APS Potomac Division

Abstracts

March 22–24, 1995 Blacksburg, VA

Alphabetized by first author's last name

DNA REASSOCIATIONS AMONG 87 XANTHOMONADS. R. Coate[1], R.L. Gherna[1], V.K. Stromberg[2], G.H. Lacy[2], J. Toth[3], and J.L. Johnson[3]. American Type Culture Collection, Rockville, MD 20852[1] and Plant Pathology, Physiology, & Weed Science[2] and Biochemistry & Anaerobic Microbiology[3], VPI&SU, Blacksburg, VA 24061

A matrix of 87 strains, containing four species and 60 pathovars, was probed with DNAs from eight strains. Sheared and denatured testor and ^{125}I-labeled probe DNAs were reassociated at high stringency. S1 nuclease digestion removed ssDNA before acid precipitation on filters and gamma emissions were measured. Reassociation (%R) was estimated for heterologous reactions by comparison with homologous reassociations. Each reaction was duplicated. Most strains fell into five species-level (≥70 %R) groups, some were affiliated with these groups below the species level (40-69 %R), and a small number were not affiliated with any group. The latter groups will be probed with different DNAs to resolve more species-level groups. A few strains reassociated at species-level with two groups.

ISOENZYMES OF *STENOCARPELLA MAYDIS* A.E. Dorrance, A.W. Way, G.H. Lacy and H.L. Warren Dept. of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0331.

Stenocarpella maydis has been reported to consist of variable strains and possibly races. This study was undertaken to document the genetic variation among geographically distinct isolates of *S. maydis* using isozyme analysis. A collection of thirty-one single spore isolates from diseased ears representing nine US geographic regions and one South African isolate were analyzed. Nine enzymes were assayed by specific activity stains. Enzyme patterns were identical for all the US strains; the South African isolate could be differentiated by hexose kinase and malate dehydrogenase patterns. These preliminary results indicate this fungus has a great uniformity in isozyme phenotypes, possibly resulting from selection pressure placed on the organism by its major host, maize. Similar patterns of uniformity have been found for biotrophs and pathovar forms, but not with facultative parasites like *S. maydis*.

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

BAS 490 F, A NEW STROBILURINE DERIVATIVE FUNGICIDE FOR CONTROL OF APPLE SCAB (VENTURIA INAEQUALIS). R.R. EVANS AND E.J. BUTTERFIELD. BASF CORPORATION, P.O. BOX 13528 RTP, NC 27709.

BAS 490 F is a new synthetic fungicide derived from the fungal secondary metabolite Strobilurine A. Research conducted in North America has demonstrated that BAS 490 F will provide excellent protectant activity against apple scab when applied on a four spray application schedule at 10-14 day intervals from 1/2 inch green through 1-2 cover. Laboratory and field trials indicate that BAS 490 F will provide excellent control of apple scab when applied up to 96 hours after infection. In addition to excellent preventative and curative control of apple scab, BAS 490 F has also provided excellent control of powdery mildew (*Podosphaera leucotricha*), sooty blotch (*Gleoedes pomigena*) and flyspeck (*Schizothyrium pomi*) in trials conducted in North America. This spectrum of activity, a unique mode of action and favorable environmental characteristics indicate that BAS 490 F will be an effective tool for the control of many important diseases which occur in apples.

PERSISTENCE AND RECOVERY OF ENDOPHYTIC ERWINIA AMYLOVORA IN APPARENTLY HEALTHY APPLE TISSUES. Quanqing Ge, Yantai Animal and Plant Quarantine Bureau, Yantai, P.R.China and T. van der Zwet, USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430.

