogen due to starvation. The tobacco gene *TobRB7* encodes a water channel protein that is expressed in the developing vascular tissue in uninfected roots and in giant cells during infection by root-knot nematodes. Analysis of the *TobRB7* promoter has revealed cis-acting elements responsible for root-specific and nematode-inducible expression of the gene. Transgenic plants have been constructed with these promoter elements driving the expression of a toxin gene, the RNAse gene *barnase*. We have obtained nematode-resistant plants using this strategy; however, the extreme toxicity of the *barnase* gene has prevented us from establishing stable resistant lines. We hope to rectify this problem by using translational control to attenuate the level of *barnase* gene expression in transgenic plants.

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FURTHER GENETIC AND BIOCHEMICAL CHARACTERIZATION OF A NEMATODE EGG-KILL FACTOR PRODUCED BY *PSEUDOMONAS AUREOFACIENS* BG33.
P.Wechter, B.Leverentz, D.C.M.Glandorf, D.Kluepfel, Dept. of Plant Pathology and Physiology, Clemson University, 120 Long Hall, Clemson, SC 29634-0377.

Five Tn5 mutants of the wildtype BG33 lack egg-kill activity are hyperfluorescent and lack protease production. From mutant 1397, a 1.8kb fragment containing a portion of a gene involved in egg-kill factor production has been cloned, radiolabelled and used to probe the BG33 genomic library. Six cosmid clones from the wildtype library strongly hybridized to the probe. The 1.8kb fragment has been sequenced and its position mapped in two of the hybridizing cosmid clones. The egg-kill factor is contained in the cell free supernatant of BG33 liquid cultures, is stable in a temperature range from -80°C to +121°C, and is resistant to protease treatment. The egg-kill activity decreases in the presence of Fe^{3+} and low pH. The initial data indicate this factor to be a polar molecule with a high molecular weight. Size fractionation of the supernatant by gel filtration is being utilized to purify the egg-kill factor.

606

BACTERIAL SOFT-ROT SYMPTOM DEVELOPMENT IN POTATO TUBERS PRIOR TO HARVEST. Gary D. Franc, Colette M-S. Beaupre and John E. Yetka. University of Wyoming, Department of Plant, Soil and Insect Sciences, P.O. Box 3354, Laramie, WY 82071.

Potato tubers (cv. Russet Burbank) exhibiting surface lesions, described as deep pits and furrows, were observed during harvest of a potato field in Montana. Symptomatic and asymptomatic tubers were examined for determination of a probable cause. Pits generally ranged in size from 5 - 23 mm diameter and 4 - 10 mm deep. Furrows presumably resulted when pits merged. Close examination of lesions showed starch and periderm residue, which indicated that tuber tissue had collapsed during lesion formation. Examination of lesion margins showed a sharp demarcation between healthy and affected tissue. Tuber soft-rot potential was determined by incubating tubers under anaerobic conditions for 6 days at 24 C. Tuber examination showed both symptomatic and asymptomatic tubers developed new lesions similar in appearance to those observed on symptomatic tubers recovered from the field. Bacterial strains recovered from lesions were characterized as *Erwinia carotovora*. Results showed original tuber symptoms were arrested soft-rot lesions that developed during the growing season and became desiccated prior to harvest. Results also showed that tubers harvested from the field had a high soft-rot potential and that soft-rot erwinias were, at least partially, involved.

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BIOLOGICAL CONTROL OF FIRE BLIGHT USING *ERWINIA HERBICOLA* Eh252 and *PSEUDOMONAS FLUORESCENS* A506 SEPARATELY OR IN COMBINATION. J. L. Vanneste, and J. Yu, Hort Research, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand.

Erwinia herbicola Eh252 and *Pseudomonas fluorescens* A506 were sprayed separately or in combination onto newly open apple or Asian pear blossoms during flowering. Treatments were applied to run off using a random block design. The day following treatment the same blossom clusters were sprayed to run off with a suspension of *Erwinia amylovora*, the fire blight pathogen. The number of healthy and diseased clusters were recorded five weeks after inoculation. In the two experiments, the level of control obtained using both strains together was not significantly different from that obtained with either strain separately. This is in agreement with results obtained on immature pear fruits in the laboratory.

608

BIOLOGICAL CONTROL OF TAKE-ALL USING ANTAGONISTIC STERILE FUNGI. N. Zriba and D. E. Mathre, Dept. of Plant Pathology, Montana State University, Bozeman, MT 59717.

Two sterile fungi isolated from a take-all suppressive soil in Montana were tested under field conditions for their ability to protect wheat from infection by *Gaeumannomyces graminis* var. *tritici* (Ggt). These two fungi (I-52 and I-58) were significantly antagonistic to Ggt *in vitro*. The two fungi were grown on two substrates (oat kernels and canola seed), added separately and combined (I-52, I-58 and I-52 + I-58), at low and high rates relative to Ggt (3:1 and 9:1 w/w), to three soils that differed in level of suppressiveness. Take-all infection and plant growth were evaluated on seedlings and mature plants. Overall, canola and the high rate of antagonist resulted in the least infection and highest plant growth among the Ggt inoculated treatments. I-52 alone was more effective in controlling take-all than I-52 combined with I-58 or I-58 alone. I-52 was more effective in its original soil than in a different suppressive soil. Antagonism of I-52 may involve nutrient competition, niche colonization or induction of plant resistance and growth.

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Biological control with *Actinoplanes* spp. of Pythium damping-off of geranium and poinsettia. A. B. Filonow and J. Dole, Departments of Plant Pathology and Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK. 74078.

Strains W57, W257 or 25844 of *Actinoplanes* spp. on clay granules applied at 5% or .5% w/w to soil-less potting mix 5 days prior to transplanting geranium or poinsettia seedlings reduced ($P < 0.05$) root rot caused by *Pythium ultimum* and increased stand compared to controls after 6 wk in the greenhouse. Damping-off in these plants was reduced at the 5% w/w rate, but not at .5% w/w in mix infested with *P. irregulare*. In mix infested with *P. ultimum*, W257 applied at 5% w/w to mix 7 days prior to transplanting reduced root rot and increased stand of poinsettia compared to the control after 6 wk. Seedling dips in a suspension of macerated mycelium of W257 (10^9 cfu/ml) only reduced root rot.

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Population dynamics and competition between wild type and mutant isolates of Colletotrichum gloeosporioides f. sp. aeschynomene on northern jointvetch in the field. Y, Luo, D.O. TeBeest, Department of Plant Pathology, University of Arkansas, 217 Plant Sci. Fayetteville, AR 72701

Population dynamics and competition among wild type (WT), benomyl resistant (B21) and nitrate reductase deficient (Nit A) isolates of C. gloeosporioides f. sp. aeschynomene were studied on northern jointvetch in the field in 1994. Three replicated competition treatments: A, WT vs B21; B, WT vs Nit A; and C, WT vs B21 vs Nit A, were tested in small pools containing 8-9 NJV plants. Two or (treatments A and B) or three lesions (treatment C) produced by mechanical inoculation of plants in each pool were used to initiate disease. Approximately 100 new lesions were collected and sampled every 7-10 days. Tissue pieces were placed on selective media and the proportion of each isolate at each sampling date was calculated. In treatments A and B, the WT isolate reached and maintained 70-80% of the population within one month after inoculation. In treatment C, mutant isolates were isolated from less than 10% of the lesions. Mutant isolates were less competitive than a wild type isolate in the field.

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STRUCTURAL CHANGES IN BACTERIAL COMMUNITIES ASSOCIATED WITH INTRODUCTION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA. W.F. Mahaffee, E.M. Bauske and J.W. Kloepper, Department of Plant Pathology, Biological Control Institute, Auburn University, AL 36849.

The bacterial communities of three habitats (rhizosphere, internal root tissue and soil) were examined for quantitative and qualitative changes associated with the introduction of a Plant Growth-Promoting Rhizobacteria (PGPR) strain and a genetically engineered microorganism. A field release on cucumber was established using wild-type PGPR strain *Pseudomonas fluorescens* 89B-27 and its bioluminescent derivative. A noninoculated control was used for comparison. Soil, and root samples were taken 0, 7, 14, 21, 35, and 75 days after planting and processed for enumeration and isolation of bacterial colonies on 5% TSA. As determined by rarefraction analysis of preliminary isolations, 25, 35, and 35 colonies from internal, rhizosphere and soil samples, respectively, were picked, streaked for purity and stored at -80 C until processed for identification by GC-fatty acid methyl ester analysis using the Microbial Identification software. More than 9,500 bacterial isolates were identified to species. Examination of the community structures of the three habitats indicated that all were significantly different ($P=0.05$) in both species number and composition, demonstrating that they are distinct habitats and not continuations of each other. Analysis of the rhizosphere community for each treatment demonstrated that the community structure of both bacterial treatments differed significantly from the noninoculated control, but they did not differ from each other. This supports previous speculations that PGPR alter the rhizosphere community, and that this alteration could be a mechanism by which pathogens are controlled. Several diversity, and similarity indices were examined and their usefulness will be discussed.

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EVALUATION OF A *TRICHODERMA* SP. AS A POTENTIAL BIOLOGICAL CONTROL AGENT FOR CHESTNUT BLIGHT. E.Y. González, M.S. Mount, P.M. Berman, and T.A. Tattar, Dept. of Plant Pathology, Univ. of Mass., Amherst, MA 01003.

A *Trichoderma* (TD) isolate from the bark of a large, apparently healthy American chestnut (*Castanea dentata*) was evaluated for potential use as a biocontrol agent against the fungus *Cryphonectria parasitica* (CP). The TD was able to stop the growth of a virulent CP on potato dextrose agar before completely overgrowing the CP. Greenhouse experiments on 2 year old American chestnut seedlings were conducted. The treatments (in random block design) were as follows: 1) TD inoculation followed 2 days later by CP inoculation; 2) CP followed by TD; 3) CP followed by sterile H_2O; 4) H_2O followed by CP; 5) TD followed by H_2O; 6) H_2O followed by TD; 7) H_2O followed by H_2O. The experiment was repeated 4 times with 5 replicates per treatment. Cankers developed and encircled the trees with treatments 3 and 4. No cankers developed with control treatments 5-7. Initial canker enlargement was slower with treatment 2, and canker development was significantly inhibited with treatment 1. Results indicate that pretreatment with TD inhibits canker development caused by CP. This may be highly useful for managed plantings of American chestnut.

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GLIOCLADIUM ROSEUM EFFECTIVELY SUPPRESSES *BOTRYTIS CINEREA* IN GREENHOUSE-GROWN BEGONIA. Li, De-Wei, John C. Sutton and Gang Peng Dept. of Environ. Biol., Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1.

In biocontrol tests, begonia was inoculated with *B. cinerea* and 24 h later with biocontrol organisms. Effectiveness of biocontrol was estimated by counting conidiophores produced by the pathogen on treated tissues. Isolates of *G. roseum* from the begonia suppressed *B. cinerea* on begonia leaf disks by 94-100%, and were as effective or more effective than 84 other microbial isolates from begonia. Effects of the inoculum concentration of *B. cinerea* (0, 10^3, 10^4, 10^5 and 10^6 conidia/mL) and of *G. roseum* (0, 10^4, 10^5, 10^6, 10^7 and 10^8) on biocontrol were examined. *G. roseum* suppressed *B. cinerea* completely in leaves and sepals, and by 75-98% in petals, when applied at the same or higher concentration as the pathogen, but was less effective at lower concentrations. In a study of temperature effects, tissues were inoculated with *B. cinerea* (10^6) alone or in combination with *G. roseum* (10^7) or with chlorothalonil (2.5 g a.i./L) and kept at 10, 15, 20, 25, and 30°C. *G. roseum* suppressed *B. cinerea* in leaves and flowers by 100% and 85-90% respectively at 20-30°C, by about 80% and 40% respectively at 10-15°C, and in each instance more effectively than the fungicide. We conclude that *G. roseum* is a powerful biocontrol agent against *B. cinerea* in begonia.

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A PROCEDURE FOR QUANTITATIVE INOCULATION OF SAFFLOWER (*CARTHAMUS TINCTORIUS*) WITH *PUCCINIA CARTHAMI*. W. L. Bruckart and D. L. Koogle, USDA-ARS, FDWSR, Frederick, Maryland 21702.

Precise quantification is needed of safflower seedling susceptibility to *P. jaceae* from yellow starthistle (*Centaurea solstitialis*). A dose-response study with the compatible safflower rust fungus, *P. carthami*, was used. Germinated safflower seeds were inoculated on the hypocotyl near the cotyledons with 1 μl drops of teliospores in water (with 0.125% v/v Silwet®) containing 2.5 to 20 μg teliospores/μl (1 μg = 62 $\pm$ 16 viable teliospores [p = 0.05]). Regression analyses of data collected 6 wk after inoculation showed a linear relationship between teliospore weight per seed and safflower height, fresh weight (wt), and dry wt. Inoculation with 20 μg of *P. carthami* spores caused reductions of 46.9%, 49.2%, and 51.5%, respectively, in plant height, fresh wt, and dry wt, compared with controls. This demonstrates the potential for precise inoculation of safflower in host range determinations and other applications.

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EGYPTIAN STRAIN OF *BACILLUS THURINGIENSIS* FOR THE CONTROL OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*. *A. M. Tohamy*, M.A.El Saedy, Y. A. Osman and M. A. Madkour. Agricultural Genetic Engineering Research Institute, Giza, and Dept. of Plant Pathology, University of Alexandria, Alexandria, Egypt.

An isolate of *Bacillus thuringiensis*, isolated from Egyptian soil, was evaluated for efficacy in controlling the root-knot nematode, *Meloidogyne incognita* race1, on tomato in a greenhouse trail. This isolate was first shown to have a potential insecticidal effect against a considerable number of economically important insect pests in *in vitro* laboratory assays. Sporulated bacterial cells (72hrs old) at the rate of 0.1g/liter were applied as soil drench one week either before or after nematode inoculation to potted tomato plants (4wks old). Treatments resulted in a significant reduction in numbers of galls and egg-masses on tomato roots when compared with nematode alone in both autoclaved and non-autoclaved soils. However, in non-autoclaved soil the effect of this isolate was superior to autoclaved soil.

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EVALUATION OF *BACILLUS* AND *PSEUDOMONAS* ISOLATES FROM TENNESSEE SOIL FOR BIOLOGICAL CONTROL OF TAKE-ALL. B.L. Clark, R.B. Reeder, and B.H. Ownley. Entomology & Plant Pathology Dept., Univ. of Tennessee, Knoxville, TN, 37996.

Bacillus and *Pseudomonas* isolates from no-till, double-cropped, wheat-soybean field soil in Tennessee inhibited growth of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) *in vitro*. *Ggt* causes take-all of wheat. Isolates were tested in the greenhouse for the ability to control take-all in a local soil. Surface-disinfested wheat seeds (cultivar FFR525) were coated with bacteria suspended in a 0.5% methylcellulose (MC) solution (1×10^8 cfu/seed) then planted into Conetainers® filled with autoclaved soil infested with *Ggt* (1%, w/w). At the 3-4 leaf stage, shoot height was measured and disease severity was rated (0 to 8 scale; 0=no lesions, 8=dead). Compared to the MC control, the greatest reduction ($P=0.05$) in disease severity was obtained with two *Pseudomonas* isolates, MF102 and MF103, and one *Bacillus* isolate, MB105, (23%, 25%, and 26%, respectively). Also, shoot height was increased ($P=0.05$) by MF102, MF103, and MB105 (22%, 17%, and 12%, respectively). MF102, MF103, and MB105 were more effective in reducing take-all than *P. fluorescens* Q2-87 (12%, NS), a strain shown to be effective against take-all in Pacific Northwest soils. These results support prior evidence that successful biocontrol of take-all will involve the use of locally isolated strains.

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PREY AGGREGATION AND FORAGING EFFICIENCY OF A MYCOPARASITIC BIOCONTROL FUNGUS. G. R. Knudsen, Plant Pathology/Soil Science, University of Idaho, Moscow.

Trichoderma harzianum is a mycoparasite of soil fungi including *Sclerotinia sclerotiorum*; colonization of sclerotia can result in biological control. Hyphal segment growth rates and branching frequency of *T. harzianum* in soil were mapped and quantified by digitizing and image analysis. Branching frequency was reduced when hyphae traversed regions between prey (sclerotia), but increased after contacts with sclerotia. A highly branched growth form may enhance encounters with aggregated prey, but may waste the foraging individual's energy with dispersed prey. It follows that the degree of aggregation of prey and the growth characteristics of a foraging mycoparasite may be partial determinants of biocontrol efficacy. An individual-based simulation model, which predicts length, location and branching of hyphal segments, was used to explore the relative efficiencies of different foraging strategies for different spatial arrangements of sclerotia. Quantifying the foraging efficiency of a mycoparasite has practical application in the design and implementation of biocontrol strategies.

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PRIMERS AND PROBES TO DETECT SOIL PSEUDOMONADS THAT PRODUCE 2,4-DIACETYLPHLOROGLUCINOL AND PHENAZINE-1-CARBOXYLIC ACID. Jos Raaijmakers, David Weller, Robert Bonsall, Linda Thomashow. USDA-ARS, Washington State University, Pullman, WA 99164.

Take-all decline (TAD) is a naturally occurring biological control of take-all of wheat. It has been postulated that TAD occurs during the monoculture of wheat because of a gradual build-up of fluorescent pseudomonads that produce antibiotics such as phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (Phl). To quantify antibiotic producers in suppressive and conducive soils we developed primers and probes directed against specific sequences within the biosynthetic loci of PCA and Phl. The primers for Phl amplify an 876-bp fragment from purified DNA and from whole cells of Phl-producing *Pseudomonas* strains, like *P. fluorescens* Q2-87. The primers for PCA amplify an 1150-bp fragment from purified DNA and whole cells of PCA-producing strains, like *P. fluorescens* 2-79. The specificity of the primers and probes will be discussed as well as their use to determine the frequency of PCA- and Phl-producing fluorescent pseudomonads in soils suppressive of take-all.

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FIELD TEST OF SATELLITE-TRANSGENIC TOMATO RESISTANCE AGAINST CMV. T. Wai[1], J. R. Stommel[2], M. E. Tousignant[1], and J. M. Kaper[1], USDA, ARS, BA, PSI, [1]Molecular Plant Pathology Laboratory, [2]Vegetable Laboratory, Beltsville, MD 20705.

Cucumber Mosaic Virus (CMV) causes disease in many crop plants, including tomato. A novel biocontrol approach employing the concept of molecular parasitism was attempted in this field test. Satellite RNAs are believed to compete for the replicase enzyme encoded by the helper virus, thereby reducing virus titers. 'UC82B', 'Lichun', and satellite-transgenic lines derived from them were challenged with CMV and transplanted to the field. Reduced levels of virus were detected by ELISA and decreased symptoms were observed for the satellite-transgenic lines. The total marketable fruit yield from the satellite-transformed UC82B line inoculated with CMV was 50% greater than that from the CMV-infected non-transformed UC82B line.

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MOLECULAR MARKERS IN OATS FOR RESISTANCE GENES TO OAT MOSAIC VIRUS AND OAT GOLDEN STRIPE VIRUS. S.L. Walker, S. Leath, J.P. Murphy, S.A. Lommel, Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

Coker 716, a hexaploid winter oat cultivar resistant to oat mosaic virus (OMV) and oat golden stripe virus (OGSV) was crossed to three susceptible oat cultivars: Brooks, Madison, and Tech. A total of 190 lines was developed from the three crosses and was evaluated in two environments in both the $F_{2:3}$ and $F_{2:4}$ generations for resistance to OMV and OGSV and yield in field plots over two years, respectively. Germplasm was evaluated in pathogen-infested plots and in non-infested plots. Yield was reduced 50 percent on average for plants grown in infested plots when compared to plants in non-infested plots for the 93-94 growing season. Correlation of yield with disease rating produced a mean value of -0.57 for the 190 lines during the 93-94 season. The phenotypic data from each line is being used to evaluate RAPD markers found in each of the four parents. Correlation of RAPD markers with resistance to OMV and OGSV will allow early generation testing of new breeding lines and reduce the advancement of disease escape lines.

622

FIELD TOLERANCE OF MELONS TO *MONOSPORASCUS CANNONBALLUS*. T.P. Alcantara[1], S.L. Rasmussen[1], D.H. Kim[1], N. Oebker[2], and M.E. Stanghellini[1], [1]Dept. of Plant Pathology and [2]Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721.

Two field trials (1993 & 1994) were conducted on melons (*Cucumis melo*) to identify genotypes with promising levels of field tolerance to vine decline caused by *Monosporascus cannonballus*. A total of 43 breeding lines and cultivars representing the major types of melon were screened in commercial fields which were uniformly infested with the fungus. Muskmelon line 'XPH-6244' (Asgrow) and several late maturing non-muskmelon genotypes demonstrated a high level of field tolerance. Commercially available muskmelon cultivars 'Gold Rush' and 'Solid Gold' were rated as moderately tolerant, whereas 'Caravelle', PMR 45', and 'Durango' were susceptible. In addition, microscopic examination of the roots of diseased plants revealed heavy occlusion of xylem vessel lumina by tyloses. No tyloses were observed in healthy plants, suggesting that extensive tylose formation may contribute to the rapidity of vine decline which is accelerated by heavy fruit load and high ambient and soil temperatures.

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PROPAGATION OF AMERICAN BEECH WITH RESISTANCE TO BEECH BARK DISEASE. M. J.Barker, D. D. Skilling, D. R. Houston, and M.E. Ostry. USDA Forest Service, 1992 Folwell Ave., St. Paul, MN, 55108, and 51 Mill Pond Rd., Hamden, CT, 06514

In NE North America, the beech bark disease (BBD), resulting from attack by the beech scale (Cryptococcus fagisuga) and subsequent infection by Nectria spp., has significantly reduced the number of larger, higher value American beech (Fagus grandifolia). Disease-free, highly resistant trees are occasionally found in severely affected stands, and may be valuable in beech management if propagated and planted in selected areas. Conventional asexual propagation systems using root sprouts, grafts, and cuttings has had limited success. However, we have successfully developed a tissue culture system using sprouts from root segments and forced buds of mature disease resistant beech trees and have brought several genotypes from culture initiation through rooting.

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RESISTANCE TO A SOLANACEOUS STRAIN OF *Crinipellis perniciosa* IN THE TOMATO MUTANT *monstrosa*. E.R. Dickstein, and L.H. Purdy, Plant Pathology Dept., University of Florida, Gainesville, FL 32611.

Crinipellis perniciosa is the causal agent of witches' broom disease in cacao (*Theobroma cacao*). The host range of another strain of *C. perniciosa*, described in 1985, is limited to plants in the Solanaceae. *Solanum quitoense, S. topiro, Lycopersicon cheesmanii, L. chilense, L. chmielewskii, L. esculentum, L. hirsutum, L. parviflorum, L. pennelli, L. peruvianum,* and *L. pimpineifolium* were inoculated with the solanaceous isolate of *C. perniciosa* to screen plants for disease resistance. A basidiospore suspension containing 75,000 spores/ml was sprayed onto plant leaves, stems and petioles using a hand held aerosol spray unit. All inoculated *Lycopersicon spp.* and *Solanum spp.* were susceptible and developed typical witches' broom disease symptoms. Various mutants of *L. esculentum* with altered growth regulator metabolism and other plant habit and size abnormalities were also inoculated with the solanaceous isolate of *C. perniciosa*. Mutants tested were *au-6 (yg-6), cana, diageotropica, gib-2, gib-3, minuta, monstrosa, procera,* and *torosa*. All mutants, except *monstrosa*, developed disease symptoms. Information on resistance in the *monstrosa* mutant and genetic material from resistant plants may provide knowledge useful for the development of disease resistance in cacao.

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OCCURRENCE OF SUDDEN DEATH SYNDROME OF SOYBEAN IN IOWA. X.B. Yang, P. Lundeen, and H. Scherm. Department of Plant Pathology, Iowa State University, Ames 50011.

Sudden death syndrome (SDS) is a new disease of soybean in Iowa. In 1993, SDS was detected in experimental plots only. In 1994, the disease was found in commercial production fields in two regions. Surveys in about 27 square miles around these fields showed that SDS was limited to fields belonging to the same owners. Disease incidence in those fields varied from 0.5% to more than 50%. No additional SDS-infested fields were detected in a random survey of 109 fields across Iowa. Pathogenicity of two isolates of *Fusarium solani* form A (causal agent of SDS) from Iowa was compared with that of an isolate from Arkansas using 20 soybean varieties. There were significant differences in incidence of leaf symptoms caused by the isolates. The isolate from Arkansas was consistently more virulent than isolates from Iowa. There were differences among the varieties, but no significant interaction between variety and isolate was found.

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SUPPRESSION OF BROWN STEM ROT IN SOYBEAN BY 'PURSUIT' HERBICIDE. E.A. Adee, C.R. Grau, R.G. Harvey, A.E. MacGuidwin, and E.S. Oplinger. University of Wisconsin, Madison, WI 53706.

In a field study with varying broadleaf weed densities, severities of foliar symptoms of BSR averaged 19% lower with the low weed density (avg. 2% biomass)/high Pursuit (imazethapyr) rate (0.072 kg a.i./ha) compared to the relatively high density (18%)/no Pursuit. Greenhouse studies were established to determine if field observations were related to the direct effect of Pursuit or weed density. Sturdy (BSR-susceptible) and BSR 101 (resistant), were grown in pasteurized soil treated (0.072 kg a.i./ha) or not treated with Pursuit. Plants were root dipped or hypocotyl injected with water or *Phialophora gregata*. Soil moisture was kept near field capacity by sub-irrigation. Foliar symptoms of BSR were rated at growth stage R1 using the Horsfall/Barratt scale (0-11). None of the plants without *P. gregata* expressed symptoms of BSR. The severity of BSR in Sturdy was greater without (4.6) compared to with Pursuit (1.4) using root dip inoculation. There was no difference in severity of BSR with (4.0) or without (4.0) Pursuit using hypocotyl inoculation ($P<0.001$, $LSD_{0.05}=1.03$). In BSR 101, there was no difference in severity of BSR with (1.0) or without Pursuit (1.0) using root dip inoculation. Plants with Pursuit were shorter (23.0 cm) than without (24.8 cm) ($P=0.08$). These data suggest that Pursuit does reduce the severity of BSR and that the suppressive effect of Pursuit on severity of BSR occurs in the roots.

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Influence of cotton variety and nematicide application on the incidence of the Fusarium wilt/root-knot nematode complex in cotton. P.D. Colyer[1], T.L. Kirkpatrick[2], and W. D. Caldwell[1], [1]Louisiana Agric. Exp. Sta., Red River Research Station, and [2]Arkansas Agric. Exp. Sta., Southwest Research and Extension Center.

Ten cotton varieties, treated with and without the nematicide aldicarb, were evaluated for their affect on the incidence of the Fusarium wilt/root-knot nematode complex in cotton. A split-plot design with a randomized complete block arrangement of treatments was used with cotton varieties as the main plots and nematicide treatments as the subplots. Yield of seed cotton, wilt and root-gall ratings, and some fiber qualities were significantly different among varieties and between nematicide treatments. Increased seed cotton yield and reduction in root-gall and wilt ratings were associated with application of aldicarb. Increased lint percent, boll weight, and length (UHM) were also associated with the application of aldicarb. Fiber micronaire, elongation, and strength were significantly different among varieties, but were not significantly different between nematicide treatments.

