Preventive and Curative Trunk Treatments for Control of Phytophthora Foot Rot of Citrus

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ABSTRACT

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To evaluate the effectiveness of fungicidal trunk paints in preventing infection by *Phytophthora parasitica*, trunks of young citrus trees were treated, and fungicidal activity was determined in a bioassay using *P. parasitica* zoospores. To attain 100% inhibition of *Phytophthora*, 60 mg/ml of captan and copper fungicides were required, whereas only 0.6 mg/ml of captafol was needed (amounts as active ingredient or metallic Cu). In longevity tests on exposed trunks painted with fungicide at 3 mg/ml, captafol was the only material that was still active after 2 wk. At 60 mg/ml, copper ammonium carbonate (CAC), cupric hydroxide (CH), and captafol were active for at least 33 wk, but captan and pyroxychlor were active for less than 17 wk. In tests on trees wrapped with the polyurethane mats presently used for freeze protection of young trees in Texas, captafol, CAC, CH, and copper salts of fatty and rosin acids at 40-60 mg/ml retained some activity for 52 wk. The standard trunk paint in current use (2.5% captan + 2.5% copper fungicide in bentonite) was ineffective at 0.12 g/ml, but was active through 29 wk at 0.48 g/ml. On trees infected by *Phytophthora*, excision of affected tissue and painting with a copper fungicide slightly improved tree recovery. Pyroxychlor applied to trunks at 240 mg/ml remained active on the bark surface for at least 8 days, penetrated the bark to the cambium, but was not translocated away from the treated area. Pyroxychlor applied as a trunk paint, but not as a soil drench, limited the expansion of foot rot lesions.

Additional key words: fungicides, grapefruit.

Foot rot, caused by *Phytophthora parasitica* Dast. [*P. nicotianae* var. *parasitica* (Dast.) Waterhouse] is the most damaging disease in Texas citrus orchards and is particularly severe on young trees (7, 8). Infection usually occurs just above the bud union and can cause extensive gummosis and bark necrosis. The disease is most severe on commercial scion cultivars, but it also may occur on tolerant rootstocks.

In the past, young trees were protected from freeze damage by banking soil around the trunks from November through March. Application of fungicidal trunk paints was recommended (3) to prevent infection of banked trees by *Phytophthora*, and paints containing 1-5% copper fungicide proved to be effective (5). Presently, trees are wrapped with polyurethane mats (11) at planting and wraps are left in place for 3-4 yr for freeze protection. Polyurethane is highly water absorbent and the wraps create a favorable environment for infection by *Phytophthora*. The fungicidal trunk paints presently used have not provided adequate long-term protection against foot rot especially under conditions favorable for infection (9).

The present study was undertaken to evaluate: (i) the protective effect of various fungicides against Phytophthora infection on young citrus trees, (ii) the effect of standard surgical treatments (7) and, (iii) the potential benefit of a systemic fungicide (4) on infected trees.

MATERIALS AND METHODS

Materials.—The following fungicides were evaluated as trunk protectants: captan {N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide}, (Captan 50 WP); captafol {*cis*-N-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2-dicarboximide}, (Difolatan 4F); basic copper sulfate (BCS), (tribasic copper sulfate, 53% copper); cupric hydroxide (CH) (Kocide 101, 54% copper); copper ammonium carbonate (CAC) (Copper-Count-N, 8% copper); copper salts of fatty and rosin acids (CSFRA) (Citcop 4E, 4% copper); and the standard trunk paint (2.5% captan plus 2.5% copper as BCS in a bentonite carrier) presently used by citrus growers.

Two formulations of pyroxychlor [2-chloro-6-methoxy-4-(trichloromethyl) pyridine] were evaluated for protective and curative effects: M4116 (240 g/liter) was used for all trunk treatments; and M4113 (60 g/liter) was used for all soil-drench treatments. Since pyroxychlor is relatively volatile (4), two materials were used to retard its evaporation from the trunk surface: (i) Estab, an emulsifiable liquid resin which solidifies to a plastic and (ii) citrus spray oil (Orchex 796) plus emulsifier, T-Mulz-A02.

