



Southern Regional Project S-302

Biological Control of Soilborne Plant Pathogens for Sustainable Agriculture

ANNUAL REPORT OF COOPERATIVE REGIONAL PROJECT S-302

Supported by Allotments of the Regional Research Fund,
Hatch Act, as Amended August 11, 1955

January 1 to December 31, 2004

Cooperating Agencies and Principal Leaders:

SAES:

Alabama	K. S. Lawrence*, J. W. Kloepper
Arkansas	C. S. Rothrock
Florida	M. L. Elliott*, L. E. Datnoff
Georgia	K. W. Seebold
Indiana	D. M. Huber
Kentucky	J. W. Hendrix
Louisiana	G. B. Padgett
Mississippi	W. E. Batson*, R. E. Baird
North Carolina	D. M. Benson*, M. A. Cubeta
Oklahoma	L. L. Singleton*, K. E. Conway
South Carolina	A. P. Keinath
Tennessee	B. H. Ownley*, C. H. Canaday
Texas	T. Isakeit

USDA-ARS:

Texas	C. R. Howell
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Administrative Advisor:

Arkansas	G. J. Weidemann
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CSREES:

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* Voting member in state

Period Covered: January 1 to December 31, 2004

Date of Report: January 3, 2005

**Minutes of 2004 Annual Meeting
of the
Technical Committee S-302 Southern Regional Project**

Saturday & Sunday, November 6-7, 2004, Jackson, TN

Chair: Monica Elliott

Secretary: Boyd Padgett

Members Present: Mike Benson (NC), Craig Canaday (TN), Monica Elliott (FL), Don Huber (IN), Anthony Keinath (SC), Bonnie Ownley (TN), Boyd Padgett (LA), Craig Rothrock (AR), and Kenneth Seebold (GA). Greg Weidemann, administrative advisor, was present November 6.

Craig Canaday hosted the meeting at the West Tennessee Experiment Station, Jackson, TN. The meeting convened November 6 at 8:20 with a welcome from Craig Canaday.

Greg Weidemann addressed the group concerning the renewal of the project. He explained the process for renewal. He also explained the differences, advantages, and benefits of a Regional Project and a Coordinating Committee. Greg recommended the group continue as a regional project.

Monica reviewed handout from Robert Nowierski, CSREES representative, and initiated the administrative meeting. Kenny Seebold volunteered to serve as secretary for 2005. The meeting site and arrangements were discussed by the group. There was discussion to meet with the Western regional group and potential meeting sites for 2005 included Estes Park; Hendersonville, NC; camp near Orlando; and Washington D.C. Monica agreed to contact the chair of the Western group to discuss the details.

Craig Canaday led a discussion on the Regional Snap Bean project. Twelve seed treatments were evaluated (six biological treatments, both with and without chemical seed treatments) on cultivars Bronco and Hialeah. Disease pressure was good at all locations except Mississippi. Three chemical reference treatments were added, but performance was not uniform across locations. He noted some biologicals may be promising, but that the best biological varied with state and with cultivar. This may be due to differences in cultivar susceptibility to root rot with Bronco being more susceptible than Hialeah. In two states (AR and TX), disease incidence was greater with some biological agents than on non-treated seed. This may be due to the carriers (molasses and yeast) used with some biological agents. A planting date effect was noted in the Tennessee trials (only participating state with multiple planting dates). It was concluded

biologicals may be most beneficial in cultivars possessing some genetic resistance to root rots, when used in combination with seed treatment chemicals, or in scenarios where moderate disease pressure occurs. Other discussion included changing the method for statistical analysis using PROC MIX.

Monica Elliott reported on isolations from biological treatments for snap bean and cotton. When biological organisms were used in combination with chemicals there was an increase in bacteria recovered relative to non-chemical treatments. However, the addition of captan was not consistent and did not result in an increase. Monica noted biological organisms were stable on cotton.

