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Project No. 00-JA2: **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).
      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

To evaluate epidemiological aspects of silver leaf disease in California and to develop new management strategies this research project was initiated. 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 indicated that the fungus grows extremely fast within the tree with an average rate of 4 inches/month during the 8-month study. To evaluate the extent of colonization, a third-leaf infected tree in a commercial orchard was dissected. The size of the decay column indicated that infection had occurred at or shortly after planting assuming a 4-inch/month constant growth rate. Myclobutanil (Laredo 2EC) was identified as a highly active fungicide against the pathogen and was injected into trees in several trials. The fungicide did not eradicate the organism but it may suppress the disease.

INTRODUCTION

Silver leaf disease has been reported on *Prunus* species in many locations worldwide. It is of major economic importance in Chile, France, and New Zealand and it has been reported on almonds in Australia and Chile. In California, 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. In 1971, the disease was implicated in two eight-year-old cling peach orchards in the Sacramento Valley (Chaney et al. 1973). Recently, silver leaf disease has become a concern for California almond growers, because several orchards have been seriously affected. The disease is caused by the fungus *Chondrostereum purpureum* (Pers.: Fr.) Pouz. This fungus is part of a complex of wood decay fungi on temperate tree fruit crops in California (Adaskaveg and Ogawa, 1991). 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 may occur that may extend into scaffold branches and 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 wounded surfaces, and the inability of previous researchers to eradicate established infections from tree trunks make silver leaf a difficult disease to control.

MATERIALS AND METHODS

Disease symptoms, pathogen description, and verification of diseased trees. Survey work was conducted to identify and confirm trees affected by silver leaf disease caused by *Chondrostereum purpureum*. For this, Roger Duncan, UCCE, coordinated almond grower reports in Stanislaus Co. for confirmation by isolation or field detection of the fungus. Verification of reports on other stone fruit crops was also conducted. Symptoms of the disease were described on almond leaves and wood. Signs of the pathogen were also described on infected trees. For this, monitoring for fruiting bodies of the fungus on diseased trees was conducted once a month throughout the year. Fruiting bodies were compared to descriptions of the pathogen. An information handout sheet was made to increase awareness among nursery personnel and growers.

Epidemiology – Spread of the disease inside trees. To obtain information on the rate of fungal spread in almond wood of trees in an experimental orchard, 40 branches on 12 trees for each cv. Carmel and Mission were inoculated in the spring of 2001 using mycelial agar plugs of a fungal culture of *C. purpureum*. In October 2001 all inoculated branches were rated for symptoms and a subset of branches was cut off for dissection. Whole trees identified during confirmation of field reports were also dissected using a chain saw. Length of discoloration was measured and extent of fungal colonization was determined by plating out discolored wood samples from within branches, trunk, and roots.

Disease management – Because sterol biosynthesis inhibiting (SBI) fungicides are very effective against fungi in the Basidiomycota (Adaskaveg et al. 1999) and because myclobutanil is the only SBI fungicide registered on almond, we evaluated the toxicity and efficacy of myclobutanil formulated as Laredo 2EC as a potential management tool. The in vitro toxicity of myclobutanil was evaluated in the laboratory using a new technology, the spiral gradient dilution method. For this, concentration gradients of the fungicide were established on a standardized volume of agar in 150-mm diameter Petri plates. Strips of agar or cellophane strips containing mycelium of the pathogen were radially placed on the plate. Mycelial growth was measured where 50% reduction occurred and the concentration was estimated using the Spiral Plate software.

In field studies, branch and trunk injections with myclobutanil were evaluated as suppressive or eradication treatments on symptomatic trees cv. Padre in two commercial orchards in the spring of 2001. Trees in the first (n = 9) and second (n = 7 trees) orchard were each treated with two or three 50-ml injections of 3% Laredo 2EC (7180 ppm a.i.) into apparently healthy tissue. Other trees (n =7) with symptoms in the second orchard were treated with Abound 2F at the same rate. Check trees with symptoms were also identified in each orchard. A "hand-pump" injector was used in all trials. At harvest, hulls and kernels of treated and non-treated trees were sampled and sent to a private lab for myclobutanil residue analysis.

