Annual Report for the Almond Research Board 2003-2004

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Project No. 00-JA2: Epidemiology and management of silver leaf of almond in central California

Principal Investigator (s): J. E. Adaskaveg
University of California
Dept. of Plant Pathology
Riverside, CA 92521

Cooperating: H. Förster and D. Thompson (UC Riverside), R. Duncan (Stanislaus Co.), and H. Andris (Fresno Co.).

Objectives

I. Epidemiology
- A. Confirmation of reported distribution of the fungal pathogen in Stanislaus and Merced Counties.
- B. Inoculation of trees to determine rates of growth and fruiting body development on almond.
- C. Sporulation cycle of fruiting bodies under California conditions (Years 2 and 3).
  1. Viability of basidiospores – Survival duration under lab conditions.

II. Disease management
- A. Laboratory evaluations of experimental and registered fungicides for almond.
- B. Preventative and eradication treatments with branch or trunk injections using fungicides including selected registered fungicides on almonds.

SUMMARY

Silver leaf disease is known to occur on *Prunus* species in many locations worldwide including reports on peach in California in the 1970s. The disease is caused by the fungus *Chondrostereum purpureum.* Surveys in almond and peach orchards and isolation of the pathogen confirmed the recent outbreak of the disease in several locations in the central valleys of California. Studies on inoculated trees in the spring of 2001 and 2002 indicated that the fungus grows extremely fast within the tree with an average rate of 10-18 cm/month. In our late summer-early fall field trials in 2003, however, growth rates of 5.3-5.4 cm were observed. Previously, myclobutanil was identified as a very active fungicide against the pathogen with EC_{50} values for in vitro mycelial growth inhibition of 0.12-0.42 ppm. Additional SBI fungicides were evaluated in 2003. EC_{50} values for two isolates of *C. purpureum* were 0.049-0.130 ppm for propiconazole (Orbit® 3.6EC), 0.004-0.010 ppm for tebuconazole (Elite® 45WP), and 0.081-0.189 ppm for tetraconazole (Eminent® 125SL) These latter fungicides, however, are currently not registered for use on almond. Tree injections with myclobutanil (Laredo® 2EC) and azoxystrobin (Abound® 2F) that were evaluated for disease management in 2001-2002 could not eradicate the organism or suppress the disease using this application method. In 2002 myclobutanil, but not tetraconazole, significantly reduced the amount of disease when applied onto freshly cut branch stubs that was followed by inoculation with the pathogen. A laboratory preparation of the biocontrol *Trichoderma viride* was the best treatment in this study reducing the amount of decay and survival of the pathogen. In 2003, these studies for silver leaf management were repeated in trials in two orchard locations. In addition to the treatments evaluated in 2002, we included a commercial formulation of the biocontrol agent *T. harzianum* (PlantShield®, BioWorks, Inc.; distributed by Wilbur-Ellis Co.). When treatments were applied to run-off using a hand-sprayer one day before inoculation, both biocontrol treatments using *Trichoderma* spp. completely prevented growth of the silver leaf pathogen into the almond wood in a late summer/fall pruning trial in Central California. *Chondrostereum purpureum* could not be isolated

from treated branches that were sampled 8 weeks after inoculation. In the Southern California trial *T. viride* completely prevented the colonization of almond branches with *C. purpureum*. *T. harzianum* was somewhat less effective, but still significantly reduced the amount of decay as compared to the control. In contrast, after treatment with myclobutanil the pathogen did get established in the wood and similar decay levels were observed as the untreated control in both trials. The commercial biocontrol formulation was also effective after application with an air-blast sprayer at a rate of 100 gal/A. Incidence and severity of decay was significantly reduced at both field locations in a trial in late summer/fall of 2003. Treatments were especially effective when inoculations were done 8 days after treatments as compared to one day after treatment. Decay incidence was 14.4 % in the Central California trial and 0% in the Southern California trial, whereas all untreated control branches were infected. In our winter pruning experiments in 2003/04 *T. harzianum* was only effective in the Southern California trial and only when inoculations with the pathogen were done after 14 days as compared to 1 day after treatment. *T. viride* was very effective at both trial locations. This difference in efficacy between *T. harzianum* and *T. viride* and between the summer and winter pruning trials could be correlated with a better tolerance to cool temperatures (4-10C) of *T. viride*. Previously, we demonstrated that wound healing was an important disease prevention strategy. When cut branches were inoculated two weeks after pruning in the spring, the amount of decay and survival of the fungus was significantly reduced as compared to inoculation 1 day after pruning. In the summer and winter trials discussed above, however, there was no difference in susceptibility of control branches that were inoculated either 8-14 days or 1 day after pruning. Laboratory studies to evaluate growth responses in competition studies between *Trichoderma* and *Chondrostereum* spp. indicated that the mode of action of the biocontrols likely is site exclusion and not antibiosis (chemical inhibition). Furthermore, inoculum concentration of the biological control and 'time of inoculation' with the pathogen were significant factors in evaluating the competitiveness of *Trichoderma* spp. in laboratory assays.

