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Abstracts

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Alphabetized by first author's last name

INCREASING OCCURRENCE AND DAMAGE OF THE NORTHERN ROOT-KNOT NEMATODE ON ONIONS IN NEW YORK STATE. G. S. Abawi and P. D. Laird. Dept. of Plant Pathology, NYSAES, Cornell Univ., Geneva, NY 14456.

The incidence and severity of infections of the northern root-knot nematode (*Meloidogyne hapla*, NRKN) in onions have greatly increased in recent years, especially in the production areas of Central New York (Oswego and Yates counties). Symptoms on severely infected onions may include stunting, delayed maturity, larger necks, smaller bulbs, and the presence of enlargements or galls on roots. In one onion field in 1991, the weight of onions harvested from heavily infested areas was 20% lower than from areas with low nematode infestations in the same field. Results from a field microplot test in 1993 showed that the bulb weight of the cultivars Norstar, Paragon, and Walla Walla Sweet was reduced by 49, 55, and 40%, respectively when grown in organic soil infested with 20 eggs of NRKN/cm^3 soil. Research is underway to determine the damage threshold density of NRKN to onions, search for resistance sources, identify appropriate rotational crops, and evaluate non-fumigant nematicides, in order to develop an integrated management program.

CHARACTERIZATION AND DETECTION OF *ERWINIA TRACHEIPHILA* ISOLATES. M. J. Blua[1], F. E. Gildow[2], F. L. Lukezic[2], S. J. Fleischer[1], and D. de Mackiewicz[2]. [1]Dept. of Entomology, and [2]Dept. of Plant Pathology, Penn State University, University Park, PA 16802.

Ten isolates of *E. tracheiphila* were compared by their ability to metabolize carbon sources, their serological reactivity and pathogenicity. In Biolog assays (Biolog Inc., Hayward, CA) isolates metabolized 15-26 of 95 carbon sources. All isolates reacted with polyclonal antibodies produced against ATCC type isolate 33245 (DAS-ELISA), and caused wilt in cucumbers. No serological reactions were observed with *E. amylovora*, *E. carotovora*, *E. herbicola* and *E. stewartii*. After 1 year in culture on nutrient agar supplemented with peptone at room temp., isolates caused wilt. On nutrient agar with additional glucose, colonies grew rapidly but lost pathogenicity. Although the isolates vary in carbon metabolism, they are serologically similar and distinguished from other *Erwinia sp.* by ELISA. Detection of *E. tracheiphila* in wild plant species and cucumber beetles in greenhouse studies implicates their importance in survival and disease outbreaks.

FACTORS RELATED TO THE MECHANISM OF GRAPE CROWN GALL BIOLOGICAL CONTROL BY *AGROBACTERIUM VITIS* STRAIN F2/5. T. J. Burr, C. L. Reid, E. Tagliati* and C. Bazzi* Department of Plant Pathology, Cornell University, Geneva NY, 14456 and *Istituto di Patologia Vegetale, University of Bologna, 40126, Bologna Italy

It was previously demonstrated that a nontumorigenic strain of *A. vitis*, F2/5, suppresses crown gall at wound sites on grape cuttings when coinoculated with tumorigenic strains of *A. vitis*. F2/5 produces an agrocin inhibitory to most *A. vitis* strains in vitro. The role of agrocin and the relative ability of F2/5 to attach to wound sites on grape were investigated as mechanisms of biological control. Agrocin-minus F2/5 strains were generated by transposon mutagenesis using Tn5 carried in *E. coli* (pSUP2021). When F2/5 mutants were coinoculated with tumorigenic *A. vitis* on grape (1:1 ratio) the same level of gall suppression was achieved as with wildtype F2/5. When F2/5 was compared to tumorigenic strain CG49 for attachment ability on cut ends of grape shoots, no significant differences were found. By using the GUS reporter gene in CG49, it was determined that F2/5 prevents transformation of grape. The mechanism of biological control appears to be specific to grape, since coinoculation of F2/5 plus tumorigenic *A. vitis* on sunflower and *K. diagremintiana* results in tumor formation.

SUMMER PRUNING INCREASES PESTICIDE COVERAGE IN APPLE CANOPIES. Daniel R. Cooley and Susan Lerner, Dept. of Plant Pathology, Univ. of Mass., Amherst, MA 01003.

Summer pruning apple trees (*Malus domestica*) reduces flyspeck (causal agent: *Zygophiala jamaicensis*) even without summer fungicide applications. However, summer pruning may further improve disease control in sprayed orchards by allowing better fungicide penetration into the tree canopy. In this experiment, mature apple trees ('McIntosh' on M.7 spaced 6 X 9 m) in randomized blocks were summer pruned using standard commercial practices on 19 - 20 July. A week later water-sensitive papers were systematically placed in the trees. A commercial air blast sprayer delivering 1305 l/ha and traveling at 4 km/h then applied water to the trees. Resulting spray deposition patterns were digitized using a flatbed scanner. Digitized images were analyzed using public domain image analysis software (Image 1.55) on a personal computer. Coverage in mid-(2.5 m) and upper canopies (3.5 m) was approx. 60% greater in pruned trees, but was not different in the lower canopies (1.5 m). Coverage was not different for inner vs. outer canopies in pruned and non-pruned tree. Thus summer pruning significantly improved spray coverage in apple trees. The simple, inexpensive image analysis techniques used should be useful for other research where spray coverage or splash dispersal are to be evaluated.

COMPARISON OF MICROORGANISMS FOR EPIPHYTIC SURVIVAL ON APPLE AND AS BIOLOGICAL CONTROLS OF APPLE SCAB. M.R. Corral Garcia, T.J. Burr, C.A. Smith, M.C. Matteson. Department of Plant Pathology, Cornell University, NYSAES, Geneva, N.Y. 14456.

Pseudomonas syringae strain 508 and two yeasts (*Rhodotorula* sp. strains 79B and 115B) were isolated from apple leaves and shown to suppress apple scab in greenhouse experiments. They were tested for epiphytic competence on apple leaves and fruits, and for control of apple scab in an orchard. Three single branches, with at least 25 fruit clusters each, were thoroughly sprayed with suspension of each microorganism (about 10^8 cfu/ml) in phosphate buffer or in half strength Potato Dextrose Broth. Captan (1.2 g/l) was included as a control treatment and each treatment was replicated 3 times. The incidence of scab on cluster leaves, but not on fruit, was significantly lower with 79B than with 508 and 115B. Adding PDB did not improve disease control nor population establishment on apple leaves over a 5-day period. Epiphytic populations were compared at 3 times during the growing season of 1994. 79B established the highest populations on apple leaves (about 10^8 cfu/g) with the least variation between individual leaves, followed by strain 115B. 508 established lower populations that were highly variable between leaves.

ELICITOR-INDUCED CHANGES OF DEFENSE-RELATED ENZYME ACTIVITIES IN TOMATO CELLS. N. Degousee,E. Blumwald and V. J. Higgins,Dept. of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2.

Intercellular fluid (IF) obtained from tomato (*Lycopersicum esculentum* L.) leaflets colonized by *Cladosporium fulvum* Cooke contains specific elicitors that induce necrosis in tomato cultivars resistant to the race of C. *fulvum* used to produce the IF. Vera-Estrella *et al.* previously reported that one race-specific elicitor, the putative product of the avirulence gene *avr*5 of C. *fulvum* induced rapid changes at the cell surface, including production of active oxygen (AO) species, increased lipid peroxidation and increased peroxidase activity, in tomato cells carrying the complementary resistance gene C*f* 5. To determine if these early events could be involved in the activation of inducible defense responses, we investigated the changes of defense-related enzyme activities in C*f* 5 cells treated with race 4 IF (containing *avr*5 product). Increased activity of chitinase, β-1,3-glucanase and peroxidase was detected by 3-5 hours following the elicitor treatment, whereas no changes in activity of phenylalanine ammonia-lyase, superoxide dismutase and catalase were observed. Preliminary results suggest that AO species are not involved in the regulation of the activity of chitinase and β-1,3-glucanase. Experiments are currently underway to study directly the regulation of the expression of a few of these defense-associated genes.

EFFECTS OF BEAN YELLOW MOSAIC POTYVIRUS (BYMV) ON WHITE LUPIN (*LUPINUS ALBUS* L.) and *BRADYRHIZOBIUM* SYMBIOSIS. R.S.Y. de Silva and J.F. Peterson, Department of Plant Science, Macdonald Campus of McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue, Québec, Canada, H9X 3V9.

We have investigated the interaction of white lupin (*Lupinus albus* L.), *Bradyrhizobium* and bean yellow mosaic potyvirus (BYMV). When host plants were infected by BYMV before inoculation with *Bradyrhizobium*, root growth was not appreciably affected but fewer nodules developed. Neither nodule size nor nitrogenase activity of individual nodules was affected but diseased plants showed lower rates of nitrogen fixation (acetylene reduction) a few weeks after viral infection. Nodules of infected plants deteriorated more rapidly than those of healthy plants. BYMV retarded aerial plant growth and delayed or inhibited flowering. The virus appears to affect the entry of *Bradyrhizobium* into roots or nodule initiation but not the nitrogenase activity of individual nodules.

