Epidemiology and Management of Silver Leaf of Almond in Central California

Project No. 04-JA-03

Project Leader: J. E. Adaskaveg, Dept. Plant Pathology, UC Riverside

Cooperating Personnel: 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 California almond and peach orchards.
   B. Inoculation of trees to determine rates of growth in almond.
   C. Effect of wound healing on infection by the pathogen at three different times of the year.

II. Disease Management
   A. Laboratory evaluation of biocontrols.
   B. Preventative treatments with biological control agents after winter pruning.

Summary

Silver leaf disease is known to occur on *Prunus* species in many locations worldwide including on peach in California where it was reported 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. Our studies on inoculated trees from 2001 to 2004 indicated that the fungus grows extremely fast within the tree. The fungicide myclobutanil and other sterol biosynthesis inhibitors were identified to be active against the pathogen in in vitro mycelial growth studies. Tree injections with myclobutanil (Laredo® 2EC) and azoxystrobin (Abound® 2F) that were evaluated for disease management in 2001-2002, however, 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. In our trials in 2003 and 2004, however, this fungicide had no effect on reducing the disease. A laboratory preparation of the biocontrol agent *Trichoderma viride* and a commercial formulation of the biocontrol *T. harzianum* (PlantShield®, BioWorks, Inc.; distributed by Wilbur-Ellis Co.) were the best treatments in previous field studies in the summer of 2003 dramatically reducing the amount of decay and survival of the pathogen. *Chondrostereum purpureum* could not be isolated from treated branches that were sampled 8 weeks after inoculation. The commercial biocontrol formulation was also

effective after application with an air-blast sprayer at a rate of 100 gal/A. The pathogen only got established in 37.5 % of the branches inoculated resulting in an average length of decay of 0.6 cm as compared to the control where 88% of the branches were infected with an average of 9.4 cm of decay. In the winter of 2004, these studies on silver leaf management were repeated at two orchard locations. Only *T. viride* was highly effective in these winter trials, especially when the biocontrol was allowed to establish for two weeks before inoculation with the pathogen. *T. harzianum* significantly reduced the disease only in the Southern California trial. This difference in efficacy between the two *Trichoderma* species at different times of the year could be explained by a better tolerance of *T. viride* to lower temperatures. We also investigated the effect of wound healing on infection and colonization of almond wood by *C. purpureum*. Wound healing after springtime pruning reduced the incidence of decay in a previous study. After summer pruning and this year's winter pruning, however, there was no difference in disease whether inoculations of pruned branches were done one day or up to four weeks after pruning, indicating that wound healing of almond wood is very slow under California conditions.

In summary, preventative treatments with either of two *Trichoderma* species effectively reduced the colonization of almond wood by *C. purpureum* after summer prunings. Only *T. viride* was highly effective after winter prunings using our severe inoculation procedure. Use of T. harzianum (PlantShield®) was less effective in the one day-after-treatment inoculation than in the 14 day-after-treatment inoculation. 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 of clean nursery stock, minimizing large wood-exposing wounds, using proper pruning practices, avoiding pruning during wet and warm environments, and orchard sanitation practices that include removal and burning of infected trees.

Introduction and summary of our research in previous years. In California, in the past, silver leaf disease 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.

Silver leaf disease is caused by the fungus *Chondrostereum purpureum.* Leaves of affected trees 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. 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 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. 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 addition, we demonstrated that the pathogen rapidly becomes established in almond wood with growth rates of 5 and 18 cm/month for summer and spring inoculations, respectively. We also found that wound healing of almond wood after pruning can be slow. Thus, in our springtime experiments in 2002, survival of the fungus and average length of wood discoloration was significantly reduced when inoculations were done 2 weeks after pruning. In our summer experiments in 2003, however, there was no significant difference in incidence of wood decay between inoculations done one day or up to four weeks after pruning.