Succulent shoots on 30 young Improved Red Jonathan/EM 7 trees were needle-inoculated with the fire blight pathogen (Erwinia amylovora) in the greenhouse in early August 1993. Shoot blight symptoms ceased spread after 60 days. During the next 8 months, all trees developed 3-4 apparently healthy side shoots below the base of the canker. In June 1994, all side shoots were removed, surface sterilized in 20% sodium hypochlorite, and 3 mm pieces were chopped at 3 cm intervals and plated on nutrient-yeast-dextrose agar. Bacterial growth originating from the pieces was streaked on Crosse-Goodman medium to confirm characteristic colony cratering of E. amylovora. Six (20%) of the trees contained the bacterium in one or more of the symptomless side shoots. Nine (33%) trees had formed terminal flower buds. Endophytic E. amylovora bacteria were recovered from shoots of four of these trees, and of these, three (75%) trees contained the bacterium in internal tissues of unopened blossom buds.

THE ROLE OF INTERPLOT INTERFERENCE IN GRAY LEAF SPOT DEVELOPMENT. A. P. Grybauskas, Plant Biology Department, University of Maryland, College Park, MD 20742-5815.

Gray leaf spot of corn (GLS) caused by *Cercospora zeae-maydis* is a disease that is sensitive to initial levels of inoculum produced by infested corn debris and thus is strongly associated with no-till production. It also produces secondary infection cycles and the wind-blown spores can travel some distance. Extrapolation of results from small plot studies to whole fields can be confounded by interplot interference. Interplot interference depends in part on the steepness of the inoculum dispersal gradient and the relative importance of secondary infection cycles. A study was conducted at a GLS endemic site where all combinations of paired plots (3 x 6 m) of a moderately resistant and a susceptible corn hybrid were grown no-till in infested corn stubble either with or without a 6 m resistant buffer in 1993 and 1994. No significant differences indicative of positive or negative

interplot interference or of an effect of buffer size could be detected. Representational error in GLS studies may not occur with a large pool of inoculum or may only be detected with considerably larger plots and buffers.

Detection of Apple Proliferation Phytoplasma in Apple by Combining Molecular and Biological Techniques. S.S. Hurtt[1], R.E. Davis[2], and R.S. Turner, Jr[1]. USDA, ARS, NGRL[1] and MPPL[2], Beltsville, MD 20705.

Apple proliferation (AP) is a severe disease of Malus spp. in western and southern Eurasia. To prevent the introduction of AP, into the U.S., imported pome germplasm is quarantined and tested for the phytoplasma (APP). To developed a rapid test for APP, Chardan apple was inoculated in September with bark chips from AP-infected or healthy apple and incubated in the greenhouse for 6-8 wk. Plants were held at 4C for 3 mo, then returned to the greenhouse. Total nucleic acids extracted from leaf veins and petioles in June were used as DNA templates in nested primer polymerase chain reactions (PCR). A universal primer pair followed by a nested primer pair designed for specific amplification of phytoplasma 16S rRNA group X (APP and related strains) were used for amplifications. A 1.1 kb product was obtained with AP-inoculated, but not healthy Chardan. Digestion of PCR product with 3 endonucleases gave the expected fragments in electrophoretic analysis. Thus APP detection was achieved in 9 mo compared to 3 yr using conventional orchard assays.

INTERACTION OF SOYBEAN RESISTANCE GENES WITH STRAINS OF SOYBEAN MOSAIC POTYVIRUS (SMV). G. Ma[1], G. R. Buss[1] and S. A. Tolin[2]. Dept. of Crop and Soil Environmental Sciences[1], and Dept. of Plant Pathology, Physiology & Weed Science[2], Virginia Polytechnic Institute & State University, Blacksburg, VA 24061.

Soybean mosaic potyvirus induces mosaic symptoms on susceptible cultivars of soybean (*Glycine max*) and, depending upon the strain, either no symptoms or local and/or systemic necrosis on resistant cultivars, most of which have an allele at the *Rsv1* locus. All *Rsv1* alleles, including two new alleles we have identified, confer immunity to less virulent strains and systemic necrosis to at least one virulent strain. However, systemic necrosis is not exclusively a response conferred by a resistance allele to virulent strains. SMV-G1 is the least virulent strain, to which all *Rsv1* resistance alleles confer resistance, and SMV-G7 is the most virulent, overcoming all alleles at *Rsv1*. In cultivars that we have shown to possess a resistance gene at a different locus, *Rsv3*, resistance is overcome by SMV-G1 but not by SMV-G7. The resistance genes at loci other than *Rsv1* may be completely dominant and epistatic to the *Rsv1* genes and thus inhibit the *Rsv1*-associated expression of necrosis.