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ASSESSMENT OF TILLAGE EFFECT ON THREE SOILBORNE SOYBEAN PATHOGENS IN IOWA UTILIZING RANDOM SAMPLING OF SOYBEAN FIELDS. F. Workneh, G. L. Tylka, and X.B. Yang, Department of Plant Pathology, Iowa State University, Ames 50011

The effect of conventional and conservation tillage practices on population densities of soybean cyst nematode (*Heterodora* glycines) and *Phytophthora sojae* and incidence of brown stem rot (*Phialophora gregata*) was investigated. In cooperation with the Iowa Department of Agricultural Statistics, a total of 102 soybean fields were randomly selected from throughout Iowa in the Fall of 1994. Composite soil samples were collected from each field in a systematic pattern and where assayed for population densities of *H. glycines* and *P. sojae*. Cysts of *H. glycines* were extracted from soil by elutriation, and eggs were extracted from cysts, then stained and counted. Population densities of *P. sojae* were determined with a leaf-disc bioassay method. Incidence of brown stem rot was determined by observation of the presence or absence of typical pith discoloration on 20 randomly sampled stem pieces from each field. *H. glycines* and *P. sojae* were detected in 69.3% and 80.4% of the samples, respectively, whereas 49% of the stem samples representing 97% of the fields had brown stem rot symptoms. Egg densities of SCN were significantly greater in samples from conventional till than those from no-till fields (χ^2=5.0478, P=0.03). Conversely, populations densities of *P. sojae* and incidence of brown stem rot were significantly greater in samples from no-till fields than those from conventionally tilled fields at P=0.005 and P= 0.10, respectively.

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VERTICAL DISTRIBUTION OF *PHYTOPHTHORA SOJAE* AND *PHIALOPHORA GREGATA* IN TILL AND NO-TILL SOYBEAN FIELDS. F. Workneh, X.B. Yang, G.L. Tylka, Department of Plant Pathology, Iowa State University, Ames 50011

The vertical distribution of *P. sojae* and *P. gregata* was investigated in two adjacent plow and no-till fields with 17 years of respective tillage history. From each field, soil samples were collected from 0 to 30 cm in increments of 5 cm, in an "X"- pattern. Population densities of *P.sojae* for each sample were determined by a leaf-disc bioassay method. To determine population densities of *P. gregata,* residues were extracted from 500 cm^3 of each sample by elutriation. Residues were then air dried and ground with a Wiley mill fitted with a 0.39 mm sieve. One gram of ground tissue was serially diluted, plated onto a semi-selective medium, and colony forming units (CFU) of *P. gregata* were counted. Population densities of *P. sojae* were significantly greater in samples from the no-till field than those of plowed field at 0-15 cm depth (P<0.01). There was no significant difference in *P. sojae* densities between the tillage systems at depths greater than 15 cm. Densities of *P. gregata* in the upper 15 cm ranged from 1×10^3 to 8.4×10^6 and 1.5×10^3 to 3×10^3 in samples from the no-till field and plowed field, respectively. However, both tillage systems had similar ranges of CFU at depths greater than 15 cm. The presence of greater inoculum densities in samples from the upper soil profile of the no-till field than that of plowed field suggests a greater risk of early infection by both pathogens in no-till fields.

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EFFECTS OF PLANTING DATE AND SOYBEAN CULTIVAR ON SOIL POPULATIONS AND ROOT COLONIZATION BY THE RED CROWN ROT FUNGUS, *CALONECTRIA CROTALARIAE*. P.U. Kuruppu and J.S. Russin, Dept. Plant Pathol. & Crop Physiol., La. St. Univ. Agr. Ctr., Baton Rouge, 70803.

Incidence of red crown rot (RCR), root colonization by *C. crotalariae*, and pathogen populations in soil were monitored in soybean cultivars Cajun (resistant) and Sharkey (susceptible) planted on 25 May (optimal), 6 June (3 wk late) and 5 July (6 wk late) in a field naturally-infested with *C. crotalariae*. Disease incidence (DI) decreased consistently following delayed planting in Sharkey but remained very low in Cajun, regardless of planting date. Root colonization in both cultivars increased over time following optimal but not delayed plantings. Colonization of Sharkey roots was greater than that of Cajun, but only during the first seven weeks after optimal planting. This suggests that root colonization during these seven weeks was critical for RCR development on susceptible Sharkey. Cajun had limited root colonization during this period, resulting in low DI. Populations of *C. crotalariae* in soil were similar in plots of both cultivars from May-August. Beginning in September, however, soil populations of *C. crotalariae* were lower in plots of Cajun than of Sharkey.

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COTTON PLANT DEVELOPMENT IN RESPONSE TO SELECTED FUNGICIDES. K. S. McLean[1] and G. W. Lawrence[2], [1]Assistant Professor, Dept. of Agriculture, NLU, Monroe, LA 71209, [2]Associate Professor, Dept. of Ent. & Plant Path, MSU, MS 39762.

Delta Coat AD, Terraclor Super X, Ridomil PC, Start 15G, Start + Temik, and SM-9 were examined for cotton seedling disease control and subsequent effects on cotton growth. Thielaviopsis basicola, Rhizoctonia solani, Fusarium spp., and Pythium spp. were isolated from diseased cotton seedling hypocotyles. Cotton seedling stand, 42 days after planting, ranged from 5.5 to 3.9 plants per foot of row in the Start and Delta Coat AD treatments, respectively. Cotton plant development followed a sigmoid growth curve. Cotton leaf area was greater in the fungicide treated plots compared to the control throughout the growing season. Plants growing in the fungicide treatments produced and retained more squares compared to the control. Lint cotton yields were greater in all fungicide treatments compared to the control.

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A NEW STEM CANKER DISEASE OF PEANUT CAUSED BY *FUSARIUM OXYSPORUM*. J. M. Mullen, A. K. Hagan, and P. E. Nelson. Department of Plant Pathology, Auburn University, AL 36849; Pennsylvania State University, University Park, PA 16802.

In Aug 1992, widespread chlorosis, wilting, and death of central stems of peanut cv. 'Florunner' were observed in 2 fields in Covington County, AL. One or more reddish to dark brown, sunken, elongated (0.5-3.0 cm) girdling lesions were found on symptomatic stems at varying distances above the soil line. *Fusarium* species sporodochia were observed on brown centers of some lesions. Damage was noted after an extended period of showers. *Fusarium oxyporum* was consistently isolated from canker margins on aPDA. Pathogenicity was tested on greenhouse-grown 13 week old 'Florunner' peanuts. A total of 15 stems on 5 plants were wounded (epidermal scalpel slice), and mycelial agar blocks were held over wounds with Parafilm. Two control plants were treated similarly with no inoculum. After 3 weeks, brown to black cankers (0.5-2.0 cm long) were present at inoculation sites. *F. oxysporum* was repeatedly isolated from lesion margins. Cankers did not form on uninoculated wounded stems.

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IDENTIFICATION OF SOYBEAN CULTIVARS FIELD TOLERANT OF MACROPHOMINA PHASEOLINA. O. N. Caryil and G. S. Smith, Plant Science Unit, University of Missouri, Columbia, MO 65211.

From 1992 to 1994, twenty four soybean cultivars were screened for field tolerance (non-specific race resistance) of M. phaseolina (MP) . Twelve cultivars were resistant to Heterodera glycines (Hg) and twelve were susceptible. The cultivars were grouped into four maturity classes (3.1 - 3.4; 3.5 - 3.8; 3.9 - 4.4; 4.5 - 4.9) and planted at two planting dates (early May and early June). Lower stem/taproots were collected at 'R7' from five plants/plot from the unifoliate node to 15 cm down the taproot. Tissue, air-dried for 1 mo and ground in an UDY mill, was plated on a semi-selective medium to quantify Mp microsclerotia (ms). Based on host suitability (Mp ms/gram), 18 of 24 cultivars were rated susceptible to Mp (mean 14,078 ms/gram), and three cultivars, DeltaPineland 3478, Hamilton, and Jackson II, were rated moderately resistant to Mp (2,040 - 2,579 ms/gram). Three cultivars, Asgrow 4715, Pioneer 9391 and Stine 3240, were intermediate in host suitability to Mp (mean 6,244 ms/gram). In 1994, the moderately resistant cultivars yielded from 4.8 to 7.0 bu/A more and from 5.0 to 10.1 bu/A more than the Mp-susceptible cultivars at the May and June planting, respectively. Resistance was independent of maturity, Hg-resistance, and the environmental conditions present over the three years.

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STUDIES OF A NEW DISEASE OF CORN OF UNKNOWN ETIOLOGY IN THE TEXAS PANHANDLE. C. M. Rush and G. J. Michels. Texas Agric. Exp. Stn., PO Drawer 10, Bushland, TX 79012.

In the summer of 1993, a new disease of corn was identified in the Texas Panhandle and has been temporarily designated as high plains virus (HPV). The disease has since been found in Colorado, Utah, Kansas, Nebraska, and Idaho on corn and wheat. The pathogen has not been identified but is naturally vectored by the wheat curl mite. A field study was initiated in 1994 to determine whether planting date and an insecticide treatment, Furadan 4F at .45 kg ai/A, affected disease incidence. A susceptible and a resistant corn cultivar was planted every four weeks beginning in the middle of April and ending in July, and plots were sprayed once, twice or left untreated. Mite populations were monitored during the season. The highest incidence of disease occurred in the May planting, and plants seemed to become resistant with age. Spray treatments had no effect on disease incidence, and wheat curl mites did not colonize the corn. Later in the growing season, the disease was identified in several native grasses and in F2 volunteer seedlings from resistant hybrids.

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PRELIMINARY IDENTIFICATION OF A DISTINCT LUTEOVIRUS AFFECTING LENTILS IN SYRIA. K. M. Makkouk, L. Katul*, S.G. Kumari and H. Naasan. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria and * Institute of Biochemistry and Plant Virology, Braunschweig, Germany.

A virus inducing yellowing and stunting of lentil plants was detected in lentil fields of northern Syria during the spring of 1994. The virus was not mechanically transmitted, but persistently transmitted by *Acyrthosiphon pisum*. The virus isolate was tested in a triple antibody sandwich ELISA (TAS-ELISA), with trapping immunoglobulins from antisera against bean leaf roll virus (BLRV), beet western yellows virus (BWYV), potato leaf roll virus (PLRV) and subterranean clover red leaf virus (SCRLV) and two detecting monoclonal antibodies (5G4, a monoclonal with luteovirus broad-spectrum activity and 4B10, a monoclonal specific for BLRV). No reaction was obtained with most antibodies used, and only weakly when using SCRLV antibodies for trapping and 5G4 antibodies for detection. By using tissue-blot immunoassay (TBIA), virus particles adsorbed on the nitrocellulose membrane were detected by 5G4 monoclonal but not with 4B10 monoclonal, or BLRV, PLRV and Chickpea luteovirus (India) polyclonal antisera, but weakly detected by BWYV and SCRLV polyclonal antisera. Serological data obtained so far with monoclonal and polyclonal antibodies suggest that the lentil isolate from Syria (SL1-94) could be a distinct legume luteovirus. Further characterization of this isolate is in progress.

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ISOLATION OF *AGROBACTERIUM TUMEFACIENS* FROM BLUEBERRY (*VACCINIUM CORYMBOSUM*). M. L. Canfield, M. L. Putnam, TJ White and L. W. Moore. Dept. of Botany and Plant Path., Oregon State Univ., Corvallis, OR 97331-2902.

A disease of unknown eitology was observed during 1993 and 1994 at a commercial field of blueberries (cv. Bluejay) in the Willamette Valley, Oregon. Plants had small contiguous eruptions along the length of branches and blackened outgrowths at plant nodes. Symptoms were similar to those reported in other areas of the Pacific Northwest on several blueberry cultivars. Radical pruning of affected tissue and application of fungicides failed to eradicate the disease. One hectare of the plants eventually had to be removed. *Phomopsis* and *Nocardia* spp. were considered as causal agents but isolations for these organisms were not successful. Even though the symptoms were not typical of crown gall, isolations were made from seven affected plants onto media selective for agrobacteria. DNA from purified isolates was tested for hybridization to three digoxygenin-labled DNA probes (*iaaM* and *iaaH; virD1* and *virD2; virFAB*) derived from the Ti plasmid of *A. tumefaciens* strain A6. DNA from 10% of the isolates hybridized to the probes and these isolates produced tumors when inoculated to tomato plants. The pathogens were identified as *Agrobacterium tumefaciens* biotype two by biochemical tests.

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IDENTIFICATION OF A DNA FRAGMENT THAT DIFFERENTIATES STRAINS OF *XANTHOMONAS CAMPESTRIS* PV. *PRUNI* BY RAPD ANALYSIS. M.C. Pagani[1], D.F. Ritchie[2], P.B. Lindgren[2], D.J. Werner[3], and C.S. Kousik[2]. [1]INIA, Las Piedras, Uruguay; [2]Dept. Plant Pathology, Box 7616; and [3]Dept. Horticultural Science, Box 7609, N.C. State Univ., Raleigh, 27695.

X. c. pv. *pruni* (Xcp) causes bacterial spot of peach. Random amplified polymorphic DNA (RAPD) analysis was used to differentiate strains of Xcp from 51 other bacterial isolates representing strains of different genera, *Xanthomonas* spp., and *X. campestris* pathovars. Among 61 primers (10-mer) screened, one primer yielded a distinctive fragment for strains of Xcp. The DNA amplification pattern generated with this primer revealed two major products of 1.5 kbp and 0.9 kbp among 53 Xcp strains tested. The 1.5-kbp fragment was present in all *Xanthomonas* and *Pseudomonas* strains examined but not in strains of *Erwinia*. The 0.9-kbp fragment associated with Xcp strains was used as a hybridization probe to confirm specificity of the fragment. Hybridization occurred to all Xcp strains tested but not to any of the other non-Xcp bacteria. This fragment may provide a sensitive and specific tool for detection and identification of Xcp strains.

638 Withdrawn

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THE EFFECT OF MULCHES AND PESTICIDE TREATMENTS ON GROWTH OF CITRUS IN A FIELD INFESTED WITH *PHYTOPHTHORA* SP. AND THE CITRUS NEMATODE. J. A. Menge[1], D. W. Freckman[2], R. Niles[2], O.J. Becker[1], E. L. V. Johnson[1] and E. Pond[1]. University of California, Riverside CA 92521[1] and Colorado State University, Fort Collins, CO 80523[2].

Nursery-grown navel orange trees on "Troyer" rootstock were planted in 1992 in a field infested with *Phytophthora parasitica* and *Tylenchulus semipenetrans*. Gypsum, a composted sewage sludge, alfalfa hay, and a combination of all three were applied under the tree canopy as mulches. Controls included no treatment, a metalaxyl + phenamiphos, and metalaxyl + phenamiphos + a combination of the three mulches. All treatments with metalaxyl + phenamiphos reduced *Phytophthora parasitica* populations by an average of 69% and increased the tree canopy volume by an average of 19% compared to the untreated control. Gypsum did not significantly affect either *P. parasitica* populations or canopy volume compared to the control. Alfalfa and sewage sludge increased *P. parasitica* populations by 277% and 127%, respectively, while reducing canopy volume by 67% and 34% compared to the untreated control. The combination of all three mulches increased *P. parasitica* populations by 72% and reduced canopy volume by 73%.

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INCIDENCE AND OCCURRENCE OF STRAWBERRY DISEASES IN DIAGNOSTIC CLINIC SAMPLES FROM 1991-1995 IN FLORIDA. A. J. Whidden, C. K. Chandler, E. E. Albregts, and D. E. Legard, University of Florida, GCREC-Dover, 13138 Lewis Gallagher Road, Dover, Fl 33527.

During the period of October 1991 through the 1994-1995 growing season, over 350 strawberry samples were analyzed at the diagnostic clinic of the University of Florida, Dover Agricultural Research Center. A majority of the samples were diagnosed with fungal pathogens (83%), although bacterial (7%), nematode (5%) and viral problems (<1%) were also found. Anthracnose (*Colletotrichum* spp.), *Phomopsis* leaf blight and fruit rot (*P. obscurans*), angular leaf spot (*Xanthomonas fragariae*), gray mold (*Botrytis cinerea*), *Phytophthora* crown rot and leather rot (*P. cactorum*), powdery mildew (*Sphaerotheca macularis*), and *Gnomonia* leaf blotch and fruit rot (*G.* spp.) were recovered from 49%, 15%, 7%, 5%, 5%, 3% and 3% of the samples, respectively. Miscellaneous fungal pathogens were implicated in 5% of the samples and 18% of the samples had abiotic/undiagnosed problems. Grower familiarity with diseases such as angular leaf spot and gray mold has resulted in fewer samples of these diseases being handled. Certain diseases such as powdery mildew, angular leaf spot, *Phytophthora* rots, *Gnomonia* leaf blotch and fruit rot were more prevalent on transplants produced in northern latitude nurseries.

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THINNED INFECTED STONE FRUIT AS AN INOCULUM SOURCE OF *MONILINIA FRUCTICOLA* IN CALIFORNIA. B. A. Holtz, R. Kolliker, and T. J. Michailides, Department of Plant Pathology, University of California, Davis/Kearney Ag Center, Parlier, CA 93648.

Immature Fantasia nectarines were found infected and sporulating with *Monilinia fructicola* conidia after they were thinned from trees and left on the ground. Experiments were conducted to determine whether the placement of thinned fruit in irrigation trenches would enhance its decomposition and reduce this source of brown rot inoculum. Of an average of 1,200 thinned fruits per tree, 36.2% showed *M. fructicola* sporulation one month after thinning. There was significantly less ($P < 0.05$) sporulation on thinned fruit in the irrigation trenches when compared to fruit on the dry berms. There was also significantly ($P < 0.05$) less conidia per infected fruit, and the % germination was also less. Latent infections of thinned fruits were determined after sterilizing (0.08% NaOCl, 1.6% EtOH, 0.05% Tween 20, 4 min), freezing (-23 C, 15 h), and incubating the fruit for 7 days (21 C, 10 h light, > 97% RH), and were positively correlated ($r^2 = 0.97$) with percent brown rot at harvest. Mummified thinned fruits, which had survived on the berms for over 3 months, were collected and incubated in moist sand (> 95% RH) in the dark for 8 wk at 2 C, and then for 2 wk at 15 C with a 12 h photoperiod. Apothecia were produced from 1% of these thinned fruit. These results suggest that infected thinned fruit can provide both primary (ascospores) and secondary (conidia) inoculum.

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DETECTION OF CITRUS BLIGHT IN THE DOMINICAN REPUBLIC. J. C. Borbon, Junta Agropresarial Dominicana, Inc. Santo Domingo, Dominican Republic, S. M. Garnsey, USDA, ARS, Orlando, Fl 32803, and K. S. Derrick, Univ. of Florida, Lake Alfred 33850.

Symptoms of citrus blight were observed in 1993 in numerous 8-yr-old Valencia sweet orange trees on Citrus volkameriana rootstock. These symptoms were not associated with other diseases or problems, and the trees grew and produced normally prior to their decline. The visual diagnosis of blight was confirmed by three diagnostic tests. Water uptake by syringe injection (Plant Disease 68:511-513) into the trunks of 12 trees with blight symptoms averaged 0.2 ml/min. compared to 2.8 ml/min. in 12 apparently healthy trees. Wood samples from the trunks of six blighted trees averaged 18 ppm Zn (9-25 ppm) while six healthy trees averaged 3 ppm (2-5 ppm). Extracts prepared from leaves of blighted trees gave a positive immunoblot reaction for the 12 kDa protein associated with blight (Proc. Fla. State Hort. Soc. 105:26-28) while extracts from healthy trees were negative. Blight was found in localized areas at two locations and may become an increasing problem since most citrus plantings in the Dominican Republic are on blight-sensitive rootstocks and are just reaching an age when blight develops.

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ALTERNATIVE STRATEGIES FOR SMALL FRUIT PRODUCTION: A SUMMARY OF A NORTHEAST S. A. R. E. PROJECT. D. R. Cooley , W. F. Wilcox, and W. J. Janisiewicz. Dept. of Plant Pathology, University of Massachusetts, Amherst, MA, 01003, Plant Pathology Dept., New York State Agric. Expt. Sta., Geneva, NY, 14456, and USDA/ARS, Appalachian Fruit Sta., Kearneysville, WV, 25430.

Fourteen cooperators representing four Universities and the USDA/ARS developed a 5 year program of research and education for small fruit production in the northeastern United States. The objectives were to develop and test alternative production practices for strawberries and raspberries; to analyze economic feasibility of these practices; to evaluate grower acceptance of alternative technology; and to deliver the knowledge based on this research to growers. Specific research areas related to plant pathology determined strawberry and raspberry cultivar susceptibility to disease, the influence of various cultural practices on fruit diseases and pesticide spray coverage, and testing potential biocontrols for *Botrytis cinerea*. Farmers indicated a willingness to adopt alternatives to standard chemically-based management practices, even if profits might be reduced. However, they were not willing to commit more management time to such technology. Information delivery channels included a newsletter, over 150 public presentations and over 100 publications.

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A LOW COST COMPUTERIZED SYSTEM TO MONITOR WEATHER AND FORECAST PLANT DISEASE INFECTION PERIODS. W.H. Shaffer, M.M. Hulse, and R.I. Parker. Dept. of Plant Pathology and Electronic Instrument Lab, University of Missouri, Columbia, MO 65211.

The Show Me Plant Disease Forecasting System™ was developed as a low cost way to monitor weather from remote sites and forecast plant disease infection periods. The computerized Show Me Weather Machine™ monitors environmental parameters including: air temperature, relative humidity, leaf wetness, rainfall, soil temperature, wind speed and wind direction every one to five minutes. It transmits this data via radio telemetry, to an IBM™/compatible computer using a Windows™ operating system and equipped with a radio receiver. The Show Me Plant Disease Forecaster ™ software displays the current weather conditions on the computer monitor. Weather summaries are stored on computer disks. Infection periods for 11 plant diseases and the potential threat of one insect are forecasted. The system is being evaluated on diseases of fruit, field, and forage crops and turf grass during 1995. A model for codling moth is also being evaluated. Results of these tests will be discussed.

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OBSERVATIONS ON THE EPIDEMIOLOGY OF SOOTY BLOTCH ON CARAMBOLA (*AVERRHOA CARAMBOLA*) IN SOUTH FLORIDA. Randy C. Ploetz, Anthony J. Dorey, and David Benscher. University of Florida, TREC, 18905 SW 280th Street, Homestead, FL 33031.

Carambola (*Averrhoa carambola*), which is also known as starfruit, is an important crop in south Florida. Within the last 5 years a gray blemish has become increasingly prevalent on fruit. Preliminary indications are that the blemish is sooty blotch, a disease caused by *Gloeodes pomigena*. Although sooty blotch blemishes are superficial, affected fruit must be washed and waxed prior to marketing, activities that significantly increase packing costs. To better understand sooty blotch behavior in production orchards, epidemiological studies were initiated. Twelve trees were chosen randomly in each of six commercial orchards of cv. Arkin, and one of cv. Kary; fruit on each tree were observed on a weekly to bi-weekly basis over two fruiting seasons in 1994. During both seasons, sooty blotch developed only on fruit at mid- to late-stages of maturity. Symptoms first appeared about 1 month into the summer rainy season (June), although this varied significantly among orchards ($P < 0.05$). The incidence and severity of sooty blotch also varied considerably among orchards on the three highest maturity classes of fruit. Preliminary trials in one of the test orchards indicated that Copper Count-N, Manzate, and Manzate + Benlate fungicides provide adequate control when used on weekly bases. As the epidemiology of sooty blotch on carambola becomes better understood, it is anticipated that less frequent, but effective applications of fungicide could be used.

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Occurrence of Microdochium Blight of Squash on the Delmarva Peninsula. Evans, T. A., University of Delaware, Newark 19717, Rouse, R. J., Ringer, C. E., Kantzes, J. G. University of Maryland, College Park, MD 20742, and Dillard, H. R., Cornell University, Geneva, NY 14456.

A blight disease of squash (Cucurbita pepo L.) has occurred at several locations on the Delmarva Peninsula, in Delaware and Maryland, each year since 1987. This disease has occurred on yellow summer squash, zucchini and pumpkin cultivars but has been most severe and caused the largest losses in the yellow summer squash cultivar Blondie. The disease is characterized by the development of tan, sunken, elongated lesions on the stems, petioles, main leaf veins, flowers, peduncles and fruit. Lesions are usually less than 5 mm long, but often coalesce to form a dry, scabby surface. Stem infection often causes defoliation and plant death within 2 wks. Disease incidence was highest for intensively managed fields where irrigation and picking often occur on a daily basis during peak production. The causal agent of this blight was determined to be Microdochium tabacinum (Van Beyma) Arx (=Fusarium tabacinum (Van Beyma) W. Gams). The disease was reported in Tennessee in 1992 and in the former U.S.S.R. previously.

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EFFECT OF FREQUENCY AND TIMING OF SPRINKLER IRRIGATION ON BACTERIAL HEAD ROT AND YIELD OF BROCCOLI. R.L. Ludy, D.D. Hemphill, Jr., and M.L. Powelson, Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Effect of frequency and timing of sprinkler irrigation on the incidence of bacterial head rot of broccoli (cv Gem), caused by *Erwinia carotovora* subsp. *carotovora*, was investigated in 1993 and 1994. Six sprinkler irrigation regimes, a factorial combination of frequency (irrigation every 2, 4 or 8-days) and timing (morning or evening), were established at head initiation. Incidence of head rot was negligible at the first harvest in both years. At the last harvest, the incidence of head rot was significantly reduced from 22 to 10% and 30 to 15% in 1993 and 1994, respectively, by the change in frequency of irrigation from every 2 to 8 days. Shorter periods (hr/day) of leaf wetness were measured under the 8-day compared to the 2-day irrigation and probably contributed to the decrease in disease incidence. In contrast, timing of irrigation had no effect on disease incidence. Head size and yield of broccoli were not affected by frequency or timing of irrigation.

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RESISTANCE TO LATE BLIGHT AND EARLY BLIGHT IN POTATO CULTIVARS AND BREEDING LINES. R. V. James, W. R. Stevenson and J. P. Helgeson, Department of Plant Pathology and USDA/ARS Plant Disease Resistance Research Unit, University of Wisconsin-Madison, Madison, WI 53706

Sixty-four cultivars and breeding lines were evaluated at Hancock WI. No fungicide was applied and conditions were very favorable for blight development. Late blight (US-8 genotype), first seen in the trial on 19 July, developed quickly and after 1 August, 42 entries could not be rated separately for early and late blight. Because early blight resistance is often associated with late maturity, the 16 of 22 lines in the early/medium maturity classes with AUDPC values (through 26 July) for early blight ≤0.06 (compared to 0.38 for Red Norland) may indicate potential early blight resistance. By 9 August, 44 entries showed >95% defoliation, but 9 of the 13 BC_1 and BC_2 lines derived from crosses of susceptible potato cultivars with *Solanum bulbocastanum* + *S. tuberosum* somatic hybrids showed good resistance (< 9% of the foliage affected by late blight). Three of these lines were also highly resistant to early blight. Several entries had significantly higher yields and a higher proportion of US#1 tubers than Russet Burbank. Significant differences in tuber susceptibility were noted when all entries were inoculated with *Alternaria solani* and *Phytophthora infestans*.

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EVALUATION OF FUNGICIDES TO CONTROL POTATO LATE BLIGHT IN WISCONSIN. W. R. Stevenson and R. V. James, University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI 53706

Weather conditions favorable to late blight development resulted in considerable damage to potato foliage and tubers in central Wisconsin during 1994. Late blight symptoms were first noted at the Hancock Agricultural Research Station on 19 July and the isolate was identified as the US-8 genotype (A2 mating type, resistant to metalaxyl). By 15 August over 95% of the foliage in unsprayed control plots was exhibiting symptoms of early and late blight. Weekly treatment with chlorothalonil, fluazinam, iprodione/chlorothalonil and combination of TPTH plus either mancozeb or metiram provided excellent control of late blight (less than 10% foliage infection through the 15 August evaluation). Total yield and yield of US#1 tubers reflected the success in controlling both early and late blight on the foliage. In spite of severe foliar disease, very little infection was seen on tubers from any treatment and there were no significant differences in tuber infection at the time of harvest. Historically metalaxyl has been highly effective for late blight control in Wisconsin but this was not the case in 1994. In the presence of the US-8 isolate, use of metalaxyl did not enhance disease control, yield or crop value over the standard mancozeb program.