Introduction. The fungus *Chondrostereum purpureum* causes silver leaf disease of *Prunus* spp. Leaves of trees affected by the disease become silvery in appearance due to a toxin produced by the pathogen and later become necrotic and abscise. Wood decay often is evident as angular to pie-shaped discolorations of cross sections of the secondary xylem. Over time, substantial white rot of the tree trunk and scaffold branches may occur that may extend into roots. Spores of the fungus that are produced in bracket-like fruiting bodies are wind-disseminated and cause new infections on fresh wood-exposing wounds. A wide range of perennial hosts, inoculum production over a long period, the difficulty of protecting all wound surfaces, and the inability of previous researchers to eradicate established infections from tree trunks make silver leaf difficult to control. In California, in the past, silver leaf has occasionally been found on temperate tree fruit crops and it is known to occur on other hosts such as willow, lilac, sycamore, rhododendron, poplar, birch, and oak. Because silver leaf disease has recently become a concern for California almond growers due to serious damage in several orchards, this research project was initiated in 2001.

Distribution of silver leaf disease in California almond orchards. We were continuing confirming reports of silver leaf disease in collaboration with farm advisors. During 2003 no major new outbreaks were found. One almond and one peach tree with silver leaf were found in Stanislaus and Yuba Co., respectively. Other reports were negative for silver leaf on *Prunus* spp. The disease has been reported from Fresno to Yuba Co. since 2001.

Sporulation cycle of fruiting bodies under California conditions and viability of basidiospores. Although fruiting bodies have been observed in several locations on naturally infected almond trees, to date, none of the trees inoculated with *C. purpureum* in our 2001 and 2002 field trials has shown any fruiting structures of the fungus. Thus, either longer times or specific environmental

conditions are needed for the fungus to sporulate. Because we were also unable to produce fruiting bodies in the laboratory no studies on the survival of the basidiospores could be conducted.

Studies on the management of silver leaf disease in 2003. ***Lab assays*** - In vitro toxicity studies were conducted in the laboratory for three additional SBI fungicides. EC_{50} values for mycelial growth for two isolates of *C. purpureum* were 0.049-0.130 ppm for propiconazole (Orbit® 3.6EC), 0.004-0.010 ppm for tebuconazole (Elite® 45WP), and 0.081-0.189 ppm for tetraconazole (Eminent® 125SL). This compares to EC_{50} values of 0.12-0.42 ppm for myclobutanil that were obtained in previous studies. Thus, tebuconazole was the most toxic material against *C. purpureum*. This fungicide, however, is currently not registered on almond.

Laboratory studies to evaluate growth responses in competition studies between *Trichoderma* and *Chondrostereum* spp. were done using a new spiral plate assay. For this, conidia of *Trichoderma* spp. were plated out using a 2.5 log dilution gradient on a Petri dish. Mycelium of *C. purpureum* on cellophane strips was radially plated on to the *Trichoderma* gradient at 0.5, 4, and 28 hr. Results indicated that the mode of action of the biocontrols likely is site exclusion and not antibiosis (chemical inhibition). Furthermore, inoculum concentration of the biological control and 'time of inoculation' with the pathogen were significant factors in evaluating the competitiveness of *Trichoderma* spp. in these assays.

