

APS Potomac Division

Abstracts

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Alphabetized by first author's last name

EFFECT OF FUMONISINS AND *FUSARIUM MONILIFORME* CULTURE EXTRACTS TO A BABY HAMPSTER KIDNEY CELL LINE. Krishanthi Abeywickrama and G. A. Bean, Dept. of Botany, University of Maryland, College Park, MD 20742.

Fusarium moniliforme was isolated from feed corn associated with leukoencephalomalacia (LEM) in horses & non-LEM corn. A baby hamster kidney cell line (BHK-21) grown in microtiter plate wells was exposed to culture extracts of 4 isolates of *F. moniliforme*, fumonisins and 1% methanol (control). The soluble protein content was measured by spectrophotometry & cell counts were also determined for 5 days. Protein in control cells increased from 167 to 360 µg/well and cells increased 9 fold (2×10^4 - 18×10^4) by the 4th day. Cells exposed to culture extracts (20-40 µl/well) or fumonisin B₁ or B₂ (5-20 µg/µl/well) showed a decrease in the protein content from 167 to between 0 & 86 µg/well indicating inhibition of proliferation and cytotoxicity to BHK-21 cells. Response of mammalian cells such as BHK-21 to *Fusarium* spp. toxins is a sensitive method to detect mycotoxins in foods and feeds.

AGGREGATIONS OF TWOSPOTTED SPIDER MITE AT BEAN RUST UREDINIA. L. R. Batra and J. R. Stavelly, Microbiology and Plant Pathology Laboratory, ARS, USDA, Beltsville, MD. 20705-2350.

Adult populations of the twospotted spider mite, *Tetranychus urticae*, were about five-fold greater on bean, *Phaseolus vulgaris*, containing multiple uredinia of the rust fungus, *Uromyces appendiculatus*, than on rust free bean. Freshly released mites went to younger leaves of rust-free plants, but to mature uredinia on older leaves of rusted plants. Mite webbing permeated uredinia to produce an amorphous appearance. Mite activity resulted in disorganization of uredinia producing a mass of dislodged urediniospores that surrounded the original uredinium for 1-2 mm. Mites became covered with urediniospores and vectored infective spores to rust free plants. Smooth teliospores seldom adhered to the mites and the mites were not attracted by telial pustules.

IDENTIFICATION OF A CARBOHYDRATE WHICH ENHANCES THE GROWTH OF A BACTERIAL ANTAGONIST AGAINST *ERWINIA AMYLOVORA*. E.W. Brown, T. van der Zwet, R.H. Bors and W. Janisiewicz. U.S. Dept. Agr., ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

A variety of carbohydrates were assayed as carbon source for *Erwinia amylovora* and a bacterial antagonist (Le-15), isolated from native honeylocust leaves. Biolog MT microplates were filled with 75 µl of 0.3 optical density suspensions of *E. amylovora* or Le-15, followed by the addition of 75 µl of various carbohydrate solutions at concentrations of 0.2, 0.4 and 0.8%. The plates were incubated at 25°C and the colorimetric absorbances of the bacterial growth were recorded at 492nm after 24 and 48 hrs. The carbohydrate 2-deoxy-D-ribose was found to be the only carbon source which greatly enhanced growth of the antagonist, but had no measurable effect on *E. amylovora*. This carbohydrate may become useful to enhance biocontrol activity of Le-15 and other naturally occurring antagonists of *E. amylovora* on blossoms and leaves of apple and pear.

LEPTOGRAPHIUM PROCERUM GROWTH IN PINE SEEDLING STEMS FOLLOWING OZONE EXPOSURE. J. A. Carlson and S. A. Alexander, Dept. of Plant Pathology, Physiology and Weed Science, VPI&SU, Blacksburg, VA. 24061-0330.

Procerum root disease (PRD), caused by the imperfect fungus *Leptographium procerum* (Kendr.) Wingf., is epidemic in eastern white pine (*Pinus strobus* L.) Christmas tree plantings in southwest Virginia. As this area is subject to ozone episodes during the summer months, a study was initiated to investigate the relationship between PRD development and ozone. This was achieved through evaluation of *L. procerum* colonization of eastern white and loblolly (*P. taeda* L.) pine seedling stem tissue following ozone exposure. Seedlings were inoculated with *L. procerum* mycelium at the base of the stem and fumigated in closed chambers 5 hours per day for 14 days with charcoal-filtered air or 200 ppb ozone. After 6 weeks, stems were sectioned above the point of inoculation and plated on selective medium to evaluate the growth of the fungus. We demonstrated that stem colonization was not significantly different between seedlings in charcoal-filtered air or ozone treatments.