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MONOSPORASCUS CANNONBALLUS ROOT ROT OF MUSKMELON: ROOT INFECTION AND SYMPTOM DEVELOPMENT IN RELATION TO SOIL TEMPERATURE. D.H. Kim, S.L. Rasmussen, and M.E. Stanghellini. Department of Plant Pathology, University of Arizona, Tucson, 85721.

Root infection and subsequent development of symptoms (both above and below ground) were monitored over a two-yr-period in commercial melon fields. In the Fall (July) planted crop, root infection occurred ca. 24 days after planting. Crusty, tan-colored non-girdling root lesions, measuring 2-4 mm in length, were first observed on small (2-4 mm in diameter) feeder roots ca. 35 days after planting. Foliar symptoms (i.e. die-back of the oldest crown leaves which exhibited diagnostic V-shaped necrotic sectors extending from the base to the outer margin of leaves) were observed ca. 47 days after planting. The first observation of perithecia on infected feeder roots occurred ca. 60 days after planting. Root infection and development of symptoms in the Spring (January - February) planted crop were delayed (relative to the Fall planted crop) ca. 30-60 days. Early infection and rapid symptom development were associated with soil temperatures greater than 25 C at the 10 cm soil depth.

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OCCURRENCE OF *SCLEROTINIA SCLEROTIORUM* ON PEPPER (*CAPSICUM ANNUUM* L.) IN OHIO. Y. Yanar and S. A. Miller, Department of Plant Pathology, The Ohio State University-OARDC, Wooster, OH. 44691.

Stem and fruit rot caused by *Sclerotinia sclerotiorum* has been considered a disease of minor importance on pepper. However, incidence of the disease has increased in processing peppers in Ohio in recent years. Eight isolates of *S. sclerotiorum* were recovered from naturally infected pepper plants collected in northwest Ohio in 1994. Pathogenicity of isolates was demonstrated by inserting autoclaved toothpicks covered with mycelia into the stem of young plants or by placing *S. sclerotiorum*-colonized flower (pepper) petals on uninjured stems. Light brown water-soaked lesions encircled the stems of all plants within 2 days of inoculation. No significant differences in virulence among the eight isolates were observed. Optimum temperature for growth of the fungus and production of sclerotia on potato-dextrose agar medium (PDA) and autoclaved carrot discs was between 15 and 20°C. Mycelial growth and sclerotia production were observed at 31°C on carrot discs but not on PDA. Mycelium was killed at 35°C.

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SPECTRAL AND TEMPERATURE MODIFYING PROPERTIES OF REFLECTORIZED SPRAY MULCH AND ITS ROLE IN MANAGEMENT OF APHID-TRANSMITTED VIRUS DISEASES OF MELONS. J. J. Stapleton[1] and C. G. Summers[2], Statewide IPM Project[1] and Dept. of Entomology, UC Davis[2], Kearney Agricultural Center, University of California, Parlier, CA 93648.

Reflectorized (silver) spray mulch can effectively deter aphid vectors from alighting on cucurbitaceous plants. In addition, the mulch usually has an unrelated, beneficial effect on plant growth. Mulch raised root zone soil temperatures 1-3 C at 6-8 cm depth, but did not affect canopy temperature. Silver mulch increased upwardly reflected light, particularly shortwave radiation, over that of bare soil. Spectral characteristics closely mimicked those of ambient, incoming radiation. Increased light intensity over that of bare soil ranged from nearly 7-fold at 330 nm (ultraviolet) to 35% at 1050 nm (near infrared). Reflectorized spray mulch delayed onset of WaMV, CMV, and ZuYMV symptoms in 'Primo F1' cantaloupe melon ca. 6 weeks under conditions of high disease pressure. Silver mulches covering 25%, 50%, 75%, and 100% of bed width increased marketable yield 9.5-, 12.5-, 13.9-, and 15.4-fold, respectively, over plants grown in bare soil.

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DECLINE AND MORTALITY OF TRIPLOID-HYBRID ASPEN IN MICHIGAN AND WISCONSIN. S.A. Enebak[1], M.E. Ostry[2], G.W. Wyckoff[1], and B. Li[1]. [1]University of Minnesota, Dept. of Natural Resources, and [2]USDA, North Central For. Exp. Station, St. Paul, MN 55108.

The fungus *Lahmia kunzei* (Korber) was associated with declining and dead triploid-hybrid aspen in six trials in MI and WI. Signs of decline included rough bark, thinning crowns, and branch dieback. The fungus and decline symptoms were found associated with trees of the *Populus tremuloides* X *P. tremula* hybrid only when the *P. tremula* tetraploid, TA-10, was used as the male with a specific female *P. tremuloides*, T-1-58. Symptoms associated with the pathogen first appeared 10 yr after planting and peaked at 20 yr with 22% of the triploid-hybrid infected. Mortality of the triploid-hybrid was 92% by age 25. Similar symptoms of decline and death, without the fungus were noted on the male which originated in an oceanic climate in southern Sweden, indicating that the tetraploid male may not be adapted to the continental climate of MN, MI and WI. While the primary cause of mortality may be the maladaptation of TA-10, *L. kunzei* has been found associated only with dying T-1-58 triploid-hybrids.

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PERSISTENCE OF SPHAEROPSIS SAPINEA ON OR IN ASYMPTOMATIC STEMS OF RED PINE NURSERY SEEDLINGS. G. R. Stanosz, D. R. Smith, M.. R. Guthmiller, and J. C. Stanosz, Departments of Plant Pathology and Forestry, University of Wisconsin, Madison, WI 53706.

Sphaeropsis sapinea (syn. Diplodia pinea) causes shoot blight and collar rot on red pine (Pinus resinosa) in Wisconsin nurseries. To investigate the possibility that this fungus persists on or in asymptomatic seedlings, five groups of 20 asymptomatic red pine seedlings were collected in November 1994 in each of two nurseries, and five groups of 20 asymptomatic jack pine (Pinus banksiana) seedlings were collected at one of the nurseries. Each shoot was divided into four subsamples: 5 needle-pairs of the most recent year's growth; 5 needle pairs of the previous year's growth; a 6 cm long stem segment of the most recent year's growth; a 4-6 cm long stem segment of the previous year's growth. Subsamples were surface-disinfested by immersion in 95% EtOH for 30 seconds, and twice in 1.05% NaClO plus Tween for two minutes. Subsamples then were placed into water agar slants and incubated for approximately 3 months. Identification of S. sapinea was based on examination of spores from pycnidia that formed on the plant tissue. The fungus was associated with 27.5% of the red pine seedlings (range 15-40%), but with none of the jack pine seedlings. With only one exception, the fungus was always associated with the stem segment of the previous year's growth. Results suggest that S. sapinea inhabits asymptomatic stems. This ability may help explain pathogen survival and rapid disease development under conditions that induce host stress.

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BACTERIA ASSOCIATED WITH WETWOOD IN SOUTHERN OAKS. S. D. McElreath[1], E. G. Porter[1], F. H. Tainter[1], and T. D. Leininger[2]. Department of Forest Resources[1], Clemson University, Clemson, SC 29634 and USDA Forest Service[2], Southern Hardwoods Laboratory, Stoneville, MS 38776.

A "sniff" test of increment borings was used to identify wetwood-affected upland and bottomland oaks. Expressed sap from borings yielded cultures of anaerobic and aerobic bacteria. From wetwood trees these included species of: (anaerobes) *Actinomyces*, *Clostridium*, *Eubacterium*, *Lactobacillus*, *Propionibacterium*, *Streptococcus*, and many unidentified species; and (aerobes or facultative anaerobes) *Bacillus*, *Enterobacter*, *Erwinia*, *Kingella*, *Klebsiella*, *Micrococcus*, *Xanthomonas*, and several unidentified species. Increment borings also were taken from oaks seemingly unaffected by wetwood. Although trees without wetwood yielded fewer genera and species of bacteria, similarity in genera between the two groups suggests that the "sniff" test may not be an accurate predictor of wetwood.

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SPONTANEOUS DECLINE OF WEEDY *MYRICA FAYA* IN HAWAI'I. Brion K. Duffy, Donald E. Gardner, National Park Service, Univ. of Hawaii, Honolulu 96822.

Recently observed spontaneous decline of *Myrica faya*, an introduced weed threatening native ecosystems in Hawai'i, may be of importance for control efforts. Decline in vigor proceeds either gradually (>2 yr) or rapidly (<2 yr) leading to tree mortality. Mature, but not senescent, trees growing in shallow soils are most affected. Principal symptoms are chronic chlorosis and defoliation affecting individual branches or the entire canopy as a unit. Wood discoloration and root decline were rarely observed. Decline occurs at several ecologically-distinct sites in and around Hawaii Volcanoes National Park and affects as many as 72% of the trees within a stand. Decline was not correlated with soil factors, deficiency/toxicity, rainfall, plant competition, or stand density. *Rhizoctonia* sp., *Pachytrype princeps*, *Hypoxylon moriforme*, *Phellinus gillvus*, 3 wood-boring beetles, and 9 genera of parasitic nematodes were collected from faya but none were consistently associated with decline. MLOs were not detected in a limited screen with DAPI. *Sophonia rufofascia*, a recently introduced leafhopper common on faya, causes widespread decline of other plants in Hawai'i and may contribute to faya decline.

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LIFE HISTORY OF *SCYTALIDIUM UREDINICOLA*, A MYCOPARASITE OF WESTERN GALL RUST. B. D. Moltzan, P. V. Blenis, and Y. Hiratsuka. Dept. of Ag., Food, and Nutritional Science, Univ. of Alberta, Edmonton, AB T6G 2P5, Canada.

Scytalidum uredinicola Kuhlman et al. is a mycoparasite which is capable of reducing the inoculum potential of western gall rust, *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka. Although the mode of parasitism has been established, seasonal development of the mycoparasite is poorly understood. The objective of this investigation was to describe the development of *S. uredinicola* on galls over time. Monthly collections of galls 3-10+ years old were made near Hinton, Alberta, from 1992 to 1995. *S. uredinicola* was isolated from the exfoliating surface of galls throughout the year and from tissues beneath the phellogen beginning in January. In both cases isolation occurred only on galls six years of age or older. Histological study demonstrated the presence of *S. uredinicola* in unruptured western gall rust sori as early as 24 April. These results suggest that *S. uredinicola* is perennial on the galls and can overwinter as viable arthrocondia on the surface of galls or as mycelium beneath developed phellogen. Further examination using SEM will be done to determine the seasonal movement of *S. uredinicola* in western gall rust sori.

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POTENTIAL PREDISPOSING FACTORS INVOLVED IN RED PINE POCKET MORTALITY. Carolyn J. Randall, John H. Hart, Forestry Department, Michigan State University, East Lansing, MI 48823.

We investigated the cause of red pine pocket mortality (RPPM), a disease that has recently appeared in red pine plantations throughout the Lake States. RPPM is restricted to 30- to 50-year old plantations of red pine (Pinus resinosa). A possible explanation is that accumulation of pine litter lowers the pH of soil, leading to increased levels of exchangeable Al^{3+}. Thus the trees are stressed and susceptible to invasion by various microbes and insects, leading to RPPM. Data on pocket decline in Michigan show soil pH of 3.9 to 5.3; Al toxicity may be a problem at these pH levels. Root tissues from declining trees had lower Ca/Al, Mg/Al and K/Al ratios than did tissues from healthy stands. The hypothesis is that nutrient stress at these sites causes mortality of fine roots and decline of red pines in plantations.

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RESPONSES OF *Cornus amomum* AND *Raphanus sativus* TO *Discula destructiva*. H. Irwin, D. E. Wedge, F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.

Responses of *Cornus amomum* and *Raphanus sativus* seedlings to spore inoculation and toxic metabolites of *Discula destructiva*, the dogwood anthracnose fungus, were studied. Seedlings of *C. amomum* and *R. sativus* were grown from seeds and maintained for six weeks in an *in vitro* tissue culture system on Murashige-Skoog media. Wounded and non-wounded leaves of both species were inoculated with spores of *D. destructiva*, partially purified culture filtrate, water, and potato-dextrose broth. Leaves were then evaluated for infection or necrosis. Spore-inoculated, wounded leaves of both species showed symptoms of infection. *D. destructiva* was recovered from the infected leaves of *C. amomum*. Necrotic responses to toxic metabolites were observed in some wounded leaves of *R. sativus*.

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DISTRIBUTION AND PATHOGENICITY OF *HETEROBASIDION ANNOSUM* FROM *ABIES ALBA* IN ITALY. M. Garbelotto and W. Otrosina. Dept. of ESPM, University of California, Berkeley, CA 94720, and USFS-Forestry Science Lab, Athens, GA 30602.

The basidiomycete *Heterobasidion annosum* contains the three intersterility groups (ISGs) S, P, and F. We have used the PCR-based random amplified polymorphic DNAs (RAPDs) to determine the ISG of 35 isolates from 17 *Abies alba* stands throughout Italy. Results provide us with an improved map of the distribution of the F ISG in Italy, including first reports for three provinces. While all isolates collected in the Appennins were classified as F, two sites in the Alps yielded both S and F isolates. *H. annosum* appeared as a destructive pathogen only in off-site planted fir stands or in silviculturally neglected natural stands. Mortality symptoms and decay patterns were quite different from those observed in *Picea excelsa* and closely resembled symptoms and patterns of S isolates on *Abies concolor* in Western North America. The mitochondrial ML5-ML6 region was amplified with the aid of the PCR. Two differently sized fragments were obtained but there was no correlation between size of the amplified ML5-ML6 region and ISG. Such correlation is observed between North American S and P ISGs; lack of it may be further evidence that the Italian F and S ISGs have only recently separated.

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INFLUENCE OF TRACHEID CONDUCTIVITY AND SOIL MOISTURE ON PROCERUM ROOT DISEASE EXPRESSION IN PINUS STROBUS. J. R. Butnor, J. A. Gray and J. R. Seiler. VPI&SU, Blacksburg, VA. 24061-0330.

We studied the effects of soil moisture stress and procerum root disease (causal agent: *Leptographium procerum*) in *Pinus strobus*. Sapwood colonized by *L. procerum* becomes impregnated with resin, resulting in foliar symptoms resembling drought stress. Trees may exhibit normal water relations despite resin occlusion, suggesting that differences in stem conductivity and/or site factors affect symptom expression. The relationships between basal resin occlusion (Oc) of tracheids, stem hydraulic conductivity (Lp), sapwood moisture content (MC), soil moisture (SM) and symptom expression (stomatal conductance, Sc) were evaluated in eight plots in two Christmas tree plantations. Sc and SM were estimated periodically while Oc, Lp, and MC were estimated at the termination of the study. Lp, MC and Sc decreased significantly ($p=0.0001$) with increasing Oc. Field observations in conjunction with on-going greenhouse studies will elucidate the role of soil moisture in disease expression.

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SPHAEROPSIS SAPINEA AND *BOTRYOSPHAERIA DOTHIDEA* ENDOPHYTIC ON PINES AND EUCALYPTS IN SOUTH AFRICA. H. Smith[1], M. J. Wingfield[1], T. Coutinho[1], and P. Crous[2], [1]Dept. of Microbiology and Biochemistry, Univ. of the Orange Free State, Bloemfontein 9300, South Africa, and [2]Dept. of Plant Pathology, Univ. of Stellenbosch, Stellenbosch 7600, South Africa.

Sphaeropsis sapinea and *Botryosphaeria dothidea* are taxonomically and ecologically similar fungi that cause serious canker and die-back diseases of *Pinus* spp. and *Eucalyptus* spp. respectively. These fungi occur abundantly on dead pine and eucalypt tissue throughout the country, and cause disease when trees are stressed or physically damaged by hail, wind, frost or insects. Until recently, we have viewed *S. sapinea* and *B. dothidea* as opportunistic pathogens that infect trees only under conducive conditions. In contrast to this view, exhaustive isolations from pine and eucalypt tissue have shown that both fungi apparently exist as symptomless endophytes in healthy trees. The enigma relating to their rapid ingress of stressed and damaged trees can thus be explained by their endophytic habit.

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FIRST REPORT OF HYPOVIRULENCE IN BRAZILIAN ISOLATES OF *CRYPHONECTRIA CUBENSIS*. L.M. Van Zyl[1], M.J.Wingfield[1], B.D. Wingfield[1], P. W. Crous[2], G.H.J. Kemp[1], and A.C. Alfenas[3], [1]Dept. of Microbiology and Biochemistry, Univ. of the Orange Free State, Bloemfontein 9300, South Africa, [2]Dept. of Plant Pathology, Univ. of Stellenbosch, Stellenbosch 7600, South Africa, and [3]Dept. of Plant Pathology, Univ. of Viçosa, MG 36570, Brazil.

Cryphonectria cubensis causes a serious canker disease of *Eucalyptus* in many tropical and subtropical parts of the world, including Brazil and South Africa. A three kb segment of double stranded (ds) RNA was associated with three of 30 isolates of *C. cubensis* from Brazil, showing morphological characteristics suggestive of hypovirulence. These dsRNA-containing isolates were significantly less virulent than those that were dsRNA free. Conversion of virulent isolates with normal morphology to hypovirulence was achieved by pairing isolates of apparently similar vegetative compatibility. Although hypovirulence in isolates of *C. cubensis* has previously been reported from South Africa, this is the first evidence of its presence from Brazil. Opportunities for exploiting this phenomenon in biological control are currently being investigated.

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EVALUATION OF WOOD DECAY FUNGI AND INSECTS IN PREDISPOSING BOTTOMLAND HARDWOODS IN THE ATCHAFALAYA BASIN OF LOUISIANA TO WIND DAMAGE CAUSED BY HURRICANE ANDREW. T.D. Leininger, A.D. Wilson, and D.G. Lester. USDA Forest Service, Southern Research Station, Southern Hardwoods Laboratory, P.O. Box 227, Stoneville, MS 38776.

Hurricane Andrew caused severe damage to over 780 sq. km. of bottomland hardwood and cypress-tupelo forests in the Atchafalaya Basin of Louisiana in August 1992. Six sites, classified as sustaining either severe or minor wind damage, were studied along the path of the hurricane. All trees within two perpendicular (east-west and north-south) transects 2 m wide located in seventeen 25-m diameter point-sample subplots were evaluated for damage to roots, stems, and canopies during early May 1994. Trees also were examined for the incidence and severity of signs, symptoms, and indicators of insects and wood decay fungi, ostensibly present before the hurricane, which may have predisposed trees to windthrow or breaks in the bole or top. Indicators and damage caused by insects and wood decay fungi were uncommon at all sites so that predisposition to wind damage by these agents was not established. There was no relationship apparent between wind direction of the hurricane, tree species, or DBH and severity of wind damage based on a damage rating scale.

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DROUGHT STRESS HAS OPPOSITE INFLUENCES ON SYMPTOM DEVELOPMENT BY POPULUS HYBRID STEMS AND EXCISED LEAF DISKS INOCULATED WITH *SEPTORIA MUSIVA*. D.L. Maxwell and G.R. Stanosz. Dept. Plant Pathology, University of Wisconsin, Madison, WI 53706.

Septoria musiva Peck (teleomorph *Mycosphaerella populorum* G.E. Thompson) causes a leaf spot and canker disease of poplars, reported to be more severe on harsh sites. To determine if drought stress predisposes hybrid poplars to colonization by *S. musiva*, we conducted a greenhouse study of clones NM-6 (nigra X maximowiczii) and NE-308 (nigra var. charkowiensis X berolinensis). We planted 6 rooted cuttings of each clone in 56x46x28 cm boxes containing 1:1 Fafard Mix #2:sandy loam field soil. Boxes were watered either 3 times/wk; or after the predawn water potential fell below -1 Mpa. Removing the fourth fully expanded leaf from the apical meristem, we placed an agar plug colonized by *S. musiva*, or a sterile plug, over the leaf scar. After 80 days, stressed trees developed significantly larger cankers than non-stressed trees. We cut two 15mm diameter disks from the first or second fully expanded leaf from two trees of each clone per box, and placed them in 24 well tissue culture plates. Each disk in a pair was inoculated with 0.1 ml of either sterile water or 10^4 spore/ml suspension. We used Optimas image analysis software to assess disease severity. In contrast to our canker data, disks from stressed trees remained less symptomatic than those from well-watered trees. These results indicate that host condition influences *S. musiva* symptom development in hybrid poplar stems and excised leaf disks.

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THE ROLE OF OXALATE DECARBOXYLASE IN pH REGULATION BY BROWN-ROT FUNGI. J.A. Micales, U.S.D.A. - Forest Service, Forest Products Laboratory, One Gifford Pinchot Dr., Madison, WI 53705.

Many brown-rot wood decay fungi accumulate large quantities of oxalic acid and dramatically decrease the pH of decaying wood. Oxalate decarboxylase, the enzyme that breaks oxalic acid into formic acid and carbon dioxide, was recently detected in several different species of brown-rot fungi including *Postia placenta* and *Gloeophyllum trabeum*. The enzyme has an extremely low pH optimum of 2.0 - 2.2 and appears to be induced under low pH conditions. Oxalate decarboxylase is loosely attached to the hyphal surface and may prevent the fungus from accumulating toxic levels of oxalic acid. As oxalic acid breaks down to the weaker formic acid, the pH of the wood increases slightly, thus permitting the pH of the wood to stabilize at a nontoxic level of acidity. Strain ME20, a low decay isolate of *Postia placenta* that fails to accumulate oxalic acid, overproduces oxalate decarboxylase. The inability of this strain to accumulate oxalic acid seems to interfere with decay development, thus preventing wood weight loss and cellulose depolymerization. Interfering with the production and regulation of oxalate decarboxylase, through the use of specific enzyme inhibitors or by modifying wood pH, could be a possible control strategy for the prevention of brown rot.

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HETEROBASIDION ANNOSUM AND BLUE STAIN FUNGI IN ROOTS OF LONGLEAF PINE ARE ASSOCIATED WITH INCREASED MORTALITY FOLLOWING PRESCRIBED BURNING. William J. Otrosina[1], Linda White, and Charles H. Walkinshaw[1]. USDA Forest Service, Institute for Tree Root Biology, 320 Green Street, Athens, GA 30602; and [1]USDA Forest Service, Alexandria Forestry Center, Alexandria, LA 71360.

Roots from experimental plots in a 40 year old longleaf pine stand (Savannah River Plant, New Ellenton, SC) that had been thinned prior to initiating prescribed fire treatments were sampled to determine extent of infection by H. annosum (Ha). Root samples (> 3.0 cm diameter) were obtained from dead and symptomatic trees from both burned and non-burned (control) plots. Additional samples of higher order lateral roots (< 3.0 mm diameter) were also obtained randomly from each treatment plot. Mortality rates of trees in the burned plots and control plots were 46 trees/ha and 16 trees/ha, respectively. About 50% of roots sampled from mortality trees in burned plots were positive for Ha compared to 15% of control roots. Also, Leptographium sp. were isolated in frequencies similar to that of Ha with respect to burned versus control plots. Histological evidence of damage to small lateral roots in burned plots was observed despite normal visual appearance.

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RELATIONSHIPS BETWEEN BLUE-STAIN FUNGI IN ROOTS OF LOBLOLLY PINE AND SOUTHERN PINE BEETLE ATTACK. William J. Otrosina, Nolan Hess, John P. Jones[a], Stanley J. Zarnoch, and Thelma J. Perry. USDA Forest Service, Institute for Tree Root Biology, 320 Green Streeet, Athens, Georgia 30602; and Louisiana State University[a], Baton Rouge, Lousiana.

Forty paired plots comprised of southern pine beetle (SPB) infested (less than 1 month since attack onset) and adjacent non-infested control plots were established in natural stands and plantations of loblolly pine. No statistical differences were found in stand parameters such as age, basal area, DBH, radial growth rate, and height between control and SPB infested plots. Bluestain fungi were isolated with a frequency of 0.875 from SPB infested plots versus 0.45 of the control plots ($P < 0.001$). Three species of blue-stain fungi identified during this study were Ophiostoma ips, Leptographium terebrantis, and L. procerum. Of these species, L. terebrantis was isolated most frequently from SPB infested plots (0.275) versus control plots (0.025), $P < 0.002$. In preliminary inoculation studies, L. terebrantis was most pathogenic to loblolly pine seedlings.

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THE SUSCEPTIBILITY OF SOUTHERN APPALACHIAN OAKS TO *Phytophthora cinnamomi*. **A. P. Jordan and F. H. Tainter. Department of Forest Resources, Clemson University, Clemson, SC 29634-1003.**

Thirty-three pole-sized white (WO), southern red (SRO), and scarlet (SCO) oaks of dominant or suppressed crown position on two sites were inoculated at 1.4 m with mycelia of either of two isolates of *Phytophthora cinnamomi* in late July, 1994. Six months later the inoculated areas were debarked and checked for canker development. Cankers formed around mycelial plugs in 100% of the trees. Mean canker length/width was 43.7/4.3 cm. From these observations, it appears that these species are very susceptible to this soil-borne pathogen. However, naturally occurring cankers on oaks have not yet been reported in the United States even though *P. cinnamomi* is common in southern Appalachian soils.

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RAPID, ACCURATE, NONDESTRUCTIVE ESTIMATION OF SYMPTOM DEVELOPMENT IN A HYBRID POPLAR LEAF DISK ASSAY OF SUSCEPTIBILITY TO *SEPTORIA MUSIVA*. D. L. Maxwell, R.N. Spear, and G. R. Stanosz. Dept. Plant Pathology, University of Wisconsin, Madison WI 53706.

Commercially available hardware and software for image acquisition and analysis can facilitate estimation of foliage disease severity. These methods can decrease labor, increase accuracy, and allow repeated, nondestructive measures. We utilized the program Optimas 4.1 (BioScan, Inc., Edmonds, WA) to quantify symptom development in a leaf-disk assay used to assess susceptibility of *Populus* hybrids to the leafspot and canker pathogen *Septoria musiva* Peck. Leaf disks (15 mm diameter) were placed in 24-well tissue culture plates with 1 ml water agar per well. They were inoculated with 0.1 ml of either sterile water or a suspension of 10^4 conidia per ml and incubated in the light at 20°C for nine weeks. Periodically after inoculation we acquired and saved JPEG compressed images of 240 leaf disks using a Sony 3CCD color video camera and a TrueVision Targa M8 frame grabber board. Later, we analyzed these images using a macro that semi-automated the process. Our macro made it possible to distinguish between green, healthy tissue and dark, necrotic tissue with a high degree of accuracy, and incorporated the percent area routine of the Optimas program to calculate relative disease severity. Data was exported directly into an Excel 4.0 (Microsoft, Inc., Redmond, WA) spreadsheet. We were able to complete image acquisition, storage, and analysis and proceed to statistical analysis in under 12 hr for 240 disks (3 min per sample).

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INFLUENCES OF DIFFUSIBLE EXTRACTS FROM SELECTED CONIFEROUS WOODS ON GERMINATION OF BASIDIOSPORES OF THE INDIAN PAINT FUNGUS. A. D. Wilson, USDA Forest Service, Southern Research Station, Southern Hardwoods Laboratory, P.O. Box 227, Stoneville, MS, 38776, and O. C. Maloy, Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.