Field tests - Because our 2002 field trial, where treatments were applied to cut branches before inoculation, had very promising results, our trials in late summer/early fall of 2003 again focused on preventative treatments. Branches 2 to 3 cm in diameter of 5-6 year-old cv. Carmel trees at two orchard locations were cut and sprayed to run-off using a hand sprayer with one of the following solutions: distilled water (control), myclobutanil (Laredo® 2EC, 12.8 fl oz), tetraconazole (Eminent® 125SL, 16 fl oz), a conidial suspension of the biocontrol *T. viride* (2×10^7 conidia/ml) in 0.5% of a gelling agent (Methocel®) to slow drying of the biocontrol, or a commercial formulation of the biocontrol *T. harzianum* (PlantShield®, BioWorks, Inc. distributed by Wilbur-Ellis Co.; 0.5 oz/gal). The branch stubs were then inoculated either the following day or 1-3 weeks later. For this, autoclaved almond wood pieces (0.5 to 1 cm in diameter, 3 to 5 mm thick) that were inoculated in the laboratory with *C. purpureum* and completely colonized by the fungus were placed on the branch stubs. The wounds were covered with Parafilm™ and with a paper towel to keep a high humidity and to prevent direct sun exposure, respectively. Inoculum was removed after two weeks. For evaluation of the treatments, inoculated branches were cut off after approximately 8 weeks, split open, and examined for wood decay. The length of the decay column was measured and the presence of the pathogen was verified by plating samples from the top and the bottom of the decay column on a selective agar medium. In another trial on cv. Mission and Sonora almond trees, branch stubs were treated with PlantShield® using a backpack sprayer at 100 gal/A. All other procedures were done the same as in the trials using a hand sprayer application.

In 2003, studies for silver leaf management were done in two orchard locations with summer and winter prunings. At evaluation time, fungal decay in the longitudinally split inoculated branches was evident as discolored, longitudinal streaks in the wood that extended down the branch from the site of inoculation. Leaf symptoms were also observed on some of the branches. As in our previous experiments, rapid growth rates of the silver leaf fungus in almond wood were observed. Thus, when water-treated branch stubs (control) were inoculated one day after treatment, the fungus was recovered from all branches (100% incidence) and it grew an average of 10.6 and 10.8 cm (5.3 and 5.4 cm per month) during the 2-month period on cvs. Carmel and Mission, respectively. This compared to an average of 36–36.7 cm/2 months for the two cultivars in the same orchard in last year's experiment. The slower growth in our 2003 trial could be explained by the fact that inoculations were done in August as compared to May in 2002. Our in vitro studies using three isolates of *C. purpureum* had demonstrated a temperature optimum for mycelial growth of *C.*

purpureum of ca. 25C (77F). At 31C (88F) growth was significantly reduced, while at 35C (95F) no or very little growth occurred. Thus, high late summer-early fall temperatures during the first part of the experimental period in 2003 probably are responsible for the reduced decay as compared to the 2002 trial.

When water-treated branches were inoculated 1 week after cutting in late summer/early fall, wood discoloration and survival of the fungus were similar to inoculations done after one day. Similarly, inoculations 2 weeks after winter prunings did not reduce wood decay as compared to the 1-day inoculation. In 2002, however, survival of the fungus and average length of wood discoloration was significantly reduced when inoculations were done after 2 weeks. Thus, based on our field trials over three seasons, wound healing of almond wood is inconsistent and most times will be ineffective in preventing silver leaf disease infections.

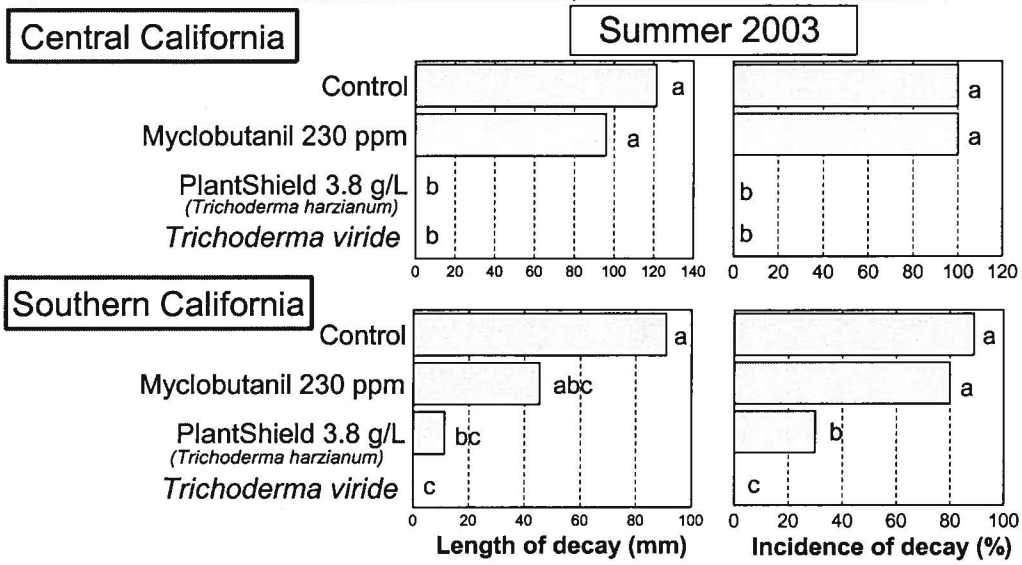
When treatments were applied to run-off using a hand-sprayer one day before inoculation, both biocontrol treatments using *Trichoderma* spp. completely prevented growth of the silver leaf pathogen into the almond wood in a late summer/fall pruning trial in Central California (Fig. 1). *Chondrostereum purpureum* could not be isolated from treated branches that were sampled 8 weeks after inoculation, thus it was no longer viable. As indicated above, the mode of action of the two biological agents likely is site exclusion. In our 2002 trial, recovery of *C. purpureum* after treatment with *T. viride* (*T. harzianum* was not included in last year's study) was only reduced to 57.1% as compared to 100% recovery in the control. In the Southern California trial *T. viride* completely prevented the colonization of almond branches with *C. purpureum*. *T. harzianum* (PlantShield®) was somewhat less effective, but still significantly reduced the amount of decay as compared to the control. In contrast, after treatment with myclobutanil the pathogen did get established in the wood and similar decay levels were observed as the untreated control in both trials. *C. purpureum* was re-isolated from all of the inoculated branches and the lengths of the decay columns were similar to those of the control. Thus, the partial efficacy of myclobutanil that we demonstrated in our 2002 spring trial could not be confirmed in the late summer-early fall trials in 2003.

