Project No. 87-X7 - Almond Diseases
Canker Complex in Almonds

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Objectives: Complete studies on Phytophthora pruning wound canker and finalize control recommendations.

Interpretive Summary: Recommendations for control of Phytophthora pruning wound canker, caused by Phytophthora syringae, have evolved from several years of laboratory and field results and observations: (1) Free moisture is needed for infection of pruning wounds and disease incidence increases during periods of high rainfall; (2) Wounds usually become resistant to infection within four to six weeks after pruning; (3) Pruning wounds on Nonpareil remain susceptible for a somewhat longer period of time than wounds on other cultivars; (4) Cankers from infections which might occur in wounds made in late winter or early spring do not cause much damage since the warm weather of May and June inactivates the pathogen. Control treatment guidelines developed impact most significantly on young nonbearing trees being trained to main scaffolds, but some controls can be applicable to mature trees in certain situations.

Cultural control measures. Pruning at a time to avoid cool, rainy periods favorable for infection in late fall and early winter is advisable. In mature bearing orchards, pruning in early fall would take advantage of the relatively warm weather and rapid development of natural resistance to infection in wounds (about 2 weeks). In young nonbearing trees, damage to limbs being trained to main scaffolds can be severe and delaying pruning until February or March is an option. If infections do occur, damage is not generally great since canker development ceases with the onset of warm temperatures of late spring. Wounds on Nonpareil remain susceptible for a longer period of time and greater attention may need to be paid to this cultivar. Most other commercially important cultivars are similar in their susceptibility to Phytophthora syringae.

It is important to be able to discriminate Phytophthora pruning wound canker from Ceratocystis canker. Ths most striking symptom of Phytophthora pruning wound canker is the association with pruning cuts and the amber colored gum and inner bark dieback surrounding the wounds during the spring. Canker surgery during midwinter to contain Ceratocystis could serve to uncover more tissue susceptible to Phytophthora syringae. Delaying surgery until February or March might be a better choice.

Chemical protection. In many orchards, altering pruning schedules is impossible. Since pruning wounds generated during the rainy periods of November and December remain susceptible for up to six weeks, chemical

protection of large wounds to the trunk and lower main scaffolds is a potential option. In most situations this would apply to wounds in the lower five to six feet of the tree.

A study was done to compare cupric hydroxide (Kocide 101 77WP) and fosetyle-Al (Aliette 80 WP) treatments for protecting wounds against Phytophthora syringae. Application of Aliette (3 and 30 g a.i. per liter of water) to pruning wounds prevented the formation of cankers, was effective during the time period that untreated wounds were susceptible to infection, and was not phytotoxic. Application of Kocide 101 (1.8 and 18.3 g a.i. per liter of water) resulted in the formation of fewer cankers than in untreated wounds for most trials, but treated wounds frequently showed at least one of the following signs of phytotoxicity: formation of clear gum, xylem discoloration, excessive inner bark dieback, and abnormal wound-healing. Fungicides were applied to the surface of pruning wounds with a paintbrush. Although Aliette is not registered for use on almond trees at this time, these results indicate that it can be very effective in protecting bark tissue from infection by Phytophthora syringae. In summary, chemical protection of larger pruning wounds to trunks and main scaffolds during cool, rainy periods when infection risk is high potentially can be an effective strategy for minimizing damage from this disease.

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Chemical Protection of Almond Pruning Wounds from Infection by *Phytophthora syringae*

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ABSTRACT

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Application of fosetyl-Al (Aliette 80 WP) (3 and 30 g a.i. L^{-1}) to almond tree (*Prunus dulcis*) pruning wounds prevented the formation of pruning wound cankers caused by *Phytophthora syringae* and was effective during the time period that nontreated wounds were susceptible to infection. Application of cupric hydroxide (Kocide 101 77 WP) (1.8, 18.3, and 740 g a.i. L^{-1}) to pruning wounds prior to inoculation with *P. syringae* resulted in the formation of fewer cankers than in nontreated wounds for most trials, but wounds treated with cupric hydroxide frequently showed at least one of the following signs of phytotoxicity: formation of clear gum, xylem discoloration, excessive inner bark dieback, and abnormal wound-induced lignification.