REPRODUCTION OF SIX PHYSIOLOGICAL RACES OF *HETERODERA SCHACHTII* ON ONE ISOLATE OF *BETA PETELLARIS* AND TWO ISOLATES OF *B. VULGARIS MARITIMA*. L. I. Miller, Dept. Plant Path., Physiol. and Weed Sci., Virginia Tech, Blacksburg, VA 24061.

Six physiological races of *H. schachtii* (C1 from tomato and C2 from sugarbeet in California, N1 from cabbage in New York, M1 from sugarbeet in Michigan and F1 and F2 from cabbage in Florida) were tested in the greenhouse to determine their ability to form egg-bearing females on 'USH11' *B. vulgaris vulgaris* (susceptible check), *B. procumbens* (resistant check), Lewellen's isolate of *B. petellaris*, the Southwald, Suffolk, England and the Loire River estuary, France isolates of *B. v. maritima*. The English isolate of *B. v. maritima* and USH11 were efficient hosts of all nematode races as was the French isolate of *B. v. maritima* to the nematode races C2, MI, N1 and F1. *B. petellaris* and *B. procumbens* were not hosts or were highly resistant to all of nematode races as was the French isolate of *B. v. maritima* to races C1 and F2.

DEVELOPMENT OF A HOST-ORIENTED DAMAGE FUNCTION FOR GRAY LEAF SPOT DISEASE OF MAIZE. Mills, D. J., Grybauskas A. P., Department of Plant Biology, University of Maryland, College Park, MD, 20742.

Gray leaf spot (GLS), induced by the imperfect fungus, *Cercospora zeae-maydis*, is among the more significant foliar diseases of maize in the Eastern U.S., often causing substantial grain yield reduction. Our objective is to quantify the relationship between disease and yield by constructing a robust damage function based on foliage quantity duration and photosynthetically active radiation (PAR) interception. In 1994, GLS epidemics of varying intensity were monitored in central Maryland. Repeated measures of disease severity, leaf area index, and PAR flux density were made to compute leaf area duration (LAD), healthy leaf area duration (HAD), and healthy leaf area absorption (HAA), which combines HAD and PAR interception. Grain yield was regressed on LAD, HAD, and HAA to test the efficacy of each measure as a predictor variable. These single-independent variable models all exhibited statistical significance ($P < .003$) with r^2 values ranging from 0.75 to 0.84.

PSEUDOMONAS AUREOFACIENS WILDTYPE AND TN*5*-MUTANT COLONIZATION OF COTTON ROOTS IN TWO SOIL TYPES. M.A. Mulesky, G.H. Lacy, and C. Hagedorn. Dept. of Plant Pathology, Physiology, and Weed Science, VPI & SU, Blacksburg, VA 24061.

Cotton seedling disease caused by *Pythium ultimum* and *Rhizoctonia solani* occurs worldwide in soils ranging from pH 5 to 8.5. Biocontrol agents of these pathogens should be capable of colonizing cotton roots over a wide pH range. A *Pseudomonas aureofaciens* strain that produces siderophores and antifungal antibiotics, and Tn*5*-induced siderophore- and antibiotic-negative mutants were evaluated in the greenhouse in two soils, a Suffolk loamy sand (pH 5.7) and a Ross fine loam (pH 8.0), under pathogen-free conditions. Conetainers with treated seed were submerged in moist sand to assess bacterial movement in the absence of downward water percolation. Root sections (upper and lower tap and upper and lower laterals) were sampled 22, 36, and 50 DAP. Migrational populations peaked at 36 DAP in both soils, but mean values overall were three-fold higher at pH 8.0. Population averages ranged from log 6.0 cfu/cm^2 on the upper tap to log 4.6 cfu/cm^2 on the lower laterals.