The effects of diffusible extracts from selected coniferous woods on germination of basidiospores of Echinodontium tinctorium (the Indian paint fungus) were tested in vitro on 1.5% water agar (WA) amended with 10 ppm Benlate. Basidiospores in spore prints collected from basidiocarps were frozen at -10 C, diluted to appropriate concentrations, and plated on WA containing two sterile decorticated wood discs from living branches of either grand fir, western hemlock, ponderosa pine, or western red cedar. The percentage of spores germinating in 20 microscopic fields (400-1000× mag.) with at least 20 spores was determined. Significantly higher percent germination was observed with spores exposed to diffusible extracts from host woods of grand fir (2.3%) and western hemlock (2.2%) than with spores exposed to extracts from nonhost wood of western red cedar (0.3%) or controls (0.4%) without wood extracts, with the exception of ponderosa pine (4.3%). Germination tended to decline for spores receiving secondary freezing prior to exposure to wood extracts.

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INCIDENCE OF ASH YELLOWS IN GREEN ASH AND ASSOCIATION WITH TREE HEALTH IN IOWA AND WISCONSIN CITIES. M.L. Gleason, P.H. Flynn, T.E. Engle, and M.A. Vitosh, Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Green ash (*Fraxinus pennsylvanica* var. *lanceolata*) in nine cities in Iowa and Wisconsin were surveyed for crown condition, radial growth, and incidence of the ash yellows (AY) phytoplasma during 1994. In each community, the survey included 12 trees ≥ 15 cm DBH in each of three health categories (1-10%, 10-30%, and >30% crown dieback) and 0-4 trees with putative witches' brooms. Root segments 0.2-0.3 cm in diameter, sampled during 30 Jul-30 Sept, were sectioned longitudinally, stained with DAPI, and examined under a fluorescent microscope for presence of the AY phtyoplasma in phloem sieve tubes. Incidence of AY per city ranged from 3 to 27%. Preliminary analysis showed no apparent relationship between AY infection and crown condition of individual trees.

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MORTALITY PREDICTION IN SOUTHERN UPLAND OAKS AFFECTED BY DECLINE AND GYPSY MOTH. S. W. Oak[1], D. A. Starkey[1], J. G. Williams[2] and F. H. Tainter[2]. USDA Forest Service[1], Southern Region, Forest Health, Atlanta, GA 30367 and Department of Forest Resources[2], Clemson University, Clemson, SC 29634.

Of 15 stand/site variables measured in 1985 and 1987, a logistic regression model was produced that included oak basal area, site index, soil depth class and stand age (R^2 = 0.65) and which correctly predicted 86% of oak decline events in western VA. Gypsy moth subsequently entered the survey area. Defoliation frequency and periodicity were considered as inciting and exacerbating decline factors. Stepwise discriminant analysis identified the number of consecutive defoliations, site index, site index/stand age and initial decline incidence as significant variables for predicting incidence change ≥15%. A logistic model (R^2 = 0.72) correctly predicted 81% of these events. Three or more consecutive defoliations usually yielded a large incidence increase irrespective of initial decline status, while only one or two consecutive defoliations produced a similar effect where decline damage already existed.

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EFFECT OF COVER-CROPPING AND FUMIGATION ON *FUSARIUM* SPECIES POPULATIONS IN BARE-ROOT NURSERY SOILS. J.Juzwik, C.M. Ocamb, and K.R. Cease, USDA Forest Service, 1992 Folwell Ave., St Paul, MN 55108.

Soilborne *Fusarium* populations were determined in two Wisconson nurseries following sudan grass cropping or bare fallow treatment, and operational methyl-bromide-chloropicrin (MBC) fumigation. Levels of *Fusarium* species were determined prior to cover crop sowing, post-cover crop incorporation but pre-fumigation, and from one month post-fumigation through the first growing season of the subsequent white pine crop. Fungal populations were not different between sudan grass and fallow areas on post-incorporation and later sampling dates. *Fusarium* species were recovered from 0-15 cm depth (0-250 cfu/g dry soil) and from 15-25 cm depth (150-775 cfu/g dry soil) one month after fumigation. Major pathogenic taxa persisted through fumigation. These taxa included *F. oxysporum*, *F. oxysporum* var. redolens, and *F. solani* in both nurseries, and the addition of *F. proliferatum* at one nursery. These results partially explain why high levels of root rot periodically occur in white pine seedlings grown in MBC-fumigated fields in these nurseries.

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CHARACTERIZATION OF THE NECROSIS TOXIN OF PYRENOPHORA TRITICI-REPENTIS STRAIN 86-124. S.W. Meinhardt[1], H-F. Zhang[1], J.G. Jordahl[2], L.J. Francl[1]. Department of Biochemistry, Department of Plant Pathology, North Dakota State University, Fargo, ND 58105.

The *Pyrenophora tritici-repentis* necrosis toxin has been isolated by a method allowing the production of milligram quantities within a single preparation. SDS gel electrophoresis and amino acid composition indicate the toxin to be the same protein isolated previously (Ballance et al. 1989. Physiol. Molec. Plant Path. **35**: 203-213). Non-denaturing isoelectric focussing indicated a basic protein with a pI near 10. The toxin was cleaved by endoproteases and 80% of the protein sequenced by gas phase protein sequencing. Second-ary structure prediction indicate a $\alpha+\beta$ protein consisting of mostly β-sheet with an α-helix near the carboxyl terminal. The predicted secondary structure is consistent with that determined from circular dichroism.

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FUSARIUM SHEATH ROT OF RICE IN ARKANSAS. R.D. Cartwright, J.C. Correll, and D.L. Crippen, Dept. of Plant Pathology, PTSC 217, University of Arkansas, Fayetteville, AR 72701.

An unusual rice disease was observed in 1993 and 1994 on the cultivar Bengal in Arkansas. Symptoms included dark brown lesions on the flag leaf sheath and blanking/discoloration of the panicle. Profuse sporulation was evident on the sheath lesions and isolations consistently yielded a fungus tentatively identified as *Fusarium proliferatum*. This fungus was also isolated from diseased tissue collected at other locations. Isolates were grown on PDA, conidia harvested in sterile distilled water and injected into stems, sheaths, unemerged panicles and sprayed on newly emerged panicles in the greenhouse. Symptoms similar to those in the field developed on inoculated plants in 1-4 wks and *F. proliferatum* was consistently isolated from symptomatic tissue. Young tillers were sometimes killed outright and spray inoculated panicles were uniformly blanked. Profuse sporulation of the fungus was noted on diseased tissue under nightly misting. *Sarocladium oryzae*, which causes sheath rot of rice, was also tested but produced different symptoms. Based on these findings, the disease appears identical to Fusarium sheath rot of rice, reportedly caused by *F. moniliforme* in India. This is the first report of the disease in the U.S.

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PATHOGENICITY OF VARIOUS STRAINS IN *FUSARIUM* SECTION *LISEOLA* TO CORN AND SORGHUM. Wang Mei, L. E. Claflin, and B. A. Ramundo. Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502.

Fusarium section *Liseola* (teleomorph *Gibberella fujikuroi*) comprises important pathogens to numerous crops including corn (*Zea mays*) and grain sorghum (*Sorghum bicolor*). Pathogens include *F. moniliforme* (*G. fujikuroi* mating populations "A" and "F"), *F. subglutinans* ("B" and "E"), and *F. proliferatum* ("D"). Three sorghum cultivars and two maize inbreds were inoculated with mating populations A, B, D, E, and F. The A and F strains were not significantly different in host preference between corn and sorghum. In general, strains B, D, and E were less virulent in corn and sorghum than strains A and F.

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EXAMINATION OF VIRULENCE AMONG ISOLATES IN MGR586 DNA FINGERPRINT GROUPS OF THE RICE BLAST PATHOGEN, PYRICULARIA GRISEA, IN ARKANSAS. J. C. Correll, J. C. Guerber, and F. N. Lee. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

DNA fingerprinting has been used to characterize regional genetic diversity of the rice blast pathogen, Pyricularia grisea. Four distinct MGR586 DNA fingerprint groups (A, B, C and D) have been found in the contemporary (after 1992) blast pathogen population with multiple haplotypes (isolates with 1-20% fingerprint variation) within each of the four groups. Virulence of 10-20 monoconidial isolates from each of the four groups were examined. Each of the fingerprint groups was represented by multiple haplotypes. Race determinations were performed on a set of Arkansas and a set of International differential rice lines in greenhouse inoculation tests. The majority of the isolates belonged to two virulence phenotype "groups", an IC1-IC17 group and an IB1-IB49 group. Isolates in the A and C fingerprint groups belonged to the IB1-IB49 virulence phenotype and isolates in the B and D fingerprint groups belong to the IC1-IC17 phenotype. Several recently recovered isolates, capable of infecting the previously resistant cultivar Katy, all belonged to MGR586 DNA fingerprint group B.

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CHARACTERIZATION OF *Verticillium dahliae* ISOLATES FROM COTTON C.A.N.OKOLI*, D. FERNANDEZ & J.P. GEIGER. Centre ORSTOM, Laboratoire de Phytopathologie, 911 Avenue Agropolis, Montpellier cedex, France

Verticillium is a fungus best known for those species within it which are pathogenic, especially on plants. Sub-specific classification has been difficult largely because of lack of host specificity, morphological variability and near absence of any recognized race structure. On cotton, *V. dahliae* is classified as defoliating or non-defoliating based on severity of symptoms on cotton plants. Isolates from cotton planted in different geographical locations were characterized by RFLPs and also by RAPDs. Three of the twenty random primers used distinguished the defoliating isolates from all the others. A *V. dahliae*-specific probe hybridized without polymorphism to all the isolates used in the study. These isolates were compared to representative members of the groups defined by Okoli et al., 1993, 1994. Results indicate that the species of *V. dahliae* as presently defined may actually consist of very-closely related, but distinct populations.

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ALTERNATE HOST RANGE OF PUCCINIA SUBSTRIATA VAR. INDICA. J. P. Wilson and S. C. Phatak, USDA-ARS Forage and Turf Unit and Department of Horticulture, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793.

The alternate host range of the pearl millet rust pathogen, Puccinia substriata var. indica was examined. Thirty-one accessions of Solanum melongena, each collected from a different country, and accessions of 27 other Solanum species were evaluated by natural infection in the field and inoculation in the greenhouse. Accessions were considered susceptible if aecia developed. All accessions of S. melongena were susceptible except one from Burkina Faso (PI 413784) and one from Ivory Coast (PI 401533), countries which are near the center of diversity of pearl millet. Newly identified alternate hosts include S. anguivi, S. ferox, S. incanum, S. linaeanum, S. gilo, S. rostratum, and S. nodiflorum. All other Solanum species evaluated were resistant. Two weed species from the United States, S. americanum and S. aviculare, were resistant and probably play no role in the epidemiology of pearl millet rust in the U.S.

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GEOGRAPHIC DISTRIBUTION OF MATING TYPES OF *SPHAEROTHECA FULIGINEA* IN THE U.S. IN 1993-94. **N. Shishkoff, M. T. McGrath, H. Staniszewska, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY, 11901.**

To determine the frequency and distribution of mating types of *Sphaerotheca fuliginea*, a powdery mildew of cucurbits whose perfect state has rarely been reported, a total of 287 isolates were collected from 27 field sites in 8 states in 1993 and 1994. They were paired with powdery mildew isolates of known mating type (MAT1-1 or 1-2) in detached leaf culture. Both mating types were present in the 8 states (AZ, CA, FL, GA, NY, NC, TX and VA) in 1993 or 1994. In 9 field sites only a single mating type was collected; in the remaining 18, both mating types were present, although one tended to predominate. The mating type predominating varied from site to site, state to state, and year to year; for example, in 1993 MAT1-1 made up 82% of isolates tested; in 1994 it was 31%. Therefore, the rare reports of the perfect state in the U.S. are not due to a scarcity of one mating type or to a geographic separation of mating types.

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THE USE OF RAPDS AND MGR586 DNA FINGERPRINTING TO CHARACTERIZE RICE BLAST ISOLATES OF PYRICULARIA GRISEA FROM A SINGLE LOCATION IN ARKANSAS. **W.J. Ross, J.C. Correll, and R.D. Cartwright. Dept. of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.**

Isolates of *Pyricularia grisea* were recovered from a single rice plot in White Co., AR, in 1994. The plot contained three replications of twenty rice cultivars commonly grown in Arkansas. The plot was located within a commercial rice field (cultivar Alan). Approximately 25 infected panicles were collected per cultivar for each replication. Isolates were examined for RAPDs using the OPX-12 primer and DNA fingerprinting using MGR586. RAPDs with OPX-12 and MGR586 DNA fingerprinting identified the four DNA fingerprint groups commonly found in the contemporary rice blast pathogen population in the state. In the White Co. plot, all four DNA fingerprint groups were recovered from the highly susceptible cultivar M204. However, the frequency of each group recovered from M204 varied from 2 - 67%. Preliminary data indicated that several cultivars were resistant to certain MGR586 DNA fingerprint groups prevalent in the local pathogen population.

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LINEAGE STRUCTURE, AVIRULENCE LOCUS POLYMORPHISM AND THE ORGANIZATION OF PATHOTYPE DIVERSITY IN THE RICE BLAST FUNGUS. Morris Levy, A. K. M. Shahjahan and Barbara Valent. Purdue University, West Lafayette, IN 47907 and DuPont Experimental Station, Wilmington, DE 19880,USA.

The rice blast fungus, *Pyricularia grisea*, exhibits high levels of pathotype polymorphism that have handicapped efforts to breed durably resistant rice cultivars. MGR-DNA fingerprints indicate that pathogen populations worldwide are typically composed of distinct genetic lineages. Each lineage has a limited cultivar range, although most express multiple pathotypes that are varied combinations of lineage-wide compatibilities. Thus, lineages typically express particular, historically conserved avirulences. Complementary evidence of this organization in a large international sampling of populations is now provided by lineage-specific RFLPs associated with *P. grisea* avirulence gene probes and by lineage-specific exclusion (incompatibility) by single resistance genes in near-isogenic testers. Based on these features we are evaluating particular resistance gene combinations that may exclude all resident lineages in various rice-growing regions. The global distribution of lineage diversity also is discussed.

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EFFECT OF BASE-SOLUBLE PROTEINS FROM CORN ON CELL-SURFACE INTERACTIONS AND GROWTH OF *ASPERGILLUS FLAVUS*
J. N. Neucere and T. E. Cleveland, USDA, ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124-4305.

Recent studies showed that a diversity of polypeptides and proteins within cereal grains are implicated in resistance to fungal infections. In this study, the base-soluble proteins from *Aspergillus flavus* resistant (Yellow Creole) and susceptible (Huffman) genotypes of corn were investigated by in vitro studies. Bioassays of fungal growth inhibition in solid media showed activity in both varieties with disparity in potency dosage. Cell-surface interactions by red blood cell agglutination assays showed hemolysis by proteins extracted from the susceptible genotype but no adverse reaction by protein from the resistant genotype. Cathodic PAGE of native proteins showed over six protein bands with differences in quantity of individual components. Native anodic PAGE showed diffused banding patterns with two bands present in the susceptible genotype not detected in the resistant genotype. SDS-PAGE showed four distinct bands in Yellow Creole that were absent in Huffman.

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GENETIC STRUCTURE AND VIRULENCE DIVERSITY OF *Pyricularia grisea* IN BANGLADESH. A. K. M. Shahjahan, C. M. Rojas and Morris Levy. Purdue University, West Lafayette, IN

282 Bangladesh field isolates of P. grisea, collected during 1988-93, were characterized for MGR-DNA fingerprints, RFLP polymorphisms for two avirulence gene probes, and for pathogenicity on a variety of rice cultivars, including near-isogenic lines (NILs) carrying single resistance genes. DNA fingerprints defined 14 distinct lineages typically found on rice hosts and one lineage each from the grass hosts Eleusine indica, Leersia hexandra and Digitaria setigera; most lineages occurred in multiple years. Some E. indica isolates were obtained from rice hosts and, rarely visa versa, but with no marker evidence of genetic recombination between these usually host-limited forms. Generally, avr-locus RFLPS also had lineage-specific distributions. Frequently detected lineages had broad but distinctive virulence spectra, each containing multiple but related pathotypes. The combination of R-genes Pi-1(t) and Pi-2(t) genes is effective against all lineages except BANG-4 and this was reflected in the field reactions of the NILs obtained under nursery conditions.

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A TECHNIQUE FOR PRODUCING CONIDIA OF *MONILINIA VACCINII-CORYMBOSI* ON ARTIFICIAL MEDIA. V. Brewster, A.W.Stretch and M.K.Ehlenfeldt. USDA-ARS, Blueberry and Cranberry Research Center, Chatsworth, NJ 08019.

Evaluation of blueberry plants for resistance to the fruit infection phase of the mummy-berry fungus, *Monilinia vaccinii-corymbosi*, has been difficult due to low production of conidia in culture. Limited numbers of spores are produced on V-8 agar(less than $1x10^3$/plate). We have modified the use of V-8 agar by growing the fungus on cellulose filter membranes placed on the surface of the medium. Conidia have been produced in quantities ranging from $2.4x10^4$ to $2.4x10^5$ spores/plate depending on the isolate. These conidia produced typical symptoms on inoculated plants thereby extending the evaluation season.

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MANAGEMENT OF PHYTOPHTHORA ROOT AND CROWN ROT AND BLIGHT OF CITRUS WITH SOIL AMENDMENTS. S. Nemec and O. Lee, USDA, ARS, 2199 South Rock Road., Fort Pierce, FL. 34945, and Route 1, Box 586, St. Cloud, FL.

Two rates of reed sedge peat, shrimp hull waste, humate, bentonite clay, gypsum, and a calcium humate were preplant strip-tilled 1.2 m deep and 1.6 m wide down the center of rows in a new citrus grove site 13 km southeast of St. Cloud, FL in August 1984. Six treatment replicates, 4 trees each of 'Valencia'/rough lemon were established in February 1985. Gypsum, calcium humate, and humate were reapplied to the soil surface annually. Cumulative ratings for *Phytophthora parasitica*-caused foot rot for each treatment from 1985 to 1988 showed that combined rates of gypsum (1120 and 2240 kg ha^{-1}) significantly ($P=0.05$) reduced foot rot, compared to the no amendment control. This may have been the result of the increase in leaf Ca and S and bark Ca in those treatments. Blight ratings begun in 1990 and biannually thereafter through April 1994 revealed that after October 1990 peat (81805 and 261775 kg ha^{-1}) significantly ($P=0.05$) suppressed the development of blight. Data from soil nutrient analysis and soil propagules of functional microflora groups did not indicate a reason for this blight suppression.

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PHYTOPATHOGENIC *FUSARIUM* STRAINS IN BIOLOGICAL CONTROL OF *CANNABIS SATIVA*. Semenchenko, G.V.[2], Tiourebaev, K.S.[2], Dolgovskaya, M.[3], Schultz, M.T.[1], McCarthy, M.K.[1], and Sands, D.C.[1] Department of Plant Pathology, Montana State University, Bozeman. [2]Institute of Zoology, Academy of Science of Kazakhstan, Almaty. [3]St. Petersburg Zoological Institute, St. Petersburg, Russia.

Strains of the fungal pathogen, *Fusarium oxysporum* f. sp. *cannabis*, were isolated from *Cannabis sativa* plants showing symptoms of yellowing, leaf curling, and stunted growth. After preliminary host range evaluation, twenty-five strains were evaluated in the field. The most virulent strain, CR-21, elicited wilt symptoms within two weeks of inoculation of field plants. Twelve of the 25 strains assessed were pathogenic on *C. sativa*. The efficacy of inoculum was dependent upon formulation, temperature, and the size of *C. sativa* plants. Pathogenic strains were efficient in causing disease in the early part of the season when temperatures were between 15 and 23°C. Additionally, plants were most sensitive to infection when they were 5-7 cm in height. At the end of the field season, 67% of the *C. sativa* plants screened were infected with *F. oxysporum*.

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THE MOLECULAR KARYOTYPES OF *PLASMODIOPHORA BRASSICAE* AND *SPONGOSPORA SUBTERRANEA*, TWO OBLIGATE, INTRACELLULAR PATHOGENS. A. T. Trese, R. J. Bryan, and J.P. Braselton, Ohio University, Athens, Ohio 45701

Economically important pathogens in the plasmodiophorid group include the causal agents of cabbage clubroot, *Plasmodiophora brassicae Wor.* and potato powdery scab, *Spongospora subterranea* f. sp. *subterranea*. These organisms have been classified as either fungi or protozoans. The uncertain taxonomic position of these organisms is due, in part, to their life cycles as obligate, intracellular parasites. There is no data available describing classical genetic crosses, molecular cloning, or gene sequencing in this group. We have obtained molecular karyotypes for *P. brassicae* and *S. s.* f. sp. *subterranea* hrough pulsed field gel electrophoresis, using plasmodia extracted directly from infected plant tissue by simple mechanical dicing and crushing. Extending this analysis to include hybridizations with selected probes will make it possible to more precisely define the relationships between members of the plamodiophorid group.

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GENETIC VARIATION AMONG ISOLATES OF *PHAEOISARIOPSIS GRISEOLA* IN SOUTHERN AFRICA. W.H.P. Boshoff[1], W.J. Swart[1], Z.A. Pretorius[1], M.M. Liebenberg[2], and P.W. Crous[3], [1]Dept. of Plant Pathology, Univ. of the Orange Free State, Bloemfontein 9300, South Africa, [2]Grain Crops Inst., Potchefstroom 2520, South Africa, and [3]Dept. of Plant Pathology, Univ. of Stellenbosch, Stellenbosch 7600, South Africa.

Angular leaf spot caused by *Phaeoisariopsis griseola* is an economically important disease of beans (*Phaseolus vulgaris*). For breeding programmes to succeed, the extent of genetic variation within the pathogen population must be determined. Twenty-eight isolates of *P. griseola* from various localities were compared using isozyme analysis by means of starch gel electrophoresis. Thirteen loci were identified in ten enzyme systems. Using UPGMA, three electrophoretic types (ETs) were detected. The most common ET (type one) included all the South African isolates, namely seven from the Gauteng and Natal Provinces, respectively, 10 from Malawi, and one from Portugal. Two isolates from Bembeke in Malawi and one from The Netherlands differed from the rest. A *Phaeoisariopsis* sp. from lemon used as an outgroup differed from the *P. griseola* isolates in all enzyme systems tested. The high homology of banding patterns among isolates of *P. griseola* from Southern Africa suggest the local population to be very uniform.

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GENETIC DIVERSITY AMONG ISOLATES OF *COLLETOTRICHUM GLOEOSPORIOIDES* INFECTING FORAGE LEGUME *STYLOSANTHES* SPP. S. Kelemu, C. X. Moreno, M. X. Rodriguez, and J. L. Badel. Plant Pathology Section, Tropical Forages Program, Centro Internacional de Agricultura Tropical, Apartado Aereo 6713, Cali, Colombia (S. A).

Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., is the most important and widespread disease of *Stylosanthes*, a diverse tropical and subtropical forage legume naturally distributed in Central and South America. The pathogen is a heterogeneous species consisting of various host specific populations. The amount of genetic diversity of 138 South American and Australian isolates in two populations of *C. gloeosporioides* was measured at molecular level by polymerase chain reaction (PCR) amplification of DNA using 11 arbitrary primers of 10 bases. The amplifications revealed scorable polymorphism among the isolates, and a total of 125 band positions was scored. Two sets of differential hosts were used to characterize pathogenic variability in 106 of the isolates. No distinct correlations existed between genetic diversity as measured by random amplified polymorphic DNA (RAPD) and pathogen race as defined by pathogenicity pattern on the differentials. However, non-pathogenic or weakly pathogenic isolates isolated from *S. guianensis* were more genetically diverse than virulent isolates from the same host, and distinctly grouped together and separated from the more virulent isolates by RAPD analysis. Generally, isolates were clustered together by their geographic origin. *S. guianensis*-specific isolates collected from Carimagua, Colombia (a *Stylosanthes* breeding and selection site) exhibited a wider-range of genetic diversity than those from a newly opened trial site in the Amazon basin of Colombia

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ANALYSIS OF INTERACTIONS BETWEEN MORPHOGENETIC MUTATIONS IN *MAGNAPORTHE GRISEA* BY GENE REPLACEMENT. Z. Shi, D. Christian, and H. Leung. Dept. of Plant Pathology, Washington State University, Pullman, WA 99164

Using chemical and insertional mutagenesis, we have identified a number of independent genes controlling various steps in the sporulation pathway of *M. grisea*. A chemically induced mutant (*con2*) and two insertional mutants (*con4* and *con7*) produce abnormal conidia. The *con2* and *con4* mutants produce appressoria but are less pathogenic than the wild type as manifested by a reduction in infection efficiency and longer latent period. The *con7* mutant does not produce appressoria and is nonpathogenic. To determine the epistatic relationship between these mutants, plasmids containing mutated *con4* and *con7* genes were introduced into a *con2* mutant to generate double mutants by gene replacement. Hybrid conidial morphology was observed in *con2*/*con4* and *con2*/*con7* double mutants. The *con4*/*con7* mutant produces appressoria but cannot infect rice. Results suggest that conidiogenesis is not determined by a linear pathway. The interactions between the morphogenetic loci influence appressorium formation, pathogenicity, and sporulation.

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LINKAGE ANALYSIS OF *GLOMERELLA CINGULATA* USING RAPDS. A.B. Thornton, C.R. Cisar, and D.O. TeBeest. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Two self-sterile isolates of *Glomerella cingulata* (anamorph: *Colletotrichum gloeosporioides*) from pecan (*Carya illinoensis*) were crossed and 45 single ascospore progeny were randomly recovered. Ten of 35 oligonucleotide primers screened for random amplification of polymorphic DNA (RAPD) fragments amplified 16 reproducible polymorphic loci in the parental isolates. Progeny were scored for the presence or absence of each RAPD locus. Chi-square analysis of the data indicated that all of the RAPD loci segrated 1:1 among the progeny. Linkage analysis of preliminary data using the computer program Mapmaker shows that none of these loci are linked. Work is continuing on construction of a RAPD-based linkage map of *Glomerella cingulata*.

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POPULATION STRUCTURE OF *MAGNAPORTHE GRISEA* IN THE CENTRAL HIMALAYAS OF INDIA . J.Kumar, R.J.Nelson and R.S.Zeigler, Division of Entomology and Plant Pathology, International Rice Research Institute, 1099 Manila, Philippines

DNA fingerprinting was used to analyze the diversity and distribution of *M. grisea* genotypes among fields from the blast-prone central Himalyan hills where traditional rice and millet cultivars are co-cultivated . *Eco*RI digests of total DNA of 170 monoconidial isolates of the blast pathogen obtained from rices, millets and weeds from 12 locations were probed using the *M. grisea* repetitive sequence MGR586. Restriction fragment length polymorphism revealed that the *M. grisea* population is highly diverse. A total of 90 haplotypes (isolates with distinct band patterns) showing an average of 60 band positions were identified from 105 rice isolates. At the level of 80 % DNA fingerprint similarity, 38 groups were identified. Distribution of the groups ("putative lineages") was largely unrelated to the cultivar of origin. However, traditional cultivars harboured the most genotypic diversity. *Eco*RI digests of the DNA from millets and weeds generally showed fewer bands, though high copy number "rice-types" fingerprints from millets and weeds were occasionally obtained. The Himalayan *M. grisea* population appears to be significantly more diverse than other populations so far studied elsewhere.

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MATING BEHAVIOUR OF *MAGNAPORTHE GRISEA* FROM CENTRAL HIMALAYAS FROM INDIA. J.Kumar and R.S.Zeigler, Division of Entomology and Plant Pathology, International Rice Research Institute, 1099 Manila, Philippines

We studied sexual fertility of the blast fungus (*M. grisea*) using about 200 isolates from rice and 6 other graminaceous hosts collected from fields in the central Himalayan hills. Each monoconidial isolate from rice and all other hosts was crossed with A and a mating types and yielded 63 fertile crosses. Isolates from ragi *(Eleusine coracana)* and foxtail millet (*Setaria italica*) showed highest fertility and both mating types were often found with in and between fields . Wide morphological variation in perithecia and ascospore morphology was observed within and among crosses. Distribution of mating types did not appear to be partitioned by geographical barriers since both mating types in ragi were found through out the region. Mating type of the rice blast fungus was determined by crossing with the most fertile tester lines from *Eleucine* and *Setaria sp.* Rice isolates were only male fertile and yielded abundant normal ascospores. Although rice and non rice isolates appear to have distinct host ranges, crosses between them in the field could occur during storage of straw bundles in which straw of all species is commonly combined.