Concentrations of all materials are expressed in mg of active ingredient or of actual copper per milliliter in the final trunk paint. All trunk treatments were applied to run-off with a paint brush over the entire trunk unless otherwise indicated.

Bioassay technique.—The protective effect of fungicidal trunk paints was evaluated with a bioassay

utilizing *P. parasitica* zoospores. Zoospores were produced as follows: *P. parasitica*, isolate S10 (8), was grown in clarified V-8 juice broth for 2-3 days at 21-27 C. The mycelial mat was rinsed several times with sterile, distilled water and incubated for 2-4 days in sterile distilled water at 21-27 C. Zoospore formation was induced by chilling sporangia at 6 C for 15-20 min and allowing them to warm to room temperature. Zoospore populations were determined by the dilution plate method or by direct counts using a hemocytometer.

Fungicide activity on treated trees was determined by cutting 9-mm diameter bark disks from the trunks with a cork borer. Disks were placed, cambial side down, on moist filter paper in a 100 × 15-mm petri dish and 30 μliters of a zoospore suspension were placed on the bark. Most experiments were conducted using about 4×10^4 zoospores/ml although the use of concentrations ranging from 3×10^3 to 1×10^5 zoospores/ml did not affect the results. The bark plugs with the zoospore suspensions were incubated for 24 hr at 30 C and then inverted onto PVP selective media (corn meal agar plus 10 mg/ml pimaricin, 100 mg/ml pentachloronitrobenzene, and 200 mg/ml vancomycin) (10). The area of the resultant colony minus that of the bark plug was calculated after 72 hr of incubation at 30 C. All data are expressed as percent inhibition of growth compared to bark disks taken from nontreated control trees. All bioassays were conducted on field-grown, 1- to 2-yr-old red grapefruit (*Citrus paradisi* Macf.) budded on sour orange (*C. aurantium* L.) rootstock. Bark disks were taken only from the grapefruit scions.

Preventive applications.—The effective dosages of the fungicides were determined by applying each concentration (0.06, 0.6, 6.0, and 60 mg/ml) of each material to the trunk of a single tree. Immediately after the fungicide had dried, six bark disks were taken from each tree and the inhibitory activity was determined by bioassay.

In experiments to determine the longevity of protectant fungicide activity, each treatment was applied to the trunks of three trees. Two bark disks were taken from each tree at each sampling date after treatment and residual fungicide activity was determined by bioassay. In one test, treated trunks were exposed to weathering and in the other, trunks were tightly wrapped with polyurethane mats 35 × 75 × 2.5 cm. Fungicides, rates, and sampling dates are given in Table 1.

Surgical treatment.—Three-yr-old Star Ruby grapefruit trees on sour orange rootstock with active foot rot lesions on the scions were selected and rated for foot rot severity. Trees were divided into four categories depending on the percentage of the trunk circumference with active gummosis and bark rot: (i) 10-20%, (ii) 20-40%, (iii) 40-60%, and (iv) 60-75%. Half of the trees in each group were treated and the other half left as nontreated controls. On treated trees, all of the infected and a small margin of adjacent healthy bark were removed and the wound was painted with a slurry of CH.

TABLE 1. Duration of inhibitory activity against *Phytophthora parasitica* on trunks of young grapefruit trees painted with various fungicides and either wrapped or not wrapped with polyurethane foam mats

