

acrylamide gel electrophoreses (PAGE). Proteins on the SDS gel with an apparent molecular weight range of 23–46 kDa were induced in the kernels by *A. flavus* infection. From in-gel (native PAGE) enzyme activity assays, three bands corresponding to chitinase isoforms and two bands corresponding to beta-1,3-glucanase isoforms were detected in the infected kernels. One chitinase isoform of 29 kDa was induced only in the infected kernels, and another one of 28 kDa was present in both infected and non-infected kernels. They were both acidic based on their migration on an acrylamide isoelectric focusing (IEF) gel. For the beta-1,3-glucanase, one isoform of 35 kDa was present in both infected and non-infected kernels, but another one, 33 kDa isoform, was induced only in the infected kernels. Both acidic and basic beta-1,3-glucanase isoforms were detected in the IEF gel. These results indicate that only particular isoforms of the two fungal degradative enzymes might be involved in combating *A. flavus* in the maturing corn kernels.

Use of SCAR primers for specific identification and monitoring of the antagonistic strain K of *Pichia anomala*. M. H. IJAKLI, S. Cognet, B. Prakoso, and P. Lepoivre. Unité de Phytopathologie, Faculté des Sciences Agronomiques, 2, Passage des Déportés, 5030 Gembloux, Belgium. Phytopathology 89:S37. Publication no. P-1999-0258-AMA.

Pichia anomala strain K was previously selected for its high and reliable protective activity against *Botrytis cinerea* and *Penicillium* sp. on post-harvested apples. The large-scale introduction of strain K requires a specific method able to monitor the population of the antagonistic agent in order to evaluate its ecological fitness and its environmental impact. In this context, RAPD was used to distinguish strain K from ten other strains of *P. anomala*. The FPA2kb fragment, with an approximately size of 2 kb, was specifically amplified by RAPD from the DNA of strain K with the use of OPN-13, one of the 28 tested RAPD primers. Two SCAR (sequence characterized amplified region) primers were designed one containing the OPN-13 primer at the 5' termini and the other resulted from FPA2kb internal sequence. These SCAR primers allowed the reproducible and specific PCR amplification of one fragment, of expected size, for the sole strain K as confirmed by Southern analysis. The possibility to transfer this method in practical conditions will be presented.

Reproduction of *Heterodera schachtii* on Bt-transgenic cabbage. R. Jin, B. S. SIPES, and D. Borthakur. College of Tropical Agriculture and Human Resources, University of Hawaii, 3190 Maile Way, Honolulu, HI. Phytopathology 89:S37. Publication no. P-1999-0259-AMA.

Plant transformation with the Bt toxin has become common to control certain insect pests but unintended side effects may arise from its use. Transgenic cabbage expressing Bt toxin Cry1Ab3 resistant to the diamondback moth were evaluated for effects on reproduction of *Heterodera schachtii*. Transformed and nontransformed cabbage plants were inoculated with *H. schachtii* eggs. Cysts and females were dislodged from the roots 6 weeks later. The number of cysts and females per plant did not differ between the transformed and nontransformed cabbage ($P > 0.05$). More eggs developed on the Bt cabbage than on the nontransformed plants, but their numbers did not differ ($P > 0.05$). The lack of differences between the Bt-transgenic and nontransformed cabbage on the reproduction of *H. schachtii* may be due to lack of ingestion by the nematode because of the toxin size, lack of activity against nematodes, or insufficient expression in the plant roots.

Effect of nitrogen fertilization and fosthiazate on control of *Globodera tabacum* subsp. *solanacearum*. C. S. JOHNSON. Virginia Polytechnic Institute and State Univ., Blackstone, VA. Phytopathology 89:S37. Publication no. P-1999-0260-AMA.

Two field experiments were conducted in 1992 and another in 1993 to examine fertilization practices and use of the nematocidal fosthiazate to control a tobacco cyst nematode (TCN – *Globodera tabacum solanacearum*) on flue-cured tobacco (*Nicotiana tabacum*). Suppression of TCN populations by fosthiazate was significant in 1993, but not in 1992. Use of fosthiazate increased tobacco yield and value in all tests. Increasing the amount of sidedress nitrogen by 20% and 40% increased midseason TCN populations in 1993 and in untreated soil at one location in 1992. Increasing sidedress nitrogen did not alter tobacco yield or value in any of the three tests. Increasing sidedress nitrogen increased average price where fosthiazate had been used at one 1992 location, but reduced average price in 1993. Increasing sidedress nitrogen fertilization does not appear to be a useful practice to improve TCN control in flue-cured tobacco.