Bill Batson was not able to attend. Therefore, Craig Rothrock reported on the results from the biological cotton seed treatments. Fourteen treatments were evaluated on two cultivars (DP 451BR and PM 1215BR). There was less disease pressure observed for DP 451BR compared to PM 1215BR. Statistical analysis revealed no location by treatment interaction; therefore results were summarized across locations. Plant stands were lowest in the non-treated control. Stands in cotton treated with Vitavax-PCNB were greater than the non-treated control. The addition of a biological organism to Vitavax-PCNB did not improve the efficacy of Vitavax-PCNB alone. The addition of *Trichoderma* to Chloroneb-Allegiance did not improve the efficacy of Chloroneb-Allegiance alone. Kodiak biological treatment did improve stand relative to the non-treated control. Craig noted that the current chemical standards were likely to change in the future.

Craig Rothrock and Bill Batson have agreed to summarize the results of the biological cotton seed treatment tests from the past years. Intentions are to evaluate the efficacy of the biological treatments, as well as, the effect of cultivars and environment. Craig and Bill will have a rough draft in the near future.

The group discussed the direction of the new project. Since interest in biologicals for seedling disease management in cotton is decreasing, it was suggested an emphasis on vegetable transplants may be warranted. Other ideas included increasing emphasis on the impact of cultural practices on seedling disease management. Several members (Seebold, Ownley, and Canaday) are already working with these crops. Tentative plans were made to explore these areas.

State reports were given by members of the group. Monica Elliott (FL) reported BioYield and *Beauveria bassiana* inhibited germination of geranium. Craig Canaday (TN) reported on interactions among tomato cultivars Celebrity, Florida 47, Mountain Fresh, and Spitfire with BioYield Concentrate and BioYield Flowable. The cultivars responded differently to these products indicating a possible cultivar effect. Mike Benson (NC) reported on using *Beauveria bassiana* to protect impatiens from *Rhizoctonia* and increase heat tolerance. In another study evaluating liquid and solid formulations of BioYield he found no difference in plant populations, but larger plants were noted with the solid formulation. Mike reported Botanigard, a commercial strain of *B. bassiana*, was phytotoxic to impatiens. In heat tolerance studies with vinca treated with *B. bassiana* Mike reported the following: (1) plants survived in all heat regimes (40, 43, 44, 46, 48 C exposed for 4 hr) five weeks after seeding; (2) vinca treated with *B. bassiana* isolate 11-98 at 10^7 CFUs grew best; (3) Botanigard isolate of *B. bassiana* did not adversely affect growth; and (4) Botanigard suppressed flowering by 50%.

Bonnie Ownley (TN) reported using brassica mulches to inhibit isolates of *Gaeumanannomyces* and *Fusarium*. She also reported on previous work evaluating various formulations of *Beauveria* for pathogen and insect control in cotton. Boyd Padgett (LA) reported on previous studies evaluating the influence of tillage and cover crops on cotton seedling disease. Excessive rains inhibited germination in conventionally-tilled plots and fungicides did not improve stand. Kenny Seebold (GA) reported on using an isolate of *Pseudomonas fluorescens* containing a gene for type III secretion system (T3SS) for managing gummy stem rot and *Phytophthora* crown rot in watermelon. Examining control of *Phytophthora capsici*, he reported Actigard provided some control, and evaluations of biocontrol agents provided similar control as tebuconazole. He will continue work with pepper and squash. Craig Rothrock (AR) updated the group on continuing work with binucleate *Rhizoctonia* for managing cotton seedling disease. Binucleates improved stand over the non-treated control and performed as well as some fungicides. Binucleates were associated more with the hypocotyls than the roots. *Trichoderma* produced inconsistent results and Actigard was phytotoxic to cotton.