EFFECTS OF MN-REDUCING BACTERIA ON FUSARIUM CROWN AND ROOT ROT OF ASPARAGUS. W. H. Elmer, Conn. Agr. Exp. Sta., Box 1106, New Haven, CT 06504.

Seven strains of bacteria that varied in their ability to reduce Mn-dioxide to Mn^{+2} were evaluated in the greenhouse for their ability to affect Fusarium crown and root rot of asparagus. Roots of 3-mo-old plants were dipped in a bacterial suspension (10^8 cells/ml) for 30 min. prior to being placed in noninfested potting mix or a mix that was infested with *Fusarium oxysporum* and *F. proliferatum*. After 10 wk, plants that were grown in infested soil and treated with Mn-reducing strains of *Pseudomonas corrugata*, *P. fluorescens* and *Rahnella aquatilis* had fewer diseased roots and greater root lengths than nontreated plants grown in infested soils. Compared to untreated plants that were grown in noninfested soils, plants that were treated with these strains had more Mn/g root. When plants that were grown in infested soil were treated with a non Mn-reducing strain of *R. aquatilis*, more disease resulted than in similarly grown plants that were not treated. Healthy plants grown in noninfested soils and treated with the non Mn-reducing strain had less Mn/g root than healthy plants not treated with this strain. A strong correlation ($r = 0.78$) was detected between the amount of Mn/g root and diseased root length.

STUDIES OF PROPHYLACTIC PROPERTIES OF SOLUBLE SILICON IN THE CUCUMBER-POWDERY MILDEW INTERACTION. A. Fawe, J. G. Menzies, R. Bel-Rhlid, C. Labbé, and R. R. Bélanger. P.A.R.C., Agassiz, B.C. VOM 1AO. CRH, Université Laval, Sainte Foy, P.Q. G1K 7P4.

Amendement of soluble silicon (Si) to nutrient solutions has be -en reported to increase the resistance of cucumber to diseases. However, the mechanism(s) by which Si would act on the plant re main unclear. It has been shown that the production of lytic en zymes and fungitoxic compounds increases during the Pythium-cucumber interaction when plants are fed with Si. In the present study, the production of toxic compounds was analysed in the leaves of plants treated or untreated with Si and infected or not with Sphaerotheca fuliginea. Using TLC and HPLC techniques, it appears that some constitutive fungitoxic compounds are produced in greater amounts in Si plants exposed to the pathogen. Futhermore, other toxic compounds are induced de novo in infected plants as a result of Si treatment. These results reinforce the hypothesis that prophylactic properties conferred by Si are greatly dependent on activation of plant metabolic processes.

RELATION OF VISIBLE AND PHYSIOLOGICAL FOLIAR INJURY FROM OZONE EXPOSURE IN HARDWOOD TREE SPECIES. T.S. Fredericksen, and J.M. Skelly. 220 Forest Resources Laboratory, Pennsylvania State University, University Park, PA 16802.

Absorbed through leaf stomata, ozone disrupts cell membranes and causes the collapse of palisade mesophyll cells producing visible symptoms on the adaxial leaf surface. Some studies indicate that physiological or "hidden" injury (e.g., decreased photosynthesis) may occur either before or without the onset of visible symptoms. Conversely, visible symptoms may not necessarily be indicative of significant physiological injury. We tested the correlation between visible and physiological foliar injury on two ozone-sensitive hardwood tree species black cherry (Prunus serotina Ehrh.) and yellow poplar (Liriodendron tulipifera L.) by conducting simultaneous measurements of visible symptoms ratings and leaf gas exchange. Visible symptoms were negatively related to net photosynthesis ($r^2 = .80$) for both species. Net photosynthetic rate decreased up to 14% before visible symptoms were evident.

RESISTANCE EVALUATION OF TRANSGENIC PLANTS EXPRESSING VIRUS COAT PROTEIN GENES AND RISK ASSESSMENT OF VIRUS SPREAD. M. Fuchs, F. E. Klas and D. Gonsalves. Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456.

Transgenic tomatoes expressing the coat protein (CP) gene of cucumber mosaic virus (CMV) and transgenic squashes containing the CP genes of CMV, zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus II (WMV II) were evaluated over a two year period under field conditions with heavy disease pressure achieved by mechanical inoculations and by natural infections by aphid vectors. Our data show that these transgenic crops have a tremendous potential to control CMV, ZYMV and WMV II infections. Therefore, demand for their commercial release is increasing. However, potential risks related to heteroencapsidation and recombination have been raised. We assessed risks of virus spread using transgenic tomato, squash and melon plants expressing the CP gene of the aphid transmissible strain CMV-WL. These transgenic plants showed variable levels of resistance when challenge inoculated with the non-aphid transmissible strain CMV-C. So far our results indicate that none of the transgenic plants studied did appear to mediate the spread of CMV-C under field, screenhouse and greenhouse conditions.

CONTINUED MIGRATION OF A2 MATING TYPE, METALAXYL-RESISTANT GENOTYPES OF *PHYTOPHTHORA INFESTANS* IN THE EASTERN UNITED STATES AND CANADA. S. B. Goodwin and W. E. Fry, Department of Plant Pathology, 334 Plant Science, Cornell University, Ithaca, NY 14853.

Severe late blight epidemics, caused by *Phytophthora infestans*, occurred on potato and tomato in eastern North America in the summer of 1994. Only three multilocus genotypes, US-1, US-7 and US-8 (determined by analyses of mating type, two allozyme and 25 DNA fingerprint loci) were identified among 89 isolates collected from nine states (FL, GA, SC, NC, VA, PA, NY, ME, ND) and one Canadian province (NB). Two genotypes were detected in seed potatoes imported into New York in Spring 1994: US-1 (A1 mating type, metalaxyl sensitive), and US-8 (A2, metalaxyl resistant). US-8, the most commonly detected genotype on potatoes in the field, was found in six states (FL, GA, NC, PA, NY, ME) plus NB. US-1 was detected in only one potato field in eastern NC. US-7 (A2, metalaxyl resistant) was the only genotype recovered from tomatoes (in FL, SC, VA). This is the first report of A2, metalaxyl-resistant isolates in GA, SC, VA and NB. There was no evidence for sexual recombination in any location. This may be due at least in part to the use of metalaxyl which probably eliminated the A1 (metalaxyl-sensitive) genotype from most locations.

MOLECULAR EPIDEMIOLOGY OF *GREMMENIELLA ABIETINA*. Richard C. Hamelin, Nicole Lecours, Per Hansson*, Magnus Hellgren**, and Gaston Laflamme. Natural Resources Canada, Ste-Foy, Qc, Canada; Swedish University of Agricultural Sciences, *Department of Silviculture,Umeå, Sweden; **Department of Forest Mycology and Pathology, Uppsala, Sweden.

Nine random amplified polymorphic DNA markers were variable within the EU race of *Gremmeniella abietina* var *abietina* in Europe. Three distinct amplitypes were correlated with geographic or ecotypic origin. The northern amplitype was present exclusively north of the 66° latitude on *Pinus sylvestris* and *P. contorta* and appeared to be adapted to the northern climate. A southern amplitype was found exclusively in the Alps at altitudes above 2500 m on *P. cembra*, *P. mugo*, *P. sylvestris* and on *Larix lyalli* and also appeared to represent an ecotype adapted to colder conditions. The third amplitype was present throughout Europe from the scandinavian countries to the Appenines mountains of northern Italy. The EU race present in North America belonged to this amplitype and the DNA profiles indicated that the origin of this introduced pathogen was central Europe. Ribosomal DNA restriction and RAPD profiles indicated that the NA race was absent from the samples from Europe.

ALTERING EXPRESSION OF THE NATIVE-INDUCIBLE ENDOCHITINASE GENE IN TRICHODERMA HARZIANUM. Christopher K. Hayes, Whachun Yoo, Gary E. Harman. Department of Horticultural Science. Cornell University, NYSAES, Sturtevant Hall, Geneva, NY. 14456.

Specific strains of Trichoderma harzianum have been shown to have biocontrol capabilities. The production of inducible, extracellular, hydrolytic enzymes, e.g. chitinases and glucanases, have been implicated as a possible mechanism of biocontrol. A gene coding for an endochitinase has been isolated from T. harzianum, strain P1. Endochitinase-minus mutants were recovered when transforming P1 with a portion of this gene. Hyperproduction of the endochitinase gene under expression of the constitutive 35S CaMV promoter was also attempted. Both P1 and an endochitinase-minus mutant of P1 were transformed with this vector. Transformants, which were grown under noninduced conditions, were recovered, with expression beginning on day one.

