

APS Northeastern Division

Abstracts

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Alphabetized by first author's last name

HOST EFFICIENCY OF SIXTEEN COVER CROPS TO THE LESION NEMATODE. G. S. Abawi and J. W. Ludwig. Dept. of Plant Pathology, Cornell University, Geneva, NY 14456.

Reproduction of the lesion nematode (*Pratylenchus penetrans*) on selected cultivars of hairy vetch, crown vetch, red clover, white clover, alsike clover, alfalfa, ryegrass, ryegrain, oat, sudangrass hybrid, buckwheat, mustard, oilseed radish, and rape were evaluated in two tests in the greenhouse at 20 to 24 C. Seeds were planted in 10-cm clay pots (5 pots/crop/test) filled with pasteurized potting soil mixture {3:1 (v:v) loam soil:sand}. After two weeks, all pots were inoculated with 1,120 or 1,000 *P. penetrans* in ca. 20 ml of water/pot in tests 1 and 2, respectively. Eight weeks after inoculation, nematodes were extracted from roots and soils by the shaker and Pie-Pan techniques, respectively; counted under a microscope; and the reproductive factor was calculated ($R = P_f/P_i$) for each crop. Only ryegrass cv. Pennant had an R value <1 (0.49 and 0.28 in test 1 and 2, respectively), indicating that it is a poor host to *P. penetrans*. Crown vetch cv. Penngift was considered as a maintenance host to *P. penetrans* (Ave. R = 1.05). Hairy vetch (M52, NK Seeds) was the most efficient host to *P. penetrans*, as it had an R value of 5.10 and 8.15 in tests 1 and 2, respectively. The other cover crops were considered as intermediate hosts.

OCCURRENCE OF *THANATEPHORUS CUCUMERIS* ON SNAP BEANS IN NEW YORK. G. S. Abawi, G. Olaya and J. W. Ludwig. Dept. of Plant Pathology, Cornell University, Geneva, NY 14456.

Hymenial layers of *Thanatephorus cucumeris* (A. B. Frank) Donk (anamorph: *Rhizoctonia solani* Kuhn) were prevalent on the lower stems and branches of snap bean cv. Labrador in a 40 acre field near Batavia, New York. Hymenia appeared as thin, dusty layers that were off-white to cream in color. Typical shallow lesions of *R. solani* were also observed within the hymenial layers and on stem and pod tissues on the same or adjacent plants. Hymenial layers were also observed on beans in two fields near Geneva, NY. Weather conditions were hot and humid with severe rain showers. Basidia, basidiospores, and typical hyphae of *R. solani* were observed with the microscope from direct preparations of hymenial layers. Isolation from hymenial layers and lesions on stem and pod tissues always yielded *R. solani*. The isolates obtained from the bean fields near Batavia and Geneva belonged to AG-4 and AG-2-2, respectively. *T. cucumeris* (AG-2-2) was previously reported on table beets in New York in 1990 (Plant Dis. 78:805-810). These findings may explain the increased occurrence of *Rhizoctonia*-incited diseases on vegetables in recent years.

Camera-ready abstracts are published as they were submitted by the Division. The abstracts are not edited or typed in the APS headquarters office.

PURIFICATION AND ACTIVITY OF A SECOND β -1,3 GLUCANASE ISOFORM FROM THE MYCOPARASITE *STACHYBOTRYS ELEGANS*. C. Archambault¹, S. Kermasha² & S. J.-Hare¹. 1. Dept. of Plant Science, and 2. Dept. of Food Science, Macdonald Campus of McGill University, 2111 Lakeshore, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 3V9.

S. elegans produces several β -1,3 glucanases when grown on synthetic medium amended with purified cell wall of the plant pathogen *Rhizoctonia solani* (AG-3) as the sole carbon source. Three isoforms of the β -1,3 glucanase were detected in the crude filtrate by activity on native and SDS-PAGE. A 94 kDa β -1,3 glucanase has been purified and was shown to lyse hyphal tips of *R. solani* (Tweddell *et al.*, 1995). A second isoform of β -1,3 glucanase (E.C.3.2.1.39; 75 kDa) was recently purified from the crude culture filtrate using ion exchange and size exclusion chromatographies and electrophoresis. Glucanolytic activity of the 75 kDa was detected on native and SDS-PAGE gels containing purified glucan from *Saccharomyces cerevisiae*. Characterization of this isoform is under way. Antibodies have been raised against the 75 kDa glucanase and a co-purified 94 kDa protein. Sequencing of internal fragments of the 75 kDa glucanases will be used for cloning. This research is funded by NSERC.

A QUICK PCR-ASSAY FOR CONCURRENT DETECTION OF BACTERIAL AGENTS OF COMMON BLIGHT, HALO BLIGHT AND BROWN SPOT IN BEAN. P. Audy, G. Saindon, A. Laroche and H.C. Huang. Agriculture and Agri-Food Canada, Research Center, Lethbridge, Alberta, T1J 4B1.

Bean common blight, halo blight and brown spot, caused by *Xanthomonas campestris* pv. *phaseoli* (Xcp), *Pseudomonas syringae* pv. *phaseolicola* (Psp) and *Pseudomonas syringae* pv. *syringae* (Pss), respectively, are major seed-borne diseases worldwide. A few infected bean seeds can generate a disease epidemic in the field, causing severe losses in yield and processing quality. Three sets of G+C-rich primers were designed from Xcp, Psp and Pss DNA sequences, and used jointly in a stringent PCR-assay using a two-step temperature profile. The primers directed the amplification of specific DNA fragments from the target organisms but not from bacterial saprophytes of bean or other plant-pathogenic species of *Xanthomonas*, *Pseudomonas*, *Clavibacter*, *Erwinia* and *Agrobacterium*. In combination, Xcp-, Psp-, and Pss-primers successfully detected either individual and mixed infection of bean common blight, halo blight and brown spot in bean tissue.

INFLUENCE OF SUBINHIBITORY DOSE OF ANTIBIOTICS FROM *SPOROTHRIX FLOCCULOSA* ON CELLULAR LIPID COMPOSITION IN FUNGI. M. Benyagoub & R.R. Bélanger. Département de Phytologie, Université Laval, Ste-Foy, Québec, Canada. G1K 7P4.

New fatty acids with antimicrobial activity were characterised from liquid cultures of *Sporothrix flocculosa*. The effects of these antibiotics on growth and lipid composition of three fungi were determined. The growth of *Cladosporium cucumerinum*, *Fusarium oxysporum* and *S. flocculosa* was suppressed by 51, 33 and 4 %, respectively, in presence of 450 μ g/ml of antibiotics. The higher sensitivity of *C. cucumerinum* was related to the presence of higher levels of phospholipid unsaturated fatty acids and cardiolipin. Following treatment, the rate of free fatty acids was higher in both sensitive fungi, while the sterol content was only slightly modified. An increase in the degree of lipid unsaturation was also noted, observation which indicates an alteration of membrane fluidity. Fluorescence anisotropy using liposomes and diphenyl hexatriene (DPH) probes confirmed that lipids from sensitive fungi were more fluid than those of *S. flocculosa*.

FUSARIUM OXYSPORUM F. SP. LOTI: A SPECIFIC WILT PATHOGEN OF BIRDSFOOT TREFOIL IN NEW YORK. G. C. Bergstrom and D. W. Kalb, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

A variant of the fungus *Fusarium oxysporum* induces vascular wilt and death of birdsfoot trefoil (BFT) (*Lotus corniculatus*) in New York. It was a principal cause of the demise of the certified BFT seed industry in the state. Wilt symptoms become apparent 50 to 60 days after seeding in infested fields. Stands are reduced severely in the seeding year and to below acceptable levels for forage production by the following year. Isolates of *F. oxysporum* from BFT in New York (Champlain Valley in 1980s and western New York in 1990s) induced wilt in all cultivars of BFT tested. The pathogenicity to legume species of *F. oxysporum* from BFT in New York was compared to that of reference cultures of *F. oxysporum* f. sp. *medicaginis* from alfalfa and f. sp. *pisi* from pea, and an isolate from red clover in Wisconsin. The BFT wilt organism appears to be a specific pathogen of BFT, for which we propose a new taxon, *Fusarium oxysporum* f. sp. *lotii*. None of 14 cultivars of BFT available commercially in North America possessed a level of resistance adequate for performance in infested New York fields. Resistant cultivars will be necessary for renewed productivity of BFT in New York.

IDENTIFICATION OF A SPECIFIC RAPD MARKER FOR THE DETECTION OF RHIZOCTONIA SOLANI (AG-3). Bounou, S¹, Jabaji-Hare², S. H., Hogue³, R. and Charest, P. M¹. ¹Département de Phytologie, Université Laval, Ste-Foy, Qué. G1K 7P4, ²Department of Plant Science, McGill University, Ste-Anne-de-Bellevue, Qué. and ³Service de Phytotechnie, MAPAQ, Ste-Foy, Qué. Canada.