Discussion was then directed at the RCU2 objective of the project. Craig Canaday (TN) commented on association among pre-plant applications of S-metolachlor and increased seedling disease compared to seedling disease where pre-emergence applications were used for snap bean production. Kenny Seebold (GA) reported that using solarization (8-10 weeks, 42-45C) reduced *Phoma* and increased yields in 2003 for onion; however, conflicting results with sour skin were obtained in 2004. Work with bio-mulches will continue. Tony Keinath (SC) observed reduced germination of sclerotia (*Sclerotium rolfsii*) using solarization. Effects decreased as depth of burial increased. In another experiment he reported no effects from two bio-fumigants (canola and mustard). Don Huber (IN) reported that glyphosate reduced manganese uptake in soybean. Deficiency can be corrected with foliar Mn applications. Other reported effects of glyphosate included: (1) reduced Mn uptake, (2) immobilization of Mn, (3) reduced root nodulations and N fixation, (4) increased drought stress, (5) earlier maturity, (6) more susceptibility to diseases, and (7) changes in soil microflora (rhizobium, fusaria, disease predisposition). He reported *Corynesporum* on dead ragweed roots and reduced growth on adjacent soybean plant sprayed with glyphosate. This was not observed on soybean plants 18 inches away.

On Sunday, November 7, the meeting was called to order at 8:30 am. The remainder of the meeting was directed toward defining new objectives for the project. It was agreed to terminate the snap bean and cotton biological seed treatment studies. The group has two years to write the new project. Tentative objectives were: 1) Evaluate biological seed treatments and biological drenches in tomato, broccoli and vinca. Pilot tests will be conducted this upcoming year. Bonnie Ownley, Craig Canaday, Tony Keinath, Kenny Seebold and Mike Benson will develop protocols. 2) Study the genetic diversity of pathogenic *Rhizoctonia* populations in two different cropping systems – probably with soybean, as it is more widely grown throughout the U.S., and an ornamental nursery plant species. Craig Rothrock will develop objective addressing genetic diversity in pathogenic *Rhizoctonia* populations. 3) Effects of cultural practices on ecological and genetic diversity of soilborne pathogens and indigenous microflora using mulches, herbicides, amendments, and long-term tillage. This objective may increase participation from the North Central region. The objective to address organic transition and changes it may have on soil diversity would be combined with objective 3.

PROGRESS OF THE WORK AND ACCOMPLISHMENTS:

Objective 1. Selection, optimization and regional evaluation of biological control agents to include application techniques and enhancement of biological control agents to control diseases caused by soilborne plant pathogens.

Regional Seed Treatment Trials:

Nine states (eight test sites) were involved in the Regional Cotton Trials (AL, AR, FL, GA, LA, MS, NC, TN, and TX) coordinated by Bill Batson. The biological treatments evaluated on two cotton cultivars (Deltapine 451B/RR and Paymaster 1218 BG/RR) included: *Bacillus subtilis* MBI 600 (Subtilex), *B. subtilis* GB03 (Kodiak), *B. pumilus* GB34 (YieldShield), *B. subtilis* GB03+ *B. amyloliquefaciens* GB99 (BioYield), *Trichoderma virens* G6, *T. virens/T. koningii* fusant TV-117, and *Trichoderma harzianum* (T-22). The *Bacillus* treatments were evaluated alone and in combination with Vitavax-PCNB+ Allegiance. The *Trichoderma* treatments were evaluated in combination with Chloroneb + Allegiance. Other treatments were nontreated control and chemical treatments alone. Significant cultivar and seed treatment effects were observed at 5 locations (AL, AR, GA, MS and NC); however, significant interactions between cultivar and treatment were only observed in AR and NC. Statistical analysis revealed no location by treatment interaction; therefore results were summarized across locations. Plant stands were lowest in the non-treated control. Stands in cotton treated with Vitavax-PCNB were greater than the non-treated control. The addition of a biological organism to Vitavax-PCNB did not improve the efficacy of Vitavax-PCNB alone. The addition of *Trichoderma* to Chloroneb-Allegiance did not improve the efficacy of Chloroneb-Allegiance alone. Kodiak biological treatment used alone did significantly improve stand count relative to the non-treated control.