EFFECT OF GROWTH PHASE AND MEDIUM ON SENSITIVITY OF RESISTANT AND SENSITIVE *PSEUDOMONAS SYRINGAE* AND *ERWINIA AMYLOVORA* TO STREPTOMYCIN. T.C. Huang, and T.J. Burr, Department of Plant Pathology, Cornell University,Geneva NY 14456

Streptomycin-resistant and sensitive strains of *Pseudomonas syringae* pv. *papulans* (Psp), and pv. *syringae* (Pss) were grown in terrific broth and cells were pelleted by centrifugation during log (OD_{600} 0.1 to 0.9) and stationary (OD_{600} 1.1 to 1.3) growth phases. Cells were then either dilution plated on Pseudomonas Agar F (PF) amended with streptomycin (50-500 µg/ml) or suspended in aqueous streptomycin sulphate of different concentrations for 2 hr and then dilution plated on nonamended PF. A very high proportion of resistant Psp and Pss in log phase were killed by 25-50 µg/ml aqueous streptomycin whereas those in stationary phase survived. The same growth phase-sensitivity relationship was observed with sensitive Psp and *Erwinia amylovora* (Ea) but not with chromosomally-encoded resistant Ea. When all strains grown in broth were plated directly on streptomycin-amended PF, growth phase did not affect sensitivity to streptomycin. Streptomycin activity was greatly reduced when dissolved in terrific broth or solutions of yeast extract (24 g/L), KH_2PO_4 or K_2HPO_4 (0.02M), or phosphate buffer (0.02M).

SPATIAL ANALYSIS AS A TOOL TO EVALUATE VIRUS RESISTANCE IN A TRANSGENIC CROP. F. E. Klas, M. Fuchs and D. Gonsalves. Department of Plant Pathology, Cornell University, NYAES, Geneva, NY 14456

Field plots comprising a solid block of transgenic squash Pavo ZW20-B expressing the coat protein genes of zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus II (WMV II), and a solid block of nontransformed Pavo were analyzed for virus spread. Both plots were surrounded with a row of ZYMV and WMV II infected plants. Rate of symptom appearance in test plants were monitored weekly. The plot with nontransformed plants showed severe infection in 30 and 93% of the plants three and six weeks after planting, respectively. In contrast, the transgenic plants did not show any severe symptoms throughout the trial period, but 12 and 34% of the transgenic plants showed only chlorotic dots on leaves three and six weeks after planting. ELISA analysis of the symptomatic transgenic plants detected virus in tissue with chlorotic dots but not in tissue between the dots nor in symptomless leaves. Geostatistical analysis of virus spread revealed a plant to plant spread of both viruses in the plot with nontransformed plants but not in the plot with transgenic plants. The analysis also showed a preferential spread for ZYMV and that spread of both viruses were preferential in a certain direction.

METHODS FOR SCLERODERRIS CANKER INOCULATION UNDER CONTROLLED CONDITIONS. **Gaston Laflamme and Guy Bussières[1]. Natural Resources Canada, Ste.Foy, QC, G1V 4C7 Canada. [1],Faculté de Foresterie, Université Laval, Ste.Foy, Qc, G1K 7P4 Canada.**

Scleroderris canker caused by Gremmeniella abietina has mainly been studied under field conditions. Marosy et al. (Phytopath. 79:1290-1293) have succeeded in inoculating red pine seedlings with G. abietina under indoor conditions and observed symptoms in less than two months. Low temperature (4°C) during a latent period of 56 days was a critical factor. After many trials with this technique, the results obtained were not consistent. Experiments conducted under variable conditions of temperature and relative humidity (RH) show that high RH is the other critical factor for infection. At 1°C, we had successful infections at 90%RH while infection failed at 80% RH. When these infected seedlings were exposed to the greenhouse conditions, pycnidia were produced on needles after few days. We were able to reproduce the complete cycle in 10 weeks.

GENETIC CONTROL OF AGGRESSIVENESS TO TOMATO IN PHYTOPHTHORA INFESTANS. T.Yun Lee and W.E.Fry, Department of Plant Pathology, Cornell University, Ithaca, NY14853.

To determine the genetic basis of aggressiveness to tomato in *Phytophthora infestans*, several sexual crosses were made among isolates aggressive and not aggressive to tomato in all possible combinations. Each cross produced more than 40 recombinant progeny [identified by allozymes (glucose-6- phosphate isomerase and peptidase) and mating type markers]. Aggressiveness to tomato and potato was measured on detached leaflets . The analysis of progeny of a cross between isolates that were aggressive toward both potatoes and tomatoes revealed little if any segregation. Similarly, analysis of a cross between isolates that were each aggressive only to potatoes also indicated that potato aggressiveness did not segregate. However, there appeared to be segregation for aggressiveness to tomatoes in progenies from two other crosses involving one parent that was aggressive toward both potatoes and tomatoes, and one parent that was aggressive primarily toward potatoes. Additional crosses are necessary to clarify the genetic control of tomato aggressiveness in *P. infestans*.

IDENTIFICATION OF COAT PROTEIN GENE AND PARTIAL GENOME ORGANIZATION OF GRAPEVINE LEAFROLL-ASSOCIATED CLOSTEROVIRUS TYPE III. Kai-Shu Ling, Roger F. Drong[1], Jerry L. Slightom[1] and Dennis Gonsalves. Department of Plant Pathology, Cornell University, Geneva, NY 14456. [1]Molecular Biology Research, Unit 7242, The Upjohn Company, Kalamazoo, MI 49007

A phage lambda ZAPII cDNA library of grapevine leafroll-associated closterovirus (GLRaV) type III RNA genome was established by cloning of the cDNA generated from GLRaV-specific double-stranded RNA. About half of the 20 kb genome has been sequenced thus far. Partial genome analysis revealed that this portion of the genome sequence has its closest relationship to another closterovirus genome, beet yellows virus (BYV). A gene region that shares identity with the heat shock protein 70 gene family was identified and sequenced. In addition, gene sequences that share identity with helicase as well as polymerase genes were also identified. By immunoscreening the cDNA library with GLRaV III-specific polyclonal as well as with monoclonal antibodies, we were able to identify three antibody positive clones. In Western blot analysis one of these clones was shown to express a fusion protein that has a molecular weight similar to the native coat protein (43 kDa).

BIOLOGICAL CONTROL OF PYTHIUM, RHIZOCTONIA, AND SCLEROTINIA INCITED DISEASES OF TURFGRASS WITH TRICHODERMA HARZIANUM (1295-22).
C.-T. Lo, E. B. Nelson, and G. E. Harman. Department of Plant Pathology and Horticulture Science. Cornell University, Geneva, NY 14456 and Ithaca, NY 14853

Trichoderma harzianum Rafai is a bioprotectant that has been tested widely against many fungi. The isolate of *Trichoderma harzianum* (1295-22) is registered for several uses with the US EPA. Its ability to control diseases of turfgrass, i.e. pythium blight caused by *Pythium graminicola*, brown patch caused by *Rhizoctonia solani*, and dollar spot caused by *Sclerotinia homoeocarpa*, was evaluated in the greenhouse. *T. harzianum* applied as granular formulation to soil reduced disease and aqueous suspensions of conidia used as a foliar spray almost completely prevented spread of all three pathogens. Thus, the rhizosphere competent bioprotectant establishes suppressive soils and conidial sprays control residual disease. Preliminary field data indicates that the fungus is highly effective when applied to a putting green. Thus, this organism has potential as a biological alternative to fungicides on highly managed golf course turf.

***IN PLANTA* EVIDENCE FOR THE INVOLVEMENT OF ACTIVE OXYGEN SPECIES IN ELICITOR-INDUCED DEFENSE RESPONSES OF TOMATO.** H. Lu and V. J. Higgins, Department of Botany, University of Toronto, 25 Willcocks St., Toronto, Ontario, Canada, M5S 3B2

Injection of intercellular fluids (IF) (containing AVR9 elicitor), obtained from tomato leaves infected with *Cladosporium fulvum* race 4, into the leaves of tomato near-isogenic line Cf9 induces necrosis. Injection of race 4 IF mixed with 0.5 mM pyranine, a fluorescent compound which can be oxidized by H_2O_2 in the presence of peroxidase, resulted in significant reduction of fluorescence by 1.5 to 3 hr, as compared to injection with control IF mixed with pyranine. This is indicative of generation of H_2O_2 upon the interaction of elicitor and leaf cells. The quenching of pyranine fluorescence was estimated both by observation under UV light and by a spectrofluorimeter. The leaf necrosis induced by elicitor was partially inhibited by co-injection with several antioxidants: 10 mM ascorbate, 5 mM α-tocopherol, or 13 mM mannitol but not by catalase, SOD, and SHAM. Peroxidase activity in elicitor-injected tomato leaves was increased over control values by 3 hr after injection. These results support previous data, based on cell cultures, which suggested that active oxygen species may be involved in defense responses of tomato plants to race specific elicitors of *C. fulvum*.

WEATHERCELL 2000 - A SOLAR-POWERED PORTABLE WEATHER STATION EMPLOYING CELLULAR TRANSMITTING TECHNOLOGY FOR USE IN THE BLIGHT-ALERT FORECAST SYSTEM FOR BOTRYTIS LEAF BLIGHT OF ONION. Sean P. Magnuson and James W. Lorbeer, Department of Plant Pathology, Cornell University, Ithaca, New York 14853.