RESULTS AND DISCUSSION

Disease symptoms, pathogen description and verification of disease reports - Diseased leaves from an infected tree are silvery in appearance (*see attached information sheet*). This results from the separation of the upper epidermis from the palisade layer that interferes with the normal reflection of light from the leaf surface. These symptoms are caused by a toxin released by the fungal pathogen *C. purpureum* growing in the wood. It is carried to leaves during water transport. Fungal infections originate from large wounds where wood is exposed. Typical wounds include pruning cuts and scaffold splits. Decay generally can extend up and down scaffold branches into the trunk and even into tree roots. Cross sections of smaller branches show "wedge-shaped" discolored wood. Fruiting bodies of the fungus often develop after substantial decay has developed inside the trunk and commonly at or near the point of infection. Fruiting bodies or basidiomes have a purple, smooth lower surface and a grayish, hairy upper surface.

The presence of silver leaf disease was confirmed in four almond orchards in Stanislaus Co. (Fig.1). Two of these orchards in the central part of the county were severely affected with many trees showing advanced symptoms, whereas in the other two orchards, located in the eastern part of the county, only few diseased trees were found. In addition, the disease was confirmed in two peach orchards in Sutter-Yuba and Fresno Co. (Fig. 1). The causal pathogen was isolated from all these locations and was positively identified as *C. purpureum* by its morphological characteristics (terminal vesicles on the hyphae). Fruiting bodies were found in one of the orchards in early spring, and they appeared to be quite short-lived once warm, dry

conditions prevailed. In addition to these confirmed occurrences, the disease was reported from numerous other locations in Stanislaus and Merced counties (R. Duncan and L. Hendricks, *personal communication*) that still need to be validated.

Epidemiology –Our results indicated that the fungus extensively colonized the branches over the 7-8 month period after inoculation (Fig. 2). Profuse gumming and cankers that extended up and down the inoculation site were present in over 50% of the branches, and there was no difference in susceptibility between the two almond cultivars. Leaf symptoms were also observed on many of the branches, but no fruiting bodies were found. When the removed branches were cut open longitudinally, fungal decay was evident as discolored, longitudinal streaks in the wood that extended in both directions from the inoculation point. An average length of decay from the inoculation point of 33.8 and 30.8 inches was measured for cv. Mission and Carmel, respectively, which corresponds to a monthly growth rate of 4.2 to 4.4 inches (Table 1).

Table 1. Disease development in branches of two almond varieties inoculated with an isolate of *Chondrostereum purpureum.*

Almond variety	Inoculation date	Average length of decay (inches)	SD	Rate of fungal growth inches/month
Mission	March 2001	33.8	8.6	4.2
Carmel	April 2001	30.8	8.5	4.4

* - Six branches of each variety were inoculated in the spring of 2001using mycelial agar plugs. Branches were cut off in October 2001 and decay was measured. Incidence of the pathogen was confirmed by isolation on agar media.

Colonization of almond wood by the silver leaf pathogen was also investigated by dissection of a 3-year-old almond tree in a commercial orchard that exhibited characteristic silver leaf symptoms. The pathogen presumably had infected this tree through a pruning cut on the trunk (ca. 1 foot above the crown), because here the decay column had its largest diameter. This was interpreted as the origin of infection. The distal end of the decay column in either direction had the smallest diameter. Discoloration of wood was found 58 inches up and 36 inches down from the presumed infection point. The presence of the pathogen in the discolored tissues was confirmed by fungal isolation and the discoloration correlated with the occurrence of the fungus within the wood. The fungus was also isolated from symptomatic roots, although at lower frequency. The implication of this latter finding is that the pathogen may still be present in root debris in the soil after diseased trees have been removed from the orchard. Thus, its survival in the soil and possible fruiting body production needs to be studied.