Nine states (eight test sites) (AL, AR, FL, GA, MS, NC, SC, TN, and TX) participated in the Regional Snap Bean Trials coordinated by Craig Canaday. Four biological control agents (*Bacillus subtilis* MBI 600 (Subtilex), *B. subtilis* GB03 (Kodiak), *Bacillus licheniformis* SB3086 (710-145F), and *Trichoderma harzianum* T-22 HC), with and without captan + streptomycin, were evaluated as seed treatments on two snap bean cultivars (Bronco and Hialeah). Additional treatments were nontreated control and captan + streptomycin used alone. A significant increase in stand was observed overall only with the chemical seed treatment. Significant cultivar effects were observed at all locations except AL. Compared to the non-treated control, T-22 significantly improved stands of Bronco in TN (April planting) and of Hialeah in TX. A significant increase over non-treated seed in stands of Bronco was also observed with MBI600 in TN (May planting). The addition of a biological treatment to the chemical control treatment resulted in significant increases in stand over the chemical only treatment in NC (for Hialeah with MBI 600) and Texas (for Hialeah with GB03, MBI 600 and T-22).

The cooperative effort of Southern Regional Project S-302 also determines the stability, population, and purity of the biological agents applied to the seed from time of seed delivery to test cooperators until the seed is actually planted. For snap bean cv Hialeah, more variation in biological seed populations throughout the planting period were observed for MB1600 alone and 710-145F with a chemical seed treatment. For snap bean cv Bronco, more variation in biological seed populations was observed for the treatment mixture of GB03 and T-22. When compared

across both cultivars and all planting dates, the addition of chemical seed treatments to the bacterial biological treatments significantly increased the seed population of the biological. The reverse was true when the biological was the fungus *T. harzianum*. For cotton seed, the seed populations of the biologicals was very stable on both cultivars (DPI 451 and PM 1218) except for BioYield (*B. pumilus* GB34 and *B. amyloliquefaciens* GB99) without the addition of chemical seed treatments.

Other Research Under Objective 1:

In TN (Canaday) on soybeans, the effects of fungicide seed treatments supplemented with biological agents [GB03 (*B. subtilis*) or GB34 (*B. pumilus*)] were evaluated for their effects on seedling diseases, plant growth, and yield and compared with use of in-furrow fungicides. Soybean stands and yields were lowest with an in-furrow spray of azoxystrobin, both with and without biological agents. The average yield of an April no-till planting of Group III soybeans in a field which had been in continuous cotton for the last 10-12 years was increased by over 570 kg/ha with an in-furrow granular formulation of *Bradyrhizobium japonicum*. Also in TN, three different formulations of plant growth-promoting rhizobacteria (PGPR) (commercially known as BioYield containing *Bacillus amyloliquefaciens* GB99 and *Paenobacillus macerans* GB122) had no observable effects on the incidence or severity of tomato diseases in two field tests. Early marketable tomato yields of Celebrity were increased by 28% with the addition of BioYield Concentrate (bacteria + chitosan) to the potting media at 28 g product/1000 plants. A higher rate (183 g product/1000 plants) failed to increase early yields of Celebrity. Early yields of Mt. Spring, Mt. Fresh, Florida 47, and Spitfire were unaffected by PGPR, further verifying previously observed interactions between tomato cultivar and PGPR.