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IDENTIFICATION OF QTLS FOR SCAB RESISTANCE IN WHEAT BY MEANS OF RAPD MARKERS. G-H. Bai[1], I. Dweikat [2] and G. Shaner[1]. [1]Department of Botany and Plant Pathology, and [2] Department. of Agronomy, Purdue University, West Lafayette, IN 47907.

Wheat scab, caused by *Fusarium graminearum*, is a destructive disease of wheat. The development of scab-resistant cultivars may be accelerated by the use of DNA markers. F6 recombinant inbred lines were derived from a cross between the resistant wheat cultivar Ning 7840 and the susceptible cultivar Clark by single-seed descent. In the greenhouse, 175 F5 and F6 lines were tested for resistance to spread of scab within a spike by injecting 1000 spores into a central spikelet. The control of resistance to spread of scab within a spike was attributed to two or three major genes and possibly some minor genes. With bulk segregant analysis, 1120 decamer primers were screened against two bulked DNAs for polymorphisms. Each bulk consisted of ten F6 families. Ten of the primers revealed polymorphisms between two resistant and susceptible bulked families and between the parents. Five of them showed significant association with scab resistance in variance analysis. These RAPD markers can be placed on two linkage groups. Two loci for scab resistance associated with these RAPD markers were identified by interval mapping. Some of the RAPD markers may be useful in screening for resistance to scab in wheat.

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DEVELOPMENT OF MAIZE NEAR ISOGENIC LINES (NILS) FOR THREE WHEAT STREAK MOSAIC POTYVIRUS (WSMV) RESISTANCE GENES. M. W. Jones[1,2], M.D. McMullen[1,3], and R. Louis[1,2]. USDA-ARS[1], OSU-OARDC, Dept. of Plant Pathology, Wooster, OH[2], and Dept. of Agronomy, Columbia, MO[3].

Three genes (*wsm1*, *wsm2*, and *wsm3*) for resistance to WSMV were identified in maize inbred Pa405 and mapped by RFLP analysis. To study the effects of these genes, NILs were developed using Pa405 as the donor parent and Oh28 as the recurrent parent. Approximately 30 cM that was delineated by the molecular markers *jc1270* and *umc65* on chromosome 6 containing *wsm1*; 50 cM from *umc92* to *bnl10.24* on chromosome 3 containing *wsm2*; and 40 cM from *umc163* to *bnl7.49* on chromosome 10 containing *wsm3* were integrated by six generations of backcrossing and then selfing. *wsm1*, *wsm2*, and *wsm3* behaved as single dominant genes (1:1 resistant:susceptible segregation in backcross progenies) when plants were airbrush-inoculated in the field. No differential reactions were observed when the NILs were rub-inoculated with five WSMV strains in the greenhouse. These NILs will be used to study the mechanism of resistance to WSMV.

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HETEROKARYON FORMATION UNDER NONSELECTIVE CONDITIONS AND ITS RESTRICTION BY VEGETATIVE INCOMPATIBILITY GENES IN *CRYPHONECTRIA PARASITICA*. D.H. Huber and D.W. Fulbright. Michigan State University, East Lansing, MI 48824-1312.

This study tested the relationship between heterokaryosis and vegetative incompatibility in *C. parasitica* without the imposition of selective growth conditions. We found that heterokaryons readily form between vegetatively compatible strains on potato dextrose agar and live chestnut tissue. Single locus mutations conferring cream (*cr*) coloration (from strain EP389) and brown (*br*) coloration (from strain 80-2) were found to complement in heterokaryons and form wild type orange pigmentation. The heterokaryotic nature of the orange mycelium was demonstrated by collecting hyphal tips, then collecting conidia from hyphal tip cultures. Hyphal tip cultures varied in color from orange to orange/brown to brown. The ratios of *cr* to *br* nuclei in the heterokaryons were inferred from counts of conidia of corresponding colors. Orange heterokaryons possessed *cr* : *br* nuclei in a 1 : 1 ratio; orange/brown heterokaryons in a 1 : 100 ratio; and brown mycelium produced only *br* nuclei. Vegetative incompatibility genes, *vic1* and *vic2*, prevented the development of orange heterokaryotic mycelium.

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DISTRIBUTION OF *GIBBERELLA FUJIKUROI* BY BIOLOGICAL SPECIES, MATING TYPE, AND FEMALE FERTILITY IN A MALAYSIAN MAIZE FIELD. Keith K. Klein, John F. Leslie and Vaishali Arjula, Dept. of Plant Pathology, Kansas State University, Manhattan, Kansas, and School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.

Samples of *Gibberella fujikuroi* (*Fusarium*, section *Liseola*) were collected along a transect through a corn field in northern Malaysia. these strains were characterized for mating population, mating type within population (where possible), and female fertility. the preponderance of the sample was found to be in mating population A. Of these, the majority (approximately 70%) were of single mating type (A+). Distribution of both mating type and species was significantly non-random, implying a highly patchy and perhaps clonal pattern of dispersal in the field. The relative effects of distribution, mating type disequilibrium, and female sterility on the effective size of these populations will be discussed.

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DETECTION OF ANTIFUNGAL COMPOUNDS IN HYDROPONICALLY GROWN SPINACH ROOTS. Roy I Konyeaso, Olufisayo A. Jejelowo, Oluwasanmi Areola, Fraline J. Castillo, Department of Biology, Texas Southern University, Houston, Texas 77004 & Dan Barta, Johnson Space Center, Houston, Texas

An assessment was made of symptom development and plant responses during interactions between *Pythium spp.* and hydroponically, aseptically cultivated spinach. Spinach plants growing inside hydroponic systems were inoculated with sterile distilled water (control) or a mycelial suspension of *Pythium spp.* (treatment) under aseptic conditions. Inoculated plants were kept inside a plant growth chamber and harvested for observation three days after inoculation. Plants showed no disease symptoms following inoculation with sterile distilled water, however, plants inoculated with *Pythium spp.* showed chlorosis of the leaves and browning of the root tips three days after inoculation. Screening for production of antifungal compounds by spinach roots in response to *Pythium spp.* was positive.

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CELL DEATH CAUSED BY HARPIN$_{Ea}$ IN TOBACCO PLANT TISSUE
L.-F. Hung, S. Pike, and A. Novacky, University of Missouri, Columbia, MO.

The hypersensitive reaction (HR) is characterized by the rapid collapse and desiccation of tissue inoculated with a high concentration of an incompatible bacterium. Harpin$_{Ea}$, isolated from *Erwinia amylovora,* elicits HR in tobacco without bacteria. We studied the effect of harpin$_{Ea}$ (a gift from S. Beer) on cell death in tobacco plant tissue using Evans blue. Dead cells were first visualized in both palisade parenchyma and subepidermal spongy mesophyll within 4 to 6 hours after infiltration with 48 μg protein ml^{-1}. The first observed dead palisade cells were surrounding stomata. The number of dead palisade and mesophyll cells increased gradually. They appeared in clusters. One hundred percent of subepidermal mesophyll were dead within 8 to 10 hours after infiltration when the intact tissue collapsed, but the percentage of palisade cells varied between experiments and usually was less. Similar results were observed when tobacco tissue was inoculated with *E. amylovora* 321. We suggest that harpin$_{Ea}$ or bacteria cause greater damage in spongy mesophyll as compared to palisade cells as a result of the larger cell surface area exposed to harpin$_{Ea}$ in the spongy mesophyll cells. As harpin$_{Ea}$ does not begin to kill the plant cells more quickly than *E. amylovora*, other factors besides harpin$_{Ea}$ may be involved in the bacterial HR. (Supported by USDA grant)

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THE EFFECT OF *ERWINIA AMYLOVORA* AND HARPIN$_{Ea}$ ON MEMBRANE POTENTIAL OF APPLE PETIOLES AND LEAVES. X. Pu, S. Pike, and A. Novacky, University of Missouri, Columbia, MO.

Harpin, the elicitor of the hypersensitive response (HR) in incompatible interactions of bacteria and plants, is thought to be also required for initiation of bacterial diseases. Cell membrane potential was measured on apple (Jonathan) petioles after inoculation with *Erwinia amylovora* wild type strains, Ea321, E9; a mutant *Ea321-K49 which lacks harpin production; and *harpin$_{Ea}$ (* Gifts from S. Beer, Cornell Univ.). Petioles inoculated with Ea321 exhibited lower initial membrane potentials: Em ~ -110 mV after 24hr; Em ~ -90 mV,after 48 hr. In contrast, the petioles inoculated with Ea321-K49 retained high initial potentials: Em ~ -140 mV, after 24 hr; Em ~ -150 mV, after 48 hr. The highly virulent strain E9 caused disease symptom 24 hr earlier than Ea321; the initial potential was around -99 mV, and after 48 hr, it was unmeasurable. In darkness additional membrane depolarization (typical of pathogenesis) occurred mainly in the pieces inoculated with Ea321 and E9. Harpin$_{Ea}$-treated petioles did not show membrane depolarization as did the wild type bacteria. However, harpin$_{Ea}$ in the bathing solution of apple leaf tissue induced membrane depolarization. These results suggest that harpin$_{Ea}$ may be responsible for the membrane depolarization during the disease process. (Supported by USDA grant)

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CLADOSPORIUM CARYIGENUM INTERACTIONS WITH HOST AND NON-HOST LEAVES. I.E. Yates[1], D.W. Maxey[1], S. Lee[2], D. Sparks[2], C.C. Reilly[3], [1]R. Russell Agr. Res. Center, ARS, USDA, Athens, GA 30604, [2]Dept. Hort., Univ. Ga., Athens, GA 30602, [3]SE Fruit&Tree Nut Lab, ARS, USDA, Byron, GA 31008

Germ tube, appressorium, and subcuticular hypha development were analyzed on host and non-host leaves for *Cladosporium caryigenum*, the pecan scab fungus. Plant features examined for competence to support fungal growth were genotype, adaxial and abaxial leaf surfaces, and leaf maturity. Germ tubes and appressoria developed on all leaves, despite the plant feature. Germ tube frequency on the susceptible host, 'Wichita', was equal to the non-host, tobacco, and significantly lower than the resistant host, 'Elliott'. Equal appressoria frequency occurred on both pecan cultivars and tobacco. Adaxial and abaxial leaf surfaces were not different within a genotype for supporting fungal development. 'Elliott', but not 'Wichita', leaf maturity influenced germ tube and appressorium frequency. Subcuticular hyphae were present only in immature leaves of 'Wichita' pecan. Thus, resistance was expressed at the plant site beneath the cuticle and at the fungal growth stage of hyphae development.

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COMPARISON OF INFECTION OF CITRUS ROOTS BY *PHYTOPHTHORA NICOTIANAE* AND *P. PALMIVORA*. T.L. Widmer[1], J.H. Graham[2], and D.J. Mitchell[1], [1]Dept. of Plant Pathology, University of Florida, Gainesville, FL 32611 and [2]Citrus Research and Education Center, Lake Alfred, FL 33850.

Phytophthora nicotianae and *P. palmivora* are two species that cause Phytophthora root rot of citrus. Although *P. nicotianae* is more prevalent in the field, *P. palmivora* is a more aggressive pathogen on citrus roots. Roots of 5-week-old citrus seedlings were inoculated with a suspension of zoospores of either *P. nicotianae* or *P. palmivora* at the root cap and zone of elongation. After 24 hours of incubation at 27 C, the inoculated root tips were excised from the rest of the root and prepared for observation. At the light microscope level after 24 hours, both species infected the cortical cells of the roots but only *P. palmivora* was observed in the stele. When observed ultrastructurally, the hyphae of both species were abundant in the intercellular spaces with direct penetration of the cortical cells. The cortical cells around the intercellular hyphae of *P. nicotianae* did not appear to be damaged because the plasma membrane was intact. In contrast, the cortical cells around the intercellular hyphae of *P. palmivora* appeared to have been disrupted; the plasma membrane was not intact and no cellular organelles were observed.

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GENETIC ANALYSIS OF CULTIVAR-SPECIFIC VIRULENCE OF *LEPTOSPHAERIA MACULANS* ON *BRASSICA NAPUS*
Patchara Pongam[1], Thomas C. Osborn[2], and Paul H. Williams[1], Depts. of [1]Plant Pathology and [2]Agronomy, Univ. of Wisconsin-Madison, WI 53706

The ascomycetous fungus *Leptosphaeria maculans*, anamorph *Phoma lingam*, is a major pathogen of crucifers, especially rapeseed (*Brassica napus*). There is a range of variation in pathogenicity among different aggressive isolates with respect to their interaction phenotype on different host cultivars. However, little is known about the genetics of virulence of the pathogen. A cross was made between two isolates of *L. maculans*, PHW 1223 and PHW 1245, which differ in pathogenicity based on their interaction phenotype on *B. napus* cv. Major. The analysis of segregating progenies and backcrosses suggests that a single major gene is involved in the interaction. Analysis of the pathogenicity of progenies from full-sib crosses are in progress.

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ACCUMULATION OF THREE DIFFERENT PHYTOALEXINS IN LEAVES OF *ARABIDOPSIS THALIANA* FOLLOWING INOCULATION WITH *COCHLIOBOLUS CARBONUM* Olufisayo Jejelowo, Department of Biology, Texas Southern University, Houston, Texas 77004 & Raymond Hammerschmidt, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Leaves of *Arabidopsis thaliana* inoculated with conidia of *Aternaria solani*, *Botrytis cinerea* or *Cochliobolus carbonum* were extracted with methanol. Extracts were applied onto thin layer chromatography (TLC) plates and developed in chlorofom:methanol, 98:2 before bioauthography with *Cladosporium*. The leaves accumulated only camalexin (R_f=0.30) in response to *A. solani* or *B. cinerea*. However, inoculation of leaves with high concentration of conidia of *C. carbonum* resulted in accumulation of camalexin and two other phytoalexins (R_f=0.15 and 0.41). The new phytoalexins flouresced purplish blue under UV light and were accumulated in small quantities in comparison to camalexin. Analysis of extracts from germinating conidia of *C. carbonum* or uninoculated leaf tissues revealed no antifungal activity. Thus, the antifungal compounds detected from leaves inoculated with *C. carbonum* are phytoalexins.

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EFFECTS OF ANTISENSE TOMATO *hmg*1 AND *hmg*2 ON DISEASE RESISTANCE OF TRANSGENIC TOBACCO AND TOMATO. Xueshu Yu, George H. Lacy, Sue A. Tolin, & Carole L. Cramer, Dept. Plant Path., Physiol. & Weed Sci., VPI & SU, Blacksburg, VA 24061-0330

3-Hydroxy-3-methylglutaryl CoA reductase (HMGR) mediates a key regulatory step in isoprenoid (including phytoalexins) biosynthesis. The tomato genome contains at least 4 differentially regulated *hmg* genes. We generated transgenic tobacco (*Nicotiana tabacum* cv. Xanthi nc) and tomato (*Lycopersicon esculentum* cv. Vendor) containing antisense tomato *hmg*1 and *hmg*2 to study their effect on disease resistance. Full-length *hmg*2 (a defense-specific isoform) and gene-specific 5' regions of *hmg*1 or *hmg*2 were inserted in the antisense orientation behind a CaMV 35S promoter. Southern and northern analyses confirmed successful transformation and antisense message expression. Tomatoes expressing the full-length *hmg*2 antisense had lower HMGR activity and were more susceptible to soft rot by *Erwinia carotovora* ssp. *carotovora (Ecc)* than control plants. This confirms previous results suggesting that tomato *hmg2* plays a critical role in disease resistance. In contrast, expression of either anit-*hmg1* or anti-*hmg2* in the heterologous tobacco system resulted in plants with enhanced resistance to *Ecc* and significantly reduced TMV lesion sizes. These results may indicate that antisense inhibition is non-specifically exerted on isogenes other than the defense-specific *hmg* gene which is quite divergent from tomato *hmg2*.

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MICROSCOPIC EXAMINATION OF PENETRATION STRUCTURES IN PATHOGENESIS OF *THIELAVIOPSIS BASICOLA* ON VARIOUS HOSTS. M.E. Hood and H.D. Shew, Department of Plant Pathology, North Carolina State University, Raleigh 27695.

Developmental events involved in penetration of host root tissue by *Thielaviopsis basicola* were examined using a variety of microscopic techniques. This study describes the dynamic interaction of fungal infection structures with host cells, including time-course morphogenesis and reaction by the host cell to penetration. Prior to penetration peg formation and throughout the infection process, cytoplasmic steaming in the infected host cell was directed to the site of penetration and resulted in aggregation of cytoplasmic constituents at the penetration site. Extensive callose deposition was observed around penetration structures, but it did not limit colonization by *T. basicola* on susceptible hosts. Similar events occurred upon penetration into adjacent cortical cells.

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EXPRESSION OF GENES ENCODING THAUMATIN-LIKE PROTEINS IS ASSOCIATED WITH RESISTANCE IN OAT AGAINST *PUCCINIA GRAMINIS*. K.-C. Lin[1], W. R. Bushnell[1], L. J. Szabo[1], and A. G. Smith[2], [1]Dept. of Plant Pathology and Cereal Rust Laboratory, USDA-ARS, and [2]Dept. of Horticulture, Univ. of Minn., St. Paul, MN 55108

Four cDNA clones encoding thaumatin-like (TL) proteins were isolated from oat infected with an incompatible isolate of *Puccinia graminis* f.sp. *avenae* (*Pga*). Northern blot analyses using gene-specific probes corresponding to each cDNA clone revealed that the expression of the four genes (*rastl-1, -2, -3, -4*) was activated in response to infection by either an incompatible isolate of *Pga* or an isolate of a nonhost pathogen, *P. graminis* f.sp. *tritici*, but not by a compatible isolate of *Pga*. The highest expression level was for *rastl-1*. The four cDNAs each encoded a polypeptide of 169 amino acids including a signal peptide of 21 amino acids, suggesting that these polypeptides were transported out of the cytoplasm. The polypeptides were similar in sequence to osmotin, PR-5, zeamatin, and TL proteins that are activated in response to stress or pathogen attack in several plant species. The abundance and the accumulation patterns of *rastl-1* gene transcript in response to infection by stem rust fungi indicate that *rastl-1* is a response gene associated with resistance in oat stem rust.

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IDENTIFICATION OF RAPD MARKERS LINKED TO MAJOR GENES FOR RESISTANCE TO POWDERY MILDEW IN WHEAT. A. Shi[1], S. Leath[1], and J. P. Murphy[2], USDA, ARS and North Carolina State University, Raleigh, NC 27695.

RAPD markers tightly linked to genes for resistance to powdery mildew in wheat are being identified in: (1) a series of 'Chancellor' near-isogenic lines (NILs) with *Pm1, Pm2, Pm3a, Pm3b, Pm3c,* and *Pm4a*; (2) bulked segregant analysis for *Pm13, Pm16,* and *Pm20* which are not available in NILs; and (3) segregating inbred or backcross populations with new resistant for lines derived from wheat progenitors. Eight markers revealed clear, specific polymorphisms associated with resistance: two for *Pm1*, three for *Pm3 (Pm3a, Pm3b, Pm3c, Pm3d,* and *Pm3f*), two for *Pm3b* alone, and two for NC92-8562 (a line with resistance derived from *Triticum tauschii*). The three markers, $OPN9_{600}$, $OPN9_{1200}$, and $OPS3_{1400}$, showed polymorphisms, not only between the NILs, but also for 18 other wheat lines with or without resistance alleles at the *Pm3* locus, and for 14 different F_1 crosses. These markers appeared to be linked with resistance alleles at the *Pm3* locus not only in NILs, but also in 18 other wheat lines and in 14 F_2 progeny of a cross between Coker 68-15 (no known *Pm* genes) and in Saluda (*Pm3a*).

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SUBSTRATE UTILIZATION BY *TYPHULA IDAHOENSIS*. **J.M. Windes and E.J. Souza. University of Idaho, 1693 S. 2700 W. Aberdeen, ID, 83210-0530.**

Cultivars of wheat tolerant to infection by *Typhula idahoensis* Remsb., a causal organism of snow mold, accumulate carbohydrates to a greater degree than susceptible cultivars. Fructans (oligo- and polyfructosyl sucrose) are the main reserve carbohydrates for cereals but may be unavailable for growth of the fungus due to the degree of polymerization. Glucose, fructose, sucrose, and two concentrations of inulin (a pentamer fraction of fructan) were incorporated into a defined medium to determine growth rate of the fungus. Carbohydrates were standardized to glucose units equal to 10g/L media. Radial growth on the mono- and disaccharide-amended media did not significantly differ from each other. Growth on both concentrations of inulin-amended media were not significantly different from the non-sugar control, Initial results indicate that *Typhula idahoensis* may be unable to utilize highly polymerized carbohydrates.

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RAPD ANALYSIS OF PHYSIOLOGICAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN SOYBEAN. D. R. Huff, B. W. Pennypacker, and O. E. Hatley, Dept of Agronomy, Penn State University, University Park, 16802.

Soybean cultivars were screened for physiological resistance to *Sclerotinia sclerotiorum* using a modification of the limited-term inoculation method. The homogeneous soybean cultivars segregated for physiological resistance, sanctioning the use of RAPD analysis to find genetic markers of physiological resistance. DNA was extracted from leaves of individual physiologically-resistant plants and from individual susceptible plants. Bulked segregant analysis was used within cultivars to find genetic markers common to the pool of physiologically-resistant plants. Following identification of a genetic marker, individual resistant plants within a cultivar will undergo additional RAPD analysis to confirm the linkage of the marker with physiological resistance. Identification of genetic markers of physiological resistance will expedite incorporation of white mold resistance into soybean cultivars by facilitating the use of recurrent phenotypic selection .

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INFLUENCE OF WATER POTENTIAL ON SURVIVAL OF SCLEROTIA AND INFECTION OF BEANS BY *Macrophomina phaseolina*. G. Olaya and G. S. Abawi. Dept. of Plant Pathology. Cornell University, Geneva, NY 14456.

Survival of sclerotia of *Macrophomina phaseolina* (Mp) was studied in a natural and pasteurized very fine sandy loam soil at matric water potentials (Ψm) of 0, -0.1, -0.3, -1, -3, -5, -15, -15 and -400 (air dried) bars. At Ψm=0 bar, viability of sclerotia was 40% and 0% after 2 and 4 wk of incubation at 30 C, respectively. Survival of sclerotia decreased with time in the soil samples adjusted from -0.1 to -15 bars, and remained about 100% viable in the air-dry soil treatment. After 20 wk of incubation, viability of sclerotia was reduced to 10% in the Ψm treatment of -0.1 bars and was more than 50% at Ψm of -5 and -15 bars. Survival of sclerotia followed a similar tendency in the pasteurized soil. The influence of osmotic water potential (Ψs) adjusted with KCl solutions on severity of infection of Mp to bean stem tissues (inoculated with sclerotia and incubated for 10 days at 30 C) was studied in small constant humidity chambers. The severity of infections was increased as Ψs decreased to -39.9 bars, then decreased and was significantly lower at -71.5 bars. The number of sclerotia of Mp produced per mm square of infected tissues was also increased as the Ψs was decreased from 0 to -53.3 bars, and was maximal at Ψs=-39.9 bars.

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RESPONSE OF BEDDING PLANTS TO PEANUT ROOT-KNOT NEMATODE, MELOIDOGYNE ARENARIA, RACE 2. ***J. T. WALKER*, J. B. MELIN, AND J. DAVIS DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF GEORGIA, GEORGIA STATION, GRIFFIN, GA 30223.**

Eighteen cultivars (cvs) of seven bedding plant species were exposed to the peanut root-knot nematode, *Meloidogyne arenaria* race 2, at initial infestation densities of 0, 1, and 4 eggs/cm^3 under greenhouse conditions. Plant height and dry weight were not significantly affected by nematodes, whereas differences in number of root-galls and egg mass indices were significant at P=0.01. Ageratum (cvs Blue Blazer, Royal Delft), salvia (cvs Rhea, Victoria Blue), marigold (cvs French Golden Gate, Inca Gold) were classified as non-hosts with no detectable root-galls or egg masses. Begonia (Cocktail Vodka, Encore White), celosia (Castle Scarlet, Kimona Cream), pansy (Padparadja, Coronation Gold, Joker Jolly), salvia (Carabiniere Red), and vinca (Little Bright Eye, Little Delicata, Cooler Grape, Dawn Carpet) were classified as hosts with mean number of galls ranging from 0.5 to 38 per replicate and egg mass indices from 0.2 to 1.5.

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RESISTANCE AND TOLERANCE TO BEAN COMMON MOSAIC VIRUS IN DRY BEANS. C. A. Strausbaugh[1], R. L. Forster[1], J. R. Myers[1], and P. McClean[2]. [1]Univ. of Idaho, Kimberly, ID 83341; [2]North Dakota State Univ., Fargo, ND 58105.

Resistance (no systemic infection) and tolerance to BCMV strains NL-3, NL-8 (ID), NY-15 (Z), and Mexican was assessed in 70 recombinant inbred lines (RILs), 10 commercial cultivars, and 7 differential cultivars. Differentiation of resistant, susceptible, and tolerant plants was based on visual symptoms, ELISA, dry weight, and vigor ratings. The RILs produced from a cross between Olathe (*bc-u bc-1*^{2}) and Sierra (no resistance genes?) should have all been susceptible to NL-3. However, 22 of 46 lines in the first study and 13 of 24 lines in a second study were tolerant to NL-3. The presence of a third gene could account for these results. Tolerance to specific BCMV strains was also found in 3 differential cultivars and 7 commercial cultivars.

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MICRO-DILUTION PLATING TECHNIQUE FOR ASSESSING POPULATION COUNTS OF MICROORGANISMS. M.M. Dee, N.B. Quigley[1], and B.H. Ownley. Entomology and Plant Pathology Dept., and [1]Microbiology Dept. Univ. of Tennessee, Knoxville, TN 37996

A cost-effective, time- and space-saving micro-dilution plating (MDP) technique was developed for assessing population counts of microorganisms in pure culture, and was compared to the standard dilution plating (SDP) method. For both methods, a ten-fold dilution series was prepared in sterile water from overnight NBY broth cultures of a *Pseudomonas* and a *Bacillus* isolate. Dilutions of the *Pseudomonas* and *Bacillus* broth cultures were plated onto King Medium B ($ 0.18/plate) or Minimal C Medium ($ 0.14/plate) in 15×100 mm petri dishes, respectively. In the SDP method, three plates were spread with 0.1 ml of each of six dilutions (10^{-3} to 10^{-8}) per bacterium (total of 36 plates, 34 minutes to perform). In the MDP method, three 10-µl droplets of each dilution were pipetted in a row on one plate and allowed to dry; three rows (one per dilution) were pipetted per plate (total of four plates, 10 minutes to perform). All plates were incubated at 28 C and colonies were counted when they were 0.5-1.0 mm in diameter. There were no differences in the total counts obtained by each method. The MDP technique required one third of the time to perform and approximately one tenth of the number of plates of culture media than the SDP method.