The commercial biocontrol formulation was also effective after application with an air-blast sprayer at a rate of 100 gal/A (Fig. 2). Incidence and severity of decay was significantly reduced at both field locations in a trial in late summer/fall of 2003. Treatments were especially effective when inoculations were done 8 days after treatments as compared to one day after treatment. Decay incidence was 14.4 % in the Central California trial and 0% in the Southern California trial, whereas all untreated control branches were infected.

In our winter pruning experiments in 2003/04 *T. harzianum* was only effective in the Southern California trial and only when inoculations with the pathogen were done after 14 days as compared to after 1 day, whereas *T. viride* was very effective at both trial locations. Myclobutanil again had no significant effect on disease control.

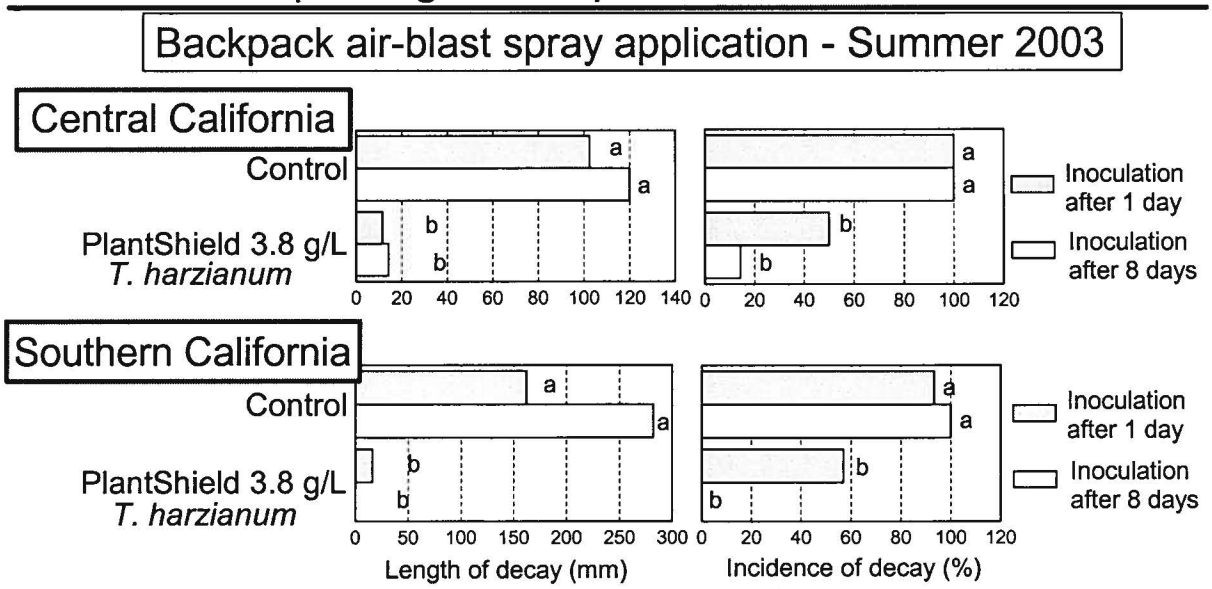
Our results indicate that preventative treatments with *Trichoderma* spp., particularly *T. viride*, can be very effective in reducing the colonization of almond wood by *C. purpureum*. A very severe inoculation method was used and thus, these treatments may be more effective at natural inoculum levels. In nature infection is by basidiospores but these spores are difficult to produce in the laboratory. Basidiospores land on pruning wounds where they are exposed to environmental conditions that may or may not favor establishment of the fungus in the wood. *Trichoderma* spp. have been used to control silver leaf disease on stone and pome fruits in Northern European countries previously. This is, however, the first time that biocontrol agents have been used on almond in a mediterranean climate such as in California. The use of *Trichoderma* biocontrols is a promising new preventative tool for the management of silver leaf disease of almond in California. Additional established cultural practices will enhance the efficacy of the biocontrol treatments. These include: planting clean nursery stock, minimizing large wood-exposing wounds, using proper pruning practices, avoiding pruning during wet and warm environments, and orchard sanitation practices such as removal and burning of infected trees.

