Epidemiology and Management of Silver Leaf of Almond in Central California

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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).
   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. The disease is caused by the fungus *Chondrostereum purpureum.* Leaves of affected hosts 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. Surveys in almond and peach orchards and isolation of the pathogen confirmed the presence of the disease in several locations in the central valley of

California. Studies on inoculated trees in 2001 indicated that the fungus grows extremely fast within the tree with an average rate of 10 cm/month during an 8-month study. In 2002, even faster growth rates of 18 cm/month were observed. Myclobutanil (Laredo 2EC) that was identified as a highly active fungicide against the pathogen and azoxystrobin were injected into trees in two trials in the spring of 2001. When trees were evaluated in the spring of 2002, however, disease severity of treated and non-treated trees was similar. Thus, the fungicides could not eradicate the organism or suppress the disease using this application method. In 2002, myclobutanil, tetraconazole, and the biocontrol *Trichoderma viride* were evaluated for infection inhibition by application onto freshly cut branch stubs followed by inoculation with the pathogen 1 or 14 days later. When treatments were applied to run-off using a hand-sprayer, the biocontrol treatment using conidia of *T. viride* was very effective, significantly reducing the amount of decay and survival of the pathogen under the severe inoculation conditions used. Decay was also significantly reduced by the myclobutanil treatment when applied by a small hand sprayer, but not when applied at a rate of 100 gal/A using an air-blast sprayer. Our study also emphasizes the importance of wound healing as a disease prevention strategy. When cut branches were inoculated two weeks after pruning, the amount of decay and survival of the fungus was significantly reduced as compared to inoculation 1 day after pruning. Thus, it remains an important management strategy to prune trees when wound healing is favored, i.e. when weather is warm and dry.

Epidemiology and management. The presence of silver leaf disease was confirmed in additional almond orchards in Stanislaus Co. in 2002. The causal pathogen was isolated and was positively identified as *C. purpureum* by its morphological characteristics (terminal vesicles on the hyphae). Fruiting bodies were again found in the spring in one orchard that was also seriously affected in 2001. These fruiting bodies were much smaller than those found in 2001, possibly because of the dryer weather conditions in the spring of 2002. No fruiting bodies were found on trees of our 2001 inoculation study. Thus, longer time periods may be needed for their formation. Alternatively, environmental conditions with dry weather may not have been conducive in 2001. In addition to confirmed occurrences of silver leaf, the disease was reported from numerous other locations in Stanislaus and Merced counties (R. Duncan, *personal communication*). Some of these reports still need to be confirmed. From several samples, however, the causal pathogen could not be isolated. This indicates that leaf symptoms similar to silver leaf can have different causes, such as other pathogens, including decay fungi or abiotic factors

As a possible management strategy, diseased trees in two orchards were injected with myclobutanil in the spring of 2001. Myclobutanil had been found to be very active against *C. purpureum* in laboratory studies with EC_{50} values between 0.1 and 0.3 ppm. When treated trees were evaluated in the spring of 2002, however, disease severity of treated and non-treated trees was similar. Thus, the fungicide could not eradicate the organism in established infections or suppress the disease. The fungicide probably did not penetrate into the diseased wood where vascular bundles are dysfunctional and do not allow the movement of liquids.

Our trials in the spring of 2002 focussed on treatments to prevent the infection of the silver leaf fungus. Branch stubs (2 to 3 cm in diameter) of 4-year-old cv. Carmel trees were sprayed immediately after cutting to run-off using a small hand sprayer with one of the following solutions: distilled water (control), myclobutanil (Laredo 2EC, 12.8 fl oz), tetraconazole (Eminent, 16 fl oz), or a conidial suspension of *T. viride* (10^7 conidia/ml) in 0.5% of a gelling agent (Methocel). The gelling agent was added to the conidia to slow the drying of the suspension once applied to the branch stubs. The branch stubs were then inoculated either the following day or 2 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 3 months, 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 myclobutanil (Laredo 2EC, 12.8 fl oz) using a backpack sprayer at 100 gal/A. All other procedures were done the same as in the first trial using a hand sprayer application.

The results of this experiment confirmed the fast growth rate of the fungus in almond wood and canker formation that was previously recorded in our trial in 2001. Fungal decay in the longitudinally split 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. When water-treated branch stubs (control) were inoculated one day after treatment the fungus was recovered from all the branches (100% incidence) and it grew an average of 55 and 54 cm during the 3-month period on cvs. Carmel and Mission, respectively. In addition, the fungus could be isolated from every inoculated branch. These growth rates with ca. 18 cm growth/month are even higher than those observed in 2001 when 11.2 cm and 10.6 cm growth was found for cvs. Carmel and Mission, respectively. It is possible that the younger trees that were used in 2002 provided a better nutrient source for the fungus than the mature trees that were used in 2001.