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Phytophthora pruning wound canker of almond trees [Prunus dulcis (Mill.) Webb] is caused by Phytophthora syringae (Kleb.) Kleb., which enters pruning wounds made during in the fall and winter (1). Wounds remain susceptible to infection for up to 6 wk after wounding (3) and need to be protected for this period. Several compounds, including copper fungicides, have been reported to control diseases caused by various Phytophthora species (7). Cupric hydroxide was effective in controlling foot rot caused by Phytophthora parasitica Dast. when applied with a paintbrush to the trunk of citrus trees (10). Some almond growers in California have attempted to control cankers caused by P. syringae by applying cupric hydroxide in linseed oil to pruning wounds, although the efficacy of this treatment under controlled conditions had not been tested. Fosetyl-Al (Aliette, Rhone-Poulenc, Inc.) is effective in controlling diseases caused by Phytophthora species and other members of the Peronosporales (8) and may be an effective wound treatment. Chemical wound treatments, however, can be detrimental to trees, as of over 40 materials applied to bark wounds, only one was not phytotoxic (5). The objective of this study was to compare cupric hydroxide and fosetyl-Al treatments for protecting wounds against P. syringae and for phytotoxicity.

MATERIALS AND METHODS

Production of Zoospores. P. syringae (isolate F-79) obtained from an almond pruning wound canker was grown on amended lima bean agar (2). The agar was cut up into pieces of approximately 2 x 2 cm squares, placed in sterile distilled water in petri dishes and kept at 15 C.

After 7 days the water was drained, sterile water was readded, and the petri dishes placed at 4 C. Two hr later the water containing zoospores was poured into test tubes. In most experiments, zoospores were used for inoculum. In several experiments, cystospores, formed by vortexing the zoospores for 90 sec and filtering them with 0.025 mm mesh filters, were applied to pruning wounds.

Protection of fresh wounds. On 10 January 1985, 20 pruning wounds were made in bearing almond trees (cv. Nonpareil) in an orchard near Davis, CA, by excising 1-2 cm diameter branches with pruning shears. A mixture containing 740 g active ingredient (a.i.) cupric hydroxide (Kocide 101 77 WP, Griffin Chemical Company) in 1 L boiled linseed oil was applied to the wound surface with a paintbrush to branches on different trees. One day later half of the wounds were inoculated with 0.3 ml of a zoospore suspension (1.7×10^4 zoospores ml^{-1}), and the remainder of the wounds were left noninoculated to investigate phytotoxicity. Signs which were used as indicators of phytotoxicity were formation of clear gum, xylem discoloration, excessive inner bark dieback, and abnormal wound-induced lignification of the inner bark. Seven wk later the inoculated branches were examined for formation of cankers as described in a previous study (1). Some noninoculated wounds were sectioned and stained using the method of Doster (3) and then examined for wound-induced lignification and periderm formation.

On 3 March 1986, a fungicide trial was performed with Nonpareil almond trees in an orchard near Davis, CA. Branches (1-2 cm

diameter) were cut transversely about 20 cm from the main branch using pruning shears. In this trial cupric hydroxide (1.8 g a.i. L^{-1}) and fosetyl-Al (Aliette 80 WP, 30 g a.i. L^{-1}) were applied as aqueous suspensions to the wounds with a paintbrush. A zoospore suspension (1 x 10^4 zoospores ml^{-1}) was applied with a paintbrush to 10 wounds for each fungicide treatment and to 49 nontreated wounds, and then plastic bags were tied around the wounds for three days to facilitate infection. After 9 wk the wounds were examined for signs of phytotoxicity and canker formation.