A portable electronic weather monitoring station named Weathercell 2000 was developed for use in the BLIGHT-ALERT forecast system for Botrytis leaf blight of onion. The primary components of Weathercell 2000 are a Davis Instruments Weather Monitor II (WMII) microprocessor and monitoring attachments, a cellular phone, a cellular modem, and a 50 watt solar panel with associated battery charging circuitry. The WMII was chosen because of its relative low cost and available Davis Instruments software program (Weatherlink) for downloading and analyzing weather data. The WMII can be directly connected to a Macintosh or IBM computer or to a modem for monitoring and downloading. The memory archive on the WMII can be set at 10 to 30 min intervals between readings allowing archive operation for one week to one month, respectively.

A LIMITED PERIOD OF SEED SUSCEPTIBILITY TO *PYTHIUM* INFECTION. A. P. Maloney, C. M. Stockwell, and E. B Nelson, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

As part of our interest in the ecology, biochemistry, and biological control of *Pythium* seed rot and damping off, we have evaluated conditions under which cucumber seeds are susceptible to *Pythium ultimum*. The aim of the current experiments was to determine when seeds become infested by the pathogen, and whether they remained uniformly susceptible during their germination and early growth. Cucumber seeds were planted in *Pythium*-infested soil with controlled temperature and soil matric water potential, and transplanted to sterile soil after various times. Seeds began to be colonized by the pathogen within 3 hours after sowing. Seedling populations sustained 80 to 100% loss of stand with 8 h exposure to infested soil. Pathogenicity levels were nearly equivalent in soil with matric potentials of -5 and -10 kPa. *Enterobacter cloacae* strain EcCT-501 was used in some experiments to suppress *P. ultimum* seed rot. In *Pythium*-infested soil (300-400 cfu/g), 0.5 ml suspensions of EcCT-501 (2×10^8 cfu/ml) controlled seed rot; 1/2 log lower concentrations were much less effective.

QUICK THAWING RATES: ANOTHER CONDITION INFLUENCING FREEZING INJURY IN RED SPRUCE. Daniel K. Manter & William H. Livingston, Dept. of Forest Ecosystem Sci., Univ. Maine, Orono, ME 04469.

Freezing injury is a major factor implicated in red spruce (*Picea rubens*) decline. Reports on freezing injury in red spruce needles have not considered the role of thawing rate. Preliminary studies showed surface temperatures of red spruce needles increased from ca. -6°C to 0°C in 20 min during exposure to sunlight after sunrise. Similar thawing rates were obtained on uncovered tissue removed from a freezer to a +3°C chamber. The latter method was used on 80 red spruce seedlings (3 yrs old) that were frozen to temperatures ranging from -5°C to -45°C. Half the seedlings were covered with plastic bags which doubled the thawing period. Freezing damage was estimated using (i) relative conductivity, (ii) chlorophyll content 10 and 21 days after freezing, and (iii) gas exchange (CO_2) 21 days after freezing. Covered seedlings were damaged at -35°C and -45°C ($P < 0.05$). Faster thawing rates on uncovered seedlings increased damage up to ca. 3 fold. Thawing rates can influence freezing damage on red spruce needles.

SUPPRESSION OF *FUSARIUM SAMBUCINUM* BY *BRASSICA* SPECIES. H. S. Mayton, R. Loria and S. F. Vaughn. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853 and USDA-ARS NCAUR, Peoria, IL 61604.

Brassica species were tested for production of volatile compounds inhibitory to a potato dry rot pathogen, *Fusarium sambucinum* (FS). Accessions of *B. juncea*, *B. carinata*, *B. nigra* and *B. napus* were screened for suppression (> 50% inhibition of radial growth) using an *in vitro* bioassay for volatile activity. Newly transferred cultures of FS were exposed for 7 days to 40 g of chopped leaf tissue and radial growth was measured. The concentration of allylisothiocyanate (AITC), a breakdown product of allylglucosinolate, was measured in the leaf tissue using gas chromatography. AITC was the most abundant glucosinolate breakdown product found in *B. nigra*, *B. juncea*, and *B. carinata*. Suppression of radial growth of FS was highly correlated with the concentration of AITC in the plant tissue. Accessions of species with the highest concentrations of AITC (> 0.10mg/g fresh tissue) were suppressive to FS in the bioassays, while those with little or no AITC were not suppressive.

FUNGICIDES PROVIDED INSUFFICIENT SUPPRESSION OF PHYTOPHTHORA FRUIT ROT OF CUCURBITS WHEN DISEASE PRESSURE WAS HIGH. M. T. McGrath, Dept. of Plant Pathology, Long Island Horticultural Research Laboratory, Cornell University, Riverhead, NY 11901.

Fields of sandy loam soil where Phytophthora fruit rot of pumpkin occurred the previous year were used to evaluate chemical control programs in 1992 - 1994. Fungicides were applied from the start of fruit formation. Metalaxyl and fosetyl-Al were applied every 14 days until plants started to senesce. Metalaxyl was also applied at planting for some treatment groups. Copper hydroxide and fluazinam were applied every 7 days. Some treatment groups included copper hydroxide combined with either metalaxyl or fosetyl-Al in one of the following 3 ways: tank mixed with the systemic fungicide, applied alone late in the growing season after 3 applications of the systemic fungicide, or both tank mixed and applied late in the season. Symptoms were observed in most plots after 4-7 days of rain, on 18 Aug 1992 (13 days after the first application), on 28 Sept 1993 (4 weeks after the last systemic fungicide application), and on 31 Aug 1994 (5 days after the third application). Non-treated plots had more rotten fruit and more defoliation than fungicide-treated plots on most assessment dates, but these differences were not significant. At least 50% of the fruit had rotted for all treatment groups by 18 Sept 1992 (85% rotten fruit for non-treated plots, 52-63% for fungicide-treated), 14 Oct 1993 (80% for non-treated plots, 50-74% for fungicide-treated), and 8 Sept 1994.

BANNER 1.1 EC PREVENTS INFECTION OF *PICEA PUNGENS* BY *CHRYSOMYXA WEIRII*. W. Merrill, 211 Buckhout Lab, University Park, PA 16802.

This study was done in an affected *Picea pungens* Christmas tree plantation in Luzerne County, PA. On 15 May 1993, when symptoms had developed on the 1992 needle complement, ten pairs of trees with approximately equal incidence of infection by *Chrysomyxa weirii* on each pair were selected with one tree of each pair in different planting blocks. One block was used as the check; the other was treated with Banner® 1.1 EC applied with a Solo® backpack mist blower at the rate of 1.17 liter f.p./H on 17 May when approximately 10% of the trees had broken some buds, 25 May, and 1 June 1993. On 13 May 1994, when symptoms were well developed but before onset of needle casting, the percentage of infected needles was determined on four 1993 internodes removed from each sample tree, one from each cardinal direction at 0.5 m from the ground. This schedule of fungicide applications reduced infection; untreated checks averaged 24.5% and treated trees averaged 1.7% of their needles infected, significantly different at $P = 0.001$.

INTERNAL SPREAD OF *ERWINIA AMYLOVORA* THROUGH SYMPTOMLESS APPLE SCION TISSUES INTO THE ROOTSTOCK M. T. Momol, E. A. Momol, J. L. Norelli, H. L. Gustafson, J. N. Cummins*, and H. S. Aldwinckle. Depts. of Plant Pathology and Horticultural Sciences*, Cornell University, Geneva, NY 14456.

Infection of susceptible apple rootstocks (e.g. M.9 and M.26) by *E. amylovora* (*Ea*) is an important problem in N. American and European orchards. An hypothesis for infection of rootstocks is by spread of *Ea* cells through internal tissues of symptomless scion shoots, down into the rootstock. Tips of potted single-shoot Empire, Golden Delicious (GD) and Summerland McIntosh trees on M.26 were inoculated in the greenhouse with *Ea* strain Ea273, and then periodically assayed for *Ea* in 1-, 2- and 3-yr-old scion tissues and in the rootstock. 1-cm long segments were surface sterilized, ground in buffer, and plated on semi-selective media. Grindates were also plated on Nutrient Agar; after 48 hr washings were assayed by PCR using 17-mer primers from the *Ea* 29kb plasmid (from K. Geider). Within 12 days of inoculation, *Ea* was detected in symptomless 1-yr-old scion tissue > 50 cm below the shoot-tip in all 3 cvs, and in 2-yr-old tissue in GD. By 22 days, it was detected in the M.26 rootstock of Empire trees.

INCREASED RESISTANCE TO *ERWINIA AMYLOVORA* OF ATTACIN E-TRANSGENIC M.26 APPLE ROOTSTOCK IN A FIELD TRIAL. M.T. Momol, J.L. Norelli, J.N. Cummins*, E.A. Momol, and H.S. Aldwinckle. Depts. of Plant Pathology and Horticultural Sciences*, Cornell University, Geneva, NY 14456.

T1 is a transgenic M.26 apple rootstock that has been transformed with the gene encoding the lytic protein, attacin E. Previously, greenhouse trials have shown that T1 has increased resistance to *Erwinia amylovora*, the incitant of fire blight. Ungrafted plants of T1, T791 (M.26 transformed with the pBI121 vector plasmid, not containing the attacin E gene), and M.7 (moderately resistant check) were planted in a field plot in May 1993. On 22 June 1994 tips of vigorously growing shoots of 2-yr old plants were inoculated by hypodermic syringe with 5×10^6 cfu/ml of *E. amylovora* strains E4001A and Ea273. Proportion of shoot length necrotized was observed over time, and area under the disease progress curve (AUDPC) and Y_{max} were calculated. After inoculation with E4001A, AUDPC was T791:1199, T1:654, and M.7:479; respective Y_{max} values were 0.62, 0.38, and 0.25. Disease progress on Ea273-inoculated plants was similar. These results indicate that T1 has increased resistance to *E. amylovora* in the field.