Studies on the distribution of the silver leaf disease confirmed its presence in orchards from Fresno to Yuba Co., but no major new outbreaks were found in 2003 and 2004. For management of the disease were have evaluated several fungicides and two biocontrol agents. Sterol biosynthesis inhibiting fungicides such as Elite (tebuconazole - Break (propiconazole - Orbit), Eminent (tetraconazole), and Laredo (myclobutanil) were shown to have a high in vitro activity. Because they are registered on almond, Eminent and Laredo were used in field inoculation studies that were conducted in the spring or summertime. When pruned almond branches were treated with the fungicides, inoculated with the pathogen, and evaluated for wood decay after several weeks, these materials, however, had little or no effect on the colonization of almond wood by the pathogen. The biocontrol agents *Trichoderma viride* and *T. harzianum* were very effective in these experiments using hand-spray or backpack spray applications. They were more effective when inoculations were done two weeks as compared to one day after treatment with the biocontrol, indicating that after establishment of the biocontrols in the almond tissues, pathogen infections are more successfully prevented. Laboratory tests indicated that the mode of action of the biocontrols likely is site exclusion and not antibiosis (chemical inhibition). To further evaluate the efficacy and potential of these preventative treatments against silver leaf disease, we conducted additional field experiments during the winter when almond trees are generally being pruned commercially.

Field tests in the winter of 2004. – Trials were set up in January of 2004 in two orchards in central and southern California. Branches 2 to 3 cm in diameter of 6-7 year-old cv. Carmel trees 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), a conidial suspension of the biocontrol *T. viride* (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 1-4 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

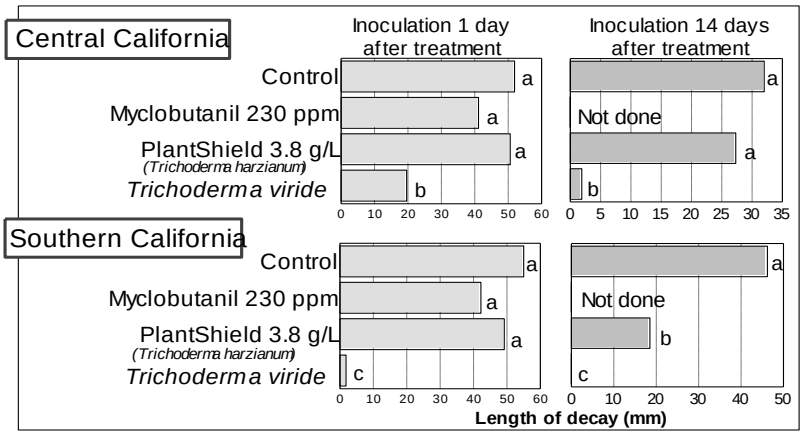
with Parafilm™ and with a paper towel to keep a high humidity and to prevent direct sun exposure, respectively. For evaluation of the treatments, inoculated branches were cut off after 8 to12 weeks, split open, and examined for wood decay. The length of the decay column was measured and the presence 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 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 using 1-day and 2-week post-treatment intervals for inoculation.

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, fast 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, *C. purpureum* was recovered from all branches (100% incidence) and it grew an average 10.5 cm per month on cv. Carmel and 14 cm per month on cv. Mission during a two-month period. This compared to an average of 5.3 and 5.4 cm per month in our summer trial in 2003 and to 18 and 18.4 cm per month in our spring trial in 2002 for the two cultivars, respectively. Thus, the slowest growth rate of the fungus was found during the summer and the fastest growth during the spring. Our previous 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. The fungus still grew at 5C (41F). Thus, high summer-early fall temperatures are most inhibitory for growth of the pathogen, explaining the reduced wood colonization that we observed in our summer experiment.