These results indicate that compared to other wood-decaying fungi *C. purpureum* is a very aggressive pathogen of almond that is capable of colonizing almond wood very quickly. Based on the estimated growth rate of the pathogen in almond wood the time when diseased trees were infected can now be deduced. For example, in the tree that was dissected in the commercial orchard the decay column was 58 inches and presuming a 4-inch growth rate per month, the tree apparently was infected 14.5 months prior to our evaluation. Thus, the tree that was in its third leaf presumably was infected during its first year in the orchard or already in the nursery assuming a slower growth rate of the fungus during the cooler dormant season. We will continue to observe the remaining inoculated trees in the experimental orchard to determine duration of time until fungal fruiting body formation and sporulation occur, to determine the effect of cooler temperatures during the dormant season on fungal development in the almond wood, and to determine if the fungus could be eradicated from the infected tree with the use of fungicides.

Disease management – Because sterol biosynthesis inhibiting (SBI) fungicides are very effective against fungi in the Basidiomycota (Adaskaveg et al. 1999) and because myclobutanil is the only SBI fungicide registered on almond, we evaluated the toxicity and efficacy of myclobutanil formulated as Laredo 2EC as a potential management tool. The in vitro toxicity of myclobutanil was evaluated in the laboratory using a new technology, the spiral gradient dilution method. With this method the EC_{50} values of four isolates of *C.*

purpureum were estimated to be between 0.12 and 0.42 µg/ml, indicating that the fungicide could be an effective control chemical. These EC_{50} values are currently being verified using conventional dilution plating on fungicide-amended media.

In field studies in 2001, no phytotoxic effects were recorded from the two fungicide treatments. In early summer of 2001, the fungus was re-isolated from the decayed wood of treated trees and was found producing basidiomes (fruiting bodies) on one treated tree several weeks after treatment. Thus, the treatment did not eradicate the fungus from the decayed wood. Presumably, little fungicide penetrated into the decayed wood where the fungus resides. Furthermore, no residues of myclobutanil were detected in hulls or kernels of treated trees. Evaluations of tree decline or recovery will be made again in the spring of 2002.

Suggested Management Practices

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 when nutrient and carbohydrate levels in the xylem sap are highest.
3) Sanitation measures include removing and burning infected wood and roots.
4) Fungicidal wound treatments have had some limited success in other countries and are currently being evaluated as eradication and preventative treatments.

References

1. Adaskaveg, J. E. and J. M. Ogawa. 1991. Wood decay pathology of temperature tree fruit crops in California. Plant Dis. 74: 341-352.
2. Adaskaveg, J.E. et al. 1999. Efficacy of sodium tetrathiocarbonate and propiconazole in managing Armillaria root rot of almond on peach rootstock Plant Dis. 83: 240-246.
3. Chaney, D. H., et al. 1973. Silver leaf of peach in California. Plant Dis. Rep. 57: 192.