Our objective is to develop a random amplified polymorphic DNA (RAPD) assay designed to identify AG-3 isolates sampled from soil, potato tubers and stems. Among forty primers (10 mers) tested, one (OPBSR37) was successful in that a 2.6Kbp DNA fragment was produced and was specific for AG-3. When this fragment was purified on agarose gel, labelled and used as probe on the Southern blot containing OPBSR37 reaction products, the isolates of AG-3 gave a positive reaction. Isolates of different AGs produced a negative reaction, rendering them distinguishable from isolates of AG-3 that produced the dominant 2.6Kbp fragment during RAPD amplification.

EFFECTS OF VARIABLE LATENT PERIODS ON DEVELOPMENT OF INFECTION BY GREMMENIELLA ABIETINA.

G. Bussièrès, ¹G. Laflamme, and ¹A. Yilmartimo. Faculté de foresterie, Université Laval, Sainte-Foy, Québec G1K 7P4, Canada; ¹Canadian Forest Service - Quebec, P.O. Box 3800, Sainte-Foy, Québec G1V 4C7, Canada

Red pine seedlings were inoculated by spraying with a conidial suspension (2.5×10^6 per mL H₂O, 3 mL per seedling) of *G. abietina*. Two isolates of the European race of the pathogen were used. The seedlings were first placed into a dew chamber at 18°C for 72 h, then kept at 1°C and 90% R.H. for periods of 29 to 56 conducive days (10 different periods were used), and finally taken to the greenhouse. After one week in the greenhouse, the symptoms were observed on all seedlings except the controls. *G. abietina* was reisolated from surface sterilized needles, even from the seedlings submitted only to 29 conducive days. The use of non-hardened seedlings may have contributed to the low number of conducive days needed for disease development.

FUNGICIDE REGISTRATION PROCESS IN CANADA. A. Carter and P. Beauchamp. Pest Management Regulatory Agency, 59 Camelot Drive, Nepean, Ontario, K1A 0Y9.

The pesticide registration system in Canada is currently in transition. The regulatory divisions from Health, Environment and Agriculture departments have been consolidated into one Agency, with the aim of developing a team approach to registration decisions. Fungicides being proposed for new registrations typically have more complete supporting data on health and environmental effects, are used in smaller quantities, and are marketed in safer formulations than products registered in previous decades. Recent initiatives in the registration system are intended to encourage better quality submissions (including fungicide efficacy), more efficient review of data, and a more transparent regulatory process. For example, under the Canada-U.S. Free Trade Agreement, regulators in the two countries are developing procedures for the joint review of data packages and harmonization of testing protocols, to make the best use of our resources.

INCREASED FREQUENCY AND SEVERITY OF SYMPTOMS OF PSEUDOMONAS SYRINGAE LEAF SPOT ON IMPATIENS UNDER GREENHOUSE CULTIVATION. M. L. Daugherty and R. L. Wick, L. I. Horticultural Research Lab., Cornell Univ., Riverhead, NY 11901 and Univ. of Massachusetts, Amherst, MA 01003.

Previous to 1994, small-diameter (2-4 mm) darkly-bordered lesions, often centering on hydathodes, were occasionally observed on impatiens (*Impatiens wallerana* Hook. f.) infected with *Pseudomonas syringae*. These symptoms were easily confused with symptoms of impatiens necrotic spot tospovirus (INSV) or Alternaria leaf spot. In 1994-95, a more extensive and damaging foliar blight was frequently observed on a number of impatiens cultivars under commercial production. The symptoms included numerous irregular leaf lesions, often involving the leaf margin and sometimes encompassing the entire blade. Lesions were initially water-soaked, drying to translucent tan with a purple border. Symptoms were most prevalent during the high-moisture conditions of plug production. Fluorescent pseudomonads were consistently isolated from lesions. Some of the strains determined to be pathogenic to impatiens by inoculation were identified as *Ps. syringae* using Biolog GN MicroPlates™ and LOPAT tests.

CONSTRUCTION OF LINKAGE AND PHYSICAL MAPS FOR THE DUTCH ELM DISEASE FUNGUS, OPHIOSTOMA ULMI (SENSU LATO). Ken Dewar, Josée Dufour, and Louis Bernier. Centre de Recherche en Biologie Forestière, Université Laval, Québec, Qc, Canada G1K 7P4.

Genetic and molecular markers were used for the systematic mapping of the chromosomes of *Ophiostoma ulmi (sensu lato)*. Meiotic analysis of chemically induced nuclear mutations and over 150 Random Amplified Polymorphic DNAs (RAPDs) in the F₁ progeny from an aggressive x nonaggressive cross suggested the occurrence of at least eight linkage groups. Electrophoretic karyotypes, obtained by Pulse Field Gel Electrophoresis (PFGE), ranged from at least five to seven chromosomal bands, depending on the strain tested. Southern hybridization of PFGE-separated DNA allowed physical identification of chromosome-specific probes for all seven chromosomes detected in one laboratory strain. None of several chromosome length polymorphisms (CLPs) observed among both wild-type and laboratory strains was correlated with aggressiveness. Inheritance of CLPs was studied in progeny from controlled crosses: a model involving fragmentation of a large ancestral chromosome could best explain the CLP data.

USE OF ADJUVANTS AND N-VIRO AGLIME FOR CONTROL OF CLUBROOT ON DIRECT SEEDED CRUCIFERS. M.A. Dubos and S.A. Johnston, Dept. of Plant Pathology, Rutgers University, New Brunswick, NJ 08903

The impact of the surfactant Induce (alkyl polyoxyalkane ether, free fatty acids, and IPA) and N-Viro Aglime (pasteurized mixture of sewage sludge and alkaline materials) was assessed on clubroot (*Plasmodiophora brassicae*) incidence in naturally infested field soils in autumn 1994 and summer 1995 field studies, respectively. Induce significantly reduced clubroot of broccoli raab compared to nontreated controls when applied as a drench in a band over the row immediately after seeding or when drenched in a band alongside of the row three weeks after seeding. N-Viro Aglime applied broadcast and incorporated prior to seeding significantly decreased disease incidence on oriental mustard compared to a nontreated control or to Terraclor 75W (pentachloronitrobenzene, a fungicide labeled for clubroot control) treatments.

SUPPRESSION OF RHIZOCTONIA CROWN ROT OF BEETS WITH CHLORIDE W. H. Elmer, CT Agr. Exp. Sta., New Haven, CT 06504.

Rhizoctonia crown rot, caused by *Rhizoctonia solani* (AG 2-2), causes major yield loss in beets. Since beets evolved in brackish soils, it was hypothesized that chloride may be suppressive to disease. 'Early Wonder' or 'Detroit Dark Red' seedlings were grown in potting mix infested with *R. solani* or in noninfested soil mix. Seedlings received weekly applications of a complete fertilizer that contained NaCl (1%, w/v). Fertilizer with no NaCl served as a control. In both cultivars, treatment with NaCl suppressed disease and improved growth compared to the control plants. Sodium chloride benefited growth in almost all treatments. The largest NaCl effect occurred on 'Detroit Dark Red' seedlings in infested soils when compared to nontreated plants. Other studies showed that NaCl was more disease-suppressive when the N-form was (NH₄)₂SO₄ as opposed to Ca(NO₃)₂, but in the absence of NaCl, the reverse was true. Field plots in infested soils treated with NaCl (560 kg/ha) produced more marketable yield than nontreated plots. In the greenhouse, other chlorides, such as KCl, CaCl₂, or MgCl₂, were equal to NaCl in suppressing disease. Chloride fertilization may be useful in management of Rhizoctonia root rot of beets.

ANALYSIS OF RISK OF PRIMARY APPLE SCAB INFECTION
Stuart P. Falk, David M. Gadoury and Robert C. Seem. Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456-0462.

We defined the relative risk of infection (*RRI*) for primary infection of apple cluster leaves by *Venturia inaequalis* as the product of cluster area (*A*), susceptibility (*S*), and ascospore dose (*D*), each on a 0-1 scale. In a repeated mist chamber study, 2-m tall columnar McIntosh trees at 4 phenophases were simultaneously exposed to ascospore inoculum. *D* was monitored by volumetric traps, and *S* was deduced from lesions/cluster (*L/C*) divided by *A* at inoculation. With *D* constant, *L/C* on a 0-1 scale was 0.02, 1.0, 0.94, 0.58 at 2-cm green (2CG), tight cluster (TC), pink (P), and bloom (B), respectively. An exponential function of *S* yielded values of 1.0, 1.0, 0.7, 0.4, 0.2, and 0.1 for green tip, 2CG, TC, P, B, and petal fall, respectively. Additional trees were sheltered outdoors within plastic tents, surrounded by a caged inoculum source, and temporarily exposed to 24 hr of sprinkler irrigation at 6 phenophases. Regression of estimated *RRI* (from measured *D* and *A* and estimated *S*) against observed *L/C* in the orchard yielded an R^2 of 0.987. We plan to develop a simple phenological model of *RRI* and explore its application in risk-based use of fungicides and anti-resistance strategies.