EFFECT OF PLANTING DATES AND PLANT DENSITY ON THE DEVELOPMENT OF GRAY LEAF SPOT OF CORN. L. M. Carrera and A. Grybasuskas, Dept. of Botany, University of Maryland, College Park, MD 20742.

Gray leaf spot, caused by *Cercospora zeae maydis*, in the susceptible corn hybrid Pioneer 3184 was studied for two years under natural inoculum conditions (NI), and under more controlled conditions for one year using artificial inoculum (AI). A split plot randomized complete block design with 4 replicates was used. Whole plots were

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planting dates (PD). NI subplots consisted of two nitrogen levels, 56 and 112 kg/ha and two plant population levels (PP), 34,593 and 69,187 pl/ha. AI subplots consisted of two shading levels, 0 and 47%, and two PP as in the NI study. Disease severity was evaluated every 7 to 10 days as % leaf area infected. On NI plots there was an interaction between PD and PP, with early PD having a higher disease severity than late PD only at low PP. There were no significant differences between low and high PP at late PD. These results do not support the hypothesis that higher PP offer a more favorable microenvironment for disease development. Furthermore, in testing the hypothesis of increased susceptibility at low PP is due to higher nitrogen availability, no differences in disease development occurred relative to nitrogen levels. In the AI study disease developed more extensively on early planted corn, and the rate of disease development and severity was greater in non-shaded plots, suggesting that cercosporin, a photoactivated toxin, may affect epidemic development.

EFFECT OF FOLIAR FUNGICIDES ON GRAY LEAF SPOT DISEASE AND YIELD OF CORN. M.R. Carter and E.L. Stromberg, Department of Plant Pathology, and Weed Science, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061-0331

Gray leaf spot (GLS) caused by *Cercospora zea-maydis*, once considered a minor late season disease, has become a significant threat to corn production in the mid-Atlantic and mid-western United States. Commercially available dent hybrids are all susceptible to some degree. Efficacy of foliarly applied fungicides to control GLS was determined by assessing leaf blighting, grain yield, and kernel weight for a hybrid, Pioneer Brand 3320, in replicated field tests over three years. Treatment units were 4-row plots (30-in spaced, 25-ft long) with one of several triazole or a benzimidazole fungicides or a water control applied to the two center rows. Treated rows were scored for leaf blight (0-5) three to five times and harvested for grain. Benlate 50DF, 8.0 oz ai/A, applied four times, was most effective 2 of 3 years, while RH-7592 2.0F, 1.8 oz ai/A, applied twice, was 1 of 3 years. Yields for non-treated controls were reduced over highest yielding treatments by 34.6-62 bu/A, depending on year, fungicide treatment, and number of applications.

VIRULENCE OF METALAXYL-RESISTANT STRAINS OF *PHYTOPHTHORA INFESTANS*. K.L. Deahl¹, R.J. Young², S.P. DeMuth¹, and D.A. Inglis³. ¹USDA/ARS, Vegetable Laboratory, Beltsville, MD 20705; West Virginia University, Morgantown, WV 26506; and ³Washington State University-NWREU, Mount Vernon, WA 98273.

Severe attacks of late blight (1989-91) in northwest Washington state on tomato and potato crops that were protected with metalaxyl prompted a survey of the efficacy of the fungicide against field isolates of *Phytophthora infestans* (PI). Sensitivity of PI to metalaxyl was tested on potato leaf disks, tuber disks, and on rye agar amended with 0-100 µg metalaxyl/ml. Thirty-two isolates that showed intermediate or high level resistance to metalaxyl were examined for virulence (pathogenic race composition) on differential potato genotypes. Simple races (single and double) were detected in a leaf disk bioassay that allowed compatible interactions of infection and sporulation. Unexpectedly, there was a high incidence of complex race combinations which may have contributed significantly to the disease epidemic.

MANAGEMENT OF TOMATO SPOTTED WILT VIRUS IN PUBLIC GARDENS. R. M. De Vries-Paterson¹, T. A. Evans¹, V. B. Steward¹. ¹Horticulture Department, Longwood Gardens, Inc., Kennett Square, PA 19348. ²Department of Plant and Soil Sciences, University of Delaware, Georgetown, DE 19947.

Tomato spotted wilt virus (TSWV) is one of the most serious diseases currently facing the greenhouse industry. Due to the nature of most horticultural display gardens, it is often difficult to implement control recommendations designed for commercial production greenhouses. Aesthetics may preclude use of barriers to suppress western flower thrips (WFT) movement. Public visitation often prevents optimum timing of pesticide applications. WFT may unknowingly be transported on visitors' clothing. Disposal of infected plants may be impossible due to rarity or historical significance of a taxa. Lack of fallow periods allows WFT to continue reproducing and increasing in number. Longwood Gardens has a strong interest in suppressing TSWV and has implemented guidelines to manage this virus-vector complex. These guidelines encompass staff education, testing plants for TSWV, quarantine procedures, movement of infected plants and/or susceptible hosts within or out of the gardens, disinfecting techniques, disposal of infected plants, use of indicator plants, and control of WFT.