Effect of Fungicides on Wound-induced lignification. Wounds were made on 4 February 1986 in branches with a 6 mm diameter corkborer through the bark to the cambium but not into the xylem. Seven almond trees (cv. Carmel), each with four wounds, received the following treatments: cupric hydroxide (1.8 g a.i. L^{-1}), cupric hydroxide (18.3 g a.i. L^{-1}), fosetyl-Al (30.0 g a.i. L^{-1}), and no fungicide (control). Each tree received all four treatments, with one treatment per wound. Twenty days later the wounded areas were removed with an 11-mm-diameter corkborer and the thioglycolic acid assay for lignin was performed on the inner bark (3).

Persistence of Fungicidal Protection. During the winter 1987, two trials were performed in an orchard near Davis, CA, using Nonpareil almond trees. In both trials, branches (1-2 cm diameter) were cut transversely 15 cm or more from the main branch using pruning shears and the fungicide treatments were applied to the wounds with a paintbrush. After various time periods of aging (see Table 3), 15

branches for each treatment were cut from the tree. The bottom half of each branch was placed in damp sand in a 12 C cold room and the treated wound surface was inoculated with P. syringae. Three wk after inoculation, the excised branches were examined for cankers and the extent of inner bark discoloration was measured. An earlier study (1) indicated this to be an accurate measure of colonization of the inner bark by P. syringae, which was consistently reisolated from the margin of the discolored zone on selective media. In one trial, the wounds were made and treated in January and mycelial plugs (V-8 medium, 12 mm diameter) were used for inoculum. In the other, the wounds were made and treated in February and inoculated with approximately 0.1 ml of a suspension containing 10^4 zoospores ml^{-1}.

RESULTS

In the 1985 trial, cupric hydroxide completely prevented infection by P. syringae, but the treated wounds usually had clear gumming around the wound (Table 1). When these wounds were sectioned, stained, and examined with the microscope, they had no lignified zone. A well-developed zone was evident in the nontreated wounded inner bark. In the 1986 trial, cupric hydroxide treated wounds had substantially fewer cankers than the nontreated but inoculated wounds; however, the treated wounds showed clear gumming and extensive discoloration of the xylem near the cambium (Table 1).

Treatment of wounds with fosetyl-Al prevented canker formation completely, while 73.5% of the inoculated nontreated wounds had cankers. No signs of phytotoxicity were observed in the fosetyl-Al treated wounds.

Substantially more lignin was detected in nontreated 20-day-old wounded tissue than in the nonwounded tissue (Table 2). Substantially more lignin was detected in the wounds treated with the high concentration of cupric hydroxide (18.3 g a.i. L^{-1}) than in the nontreated wounds, but there was no significant difference among the wounds treated with the low rate of cupric hydroxide (1.8 g a.i. L^{-1}), fosetyl-Al, and the nontreated wounds (Table 2).

Although inoculation of all fresh nontreated wounds resulted in cankers, as these wounds aged fewer and smaller cankers developed. Cankers did not develop after inoculation of 6-wk-old wounds in trial #1 and of 24 day-old wounds in trial #2. In the fosetyl-Al treated wounds, cankers were not observed except in two branches (Table 3). Cupric hydroxide treated wounds developed substantially smaller and slightly fewer cankers than the nontreated aged wounds in trial #2, but there was little difference in trial #1.

DISCUSSION

The treatment of wounds with fosetyl-Al was effective in preventing infection by P. syringae, while treatment with cupric hydroxide usually decreased the number of cankers formed but resulted in some phytotoxicity (Tables 1 and 3). During winter, wounds remained susceptible to P. syringae for up to 6 wk (3). In this

study, fosetyl-Al protected the wounds in almond trees from infection for up to 6 wk indicating that fosetyl-Al should provide adequate protection under conditions where application of a fungicide to bark injuries is appropriate. Protection of fresh pruning wounds in scaffold limbs, in other large branches in mature trees, and in branches being trained for scaffold limbs in young trees is especially important during periods of cool, wet weather in late fall and winter.