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EVALUATION OF ISOLATION PROCEDURES AND SOIL SOURCES IN SELECTING PATHOGEN-SUPPRESSIVE *Streptomyces* STRAINS. D. Liu, B. E. Paulsrud, L. L. Kinkel, N. A. Anderson, Dept. of Plant Pathology, Univ. of Minn., St. Paul 55108

Two methods were used to isolate pathogen-suppressive *Streptomyces* strains from naturally occurring potato scab-suppressive and scab-conducive soils. In the soil dilution method, a semi-selective medium was used to isolate strains. In the Andersen Air Sampler (AAS) method, 2% water agar (WA), or Potato Dextrose/Czapeks (PDA/CZ) medium was separately pre-lawned with potato pathogens *S. scabies* strain RB4, *Verticillium dahliae*, *V. albo-atrum*, or *Helminthosporium solani* and then impacted with soil using the AAS. *Streptomyces* strains that inhibited the lawn pathogen were isolated. All strains were then co-plated separately with RB4 and 87 on oatmeal medium. In selecting for powerful *Streptomyces* antagonists, the AAS method, WA medium, and suppressive soil were superior to the soil dilution method, PDA/CZ medium, and conducive soil, respectively. In summary, the AAS method is an inexpensive and efficient strategy for isolating pathogen-suppressive microorganisms from soil, and disease-suppressive soil provides a superior source of pathogen-suppressive *Streptomyces* strains.

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TIMELY DELIVERY OF ORNAMENTAL AND TURF DISEASE INFORMATION IN OHIO. N.J. Taylor[1], J.A. Chatfield[2], and J.F. Boggs[3]. [1]Plant Pathology Dept., 201 Kottman Hall, 2021 Coffey Rd., Columbus, OH 43210; [2]Northeast District Extension, OARDC, 1680 Madison Ave., Wooster, OH 44691; [3]11100 Winton Rd., Cincinnati, OH 45218.

Delivery of current information on plant diseases, insects, and other problems of ornamentals and turf is being accomplished in Ohio through a partnership among departments, extension agents, and industry. An interdisciplinary team, formed in 1992, facilitates information exchange and delivery. From weekly teleconferences a newsletter is developed and distributed electronically to extension, research, government, and industry personnel and over the Internet. Current information is in the hands of the user within 48 hours.

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TREEMAPPER: A COMPUTER PROGRAM FOR CALCULATING AND MAPPING TREE POSITIONS IN COORDINATE FIELD PLOTS. D. G. Lester, and A. D. Wilson. USDA Forest Service, Southern Hardwoods Lab, P.O. Box 227, Stoneville, MS, 38776.

Accurate maps of forest plots are required for many aspects of forest research. Field measurements of horizontal distance and azimuth between trees or reference points, collected with compass and tape or an electronic measuring device (EMD), are usually plotted manually, with computer assisted drawing programs, or GIS software to produce maps of forest research plots. These methods are tedious, time consuming, or expensive. TreeMapper for DOS (written in Visual Basic) converts these field measurements to coordinates and graphically plots tree positions (to scale) on screen in rectangular coordinates relative to plot center. Data may be entered manually or imported from a datalogger or EMD. Plots of tree positions may be rotated up to 90 degrees using Polar X or Polar Y plotting methods to improve fit within the map frame. Horizontal distance and azimuth between any two points not directly measured may be calculated. Maps may be printed with high resolution LaserJet-compatible printers or saved to disk in graphics file formats easily imported into other computer graphics software. TreeMapper requires a 386 or higher IBM compatible computer, VGA monitor, a minimum of 2 MB of RAM, and 2 MB of free hard disk space.

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BICARBONATES AND BOTRYTIS: VII. CALCEIN AM AND ETHIDIUM HOMODIMER I INDICATE VIABILITY OF FUNGAL CONIDIA. C. L. Palmer, R. K. Horst, H. W. Israel, and R. W. Langhans. Departments of Floriculture and Ornamental Horticulture and of Plant Pathothology, Cornell University, Ithaca, NY, USA 14853.

Viability of *Botrytis cinerea* Pers. conidia has been determined using Calcein AM (CaAM) and Ethidium Homodimer I (EtH I) (Phytopathology 84:1065). To confirm validity of this technique, conidia of *Alternaria solani* and *Sphaerotheca pannosa* f. sp. *rosae* were placed onto agar/collodion coated glass microscope slides previously treated with water, 50 mM $KHCO_3$, or 50 mM NH_4HCO_3. After incubation for 24 h at 100% RH, conidia were stained with CaAM and EtH I. Total and germinated conidia were counted; ungerminated conidia were placed into the following categories: alive, dying, dead, and impermeable. For *S. pannosa* f. sp. *rosae*, conidial viability was also determined using morphological distortions. Fluorescent results were similar to those obtained for *B. cinerea*. Therefore, the CaAM/EtHI stain provides a rapid method to assess viability of fungal conidia. (Supported by H&I Agritech Inc., Ithaca, NY 14850.)

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DETECTION AND QUANTITATION OF NEOMYCIN PHOSPHOTRANSFERASE II IN PLANT BY IMMUNOASSAY. J. Q. Xia, C. Sutula, and C. Mueller. Research Dept., Agdia, Inc., Elkhart, IN 46514.

An ELISA has been developed to detect and quantitate neomycin phosphotransferase II (NPT II) in transgenic plants. Plant tissue is simply ground with sample extract buffer and incubated with antibody-enzyme conjugate in an antibody coated well. A standard curve is prepared for each test run to calculate NPT II concentration for each sample examined. The test is effective in detecting NPT II in plant leaves and seeds for more than ten different crops. Sensitivity of the test is at least 0.1ng per ml, or 10pg per well of NPT II in plant sap. The test procedure can be finished in 4 hours, or overnight if more convenient. The simple and rapid method provides a reliable way to detect and quantitate NPT II in plants.

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APOTHECIA PRODUCTION OF *SCLEROTINIA SCLEROTIORUM* AND *SCLEROTINIA MINOR*. W. Gutierrez and H. D. Shew, Department of Plant Pathology, N.C. State University. Raleigh 27695

A method for induction of apothecia from sclerotia of *S. sclerotiorum* and *S. minor* was developed. Sclerotia of *S. sclerotiorum* and *S. minor* were harvested from isolates grown on sterile carrot discs for 3 weeks. Sclerotia were separated from the medium on 0.5 and 2.0 mm sieves under running tap water, disinfested with 0.6 % NaOCl for one minute, rinsed with sterile deionized water, and dried for 24 hours at room temperature. Ten to 20 sclerotia from each isolate were placed into glass jars (118 ml) containing sterile sand and water (4:1 w/v), covered with a plastic lid and sealed with parafilm. Jars were incubated at 2° C in the dark for 30 days, then moved to 12° C until stipes were visible (5 mm long). Jars were then moved to a growth chamber at 16° ± 2° C with 12 hours of light until apothecia were fully developed. Ascospores were collected for 1 to 2 weeks after the apothecia were mature. Most isolates of *S. sclerotiorum* produced from 2 to 8 apothecia per sclerotium. Apothecia of *S. minor* was observed only on sclerotia larger than 2.0 mm in diameter and fewer apothecia per sclerotium was produced.

725

INDUCING FRUITING BODIES OF SHIITAKE MUSHROOM ON HARDWOOD SAWDUST INSIDE THE GREENHOUSE. R. P. Pacumbaba, Department of Plant and Soil Science, Alabama A&M University, Normal, AL 35762.

Propagule of shiitake mushroom, S1 and S2 strains, was isolated from the buttons (unopened fruiting bodies) and plated in Pacumbaba's medium # 48*. Mycelial growth with flocculent consistency was noted in petri dishes within 20 days after inoculation. Pure culture of the mycelium inoculated to autoclaved hardwood sawdust in culture containers plus Pacumbaba's broth # 51*, attained spawn consistency within 20 days after inoculation. Fruiting bodies (pins) of shiitake strain # S2 appeared in Pacumbaba's medium # 48* in petri dishes and in the culture vessels within 30 days after inoculation. The sides of the culture container were squeezed to induce production of pins on shiitake strain # S2 40 days after inoculation and on strain # S1 101 days after inoculation. Two to 4 days later, fruiting bodies (pins) started to appear. The spawns with fruiting bodies were transferred to the greenhouse, where more fruiting bodies were induced, and allowed to continue to produce shiitake mushrooms.

*Patent pending for Pacumbaba's media and broth.

726

PRODUCTION OF MONOCLONAL ANTIBODIES TO THE GLYCOPROTEINS OF TOMATO SPOTTED WILT TOSPOVIRUS (TSWV) USING ANTIGENS ELUTED FROM CHROMOPHOR STAINED GELS. M.D. Bandla and J.L. Sherwood. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

The virion associated glycoproteins (gps) of TSWV can be isolated from unstained gels of virus preparations separated on polyacrylamide gels for antibody production, but yields can be low and the proteins cross contaminated making antibody production difficult. These problems can be avoided using a chromoPhor protein recovery method (Larson and Shultz 1993, BioTechniques 15: 316-323). Gp 1 (p78) and gp 2 (p58) of TSWV were isolated from SDS-PAGE gels using the chromoPhor method and monoclonal antibodies (Mabs) produced. The specificity of the Mabs were compared by DAS-ELISA, western blot and immunoelectron microscopy to that of a polyclonal antiserum to the glycoprotein fraction of TSWV (courtesy of D. Gonsalves, Cornell University). The use of these antibodies for the study of TSWV-vector relationship is being evaluated. This appears to be the first report of using chromoPhor recovered antigens to produce Mabs.

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TAQMAN™ FLUORESCENT PCR ASSAY FOR GEL-FREE DETECTION OF PLANT PATHOGENS. DA Knorr, A Rowhani, and DA Golino, Applied Biosystems, Foster City, CA, 94404, and Plant Pathology Department, University of California, Davis, CA 95616.

TaqMan™ PCR is a recent advancement for detecting specific PCR amplification products using emitted fluorescence from sequence-specific probes rather than gel electrophoresis. We have applied this technology towards detecting a variety of plant pathogens. Systems under investigation include RNA viruses (grapevine fanleaf virus, tomato ringspot), phytoplasmas (*Xylella fastidiosa*, Western X), and viroids (potato spindle tuber viroid, and grapevine yellow speckle viroids A & B). Advantages of TaqMan over current screening methods are an ability to reliably detect low titres of pathogens and a common, high-throughput format. This technique should be useful for constructing panels of compatible PCR primers and TaqMan probes to screen for important pathogens in any crop.

728

A RAPID, SMALL SCALE PROCEDURE FOR EXTRACTING VIRUS, VIROID, MLO AND BACTERIAL NUCLEIC ACIDS FROM PLANTS FOR ANALYSIS BY PCR. Yun-Ping Zhang[1], Jerry K. Uyemoto[2], and Bruce C. Kirkpatrick[1]. [1]Department of Plant Pathology and [2]USDA-ARS, University of California, Davis CA 95616.

The polymerase chain reaction (PCR) is a rapid and powerful tool for detecting and identifying plant pathogens. However the complexity of most nucleic acid extraction procedures limit the numbers of samples that can be easily processed for analysis by PCR. We have developed a simple, small scale, nucleic acid extraction procedure that is performed entirely in 1.5 ml microfuge tube. Plant tissues are frozen in liquid nitrogen, pulverized, and the tissue incubated with hot CTAB buffer. The buffer is extracted once with chloroform, nucleic acids are precipitated with isopropanol and suspended in water. Viral dsRNA was isolated from the total nucleic acid mixture using CF-11 cellulose and used as templates in RT-PCR. The total nucleic acid fraction was used as template in PCR to detect viroid RNA and bacterial/MLO DNA. This procedures was used to detect green ring mottle virus, apple scar skin viroid, western X-disease MLO and *Xylella fastidosa* in woody plant hosts.

729

AN ALGINATE FILM TECHNIQUE FOR STUDYING THE ECOLOGY OF *PLEIOCHAETA SETOSA* IN SOIL. D.J. Collins[1], K. Burch[1], R. Rodríguez-Kábana[1], A.R. Howlider[2] and P. Hughes[1], [1]Department of Plant Pathology, Auburn University, AL 36849, and [2]Bangladesh Institute of Nuclear Agriculture, P.O. Box-4, Mymensingh, Bangladesh.

Pleiochaeta setosa (Kirchn.) Hughes, is the causal agent of brown leaf spot as well as Pleiochaeta root rot of lupin. Conidia of the fungus are the primarily source of inoculum for both diseases. Little is known about the behavior of conidia of *P. setosa* in soil. An alginate film technique was developed to study the ecology of *P. setosa* in soil. 6 X 2 cm fiberglass screens with a 1.5 mm mesh openings were dipped into a 2% sodium alginate solution containing conidia of *P. setosa*. Inoculum concentration was adjusted to give 2-3 conidia per mesh opening. The fiberglass screens with conidia embedded in alginate were then dipped in a 0.25M $CaCl_2$ solution to form a solid alginate gel. In a preliminary experiment fiberglass screens were buried in raw soil or placed in a moist chamber, and incubated at 15 C for four days. Percent germination of conidia was determined by scanning the gels with a light microscope. Eighty per cent of conidia of *P. setosa* remained ungerminated in natural soil as compared to 80% germinated in the moist chamber. Conidia placed in autoclaved soil readily germinated. Alginate films provide a quick method for measuring soil fungistasis, perhaps serving as a model for other pathogen-soil systems.

730

DETECTION OF BARLEY YELLOW DWARF VIRUS-PAV-IL BY USING DIFFERENT TECHNIQUES. Figueira, A. R[1]; Domier, L.L.[2,3] and D'Arcy, C.J.[3], [1]UFLA/Depto de Fitossanidade, cx. postal 37, 37200-000-Lavras-MG Brazil; [2]USDA-ARS-CPRU and [3]Department of Plant Pathology, University of Illinois,Urbana IL 61801

The sensitivity of an improved nucleic acid hybridization technique, using chromogenic and chemiluminescent substrates, was compared to PCR, DAS and TAS-ELISA for the detection of BYDV-PAV-IL in extracts of infected leaves and in purified virus preparations. The sensitivities of DAS and TAS-ELISA were similar, detecting 1 ng of purified PAV and the equivalent of 78 ng of infected tissue. Nucleic acid hybridization technique, using both substrates, also detected 1 ng of purified PAV amd virus om the eqiova,emt of 25 ng of infected plant tissue. PCR was the most sensitive of the three techniques and detected 0.1 pg of RNA extracted from purified virus and the RNA in the equivalent of 0.5 pg of infected leaf tissue.

731

GREENSKEEPER, A VISUALIZATION TOOL TO ENCOURAGE THE ADOPTION OF GREEN-SPECIFIC MANAGEMENT PRACTICES ON BENTGRASS PUTTING GREENS. B. S. Corwin and P. A. Donald, Dept. of Plant Pathology, University of Missouri, Columbia, MO 65211

Applying pesticides on a green-specific basis is a management practice that can reduce pesticide inputs on bentgrass putting greens. Before superintendents will adopt this practice, its reliability must be demonstrated. Histories for each of six greens on five courses in the St. Louis, MO area were compiled. The history includes construction type, % infestation with Poa annua, pesticide use, and other management inputs. Disease occurrences, plant parasitic nematode populations, root depths, and greens' quality were monitored over the 1994 season. The project continues in 1995 with an additional three courses. Historical information, current season data, and photographs have been incorporated into GreensKeeper©, an interactive visualization tool. The application is a Windows®- based application constructed with Toolbook®. Superintendents, greens committees, and club managers can use GreensKeeper© to evaluate pest management strategies, cultural practices, and other factors that impact greens' quality.

732

EFFECT OF LONG-TERM CORN/SOYBEAN ROTATION ON THE PATHOGENICITY OF *PYTHIUM* POPULATIONS ON SOYBEAN. Ben Q. Zhang, X.B. Yang, Dept. of Plant Pathology, Iowa State University, Ames, IA 50011

Effect of corn/soybean rotation on *Pythium* pathogenicity was studied at Iowa State University research farms on which long-term cropping experiments were conducted. *Pythium* spp. were isolated from continuous soybean (SS), continuous corn (CC), and corn/soybean rotation (CS) fields with cropping history ranging from 17 to 30 years. Thirty to forty isolates were randomly selected from each field and the virulence of those isolates on soybean was tested. *Pythium* isolates from SS fields were significantly more pathogenic on soybean than those from CC fields. *Pythium* populations from 17-year CS fields were significantly less pathogenic than the populations from 17-year SS fields. Frequency of isolates which were highly pathogenic to soybean was greater in SS fields than those from CS fields. No significant differences in pathogenicity were found between *Pythium* populations from SS and CS fields when the cropping histories were over 30 years.

733

DIFFERENTIATING BOTRYOSPHAERIA SPECIES UTILIZING MORPHOLOGICAL, CULTURAL AND MOLECULAR CHARACTERS. K.A. Jacobs[1], S.A. Rehner[2], and G.R. Johnson[1]. [1]FNPRU, U.S. National Arboretum, Washington, DC 20002. [2]SBML, Beltsville, MD 20705.

Botryosphaeria species cause diseases on over 100 woody plant genera. The taxonomy of the genus is based primarily on anamorphic characters and remains confused. Twenty-two isolates representing several *Botryosphaeria* species and related taxa were compared utilizing morphological and cultural characters and sequence homology in the internal transcribed spacers (ITS) of the 5.8S nuclear rDNA gene. ITS data resolved 8 groups, one that consisted of all *Lasiodiplodia theobromae* isolates and shared consensus with other characters. The remaining 7 groups shared only partial consensus with groups defined by growth rate, colony and conidial morphology. *B. ribis* was grouped with *B. dothidea*, but the latter species was polymorphic in several characters. Results indicate that morphological, cultural and molecular characters varied in identifying intraspecific and interspecific relationships.

735

Using single strand conformation polymorphism to differentiate vegetative compatibility groups of *Fusarium oxysporum f. sp. cubense*. Naomi D'Alessio and David Kuhn. Biological Sciences, Florida International Univ., Miami FL 33199

Fusarium oxysporum f. sp. cubense (Foc), a deuteromycete that causes banana wilt, is classified into 14 vegetative compatibility groups (VCGs) and three races. We have developed a molecular test which distinguishes some of the VCGs and provides data for inferring relationships among them. A set of primers was developed that amplifies an intergenic region of mitochondrial DNA. When amplified, digested and electrophoresed under conditions that distinguish single strand conformation polymorphism (SSCP), this region varied sufficiently to distinguish seven mitochondrial types (mitotypes) which differ by length, RFLP and SSCP. These data suggest a strong correlation between VCGs and mitotypes and no correlation between races and mitotypes. Other forma species of *Fusarium oxysporum (conglutinans, lycopersici, pisi* and *raphani*) were similar when analysed by this method.

736

SEPARATION OF THE FAMILY LEPTOSPHAERIACEAE FROM THE FAMILY PLEOSPORACEAE (LOCULOASCOMYCETES): MOLECULAR EVIDENCE FROM rDNA SEQUENCES. Jiaowang Dong, Weidong Chen, and J. L. Crane, Illinois Natural History Survey and University of Illinois, Urbana, IL 61801

The members of the Leptosphaeriaceae had long been included in the Pleosporaceae. Barr (1987) separates them on basis of the differences in their anamorph and shape of asci. Eriksson and Hawksworth (1988 & 1990) accept the separation, but maintain that other criteria, especially molecular data, should be studied in order to claim a reliable natural classification. Five taxa of the Leptosphaeriaceae (*Leptosphaeria* and *Ophiobolus*) and four taxa of the Pleosporaceae (*Pleospora, Clathrospora,* and *Lewia*) were studied to verify Barr's classification of the Leptosphaeriaceae and the Pleosporaceae, and their nuclear rDNA was amplified and sequenced. The partial 18S rDNA sequences were analyzed using PAUP. The phylogenetic analyses showed the members of the Leptosphaeriaceae formed a clade, and the members of the Pleosporaceae produced another distinct clade. Our results generally supported Barr's separation of the Leptosphaeriaceae from the Pleosporaceae.

737

DIFFERENTIATION OF GEOGRAPHIC ISOLATES OF *ANISOGRAMMA ANOMALA* USING RAPDs. N.K. Osterbauer, T. Sawyer, and K.B. Johnson, Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, 97331.

Anisogramma anomala causes eastern filbert blight, a canker disease on European hazelnut (*Corylus avellana*) in the Pacific Northwest (PNW). This fungus is indigenous to the eastern U.S. and was introduced to the PNW. To determine the relative genetic relatedness of the PNW and eastern U.S. populations, DNA from the ascospores of 5 isolates each collected from the PNW, Illinois (IL), Minnesota (MN), and New York (NY) was amplified using the randomly amplified polymorphic DNA (RAPD) technique. Fifty 10-base pair oligomer primers were screened, with six producing unique amplification patterns in each isolate. Cluster analysis indicated that the PNW isolates were more similar to the IL isolates than those from NY and MN. Isolates from NY and MN exhibited the greatest variation. Additional isolates from these regions and other regions of eastern North America are being analyzed.

738 Withdrawn

739

Development of an Ectomycorrhizal Model System for Identification and Characterization of "Symbiosis" Associated Genes. S.J. Kim and G.K. Podila, Michigan Technological University, Houghton, MI 49931

We have developed a semi-in vitro system for *Laccaria bicolor* x *Pinus resinosa* to study symbiosis related genes associated with the ectomycorrhizae formation. This system allows us to manipulate the fungus and the red pine seedlings to interact for different time periods, reisolate the fungus and seedlings separately, and extract mRNA from each of the components. The mRNA is then used to perform novel DDRT-PCR technique to identify *L. bicolor* cDNA clones that are differentially expressed in response to the signals from red pine seedlings. We have also developed a transformation system for *L. bicolor*, which can be used, to determine the functional significance of cDNA clones isolated from *L. bicolor*, for their role in the symbiosis and the formation of ectomycorrhizal association with red pine. Progress made in the above mentioned areas will be presented.

740

THE EFFECTS OF CELL WALLS AND CELL WALL-ASSOCIATED PHENOLICS OF HOST AND NONHOST ROOTS ON THE GROWTH OF TWO SPECIES OF VAM FUNGI. D.D. Douds, Jr.*, G. Nagahashi, and G. D. Abney; USDA-ARS ERRC; 600 E. Mermaid Lane; Philadelphia, PA 19118 USA.

Purified cell walls, crude cell wall extracts, and cell wall-associated phenolic compounds from Ri T-DNA transformed roots of host (*Daucus carota*) and nonhost (*Beta vulgaris*) plants were incorporated into growth medium to determine their effects on the growth of vesicular-arbuscular mycorrhizal [VAM] fungi. Purified cell walls of both plants had little effect on *Gigaspora gigantea* but inhibited the growth of *Gigaspora margarita*. Hyphae produced appressoria-like structures in the presence of *D. carota* cell walls. Para-hydroxy benzoic acid, a constituent of *D. carota* roots, stimulated growth of *G. margarita* hyphae, but did not affect hyphal growth of *G. gigantea*. Ferulic acid, a major constituent of nonhost root, depressed the growth of both fungi.

741

MODIFICATIONS AND REFINEMENTS TO SOILLESS AND SOIL:SAND MEDIA SYSTEMS FOR PRODUCING INOCULUM OF ARBUSCULAR MYCORRHIZAL FUNGI. J.E. Kurle, and F.L. Pfleger. Dept. of Plant Pathology. Univ. of Minn., St. Paul MN 55108

Host plant and media were investigated to increase production of inocula of arbuscular-mycorrhizal (AM) fungi. Big bluestem was an ideal host plant in either system because it consistently yielded greater numbers of AM spores. An automatically watered system using coarse sand (1mm>60%> 0.850mm, 0.600mm>40%>0.425mm) as media was modified to produce AM fungal spores and hyphae for production of monoclonal antibodies and fatty acid analysis. Maximum spore production (99,000 spores/pot) occurred when 42 ppm P was applied to plants in a half strength Hoagland's solution 4X/day @ 50 ml/pot with natural lighting of 2000 μmol m^{-2} peak (14 hour day/10 hour night) at 28°C during July. In winter supplemental lighting (peak light levels of 1000 μmol m^{-2}) and reduced nutrient applications (4X/day @ 30 ml/pot) were required to produce ~10,000 spores/pot. A soil:sand solid media system was developed to produce inoculum for field studies. Soil pH (6.0 and 7.0) was maintained with a flowable lime compound and fertility (21 and 42 ppm P) adjusted with slow release fertilizer. This system yielded spore numbers similar to those obtained from the soilless media.

742

CHITINASE ACTIVITY IS ELEVATED IN TOMATO ROOTS FOLLOWING INOCULATION OF MELOIDOGYNE INCOGNITA. Q. Yu and J. Potter, PMRC, Agriculture Canada, 4902 Victoria Ave, Vineland Station, Ontario, L0R 2E0, Canada.

Chitinase activity was measured in tomato roots (cv. Moneymaker) during colonization by M. incognita. The tomato plants were grown in sterile soil under controlled environment (light/dark period 16/8; temperature 23°C). At four leaf stage, 15000 larvae of mixed growth stages were inoculated in each plant. There were 40 plants/treatment, control plants were uninoculated. Higher level of chitinase activity in inoculated plants over control was observed one week after inoculation. This level of activity differed slightly thereafter within the eight week test period.

743

ROOT-KNOT NEMATODE MANAGEMENT IN A KENAF-SOYBEAN-COTTON ROTATION. G.W. Lawrence[1], K.S. McLean[2], J.J. Cornelius[1], and J.W. Barnett[1]. [1]Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762 and [2]Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209.

Rotation plots were established in 1993 to examine the influence of alternate crops on root-knot nematode (*Meloidogyne incognita* race 3) population development and kenaf (*Hibiscus cannabinus*) yields. Rotation crops included kenaf cv. Everglades 41 (root-knot susceptible), soybean cv. Forrest (root-knot resistant), and cotton cv. Delta and Pineland 20 (root-knot susceptible). The 1994 growing season represented the second year of a planned three-year study. *Meloidogyne incognita* populations averaged 4,558 nematodes/250 cm^3 of soil from kenaf plots at 142 days. Nematode populations in plots planted with cotton and soybean were 1,085 and 155 nematodes/250 cm^3 of soil, respectively. Kenaf yields were 2,727.3 kg/ha in *M. incognita* infested plots compared with 29,318.2 kg/ha in the untreated control.

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MEASUREMENT OF THE ACTIVITY OF SOIL-BORNE MICROORGANISMS ANTAGONISTIC TOWARD ROOT-KNOT NEMATODES IN FIELD SOIL. Kiewnick,S. and R.A.Sikora, Institute of Plant Pathology, Dept. of Soil Ecosystem Phytopathology, Nussallee 9, D-53115 Bonn, Germany

Juveniles of *Meloidogyne incognita* were added to soil samples at different inoculum densities. After 3 days incubation the antagonistic potential was estimated by (1) extraction of juveniles from soil samples using modified Oostenbrink dish techniques or (2) by estimating galling index 2 weeks after introduction of cucumber seedlings. The potential was determined by comparing the density of juveniles extracted from soil with the number extracted from sterilized controls. The ability to reduce an introduced inoculum depended on initial density and on the antagonistic potential within the soil sample. The technique allowed good estimation of activity of antagonistic microorganisms in different soil samples. The reduction in the number of juveniles extracted from a soil was related to reductions observed in galling in the same soil sample. These results demonstrate that this method can be used effectively to measure a part of the antagonistic potential in soils.

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GEOGRAPHICAL DISTRIBUTION OF NEMATODES OF THE FAMILY LONGIDORIDAE (THORNE 1935) MEYL 1960 IN MOLDOVA VINEYARDS. Alex Polinkovsky, 2380 Warrensville Center Road, Cleveland, Ohio 44118

Vineyards' investigations in 21 districts of Moldova showed that nematodes of the family Longidoridae are wide-spread. They were found in 89.5% from all soil samples. Genus Xiphinema has more prevalent than genus Longidorus. Nematodes of genus Xiphinema displayed in all investigated districts, but the genus Longidorus, were comparatively rare (in 5 from 21 districts).In all nine species were discovered: X. index, X. mediterranium, X. vuittenezi, X. italiae, X. diversicaudatum, X. turcicum, L. elongatus, L. sp., L. sylphus. Two species X. italiae and L. sp. were described as new for Moldova, and L. sylphus were new for the former Soviet Union. A male specimen of X. index was registered for the first time in Europe. Four species (X. index, X. italiae, X. diversicaudatum, L. elongatus) are know as vectors of plant viruses. Study of the dynamics of quantity and vertical distribution showed that larger numbers of nematodes were displayed in the 30-80 cm. level and maximum height had two peaks, one in spring and one in fall.