FURTHER CHARACTERIZATION OF ACTIVE OXYGEN PRODUCTION DURING RECOGNITION OF INCOMPATIBLE BACTERIA BY TOBACCO SUSPENSION CELLS. J.A. Glazener and C.J. Baker. MPPL, USDA ARS, Beltsville, MD 20705.

Pseudomonas syringae pv. *syringae* causes a hypersensitive reaction (HR) on tobacco and early in the interaction there is a burst of active oxygen (AO) production. In this study HR causing bacteria were added to tobacco suspension cells and characteristics of AO production were studied using luminol dependent chemiluminescence. Increases in bacterial concentration decreased AO detection, possibly due to the presence of catalases and/or other mechanisms. Stimulation of AO by bacteria could be blocked by addition of streptomycin within 2 h after inoculation. Pretreatment of plant cells with heat-killed *P. fluorescens* stimulated increased AO production, while live bacteria reduced and delayed the onset of AO production. The results indicate that the level of AO detected during plant/bacterial interaction is dependent on various bacterial factors.

INCIDENCE OF OZONE-INDUCED INJURY ON CANOPY HARDWOOD SPECIES IN SHENANDOAH NATIONAL PARK, VA. E. S. Hildebrand, J. M. Skelly, Penn State University, Univ. Park, PA 16802 D. R. Mangis, Air Quality Div., Nat'l. Park Serv., Lakewood, CO 80215, and J. F. Karish, Mid-Atlantic Region Nat'l. Park Serv., Univ. Park, PA 16802.

To assess the incidence of ozone-induced symptoms within the crowns of mature canopy hardwood species, trend plots were established in the forest stands immediately adjacent to the Dickey Ridge (DR), Big Meadows (BM), and Sawmill Run (SR) air quality monitoring stations. Species studied included black cherry, yellow-poplar, white ash, and sassafras; 245 trees across the 3 plots were climbed and 3 uppermost sun-exposed branches sampled and evaluated for adaxial stipple. For black cherry, 12 of 30 (40%), 52 of 60 (87%) and 2 of 30 (7%) trees were symptomatic at DR, BM, and SR, respectively. Yellow poplar had 19 of 30 (63%) and 20 of 30 trees (67%) symptomatic at DR and SR, respectively. For white ash, 13 of 30 (43%) and 19 of 30 trees (63%) were symptomatic at DR and BM. Of 5 sassafras trees sampled at SR 2 (40%) were symptomatic.

EFFECT OF FUSARIUM ROOT AND CROWN ROT ON ALFALFA CULTIVARS IN DELAWARE. E. R. Jones¹, R. B. Carroll², R. H. Swain¹ and D. P. Whittington³, Dept. of Agriculture and Natural Resources, Delaware State College, Dover, DE, 19901, Department of Plant and Soil Sciences, Univ. of Delaware², Newark, DE 19717-1303.

A study was made to determine alfalfa (*Medicago sativa* L.) cultivar response to Fusarium crown and root rot and its effect on stand longevity. In August 1985, 34 cultivars were established and fertilized annually for a 22 Mg ha⁻¹ forage yield with 0-85-335 kg ha⁻¹ + boron. Each season, 1986-1989, 5 harvests were made. A 0.28 m² area of each plot was excavated in May 1990 to determine the number of producing plants and evaluate crown and root rot. The effect of disease on stand density and forage production was not clearly defined. Neither stand density nor first harvest forage yield in 1990 were correlated with disease ratings. Disease ratings were closely related to stand and forage production for some cultivars but not others. The predominant species of Fusarium isolated from infected root and crown tissues were *F. solani* and *F. oxysporum*.

RESISTANCE OF EUONYMUS KIAUTSCHOVICUS 'MANHATTAN' TO PHYTOPHTHORA STEM ROT. S. H. Kim, T. N. Olson, and E. M. Dutky¹, PA Dept of Agr, Harrisburg, 17110 and ¹Dept of Botany, Univ of MD, College Park 20742

Phytophthora citrophthora (Pc) was isolated from shoot blight, stem dieback, crown rot, and root rot of *Euonymus fortunei* 'Emerald Gaiety' (EfEG). Pc isolates from two EfEG and a *Pieris* sp. caused stem lesions, 8 mm in two weeks on EfEG near soil line when an intact rooted cutting was transferred to a larger container by filling with Pc inoculum grown in a vermiculite V8-200; however disease symptoms were not observed on *E. kiautschovicus* 'Manhattan' (EkM). The EfEG with the lesions died, 20 out of 45, or recovered from the symptoms during 17 months incubation in a greenhouse. Pc isolates were recovered from the asymptomatic EfEG and EkM plants two years after inoculation. Virulence differences among three isolates of Pc were not observed.