DETECTION OF PHYTOPHTHORA SPECIES CAUSING ROOT ROT OF RASPBERRY IN QUEBEC. C. Garand¹, C. Beaulieu², P.-M. Charest¹, R. Hogue³ and S. Labege⁴. ¹Dept. de Phytologie, Université Laval, Ste-Foy; ²Dept. de Biologie, Université de Sherbrooke, Sherbrooke; ³Service de Phytotechnie, MAPAQ, Ste-Foy; Centre de Recherche, ⁴Agriculture et Agro-alimentaire Canada, Ste-Foy, Québec, CANADA

Several *Phytophthora* species cause root rot disease in raspberry plants. This work has been undertaken to develop a PCR-RFLP test to detect *Phytophthora fragariae* var. *rubi*, an highly pathogenic in eastern Canada, in raspberry roots and soil. Both internal transcribed spacers (ITS1, ITS2) between nuclear ribosomal DNA genes of *Phytophthora fragariae* species have been amplified and sequenced. The sequence of ITS I region have been compared with known ITS sequences of ten others *Phytophthora* species. A set of primers and 8 restriction enzymes (AluI, BanII, DraI, HaeIII, HhaI, MboI, RsaI, TaqI) have been selected to amplify an ITS1 region and to produce RFLP profiles which differentiated *Phytophthora fragariae* species from others *Phytophthora* species as well as with other *Pythium* spp.

ECONOMICS OF FUNGICIDE REGISTRATIONS AND CANCELLATIONS. Leonard P. Gianessi, National Center for Food and Agricultural Policy, 1616 P Street, N.W. First Floor, Washington, D.C. 20036

The use of synthetic fungicides has contributed greatly to increases in crop yields in North America. Potato yields improved dramatically following the widespread use of EBDC fungicides in the early 1950's. Recently, certain registrations of fungicides have been canceled, either through government regulatory actions or voluntary actions of corporations. Cherry yields declined following the loss of Captafol for leafspot control. The loss of six fungicide registrations for cranberries has resulted in a 50% decline in the production of fresh cranberries from Massachusetts. Potential regulatory actions (such as strict enforcement of the Delaney Clause) could dramatically reduce fungicide registrations and negatively impact crop yields.

INFECTION OF NON-TARGET TREES BY CHONDROSTEREUM PURPUREUM USED AS A MICROBIAL PHYTOCID. Lyne Gosselin, Robert Jobidon*, Louis Bernier. Centre de recherche en biologie forestière (CRBF), Université Laval, Sainte-Foy (Québec), Canada G1K 7P4; * Ministère des Ressources Naturelles du Québec (MRN), Complexe Scientifique, Sainte-Foy (Québec), Canada G1P 3W8.

The fungal pathogen *C. purpureum* (Cp) is being tested as an alternative to chemical herbicides to control stump sprouting of commonly found broad-leaved species in northeastern America. As part of an epidemiological study conducted to determine the environmental risk of such treatment, occurrence and origin of infection of non-target trees are investigated at two different sites. Two tree species cut while spores were being emitted from treated areas were studied. Sampling was realized within five plots (50 m²) located from 0 to 400 m downwind of the area, treated with two native strains of Cp. For each plot, 20 stumps were gathered and analysed to detect the presence of Cp and to assess the proportion of stumps infected. RAPD fingerprints of the recovered isolates were compared to those of the strains used for treatment and two RAPD markers specific to each inoculant strain were analysed. Results indicate that infection rate was relatively low, varying from 0 to 45% among plots, without any evidence of a gradient of infection according to distances from inoculated area. Moreover, less than 20% of the infected stumps were colonized by progeny of the inoculant strains. These results suggest that spores emitted from the resident population of *C. purpureum* were mainly responsible for the infection of non-target trees.

INHIBITION OF BOTRYTIS BLIGHT ON WHOLE PETUNIA FLOWERS BY PSEUDOMONAS FLUORESCENS. A. B. Gould, D. Y. Kobayashi, and M. S. Bergen, Department of Plant Pathology, Rutgers University, PO Box 231, New Brunswick, NJ 08903.

A total of 172 bacterial isolates from petunia leaves and flowers were evaluated for the ability to suppress Botrytis blight of petunia using a rapid, small-scale petal disk assay. One isolate, *Pseudomonas fluorescens* PB92B10E, consistently inhibited *Botrytis* sporulation on petal disks by 100% and was tested for control of the disease on the whole plant level. In seven different trials in the mist chamber, PB92B10E reduced disease incidence by an average of 77% on whole flowers inoculated with *B. cinerea* conidia. Populations of a rifampicin-resistant mutant of PB92B10E increased by 10⁴ g⁻¹ tissue (fresh weight) over a seven day period on whole flowers. The identification of *P. fluorescens* PB92B10E demonstrates the utility of the petal disk assay for the development of potential antagonists for management of greenhouse crop diseases.

IDENTIFICATION OF ROOT-ROT ORGANISMS IN NURSERY SEEDLINGS BY TWO-STEP NESTED PCR. Richard C. Hamelin, Pierre Bérubé, Manon Gignac, and Martin Bourassa. Canadian Forest Service-Québec, P. O. Box 3800, Sainte-Foy, Québec G1V 4C7, Canada

The internal transcribed spacer (ITS) of the ribosomal DNA subunit repeat was sequenced in 9 isolates of *Cylindrocladium floridanum* and 11 isolates of *Cylindrocarpon destructans*. Some intra-specific variability was present in *C. destructans* but not in *C. floridanum*. Three variants were identified, but there was no apparent association between ITS variants and host or geographic origin. Primers were designed to amplify portions of the ITS of *C. floridanum* and *C. destructans* and were tested with cultures from white, black, red, and Norway spruce, from jack and red pine from 11 nurseries in Québec, and with 20 common contaminants of conifer roots. No amplification resulted in PCR reactions with non-target fungi. For amplifications from infected tissues, the ITS was first amplified with a universal primer and a fungus-specific primer; a second amplification with species-specific primers resulted in the amplification of species-specific amplicons in all infected seedlings.

SURVIVAL OF ASCOSPORES AND GERMLINGS OF VENTURIA INAEQUALIS ON APPLE LEAVES UNDER NATURAL DRY CONDITIONS. B. H. Hara-Kaonga and W. E. MacHardy, Department of Plant Biology, Univ. of New Hampshire, Durham, NH 03824.

Viability of ascospores and germlings of *Venturia inaequalis* was quantified with epi-UV fluorescence microscopy using fluorescein diacetate. Inoculated McIntosh apple seedlings were exposed to sun or shade for 2, 6, or 10 h on 13 days between 27 April and 3 August, 1994. Leaf surface temperature (LST) was compared to ambient temperature (AT). The LST averaged 3.5 C higher than AT (sd 3.0). For all test conditions (13 to 34 C LST and 39 to 71% RH, based on AT), the percentage of viable ascospores was 62, 47, and 40% at 2, 6, or 10 h sun exposure, respectively. In the shade, viability was 63% after 2 h and remained unchanged through 10 h. Germlings responded similarly. Under the most stressful conditions (10 h in sun, 34 C LST), the average percent viability of ascospores and germlings was 20 and 13%, respectively, and for 10 h in sun at all temperatures was 40 and 36%, respectively. The percent viability of ascospores and germlings not subjected to drying (controls) was 77 and 70%, respectively. The high survival rate under the stressful test conditions raises doubts concerning decision-rules for determining an infection period during discontinuous wetness periods.

MANAGEMENT OF APPLE DISEASES WITH FUNGICIDE PROGRAMS APPLIED AT TREE-ROW-VOLUME RATES. Kenneth D. Hickey, Department of Plant Pathology, The Pennsylvania State University, Fruit Research and Extension Center, Biglerville, PA 17307-0309.

Efficacy of fungicide programs applied with an airblast sprayer at rates to match tree size (tree-row-volume (TRV) rates) was determined over 3 years (1993-95) for control of apple scab, *Venturia inaequalis* and powdery mildew, *Podosphaera leucotrica* in southcentral PA. The fungicide mixtures consisted of fenarimol or myclobutanil with captan, EBDC, or ziram applied in replicated seasonal spray programs. Rates were proportionately adjusted for tree size in each orchard based on a standard orchard with a TRV of 40,232 cu m/ha equaling the full rate (100%). Over the 3 year period, treatments with TRV rates of 40,50, or 66% of the standard rate provided levels of scab and p. mildew control at commercially acceptable levels. Rates adjusted for trees measuring 32% of the standard provided inadequate scab control on fruit under high disease pressure, fair control under moderate and adequate control under low pressure.

CLONING OF β -1,3-GLUCANASES FROM THE BACTERIAL BIOCONTROL AGENT, N4-7. M.A. Holtman, D. Kobayashi. Rutgers University, Dept. Plant Pathology, New Brunswick, NJ 08903

N4-7, a gram-negative soil bacterium, is an effective biocontrol agent of summer patch disease of Kentucky bluegrass caused by *Magnaporthe poae*. N4-7 is unique in that it produces all common degradative enzymes such as chitinase, glucanase, lipase and protease. Six cosmid clones which produce β -1,3-glucanase activities were isolated from the wildtype genomic library expressed in an *Enterobacter* sp. The clones shared several common restriction fragments but were varied in substrate specificity for laminarin (soluble β -1,3-glucan), zymosan (insoluble β -1,3-glucan) and 4-methylumbelliferyl- β -glucoside. Two clones were active against all three substrates and contained both 3 and 8 kb *Bam*HI fragments. Preliminary mapping suggests that the 3 kb region is responsible for laminarinase activity whereas the linked 8 kb region confers β -glucosidase and zymosanase activity.