BEAN ANTHRACNOSE DEVELOPMENT IN DIFFERENT TILLAGE PRACTICES. N. Ntahimpera, H. R. Dillard, A. C. Cobb, and R. C. Seem, Cornell University, New York State Agricultural Experiment Station, Dept. of Plant Pathology, Geneva, NY 14456.

Bean anthracnose, caused by *Colletotrichum lindemuthianum*, overwinters in infested bean plant debris in New York State. Three tillage practices - chisel, moldboard plow, and rotovator - were evaluated to determine their impact on initial disease development and disease progression over time. The treatments were superimposed in spring in a field previously infected with *C. lindemuthianum*, race β. The susceptible light red kidney bean cultivar, Horizon, was planted in plots of the 3 treatments in 3 replications. The highest initial disease level was observed in chiseled plots, where more bean debris was left on the surface, than in the other treatments. It was also noted that the chisel treatment developed higher final disease and lower yield than the treatments where debris were buried 10 or 20 cm in soil. Ordinary runs analysis showed that the disease occurred randomly early in the season, confirming that initial inoculum of bean anthracnose was from bean debris from the previous season.

AN UNEXPECTED MATING TYPE BIAS IN ROADSIDE POPULATIONS OF THE ANTHER SMUT FUNGUS, *USTILAGO VIOLACEA*. P. Oudemans and H.M. Alexander, Rutgers Blueberry and Cranberry Research Center, Oswego Lk. Rd. Chatsworth, NJ 08019

The anther smut fungus, *Ustilago violacea*, has a bipolar sexual compatibility system governed by two alleles, A1 and A2. In a survey of 6 populations of *U. violacea* near Mtn. Lake, VA, 252 infected flowers were sampled. From each flower, 20 single sporidial lines were isolated and the mating types determined. Of these, 48% produced sporidia of the A1 mating type only. All other samples produced equal ratios of A1 and A2. Biased mating type ratios were found in 5 of the 6 populations sampled and ranged in frequency from 8% to 98%. Analysis of the spatial distribution of biased individuals revealed a nonrandom distribution, suggesting a genetic basis. Matings between biased A1 and non-biased A2 sporidia always resulted in non-biased segregation of mating type. Microscopic examination of germinating biased teliospores revealed conjugation occurring between the two most basal cells of the probasidium leaving the terminal cell to produce sporidia. Thus a new mechanism for inbreeding is proposed.

BICARBONATES AND BOTRYTIS: IV. ADDITIONAL CHARACTERIZATION OF EFFECTS ON GERMINATION OF *BOTRYTIS CINEREA* CONIDIA. C. L. Palmer, R. K. Horst, H. W. Israel, and R. W. Langhans. Depts. of Flor. & Orn. Hort. and of Plant Path., Cornell University, Ithaca, NY, USA 14853.

Bicarbonate-treated *Botrytis cinerea* Pers. conidia after incubating 24 h have been shown to germinate significantly less frequently than untreated conidia. To test whether bicarbonate activity occurs with less exposure time, we quantitatively contrasted germination at 0, 1, 9, and 24 h. Conidia were placed onto agar/collodion coated glass microscope slides sprayed with 0 or 50 mM NH_4HCO_3 in 2% potato dextrose broth. After incubation at 100% RH, inoculated slides were stained with a solution containing Calcein AM and Ethidium homodimer I to determine spore viability. Total spores and germinated spores were counted; ungerminated conidia were placed into the following categories: alive, dying, dead, and impermeable. Over time, germination increased for both treated and untreated conidia. Percent ungerminated conidia in each category remained constant for 0 mM. With NH_4HCO_3, percent alive conidia decreased while dying, dead, and impermeable conidia increased. We hypothesize that for *B. cinerea* conidia NH_4HCO_3 prevents germination via fungicidal and fungistatic activities.
(Supported by H&I Agritech Inc., Ithaca, NY 14850.)

A SIMPLIFIED RAPD METHOD FOR FUNGI. Zheng Pan and R. L. Wick, Department of Plant Pathology, University of Massachusetts Amherst, MA 01003.

Random amplified polymorphic DNA (RAPD) analysis has been recently introduced as an aid for classification of species and disease diagnosis. Usually the detergent hexadecyltrimethylammonium bromide (CTAB) and CsCl gradient centrifugation are used to get high quality genomic DNA. We cultured *Phytophthora capsici* directly in 1.5 ml microcentrifuge tubes, isolated genomic DNA by the phenol/chloroform method, eliminated the CTAB and CsCl steps, diluted the DNA, and carried out RAPD with reproducible results. Dilution of the sample DNA allowed the elimination of the CTAB and CsCl steps. The optimum amount of DNA in the reaction volume was the maximum amount of sample DNA which did not inhibit PCR (40-80 pg/12.5 μl). This procedure was also used successfully for *Pythium* sp. and *Fusarium oxysporum* f. sp. *basilicum*.

DISSECTION OF ENGINEERED RESISTANCE TO TOSPOVIRUSES. S.-Z. Pang, C. Gonsalves, J.L. Slightom[1] and D. Gonsalves. Department of Plant Pathology, Cornell University, NYSAES, Geneva, NY 14456, USA. [1]Molecular Biology Research, Unit 7242, The Upjohn Company, Kalamazoo, MI 49007. USA

Transgenic tobacco plants can be protected against tospoviruses either by the transcripts of their nucleocapsid (N) protein genes or by their translated N proteins. The N gene RNA, when expressed at low levels, completely protects against the homologous virus and close relatives whereas high levels of N protein are required for inoculum strength-dependent resistance to the homologous and distantly related viruses. The present study expands our previous results by testing plants expressing various short N gene fragments for resistance to several related viruses. This and other relevant results provide some useful mechanistic insights for engineered resistance to viral infection. We will also discuss the correlation that we previously found between the N gene expression level and resistance in other crop systems.

DEVELOPMENT OF TOLERANT PAPAYA CULTIVARS TO CONTROL PAPAYA RINGSPOT VIRUS IN NORTHEAST THAILAND, AND MEASUREMENT OF THE SOCIOECONOMIC IMPACT ON LOCAL VILLAGE FAMILIES. Vilai Prasartsee, Carol Gonsalves[1], Somsak Wichainun, Ahtit Fukiapaiboon, Waewchark Kongpolprom, Kanokwan Theerasart, Chalermchai Prasartsee and Dennis Gonsalves[1]. Northeast Regional Office of Agriculture, Tha Pra, 40260, THAILAND. [1]Dept. of Plant Pathology, Cornell University, Geneva, NY 14456 USA.

Papaya ringspot virus (PRV), first reported in Northeast Thailand in 1975, is now the major limiting factor to papaya production in this region. Efforts to control PRV by eradication and cross-protection have had limited success. Therefore, PRV-tolerant cultivars were developed from crosses of 'Kaekdum', the susceptible local Thai papaya with a dioecious papaya, 'Florida Tolerant', that is polygenic for tolerance. Three tolerant lines, 'Tha Pra Line 1', 'Tha Pra Line 2', and 'Tha Pra Line 3' were developed. These have desirable horticultural characteristics and good PRV-tolerance under severe disease pressure. Surveys monitoring the impact of PRV-tolerant cultivars on the socioeconomic status of villagers will be done prior to a mass seed distribution in 1995, and for 2 years after fruit production.

PHYSIOLOGICAL AND GENETIC CHARACTERIZATION OF *STREPTOMYCES* SPECIES PATHOGENIC ON POTATO. James P. Prince and Rosemary Loria, Department of Plant Pathology, Cornell University, Ithaca NY 14853.

Thirty *Streptomyces* strains from diverse geographic regions were compared for phytotoxin and extracellular enzyme production. Pathogenicity to potato was determined on minitubers produced on stem cuttings and on tuber slices. Thaxtomin production varied greatly among pathogenic strains. Nonpathogenic strains did not produce thaxtomin. Pathogenic strains also varied in cellulase and amylase activity. The mean amylase activity was significantly less ($p<0.05$) for pathogenic strains than for nonpathogens, but pathogens did not differ from nonpathogens in cellulase activity. RFLP analysis of 16S rDNA was used to generate a dendrogram of genetic distance, indicating the clustering of highly virulent strains.

CHANGES IN PEROXIDASE ACTIVITY OF TISSUE-CULTURED PLANTLETS OF POPULUS TREMULOIDES INDUCED BY INOCULATION WITH ASCOSPORES OF HYPOXYLON MAMMATUM. B.A. Race and P.D. Manion. SUNY College of Environmental Science and Forestry, Syracuse, NY 13210.