746

DETERMINING SUSCEPTIBILITY OF SOYBEAN CULTIVARS TO WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN THE NORTH CENTRAL REGION. A. F. Olah and A. F. Schmitthenner, Dept. of Plant Pathology, OARDC, The Ohio State University, Wooster, OH 44691.

Constraints to assessing reaction of soybean cultivars to white mold (*Sclerotinia sclerotiorum*) include unreliability of disease-conducive weather and uneven natural infestation in field plots. Ascospores were produced and sprayed (2.4×10^4 spores/row ft) on flowering soybeans in OH, MI, WI and MN. Moisture was maintained after spraying with overhead irrigation. Yield loss, disease severity and weight of sclerotia produced in diseased plants were determined on locally-adapted cultivars. In OH, the correlation between disease severity ranks in sprayed plots with naturally infested plots was 0.61, while in MI it was 0.46 and in WI 0.70. MN had no disease. Data from common cultivars in MI and WI had acorrelation of disease severity ranks of 0.40, while in OH and MI, it was 0.84. Regression analysis indicated % diseased plants was a poor predictor of yield loss.

747

Impact of ergot in Kentucky bluegrass grown for seed in eastern and central Oregon. S. C. Alderman and D. D. Coats, USDA, ARS NFSPRC, Corvallis, 97331 and COARC, Madras, OR, 97741.

Samples of Kentucky bluegrass grown for seed in eastern and central OR were cleaned to remove debris but retain seed and sclerotia (ergot) of *Claviceps purpurea*. Ten-gm subsamples were examined under 5X magnification and ergot were counted. In central OR in 1992 and 1993 and in eastern OR in 1991-1993, 79, 104, 74, 56, and 67 samples, respectively, were examined. Ergot were found in 38-76% of samples; >1 ergot/gm seed occurred in 19-38% of samples and >10 occurred in 2-10% of samples. Although <1% seed were infected, 1-10% of seed lots required recleaning. Cost of recleaning seed in 1995 to meet purity standards was about 44 cents/kg seed. Additional seed loss may occur during combining or cleaning when honeydew, a sugary, sticky exudate occurring during floral infection, aggregates seed and debris.

748

DECREASED NET PHOTOSYNTHETIC RATE OF BEAN LEAVES ASSOCIATED WITH INCREASED CHLOROSIS FROM RUST AND NUTRITIONAL DEFICIENCY. M. T. Iamauti, T. A. Davoli, and R. D. Berger, Departamento de Fitopatologia, USP/ESALQ, Piracicaba, SP, Brazil and Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

A range of colors for leaves of bean (Phaseolus vulgaris) occurred either after inoculation with different concentrations of urediniospores of Puccinia appendiculatus, or after irrigation with different concentrations of soluble fertilizer. The relative distance in the 16^3 red-green-blue color cube for the various digitized color images from dark green was determined by the Pythagorean Theorem applied twice. The net exchange of CO_2 for plants grown under low light (50-75 lumens) was determined by a LI-6200 Photosynthesis System. A 6-8% decrease in the net exchange of CO_2 occurred for each unit decrease in color (maximum = 14 units) from the darkest green (on which maximum photosynthesis occurred) regardless of whether the change in color was from rust or from nutritionally induced chlorosis. Thus, it may be possible to predict yield (or loss) from color-image analysis of the crop canopy over time.

749

ESTIMATION OF YIELD LOSSES CAUSED BY SOYBEAN CYST NEMATODE IN THE NORTH CENTRAL REGION IN 1993 AND 1994. P. R. Thorson[1], G. L. Tylka[1], W. C. Stienstra[2], and the North Central Soybean Cyst Nematode Project Investigators. [1]Department of Plant Pathology, Iowa State University, Ames, IA 50011, [2]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108.

Experiments were conducted at 27 and 32 locations in 1993 and 1994, respectively, by scientists from IL, IN, IA, KS, MI, MN, MO, NE, OH, and WI to determine the effects of soybean cyst nematode, *Heterodera glycines*, on yield of susceptible and resistant soybean varieties. Four replications of resistant and susceptible soybean varieties in maturity groups I, II, III, and IV were planted at each location. Soil samples were collected from each plot at the beginning and end of each growing season, and *H. glycines* egg densities were determined at Iowa State University. Plots were harvested, relative yields were calculated, and yield loss estimates due to *H. glycines* were made by comparing relative yields from noninfested sites and infested sites. In 1993, losses of $1.48/ha, $82.45/ha, and $57.40/ha were estimated for soybeans grown in the North Central Region in maturity group zones I, II, and III, respectively. For 1994, estimates of losses were $248.06/ha, $116.56/ha, and $52.31/ha for maturity group zones I, II, and III, respectively.

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BIOLOGICAL CONTROL OF BROWN ROT OF CITRUS FRUIT CAUSED BY *PHYTOPHTHORA CITROPHTHORA*. S. Droby, B. Horev, L. Chalupovicz and E. Cohen. Dept. of Postharvest Science of Fresh Produce, ARO, The Volcani Center, P.O.Box 6, Bet Dagan 50250, ISRAEL.

Phytophthora citrophthora, the cause of brown rot of citrus fruit, usually infect fruit on the lower branches of the tree as a result of splashing zoospores in drops of rain water from the soil. Harvested fruit may be infected with invisible symptoms and subsequently rot during transport and storage. As a result of mounting concerns regarding the presence of fungicide residues in the fruit, the loss of fungicide efficacy due to development of resistant fungal strains and withdrawal from the market of key fungicides for postharvest use, we have been developing a biological control strategy for the control of brown rot of citrus fruit. The efficacy of various Trichoderma isolates in inhibiting infection and development of brown rot was evaluated by three different inoculation techniques: (1) zoospore suspension, (2) contact with infested soil, (3) agar plug. Results from our study show that one of the *Trichoderma* isolates tested significantly reduced infection of grapefruit by the pathogen. In addition, the effective *Trichoderma* isolate inhibited the development of green mold decay on surface wounds of grapefruit.

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EFFECTS OF NEEM OIL ON FIRMNESS AND STORAGE DECAY OF 'GOLDEN DELICIOUS' APPLES. Harold E. Moline, USDA, ARS, PSI, HCQL, Beltsville, MD 20705.

The antifungal effects of an emulsifiable formulation of neem seed oil were tested on 'Golden Delicious' apples. One lot of fruit were treated by dipping in a 2% water emulsion of 'Neem Guard' at harvest, stored for 4 months at 0C, removed from storage and inoculated with *Botrytis cinerea* spores. Another lot was treated after inoculation with fungal spores at the end of 4 months storage. Decay readings and firmness measurements were compared with untreated inoculated fruit after 1 week storage at 20C. Fruit dipped in 'Neem Guard' at harvest had 30% less decay and were significantly firmer than untreated control fruit (62.1 Newtons vs. 48.4 Newtons for control). Fruit dipped in 'Neem Guard' after inoculation had 49% less decay than control fruit, however these fruit were no firmer than control fruit after ripening 1 week at 20C. A third treatment dipped in 'Neem Guard' at harvest and again after inoculation had 52% less decay than control fruit and were as firm as those treated with 'Neem Guard' at harvest (63.3 Newtons).

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INHIBITORY COMPOUNDS TO AFLATOXIN BIOSYNTHESIS IN THE CORN INBRED LINE TEX6. Zheng-Yu Huang[1], Donald G. White[2], and Gary A. Payne[1]. [1]North Carolina State University, Raleigh 27695-7616, and [2]University of Illinois, Urbana 61801

Preharvest aflatoxin contamination of corn continues to be a concern. Corn lines have been identified with heritable resistance to aflatoxin accumulation, but selection for the resistance within desirable genotypes is laborious and expensive. An objective of this research was to determine if it were possible to identify compounds in corn kernels responsible for resistance to aflatoxin contamination in order to enhance breeding programs. We examined corn line B73, whose derivatives are widely used commercially and considered susceptible to aflatoxin contamination, and a resistant line, Tex6, developed at the University of Illinois. Corn kernels were used as a substrate for a strain of *Aspergillus flavus* that harbors a construct with the *ver1* promoter fused to the *E. coli uidA* gene (Flaherty et al., 1995). In the assay, the fungus produced significantly less aflatoxin and GUS activity on Tex6 than B73. Further, the addition of Tex6 extracts to a defined medium resulted in less aflatoxin accumulation, suggesting that Tex6 contains an inhibitor of aflatoxin synthesis. The active compound(s) is water soluble, heat labile (100C), and can be precipitated by $(NH_4)_2SO_4$. Extracts from Tex6 had no significant effect on fungal growth. Chemical characterization of this compound(s) is in progress.

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USE OF A NORSOLORINIC ACID PRODUCING ASPERGILLUS PARASITICUS MUTANT TO IDENTIFY RESISTANCE TO PREHARVEST AFLATOXIN CONTAMINATION IN CORN.
David M. Wilson and Neil W. Widstrom, University of Georgia and USDA/ARS, Coastal Plain Station, Tifton, GA 31793.

Aflatoxin synthesis is only partially blocked in the norsolorinic acid (nor) producing A. parasiticus mutant, ATCC 24690. When corn is wound inoculated the fungus infects corn and produces both nor and aflatoxin. Many infected kernels can be visually identified because of the reddish color of nor. In 1994, eight corn entries were wound inoculated 20 days after full silk. Ears were rated for the number of red kernels and aflatoxin content at physiological maturity. The red kernels occurred randomly on the ear and were not associated with the inoculation site or insect damage. There was a highly significant correlation between aflatoxin content and number of red kernels. Resistance was highly related to both the paucity of red kernels and low aflatoxin content. The use of this mutant may prove useful in developing inexpensive breeding strategies for resistance to the A. flavus group and aflatoxin contamination.

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ANTIFUNGAL AND ANTITOXIGENIC ACTIVITY OF PROTEIN EXTRACTS OF MAIZE KERNELS VARYING IN RESISTANCE TO AFLATOXIN PRODUCTION BY ASPERGILLUS FLAVUS. R.L. Brown, A.J. DeLucca, T.E. Cleveland, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, LA 70179

Kernels of three maize inbreds, two that were resistant and one that was susceptible to aflatoxin production by Aspergillus flavus in field trials and in laboratory assays, were extracted for proteins with buffers at pH 2.8 and at pH 7.5. Kernels extracted were either water-imbibed, dry, wounded in the endosperm or embryo region, or infected with A. flavus prior to extraction. Protein extracts were tested against both fungal growth and aflatoxin accumulation in a bioassay which used an A. flavus tester strain that had been transformed with a GUS reporter gene linked to an A. flavus β-tubulin gene promoter. Spore germination and fungal growth were investigated using both a quantitative GUS assay, and histochemical GUS detection. Results suggest that a protein may be involved in the resistance response of tested maize genotypes.

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USING BRINE SHRIMP BIOASSAY TO MEASURE TOXICITY OF *FUSARIUM* TOXINS. R.A. Shelby, D. Zhang, and L.W. Dalrymple, Department of Plant Pathology, Auburn University, AL 36849.

Brine shrimp (*Artemia* sp.) were used in a bioassay of various *Fusarium* toxins and aqueous extracts of *F. moniliforme* culture material. Toxins were dissolved in saline solution and brine shrimp hatchlings were incubated for 24 hr at 30C in 96-well polystyrene plates. By making serial dilutions of toxins, it was possible to calculate the concentration required to cause 50% mortality (LC50 values). Using pure compounds, LC50 values of 0.069, 0.250, 32.7, 21.5, and 130.8 ppm were observed for T-2 toxin, Diacetoscirpenol, Zearalenone, Fumonisin B1, and Fumonisin B2, respectively. *F. moniliforme* cultured on corn was extracted with water, similarly tested by the brine shrimp bioassay and simultaneously analyzed for fumonisins by liquid chromatography. By comparison, culture extracts were approximately 10 times more toxic than could be accounted for on the basis of fumonisin alone. Brine shrimp are good bioindicators of acute toxicity due to their universal availability, ease of handling, and reproducibility of results.

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ISOLATION AND CHARACTERIZATION OF PLANT-MODULATED BACTERIAL PROMOTERS/GENES IN ROOT COLONIZING PSEUDOMONADS.
J.J. Kim [1], J.E. Lawrence [2], D.A. Kluepfel [2]. [1] Department of Microbiology and [2] Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Approximately 3,000 random gene fusion mutants in the rhizobacterium *Pseudomonas aureofaciens* were generated using a promoterless Tn5::*xylE* reporter gene. Wheat seedlings were germinated and then grown on 0.5X Minimal M9 agar plates for 2 days. Each healthy seedling was inoculated with a single mutant, incubated for 3 days (20° C; 12h light:12h dark) and examined for root exudate induced *xylE* expression. Four root exudate inducible bacterial promoters, along with numerous constituitively expressed promoters, were identified. Further characterization of plant inducible bacterial promoters and the genes controlled by these promoters will aid in determining their function(s) in bacteria colonizing the rhizosphere.

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IN SITU MOBILIZATION OF A NON-CONJUGATIVE PLASMID INTO A ROOT-COLONIZING STRAIN OF *PSEUDOMONAS FLUORESCENS*. Padma Sudarshana and G. R. Knudsen, Plant Pathology Division, University of Idaho, Moscow.

Transfer frequencies of the non-conjugative *lac*ZY plasmid pMON5003 from *Escherichia coli* to *P. fluorescens* were determined in pea spermosphere and rhizosphere microcosms. Pea seeds were inoculated with *P. fluorescens* 2-79 (recipient) at 10^8 CFU/seed and planted in sterile silt loam soil. *E. coli* M182 (pMON5003) (donor) and *E. coli* TB1(containing the conjugative helper plasmid pRK2013) were uniformly distributed in the surrounding soil. Populations of *P. fluorescens* on pea root segments increased over 11 days and averaged 1.4×10^7 CFU/cm of root at distances of 1-5 cm from the seed. Mean numbers of transconjugants were 20 CFU/cm of root over the 11-d period and were proportional to recipient cell numbers. Plasmid transfer was not observed in the absence of TB1(pRK2013). On seed and root surfaces, mobilization frequencies were 10 to 100-fold lower than previously observed on agar surfaces.

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CHARACTERIZATION OF ORGANIC WASTES AND WASTE AMENDED SOILS SUPPRESSIVE AND NONSUPPRESSIVE TO PYTHIUM ROOT ROT OF SUGARCANE. N. Dissanayake, J.W. Hoy, and G.A. Breitenbeck. Dept. of Plant Path. and Crop Phys., Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Organic wastes and composts were characterized for chemical and biological properties that reflected potential to suppress Pythium root rot of sugarcane when used as soil amendments. All amendments stimulated soil microbial activity and increased organic matter and plant nutrient contents of soils in greenhouse experiments. Only sewage sludge, sugar mill filter press cake and cotton gin trash compost consistently suppressed Pythium root rot. Composts derived from municipal solid waste (MSW), yard waste or various tree barks also were compared. MSW composts enhanced disease severity, whereas the others had variable effects. Suppressive materials had the highest microbial activity levels, contained higher levels of N and P and had lower C:N ratios than nonsuppressive materials. MSW and soils amended with MSW contained high salt concentrations. Extracts obtained from the organic materials studied promoted mycelial growth and formation of reproductive structures by Pythium in vitro, suggesting that suppressiveness is not associated with diffusible substances.

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EFFECT OF ELEVATED GROWTH TEMPERATURE AND CYCLOHEXIMIDE ON DSRNA AND CULTURE MORPHOLOGY OF *MONOSPORASCUS CANNONBALLUS*. Y. J. Park, R. D. Martyn, and M. E. Miller, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843 and Weslaco 78596.

Monosporascus cannonballus is a soilborne ascomycete that causes a severe root rot and vine decline of curcubits. Some isolates have shown variability in both colony morphology on standard growth media and aggressiveness in greenhouse pathogenicity tests which have been correlated with the presence of dsRNA. Less aggressive isolates that contained dsRNA formed fewer perithecia, had reduced growth, produced heavy pigmentation, and appeared to degenerate in culture. While there is a high correlation between the presence of dsRNA and culture degeneration, no definitive proof for a "cause and effect" relationship exists. Attempts to cure an isolate of its dsRNA by culturing on cycloheximide-amended medium and growing at elevated temperatures (37 C) for several successive generations were only partially effective. Elevated temperature, but not cycloheximide, caused a change in dsRNA banding patterns in most subcultures, but no specific and consistent changes were observed. However,in one subculture, all dsRNA bands were eliminated and that "cured" subculture reverted to a wild type morphology.

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IMPORTANCE OF AMINO ACIDS AS SOURCES OF REDUCED CARBON FOR SPERMOSPHERE PROLIFERATION OF *ENTEROBACTER CLOACAE*. D. P. Roberts[1], P. D. Dery[1], and J. S. Hartung[2]. [1]Biocontrol of Plant Diseases Laboratory and [2]Fruit Laboratory, USDA-ARS Beltsville, MD 20705.

The biocontrol bacterium *Enterobacter cloacae* grew *in vitro* and in natural soil on seven amino acids commonly found in seed exudates when these compounds were supplied as sole sources of reduced carbon. Strains M2 and M59, mini-Tn*5* Km mutants of *E. cloacae* strain 501R3, were incapable of growth or had reduced growth on all seven of these amino acids. However, these two strains were similar to strain 501R3 in growth on amino acids supplied as nitrogen sources and in other nutritional tests. In corn and cucumber spermosphere proliferation assays, populations of strains M2 and M59 were significantly lower (P=0.06) than populations of strain 501R3 in pea spermosphere. However, proliferation by strains M2 and 501R3 was similar in bean, cowpea, radish, and sunflower spermospheres, whereas proliferation by strains M59 and 501R3 was similar (P=0.05) in pea and radish spermospheres.

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EFFECT OF CROP ROTATION ON SUDDEN DEATH SYNDROME OF SOYBEAN AND SOIL POPULATIONS OF *FUSARIUM SOLANI AND HETERODERA GLYCINES* . J.C.Rupe, R.T. Robbins, and C.M. Becton, University of Arkansas, Fayetteville.

Five rotations were compared for their effect on SDS and soil populations of *F. solani* and *H. glycines* from 1989-92: continuous susceptible soybean (SSSS),or two years of a resistant soybean (SRRS), sorghum (SSgSgS), fescue (SFFS), or wheat (SWWS). The test was a randomized complete block with six replications. Soil populations of *F. solani* and *H. glycines* were determined at planting, flowering and harvest. SDS was rated weekly in August and September and yields taken. All rotations significantly reduced egg and cyst populations of *H. glycines* when compared to the SSSS. No rotation effected soil populations of *F. solani* or SDS development, but yields were higher with SFFS and SWWS than SSSS.

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HERBICIDE EFFECTS ON PYTHIUM ARRHENOMANES, PYTHIUM ROOT ROT, AND GROWTH OF SUGARCANE. N. Dissanayake, J.W. Hoy, and J.L. Griffin. Dept. Plant Path. and Crop Phys., La. State Univ. Agric. Center, Baton Rouge, LA 70803.

Greenhouse tests were conducted to evaluate effects of herbicides applied postemergence to sugarcane on the severity of Pythium root rot. Terbacil and glyphosate consistently increased root rot severity and reduced sugarcane shoot and root growth in Pythium-infested soils 8 to 10 weeks after herbicide treatments. Similarly, they reduced shoot and root growth in steam-sterilized field soils. Although pendimethalin caused clubbing of roots, effects on shoot and root growth and root rot severity were variable. Asulam had variable effects on shoot and root growth and root rot severity in Pythium-infested soils. In general, herbicides injurious to sugarcane were associated with reduced plant growth and enhanced root rot severity. Metribuzin and atrazine did not affect plant growth or root rot severity in Pythium-infested field soils. In pathogenicity tests, all herbicides caused increased root rot severity and reduced plant growth. In vitro studies showed that pendimethalin reduced colony growth of Pythium arrhenomanes with increasing concentration, and atrazine reduced growth at the highest concentration. The other herbicides did not affect mycelial growth.

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SPACE SOLARIZATION FOR SANITATION OF INOCULA OF PLANT PATHOGENS IN THE GREENHOUSE STRUCTURE. E. Shlevin, J. Katan , Y. Mahrer, Faculty of Agriculture, Rehovot 76100, ISRAEL and G. Kritzman, ARO, Bet Dagan 50250, ISRAEL.

Large amounts of inocula persist in the soil and on the greenhouse structure. Inocula adhering to the greenhouse structure are usually not effectively controlled, due to technical difficulties in reaching them. Closing the greenhouse during the period between crops, in the summer time in Israel, raises air temperatures to 65°C and higher (space solarization). This heating occurs under dry conditions, which is less effective than wet ones. The inoculum of *Clavibacter michiganensis* was the most sensitive and that of *Fusarium oxysporum* f. sp. *radicis-lycopersici* was the least sensitive to space solarization; *Pythium* sp. and *Sclerotium rolfsii* were intermediate. Wetting the greenhouse structure did not improve pathogen control, since this resulted in temporary cooling due to evaporation. A model for predicting the effectiveness of space solarization is in being developed. Space solarization is a potential inexpensive and nonchemical sanitation method. Combining space and soil solarization greatly improves pathogen control.

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IMAGING OF BARLEY YELLOW DWARF VIRUS BY CRYO-TRANSMISSION ELECTRON MICROSCOPY AND HIGH RESOLUTION CRYO-SCANNING ELECTRON MICROSCOPY. S.-L. Cheng, Y. Chen, R. F. E. Crang, M. Yeager, L. L. Domier, and C. J. D'Arcy. University of Illinois and USDA-ARS, Urbana, IL, University of Wisconsin, Madison, WI, and The Scripps Research Inst., La Jolla, CA.

The 50kDa protein of BYDV is a structural protein, which has been proposed to be on the external surface of virus particles. To observe fine surface structure, BYDV-PAV-IL particles were examined by cryo-transmission electron microscopy (cryo-TEM) and high resolution cryo-scanning electron microscopy (cryo-SEM). Both cryo-TEM and cryo-SEM confirmed an average particle diameter of 28 nm. Cryo-SEM revealed hexagonal particles with knob-shaped structures on the surface. In cryo-TEM preparations, spike-like protrusions with an average length of 7 nm were observed on the surface of the hexagonal virus particles. Many virus particles had one or more of these structures, but some particles had none. After treatment with 400 ng trypsin/µg virus for 1.5 hr, the number of particles with knob-shaped structures in cryo-SEM was reduced from 46% to 5%. We propose that these structures are the 50kDa protein.

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ABILITY TO OVERCOME ZUCCHINI YELLOW MOSAIC VIRUS COAT PROTEIN MEDIATED RESISTANCE IN MELONS IS ASSOCIATED WITH ALTERED HOST RANGE
R. Grumet, G. Akula, E. Kabelka, R.C. Yadav, Dept. of Horticulture, Michigan State Univ., E. Lansing MI 48824 and R. Provvidenti, Dept. of Plant Pathology, Cornell Univ., Geneva NY 14456.

Transgenic melons expressing the zucchini yellow mosaic virus (ZYMV) coat protein (CP) gene were tested for resistance against a range of ZYMV strains and related cucurbit potyviruses. The transgenic melons showed very strong resistance against the strain from which the CP gene was derived (ZYMV-CT), and against several other strains varying in severity from mild to highly severe. The transgenic melon plants also exhibited some resistance to infection by watermelon mosaic virus, but not papaya ringspot virus. Two ZYMV strains were able to overcome the ZYMV-CP mediated resistance: a severe, non-aphid transmissible strain from Israel (NAT) and a mild strain from California (CA). ZYMV-NAT also was able to overcome naturally occurring ZYMV resistance gene(s) in the cucumber cvs. TMG-1 and Dina. Unlike many strains of ZYMV, including ZYMV-CT, the resistance-breaking strains were able to systemically infect *N. benthamiana*, and ZYMV-CA also was able to systemically infect *Phaseolus vulgaris* cv. Black Turtle 2. Comparisons among predicted amino acid sequences suggest that the ZYMV-CP resistance-breaking property is not due to mutations in the CP gene itself.

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LONG-DISTANCE SPREAD OF PEPPER MOTTLE POTYVIRUS AND CUCUMBER MOSAIC CUCUMOVIRUS WITHIN *Capsicum annuum* L.
M. Andrianifahanana, Edward J. Sikora, and John F. Murphy, Department of Plant Pathology, Auburn University, AL, 36849-5409

The spread of pepper mottle potyvirus (PeMV) and cucumber mosaic cucumovirus (CMV) through time in *Capsicum annuum* L. 'Early Calwonder' is being investigated. Virus was applied to the first true leaf of plants that were at the 8-10 true leaf stage. Viral antigen was detected using immuno-tissue blot analysis. PeMV antigen was detected in the inoculated leaf by 3 days post-inoculation (dpi) and in the stem below the inoculated leaf at 3.5 dpi. PeMV antigen was detected in uninoculated leaves 6-10 and all stem sections above the inoculated leaf by 4.5 dpi. CMV appeared to follow a similar pattern of spread as PeMV, but CMV antigen was not detected in the inoculated leaf until 4 dpi. Subsequent spread of CMV was comparable to that of PeMV. Of particular interest was the observation that each virus appeared to spread in the outer portion of the stem from the inoculated leaf to the roots, while spread up the stem from the roots to uninoculated leaves occurred in the inner portion of the stem. Examination of the effect on systemic spread of PeMV by excision of the inoculated leaf at specific times post-inoculation revealed that PeMV had moved out of the inoculated leaf between 2-3 dpi , and removal of the inoculated leaf had no effect on the rate of systemic spread or accumulation of PeMV antigen in uninoculated tissues.

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SPECIFICITIES OF MOUSE MONOCLONAL ANTIBODIES TO CUCUMBER MOSAIC VIRUS.
H. T. Hsu, L. Barzuna and W. Bliss, USDA, ARS, Beltsville, MD; University of Costa Rica; and Agdia, Inc., Elkhart, IN.

Hybridomas secreting monoclonal antibodies (McAbs) to cucumber mosaic virus (CMV) were produced. BALB/c mice were immunized with an immunogen preparation composed of a mixture of purified CMV from different geographical origins. Immune splenocytes were fused with myeloma cells FOX-NY. CMV antibody secreter hybrids were selected using a similar immunogen mixture adsorbed either directly on plates at pH 9.6 or on rabbit anti CMV-S and CMV-D sera coated plates. A total of 23 hybridoma cultues were identified and cloned; all produced McAbs that reacted positively with antigens trapped on antiserum coated plates. Twenty of them also reacted on antigen coated plates. Examination with members of known subgroups revealed that 8 cloned hybridomas produced subgroup I-specific antibodies and 4 secreted subgroup II-specific antibodies. The remaining cell lines produced antibodies that reacted with both serotypes. Tests of various isolates with McAbs showed that the Ixora isolate from the Philippines, C310 from Georgia and both NT9 (tomato) and BCM (banana) from Taiwan are in subgroup I. S390 from Texas reacted to both subgroup I and subgroup II specific McAbs.

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MONOCLONAL ANTIBODIES THAT DISCRIMINATE A CANADIAN PVY-N FROM OTHER PVY-N AND PVY-NTN ISOLATES.
J.A. Abad, J.B.Young, O.W. Barnett, and S.A. Lommel. Dept. of Plant Pathology, NCSU Raleigh, NC 27695-7616.