SOUR CHERRY ISOLATE OF PLUM POX VIRUS IS A UNIQUE MEMBER OF THE VIRAL M STRAIN AND IT IS TRANSMITTED BY CHIP BUDDING TO SOUR AND SWEET CHERRY. L.Nemchinov, and A.Hadidi. National Germplasm Resources Laboratory,ARS-USDA, Beltsville, MD, 20705

Characterization of the sour cherry isolate of plum pox virus (scPPV) has been investigated by RT-PCR,nucleotide sequencing, and viral transmission to sour and sweet cherry. A DNA fragment of 220 bp was amplified from infected tissue using DNA primers for PPV RNA 3'non-coding region. Its nucleotide sequence is at least 95% homologous to that of other PPV isolates. RT-PCR assays as well as molecular hybridization tests demonstrated that PPV is systemically distributed in infected trees. RFLP analysis of amplified products from infected tissue using PPV CP-primers showed that the scPPV is a unique M-strain,which contains neither Rsa1 nor Alu1 site.This information was also confirmed by nucleotide sequencing of several clones of the amplified product. Attempts to transmit scPPV to sweet cherry (Prunus avium), and sour cherry (Prunus mahaleb) by chip budding resulted in systemic viral infection in both plant species.The infection was confirmed by RT-PCR using both sets of primers as well as by RFLP.

UTILIZATION OF PCR TECHNOLOGY FOR THE DETECTION AND IDENTIFICATION OF APPLE STEM PITTING RELATED VIRUS FROM PEACH L.Nemchinov, A. Hadidi, Ye. Zemchik*, and T. Verderevskaya* National Germplasm Resources Laboratory, ARS-USDA,Beltsville, MD, 20705, and *Horticultural Research Institute, 14 Costiujeni Street, Kishinev, Moldova, 277072.

Recently, a latent agent in apricot variety Silistra was graft-transmitted to peach seedlings in which it caused bright yellowish-green spots on infected leaves (Zemchik and Verderevskaya, 1993, Sel'skohoziaystvennaya Biologia,v.3.130-132).Filamentous virus particles were identified in extracts of infected peach which reacted positively with apple stem pitting virus(ASPV) antisera. To determine whether the agent in peach is indeed ASPV, we have utilized ASPV DNA primers specific for the viral 3'end of ASPV coat protein gene and the adjacent portion of the 3'non translated region in RT-PCR or IC-RT-PCR assays. The expected size of the amplified ASPV cDNA product was 290 bp. ASPV isolate B-39 from tobacco was used as a positive control.The size of IC-RT-PCR or RT-PCR amplified products from infected peach or tobacco was 290 - 300 bp. The nucleotide sequence of the PCR products from tobacco and peach will be determined and discussed.

AN EXAMINATION OF THE NATURAL VARIATION OF PHYTOPHTHORA INFESTANS ISOLATES TO CYMOXANIL, CHLOROTHALONIL, AND METALAXYL. R.J. POWER[1,2], A.L. Morehart[1] & R.A. Hamlen[2]. [1]Dept. of Plant and Soil Sciences, Univ. of Delaware, Newark, DE 19711; [2]E.I. DuPont de Nemours, Stein Laboratory, Newark, DE 19717.

Fifty Phytophthora infestans (Mont.) deBary isolates from around the world were tested for fungicidal sensitivity by colony radial growth, sporangial germination, detached leaf assay, and on whole plants. Isolates were identified as gentically unique prior to testing to avoid redundancy. Cymoxanil and chlorothalonil yielded comparable mean EC_{50} values of 0.23 and 0.25 μg/ml, respectively, in the radial growth assay. Two isolate clusters with mean EC_{50} values of 0.20 and 47μg/ml were produced by metalaxy treatment. Both metalaxyl and cymoxanil failed to inhibit sporangia and zoospores in vitro. Inhibition of germination by chlorothalonil correlated well with the radial growth results based on overall population means and ranges. In vitro results correlated well with whole plant data (R^2=.99), due in part to the presence of sufficient variation provided by metalaxyl resistance.