Peroxidase activity increases as a general response of plants to pathogen infection. We hypothesized that peroxidase levels change upon ascospore infection of unwounded tissue-cultured P. tremuloides plantlets. To test the hypothesis we chose four clones of varying susceptibility. The procedure was to quantify peroxidase activity in the P. tremuloides clones post inoculation and to evaluate the difference between inoculated and uninoculated tissue-cultured plantlets exposed to light and dark conditions. Peroxidase activities of inoculated clones grown under light conditions did not differ from uninoculated clones except for one highly resistant clone. Three inoculated dark-grown clones changed the peroxidase activity relative to uninoculated clones. Because disease rating increased only in the dark-grown clones, changes in the levels of peroxidase verify the interaction.

EFFECTIVENESS OF IPRODIONE FOR CONTROLLING *PENICILLIUM EXPANSUM* ON APPLES. D. A. Rosenberger, F. W. Meyer, and C. A. Engle, Cornell's Hudson Valley Lab, P.O. Box 727, Highland, NY 12528.

Iprodione, thiabendazole (TBZ), and thiophanate-methyl were compared in three postharvest fungicide trials. Wounded fruit were immersed first in spore suspensions containing 5,000 or 10,000 conidia/ml of an benzimidazole-sensitive isolate of *Penicillium expansum*, then in fungicide solutions. In two of the three trials, iprodione at 1200 µg/ml was less effective than TBZ at 530 µg/ml and TBZ was less effective than thiophanate-methyl at 765 µg/ml. Residual activity in treated fruit (as judged by decay control) was greatest for thiophanate-methyl and least for iprodione. When treated Empire fruit were held 45 days at 2 C followed by an additional 10 days at 18 C, the incidences of decay after three and 10 days of the shelf-life test were 61/92% for the control, 0/0 for thiophanate-methyl at 765 µg/ml, 0/7% for TBZ at 530 µg/ml, 0/15% for iprodione at 1200 µg/ml, and 3/47% for iprodione at 600 µg/ml. In a fourth trial where fruit were inoculated with a combination of benzimidazole-resistant and sensitive isolates, TBZ was ineffective and combinations of TBZ plus iprodione were no more effective than iprodione used alone.

FUNGICIDES FOR CONTROLLING BLACK KNOT ON PLUMS. D. A. Rosenberger, F. W. Meyer, and C. A. Engle, Cornell's Hudson Valley Lab, P.O. Box 727, Highland, NY 12528.

Fungicide treatments were applied to 10-yr-old plum trees (*Prunus domestica)* on 28 April (bud burst); 6 (bloom), 13 (petal fall), 21 (shuck split), and 29 May 1992 to evaluate their effectiveness against *Apiosporina morbosa.* Inoculum in test trees was adjusted to four black knots per tree on 21 April 1992 by removing knots from other plum trees and tying them into the test trees. Most of the infections observed probably occurred during a 39 hour, 55 F wetting period which began 15 May 1992. No fungicides were applied in 1993. Black knots in each tree were counted in December 1993. In the unsprayed plots, the cultivar Oullins averaged 112 knots per tree with infection over 8.6% of susceptible shoot growth whereas NY-56.713.1, a blue prune-plum, had 232 knots per tree with 7.8% of shoot growth affected. Fungicides showed similar effectiveness on both cultivars. The mean number of knots per Oullins tree was 4, 3, and 9 for chlorothalonil at 1,235 µg/ml, mancozeb at 1,438 µg/ml, and ziram at 1,366 µg/ml, respectively. Thiophanate-methyl at 509 µg/ml plus captan at 1195 µg/ml was significantly less effective with 35 knots per tree. Myclobutanil at 40 µg/ml and fenbuconazole at 37 µg/ml were ineffective.

INHERITANCE OF RESISTANCE TO THE COWPEA RUST FUNGUS. D. E. Ryerson and M. C. Heath. Department of Botany, University of Toronto. 25 Willcocks St. Toronto, Ontario Canada. M5S 3B2

Crosses were made between cowpea (Vigna unguiculata L. [Walp.]) cultivars California Blackeye (susceptible to the cowpea rust fungus Uromyces vignae [Barcl.], race 1) and Calico Crowder (resistant). Each plant was characterized for resistance by light microscope observation of 100 infection sites (50 monokaryotic and 50 dikaryotic) at six days after inoculation. The first filial generation all displayed a resistant phenotype not unlike the Calico Crowder parental. Of the 62 F_2 plants examined, 39 showed a highly resistant phenotype indistinguishable from the F_1 plants. Seventeen showed fairly high resistance, although less than that shown by the resistant parental plant. Four F_2 plants were partially resistant while two were found to be susceptible. The two susceptible individuals were found in subsequent generations to harbour a recessive resistance gene. Some of the plants showed slightly different responses to the monokaryon than to the dikaryon. Cytological examination of 147 F_3, 186 F_4 and 88 F_5 plants revealed a complex mixture of phenotypes, ranging from full resistance to full susceptibility. Analysis of the phenotypes suggests that resistance to the cowpea rust fungus in cowpea cultivar Calico Crowder is complex, involving at least three genes for resistance. This observed complexity may be in part due to the fact that North American cowpeas have not been directly bred for resistance to the cowpea rust fungus.

RELATION OF TEMPERATURE, AERATION, AND MOISTURE ON SPOROGENIC GERMINATION OF SCLEROTIA OF *COLLETOTRICHUM COCCODES* . S. Sanogo, S.P. Pennypacker, and R. Stevenson. Department of Plant Pathology, The Pennsylvania State University, PA 16802.

The effects of temperature and aeration, and temperature and moisture on sporogenic germination of sclerotia of *Colletotrichum coccodes* was assessed in two separate experiments in incubators set at 14, 22,26, 30, and 34 C. Glass petri plates (9 cm), containing sclerotia placed onto moistened filter papers, were maintained in each temperature treatment chamber for 5 days. In the first experiment (temperature-aeration), plates were unsealed or sealed with parafilm. In the second experiment (temperature-moisture), three levels of moisture were generated by adding 1.5, 3, and 5 ml of sterile distilled water to filter papers in unsealed plates. Preliminary results indicate that conidia and setae are profusely produced on sclerotia in unsealed plates at all temperature levels except for 14 C. In contrast, mycelium is mostly produced in sealed plates. A significant temperature-moisture interaction was detected ($P<0.0001$) and suggested that sporogenic germination of sclerotia at each temperature level was dependent on moisture levels.

CELLULAR MECHANISMS INVOLVED IN COWPEA RUST FUNGUS-TRIGGERED CALLOSE SYNTHSESIS IN COWPEA PLANTS. - D. Škalamera and M.C. Heath. Department of Botany. University of Toronto, Toronto, Canada M5S 1A1

Cellular mechanisms involved in fungus-triggered callose synthesis were investigated by injecting cowpea leaves with metabolic inhibitors prior to inoculation with urediospores of the cowpea rust fungus, *Uromyces vignae,* race 1. Inhibitors of protein synthesis reduced the incidence of callose deposits in both the resistant and the susceptible cowpea cultivars. They also inhibited boric acid-induced callose synthesis in both cultivars. Inhibitors of protein glycosylation and antimicrofilament agents reduced only fungus-triggered callose deposition in the resistant cultivar, suggesting that callose deposition may be controlled by different mechanisms in the resistant and the susceptible cultivars. One of the antimicrofilament agents that inhibited callose deposition in the resistant cultivar did not affect callose deposition after the fungal haustoria were killed with polyoxin D. This suggests that the callose deposition during the resistance response involves different cellular processes than wound callose. Inhibitors of transcription and dictyosome function and an antimicrotubule agent had no effect on fungus-triggered callose deposition in either cultivar.

EFFECT OF TWO FORMULATIONS OF CHLOROTHALONIL ON APPRESSORIAL FORMATION BY *COLLETOTRICHUM COCCODES* . R. Stevenson, S. Sanogo, and S.P. Pennypacker. Department of Plant Pathology, The Pennsylvania State University, PA 16802.

The effective dosage level was established for two formulations of the fungicide chlorothalonil (Bravo 500 and Bravo 720) using a cellophane bioassay procedure. Filter paper discs (18 mm) were saturated with fungicide solution at concentrations of 0.01, 0.1, 0.2, 0.4, 0.8, 1 (100% a.i.), and 0 ppm (no fungicide) and then placed in individual wells of a spot plate. Three cellophane discs (5 mm) then were placed on each of the filter paper dics and a 0.5 µl drop of a conidial suspension (about $5x10^5$ conidia/ml) was placed in the center of each cellophane disc. The spot plates were maintained for 24 hr in darkness in a high humidity chamber loaded within an incubator set at 22 C. Then, appressorial formation was evaluated on the first 100 conidia observed on each cellophane disc. Differences in appressorium formation were detected over the range of chlorothalonil concentrations of 0.01 to 0.2 ppm. No appressoria formed at or above 0.4 ppm. Significant statistical differences ($P<0.0001$) were detected between the two chlorothalonil formulations.

DETECTION OF *XANTHOMONAS CAMPESTRIS* PATHOVAR *PELARGONII* BY HYBRIDIZATION-SPECIFIC PCR. M. A. Sulzinski[1], B. Schlagnhaufer[2], G. W. Moorman[2], and C. P. Romaine[2]. [1]Department of Biology, University of Scranton, Scranton, PA 18510 and [2]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802.