Wrapping and fungicide treatments	Conc. (mg/ml)	*P. parasitica* inhibition[w] after treatment				
		2 wk	17 wk	33 wk	42 wk	52 wk
Nonwrapped trees:						
captan	60	99 a[x]	12 c	...	...	...
captafol	3	83 a	0 c	...	...	...
captafol	60	100 a	100 a	93 a	83 a	0 a
copper ammonium carbonate	60	100 a	100 a	65 b	73 a	25 a
cupric hydroxide	60	100 a	70 b	68 ab	27 b	0 a
pyroxychlor	60	16 b	0 c	...	...	...
standard trunk paint[y]	3+3	21 b	0 c	...	...	...
no treatment	...	0 b	0 c	0 c	0 b	0 a
		16 wk	29 wk		41 wk	52 wk
Wrapped trees:[z]						
captan	60	89 a	0 b		15 cd	...
captafol	60	100 a	100 a		100 a	81 a
copper ammonium carbonate	60	100 a	70 a		90 ab	78 a
cupric hydroxide	60	89 a	77 a		52 bc	55 a
basic copper sulfate	60	12 bc	0 b		...	...
copper salts of fatty rosin and acids	40	100 a	81 a		51 bc	68 a
standard trunk paint[y]	3+3	28 b	0 b		...	...
standard trunk paint[y]	12+12	95 a	85 a		0 d	...
no treatment	...	0 c	0 b		0 d	0 b

[w]Average percent reduction in colony area compared to the nontreated control in a bioassay using *P. parasitica* zoospores.

[x]Mean separation by Duncan's multiple range test ($P = 0.05$). Within the nonwrapped and wrapped groups, differences between means in the same column and followed by the same letters are not statistically significant.

[y]A mixture of 2.5% captan and 2.5% copper as basic copper sulfate in a bentonite carrier. Concentrations indicated are those of captan and copper in the final tree paint.

[z]Trunk wrapped tightly with polyurethane mats (about 35 × 75 × 2.5 cm).

All trees were rated for foot rot severity 2 and 5 mo after treatment on the scale described in Table 2 and the number of surviving trees was determined after 1 yr.

Evaluation of pyroxychlor.—The longevity of pyroxychlor on treated trunks was determined by applying (i) 15 ml fungicide/tree alone, (ii) 15 ml fungicide/tree plus citrus spray oil immediately after application, and (iii) citrus spray oil alone (control). Each treatment was applied to three trees and two bark plugs were taken for bioassay from each tree on each sample date. The experiment was repeated and the data presented are the average of the two experiments (Table 3).

To determine the distribution of inhibitory activity in pyroxychlor-treated trees, 15-cm bands around the trunks of three trees were treated. After 4 days, two bark disks were taken for bioassay from each tree: in the treated band, the trunk below the treated band, the tap root, the trunk above the treated band, and the primary branches. At some locations, the inner cambial surface as well as the outer bark surface was assayed for activity. The experiment was repeated and the data presented are the average of the two experiments (Table 4).

Three experiments were established to evaluate pyroxychlor as a possible curative treatment for existing foot rot lesions. In one experiment, scion or rootstock lesions on 42 2-yr-old Valencia orange [*C. sinensis* (L.) Osbeck] trees on sour orange rootstock were rated for foot rot severity by estimating the percentage of the circumference with active gummosis and bark rot. Trees were paired according to foot rot severity and one tree in each pair was treated by painting the trunk with a 1:1 (v/v) mixture of pyroxychlor and Estab, and the other received Estab alone. In a second experiment, 45 2-yr-old Valencia orange trees on sour orange rootstock were divided into three equal groups according to foot rot severity, and basins were formed around the trees with soil. Pyroxychlor was applied to one group at 1.25 ml/liter, to a second at 5 ml/liter, and the third received water only. A third experiment was established on 2-yr-old nucellar red grapefruit on sour orange rootstock. Twelve trees with severe foot rot on the scion were selected and paired according to severity. One tree of each pair was treated by painting each trunk with 15 ml of pyroxychlor and the other was not treated. In all experiments the percent of the trunk circumference with