Controlling secondary spread of fire blight with prohexadione calcium. A. L. JONES (1), W. G. D. Fernando (2), and G. R. Ehret (1). (1) Michigan State University, East Lansing, MI; (2) University of Manitoba, Winnipeg, Canada. Phytopathology 89:S37. Publication no. P-1999-0261-AMA.

Apple trees are highly susceptible to fire blight when actively growing, but this period of susceptibility can be reduced to 1 to 2 weeks with a single high-rate application of the growth retardant prohexadione calcium (BAS 125W, Apogee). Prohexadione calcium inhibits the biosynthesis of gibberellin. Inhibition of gibberellin biosynthesis results in an early cessation of growth and reduced risk of infection by *Erwinia amylovora*. Trees treated with 250 mg/L prohexadione calcium late in the bloom period were found to have a significantly lower incidence of secondary shoot blight than unsprayed trees. Also, infections were suppressed on shoots inoculated 7 days after treatment with 250 mg/L prohexadione calcium. Two applications of 125 mg/L prohexadione calcium were not as effective as one 250 mg/L application in suppressing infection or secondary spread. Prohexadione calcium appears to offer a new and novel approach to fire blight control that does not involve the use of antibiotics.

Investigations into coconut diseases of uncertain aetiology. P. JONES (1), C. P. R. Cronjé (2), and J. S. Warokka (3). (1) IACR Rothamsted, Harpenden, Herts., AL5 2JQ, UK; (2) Pathology Division, SASEX, PB X02, Mt. Edgecombe, KwaZulu Natal, South Africa; (3) Research Institute for Coconut and Palmae, Manado, Indonesia. Phytopathology 89:S37. Publication no. P-1999-0262-AMA.

For most of the 20th Century there have been sporadic outbreaks of lethal diseases in coconuts grown in South East Asia. In Indonesia Natuna wilt and Kalimantan wilt are two such diseases. Phytoplasmas cause some of the most devastating diseases of coconuts in the Americas and Africa and we have compared symptoms and used DNA amplification to determine if either of these diseases is associated with a phytoplasma. Amplification of the 16S–23S rRNA genes by a nested-PCR gave products of 1250 bp in size. The restriction fragment length polymorphism of these amplimers suggested that at least one type of phytoplasma was present in coconut palms in Indonesia. Sequencing of the 16S–23S rDNA intergenic spacer region revealed a single copy of the characteristic t-isoleucine gene in all samples, confirming the phytoplasma status of the amplified sequences. Our discovery that phytoplasmas are active in coconut disease epidemics in Indonesia has implications for the sustainability of coconut farming systems in the region.

Rhizosphere bacterial communities associated with hydroponic lettuce: Potential role in biological control of *Pythium*. D. D. JURKONIE and E. B. NELSON. Cornell University, Ithaca, NY. Phytopathology 89:S37. Publication no. P-1999-0263-AMA.

Roots of hydroponically grown plants provide a simplified habitat for analyzing population dynamics among rhizosphere bacteria suppressive to *Pythium*. In preliminary experiments, a limited number of bacteria were isolated from roots of hydroponically grown lettuce and identified based on carbon utilization patterns and rDNA sequences. Culture-based community composition was compared with that obtained from community DNA analyses. Total bacterial DNA was extracted from lettuce roots and amplified using three primer sets targeting microbial 16S rDNA sequences. Amplified rDNA from two primer sets was separated by denaturant gradient gel electrophoresis (DGGE). Resolved DGGE bands were excised and re-amplified for sequencing and analysis. Full-length 16S rDNA from the third primer set was cloned and digested with restriction enzymes for selection of unique clones for sequencing and analysis. Preliminary results have demonstrated the feasibility of using this multifaceted approach for examining the qualitative nature of rhizosphere bacterial communities which may impact the biology and ecology of *Pythium*.

★ **Effects of incorporation implement and application rate on dazomet efficacy.** J. Juzwik (1), J. T. BLODGETT (1), D. L. Stenlund (2), and R. R. Allmaras (3). (1) USDA Forest Service, (2) MN Department of Transportation, and (3) USDA-ARS, St. Paul, MN. Phytopathology 89:S37. Publication no. P-1999-0264-AMA.

Dazomet (DAZ) is a non-ozone depleting alternative to methyl bromide for preplant soil fumigation in forest nurseries. Two factors that affect efficacy of DAZ fumigation are the uniformity and depth of incorporation of the surface-applied, granular product. Trials were conducted in a Minnesota and a Wisconsin (WI) nursery with five tillage implements and two DAZ rates to determine treatment effects on fungal populations by soil depth. Greatest depths of biocidal activity were found with spading machine treatment in both nurseries based on lettuce bioassays. DAZ incorporated by all imple-

* ments was equally effective in reducing *Fusarium* spp. populations to 12 cm in both locations with both chemical rates. Reductions of *Fusarium* spp. below 18 cm in WI were greatest using the spading machine with either chemical rate. Reductions of *Cylindrocladium* spp. below 24 cm in WI were greatest following spading treatment with the high DAZ rate. Results suggest selection of incorporation implement and DAZ rate should be based on vertical distribution of target pathogens.