Fosetyl-Al provides an alternative to cupric hydroxide which exhibited some phytotoxicity even at the relatively low rate of 1.8 g a.i. L^{-1} after application to pruning wounds. The low concentration (1.8 g a.i. L^{-1} = 2 lb actual per 100 gal) was less than or equal to the spray rate commonly used in stone fruit orchards. Phytotoxicity observed at such low rates on fresh pruning wounds but not on the foliage may be explained by the absence of a barrier to the fungicide such as the cuticle of leaves or outer bark on branches. Furthermore, the xylem of fresh wounds in winter rapidly draws in the fungicide suspension (personal observation), and painting wounds may introduce more of the compound than spraying.

The thioglycolic acid assay quantifies the amount of lignin in wounded inner bark tissue (3), and provides a measure of the progression of wound periderm development. The assay was used in this study as a sensitive test for an abnormal response of the inner bark in treated wounds (Table 2). The wounds treated with cupric hydroxide at the concentration 18.3 g a.i. L^{-1} had substantially more

lignin than the nontreated wounds indicating an alteration of the normal wound response, while the wounds treated with fosetyl-Al or cupric hydroxide at 1.8 g a.i. L^{-1} had about the same amount of lignin as the nontreated wounds (Table 2). However, in the 1986 trial (Table 1) we observed extensive discoloration of the xylem near the cambium, but not of the inner bark, in wounds treated with the low rate of cupric hydroxide suggesting that parts of the xylem were more sensitive than the inner bark. The signs of phytotoxicity observed in cupric hydroxide treated wounds were clear gumming, xylem discoloration, occasionally additional bark dieback, and abnormal lignification. Application rates recommended for foliage or branches with intact outer bark may not be appropriate for fresh wounds.

Although bark wound treatments have been reported to be ineffective both as physical and chemical barriers to entry by many wood-decay fungi (9), treatment of pruning wounds with benzimidazole fungicides was effective in the protection of apricot trees against Eutypa dieback (6). Our results indicate that a treatment containing a fungicide such as fosetyl-Al, which does not impair wound closure and protects bark tissue during the period of wound resistance development, can be very effective against P. syringae.

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LITERATURE CITED

1. Bostock, R. M., and Doster, M. A. 1985. Association of Phytophthora syringae with pruning wound cankers of almond trees. Plant Dis. 59:568-571.

2. Bruck, R. I., Fry, W. E., and Apple, A. E. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of Phytophthora infestans. Phytopathology 70:597-601.

3. Doster, M. A. 1987. Epidemiology of Phytophthora Pruning Wound Canker in Almond Orchards and Studies on the Processes Related to the Development of Disease Resistance in Wounded Almond Bark. Ph.D. Dissertation. University of California, Davis, CA. 144 pp.

4. Finney, D. J., Latscha, R., Bennett, B. M., and Hsu, P. 1963. Tables for Testing Significance in a 2 x 2 Contingency Table. Cambridge University Press. London. 103 pp.

5. Mercer, P. C. 1979. Attitudes to pruning wounds. Arboric. J. 3:457-465.

6. Moller, W. J., Ramos, D. E., and Sanborn, R. R. 1977. Eutypa dieback in California apricot orchards: Chemical control studies. Plant Dis. Reptr. 61:600-604.

7. Ribeiro, O. K. 1978. A Source book of the Genus Phytophthora. J. Cramer, Vaduz. 417 pp.

8. Schwinn, F. J. 1983. New developments in chemical control of Phytophthora. Pages 327-334 in: Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul. 392 pp.

9. Shigo, A. L. 1982. Tree decay in our urban forests: what can be done about it? Plant Dis. 66:763-768.

10. Timmer, L. W. 1977. Preventive and curative trunk treatments for control of Phytophthora foot rot of citrus. Phytopathology 67:1149-1154.

Table 1. Percentage of wounds inoculated with P. syringae that developed cankers and the presence of phytotoxicity following application of fungicides in 1985 and 1986.