Also in TN a tomato field experiment conducted in 2003 was repeated in 2004. Four pest control methods (a transplant drench of imidacloprid followed with standard pesticide sprays, seeds treated with *Beauveria bassiana* Botanigard, seeds treated with *B. bassiana* 11-98, and an untreated control) were evaluated with and without the addition of PGPR (BioYield) to the transplant potting mix for their effects on insect pests, tomato diseases, plant growth, and yield of two tomato cultivars (Celebrity and Mt. Spring). Treating seeds with Botanigard or 11-98 had no apparent effect on insect infestations. Insecticides and use of PGPR appeared to reduce early yield losses due to fruit worm on Mt. Spring but not Celebrity. Late season aphid infestations were essentially eliminated with the pesticide treatments. The severity of foliar diseases (early blight + Septoria leaf spot) was highest with the 11-98 + PGPR combination. Foliar diseases were lowest with pesticides + PGPR. The combination of Botanigard with PGPR lead to the highest early yields for both cultivars and increased yields to levels comparable to pesticide use. Overall yields were highest with pesticides + PGPR and were significantly higher than with Botanigard + PGPR. Tomato yields were lowest with the combination of 11-98 + PGPR.

In FL a negative interaction resulted when geranium seed was treated with *B. bassiana* isolate 11-98 and pure cultures of the bacterial isolates in the PGPR product BioYield (GB99 and GB122). Seed treated with *B. bassiana* alone resulted in radicles that were not significantly different in length from the water controls. Seed treated with either of the BioYield isolates significantly reduced radicle length compared to the control, but combining the BioYield isolates with *B. bassiana* significantly reduced radicle length even more.

In NC there was a slight inhibition (8-10%) in seedling emergence and stand establishment of impatiens grown in potting mix with either formulation of BioYield (concentrate containing chitosan vs. flowable) compared to the untreated control. Leaf widths were 124 to 132% larger on seedlings in potting mix amended with BioYield compared to the untreated control. Thus, the slight inhibition of seed germination and emergence in impatiens was off-set by an increased growth response once seedlings emerged. NC examined effect of *B. bassiana* isolates 11-98 and Botanigard for biocontrol of pre-emergence damping-off caused by *Rhizoctonia solani* on two bedding plants, impatiens and vinca. Compared to stand counts in the non-infested control or in the Medallion (fungicide)-treated trays, Botanigard, with or without BioYield, was highly phytotoxic to seed germination and emergence, particularly at the high population rate of seed treatment. Compared to the untreated, infested control, strain 11-98 did not improve stand count, although Medallion did. Addition of BioYield amendment to the potting mix did not improve stand counts in combination with either *Beauveria* strain.

In GA a PCR protocol was successfully used to identify bacteria for the biological control of Phytophthora crown and fruit rot (PCFR) of summer squash and gummy stem blight (GSB) of watermelon and cantaloupe. The underlying mechanism behind the reductions in disease observed is not understood at this time. Two of the four strains used in this study, R6 and R10, exhibited only antibiotic activity in vitro against *P. irregulare* and *D. bryoniae*, while two of the four strains, R8 and B20, tested positive for the Type III secretion system (T3SS). R8 also exhibited in vitro activity against fungal pathogens. In the case of R8 and B20, it is possible that SAR played a role in reducing the severity of PCFR and GSB. The biological control agents tested in this study could be used potentially to reduce fungicide inputs if applied prior to transplant of seedlings. It appears that screening potential biocontrol agents for the T3SS could be a useful tool in the search for alternative control measures for diseases of cucurbits.

In AR an experiment was repeated to evaluate three binucleate *Rhizoctonia* fungi (BNR) and two *Trichoderma virens* isolates for control of *Pythium* spp. and *Rhizoctonia solani* seedling disease on cotton. Experiments were conducted at artificially and naturally infested field sites. Allegiance chemical control seed treatment was included. Cotton stand was significantly higher in the artificially infested site only for the fungicide treatment and BNR treatments for both the 13 and 31 day stand counts. In the naturally infested site, the highest stand count was found in the fungicide treatment for both stand count dates. The BNR treatments and the fungicide treatment had lower isolation of *R. solani* in general compared to the control.

Objective 2. Improve understanding of mechanisms and applicability of biological control agents across different cultivars, environments, and cropping systems.