Nitidulid species associated with fresh wounds on red oaks during spring in Minnesota. J. JUZWIK (1), T. C. Skalbeck (2), and M. F. Neuman (2). (1) USDA Forest Service, St. Paul, MN; (2) University of Minnesota, St. Paul, MN. *Phytopathology* 89:S38. Publication no. P-1999-0265-AMA.

Nitidulids (Coleoptera: Nitidulidae) are considered the primary vectors of *Ceratocystis fagacearum* in north central USA. The species within this family most closely associated with the oak wilt disease cycle are being investigated. Beetles were collected from fresh wounds made on healthy red oaks in two east-central Minnesota locations during April, May, and June 1998. Highest numbers of nitidulids were collected within 3 days of wounding during May in both locations. *Colopieris truncatus* and *Carpophilus sayi* accounted for 97% of the total nitidulids collected within 7 days of wounding in May. *C. truncatus* was predominant on wounds in one location while *C. sayi* was predominant in the other. A high frequency of the insects (78%) carried viable *C. fagacearum* propagules based on serial dilution plating of macerated beetles. These findings, coupled with study results of nitidulid species predominant on oak wilt mats in these same locations, suggest the principal nitidulid species transmitting the pathogen from diseased to healthy oaks in Minnesota are *C. truncatus* and *C. sayi*.

Characterization of a new naturally-occurring defective RNA 3 of cucumber mosaic virus. I. B. KAPLAN, S.-M. Wong, and P. Palukaitis. Cornell University, Ithaca, NY. *Phytopathology* 89:S38. Publication no. P-1999-0266-AMA.

Passaging the Fny strain of cucumber mosaic virus (CMV) in tobacco sometimes results in the formation and accumulation of an additional viral RNA which is encapsidated along with the three genomic RNAs of CMV. The additional RNA is a deleted form of RNA 3 designated D RNA 3-1. The deletion occurs within the 3a gene, which promotes viral movement. The deleted region encompasses nucleotides 296(7)-634(5) from the 5'-end of Fny-CMV RNA 3 and it is an in-frame deletion. D RNA 3-1 was cloned and transcripts for RNA 1, 2 and D RNA 3-1 were inoculated onto transgenic plants expressing the CMV movement protein. D RNA 3-1 was able to replicate and to provide coat protein for encapsidation of the CMV RNAs. The presence of D RNA 3 1 had no effect on CMV RNA accumulation in tobacco. In contrast to tobacco, squash plant would not support replication of D RNA 3-1, neither in the systemic leaves nor in the inoculated cotyledons. Various D RNAs 3 were compared for their ability to co-exist during passage, and they displayed a hierarchy of selection and accumulation, which will be described.

Identifying and mapping Fusarium wilt resistance in melon (*Cucumis melo* L.). T. KARSIES (1), Y. Wang (1), C. E. Thomas (2), and R. A. Dean (1). (1) Clemson University, Clemson, SC; (2) USDA-ARS, U.S. Vegetable Lab, Charleston, SC. *Phytopathology* 89:S38. Publication no. P-1999-0267-AMA.

Fusarium wilt is a very destructive disease in melon production worldwide. The disease can be effectively controlled only by host resistance. There are four races as differentiated by host varieties. Resistance to races 0 and 1 is controlled by a single dominant gene *Fom-2* while resistance to races 0 and 2 is controlled by another single dominant gene *Fom-1*. Resistance to race 1,2 is polygenically controlled. We have identified DNA markers closely linked to *Fom-2* and converted closely linked markers to codominant markers. Screening melon genotypes of diverse origins shown these markers are robust and can be useful in marker-assisted selection. Further progress on identifying markers linked to *Fom-1* and mapping *Arabidopsis* genes onto melon genetic map will be presented.

Antifungal activity of chitosan and chitosan fragments with different molecular weights on postharvest pathogens. Mohammad R. KASAAI (1), M. V. Bhaskara Reddy (1), Gérard Charlet (2), and Joseph Arul (1). (1) Dept. Food Science and Nutrition and Horticultural Research Center; (2) Department of Chemistry and CER-SIM, Université Laval, Sainte-Foy, Quebec, Canada G1K 7P4. *Phytopathology* 89:S38. Publication no. P-1999-0268-AMA.