EVALUATION OF ALTERNATIVE PEST CONTROL MATERIALS FOR HOME GARDEN ROSE MAINTENANCE. J. C. Locke and D. L. Clement. USDA, ARS, Beltsville, MD 20705 and CES, Univ. of Maryland System, 12005 Homewood Drive, Ellicott City, MD 21042.

Six materials or combinations were compared to a fungicide spray program as part of an IPM demonstration on floribunda rose (cv. Iceberg) in 1991. The materials were targeted at the most common rose diseases; blackspot and powdery mildew. Safer Insecticidal Soap, Wilt Pruf, Volck Oil, Volck Oil + baking soda, neem oil, and neem wax were applied weekly (22 applications) from May 8 to October 1. The standard fungicide comparison was Funginex. Sevin and malathion were applied as required for insect and spider mite control. Rose bushes in 2-gal containers were spaced on a mulched plot with drip irrigation. The most effective material for blackspot reduction was Funginex but neem wax, neem oil, Safer Insecticidal Soap, and Volck Oil + baking soda showed marked reduction. No powdery mildew developed for evaluation. The neem oil and neem wax treatments had noticeably fewer spider mites during the summer, especially compared to Funginex.

POWDERY MILDEW MANAGEMENT IN SUMMER SQUASH WITH HOST RESISTANCE, FUNGICIDES, OR AN INTEGRATED PROGRAM. M. T. McGrath¹ and M. G. Hutton², ¹Dept. of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY 11901-1098 and ²Petoseed Co., RR2 Box 80A, Slade Lane, Bridgeton, NJ 08302-8723.

Development of *Sphaerotheca fuliginea* was suppressed in resistant PSX 2287 as compared to susceptible Goldbar for late-season crops (transplanted 23 July). Average powdery mildew severities (% symptomatic leaf area) on adaxial and abaxial leaf surfaces were 3.2% and 0.4% for PSX 2287 and 22.2% and 9.4% for Goldbar on 11 September. Severity was less than 0.1% for both cultivars sprayed 8 times with chlorothalonil (7-day), triadimefon (14-day), and benomyl (14-day) in a preventive program beginning 31 July. Good control also was achieved with 3 or 5 sprays initiated on 23 August after disease detection. Yield during the last third of the harvest period (September 16-30) was reduced; the average cumulative fruit weights per plant were 606 and 563 g for non-treated PSX 2287 and Goldbar and 1037 and 983 g for fungicide-treated PSX 2287 and Goldbar. An integrated management program utilizing resistance and fungicides is recommended.

MALE DIMENSIONS OF *GLOBODERA TABACUM VIRGINIAE* AND *G. I. SOLANACEARUM* CULTURED ON *SOLANUM CAROLINENSE* AND *NICOTIANA TABACUM*. L.L. Miller. Dept. of Plant Path., Phys. and Weed Sci., VP I&SU, Blacksburg, VA 24061.

Comparisons were made of certain characters of males of type locality isolates of *Globodera tabacum virginiae* (N1) and *G. i. solanacearum* (N2) when cultured on horse nettle (P1), *Solanum carolinense*, and tobacco (P2), *Nicotiana tabacum* cv VA 312. P1 and P2 were efficient hosts for N1 and N2. Mean dimensions in μm of 115 specimens were as follows-length (LTH): N2P2 1099, N2P1 1109, N1P2 1138, N1P1 1164; stylet length (STL): N2P1 26.0, N2P2 26.4, N1P2 26.5, N1P1 26.6; spicule length (SPC): N1P1 32.9, N2P1 32.9, N1P2 33.1, N2P2 33.7. Comparisons of LTH dimensions between nematode subspecies on P1 and P2, the STL dimensions on P1 and the SPC dimensions on P2 were significantly different ($P=0.01$). The LTH dimensions of N1 on P1 and P2 were greater ($P=0.01$) than for N2 on P1 and P2, but the LTH dimensions within subspecies on P1 and P2 were not significantly different.

Developmental stages in the life cycle of *Sporidesmium sclerotivorum*: a biocontrol agent for *Sclerotinia* diseases. A. Mintz and A. Paa. Washington Research Center, W.R. Grace & Co.-Conn., Columbia, MD 21044.