PROGRESS TOWARD MOLECULAR ANALYSIS OF SOYBEAN MOSAIC POTYVIRUS (SMV) STRAINS. S. Qusus[1], C. L. Cramer[1], M. A. Saghai Maroof[2], and S. A. Tolin[1]. Dept. of Plant Pathology, Physiology & Weed Science, and Dept. of Crop, Soil & Environmental Sciences. Virginia Polytechnic Institute & State Univ. Blacksburg, VA 24061.

Understanding the interaction between soybean and SMV strains will be enhanced by molecular analysis of viral genomes. We describe new modifications of a PCR-based method to compare SMV strains that do not require virus purification and RNA isolation. Following partial clarification and concentration, pellets containing SMV-G1/VA were resuspended in reverse transcriptase buffer, then enzyme and oligo dT primer were added. For cDNA synthesis, the mixture was subjected to freeze-thaw cycling. Second strand synthesis was by PCR using 1 µl of cDNA reaction mixture. Primers (15-20mers) deduced from conserved regions within the 3' end of published sequences of SMV-G2 and SMV-G7 generated expected PCR product sizes from SMV-G1. Partial sequences from clones confirmed that SMV sequences had been amplified. This method will be used to seek variation among additional strains and isolates that interact with specific resistance genes.

SENSITIVE DETECTION OF BANANA BUNCHY TOP VIRUS FROM INFECTED LEAVES, TISSUE CULTURES AND VIRULIFEROUS APHIDS USING POLYMERASE CHAIN REACTION. A.M. Shamloul, S.I. El-Afifi, and A. Hadidi National Germplasm Resources Laboratory, ARS-USDA, Beltsville, MD 20705

Banana bunchy top is the most devastating virus disease of bananas worldwide. Banana bunchy top virus (BBTV) is phloem limited, occurs in extremely low concentrations and it is not transmitted mechanically. It is transmitted by aphids in a persistent manner. BBTV contains at least six circular ssDNA components, each about 1 Kb in size. DNA primers for BBTV were constructed based on the nucleotide sequence of DNA component 1 which contains the viral putative replicase gene. Three pairs of primers were utilized for polymerase chain reaction (PCR) or immunocapture (IC)-PCR amplification of 439 bp, 446 bp and 476 bp fragments from extracts of BBTV-infected leaves or tissue cultures or from viruliferous aphids. The amplified DNA fragments were identified by Southern and dot-blot hybridization analyses with a cRNA probe of cloned BBTV DNA. Viral specific DNA fragments were absent from amplified extracts of uninfected banana leaves or tissue cultures or from nonviruliferous aphids.

EVALUATION OF EGYPTIAN SOYBEANS FOR RESISTANCE TO ANTHRACNOSE CAUSED BY *COLLETOTRICHUM DEMATIUM* VAR *TRUNCATUM*. M.M. Soliman and R.B. Carroll, Dept. of Plant & Soil Sciences, University of Delaware, Newark DE 19717-1303.

Eight Egyptian soybean lines were tested under greenhouse conditions for resistance to three sclerotia-forming isolates of *Colletotrichum truncatum* (DE, Ct3 and Ct4). Six seedlings of each line were grown in 12-cm-diameter pots containing pathogen-free sand. Two experiments with four replicates each were conducted using two types of inoculation (soil infestation at 80 sclerotia/g sand and foliar inoculation at 3-5 x 10^3 sclerotia/ml suspension). Seedlings were inoculated at the V1 growth stage and evaluated 45 days later for anthracnose symptoms on foliage and roots along with shoot length (cm), shoot dry weight (g) and root dry weight (g). The DE isolate was more pathogenic than Ct3 and Ct4 on all soybean lines. Lines H1L70, H1L82, and H2L21 exhibited resistance to *C. truncatum*. Lines H2L13 and H15L4 were susceptible, and H1L68, H2L35, and Dr-1 were intermediate in resistance to this pathogen. The two inoculation methods provided identical results for resistance screening, but higher disease ratings were obtained via foliar inoculation.