EFFECTS OF PARA-FORMALDEHYDE ON SUGAR MAPLES TAPPED FOR SAP. II. TEMPORAL DEVELOPMENT OF DISCOLORATION AND DECAY. David R. Houston, USDA Forest Service, Hamden, CT 06514.

Paraformaldehyde (PF) is placed in tapholes (TH's) to increase sap yield. I examined the effects of PF and different tapping and spout removal times on development of discoloration (DIS) and decay (DEC). Trees (162) were tapped (2/tree) either before, after or at onset of sapflow in 1991; each TH received either no PF, a PF pellet or PF flushed with water when spouts were pulled early (April) or late (May). Trees (54) were cut after 2, 3 or 4 growing seasons and a 2 m bolt centered on the TH's was dissected. Measures of DIS and DEC columns (COL) were used to calculate COL volumes. DIS COL, longest around PF TH's increased over time. By 4 seasons, 60-75% of PF TH's were decayed vs only 25% of controls. Flushed TH's had more and larger DEC COL. DIS COL were smaller; DEC COL were both fewer and smaller around PF TH's made at onset, but were especially large around TH's made prior to sapflow. TH's with spouts pulled late had fewer and smaller DEC COL. Distribution of the decayed TH's will determine their importance.

CONTROL OF GREY SNOW MOULD WITH TYPHULA PHACORRHIZA. Tom Hsiang, Chunren Wu, Lin Yang and Leon Liu, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Isolates of *Typhula phacorrhiza* were collected from corn fields throughout southern Ontario in the spring of 1994. Based on growth rate and sclerotia production among 170 isolates, 46 of them were field tested in winter 1994 to evaluate suppression of grey snow mould disease of creeping bentgrass caused by the pathogens *T. ishikariensis* or *T. incarnata*. In field trials, isolates of *T. phacorrhiza* varied significantly in their ability to suppress grey snow mould. Effects ranged from 70% suppression (no significant difference from fungicide-treated control) to 0% suppression (no significant difference from *T. ishikariensis* or *T. incarnata* inoculated checks). Most isolates inhibited both *T. ishikariensis* and *T. incarnata* equally. A weak negative correlation was found between sclerotial production on BASM or mixed grain medium and snow mould suppression in the field plots.

THE IMPORTANCE OF PLANT DISEASE MANAGEMENT IN U.S. PRODUCTION OF LEAFY GREEN VEGETABLES. S.A. Johnston, M.A. Dubos, J.K. Springer, G.C. Hamilton, R.A. Davis, K.L. Smith and C. Osteen. Rutgers Univ., Bridgeton, NJ 08320; USDA, Beltsville, MD 20705

The following results were obtained from a National Agricultural Pesticide Impact Assessment Program project. The incidence of leaf spotting diseases of collards, kale, mustard greens, spinach, and turnip is high in southeastern states, whereas downy mildew and seedling diseases are common in all production areas. Foliar fungicides are used on 93% of all lettuce acreage and are applied to 49 to 68% of acreage planted to other leafy greens. Use of seed treatment fungicides range from 7% for lettuce to 89 to 100% for other leafy greens. The ban of fungicides used on leafy green crops would result in production losses large enough to noticeably increase price. For example, restriction in foliar fungicides use would result in yield decreases that range from 16% for mustard greens to 46% for turnip greens. In addition, loss of seed treatment fungicides would result in yield decreases of 5 to 10% for all leafy greens.

SELECTION OF EFFECTIVE ANTAGONISTS AGAINST *RHIZOCTONIA SOLANI* (AG-3). N.Z. Kabir¹, P.M. Charest² & S. J. Hare¹; 1- Plant Science Dept., McGill University, 21,111 Lakeshore Rd., Ste.-Anne-De-Bellevue, Quebec. 2- Dépt. of Phytologie, Université Laval, Ste.-Foy, Québec, Canada.

A total of 256 soil fungi were isolated from sclerotia of *R. solani* infested potato tubers (cultivars: Norland, Atlantic and Souris). Fifty fungal isolates were selected and tested for their antagonistic ability against sclerotia. Twenty-four antagonists decreased the sclerotial viability by 70% to 100%. Pathogenicity of *R. solani* on beet seedlings grown in infested soil with *R. solani* and amended with conidial suspension of each of the antagonist significantly decreased. Six antagonists (F2, F11, F132, F158, F216, F258) significantly increased tap root length and number of secondary roots as compared to those that were infected with *R. solani*. Evaluation and rapid screening of these antagonists for their ability to produce the extracellular enzymes glucanases, chitinases, cellulases, amylases, mannses, proteases, pectinases and lipases was carried out using the agar-plate assay. F2, F11, F132 and F258 showed pronounced chitinase, glucanase, cellulase, lipase and amylase activities. The mycoparasitic ability of these 4 antagonists on *R. solani* hyphae was examined microscopically. F2, F11 and F132 infected and colonized *R. solani* hyphae. Experiments are in progress to test the efficiency of these antagonists to control stem canker of potato.

COMPETITIVE FITNESS OF A NEW AND OLD LINEAGE OF *PHYTOPHTHORA INFESTANS* IN THE USA. M. Kato, and W. E. Fry. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

Competitive fitness of two clonal lineages of *Phytophthora infestans* was examined in the laboratory to test the hypothesis that a new lineage (US-8) of this fungus is displacing a pre-existing lineage (US-1) due to superior competitive fitness. Potato leaflets were inoculated initially with a mixture (1:1) of US-1 and US-8 sporangia and sporangia from the resulting sporulating lesions were bulked and used to inoculate another set of leaflets after 5-6 or 6-7 days. The ratios of the lineages were monitored for up to seven generations by determining glucose-6-phosphate isomerase genotype of each isolate causing each lesion by electrophoresis. 65% to 95% of lesions were produced by US-1 after the initial inoculation, indicating that US-1 has a higher infection efficiency than US-8. When the inoculation cycle was 5-6 days, US-8 subsequently predominated. When 6-7 days, there were no consistent trends in the subsequent ratios of the two lineages. These results suggest that despite the higher infection efficiency of US-1, shorter latent period and/or higher sporulation in the early stages of lesion development in US-8 enables it to overcome this fitness disadvantage when the inoculation cycle is shortened.

EVIDENCE OF A DIRECT ROLE OF SILVER THIOSULPHATE ON GERANIUM DEFENSE METABOLISM AGAINST *PYTHIUM ULTIMUM*. Caroline Labbé & Richard Bélanger, Département de Phytologie, Université Laval, Qc, G1K 7P4.

An experiment was carried out to determine by which mechanism geranium plants (*Pelargonium X hortorum*) lose their resistance against *Pythium ultimum* following an application of silver thiosulfate (STS), a treatment used to prevent early petal abscission. Secondary metabolites from different chemical classes were extracted from both control and treated plants and were analysed for their toxicity against *Cladosporium cucumerinum* and *P. ultimum*. Two molecules with strong antifungal activity were found to be constitutively present in control plants while they could not be recovered from STS-treated plants. It thus appears that geranium has some constitutive chemical weapons to fend off *Pythium* attacks, and that the activity or synthesis of such weapons is altered following a STS treatment. Investigations into the chemical nature of these compounds indicate that they are not of phenolic nature but would rather belong to a more complex class of molecules.

EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON MATURATION OF ASCOSPORES OF *SCHIZOTHYRIUM POMI*, FLYSPECK OF APPLE. S. Lemer, D.R. Cooley, Dept. of Plant Pathology, University of Mass., Amherst, MA 01003.

Schizothyrium pomi, causal agent of flyspeck disease, grows on the waxy cuticle of apples. Growth of the fungus on its host is not apparent until pseudothecia form as overwintering structures in late summer, lowering fruit quality. Scheduling fungicide applications based on knowledge of time of infection is therefore not yet practical. We have observed a single discrete period of ascospore development and release in each of three growing seasons and would like to determine the environmental conditions influencing the maturation of inoculum, relating it to apple phenology. The effects of temperature and relative humidity on the maturation of *S. pomi* ascospores from pseudothecia on wild *Rubus* spp. canes were studied in the laboratory. Sections of infected canes were incubated at 7, 14 and 21°C and in low, medium and high relative humidity (RH) environments. No development of asci or spores was observed at the start of the experiment. At 48 hours, mature spores were observed at 21°C in the high RH treatment. At 72 hours immature spores were observed at 14°C, but only at high RH. By the end of the experiment at 8 days, no spores had developed at 7°C even at high RH. It appears that both temperature and relative humidity have an effect on spore development.

