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 80WP) (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 77WP) (1.8, 18.3, and 740 g a.i. L^{-1}) to pruning wounds before 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.

Phytophthora pruning wound canker of almond trees (*Prunus dulcis* (Mill.) Webb) is caused by *Phytophthora syringae* (Kleb.) Kleb., which enters pruning wounds made during the fall and winter (1). Wounds remain susceptible to infection for up to 6 wk after wounding (3) and need to be protected during this period. Several compounds, including copper fungicides, have been reported to control diseases caused by various *Phytophthora* spp. (7). Cupric hydroxide was effective in controlling foot rot caused by *P. 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) is effective in controlling diseases caused by *Phytophthora* spp. and other members of the Peronosporales (8) and may be an effective wound treatment. Chemical wound treatments, however, can be detrimental to trees; of more than 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

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treatments for protecting wounds against *P. syringae* and for phytotoxicity.

MATERIALS AND METHODS

Production of zoospores. Isolate F-79 of *P. syringae*, obtained from an almond pruning wound canker, was grown on amended lima bean agar (2). The agar was cut into approximately 2 × 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 added, and the petri dishes were placed at 4 C. Two hours 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- to 2-cm-diameter branches with pruning shears. A mixture containing 740 g a.i. of cupric hydroxide (Kocide 101 77WP) in 1 L of boiled linseed oil was applied with a paintbrush to the wound surfaces. One day later, half of the wounds were inoculated with 0.3 ml of a zoospore suspension (1.7×10^4 zoospores ml^{-1}); to investigate phytotoxicity, the remainder of the wounds were not inoculated. Signs 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 weeks 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), 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 in 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 80WP, 30 g a.i. L^{-1}) were applied as aqueous suspensions to the wounds with a paintbrush. A zoospore suspension (1×10^4 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 3 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 and 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 winter 1987, two trials were performed in an orchard near Davis, CA, using Nonpareil almond trees. In both trials, branches 1–2 cm in 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 periods of aging, 15 branches for each treatment were cut from the tree. The bottom half of each branch was placed in damp sand in a cold (12 C) room, and the treated wound surface was inoculated with *P. syringae*. Three weeks 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, wounds treated with cupric hydroxide had substantially fewer cankers than nontreated inoculated wounds. Treated wounds, however, showed clear gumming and extensive discoloration of the xylem near the cambium (Table 1). Treatment of wounds with fosetyl-Al prevented canker formation completely, whereas 73.5% of nontreated inoculated wounds had

Table 1. Percentage of wounds inoculated with *Phytophthora syringae* that developed cankers and presence of phytotoxicity after application of fungicides in 1985 and 1986

Treatment	Rate of fungicide (g a.i.L^{-1})	Wounds developing cankers (%)	Presence of phytotoxicity[v]
		1985	
Nontreated	···	40	−
Cupric hydroxide	740[w]	0[x]	+
		1986	
Nontreated	···	73.5	−
Cupric hydroxide	1.8	20.0[y]	+
Fosetyl-Al	30.0	0[z]	−

[v] + = One or more of the following signs observed in noninoculated wounds: clear gumming, xylem discoloration, excessive inner bark dieback, and abnormal wound response; − = no signs of phytotoxicity observed.
[w] Mixed in 1 L of boiled linseed oil, with no water added; all other fungicides in water only.
[x] Significantly fewer cankers than nontreated according to Fisher's exact test ($P = 0.043$).
[y] Significantly fewer cankers than nontreated according to Fisher's exact test ($P = 0.002$).
[z] Significantly fewer cankers than nontreated according to Fisher's exact test ($P = 0.000$).

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

Treatment	Rate of fungicide (g a.i. L^{-1})		LTGA (μg mg^{-1} dry wt)[z]
Cupric hydroxide	18.3		4.58
Nontreated	···		3.02
Fosetyl-Al	30.0		2.97
Cupric hydroxide	1.8		2.34
Nonwounded	···		0.92
		$LSD_{0.05}$	0.76
		$LSD_{0.01}$	1.03

[y] Tissue between cambium and outer bark.
[z] LTGA = ligninthioglycolic acid, determined 20 days after treatment of wounds and expressed as μg/mg dry weight methanol-extracted inner bark tissue.

Table 3. Persistence of fungicidal protection of wounds in almond trees from infection by *Phytophthora syringae* during winter 1987

Treatment	Rate of fungicide (g a.i. L^{-1})	Percentage of cankers (discoloration length [mm])[x] Trial 1 Wound age (wk) 0	1	3	6	Mean	Trial 2 Wound age (days) 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	3.0	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	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)
						$LSD_{0.05}$ 3					$LSD_{0.05}$ 3

[x] Percentage of inoculated wounds with discoloration longer than 15 mm. Numbers in parentheses are mean discoloration lengths (mm) of inner bark, measured 3 wk after inoculation. In nontreated noninoculated wounds, mean discoloration length was 5 and 4 mm for fresh and aged wounds, respectively, in trial 1 and 5 mm for both fresh and aged wounds in trial 2. Wounds in trial 1 were inoculated with mycelial agar plugs, and those in trial 2, with a zoospore suspension (10^4 ml^{-1}).

[y] Made at time of inoculation for each wound age period.

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

cankers. No signs of phytotoxicity were observed in wounds treated with fosetyl-Al.

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

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

DISCUSSION

Treatment of wounds with fosetyl-Al was effective in preventing infection by *P. syringae*, whereas 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 showed 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, whereas 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). In the 1986 trial (Table 1), however, 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 wounds treated with cupric hydroxide 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 physical and chemical barriers to entry by many wood-decay fungi (9), treatment of pruning wounds with benzimidazole fungicides was effective in protecting 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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