Sporidesmium sclerotivorum parasitizes the sclerotia of plant pathogenic *Sclerotinia* spp. Plant diseases such as lettuce drop, caused by *S. minor* or *S. sclerotiorum* are often difficult to control due to the resistant nature of the sclerotia, and to the regulatory constraints on the application of chemical pesticides. *Sclerotinia* diseases of high-value cash crops therefore, can result in economically significant losses. In field trials conducted in 1987-1989, *Sporidesmium sclerotivorum* suppressed 63-83% of *Sclerotinia* diseases in lettuce (Adams and Ayers, 1982). Various stages in the life cycle of *Sporidesmium sclerotivorum* have been observed, including the development of the non-infective *Selenospora* state. A photographic morphological description of *Sporidesmium sclerotivorum* is presented.

A POLYMERASE CHAIN REACTION ASSAY FOR THE DETECTION OF POTATO LEAFROLL VIRUS. M.S. Montasser, A. Hadidi, L. Levy and R.W. Goth. USDA, ARS, National Germplasm Resources Laboratory, Beltsville, MD 20705-2350.

Reverse transcription-polymerase chain reaction (RT-PCR) assays were developed for the detection of potato leafroll luteovirus (PLRV) from nucleic acid extracts of infected potato leaves and tubers as well as of viruliferous aphids. DNA primers (22 nucleotides in length) specific for a major portion of PLRV coat protein gene were used for cDNA synthesis and specific PCR amplification of a 487 bp DNA fragment from infected or viruliferous tissue. The viral origin of this DNA fragment was confirmed by hybridization with ³²P-labeled PLRV cDNA probe specific for the viral coat protein gene. This DNA was absent from amplified extracts of uninfected or nonviruliferous tissue. The RT-PCR assay for PLRV is more sensitive than existing detection methods and provides information about PLRV detection from host or insect vector without requiring large samples or molecular hybridization.

PATHOTOXIC PRINCIPLE IN FILTRATE OF *STENOCARPELLA MACROSPORA* WHICH INDUCE *STENOCARPELLA MAYDIS* TO INCITE BLIGHT OF MAIZE LEAVES. M.A. Morant, University of Maryland Eastern Shore, Princess Anne, MD 21853 and H.L. Warren, Department of Plant Pathology, Physiology and Weed Science, VPI and SU, Blacksburg, VA 24061.

The crude culture filtrate of *Stenocarpella macrospora* induced lesions similar to those of the pycnidiospores, on leaves of maize inbreds H84, B73, OH514 and OH43. Pycnidiospores of *S. maydis* were able to infect leaves only when resuspended in the filtrate of *S. macrospora* and injected into the hosts. Infection did not occur when inoculum was atomized onto the leaves nor pipetted into leaf whorls. Regardless of the method of inoculation inbred H84 was resistant to *S. maydis*. Symptoms were delayed for 42 hr when pycnidiospores of either fungus was unwashed compared to 24 hr for washed pycnidiospores. These results indicate that *S. macrospora* produces a toxic substance which enables it to infect maize leaves while a similar toxin is lacking in *S. maydis*.

FUNGI ASSOCIATED WITH BOTRYOSPHERA DIEBACK OF OAK TREES IN DELAWARE WOODLOTS. A. L. Morehart, Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19717-1303.

Oak trees exhibiting dieback symptoms were studied at 75 locations during 1990-1991. Crown dieback, necrotic twigs and leaves were associated with *Botryosphaeria quercuum* (Schwein.) Sacc. (22%), insect damage (23%), multiple agents or unidentified causes (55%). *B. quercuum* was isolated from 25% of 138 red oaks (*Quercus rubra* L.) and 19% of 129 white oaks (*Quercus alba* L.) cultured. Disease intensity, in terms of the number of cankers per 30 cm of defoliated branch, was equal in both oak species. *Nectria galligena* Bres., three other canker-forming fungi, and three antagonists, *Calcarisporium parasiticum* Barnett, *Gliocladium virens* Miller et al., and *Trichoderma viride* Persoon:Fr. were found sporadically on bark from the vicinity of cankered tissue. *Cladosporium cladosporioides* (Fr.) deVries was present in or near cankers during every month of the year. Ten other epiphytic fungi were identified with *B. quercuum* infected trees.