DROUGHT ASSOCIATED WITH BROWN ASH DIEBACK IN MAINE. W. H. Livingston, A. Hager, and A.S. White. Dept. Forest Ecosystem Science, Univ. Maine, Orono, ME 04469, and D. Hobbins, Univ. Maine at Ft. Kent, ME 04743.

Surveys by the Maine Forest Service have demonstrated a severe crown-dieback problem in the state's brown (black) ash (*Fraxinus nigra*) population. Measurements on 57 plots in 1993 from throughout the state showed an average crown dieback of 45%. Six of these plots were sampled in the fall of 1994 for dendrochronological studies and for phytoplasmas, which were not detected. Since 1948, 12 out of 16 flood/freeze events in early winter were associated with initiation or continuance of growth declines in all six plots ($P < 0.001$ for binomial test). However, 1987 was the only year since 1948 in which all six plots were in growth decline, and flood/freeze events were not widespread at this time. Average stream flows for May were used as a drought indicator, and they were the lowest since 1948 for the six plots from 1985-87. The first reports of widespread dieback in northern Maine were recorded in 1987. Apparently, severe drought in the mid 1980's initiated the recent episode of brown ash dieback.

BIOLOGICAL AND MOLECULAR DIVERSITY WITHIN THE RMV STRAIN OF BARLEY YELLOW DWARF VIRUS. Eglantina Lucio-Zavaleta and Stewart Gray, Dept. Plant Pathology, Cornell University and USDA-ARS, Ithaca, NY.

Recent collections of alate *Rhopalosiphum padi* in NY indicated that a significant number were transmitting the RMV strain of barley yellow dwarf virus (BYDV); a strain normally vectored by *R. maidis*, but not *R. padi*. Studies were then initiated to investigate the diversity of RMV isolates and the ability of field collected *R. padi* biotypes to vector RMV. Sixteen RMV isolates were obtained from viruliferous aphids, two transmitted by *R. maidis* and 14 transmitted by *R. padi*. All tested positive for RMV in ELISA. Reverse transcription-polymerase chain reaction (RT-PCR) amplification of the coat protein gene and subsequent restriction enzyme digestion identified six unique RFLP patterns. Four field isolates could not be amplified by RT-PCR using the same primer. *R. maidis* transmitted all the isolates, a laboratory maintained biotype of *R. padi* did not transmit any, and field-collected *R. padi* transmitted isolates from one RFLP group and two isolates not amplified by RT-PCR. The data confirm variability among RMV isolates both in terms of virus characteristics and vector associations.

FREEZE/THAW EVENTS RESULT IN WINTER INJURY ON RED SPRUCE. Anne E. Lund & William H. Livingston, Dept. of Forest Ecosystem Science, University of Maine, Orono, ME 04469.

Detached branches from 17 mature red spruce (*Picea rubens* Sarg.) were used to determine if multiple freeze/thaw events could result in winter injury. Samples from each tree consisted of three branch tips per freeze/thaw treatment. Treatments consisted of zero (held at +5°C), one, two or four freeze/thaw cycles of 24 hrs per cycle. For each cycle, samples were frozen to -35°C at a rate of 5°C/hr. The tissue was held at this minimum temperature for 2 hrs then thawed quickly by placing it in a +5°C chamber. Of the 17 trees sampled, 13 exhibited visible symptoms of winter injury (needle reddening) after four freeze/thaw cycles. On these symptomatic trees, chlorophyll absorbency of needle extract was significantly reduced from nonfrozen samples by 7.7% and 22% for two and four cycle treatments, respectively ($P \leq 0.01$). Our results indicate that frequency of freeze/thaw events appears to be a factor in winter injury on red spruce.

AUTUMN DEVELOPMENT OF FOLIAR SCAB CAUSED BY VENTURIA INAEQUALIS. W. E. MacHardy and D. K. Sutton. Department of Plant Biology, Univ. of New Hampshire, Durham, NH 03824.

A late-autumn assessment of foliar scab provides a critical input into the equation to predict potential ascospore dose in an apple orchard, but disease expressions other than the characteristic olivaceous velvety lesion confound the assessment. These include (i) small, round spots covered with a tan to dark-brown mycelial growth of *V. inaequalis* indistinguishable macroscopically from those of several other fungi common on apple leaves in autumn, (ii) extensive but not easily-recognized growth of *V. inaequalis* along veins, (iii) small red spots similar to red spots due to other causes, and (iv) microscopic tufts of *V. inaequalis* that emerge without the development of symptoms. Dentrifical mycelial growth or tufts of conidiophores bearing conidia of *V. inaequalis* are evident when these 'atypical' expressions are observed under a hand lens or microscope. In a commercial orchard in which 'atypical' lesions occurred on 2.6% and 'typical' lesions (plus 'atypical' expressions) were on 1.3% of the leaves examined, pseudothecia developed on a minimum of 24% of leaves with symptoms, and leaves with 'atypical' symptoms often discharged an abundance of ascospores in a trapping study. Unrecognized infections may account for some instances in which pseudothecia developed on a leaf with a single lesion.

RUSSET OF APPLE FRUIT INDUCED BY ISOLATES OF RHODOTORULA GLUTINIS AND AUREOBASIDIUM PULLULANS. M. C. Matteson, T. J. Burr, M. R. Corral-Garcia, D. Parker, W. Koeller, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY, 14456.

Apple fruit russet sporadically causes reduced fruit quality and economic losses to growers. It is associated with breaks in the cuticle and death of floral and young fruit epidermal cells. Russet has been attributed to various causes including powdery mildew infections, cultural and environmental conditions, fruit cultivar, mechanical injury, and certain pesticides. By spray-inoculating McIntosh fruit in the field weekly, beginning at pink, we have determined that russet can be induced by two common foliar epiphytes of apple identified as *Rhodotorula glutinis* and *Aureobasidium pullulans*. These isolates and fifty-two other yet unidentified fungal epiphytes from apple were screened for their ability to produce cutinase. Seventeen of the isolates produced detectable levels of the enzyme as determined with an esterase assay using p-nitrophenyl butyrate as the model substrate. Although the two isolates that induced russet produced cutinase, another cutinase-positive epiphyte did not cause russet. Our results may elucidate the mechanism by which certain fungicides are associated with reduced fruit russet.

Efficacy Of Section 18 (1995) And Other Fungicides In Suppressing Potato Late Blight Caused By The US-8 Genotype Of Phytophthora infestans. H. S. Mayton, and W. E. Fry, Department of Plant Pathology, Cornell University, Ithaca, New York 14853.

Epidemics of potato late blight in small plots of the susceptible potato cultivar Norchip were suppressed to different degrees by different fungicides. Epidemics were initiated by an isolate of the US-8 clonal lineage of *Phytophthora infestans*, obtained in New York State. Fungicides were applied every 7 days in a volume of 200 l water/ha. Untreated plots were killed by late blight within 30 days of inoculation. The efficacy of the emergency labeled fungicides and the standard, chlorothalonil, was (in order of decreasing efficacy): propamocarb, chlorothalonil, dimethomorph and cymoxanil. A mancozeb and tin fungicide mixture was equivalent to chlorothalonil and propamocarb. A single unreplicated plot of potatoes drenched (19,000 l/ha) with a dilute (0.04%) solution of copper sulfate had the least disease.

EVALUATION OF JMS STYLET-OIL® FOR CUCURBIT POWDERY MILDEW UNDER GREENHOUSE AND FIELD CONDITIONS M. T. McGrath and N. Shishkoff, Department of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY 11901.

JMS Stylet-Oil® (0.75%) applied once to greenhouse-grown summer squash either 4 hrs before transfer of *Sphaerotheca fuliginea* conidia to inoculation sites or 5 days after inoculation suppressed the size of the area infected by 48-60%. An application 5 days before inoculation was not effective. A 4-day spray program beginning 5 days after inoculation was effective; compared with nontreated plants, oil-treated plants 20 days after inoculation had fewer affected leaves above the inoculated leaf (34% vs 71%) and fewer colonies (26 vs 244). Under field conditions, however, JMS Stylet-Oil® was not adequately effective when applied to melon on a 7-day schedule with a tractor-mounted boom sprayer that delivered 692 L·ha⁻¹ at 1724 kPa. The first application was made on 28 July 1995, 4 days after symptoms were detected on old leaves and 36 days after transplanting. Average severities on 17 August on adaxial/abaxial leaf surfaces were 66%/80% for nontreated plants, 43%/44% for plants treated with JMS Stylet-Oil®, and 3%/13% for plants treated with chlorothalonil (7-day) plus myclobutanil (14-day).