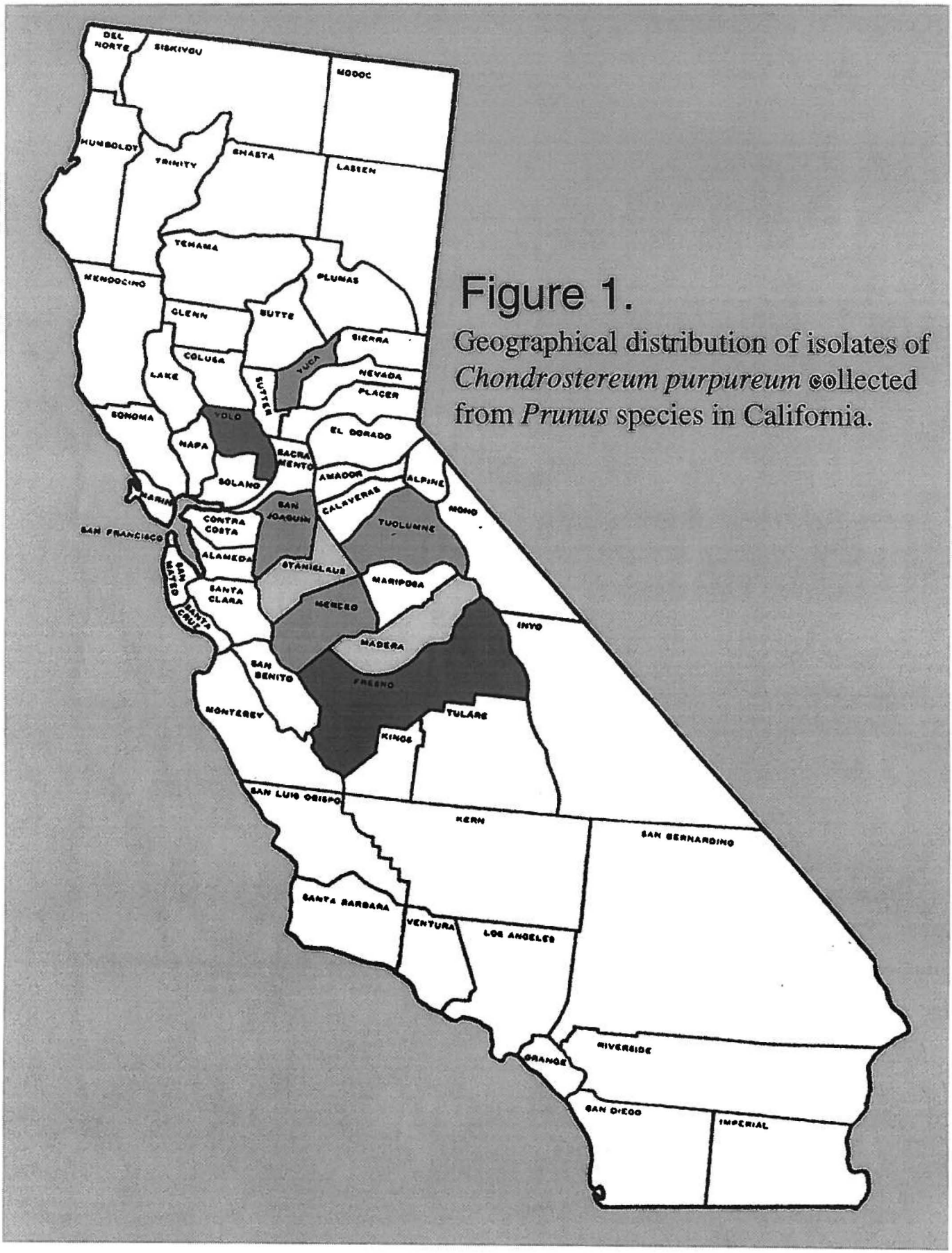
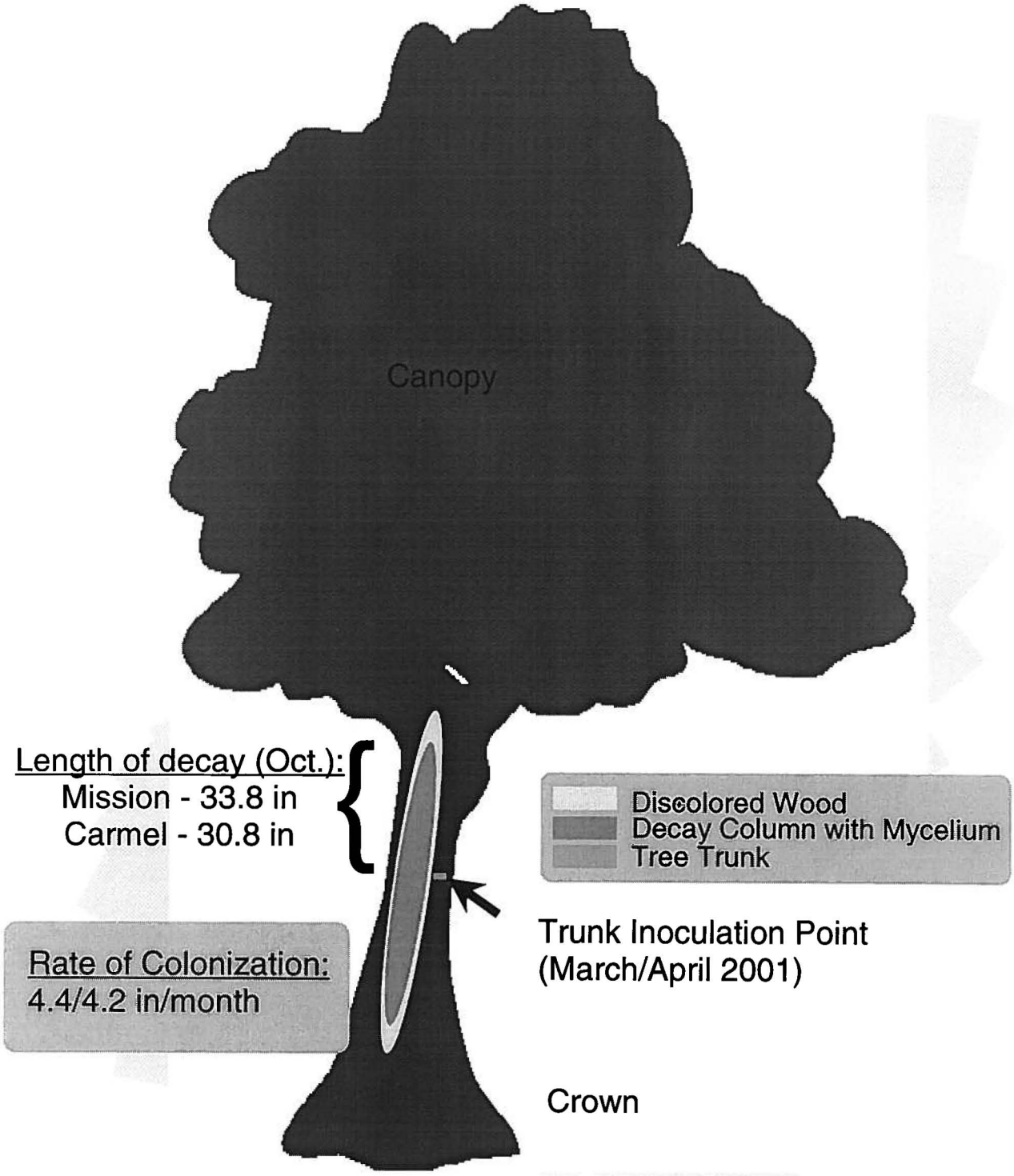