Enterobacterial repetitive intergenic consensus (ERIC) DNA sequences were used to prime PCR products from genomic DNA of *Xanthomonas campestris* pv. *pelargonii*, the causal agent of bacterial blight of geraniums. These products were separately cloned using the Stratagene pCR-Script SK(+) kit. Biotinylated primers were used to generate labelled probes from the cloned DNA templates. Using ERIC primers, DNA of various bacterial species and pathovars was amplified by PCR and the resulting amplified DNA was Southern blotted and hybridized to the biotinylated probes. One probe showed specificity for *X. campestris* pv. *pelargonii*, hybridizing to amplified DNA of 18 geographically-diverse isolates. In contrast, this probe failed to hybridize to amplified DNA of 24 isolates representing 10 other pathovars of *X. campestris* or of six isolates representing four other bacterial genera.

TRANSGENIC AND CLASSICALLY CROSS PROTECTED PAPAYA SHOW LIMITED PROTECTION AGAINST PAPAYA RINGSPOT VIRUS ISOLATES FROM DIFFERENT GEOGRAPHICAL REGIONS. P. Tennant[1], C. Gonsalves[1], K. Ling[1], M. Fitch[2], R. Manshardt[3], J. Slightom[4], and D. Gonsalves[1]. [1]Dept. of Plant Pathology, Cornell University, Geneva, NY 14456. [2]USDA-ARS, Aeia, HI 96701. [3]Dept. of Horticulture, University of Hawaii, Honolulu, HI 96822. [4]Upjohn Company, Kalamazoo, MI 49001.

Transgenic papaya expressing the coat protein (CP) gene of the mild papaya ringspot virus strain from Hawaii (PRV HA5-1) showed high levels of resistance against severe PRV isolates from Hawaii. Similarly, PRV HA5-1 cross protected papaya offered high levels of protection against two severe isolates from Hawaii. However, neither transgenic nor PRV HA5-1 inoculated papaya showed good levels of protection against PRV isolates from 11 geographical regions that were serologically related to PRV HA5-1. For example, there was complete resistance, delay in symptom development with symptom attenuation to PRV isolates from the Bahamas and Mexico and a shorter delay in symptom development but no symptom attenuation to isolates from Brazil or Thailand. Investigations with CP genes of other PRV isolates are underway to possibly broaden resistance to PRV.

INTERACTIVE EFFECTS OF OZONE AND NITROGEN FERTILIZATION ON FUNGAL LEAF PATHOGENS ON WHEAT. Andreas v. Tiedemann, Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853

Nitrogen fertilization is a major factor in conventional cereal production in Germany and its physiological effects on the plant represent a significant impact on potential ozone damages. Spring wheat was grown in pots at four different levels of nitrogen representing degrees from deficient (N1) to moderate (N2), optimal (N3) and over-supply (N4) and exposed to four different levels of ozone (0, 40, 80 and 120 ppb ozone, 5 days, 7 h daily). Leaf sensitivity to ozone was least on plants grown at optimal N supply. Leaf damage increased significantly on plants at low (N1) or high (N4) nitrogen supply. Following exposure to ozone, plants were inoculated with *Septoria nodorum* (leaf and glume blotch), *Erysiphe graminis* (powdery mildew) or *Puccinia recondita* (leaf rust). Ozone enhanced the formation of *Septoria* lesions, reduced the development of powdery mildew and increased leaf rust infection. The predisposing effect of ozone on *Septoria* and leaf rust severity was strongest on plants optimally supplied with nitrogen. The antagonistic effect of ozone against mildew was least pronounced at optimal nitrogen conditions. It is concluded that optimal N fertilization improves wheat plant resistance against primary damages of ozone but increases secondary damages caused by predisposing effects on leaf pathogens.

PURIFICATION AND CHARACTERIZATION OF A ß-1,3-GLUCANASE SECRETED BY *STACHYBOTRYS ELEGANS*, A MYCOPARASITE OF *RHIZOCTONIA SOLANI*. R. J. Tweddell[1], P. M. Charest[2], and S. H. Jabaji-Hare[1]. 1. Department of Plant Science, Macdonald Campus, McGill University, Ste-Anne-de-Bellevue, Québec, Canada, H9X 3V9. 2. Pavillon C.-E. Marchand, Université Laval, Ste Foy, Québec, Canada, G1k 7P4.

Ultrastructural studies of the interaction between the mycoparasite *Stachybotrys elegans* and its host *Rhizoctonia solani*, and studies on the production of hydrolytic enzymes by *S. elegans* conducted in our laboratories strongly suggest the implication of ß-1,3-glucanases in the parasitism process. The objectives of this work were to 1- purify and characterize β-1,3-glucanase secreted by *S. elegans*, and 2- demonstrate its lytic effect on *R. solani* mycelium. ß-1,3-glucanase (E.C. 3.2.1.39) secreted by *S. elegans*, when grown on minimal synthetic medium containing *R. solani* cell-wall fragments, was purified to homogeneity. The purification method involved ammonium sulfate precipitation, ion-exchange and gel-filtration chromatography. The molecular weight was 67 Kda as determined by gel-filtration. The enzyme has an optimum pH of 5.0 and is most active between 40°C and 50°C. The enzyme activity is not sensitive to metal ions and a Km of 0.18 mg/mL was estimated for laminarin as a substrate. Application of purified ß-1,3-glucanase to actively growing *R. solani* hyphae caused the induction of morphological changes such as hyphal tip swelling and bursting.

NEEDLE BLIGHT OF *PINUS STROBUS* IN THE NORTHEAST. N.G. Wenner, and W. Merrill. 211 Buckhout Lab, University Park, PA 16802.

The tip necrosis and blighting of succulent, elongating, current-year needles of *Pinus strobus* in the northeastern US, which is attributed by some to "ozone-injury," is not due to ozone. The pathological anatomy of affected needles does not resemble that described for ozone injury but rather is virtually identical to that described by Linzon in 1965 as "semimature-tissue needle blight." Although several organisms may produce similar gross symptoms, throughout the northeastern US the syndrome on affected trees is consistently associated with the presence of an as yet undescribed species of needlecast fungus [Ascomycetes: Rhytismatales]. This fungus is present in the mesophyll of healthy-appearing and dying tissues of affected needles before these needles have elongated to half their eventual mature size. Its life cycle, especially timing of fruiting body maturation and spore release, is consistent with the apparent timing of infection and symptom development. The pathological anatomy of needles infected by this fungus agrees with that described for other needlecast fungi beginning with Hartig's pioneering work in 1874.

COMPARISON OF DOUBLE-ANTIBODY SANDWICH MULTIWELL ELISA WITH INDIRECT TISSUE BLOT ELISA FOR THE DETECTION OF INSV IN IMPATIENS. R. L. Wick and P. Haviland. Dept. of Plant Pathology, UMass, Amherst, MA 01003.

A DAS peroxidase-based ELISA kit from Agdia inc. was compared with indirect ELISA tissue blot on nitrocellulose membranes for the detection of impatiens necrotic spot virus in single-flowered impatiens, double-flowered impatiens and New Guinea impatiens. For each type of impatiens, a single diseased plant was used from which symptomatic and asymptomatic leaves were tested. Plants free of the virus of each type of plant were used as negative controls. To compare the two ELISA formats, a single leaf was rolled, cut in half, and pressed onto the nitrocellulose membrane. The same entire leaf was then processed by multiwell ELISA. There were 13 replications per treatment of the diseased plants and 5 replications of the healthy controls. The experiment was repeated with a new set of plants. Results from multiwell ELISA were consistent with expectations based on symptoms. Tissue blot ELISA results were very inconsistent.

SURVEY OF GREENHOUSE PLANTS AND THRIPS FOR IMPATIENS NECROTIC SPOT VIRUS IN MASSACHUSETTS. R. L. Wick, P. Haviland, Dept. of Plant Pathology, UMass, Amherst, T. Smith and P. Lopes, Cooperative Extension UMass, Amherst, MA 01003.

Twenty-eight of 49 plants tested were found to be positive for INSV by multiwell ELISA. These included single and double-flowered impatiens, New Guinea impatiens, *Primula, Lycium, Browallia, Streptocarpus, Evolvulus, Chrysanthemum* x *superbum, Iberis, Capsicum annuum*, and the weed *Galinsoga*. 173 out of 700 individual thrips tested positive for INSV and in some cases resulted in absorbance readings above 1.999 at 405 nm.; many of the thrips negative for INSV were collected from greenhouses that were not suspected of harboring the virus. No appreciable differences were found when thrips were tested immediately after being removed from plants (18/18 positive), compared to those placed on sticky cards before the assay (31/36 positive). INSV could be detected in thrips removed from sticky cards stored in the greenhouse for a minimum of 16 days.