Fungicide	Concentration applied (g ai L^{-1})	Wounds developing cankers(%)	Presence of phytotoxicity[v]
	1985		
Nontreated	none	40	-
Cupric hydroxide	740[w]	0[x]	+
	1986		
Nontreated	none	73.5	-
Cupric hydroxide	1.8	20.0[y]	+
Fosetyl-Al	30	0.0[z]	-

[v] + indicates one or more of the following phytotoxicity signs were observed in noninoculated wounds: clear gumming, xylem discoloration, excessive inner bark dieback, and abnormal wound response; - indicates no signs of phytotoxicity were observed.

[w] Mixed in 1 L linseed oil, with no water added. All other fungicides were in water only.

[x] Significantly fewer cankers than untreated according to Fisher's Exact Test (P = 0.043).

[y] Significantly fewer cankers than untreated by Fisher's Exact Test (P = 0.002).

[z] Significantly fewer cankers than untreated by Fisher's Exact Test (P = 0.000).

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Table 2. The effect of fungicides on wound-induced lignification in almond inner bark tissue[y].

Wound treatment	Rate (g a.i. L^{-1})	LTGA ($\mu g\ mg^{-1}$ dry wt)[z]
Cupric hydroxide	18.3	4.58
Nontreated	none	3.02
Fosetyl-Al	30.0	2.97
Cupric hydroxide	1.8	2.34
Nonwounded	none	0.92
		$LSD_{.05}$ = 0.76
		$LSD_{.01}$ = 1.03

[y] The inner bark is comprised of the tissue between the cambium and the outer bark.

[z] LTGA = ligninthioglycolic acid, and is expressed as μg per mg dry weight methanol-extracted inner bark tissue. LTGA was determined 20 days after treatment of the wounds.

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Table 3. T persistence of fungicidal protection of wounds i lmond trees from infection by Phytophthora syrin during the winter 1987.

Treatment	Concentration applied (g a.i. L^{-1})	Percentage cankers (Discoloration length (mm))[w]									
		Trial 1[x]					Trial 2[x]				
		Wound age (weeks)					Wound age (days)				
		0	1	3	6	Mean	0	8	16	24	Mean
Fresh wounds[y]	-	100(51) a[z]	100(56) a	100(54) a	100(45) a	100(51)	100(50) a[z]	100(37) a	100(37) a	100(41) a	100(41)
Aged wounds	-	100(51) a	100(56) a	73(17) a	0(4) b	68(32)	100(50) a	87(33) a	93(33) ab	0(4) b	70(30)
Cupric hydroxide	1.8	100(57) a	100(50) a	73(22) a	42(13) b	79(37)	87(36) a	20(12) b	60(19) b	0(5) b	42(18)
Fosetyl-Al	3	7(8) b	0(5) b	0(5) b	0(4) b	2(6)	0(4) b	0(4) b	0(5) c	0(4) b	0(4)
Fosetyl-Al	30	0(6) b	0(6) b	0(5) b	0(4) b	0(5)	0(4) b	0(8) b	7(7) c	0(4) b	2(6)
						($LSD_{.05}$=3)					($LSD_{.05}$=3)

[w] Percentage cankers refers to the percentage of inoculated wounds which resulted in discoloration lengths greater than 15 mm. The numbers in parentheses are the mean discoloration lengths (in mm) of the inner bark as measured 3 weeks after inoculation. For nontreated, noninoculated wounds, the mean discoloration length was 5 mm and 4 mm for fresh and aged wounds, respectively, in trial #1 and 5 mm for both fresh and aged wounds in trial #2.

[x] Trial #1 was inoculated with mycelial agar plugs and trial #2 with a zoospore suspension (10^4 ml^{-1}).

[y] Fresh wounds were not aged but made at the time of inoculation for each wound age period.

[z] Percentage cankers for different treatments for a given wound age followed by different letters are significantly different at P = 0.05 when considered as a 2 x 2 contingency table according to the tables compiled by Finney et al. (4).