CHEMILUMINESCENT NUCLEIC ACID PROBES AS AN ALTERNATIVE TO ³²P FOR DETECTING PLANT PATHOGENS. E. V. Podleckis and R. W. Hammond, USDA, ARS, PSI, National Germplasm Resources Laboratory and Microbiology and Plant Pathology Laboratory, Beltsville, MD 20705

A chemiluminescent hybridization-based assay for detecting potato spindle tuber viroid (PSTVd) is described. Labeled cRNA probes for PSTVd were generated by SP6 RNA polymerase transcription of a plasmid vector using digoxigenin-11-UTP or α -³²P UTP. Dot blot hybridizations of purified PSTVd and sap extracts from infected potato plants demonstrated that the chemiluminescent detection of the nonisotopic probe was as sensitive as autoradiography using the ³²P probe. Both probes were able to detect as little as 2 pg of purified PSTVd and both probes could detect viroid in extracts from infected potato leaves diluted as much as 1/2000 with healthy potato leaf extracts. The probes were both successful in detecting PSTVd in direct tissue blots of infected tomato leaves, stems and roots and potato tubers. In a series of consecutive blots, the same digoxigenin-labeled probe solution was reused ten times with no loss of sensitivity. Chemiluminescent detection of digoxigenin-labeled cRNA probes is a sensitive, specific, safe and easy alternative to radioisotopes for detecting PSTVd.

Proliferation of *Enterobacter cloacae* strains A-11 and 501R3 in cucumber and pea spermosphere. D. P. Roberts and A. M. Marty. Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, MD 20705.

Enterobacter cloacae strain A-11, a mutant with a generalized deficiency in carbohydrate utilization, was isolated after mutation of strain 501R3 with transposon mini-Tn5 Km. Strain A-11 lost the ability to grow on or had a reduced growth rate on twelve carbohydrates but was similar to strain 501R3 in all other nutritional tests. In cucumber spermosphere proliferation assays populations of strains 501R3 and A-11 were significantly different ($P = 0.001$) at 20 and 45 hours. Strain A-11 did not proliferate in cucumber spermosphere while strain 501R3 increased in number 21-fold after 45 hours. In pea spermosphere, strains A-11 and 501R3 increased in number 345-fold and 690-fold, respectively, after 45 hours (significantly different at $P = 0.1$). These data suggest that spermosphere carbohydrates are used as nutrients during proliferation by the biocontrol-bacterium E. cloacae in cucumber and pea spermosphere.

EFFICACY AND RISK ASSOCIATED WITH PUCCINIA JACEAE AS A BIOCONTROL AGENT OF YELLOW STARHISTLE. Nina Shishkoff and William L. Bruckardt. USDA/ARS, Bldg. 1301, Fort Detrick, Frederick, MD, 21702.

Pustule counts were not the best way to evaluate the efficacy of rust isolates against target weeds or the best way to evaluate the risk posed by the rusts to nontarget plants. Measuring the root biomass reduction caused by 5 isolates of Puccinia jaceae on their respective target hosts (yellow starthistle, purple starthistle, and diffuse knapweed) showed that only an isolate from yellow starthistle caused significant biomass reduction of the thistle. When cornflower, susceptible to each isolate, was inoculated, no significant biomass reduction was observed with any isolate. Observations on the effect of infection on leaf lifespan led to the conclusion that plant characteristics such as reproductive strategy should be taken into account when evaluating a biocontrol system for efficacy and risk to nontarget plants.

SURVIVAL OF LAETISARIA ARVALIS IN SOIL AND SUPPRESSION OF RHIZOCTONIA STEM CANKER AND BLACK SCURF OF POTATO. Jasmit Sidhu and R. J. Young. Dept. of Plant Pathology, West Virginia University, Morgantown, WV 26506-6057.

Soil amendments with Laetisaria arvalis, a mycoparasite, effectively controlled stem canker and black scurf of potato caused by Rhizoctonia solani. Soil application of L. arvalis in greenhouse experiments significantly reduced disease severity in three years of subsequent potato crops. Population densities of L. arvalis as assayed by sugarbeet seed colonization method fluctuated during this period. The fungus survived in soil exposed to a low temperature of -5°C and high temperatures, $30-35^{\circ}\text{C}$. In field trials, L. arvalis grown on oat kernels and applied at 5, 10, and 20 gm/2.25 m row significantly suppressed disease severity caused by seedborne or soilborne inoculum of R. solani as compared to no soil amendments. Viability of seedborne inoculum, determined by retrieving sclerotia from seed pieces after different intervals, was reduced 40-60 percent. Sclerotial intensity and number of progeny tubers with sclerotia were also reduced.

DETECTION OF THREE VIRUSES OF CLOVERS BY DIRECT TISSUE IMMUNOBLOTTING. Indira Srinivasan and Sue A. Tolin. Dept. of Plant Pathology, Physiology and Weed Science. Virginia Polytechnic Institute & State University, Blacksburg, 24060.