In Texas seed treatment of DP 451 B/RR, a seedling disease susceptible cotton cultivar, with preparations of “P” and “Q” strains of *Trichoderma virens*, has shown that “P” strains are ineffective as biocontrol agents, while “Q” strains give good disease control. In an effort to determine what characteristics make the strains of one group biocontrol effective and the other not, the strains of the two groups were assayed for pathogenicity to cotton seedlings in non infested soil, phytotoxin production, lytic enzyme activity, induction of phytoalexins in cotton roots, and metabolism of pathogen propagule stimulants released by germinating seedlings. The results indicated the “P” strains were indeed pathogenic to the susceptible cultivar in non infested

soil, whereas the “Q” strains were not. Both “P” and “Q” strains synthesized the phytotoxin viridiol on certain substrates, and mutant of “P” strains deficient for viridiol production were still pathogenic to cotton. This indicates that viridiol production was not active in the disease syndrome. Both groups of strains were equally capable of synthesizing lytic enzymes such as cellulase, polygalacturonase, and protease, so this could not account for differences in pathogenicity. Assay of “P” and “Q” strains for metabolism of germination stimulants also indicated that both were equally capable of metabolizing these compounds. Therefore, this phenomenon could not be responsible for the observed differences. However, when the strains in the two groups were compared for the capacity to induce phytoalexin synthesis in cotton radicles, the “P” strains proved to be only weak inducers of the terpenoids hemigossypol and desoxyhemigossypol in cotton roots, whereas “Q” strains were very active inducers of the phytoalexins. These results indicate that although both “P” and “Q” strains are low grade pathogens of susceptible cotton cultivars, induction of high concentrations of phytoalexins in cotton roots by “Q” strains suppresses their further development in the cotton root and subsequent infection by other pathogens. Failure of the “P” strains to induce phytoalexin synthesis allows them to parasitize the cotton root, destroy root tissue and ultimately kill the seedling.

Solarization studies were conducted in GA and SC. In GA soils with a history of 10 years of onion production were solarized using clear plastic (3.0 mil thickness) for a minimum of 10 wks in mid-summer in Tifton, Georgia in 2002-2004. In 2003, total yield, mean bulb weight, and number of jumbo grade onions were significantly higher and the severity of pink root, caused by *Phoma terrestris*, was significantly lower in solarized plots. In 2004, trends were similar for total yield, mean bulb weight, number of jumbo grade bulbs, but were not significantly different. The number of small grade bulbs and pink root severity were significantly lower in solarized plots in 2004. Significantly fewer bulbs with Fusarium basal rot occurred in solarized plots in 2003, but because of lack of disease pressure in 2004 there were no significant differences among treatments. However, solarization had a long-term impact on reducing populations of soil saprophytes (cfu/g of soil) in both years. Solarization significantly reduced the numbers of weeds, particularly yellow nutsedge, in onion plots in 2004. Although having no impact on bacterial diseases in the 2002-2003 season, a second year of solarization reduced the number of CFUs of *Burkholderia cepacia* per gram soil a thousand fold in the 2003-2004 onion season. Although not significant, solarized plots yielded a lower percentage of onions with sour skin in 2004, a stark contrast from the results obtained in 2003.

A three-year project on soil solarization was continued in SC. Treatments included solarization for one, two, or three summers with or without cropping immediately after solarization. Plots were solarized for 10 weeks from early June to mid-August. Population dynamics of *Pythium* spp. differed between solarized and nonsolarized soil. Sclerotia of *Sclerotium rolfsii* did not germinate in response to methanol and did not cause any lesions on snap bean stems 2 months after burial at 5 cm in solarized soil. Viability and virulence also were reduced 1 month after burial. Sclerotia buried at 15 cm were not affected significantly by solarization.