In 1991, the USDA stopped shipment of potatoes from Prince Edward Island, Canada because they found potatoes infected with a new isolate of PVY-N that was highly virulent on tobacco. In order to facilitate the diagnosis of this virus, two monoclonal antibodies (MAbs), one specific to the PVY-N isolate from Canada (PVY-N 204) and the other with a broad spectrum reactivity against other PVY isolates, were selected after screening >400 hybridoma culture supernatants. A mixture of three different PVY-N isolates: PVY-204 and PVY-Foster from Canada, and PVY-178 from Chile were used as antigens. In indirect ELISA tests, *PVY-204N MAb* reacted specifically against the PVY-N from Canada. *PVYbs MAb*, with a broader spectrum, detected all the PVY isolates belonging to the PVY-O and PVY-N groups of strains tested so far with the exception of two isolates belonging to PVY-NTN, a newly recognized group of PVY strains. A simple assay to detect and discriminate PVY-N 204 from other necrotic PVYs and PVY-NTN using the two MAbs and a polyclonal antiserum that detects all PVY isolates is currently being developed.

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PRODUCTION AND EXPRESSION OF A FUNCTIONAL SINGLE-CHAIN ANTIBODY (ScFv) AGAINST TOMATO SPOTTED WILT VIRUS N PROTEIN.
K.D. Chenault, M.D. Bandla, and J.L. Sherwood. Department of Plant Pathology, Oklahoma State University, Stillwater, OK 74078.

Total mRNA was isolated from a monoclonal cell line producing a MAB against the tomato spotted wilt virus (TSWV) N protein. Double-stranded circular cDNAs were produced and used as template for PCR amplification of the variable regions of the heavy and light chain antibody genes using primers specific for those regions. Once cloned, the VH and VL regions were fused by a flexible peptide linker and the construct was expressed using the pET 14b expression system. The ScFv was renatured by dialysis in PBS and found to bind to TSWV N protein by ELISA. The ScFv construct is being used for transformation of *Nicotiana tabacum L.* to evaluate expression and ScFv-antigen binding *in planta*.

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SEROLOGICAL DETECTION OF TOMATO MOTTLE VIRUS NONSTRUCTURAL PROTEINS.
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Tomato mottle virus (TMoV) is a whitefly-transmitted geminivirus which is an important pathogen of tomato in Florida. The TMoV genome is composed of two circular, single-stranded DNA molecules that together contain at least 7 open reading frames (ORFs). To study the functions of its nonstructural proteins, we cloned and expressed each of the AC4, BC1 and BV1 ORFs in *E. coli*. Polyclonal antisera to each of these proteins were prepared in rabbits. The antisera were used in western blots to index AC4, BC1 and BV1 proteins in both TMoV-infected and transgenic plants. As reported for other bipartite geminiviruses, the BC1 movement protein (MP) (33-kD) was detected both in the membrane and cell wall fractions of leaf extracts, whereas BV1 MP (29-kD) was found in both the cytoplasm and membrane fractions. The AC4 protein (10-kD) was also detected in the membrane fraction. This is the first report that the AC4 protein is readily detectable in plants infected with a bipartite geminivirus.

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SINGLE STRANDED CONFORMATIONAL POLYMORPHISM ENABLES THE DIFFERENTIATION OF CITRUS TRISTEZA CLOSTEROVIRUS STRAINS.
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Single stranded conformational polymorphism (SSCP) permits the separation of DNA molecules that are similar in size but differ in sequence. The sequence differences result in conformational changes, which in turn lead to differential migration of single stranded DNA in nondenaturing polyacrylamide gels. Reverse transcription and polymerase chain reaction were coupled to SSCP to identify sequence differences among several strains of citrus tristeza closterovirus (CTV). Two CTV genes were used for this purpose: the diverged copy of the coat protein gene (p27), 720 nucleotides in length, and a highly expressed gene of unknown function (p23), 630 nucleotides long. Differences were found among the strains for both genes. The mild strains examined all produced a distinct pattern, as did some of the stem pitting strains. This technique is potentially useful in rapidly and inexpensively screening and differentiating CTV strains.

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Development of Hybrid Strains of Bean Common Mosaic Potyvirus.
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When opposite primary leaves of incompatible (susceptible to primary leaf but resistant to trifoliate leaf infection) or compatible (totally susceptible) bean cultivars were inoculated with the NL-8 strain (serotype A) of bean common mosaic necrosis virus (BCMNV) and the US-5 strain (serotype B) of bean common mosaic (BCMV), respectively, systemic movement occurred mainly in the compatible host-virus combinations. Occasionally, however, an "incompatible" virus was detected in a few trifoliate leaves. A total of 16 virus isolates were separated by repeated transfers through differential hosts. All 16 expressed biological properties different from either parental isolate. Seven of these infected bean cv Pinto UI 114, a cultivar resistant to both parents or a mixture of both. Applying the "gene-for-gene" concept used to evaluate genetics of BCMV resistance in beans and to define pathogenicity genes for BCMV, these seven isolates appear to be hybrids because they contain the genes for pathogenicity of both parents presumably in a single particle. Similar isolates appear to have been found recently in nature in the US and Africa.

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REPLICATION OF CITRUS TRISTEZA VIRUS IN *NICOTIANA BENTHAMIANA* PROTOPLASTS. J. Navas-Castillo[1], S. Gowda[1], M.E. Hilf[2], S. M. Garnsey[2], and W.O. Dawson[1]. [1] University of Florida, CREC, Lake Alfred, FL 33850; [2]USDA-ARS, Orlando, FL 32803.

Citrus tristeza virus (CTV) is an aphid-transmitted and phloem-limited closterovirus that causes a major disease of citrus. The host range of CTV is restricted to woody species of *Citrus* and related genera. A system to examine the replication of this virus has been unavailable. We present the results on the development of a protoplast system able to support efficient CTV replication. *Nicotiana benthamiana* mesophyll protoplasts were inoculated with partially purified virions or RNA of several Florida isolates, using polyethylene glycol. CTV replicated in these protoplasts, as shown by western- and northern-blot analysis, and electron microscopy. Data on the time-course of the CTV genome replication are presented as an example of the new possibilities offered by this system for the study of closterovirus.

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EVIDENCE AGAINST BINDING OF POTYVIRUS HELPER COMPONENT TO VIRIONS *IN VITRO*. D. W. Thornbury, R.Y. Wang and T.P. Pirone. University of Kentucky, Lexington, KY

Immunogold labeling and electron microscopy (ImG-EM) were used to study the putative association of potyvirus helper component (HC) with virions of potato virus Y, tobacco vein mottling virus and tobacco etch virus. Purified preparations of HC and virions were allowed to associate *in vitro*. These mixtures, as well as leaf-dip preparations from infected plants, were examined by ImG-EM using monoclonal antibodies to HC and polyclonal antibodies to virions followed by either goat anti-mouse or goat anti-rabbit antibodies conjugated to 10nm and 5nm diameter gold particles, respectively. No attachment of HC to virions was observed with either treatment; antibodies to virions labeled virions specifically, whereas antibodies to HC produced a random distribution of gold particles. *In vitro* virion-HC mixtures were also centrifuged through a sucrose cushion or passed through a Sepharose 4B column to separate unbound HC from virions. No transmission was detected in aphid bioassay of the virion-containing fractions and ImG-EM examination showed no evidence of an HC-virion association. Previous reports have indicated an association of HC on virions in the aphid's food canal; our results suggest that this occurs only in the aphid and not *in vitro* or *in planta*.

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TWO AMINO ACID CHANGES IN THE COAT PROTEIN SEQUENCE OF THE M STRAIN OF CUCUMBER MOSAIC VIRUS EFFECT ITS MOVEMENT IN MAIZE. CHUNG-HO KIM, LEE ZHANG AND PETER PALUKAITIS. DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY, ITHACA, NY 14853

The M strain of cucumber mosaic virus (M-CMV) cannot infect maize. In contrast, the Fny strain of CMV can infect maize. Using pseudorecombinants constructed between M-CMV and Fny-CMV genomic RNAs, the maize plants could be infected when the inoculum consisted of RNA 1 and RNA 2 of M-CMV and RNA 3 of Fny-CMV. Chimeras formed between M- and Fny-CMV were used to demonstrate that the inability of M-CMV to infect maize mapped to two regions of the coat protein (CP) gene. Amino acid changes were introduced into regions of CP gene of M-CMV. Two nonconservative changes in the CP gene of M-CMV at positions 129 (Leu to Pro) and 162 (Thr to Ala) altered the CP sequences to those in Fny-CMV at these two positions and enabled the resulting M-CMV mutant to infect maize systemically. These results, together with those from molecular hybridization analyses, indicate that the host range restriction for M-CMV in maize is due to the inability to systemically move in this plant.

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INVOLVEMENT OF CARBOXYLATE GROUPS IN THE DISASSEMBLY OF TOBACCO MOSAIC TOBAMOVIRUS. B. Lu[1], G. Stubbs[3], and J. N. Culver[2], [1]Department of Plant Biology, University of Maryland, College Park, MD 20742; [2]Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, MD, 20742; [3]Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235

Structural studies of TMV have identified inter-subunit carboxylate groups whose mutual repulsion would predictably drive viral disassembly. To test the role of each carboxylate group in virion disassembly, site-directed mutagenesis was utilized to change group members to their amide forms. Amide exchange should maintain the hydrogen binding capacity of each residue but remove the repulsive negative charge. Single site mutations include E50Q, D77N, E95Q, E106Q, D109N, and E116Q, and multiple site mutations include 50Q/77N, 95Q/97Q, 97Q/109Q, and 95Q/97Q/109Q. Infectious RNAs from these mutants showed reduced infectivity and slower long distance movement as compared with the wild type. Virion stability will be tested using *in vitro* alkaline disassembly to confirm that these carboxylate group interactions are important in the disassembly of TMV. It is suggested that transgenic plants expressing such coat proteins could have enhanced resistance to virus infection.

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FULL-LENGTH cDNA CLONING OF POTATO VIRUS Y STRAIN VAM-B. R. Acosta-Leal, and Z. Xiong. Dept. of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Potato virus Y (PVY) strain VAM-B is a North American isolate capable of systemically infecting PVY-resistant tobacco 'Virgin A Mutant' (VAM). This strain is similar to PVY-NN which is unable to infect intact tobacco VAM plants. As a first step to identify the viral mutation(s) which confers the ability of PVY VAM-B to overcome the tobacco VAM resistance, full-length infectious cDNA clones of this strain are being constructed. Purified viral genomic RNA was used to synthesize a cDNA library according to the Gubler and Hoffman procedure (BRL cDNA synthesis systems). Several large cDNA clones which hybridized with PVY cDNA probes were sequenced at both the 5' and the 3' termini. Two overlapping large clones, pVAM-105 and pVAM-184 covered the entire PVY genome with the exception of the first 17 nucleotides at the 5' terminus. These clones will be used to construct an infectious cDNA clone of PVY VAM-B strain as well as to create recombinant full-length cDNAs with PVY VAM-B/NN specific sequences.

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COINCIDENCE OF TRANSMISSION OF DOUBLE-STRANDED RNA (dsRNA) AND PHENOTYPIC CHANGES IN *RHIZOCTONIA SOLANI*. Jianhua Jian, Dilip K. Lakshman, and Stellos M. Tavantzis. Department of Plant Biology and Pathology, Universiry of Maine, Orono, ME 04469.

Three hypovirulent cultures, Rhs 1A1, Rhs 1A2, and Rhs 1A3, originated as sectors of the virulent AG 3 isolate Rhs 1AP of *R. solani*. Rhs 1AP contains 2 dsRNAs of 23 kb and 6.4 kb. Rhs 1A1 has the above 2 plus 3 new dsRNAs of 25 kb, 3.6 kb, and 1.2 kb. The 5 dsRNAs are genetically unrelated. Rhs 1A2 has the 3.6-kb and 1.2-kb, whereas Rhs 1A3 contains the 25-kb, and 1.2-kb dsRNAs. Hyphal anastomoses were conducted in which the 4 cultures were paired in all possible combinations. Results showed that particular dsRNAs were transmitted to cultures in which they did not occur previously, and their transmission coincided with changes in the virulence and culture morphology of the recipient.

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SEQUENCE ANALYSIS OF A HYPOVIRULENCE-ASSOCIATED DOUBLE-STRANDED RNA (dsRNA) FROM *RHIZOCTONIA SOLANI*. D.K. Lakshman and S. M. Tavantzis, Dept. of Plant Biology and Pathology, Univ. of Maine, Orono, ME 04469.

A 3569-bp cytoplasmic dsRNA (M2) replicates in hypo-virulent, sector-originated cultures but not in the parental virulent isolate Rhs 1AP (AG 3). Both strands possess open reading frames (ORF) longer than 130 amino acids (aa). One strand has two overlapping ORFs of 414 aa (M2F1A) and 361 aa (M2F3A), respectively. M2F3A, lacks a methionine initiation codon and is likely to be translated by -1 frame shifting. M2F3A has all 4 motifs characteristic of a dsRNA viral RNA-dependent RNA polymerase (RDRP) and is phylogenetically related to the RDRP of a hypovirulence-associated, mitochondrial dsRNA from *Cryphonectria parasitica*. Southern analysis showed that genomic DNA of *R. solani* contains copies of M2.

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MOLECULAR CHARACTERIZATION OF A VIRAL PATHOGEN INFECTING MAIZE AND WHEAT IN THE HIGH PLAINS. S. G. Jensen and J. S. Hall. USDA-ARS and the University of Nebr. Lincoln, NE 68583

A disease of maize and wheat has been found in the high plains of central and western US. We have attempted to purify and characterize the pathogen which we have termed the high plains virus. Large particulates were removed from the sap of mite inoculated maize and wheat by low speed centrifugation and the virus-size particulates were concentrated by ultracentrifugation of the supernatant. Resolution of viral proteins on 5-12 % gradient polyacrylamide gels revealed two proteins of approximately 32 kDa in size. During sucrose density gradient centrifugation the 32 kDa proteins were associated with a diffuse, slow moving band of nucleoproteins. The slow sedimentation indicated an asymmetric filamentous shape or low density or both. No characteristic particles could be identified by electron microscopy. Extraction of purified nucleoproteins, or of infected plant sap, yielded six species of dsRNA. The dsRNAs range in size from about 700 to 2500 bp. Molecular characterization suggests a new virus, possibly related to the bunyaviradae.

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MOLECULAR CLONING, SEQUENCING, AND RT-PCR DETECTION OF POINSETTIA MOSAIC TYMOVIRUS. J.A. Abad, C.L. Hemenway, and J.W. Moyer. Depts. of Plant Pathology and Biochemistry NCSU Raleigh, NC 27695-7616.

Poinsettia mosaic virus (PnMV) is a putative member of the Tymovirus group. An isolate of this virus was obtained from Poinsettia plants showing mosaic symptoms. Partial sequence was determined from a 1.6 kb cDNA clone obtained after reverse transcription of the viral RNA. This sequence corresponds to the replicase open reading frame of Tymoviruses. Identities ranging from 66% to 48% at the amino acid level were found with onion yellow mosaic, erysimum latent, eggplant mosaic, and turnip yellow mosaic Tymoviruses. Primers to amplify a 0.6 kb fragment from the viral genome were designed and used successfully to diagnose this virus in Poinsettia and *Nicotiana benthamiana* infected plants via RT-PCR. These results confirm the putative assignment of PnMV to the Tymovirus group previously based upon particle morphology, genome size and serology.

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MOLECULAR ANALYSIS OF COAT PROTEIN GENE SEQUENCES OF WHITEFLY-TRANSMITTED GEMINIVIRUSES FROM THE CARIBBEAN BASIN. G. K. Banks[1], M. Sosa[2], J. Bird[2], and J. K. Brown[1]. [1]Dept. of Plant Sciences, Univ, of Arizona, Tucson, AZ 85721; [2]College of Agriculture Sciences, Univ. of Puerto Rico, Rio Piedras, PR 00928.

Whitefly-transmitted geminiviruses exist in weed hosts which are potential reservoirs of economically important geminiviruses throughout the Caribbean Basin. The coat protein (CP) gene is the most highly conserved among Subgroup III of the Geminiviridae. The core region of the CP gene was amplified by PCR and sequenced from a suite of geminiviruses from the Caribbean Basin. The core region sequences from Caribbean Basin geminiviruses were compared to those in a database containing CP gene sequences from biogeographically diverse geminivirus isolates. Results indicated a high degree of similarity between the Caribbean viruses and other New World geminiviruses studied to date, and delineated several distinct subclusters within the Caribbean subgroup. Geminiviruses within Caribbean subclusters, in some cases, exhibit similar symptomatologies and have similar host range phenotypes.

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MOSAIC SYMPTOM DETERMINANTS IN THE TMV 126 kDa OPEN READING FRAME AND THEIR EFFECTS ON SURROUNDING SEQUENCES: THE UNIT HYPOTHESIS. S.A. Carter, M.H. Shintaku, P. Derrick, R.S. Nelson, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

The *Masked* strain of TMV produces attenuated symptoms in systemically-infected tissue of tobacco when compared with the U1 strain. Recently we produced site-directed mutants within cDNAs of each strain from which infectious RNA can be transcribed. Eight nucleotides differences, resulting in 8 amino acid changes within the 126/183 kDa ORF are solely responsible for the attenuation. Two nucleotides, positions 2 and 6, independently controlled the appearance of the mosaic or attenuated phenotype when placed in the *Masked*-TMV or U1-TMV background, respectively. A third nucleotide, position 4, was responsible for attenuated symptoms regardless of background, but exacerbated symptoms in the *Masked*-TMV background in the presence of the mosaic-determinant nucleotides. The progeny of some of the site-directed mutants have further mutated specifically within the 8 positions, suggesting that these nucleotides, or the corresponding amino acids, perform their function best when present as a uniform, parental sequence unit.

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IDENTIFICATION OF THE GENE(S) OF TOBACCO ETCH VIRUS RESPONSIBLE FOR THE WILTING RESPONSE OF TABASCO PEPPER. M.H. Chu, D.W. Thornbury, and T.P. Pirone. Dept. of Plant Pathology, Univ. of KY, Lexington, KY 40546.

Infection of Tabasco pepper by tobacco etch virus (TEV) typically causes wilting, due to root necrosis. A strain of TEV, designated TEV non-wilting (TEV NW), causes mosaic symptoms but not wilting in Tabasco pepper. In order to find the mutation(s) responsible for the inability of TEV NW to cause wilting, the complete nucleotide sequence of TEV NW was determined. TEV NW has 9496 nucleotides and a poly-adenylated tail; translation of a single putative polyprotein can be deduced. Compared to the published sequence of a TEV isolate that causes wilting, there are 767 nucleotide and 126 amino acid changes scattered throughout the TEV NW genome. The location of the responsible gene(s) is being pursued by inserting regions of TEV NW cDNA into a full-length TEV cDNA construct, whose infectious transcripts can cause wilting on Tabasco pepper. Thusfar, substitutions of the individual P1, HC, P3 and CP genes of TEV NW into TEV have not altered the wilting response. Replacement of the remaining portions of the TEV genome with TEV NW is currently being undertaken.

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THE TRIPLE GENE BLOCK OF BARLEY STRIPE MOSAIC VIRUS IS DISPENSABLE FOR LOCALIZED CELL-TO-CELL MOVEMENT IN INOCULATED OAT AND BARLEY LEAVES. John J. Weiland and Michael C. Edwards. Dept. of Plant Pathology, North Dakota State University, & USDA-ARS Northern Crop Science Laboratory, Fargo, ND 58105.

Genetic determinants of barley stripe mosaic virus (BSMV) oat pathogenicity were recently mapped to the αa gene, whose product is a putative component of the viral replicase (Weiland & Edwards, *Virology* **201**,116-126[1994]). Alpha-a gene mutants that cannot detectably infect oat plants replicate in oat protoplasts to levels similar to BSMV strains that are systemic pathogens of oat. In order to ascertain whether the mutants that are non-pathogenic to oat are capable of replicating in single cells *in planta*, a recombinant BSMV RNAβ (18βgus) was constructed in which the triple gene block was replaced with the *E. coli* β-glucuronidase gene. 'Rodney' oat and 'Black Hulless' barley plants were inoculated with infectious transcripts of BSMV RNAα, RNAγ, and 18βgus. Surprisingly, macroscopic blue foci were observed on the inoculated leaves 24 hr post-inoculation following histochemical tissue staining for β-glucuronidase. A single focus encompassed an ave. of ~50 cells. Electron microscopic examination of cells within and adjacent to the blue foci revealed the presence of typical BSMV rod-shaped virions. The data suggest that the BSMV triple gene block is not required for limited cell-to-cell movement in cereal hosts.

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HYBRIDIZATION GROUPS OF SQUASH MOSAIC VIRUS. James S. Haudenshield and Peter F. Palukaitis, Department of Plant Pathology, Cornell University, Ithaca, New York 14853-4203.

Squash mosaic virus (SqMV) is a seed-borne, beetle transmitted comovirus, infecting a wide range of cucurbits (Freitag, Phytopathology 46:73-81, 1956). Earlier workers have utilized host-range and symptomatology, but mainly serology to identify the virus and delimit two groups of SqMV from North America. More recently, the gene for the two capsid proteins has been mapped to RNA-2 ("M" particles), and the corresponding nucleotide sequences for one virus isolate has been determined (Hu et al., Arch. Virol. 130:17-31, 1993). We have purified RNA from six other isolates of SqMV, and prepared cDNA clones from RNA-2 of two. In Northern blot experiments, DNA probes made from these clones define two SqMV hybridization groups. This grouping was verified by reciprocal Northern blots of the source RNA, probed with cDNA made from the opposite group. Preliminary analysis of the cDNA clones indicates 70%–80% sequence similarity in RNA-2 between these groups. The two hybridization groups identified here may correlate to the earlier serological groupings.

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CLONING AND SEQUENCE ANALYSIS OF THE N GENE OF SEVERAL MORPHOLOGICALLY AND SEROLOGICALLY DISTINCT ISOLATES OF IMPATIENS NECROTIC SPOT TOSPOVIRUS. Ramon Jordan. USDA-ARS, US National Arboretum, Floral & Nursery Plants Research Unit, Beltsville, MD 20705.

The structural nucleoprotein (N) gene and intergenic region of four serologically and morphologically distinct isolates of impatiens necrotic spot (INSV) tospovirus was investigated. Isolates under study included a defective gloxinia isolate (INSV-lgg), two high temperature variants derived from lgg (HT1 and HT2; producing abnormal virions), and a non-defective isolate from *Schizanthus* (INSV-B). Total RNA purified from virus-infected *Nicotiana benthamiana* was amplified using degenerate primers and a reverse transcription and polymerase chain reaction (RT-PCR). Primers were designed to flank the N gene and portion of the intergenic region of the tospovirus S RNA having the greatest nucleotide sequence homology among isolates of INSV. PCR products of ~1kbp were generated and cloned into a pBluescript vector. Clones could be differentiated by DNA restriction map analysis and immunoassay of N protein-expressing clones using virus-specific N protein antisera. Nucleotide sequence comparisons of portions of the N gene protein and intergenic regions indicate a range of 84-95% similarity to published INSV sequences, compared to the 94-99% sequence homology between the published virus sequences.

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Distribution of Epitopes Among Viruses in the Bean Common Mosaic Virus Subgroup and the Location of Three on the N-terminal End of the Capsid Protein. G.I. Mink, Washington State University and M.J. Silbernagel, USDA-ARS, Prosser, WA99350, H.J. Vetten, IBP, Braunschweig,Germany, S.D. Wyatt and Roshan Abdallah, WSU, Pullman, WA 99350, and P.H. Berger, University of Idaho, Moscow, Id 83844.

Monoclonal antibodies specific for up to eight epitopes located on the N-terminal of the coat protein of several viruses in the bean common mosaic (BCMV) subgroup were reported earlier. While none of the eight were found on strains of bean common mosaic necrosis virus (formerly BCMV serotype A) or on cowpea aphid-borne mosaic virus, one or more were detected on all of the serotype B viruses which includes adzuki bean mosaic (AzMv), BCMV, blackeye cowpea mosaic, and peanut stripe viruses. Three epitopes (tentatively identified as B/E3, B/3A, and B/E4) were found to be located within the 50 N-terminal amino acids of the capsid protein. All serotype B viruses except AzMV possess either B/E3 or B/4, but never both. Using commercially prepared polypeptides based upon the N-teminal sequences of BCMV strains US-1 (B/E3) and US-6 (B/ 4) and a competitive ELISA procedure, we have identified the specific amino acids essential for each epitope.

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THE 17 KDA PROTEIN OF BARLEY YELLOW DWARF VIRUS STRAIN PAV IS NOT REQUIRED FOR REPLICATION. Jaesun Moon, Dept. of Plant Pathology and Leslie L. Domier, USDA-ARS, Dept. of Plant Pathology, University of Illinois, Urbana, IL 61801.

Barley yellow dwarf virus (BYDV) is a member of luteoviruses, aphid-transmitted in a persistant manner, and phloem-limited in host plants. The full-length BYDV-PAV-IL cDNA clone (pGP11) flanked by CaMV 35S promoter and nopaline synthase transcription terminator was constructed in pTZ19R. The mutant clone (pGP12) was constructed by site-directed mutagenesis in which the start codon of ORF4 was replaced by ACG without changing amino acid sequence of coat protein. When oat protoplasts were transfected with linearized pGP11 by electrophoration and cultured 5 days, the 22 KDa coat protein accumulated to essentially to the same levels as in protoplasts transfected with purified BYDV RNA. To determine if the 17 KDa protein was necessary for replication, pGP12 was transfected with as described above. While pGP12 was infectious, the 22 KDa coat protein accumulated to lower levels in protoplasts transfected with pGP12 than in protoplasts transfected with pGP11. The result indicated that the 17 KDa protein was not essential for viral replication.

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SUBSTANTIAL SEQUENCE DIFFERENCES AMONG RNA-2 MOLECULES OF INDIAN PEANUT CLUMP FUROVIRUS. R.A.Naidu, J.S. Miller[1], M.A.Mayo[1], and A.S. Reddy. ICRISAT Asia Center, Patancheru, India 502 324; [1]Scottish Crop Research Institute, Dundee DD2 5DA, UK.

Indian peanut clump virus (IPCV) is a Furovirus which causes an economically important disease of peanut crops in India. The IPCV genome is bipartite; RNA-2 encodes the coat protein. IPCV occurs in at least three distinct serotypes(H, L and T) and resembles peanut clump virus (PCV) which occurs in West Africa. The coat proteins of IPCV-H and PCV are 61% identical in aminoacid sequence. Nucleotide sequence analysis of RNA-2 of IPCV-L revealed ORFs for 5 proteins including coat protein. IPCV-L coat protein is 65% and 67% identical in aminoacid sequence with those of IPCV-H and PCV respectively. Thus, the two serotypes of IPCV are related to each other only as closely as each is to PCV suggesting they are different viruses.

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An Oil/Starch/Sugar Encapsulation Method Suitable for Gram-Negative Bacteria and Other Microbes. *N.K. Zidack*, P.C. Quimby, Jr., and A.J. Caesar, USDA/ARS Rangeland Weeds Lab, Montana State University, Bozeman, MT 59717.

The development of biological control agents into commercial products is dependent on stable, efficacious and economical formulations. An oil/starch/sugar encapsulation method for microbes has been developed that provides excellent storage life for gram-negative bacteria. It is inexpensive and does not require specialized equipment. A fluorescent, pathogenic bacterium isolated from leafy spurge (*Euphorbia esula*) was formulated using this method. There has been no decrease in viability at 2 C after 6 months and a 0.83 $\log_{10}$ cfu/g loss at 22 C. This method has also been used successfully for the formulation of fungi including *Fusarium, Rhizoctonia* and *Sclerotinia* spp. as well as the nematode *Subanguina picridis*.

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