We have modified the techniques of Lin et al. (Phytopathology 80:824, 1990) to detect peanut stunt (PSV), bean yellow mosaic (BYMV) and clover yellow vein (CYVV) viruses in Trifolium repens, T. subterraneum and other hosts. Blots were made by gently pressing freshly torn surfaces of leaves, petioles or stolons onto nylon and cellulose membranes or various types of paper. Residual green color was removed by rinsing in 5% Triton X-100 prior to blocking with milk. Primary antibody was viral-specific polyclonal antisera diluted 10^{-4} - 10^{-6} , and secondary antibody was goat anti-rabbit conjugated with alkaline-phosphatase. Blotted membranes could be held at least 1 month before processing. In terms of sensitivity and specificity, immunoblots gave results equal or superior to indirect ELISA. Direct tissue blotting is also less laborious and cheaper than ELISA.

RECENTLY INTROGRESSED RUST RESISTANCES FOR MAJOR UNITED STATES CLASSES OF COMMON BEAN. J.R. Staveland, Microbiology and Plant Pathology Laboratory, ARS, USDA, Beltsville, MD 20705-2350.

By 1992, 58 pathogenic races of the bean rust fungus, Uromyces appendiculatus, isolated from many production areas of North America and other continents, were maintained at Beltsville for use in developing rust resistant germplasm lines for commercial classes of Phaseolus vulgaris beans grown in the United States. Since 1984, germplasm lines have been developed and released that combine resistances to all of the races. In the past five years, resistance to all races has been identified in 35 of 3,432 plant introductions (PIs). In 1991, the first improved germplasm lines having resistance to all races from PIs 151385, 151395, and 181996 were released for processing and market green snap beans and navy dry beans, respectively. These lines also have other independent rust resistances.

SURVIVAL OF BINUCLEATE RHIZOCTONIA ON TOBACCO UNDER FIELD CONDITIONS. S.K. Walker, C.S. Johnson and E.L. Stromberg, Dept. PPWS, VPI & SU, Blacksburg, VA 24061-0331.

Tobacco seedlings were inoculated with one of five isolates of binucleate Rhizoctonia before transplanting to a field at Southern Piedmont Ag. Exp. Sta., Blackstone, VA. Plots consisted of a treatment row amid two rows of non-inoculated plants. Root and hypocotyl samples were collected once monthly and plated onto water or potato dextrose agar. Isolates showing morphological characteristics of Rhizoctonia were transferred. Mycelia from cultures were stained with a fluorescent dye to determine if cells were binucleate (BN) or multinucleate (MN). Month one isolates from treated rows (TR) were 3BN:1MN and border rows (BR) were 1BN:1MN. Month two isolates from TR were 2BN:1MN and BR were 1BN:2MN. Month three isolates from TR were 1BN:1MN and BR were 1BN:5MN. Binucleate Rhizoctonia isolations declined over the growing season. Multinucleate Rhizoctonia isolations from non-inoculated plants increased each month.

DIALLEL EVALUATION OF MAIZE INBREDS FOR RESISTANCE TO Colletotrichum graminicola (Ces.) Wils. T. Weldekidan and J.A. Hawk, Dept. of Plant and Soil Sciences, University of Delaware, Newark, DE 19717-1303.

Nine maize inbreds and their diallel hybrids were evaluated for resistance to C. graminicola in 1991. Plots were 3.1 m double-rows arranged in a randomized complete block with four replications. Plants were inoculated one to two weeks after mid-silk by injecting 2 ml conidia suspension into the first elongated internode. Ratings were made by counting the total number of internodes infected and the number of internodes with $>75\%$ infection. Significant general combining ability (GCA) effects were obtained from crosses of resistant inbreds ($P=0.01$). The inbreds DEB11 ASR, RD6501, and LH123 had negative GCA effects for both ratings. These lines would be a good source for resistance to C. graminicola. Inbreds LH132, OF9, PA91, and DEB11 had positive GCA values indicating susceptibility to the pathogen. Specific combining ability effects were significant for particular hybrids.

COAT PROTEIN-MEDIATED RESISTANCE TO SUNN-HEMP MOSAIC VIRUS. R. A. Welliver and G. A. de Zoeten. Pennsylvania Department of Agriculture, Harrisburg, PA 17110 and Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824.

A chimeric gene was constructed using the sunn-hemp mosaic virus (SHMV) coat protein coding region and the cauliflower mosaic virus 35S promoter. The gene was introduced into the SHMV local lesion host Nicotiana tabacum cv Xanthi-nc via an Agrobacterium tumefaciens binary vector. Transgenic plants accumulated coat protein to 0.1% of total extractable protein. Expression of the gene resulted in resistance to SHMV infection, but did not provide resistance to infection by SHMV RNA. This differs from SHMV cross protection, in which an established infection provides resistance to superinfection by either SHMV or SHMV RNA. (T.M. Zinnen and R.W. Fulton. J. Gen. Virol. 67:1679, 1986.)