The effects of brassica mulches or meal on soilborne pathogens were evaluated in SC and TN. In SC, *Brassica juncea* cv. Cutlass and *B. napus* cv. Dwarf Essex were compared to fallow soil and treatment with 75% methyl bromide-25% chloropicrin for control of Fusarium wilt of watermelon in field plots replicated six times. Brassica cover crops were incorporated one month

prior to transplanting seedless watermelon cv. Tri-X 313. At 41 days after incorporation, soil populations of *Fusarium*, as determined by dilution plating on Komada's medium, did not differ among treatments except that populations in fumigated plots were lower than in plots cropped to brassicas. Yield (kg/ha) of watermelon did not differ among treatments.

In TN the effects of *Brassica* spp. mulches or meal on growth of several isolates of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) and *Fusarium* spp. were studied to determine if inhibition was fungicidal. Exposure of fungal pathogens to mulches of canola and Dwarf Essex rape inhibited growth of the pathogens but mycelial growth resumed following removal of the mulch treatments. In contrast, growth of *Ggt* and *Fusarium* spp. did not resume following exposure to mustard meal and mulch, indicating that these treatments were fungicidal. Phytotoxicity of mustard mulch to wheat seedlings and the efficacy of mustard mulch for suppression of take-all caused by *Ggt* was evaluated. Wheat seeds were planted into soil in containers with and without inoculum of *Ggt*. After 28 days, shoots were excised and roots were left in the soil. Soil with healthy roots and diseased roots were covered with mustard mulch for 21 days. Mulch was not applied to controls. Wheat seeds were replanted into the soil. Disease severity and shoot height were determined after 28 days. The main effects of mulch treatment and pathogen, and the interaction of these factors were significant at $P = 0.0001$. Treatment of soil containing *Ggt*-infected roots with mustard mulch significantly reduced take-all disease severity in the subsequent wheat planting in two tests. In one test, height of seedlings from mustard mulch-treated soil was reduced.

In IN research has established that manganese oxidation is a virulence factor for the wheat take-all pathogen, *Gaeumannomyces graminis*, and other plant pathogens. Culture assays and spectrophotometric analysis of crude culture extracts derived from *Gaeumannomyces* support the involvement of a multi-copper oxidase such as laccase, and possibly other extracellular oxidative enzymes, in manganese oxidation, which reduces the plant's ability to respond to infection by the pathogen. Twelve putative laccase genes were found in *Magnaporthe grisea*, a fungus closely related to *Gaeumannomyces graminis*, and we found strong expression of *lcc 15* under manganese oxidizing conditions. Two genes with homology to manganese peroxidase also were identified in *Magnaporthe grisea*, and targeted deletion of these two genes resulted in greatly reduced virulence. It appears that there are at least two separate enzymes involved in manganese oxidation by these fungi that could influence their virulence and rhizosphere interactions via biological control.

Common sources of Mn fertilizers (sulfate, carbonate, and oxide salts) are immobilized by glyphosate, and herbicidal efficacy is also reduced. It was observed in IN that there was an increase in take-all of wheat following glyphosate resistant soybeans and increased colonization of necrotic soybean roots by *Corynespora*. Application of Mn a few days prior to, at the same time, or within a few days after the application of glyphosate has not been efficiently translocated by soybean plants. Tank-mixing the micronutrient with the glyphosate can reduce herbicidal efficacy from 5-50% on specific weed species. The glyphosate formulation can be as important as micronutrient source in these interactions, with the potassium salt being less interactive with micronutrients than other herbicide formulations. Unlike most inorganic sources of micronutrients, specific nutrient chelates or micronutrient complexes (amino acid or protein complexes) are compatible with glyphosate and readily translocated to new soybean tissue from tank-mix combinations. Although increased translocation of micronutrients in compatible

micronutrient-glyphosate formulations was observed with soybeans grown on a nutrient sufficient soil, no additional yield increase was observed to indicate that excess micronutrient availability is of little value in increasing yield.