Table 1. Disease development in branches of two almond varieties inoculated with an isolate of Chondrostereum purpureum either 1 or 14 days after branch cutting.

Almond variety	Inoculation	Average length of decay (cm)	SD	Rate of fungal growth (cm/month)
Mission	1 day after cutting	54.0	30.1	18
Carmel	1 day after cutting	55.0	33.4	18.3
Mission	14 day after cutting	13.8	25.8	4.6
Carmel	14 day after cutting	7.0	11.3	2.3

- A minimum of eight branches of each variety was inoculated in May of 2002 using wood pieces colonized by the pathogen. Branches were cut off in August 2002 and decay was

measured. Presence of the pathogen was confirmed by isolation on agar media.

When water-treated branch stubs were inoculated after 2 weeks, wood discoloration and survival of the fungus was much reduced. The fungus only could be recovered from 41.7 and 64.3% of the two cultivars, respectively, indicating that some of the wood discoloration was not due to fungal wood decay. The average length of wood discoloration was 7 cm and 13.8 cm for cvs. Carmel and Mission, respectively (Table 1). These data indicate that almond wood during dry, warm weather heals quickly and then becomes less susceptible to fungal infection. It emphasizes one of our previous recommendations for management of the disease to avoid pruning during wet and warm environments when fungal inoculum is more likely to establish infections.

When treatments were compared that were applied to run-off using a hand sprayer one-day before inoculation, myclobutanil and the biocontrol application with conidia of *T. viride* sp. significantly reduced the amount of wood decay. The lengths of the decay columns in branches were 55.1 cm in the control, 42.3 cm in the tetraconazole, 24.8 cm in the myclobutanil, and 16.1 cm in the *Trichoderma* treatment (Table 2). Recovery rates of the fungus from the discolored wood was 100% for the control and the tetraconazole and myclobutanil treatments, and 57.1% for the *Trichoderma* treatment. When branches were inoculated two weeks after treatments, the treatments reduced the amount of decay from 7 cm in the control to 2.9 cm for tetraconazole, 1.5 cm for myclobutanil, and 0 cm for the biocontrol treatment (Table 2). Myclobutanil applied using an air-blast sprayer did not significantly reduce the disease as compared to the control at both inoculation dates. Thus, infection by the pathogen could not be prevented in these studies, but the results are still encouraging. A reduction in disease was observed, although we used a very severe inoculation method. Colonized wood pieces were sealed onto the stub wounds, thus providing optimum conditions for infection. In nature, however, infection is by basidiospores that are difficult to produce in the laboratory. They land on pruning wounds where they are exposed to the environment. Therefore, infections are less likely to establish. Under natural field conditions the efficacy of our treatments is expected to be better. Rates of fungicides were selected based on existing or planned registrations of the fungicides to help in the registration process for this use. The biocontrol is not commercially available but could be developed.

Table 2. Efficacy of fungicides and a biocontrol agent in protecting branch stub

cuts inoculated with an isolate of *Chondrostereum purpureum*.

Inoculation Time (day)	Treatment	Average length of decay (cm)	LSD
1 day	Control	55.1	A
	Tetraconazole	42.3	AB
	Myclobutanil	24.8	B
	Trichoderma	16.1	B
14 day	Control	7.0	A
	Tetraconazole	2.9	A
	Myclobutanil	1.5	A
	Trichoderma	0	A

- A minimum of eight branches on four Carmel trees for each treatment were cut, treated with a fungicide or the biocontrol agent, and were inoculated in May of 2002 using wood pieces colonized by the pathogen. Branches were removed from trees in August 2002 and decay was measured. Presence of the pathogen was confirmed by isolation on agar media.

Suggested Management Practices – 2002 Update

1) Start with clean nursery stock
2) Prevention of the establishment of the fungus by:
    a. Minimize large wood-exposing wounds
    b. Use proper pruning practices (e.g., avoiding stub and bench cuts)
    c. Avoid pruning during wet and warm environments and during periods of high host susceptibility. Trees are least susceptible in the summer (June, July, and August) and early fall (September and October) months. They are most susceptible during late winter and early spring.
3) Sanitation measures include removing and burning infected wood and roots.
4) Fungicidal and biological wound treatments look very promising as protective treatments of large pruning wounds based on this year's data. Eradication is not possible unless the branch is removed below any wood discoloration.