DETECTION OF BEAN VIRUSES AND THEIR EFFECT ON YIELD. H. L. Warren, A. W. Way, and S. A. Tolin. Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Snap beans were tested for bean yellow mosaic virus (BYMV) and peanut stunt virus (PSV) using indirect ELISA or the tissue blot test, both with polyclonal antibodies. Samples were collected from four cultivars naturally infected in replicated plots. White Half Runners, Earliserve, Kentucky Wonder and Blue Lake, exhibiting virus symptoms and testing positive with tissue blot test, sustained yield losses of 50, 50, 32, and 26 percent compared to virus-free plants. In another test, Blue Lake beans inoculated with BYMV, PSV, BYMV + PSV sustained yield losses of 90, 98, and 99 percent, respectively, compared to virus-free plants. The bean cultivars used had a significant effect on yield obtained although disease severity and prevalence were similar.

TOMATO *hmg*2 OVEREXPRESSION IN TOBACCO AFFECTS RESISTANCE TO BACTERIAL SOFT ROT AND TMV. Xueshu Yu, George H. Lacy, Sue A. Tolin, & Carole L. Cramer, Dept. Plant Path. Physiol. & Weed Sci., VPI & SU, Blacksburg, VA 24061-0330

3-Hydroxy-3-methylglutaryl coA reductase (HMGR) is the key enzyme in the biosynthesis of isoprenoids including defense-related phytoalexins. The tomato genome contains at least four *hmg* isogenes. Among them, *hmg*2 appears to be specifically associated with plant defense responses. Tomato *hmg*2 cDNA was cloned, expressed in *E. coli* to confirm its HMGR activity, inserted behind the CaMV 35S promoter, and engineered into tobacco (*Nicotiana tabacum* cv. Xanthi nc). Southern and northern analyses confirmed transformation and message expression. Enzyme activity was enhanced compared to nontransformed plants. Analysis of selected transgenic plants revealed significantly reduced tissue maceration by *Erwinia carotovora* subsp. *carotovora*. The mean size of necrotic lesions induced by TMV was also significantly reduced. Thus, genetic manipulation of the rate-limiting step in a major defense pathway could provide a novel strategy for enhancing disease resistance.

SINGLE AND DOUBLE INFECTION OF APPLE SCAR SKIN AND PEAR RUSTY SKIN VIROIDS IN ASIAN PEARS. S.F. Zhu , X. Yang, and A. Hadidi. National Germplasm Resources Laboratory, ARS-USDA, Beltsville, MD 20705

Two-dimensional gel electrophoretic analysis of nucleic acid extracts from diseased Asian pear fruits with rusty skin symptoms or no symptoms revealed one circular RNA. Occasionally two circular RNAs were detected from fruits with symptoms. All viroid RNAs hybridized with cRNA probe of apple scar skin viroid (ASSVd). No circular RNA or positive hybridization was obtained from healthy fruits. DNA primers for ASSVd were used in RT-PCR assays to amplify viroid cDNA from nucleic acid extracts of symptomless pears and pears with rusty skin symptoms. Gel-purified cDNA products were either directly sequenced or cloned into the vector pUC18 or pCR™II then sequenced. Nucleotide sequence analysis showed that symptomless pears were infected with ASSVd. This viroid is 229 nt and differs from the Japanese ASSVd type strain at 3 sites. Pear fruits with rusty skin symptoms were infected with pear rusty skin viroid (PRSVd) or with PRSVd and ASSVd. In single infection, PRSVd consists of 334 nt and differs from ASSVd type strain in 25 sites. In double infection, PRSVd is 332 nt and ASSVd is 330 nt. PRSVd and ASSVd differ from the ASSVd prototype at 20 and 8 sites, respectively.