PEDIGREE ANALYSIS OF THE TRANSMISSION OF AN UNUSUAL DOUBLE-STRANDED RNA IN BARSOY BARLEY. R. J. Williams, I. A. Zabalgoceazcoa, and F. E. Gildow. Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

In 1988, a ds-RNA molecule was isolated from Barsoy barley (*Hordeum vulgare* L.). The ds-RNA consists of approximately 13,120 bp in a completely base paired linear molecule resistant to S1 nuclease, and is sexually transmitted via egg and pollen. The distribution of the ds-RNA in pedigree-related cultivars was analyzed using cellulose chromatography and agarose gel electrophoresis. Thirteen of 34 pedigree related cultivars tested positive for the ds-RNA. We were able to trace the line of inheritance of this molecule through six crosses made over a 90 year period. The earliest known positive ancestor is Nakano Wase, which was selected from a landrace in Japan around 1900. Consistent transmission of this ds-RNA was verified by the presence of the molecule in the recently released Barsoy-derived cultivar Venus. The genetics and function of this ds-RNA are now under study.

OCCURRENCE OF HIBISCUS CHLOROTIC RINGSPOT VIRUS IN SINGAPORE. S.M. Wong, and C.G. Chng. Dept. of Botany, National University of Singapore, Kent Ridge, Singapore 0511, Republic of Singapore.

In 1991, symptoms of chlorotic ringspots, mottling and vein-banding of leaves were observed in many *Hibiscus rosa-sinensis* cultivars in Singapore. Some plants showed severe flower distortion and stunting. An isolate of a virus was obtained through 3 successive single-lesion-passage using *Chenopodium quinoa*. Virus purification and dsRNA extraction were carried out according to Hurtt (*Phytopathology* 77: 845-850, 1987). A single component spherical virus particle measuring about 28 nm in diameter was observed under the transmission electron microscope. Three dsRNA bands of molecular weight approximately 2.6, 1.2, and 1.1×10^6 were obtained. The purified virus reacted strongly to both antisera of the fast and slow components of hibiscus chlorotic ringspot virus (HCRV) (ATCC PVAS 436a & 436b). Based on the virus particle morphology, dsRNA pattern, and serology, this virus was identified as an isolate of HCRV.

A NEW VIRUS IN ALSTROEMERIA IN THE U.S.A. S.M. Wong, Dept. of Botany, National University of Singapore, Kent Ridge, Singapore 0511, Republic of Singapore, R.A. Reiser, Dept. of Floriculture and Ornamental Horticulture, and R.K. Horst, Dept. of Plant Pathology, Cornell University, Ithaca, N.Y 14853.

Stunting, leaf chlorosis, necrosis, and streaking were observed in alstroemeria (*Alstroemeria* sp.) Endowment Series maintained in the Kenneth Post Laboratory, Cornell University, in March 1989. Flexuous rods 780 nm long x 12 nm wide, which were not decorated by antisera against chrysanthemum virus B, lily symptomless, and carnation latent virus, were seen by transmission electron microscopy in crude leaf extracts. Indirect ELISA tests with potato virus X, potato virus S, potato virus M, potato virus Y, and a broad spectrum potyvirus monoclonal antibody were all negative. Only laminated inclusions were found in the cytoplasm of leaf tissues. This virus, which can provisionally be assigned to the potyvirus group based on cytopathology and particle morphology is characteristically different from the alstroemeria mosaic potyvirus reported in the U.K. and the Netherlands.

MOLECULAR CLONING OF THE COAT PROTEIN GENE OF A SINGAPORE ISOLATE OF ZUCCHINI YELLOW MOSAIC VIRUS. M. Wu, Lee, S.C., and S.M. Wong. Dept. of Botany, National University of Singapore, Kent Ridge, Singapore 0511, Republic of Singapore.

A viral coat protein (CP) gene of a Singapore isolate of zucchini yellow mosaic virus (ZYMV-S) was isolated and its nucleotide sequence was determined. The CP gene mapped to the 3' terminal region of viral genome revealed a single reading frame of 1035 nucleotides (nts) followed by a 3' non-coding region of 215 nts. The proposed protease cleavage site for the release of the CP was deduced to be at Glu-Ser (at amino acid position 66-67). A point mutation near the N-terminal of the CP which changes the amino acid triplet DAG to GAG at position 75 is probably responsible for its non-aphid transmissibility. The expressed CP in both prokaryotic and eukaryotic systems was detectable by immunoblotting.

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