When treatments were compared that were applied to run-off using a hand sprayer one day before inoculation in these winter trials, the fungicide myclobutanil had no effect on either survival of the pathogen or on wood decay at both trial sites (Fig. 1). After fungicide treatments, *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. These results were similar to our late summer-early fall trials in 2003, and do not confirm the partial efficacy of myclobutanil that we demonstrated in our 2002 spring trial. In contrast to our previous trials, the commercial preparation of *T. harzianum* (PlantShield®) was ineffective in preventing wood decay when pruned branches were inoculated one day after treatment. *T. viride*, however, significantly reduced the incidence and severity of the disease in both trials. In the central California experiment disease severity was reduced from 51.9 cm decay in the control to 19.6 cm in the *T. viride* treatment. In the Southern California trial this biocontrol was much more effective in preventing the establishment of the fungus and in preventing wood decay. Disease severity was reduced from 55 cm in the control to 1.8 cm and there was a 90% reduction in disease incidence. When treated branches were inoculated after 14 days, *T. viride* was highly effective at both locations and *T. harzianum* significantly reduced the severity of wood decay in the southern California trial (Fig. 1). Thus, *T. viride* has been highly effective in

our spring, summer, and winter field trials, whereas *T. harzianum* provided good control only in the summer trials (this biocontrol was not tested in the spring trial in 2002).

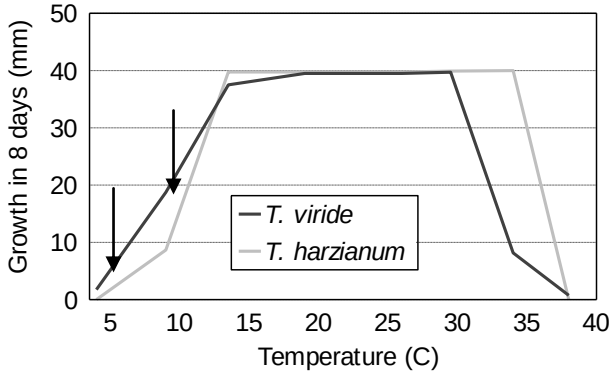
Figure 1. Evaluation of pruning wound protection treatments using fungicides and biocontrols



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* after 1 or 14 days. Branches were removed from trees 8 weeks after inoculation and decay was measured. Presence of the pathogen was confirmed by isolation on agar media.

Both *Trichoderma* species were re-isolated from treated branches after the 2-month incubation time after treatment, demonstrating their persistence in the environment and suggesting that only their competitiveness was affected at different times of the year in our field experiments. To find a possible reason for this inconsistent efficacy, we determined the in vitro temperature-growth relationships for the two biocontrols. As indicated in Fig. 2, *T. viride* grows better at 4 to 10 C (growth curve between arrows) than *T. harzianum*. This may explain the higher efficacy of *T. viride* in our winter pruning experiments. We also found in these studies that *T. harzianum* is more tolerant to high temperatures and still grows well at 35C where growth of *T. viride* is greatly reduced. This difference in high-temperature sensitivity between the two species of *Trichoderma*, however, did not compromise the efficacy of *T. viride* in our summer experiments.

Fig. 2. In vitro temperature-growth relationships for *Trichoderma harzianum* and *T. viride*



Isolates of *Trichoderma* were grown on potato dextrose agar at selected temperatures and growth was measured after 8 days.

The effect of pruning wound healing was again evaluated in our winter trials in 2004. At both sites, when branches were inoculated up to 4 weeks after pruning, still 100% of branches were infected by *C. purpureum*, wood discoloration was evident and the pathogen was re-isolated from all inoculated branches. The severity of wood decay, however, was significantly reduced from 32 cm in inoculations 4 weeks after pruning to 26.6 cm in inoculations one day after pruning in the central California trial. No differences were observed in severity of disease in the southern California trial.

Our studies demonstrate that biocontrols can be effective protective treatments for the management of silver leaf disease of almond. Although *Trichoderma* biocontrols have been described to be effective in other countries, this was never tested before under California growing conditions. Only the less effective *T. harzianum* is currently commercially available. It has, however, to be emphasized that we used a very severe inoculation method. Colonized wood pieces were sealed onto the stub wounds, thus providing optimum conditions for infection. Under natural field conditions, however, 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. Therefore, under natural field conditions the efficacy of our treatments may be improved.