SECONDARY FUNGI ASSOCIATED WITH NEEDLECASTS OF PINUS STROBUS. W. Merrill and N.G. Wenner, 211 Buckhout Lab, University Park, PA 16802

Several fungi are associated with needlecasts of *Pinus strobus* throughout the Northeast. Often several of these fungi occur on the same needle. The most common is *Hendersonia pinicola*, which causes a "pepper-spotting" of the dorsal and ventral surfaces of dead needle portions. These spots superficially resemble the *Leptostroma* stages of needlecasts. Another is *Pezizella minuta* which resembles *Nothophacidium phyllophilum*, but with smaller asci and different hosts and associated primary fungi. An as yet undetermined *Sarcotrichia* sp. occurs occasionally; its J+ ascus pores and long ascospores distinguish it from the previous two species. Three other Sphaeropsidales are common. One, dubbed the "corner fungus", forms rows of minute pycnidia along corners between the dorsal and ventral sides of the needles. Another forms pycnidia in the inner mesophyll, and a third forms pycnidia in the outer mesophyll. We have been unable to place these three species into existing genera. High incidence of these fungi prevent sporulation of the associated needlecast fungi, easily leading to misdiagnosis.

COMPARATIVE ANALYSIS OF DNA SEQUENCES BETWEEN THE 16S AND THE 23S RIBOSOMAL RNA GENES OF *AGROBACTERIUM VITIS*. E.A. Momol¹, W.F. Lamboy², C.L. Reid¹, L. Otten³, and T.J. Burr¹.
¹Departments of Plant Pathology and ²Horticultural Sciences and USDA-ARS, PGRU, Cornell University, Geneva, NY and ³C.N.R.S. Institute of Plant Molecular Biology, Strasbourg, France.

DNA sequences of the spacer region between the 16S and the 23S rRNA genes were used to evaluate the genetic relationships between seven *Agrobacterium vitis* strains. Three were nontumorigenic strains that provide biological control of crown gall on grape and were isolated from *Vitis riparia*. Four tumorigenic strains were isolated from different geographical regions and represented strains with octopine, nopaline, and vitopine Ti plasmid types. DNA of the spacer region was amplified using two primers derived from 16S and 23S rRNA conserved regions and was then sequenced. Multiple alignment of the spacer sequences revealed sufficient variation for designing PCR primers which can distinguish between related groups of *A. vitis*.

MICROHYPHAE AS PART OF COATING ON VESSEL WALLS IN CARNATION AND SUMAC INFECTED BY *FUSARIUM* WILT PATHOGENS. G.B. Ouellette¹, R.P. Baayen², L. Bernier¹, and M. Cherif¹.
¹ Canadian Forest Service - Quebec, P.O. Box 3800, Sainte-Foy, Quebec G1V 4C7, Canada; ² IPO-DOL, P.O. Box 9060, NL-6700 Wageningen, The Netherlands; ³ Université Laval, Pavillon C.-E. Marchand, Sainte-Foy, Quebec G1V 7P4, Canada.

TEM observations of a susceptible carnation cv. and staghorn sumac infected respectively by *Fusarium oxysporum dianthi* and *F. oxysporum* f. sp. *rhois* have shown, appressed to vessel walls, some cytoplasm-containing structures limited by only a thin opaque layer, and at times continuous with typical fungal cells, indicating their analogy with microhyphae. Using a labeled probe, chitin was detected in a translucent wall layer present in typical cells but exceptionally in microhyphae; labeling was erratic in other microhyphae. These as well as larger fungal cells were surrounded by an extracellular, fibrillar, opaque sheath that permeated directly host cell walls, including those of vasicentric parenchyma cells, as shown by labeling for cellulose.

STRUCTURE AND ORIGIN OF EXTRACELLULAR SHEATHS AROUND CELLS OF *OPHIOSTOMA ULMI* AND *O. NOVO-ULMI*. G.B. Ouellette¹, H. Chamberland², and J.-G. Lafontaine².
¹ Canadian Forest Service - Quebec, P.O. Box 3800, Sainte-Foy, Quebec G1V 4C7, Canada; ² Université Laval, Pavillon C.-E. Marchand, Sainte-Foy, Quebec G1V 7P4, Canada.

Growing on various substrates (agar medium, elm tissues, millipore membranes), cells of these pathogens produce an often thick extracellular sheath; it was shown to be formed of tubular-like structures proximally to the cells and of loose fibrils distally, particularly in samples from millipore membranes, and sterilized wood sections fixed by high-pressure freezing. Sheath structures appeared to be continuous from within the cell through the thin irregular wall layers (with labeled probes shown to contain cellulose but not chitin) delimiting the generally pleomorphic cells in these samples. The sheath contained β ,1-3 glucans, possibly actin and other constituents but not chitin and cellulose. In addition to being involved in host wall alterations, sheaths appeared to form part of the coating material along vessel walls.

BUSINESS CONSIDERATIONS IN MINOR USE REGISTRATIONS FOR FUNGICIDES. Elizabeth D. Owens, ISK Biosciences Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, OH 44061-8000. Pesticides are increasingly costly to develop and register. Therefore, most companies target markets that will provide returns on these investments quickly. This means that minor use opportunities are scrutinized for their market potential prior to a decision on application for registration of the use. Some minor uses offer significant economic opportunities and others cannot support registrations of new materials. However, there are other issues that a company examines prior to making a decision on the best approach to registration.

DEVELOPMENT OF A DNA PROBE FOR DETECTION OF *FUSARIUM OXYSPORUM* F. SP. *BASILICUM* IN DISEASED PLANTS. Zheng Pan 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* (Fob) is becoming increasingly important internationally. Early detection of Fob in seeds and plants is critical to disease management. Fifteen isolates of Fob were subjected to RAPD analysis and compared with other fusaria to recover a unique DNA fragment useful for detection of Fob. All 15 isolates of Fob had identical DNA band patterns following PCR with a 10-mer random primer (OPA02) and electrophoresis. A 0.7 kb fragment unique to Fob was eluted from agarose gel, cloned into plasmid vector, and labelled with nonradioactive digoxigenin-dUTP. This probe specifically bound to amplified DNA from Fob but not from other fusaria tested. DNA extracted from Fob-colonized basil plants were amplified with primer OPA02 and successfully detected with the probe.

POTATO ANTHRACNOSE (BLACK DOT) EPIDEMICS OBSERVED IN RESEARCH AND COMMERCIAL FIELDS IN PENNSYLVANIA DURING THE 1995 GROWING SEASON. S. P. Pennypacker and S. Sanogo. The Pennsylvania State University, University Park, PA. 16802.

The occurrence of 'natural early dying' attracted grower attention early in the 1995 season. The initial yellowing and rolling of upper leaflets was attributed to prevailing statewide drought conditions. Fungicides applied throughout the season for the control of early and late blight had no effect on alleviating the problem. The affected plants were often stunted and their leaves eventually died. Above ground stems of most affected plants possessed silver-like lesions of various shapes encrusted with black sclerotia. Sclerotia were also present in vascular tissue and within the broken down cortical portions of the lower stems and stolons. Black sclerotia also occurred on tubers. These signs and symptoms indicated the fields were heavily infested with *Colletotrichum atramentarium* or *C. coccodes*, the causal agent of anthracnose or black dot.

THE *IN VITRO* PRODUCTION OF PSEUDOTHECIA AND HARVEST OF AXENIC ASCOSPORES OF *VENTURIA INAEQUALIS*. V. Phillion, O. Carisse and T. Paulitz. Agriculture and Agrifood Canada, 430 Gouin Blvd., St-Jean, Qc, J3B 3E6; Dept. of Plant Science, McGill University, 21 111 Lakeshore Road, Ste-Anne-de-Bellevue, Qc, Canada, H9X 3V9.

A method for the *in vitro* production of pseudothecia of *Venturia inaequalis* was adapted to eliminate the need for conidia by using a mycelial suspension. This method reduced the risks of sterile matings by using a mixture of strains. Furthermore, the maturation time was shortened by varying the temperature during the incubation. The ascospores were harvested by forcing their ejection in a bubbler type apparatus in one hour and their production was then estimated. This approach can be used to evaluate the potential of antagonists to inhibit the ascospore production of *Venturia inaequalis*. Variations of this method could be used to evaluate pseudothecial maturity and forecast the first ascospore releases in the spring. Finally, axenic ascospores could be harvested and used to test fungicides, biocontrol agents, for controlled infections, or in genetic studies.

GENETICS AND BIOCHEMISTRY OF VIRULENCE IN *NECTRIA GALLIGENA*. Daniel Plante and Louis Bernier. Centre de Recherche en Biologie Forestière, Université Laval, Québec, Qc, Canada G1K 7P4.

The genetic basis of virulence was investigated in the homothallic fungus *Nectria galligena*, causal agent of perennial Nectria canker. Single ascospore progeny (F₁) were first recovered from individual perithecia found on cankers occurring naturally on yellow birches. In one set of progeny, virulence towards Golden Delicious apples segregated in a near normal distribution pattern, suggesting this trait was variable and under polygenic control. Selected F₁ strains, representing the range of variation for virulence in this progeny, were further crossed. RAPD markers were used to verify that the resulting F₂ progeny originated from true crosses rather than from selfing. One cross between a moderately and a highly virulent strains yielded F₂ progeny in which the two classes of virulence (assessed both on apples and on yellow birch seedlings) segregated 1:1, suggesting that this difference was controlled by a single locus. Although the two F₁ parents also differed in their production of pectin lyase *in vitro*, there was no correlation between virulence and enzyme production in the F₂ progeny.