Antifungal activity of chitosan with different molecular weights (4.21 × 10⁵ to 1.96 × 10⁶ Da) was investigated on postharvest pathogens *Rhizopus sto-*

lonifer, *Botrytis cinerea*, *Alternaria alternata* and *Penicillium digitatum*. Chitosan fragments were prepared by oxidative degradation with sodium nitrite, and the molecular weights were determined by viscometry. The antifungal activity was assessed by inhibition of radial growth of the fungus on PDA at chitosan concentrations ranging from 0.75–6.0 mg/ml and at pH 5.0. Both original and fragments of chitosan inhibited the growth of all fungi tested, with a greater effect at higher concentrations. Among the fungi *Rhizopus stolonifer* was the least sensitive and, where as *Alternaria alternata* was the most sensitive to chitosan. The fragments with lower molecular weights exhibited higher antifungal activity at lower chitosan concentrations and this difference between molecular weights diminished as chitosan concentration was increased.

Evaluating collard and kale for resistance to yellows. A. P. KEINATH (1) and M. W. Farnham (2). (1) Clemson University, Charleston, SC; (2) USDA-ARS, Charleston, SC. *Phytopathology* 89:S38. Publication no. P-1999-0269-AMA.

Collard and kale cropped during the summer may be infected by *Fusarium oxysporum* f. sp. *conglutinans*. Seedlings were root-dipped in 10⁷ microconidia/ml and grown at 25/25 or 30/25 C day/night temperatures. In another test, seedlings were transplanted into naturally-infested sandy soil in Lexington County, SC in June 1997 and 1998. Symptoms were rated visually five times in the growth chamber and three times in the field. Yellows was more severe at 30 than at 25 C but there was no cultivar-by-temperature interaction. Based on final disease incidence (DI) in the field, all five kale and seven of 17 collard lines were more susceptible ($P = 0.01$) than Bravo cabbage, which has well-characterized resistance. DI was 73 to 21% for susceptible lines, 19 to 5.6% for resistant lines, and 4% for Bravo. Cultivar means from growth chamber and field experiments were highly correlated ($P = 0.0003$), although Top Bunch collard was susceptible in the growth chamber but resistant in the field. Uncharacterized resistance to yellows in certain collard cultivars and landraces effectively decreased DI under disease-conducive conditions.

Genetic and pathogenic diversity of *Colletotrichum gloeosporioides* isolates infecting *Arachis pintoi*. S. KELEMU, F. Muñoz, and M. X. Rodriguez. Centro Internacional de Agricultura Tropical, A. A. 6713, Cali, Colombia. *Phytopathology* 89:S38. Publication no. P-1999-0270-AMA.

Anthrachnose, caused by *Colletotrichum gloeosporioides*, is a disease of increasing importance on *Arachis pintoi*. The pathogenicity of 91 isolates of *C. gloeosporioides* isolated from *A. pintoi* in four regions of Colombia was studied on five accessions (CIAT 17434, CIAT 18744, CIAT 18748, CIAT 22160, and the original host accession corresponding to each isolate). Eighty-four of the isolates were pathogenic on at least one host accession, whereas the remaining seven were non-pathogenic. Differential reactions were observed indicating the existence of pathogenic specialization in *C. gloeosporioides* on *A. pintoi*. The genetic variability among these isolates was measured at molecular level by random amplified polymorphic DNA (RAPD), and Southern blot analysis using a repetitive DNA probe (termed CgT1: *C. gloeosporioides* Transposon 1) generated from an isolate of the pathogen infecting the forage legume *Stylosanthes guianensis*. A total of 81 band positions were scored and analysis of the RAPD data revealed at least five groups, with the isolates from one region distributed in all the groups.

Modification of aphid choice by a plant virus. S. E. KELLEY. Emory University, Atlanta, GA. *Phytopathology* 89:S38. Publication no. P-1999-0271-AMA.

Previous studies have shown that aphids, an important vector, are attracted to the reddened and yellowed leaves of virally-infected crop plants. Aphid preference for symptomatic plants is paradoxical, because (1) infections in wild plants with these same viruses are most often asymptomatic, and (2) vector preference for infected plants limits the spread of infection. We report here experimental evidence that wild-collected aphids, *Schizaphis graminum* detected apparently asymptomatic infection by an economically significant, aphid-transmitted luteovirus, barley yellow dwarf (BYDV-variant SGV), in a natural host, *Anthoxanthum odoratum*. Moreover, feeding on BYDV-infected plants altered aphid preference. In laboratory choice experiments, aviruliferous aphids strongly preferred BYDV-infected plants, but viruliferous aphids strongly preferred uninfected plants. While manipulation of aphid preference can enhance viral spread, it did not improve aphid fitness. Viruliferous and aviruliferous aphid colonies, initiated with single aphids, grew to a similar size on BYDV-SGV infected and uninfected plants. Pathogen manipulation of vector behavior may be an important and widespread phenomenon.