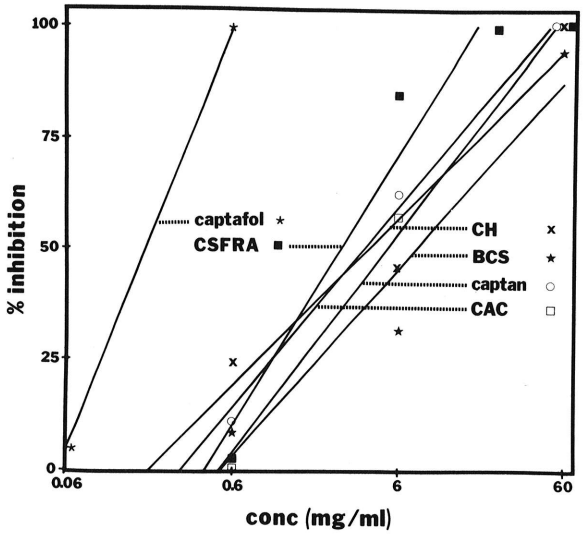

Fig. 1. Dosage response of *Phytophthora parasitica* to various concentrations of fungicides applied as trunk paints. Inhibition expressed as percentage of control in a bioassay using Phytophthora zoospores. Cupric hydroxide, CH; basic copper sulfate, BCS; copper ammonium carbonate, CAC; copper salts of fatty and rosin acids, CSFRA.

TABLE 2. Effect of surgical removal of infected bark on the recovery of Star Ruby grapefruit from Phytophthora foot rot

Treatment	No. of trees	Initial[w] severity rating	Average severity rating[x] months after treatment 2	Average severity rating[x] months after treatment 5	Trees surviving 1 yr after treatment (no.)
Treated[y]	7	1	0.9	0.7[z]	3
	8	2	1.3	0.8[z]	5
	9	3	1.6	1.4[z]	2
	4	4	3.3	3.0	0
			Avg. 1.6	1.3[z]	
Untreated control	7	1	1.9	1.9	3
	8	2	2.1	2.0	3
	9	3	2.6	2.7	2
	4	4	3.0	4.0	0
			Avg. 2.3	2.5	

[w]Trees rated from 1 to 4 according to amount of active gumming from slight to severe.

[x]Trees rated after treatment on a scale of 0 to 4 with 0 = lesion healed; 1, 2, and 3 slight, moderate, and severe gumming, respectively, and 4 = tree girdled.

[y]Infected bark removed and wound painted with a slurry of cupric hydroxide.

[z]Significantly different from the corresponding control according to Duncan's multiple range test ($P = 0.05$).

active gumming was rated 1, 2, and 3 mo after treatment (Table 5).

RESULTS

Protectant fungicides.—When fungicides were applied to trunks and assayed immediately, captan and all copper fungicides were about equally active at 6 mg/ml, but there was little or no activity at 0.6 mg/ml (Fig. 1). Captafol was substantially more active than the other materials tested and completely inhibited growth of *P. parasitica* at concentrations as low as 0.6 mg/ml.

Duration of activity of the fungicides on nonwrapped trees varied greatly. Pyroxychlor at 60 mg/ml, the standard trunk paint at the lowest recommended rate (Table 1) and CH, CAC, and captan at 3 mg/ml (not included in Table 1) had no activity after only 2 wk. Captan at 60 mg/ml and captafol at 3 mg/ml were active initially, but had no activity 17 wk after treatment. Cupric hydroxide was active for 33 wk and captafol and CAC for 42 wk at 60 mg/ml.

Results with treated trees wrapped with polyurethane mats were generally similar, but the persistence of many fungicides was increased. Captafol, CAC, CH, and CSFRA retained some activity for 52 wk. The persistence of captan, captafol, CH, and the low rate of the standard trunk paint was extended beyond that on nonwrapped trees. The standard trunk paint at the highest recommended rate retained activity for 29 wk, and compared well with other fungicides used at higher rates. Basic copper sulfate alone had little activity and had apparently been washed from the trunk by the 16-wk sampling date.

Superficial necrosis and considerable gummosis occurred on trunks treated with CSFRA. None of the other fungicides was phytotoxic.

Curative treatments.—Foot rot severity was not significantly reduced by surgical treatment after 2 mo, but it was reduced after 5 mo (Table 2). Foot rot was more effectively controlled by treatment of trees with low initial severity ratings. However, many of the trees eventually died whether treated or not.