DNA POLYMORPHISMS IN *LEPTOSPHAERIA KORRAE*, THE CAUSAL AGENT OF NECROTIC RING SPOT. Y. L. Raffle and T. Hsiang, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Leptosphaeria korrae is a homothallic Ascomycota that causes necrotic ring spot in turfgrass, principally Kentucky bluegrass. It is not known if this disease is spread primarily by mycelia or ascospores. To evaluate the role of ascospores in dissemination, it first needed to be determined if progeny from self fertilization could be differentiated since polymorphisms are expected to be limited in isolates of such an organism. Eight sibling single spore progeny were examined for the presence of DNA polymorphisms using random amplified polymorphic DNA markers. No polymorphisms were observed with any of the five primers tested. Due to this lack of polymorphism, the study was expanded to look for differences between isolates on a regional level. Isolates of *L. korrae* from British Columbia, Ontario, Quebec and the state of Washington were then examined using the same primers. Two of the primers revealed polymorphisms within and between these regions.

IMMUNOCYTOCHEMICAL EVIDENCE THAT A SIMILAR SECRETION PROCESS OF PECTIC MATERIAL OCCURS DURING TYLOSIS AND GUM (GEL) FORMATION.

Rioux, D.¹, Nicole, M.² and Ouellette, G.B.¹. ¹ RNCAN, Canadian Forest Service-Quebec, P.O. Box 3800, Sainte-Foy, Quebec G1V 4C7, Canada. ² Institut français de recherche scientifique pour le développement en coopération, Laboratoire de phytopathologie, B.P. 5045, 34032 Montpellier, France.

When tyloses expand within vessel elements of trees, some areas such as pit chambers, three-way junctions between cells, and the border of vessel rims are not completely occluded by the tylosis primary wall. However, the tylosis secretes material intensely positive for pectin across its primary wall to completely obstruct such areas, as revealed by labeled monoclonal antibodies to pectin. In other species, the same labeling reveals that gums are partly composed of pectic material secreted by parenchyma cells via their protective layer. As the tylosis primary wall is an extension of this protective layer, the secretion of pectin by tyloses and parenchyma cells appears analogous.

REDUCING FUNGICIDE USE IN AGRICULTURE: BENEFITS, RISKS, AND UNEXPECTED OUTCOMES. D. A. Rosenberger, Cornell University's Hudson Valley Lab, P. O. Box 727, Highland, NY 12528.

During the past 15 years, academic scientists and government policy makers have generally assumed that reducing fungicide use in agriculture is a worthy objective that serves the public interest. Reducing fungicide use sometimes, but not always, results in reduced environmental impacts, public relations benefits for agricultural producers, and reduced expenses for disease control. However, reducing fungicide use may increase the risks of crop failure, promote development of fungicide-resistant pathogens, and reduce the economic incentives for development of newer and safer fungicides. Fungicides remain critically important for production of horticultural and other high-value crops. Most disease-management systems, including those based on IPM, biological controls, and use of disease-resistant germplasm, still depend on fungicides for some aspects of disease control. In some crop systems, fungicides are providing hidden benefits that may include micro-nutrient effects, control of "minor" non-target diseases, and suppression of microflora that adversely affect plant health and productivity. Examples are provided from research with apple diseases and scab-resistant apple cultivars.

DISPERSAL OF PYCNIDIOSPORES OF *STAGONOSPORA NODORUM* FROM MIDDLE LEAVES RESULTS IN INFECTION OF WINTER WHEAT SEED. Denis A. Shah¹, Gary C. Bergstrom¹, and Keith L. Eggleston². ¹Department of Plant Pathology and ²Northeast Regional Climate Center, Cornell University, Ithaca, NY 14853.

Seed infection by *Stagonospora nodorum*, a rain splash-dispersed pathogen, commonly occurs in New York winter wheat, even in the absence of pycnidia on the upper leaves. In a plot of 'Geneva' wheat in Ithaca, New York in 1995, no pycnidia were observed on flag leaves during grain development, yet 20% of the harvested seed were infected by *S. nodorum*. Pycnidia did occur on the F-2 leaves, and vertical rain splash (measured with a splashmeter designed at the Long Ashton Experiment Station in Bristol, UK) was sufficient during several rainfall events to account for dispersal of pycnidiospores from F-2 leaves to spikes. Height of leaves and spikes, disease development, and canopy structure measurements from adjoining plots of 43 other wheat varieties were analyzed for their relative contribution to seed infection.

IN VITRO CONTROL OF *SPHAEROTHECA FULIGINEA* ON SQUASH LEAVES USING JMS STYLET-OIL® OR TWEEN 20 DETERGENT. N. Shishkoff, M.T. McGrath, LI Horticultural Res. Lab, Cornell Univ., Riverhead, NY, 11901.

To determine whether JMS Stylet-Oil® or Tween 20 (polyoxyethylene sorbitan monolaurate) treatments reduced powdery mildew on squash in detached leaf culture, leaves were sprayed with a 0.75% or 1.5% oil emulsion one or more times before or after inoculation with *Sphaerotheca fuliginea*. Oil at 0.75% did not have a significant effect, but oil at 1.5% significantly reduced colony size, apparently by reducing the rate of colony expansion. An application of oil every 4 days was more effective than a single spray. Oil was not a significant eradicator of preexisting mildew colonies, nor did it appear to affect colony initiation. There was no indication that oil significantly reduced spore viability, since spores taken from sprayed colonies readily formed new colonies. Although oil appeared to cause abnormalities of conidiophores and spores immediately after application, this effect was temporary and its significance is not clear. Tween 20 also had an inhibitory effect on mildew growth, and combined applications of oil and Tween 20 were more effective than either alone.

GREEN ASH FAMILY VARIATION IN RESPONSE TO ASH YELLOW S PHYTOPLASMAS. W. A. Sinclair¹, T. H. Whitlow², and H. M. Griffiths¹, Departments of Plant Pathology¹ and Floriculture & Ornamental Horticulture², Cornell Univ., Ithaca, NY 14853

Twenty 1-yr-old seedlings of each of 35 half-sib families of green ash (*Fraxinus pennsylvanica*) from 15 sources (38-50° N, 70-98° W) were planted in pairs in a randomized complete block design at Ithaca, NY. One tree of each pair was inoculated at age 2 with two New York strains of ash yellows phytoplasmas by bark-patch grafting. DAPI tests revealed 98% of inoculated trees and 2% of controls diseased after six growing seasons. Analyses (ANOVA) after seven seasons revealed significant ($P < 0.01$) disease-associated suppression of height, stem diameter, and rate of volume growth (diam² x height). Average growth rates of diseased trees in the 7th season, as a proportion of growth of healthy trees, varied among families from 0.06 to 0.70 without relationship to geographic sources. Family-x-treatment interaction on growth was significant ($P = 0.001$), indicating possible heritable control of phytoplasma tolerance.

CHANGES IN THE PLANT ENDOMEMBRANE SYSTEM ASSOCIATED WITH CALLOSE DEPOSITION DURING THE INFECTION OF COWPEA BY THE COWPEA RUST FUNGUS. - D. Škalamera and M.C. Heath. Department of Botany. University of Toronto, Toronto, Canada M5S 1A1

Electron microscopy and stereological analysis of cowpea (*Vigna unguiculata*) leaf tissue infected by the cowpea rust fungus (*Uromyces vignae*) was used to evaluate changes in plant membranes associated with callose synthesis that typifies an unusual form of rust resistance. Our data suggest that both callose synthesis and fungal presence in the plant cell are associated with *de novo* synthesis of membranes. Furthermore, callose deposition may require an increase in smooth membrane surface of uncertain origin, while the establishment of a haustorium may be dependent on increased synthesis of rough ER in the plant cell.

INFLUENCE OF SOIL MOISTURE ON THE ABILITY OF THE LUMBRICID EARTHWORM *APORRECTODEA TRAPEZOIDES* TO REDUCE THE DISEASE SEVERITY OF TAKE-ALL. Stephens P.M. and Davoren C.W., Cooperative Research Centre for Soil & Land Management, PMB 2, Glen Osmond, South Australia

Field trials in 1993 demonstrated that the earthworm *Aporrectodea trapezoides* can reduce the disease severity of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) on wheat (Soil Biol & Biochem 26:1291). However, in 1994 (when rainfall represented ca. 54% of that which fell in 1993), *A. trapezoides* did not reduce the disease severity of *Ggt* in a field trial at one of the same locations. In subsequent pot trials, *A. trapezoides* only reduced the disease severity of *Ggt* (measured by a significant reduction in root disease rating) at a soil matric potential of 11 kPa, but not at 27, 56, or 357 kPa. These results suggest that the inability of *A. trapezoides* to reduce *Ggt* disease severity in 1994 may have been due, in part, to the relatively lower rainfall.

MANAGEMENT OF *MELOIDOGYNE HAPLA* ON LETTUCE IN ORGANIC SOIL WITH SUDAGRASS AS A COVER CROP. N. M. Viaene and G. S. Abawi. Dept. of Plant Path., Cornell Univ., Geneva, NY 14856.