INFLUENCE OF CULTURAL PRACTICES ON DEVELOPMENT OF STRAWBERRY GRAY MOLD W. F. Wilcox, R. C. Seem, and M. P. Pritts*, Cornell University, Dept. Plant Pathology, NYState Agr. Expt. Sta., Geneva 14456 and *Dept. Fruit and Vegetable Science, Ithaca, NY 14853

The influence of cultural practices on development of gray mold (*Botrytis cinerea*) was examined in replicated plots of 'Honeyoye' and 'Allstar' strawberries. Experimental variables were raised vs. flat planting beds; narrow (30 cm.) vs. wide (45 cm) fruiting rows; canopies mowed vs. nonmowed during summer renovation; and supplemental spring applications of nitrogen (33.6 and 67.2 kg/ha of N) vs. lack thereof. Each year, all treatments received a standard herbicide program, 85 kg/ha of N at renovation, and no fungicide. During three fruiting years, bed height, row width, and mowing had a negligible effect on disease incidence, whereas spring N applications had a pronounced impact. Mean combined (harvest plus postharvest) gray mold incidences for flat bed/wide row treatments with supplemental N applications of 0, 33.6, and 67.2 kg/ha were 14, 36, and 37% in 1992; 16, 31, and 38% in 1993; and 13, 37, and 53% in 1994, respectively. These data were correlated with differences in microclimate within treatment canopies, e.g., during the 1993 fruiting season, cumulative leaf wetness periods were 122, 209, and 185 h and mean relative humidities were 74, 79, and 82%, respectively.

A SINGAPORE ISOLATE OF BROAD BEAN WILT VIRUS (BBWV) BELONGS TO SEROTYPE II. S. M. Wong, T. Gan, C. G. Chng, and P. L. Chong. Botany Dept., National University of Singapore, Kent Ridge, Singapore 0511. Republic of Singapore.

A Singapore isolate of BBWV was isolated from the Brazilian Red Cloak Megakepasma erythrochlamys L., which exhibited leaf distortion and mosaic symptoms. The virus isolate was designated as BBWV-ME. It can be mechanically transmitted to members of the Aizoaceae, Amaranthaceae, Chenopodiaceae, and Solanaceae. Both amorphous and crystal-like inclusions were observed in the ultrathin sections of infected Chenopodium quinoa leaf cells. The virions measured 27 nm in diameter. Two components were purified using sucrose density gradient centrifugation. Two antisera were raised against two coat protein polypeptides of molecular weight 24 kDa and 41 kDa, respectively. By immuno-electron microscopy tests, BBWV-ME is found to be more closely related to BBWV serotype II than serotype I.

MOLECULAR CHARACTERIZATION OF A NATURALLY OCCURRING DEFECTIVE RNA (D1-RNA) DERIVED FROM THE Fny STRAIN OF CUCUMBER MOSAIC VIRUS. S. M. Wong, I. Kaplan and P. Palukaitis. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.

A naturally occurring defective RNA (D1-RNA) was observed after several passages of the Fny strain of cucumber mosaic virus (Fny-CMV) on tobacco (*Nicotiana tobacum* Xanthi nc). Using RNA probes specific to the coding regions corresponding to Fny-CMV RNAs 1, 2 and 3, it was demonstrated that the D1-RNA was derived from RNA 3. We cloned the D1-RNA employing the RT-PCR technique with primers corresponding to sequences from the 5' end and the coat protein region. Restriction enzyme mapping and sequence analysis showed that the deletion was in frame and within the 3a gene, from nucleotide 296-634 or nucleotide 297-635. Northern blot analysis revealed that tobacco plants inoculated with RNA transcripts obtained from the D1-RNA cDNA clone mixed with those from Fny-CMV cDNA clones representing RNAs 1, 2 and 3 would produce a similar-sized, defective RNA. We will determine if the D1-RNA interferes with the replication and movement of other cucumoviruses.

TWO AMINO ACID SEQUENCE CHANGES IN THE COAT PROTEIN OF THE M-STRAIN OF CUCUMBER MOSAIC VIRUS (M-CMV) ALTERED ITS SYSTEMIC MOVEMENT IN SQUASH. **S. M. Wong, M. H. Shintaku and P. Palukaitis. Dept. of Plant Pathology, Cornell University, Ithaca, NY 14853.**

While most CMV strains infect cucurbits systemically, the M-CMV can not spread into systemic leaves of inoculated zucchini squash (*Cucurbita pepo*). This phenomenon was mapped to RNA 3 using pseudorecombinants constructed between M-CMV and other CMV strains. Furthermore, chimeras formed between cDNA clones of M-CMV RNA 3 and Fny-CMV RNA 3 showed that the phenotype was delimited to two domains. By sequence comparison, the coat protein amino acids positions 129 and 214 were delimited. Site-directed mutagenesis revealed that by changing Leucine to Proline at position 129 and Arginine to Glycine at position 214, the mutated pseudorecombinant F1F2M3 could move as efficiently as the wild type Fny-CMV (F1F2F3). A squash cotyledon tissue blot technique was used in studying the local movement of the viruses.

VARIABILITY OF MYCOPLASMALIKE ORGANISM (MLOs) ASSOCIATED WITH BLUEBERRY STUNT DISEASE. B.Y. Xu and T.A. Chen, Dept. of Plant Pathology, Rutgers University, New Brunswick, N.J. 08903.

The 16S rRNA genes of MLOs associated with blueberry stunt (BS) disease of different cultivars grown in New Jersey and Michigan were amplified and sequenced. When compared with 12 other plant pathogenic MLOs the homology of 16s DNA between BS-MLO and that of Western severe aster yellows and apricot leafroll was 98.7%, indicating that they could be placed within the aster yellows strain cluster. The homology of 16s DNA between BS-MLO and the other 10 MLOs ranged from 88.9 to 95.5%. Based on the sequence analyses of 16s rRNA genes and PCR amplification using various primer combinations, we have found that genetical differences existed between BS-MLOs isolated from NJ and MI. In addition, two strains of BS-MLO were identified from a single diseased plant from Michigan when Alu I was used for RFLP analysis. These results suggested that detection of BS disease could be quite complex since more than one set of primers may be required in PCR for accurate diagnosis of BS-MLO strains.

ENHANCED PR PROTEINS ACTIVITY IN PROTECTED BEAN SEEDLINGS BY NON-PATHOGENIC BINUCLEATE *RHIZOCTONIA* SPECIES. L. Xue[1], P. Masilamany[1], P. M. Charest[2] & S. J.-Hare[1]. 1. Dépt. of Plant Science, McGill University, Ste.-Anne-de-Bellevue, Québec 2. Sciences de la Vie et de la Santé, Université Laval, Ste. Foy, Québec, Canada.

Inoculation of bean hypocotyls with a non-pathogenic isolate of binucleate *Rhizoctonia* (BNR) induced the production of pathogenesis-related proteins (PR). Chitinase and ß-1,3 glucanase activities were determined in bean hypocotyls that were (i) protected with BNR, (ii) protected with BNR and then challenged with a virulent isolates of *Rhizoctonia solani*; (iii) challenged with *R. solani*; and (iv) untreated (control). Significantly higher exo- and endochitinase activities ($P<0.05$) were detected in protected bean hypocotyls as compared to those either infected with *R. solani* or untreated. Activity of endochitinase in protected hypocotyls significantly increased with time. Neither protected nor challenged bean hypocotyls showed significant difference in ß-1,3 glucanase activities. Disease severity and incidence in bean seedlings protected and then challenged with *R. solani* decreased significantly and were highly correlated with enhanced activities of endochitinases [$p=0.0091$]. These results suggest that the BNR species activate host defense mechanisms.

INOCULUM CONCENTRATION OF *BOTRYTIS CINEREA* AND OF THE BIOCONTROL AGENT *GLIOCLADIUM ROSEUM* IN RELATION TO SUPPRESSION OF THE PATHOGEN IN RASPBERRY. H.Yu and J.C.Sutton. Dept. of Environmental biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Detached raspberry shoots were inoculated with *B. cinerea* (10^5, 10^4, 10^5, and 10^6 conidia/mL) and 24 h later with *G. roseum* (10^4, 10^5, 10^6, 10^7, and 10^8 conidia/mL) in all combinations. Sporulation incidence of *B. cinerea* was estimated on host tissues that were incubated on paraquat-chloramphenicol agar medium. Sporulation incidence of *B. cinerea* reached 100% on leaves, stems, stamens, and stigmas inoculated with the pathogen only at 10^5, 10^6, 10^4, and 10^5 conidia/mL, respectively. Combinations of inoculum concentration of *G. roseum* (*G.r.*) and *B. cinerea* (*B.c.*) that suppressed the pathogen by 90-100% were: in leaves *G.r.* 10^4-10^6/*B.c.* 10^3-10^6 (all combinations); in stems *G.r.* 10^7-10^8/*B.c.* 10^3-10^6 and *G.r.* 10^6/*B.c.* 10^3-10^4; in stamens *G.r.* 10^7/*B.c.* 10^3-10^5, *G.r.* 10^6/*B.c.* 10^3-10^4, and *G.r.* 10^5 and 10^6/*B.c.* 10^3; in stigmas *G.r.* 10^7/*B.c.* 10^3-10^5 and *G.r.* 10^6/*B.c.* 10^3-10^4. In stamens and stigmas, *G. roseum* at 10^8 was often less effective than at 10^7 or 10^6. We concluded that *G. roseum* effectively suppressed *B. cinerea* in stems, stamens, and stigmas only when the inoculum concentration was at least 10^1-10^2 times greater than that of the pathogen and did not exceed 10^7 conidia/mL.