Figure 1.

Geographical distribution of isolates of *Chondrostereum purpureum* collected from *Prunus* species in California.

Figure 2. Wood colonization of almond trees by *Chondrostereeum purpureum* after inoculation.



Silver Leaf Disease of almond caused by *Chondrostereum purpureum*

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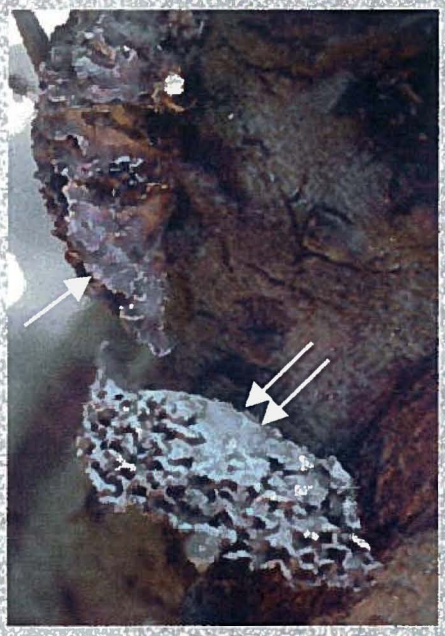

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Disease symptoms of silver leaf: 1) Healthy tree (right) and diseased tree (left). Note the discoloration of the foliage of the diseased tree; 2) Diseased leaves from an infected tree (right) have a silvery appearance compared to healthy green leaves from a non-infected tree; 3) Infections originate from large wounds and decay extends into scaffold branches (Arrow points to basidiome); 4) Fruiting bodies of the fungus often develop at the infection site after substantial decay has developed inside the trunk. Purple, smooth lower surface of basidiome (arrow) and grayish upper surface (double arrow); 5) Cross section of discolored wood of smaller scaffold branches; and 6) Cross section of trunk showing discolored, white-rotted wood.