Fig. 1. Evaluation of pruning wound protection treatments using fungicides and biocontrols



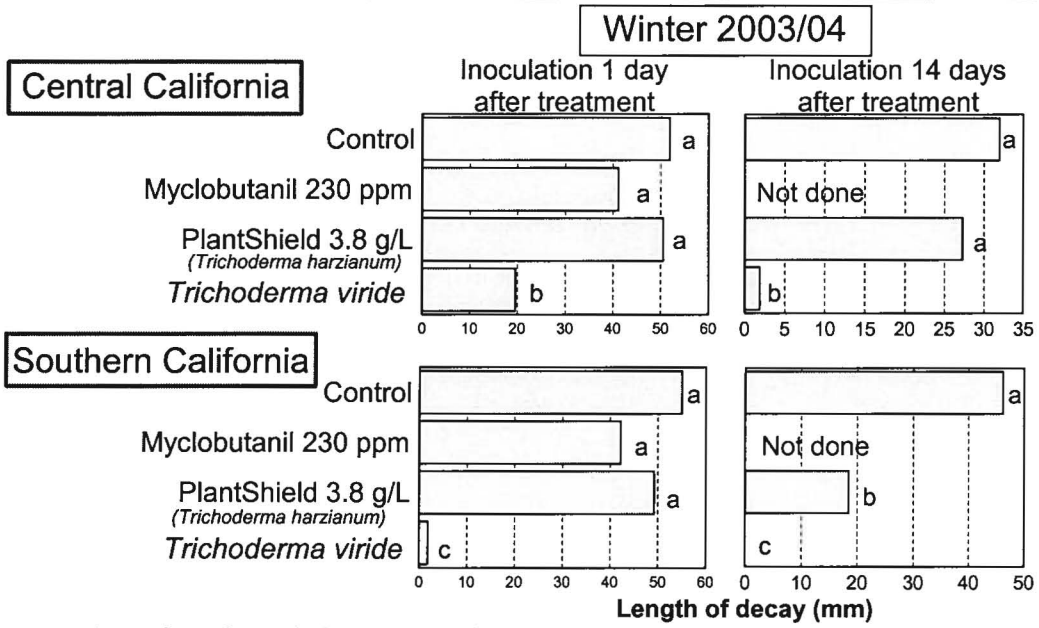
Branches of cv. Carmel almond trees (0.5 -1 in in diameter) were cut, treated with a hand sprayer, and were inoculated with *C. purpureum* the following day. Trees were evaluated after 6-8 weeks. The presence of the fungus was confirmed by isolation.

Fig. 2. Evaluation of the biocontrol product PlantShield as a pruning wound protection treatment



Branches of almond trees (0.5 -1 in in diameter) were cut and trees were treated with PlantShield using a back-pack sprayer. Branches were inoculated with *C. purpureum* either the following day or after 8 days. Trees were evaluated after 6-8 weeks. The presence of the fungus was confirmed by isolation.

Fig. 3. Evaluation of pruning wound protection treatments using fungicides and biocontrols



Branches of cv. Carmel almond trees (0.5 -1 in in diameter) were cut, treated with a hand sprayer, and were inoculated with *C. purpureum* the following day or after 14 days.