IMPACT:

Pesticides in the environment, and public concern over food safety, have led scientists in Southern Regional Research Project S-302 to look at biological control and cultural control practices as central components of an ecologically based approach to Integrated Pest Management for plant diseases. Identification of biological controls or combinations of biologicals and chemicals that reduce losses due to seedling diseases will provide alternative management strategies for growers and reduce dependence on chemical treatments alone, thus reducing the risk of pathogen resistance. For example, currently, cotton seed is planted at five times the rate needed to compensate for plant losses due to seedling diseases that occur even with chemical fungicides applied to seed. Transgenic cotton cultivars, with higher seed costs, are widely used and have spurred a need to develop more effective seed treatments. Evaluations of seed treatments on a regional scale provide information that cannot be gained by a single state.

Identification of biologicals, such as antibiotic-producing bacteria or bacteria with Type III secretion systems, provide new sources for development of biocontrol agents. Cultural controls such as in-furrow applications of meal prepared from *Brassica* spp., or cropping schemes that incorporate *Brassica* mulches into soil have the potential to be effective, economical alternative management strategies to chemical treatments for protection of plants against soilborne pathogenic fungi.

WORK PLANNED FOR NEXT YEAR:

Work will continue on both project objectives. Regional testing of biological control agents will be altered to conduct regional preliminary experiments on tomato, broccoli and selected ornamental bedding plants. Researchers in several states will work together to better understand the mechanisms of biocontrol and the complex interactions between host, pathogen, biocontrol agent, and the environment.

PUBLICATIONS ISSUED AND MANUSCRIPTS APPROVED DURING THE YEAR:

Refereed Journal Articles:

Abad, J.A., Moyer, J.W., Kennedy, G.G., Holmes, G.A., and **Cubeta, M.A.** 2004. An epidemic of *Tomato spotted wilt virus* on potato in eastern North Carolina. *Amer. J. Potato Res.* (accepted).

Bush, B.J., Carson, M.L., **Cubeta, M.A.**, Hagler, W.M., and Payne, G.A. 2003. Infection and fumonisin production by *Fusarium verticillioides* in developing maize kernels. *Phytopathology* 94:88-93.

Ceresini, P.C., Shew, H.D., Vilgalys, R., U.L. Rosewich-Gale and **Cubeta, M.A.** 2003. Detecting

migration in populations of *Rhizoctonia solani* AG-3 from potato in North Carolina using multilocus genotype probabilities. *Phytopathology* 93:610-615.

Elliott, M. L., E. A. Guertal, and H. D. Skipper. Rhizosphere bacterial population flux in golf course putting greens in the Southeastern United States. *HortScience* 39:1754-1758.

Fichtner, E. J., **Benson, D. M.**, Diab, H. G. and Shew, H. D. 2004. Abiotic and biological suppression of *Phytophthora parasitica* in a horticultural medium containing composted swine waste. *Phytopathology* 94:780-788.

Harrison, H. F., Jackson, D. M., **Keinath, A. P.**, Marino, P. C., and Pullaro, T. C. 2004. Broccoli production in cowpea, soybean, and velvetbean cover crop mulches. *HortTechnology* 14:484-487.

Hollowell, J.E., Shew, B.B., Wilcut, J.W., and **Cubeta, M.A.** 2003. Weed species as hosts of *Sclerotinia minor* in peanut fields. *Plant Dis.* 87:197-199.

Ownley, B.H., B.K. Duffy, and D.M. Weller. 2003. Identification and manipulation of soil properties to improve biological control performance of phenazine-producing *Pseudomonas fluorescens*. *Appl. Environ. Microbiol.* 69: 3333-3343

Technical Articles:

Batson, Jr., W.E., Caceres, Elliott, M.L., Huber, D.M., Hickman, M.V., McLean, K.S., Ownley, B., Newman, M., Padgett, G.B., Rushing, K.W., Kenny, D.S., Rothrock, C.S., Seebold, K., and Thaxton, P. 2003. Biological seed treatment evaluations for control of the cotton seedling disease complex, 2001. *Biological and Cultural Tests for Control of Plant Disease* Vol. 18:F002 (electronic).

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