Field microplots were filled with organic soil and infested with 0, 6 or 24 eggs of *M. hapla* (Mh)/cm³ soil. Half of the microplots were planted to sudangrass hybrid Trudan 8, while the other half was left fallow. After 50 days, in mid-October, sudangrass was incorporated into the soil as a green manure. Lettuce cv. Montello was planted in all microplots the next spring and harvested 9 weeks later. Microplots were reinfested with 0, 2 and 4 eggs of Mh/cm³ soil in plots that previously contained zero, low and high nematode densities, respectively, to compensate for nematode eggs removed with harvested lettuce roots. Plots were again planted to sudangrass or left fallow, and planted to lettuce the following spring. The experiment was repeated a third time. Generally, lettuce yield was significantly higher in Mh-infested soils amended with sudangrass. In the first year, egg production of Mh on lettuce roots was not affected by the sudangrass amendment. However, in the following two years, egg production was significantly lower (27 to 94 %, depending on Mh infestation and year) on lettuce roots grown in sudangrass-amended microplots, except in the plots infested with the low nematode density in the third year.

NEMATOPHAGOUS FUNGI ASSOCIATED WITH *MELOIDOGYNE HAPLA* IN ORGANIC SOILS IN NEW YORK. N. M. Viaene and G. S. Abawi. Dept. of Plant Pathology, Cornell University, Geneva, NY 14856.

Organic soils, collected from five production areas in New York, were added to pots (500 cm³), infested with 2,000 eggs of *M. hapla*/pot and planted to lettuce for 4 months. Egg masses (EM) were removed from lettuce roots, surface-disinfested, incubated on water agar (WA) and observed over a period of 30 days. Assay juveniles (J2) of *M. hapla*, hatched from clean eggs, were added to cups filled with these soils and retrieved after 5 to 7 days by centrifugal flotation. Retrieved J2 were surface-sterilized, plated on WA and examined after 7 days. Fungal growth was observed on 22% of the 311 EM and on 13% of the 1,026 J2 examined. A total of 41 isolates were selected for characterization. The isolates were considered pathogenic to *M. hapla* when they were reisolated from added assay EM or assay J2. Thirteen fungal isolates were pathogenic to EM and included species of *Fusarium* and *Alternaria*. Nine isolates, mostly nematode-trapping fungi, were considered J2 pathogens. These included *Monacrosporium ellipsosporum*, *M. cionopagum*, *Arthrobotrys oligospora* and *A. dactyloides*. Sixteen isolates failed to infect EM or J2 and were considered nonparasitic to *M. hapla*.

SCLEROPHOMA SEMENOSPORA CANCKER ON *ABIES FRASERI* CHRISTMAS TREES IN PENNSYLVANIA. N.G. Wenner and W. Merrill, 211 Buckhout Lab, University Park, PA 16802

In April 1995 basal stem cankers were found killing young *Abies fraseri* Christmas trees in Schuylkill County, PA. In June 1995 girdling branch cankers and terminal dieback were found on young *A. fraseri* Christmas trees in Centre County, PA. Masses of non-ostiolate erumpent pycnidia with immersed stipes of *Sclerophoma semenospora* (teleomorph = *Sydowia semenospora*) occurred in the bark over cankered areas of all trees. Single spore cultures from these pycnidia yielded the *Hormonema* anamorph of this fungus, distinguishable by its large, septate, brown conidia. In North America *S. semenospora* has been previously reported to cause dieback of drought-stressed *Pseudotsuga menziesii*, small discrete stem cankers on *A. amabilis*, and leader dieback on *A. lasiocarpa* in the Pacific Northwest. This is the first report of this pathogen from eastern North America, and the first report of it on *A. fraseri*.

OCCURRENCE OF FOLIAR NEMATODES ON WOODLAND PLANTS IN A MASSACHUSETTS NATIVE PLANT GARDEN. R. L. Wick University of Massachusetts, Amherst, MA 01003.

Native woodland plants from the New England Wild Flower Society's Garden in the Woods in Framingham MA, were submitted to the University of Massachusetts for confirmation of the occurrence of foliar nematodes. The problem was especially prevalent in the nursery, where *Actaea* and *Waldsteinia* were severely affected. A nematode, which was consistent

morphologically with *Aphelenchoides fragariae*, was found in several locations throughout the woodland site on the following plants: *Actaea pachypoda*, *A. rubra*, *Adiantum pedatum*, *Caltha palustris*, *Crysozomum virginianum*, *Cimicifuga acerina*, *Cornus canadensis*, *Diphylleia cymosa*, *Disporum lanuginosum*, *D. maculatum*, *Geranium maculatum*, *Hepatica acutiloba*, *Hydrophyllum virginianum*, *Jeffersonia dubia*, *Lilium canadense*, *Polystichum braunii*, *Smilacina racemosa*, *Thelypteris hexagonoptera*, *Trientalis borealis*, and *Waldsteinia fragarioides*.

ULTRASTRUCTURE AND CYTOCHEMISTRY OF INFECTION BY *GREMMEIELLA ABIETINA* ON PINE.

A. Ylimartimo, G. Laflamme, M. Simard, and D. Rioux. Canadian Forest Service - Quebec, P.O. Box 3800, Sainte-Foy, Quebec G1V 4C7, Canada.

Colloidal gold techniques were applied to elucidate the infection processes of *Gremmeniella abietina* in *Pinus resinosa* and *P. sylvestris* seedlings. The pathogen cells, surrounded by the fibrillar material of extracellular fungal sheath (EFS), colonized sparsely both intercellular and intracellular spaces of host bracts and short shoot tissues. Based on our results from gold-labelings using exoglucanase and antibodies against pectin or fungal laccase, the hyphae, except EFS, do not appear to contain cellulose or pectin, and we have indications that phenoloxidases secreted by the pathogen are involved in host cell wall degradation, likely increasing binding sites for gold probes. It is suggested that EFS of *G. abietina* may be implicated in host-pathogen interactions such as attachment of hyphae to the host surface and degradation of cellulose and pectin in host cell walls inside host tissues.

STRATEGIES FOR USING *GLIOCLADIUM ROSEUM* TO MANAGE GRAY MOLD IN CONTAINER-GROWN SEEDLINGS OF BLACK SPRUCE IN GREENHOUSES. P.G. Zhang, J.C. Sutton, and A.A. Hopkin*, Dept. of Environmental Biology, University of Guelph, Ontario N1G 2W1, Canada, and *Great Lakes Forestry Centre, Sault Ste. Marie, Ontario P6A 5M7, Canada.

Inoculum of *G. roseum* and the fungicide chlorothalonil were applied four times to seedlings of black spruce (*Picea mariana*) at intervals of 2- to 4-wk starting when the seedling canopies closed. Incidence of seedlings killed by the gray mold pathogen, *Botrytis cinerea*, was suppressed by 40, 42, 63, and 81% by *G. roseum* at inoculum concentrations of 10², 10⁴, 10⁶, and 10⁸ conidia/mL water plus surfactant respectively, and by 56% by chlorothalonil. Incidence of killed seedlings in the checks was 40%. Programs of 1 to 6 applications of *G. roseum* (10⁶ conidia/mL) and 6 of chlorothalonil, at 1- and 2-wk intervals, starting when the canopies closed, also were compared for biocontrol effectiveness. One application of *G. roseum* suppressed gray mold as effectively as did the fungicide program and 2 to 6 applications of the antagonist. We conclude that one application of *G. roseum* at canopy closure effectively suppresses gray mold for at least 12 wk.

BIORATIONAL COMPOUNDS IN RELATION TO BIOLOGICAL CONTROL OF *BOTRYTIS CINEREA* IN BLACK SPRUCE SEEDLINGS. P.G. Zhang, J.C. Sutton, and W. Tan*, Dept. of Environmental Biology, University of Guelph, Ontario N1G 2W1, Canada, and *Great Lakes Forestry Centre, Sault Ste. Marie, Ontario P6A 5M7, Canada.

Twenty-two inorganic salts, 18 carbohydrates, and 24 nitrogenous compounds (50 mM, 100 mg/L and 2 mM in water agar, respectively) were evaluated for their effects on *B. cinerea* and the biocontrol agent *Gliocladium roseum*. Calcium carbonate and dibasic potassium phosphate each suppressed germination, germ tube elongation and colony growth of *B. cinerea*, but did not of *G. roseum*. D-arabinose, L-alanine, L-glycine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-serine, L-threonine, L-tryptophane, and L-valine suppressed colony growth of *B. cinerea* but stimulated that of *G. roseum*. Black spruce seedlings that were environmentally predisposed to infection by *B. cinerea* were treated with the salts (50 mM) and *G. roseum* (10⁶ spores/mL) in water plus surfactant or with the conidial suspension only, kept in high humidity for two days, and challenge-inoculated with *B. cinerea*. In comparison to the *G. roseum* check, *G. roseum* plus ammonium carbonate and calcium carbonate suppressed sporulation incidence of *B. cinerea* on the needles by 61 and 76% and on the shoots by 20 and 39% respectively. These carbonate salts may have value in enhancing biocontrol of *B. cinerea* by *G. roseum*.