Epidemiology and management of silver leaf of almond in California

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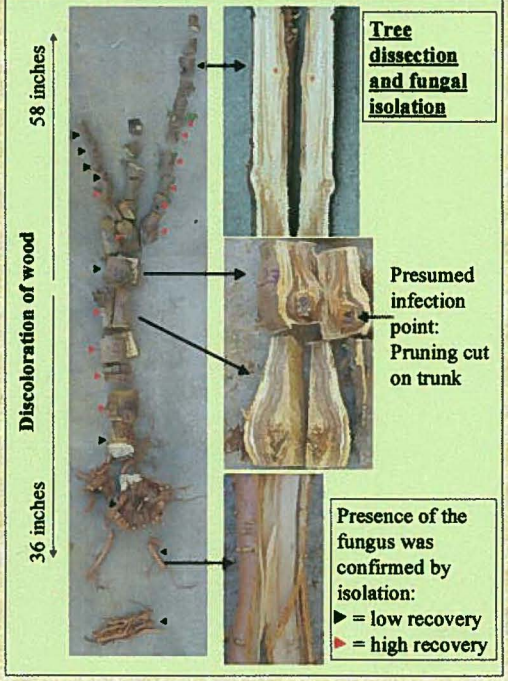
Disease symptoms of silver leaf caused by the fungus *Chondrostereum purpureum*: 1) Diseased leaves from an infected tree; 2) Infections originate from large wounds and decay extends into scaffold branches (Arrow points to basidiome); 3) Fruiting bodies of the fungus often develop after substantial decay has developed inside the trunk. Purple, smooth lower surface of basidiome (arrow) and grayish upper surface (double arrow); 4) Cross section of discolored wood of smaller branches.

Geographical distribution of silver leaf disease in California.

The map indicates counties where silver leaf disease was confirmed by fungal isolations.
Reports from other locations still need to be validated.

Wood colonization by *C. purpureum* of a 3-year-old, naturally infected almond tree

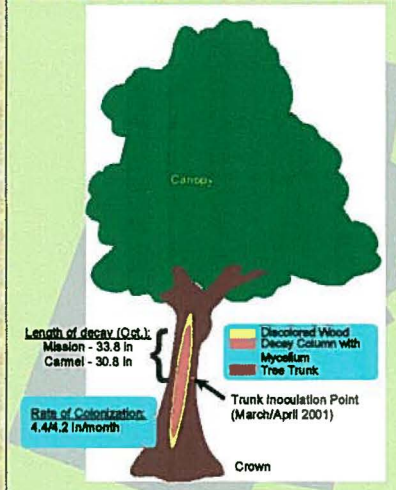


Tree dissection and fungal isolation

Presumed infection point: Pruning cut on trunk

Presence of the fungus was confirmed by isolation:
▶ = low recovery
▶ = high recovery

Wood colonization by *C. purpureum* of almond trees after inoculation



Fungal growth over the 7 to 8-month period was extensive with a calculated rate of ca. 4 in/month. Profuse gumming and cankers were evident on branches.

The trees will be observed for fruiting body formation by the fungus and treatment methods will be evaluated.

We conclude from our tree dissection and inoculation studies that *C. purpureum* is a very aggressive pathogen that is able to colonize almond wood very quickly

Fungicide studies in the laboratory

Sterol biosynthesis inhibiting fungicides are known to be very effective against basidiomycetes. Because myclobutanil (Rally, Laredo) is registered on almonds, this chemical was evaluated for its toxicity against *C. purpureum* using 2 methods:
- Amended agar media with a fungicide dilution series
- Spiral gradient endpoint dilution.

Using both methods, EC50 values were calculated to be between 0.2 and 0.4 ppm.

Thus, myclobutanil could be used as an effective control chemical.

Spiral gradient endpoint dilution

Studies for disease management in the field

In two orchards, symptomatic 'Padre' trees were pressure-injected with Laredo (myclobutanil) or Abound (azoxystrobin) in the spring of 2001. In the summer of 2001, however, the fungal pathogen was re-isolated from decayed wood of treated trees. Thus, the treatments apparently did not penetrate into the decayed wood and the fungus could not be eradicated. We will determine if continued injections can stop fungal spread in the tree. Preventative and pruning treatments will be evaluated in 2002.