The inhibitory activity of pyroxychlor on treated trees lasted for at least 8 days, but less than 13 days (Table 3). Treatment of trunks with citrus spray oil following application of pyroxychlor did not enhance fungicidal activity and may have decreased it slightly.

No fungicidal activity was detected in pyroxychlor-treated trees outside of the area actually treated. No fungicidal activity was detected on the outer surfaces of roots, bark from primary and secondary branches, or on the cambial or outer surface of bark just below the treated area. Fungicidal activity was detected only on the outer bark and cambial surfaces of disks taken directly from the treated area (Table 4).

Pyroxychlor trunk paints had some curative effect on existing foot rot lesions. Hot, dry weather following the application of pyroxychlor was favorable for natural recovery and most of the treated and control trees eventually recovered. However, trunk treatment of infected Valencia oranges (treatment A, Table 5) significantly increased the rate of recovery. In tests on severely affected grapefruit trees, there was no natural recovery and pyroxychlor (treatment F, Table 5) significantly reduced the percentage of the circumference with active gummosis 3 mo after treatment. Drench treatments failed to speed the recovery of the foot rot-affected Valencia orange trees.

TABLE 3. Duration of inhibitory activity against *Phytophthora parasitica* on trunks of young grapefruit trees treated with pyroxychlor

Treatment	*P. parasitica* inhibition (%)[w] after treatment:			
	1 day	3 days	8 days	13 days
Pyroxychlor[x]	100 a[z]	94 a	73 a	24 a
Pyroxychlor[x] + oil[y]	94 a	65 b	48 a	20 a
Oil only[y]	0 b	0 c	0 b	0 a

[w]Percent reduction in colony area compared to the oil-treated control in a bioassay using *P. parasitica* zoospores.
[x]Trunks painted with 15 ml each of pyroxychlor (formulation M-4116) at 240 g/liter.
[y]Trunks painted with citrus spray oil alone or immediately after application of pyroxychlor.
[z]Mean separation by Duncan's multiple range test ($P = 0.05$). Means in the same column followed by the same letter are not significantly different.

TABLE 4. Inhibition of growth of *Phytophthora parasitica* in different locations on trunks of young grapefruit trees treated with pyroxychlor

Treatment	Trunk location sampled	Surface tested	Inhibition[y]
Pyroxychlor	Treated area	Outer bark	79 a[z]
Pyroxychlor	Treated area	Cambial	49 b
Pyroxychlor	Below treated area	Outer bark	19 c
Pyroxychlor	below treated area	Cambial	13 c
Control	Trunk	Outer bark	0 c

[y]Average percent reduction in colony area compared to the nontreated control in a bioassay using *P. parasitica* zoospores 4 days after treatment with pyroxychlor (formulation M-4116, 240 g/liter) at 15 ml/tree.
[z]Mean separation by Duncan's multiple range test ($P = 0.05$). Means followed by the same letter are not significantly different.

TABLE 5. Effect of pyroxychlor trunk paints and soil drenches on the development of existing foot rot lesions on young citrus trees

Treatment	Type	Rate	Circumference gumming (%)				
			Pre-treatment	Months after treatment			
				1	2	3	avg.
A[w]-pyroxychlor + Estab	Paint	15 ml/trunk	33	8[z]	9	2	6[z]
B[w]-Estab control	Paint	15 ml/trunk	33	24	19	10	18
C[x]-pyroxychlor	Drench	5 ml/liter	29	17	17	5	13
D[x]-pyroxychlor	Drench	1.25 ml/liter	29	15	19	3	12
E[x]-water control	Drench	...	29	16	15	4	12
F[y]-pyroxychlor	Paint	15 ml/trunk	55	57	62	15[z]	45
G[y]-untreated control	...	...	55	53	62	52	52

[w]Each treatment was applied to 21 2-yr-old nucellar Valencia orange trees. Pyroxychlor (formulation M-4116, 240 g/liter) was applied to trunks in a 1:1 mixture with Estab to retard volatilization of the fungicide.

[x]Each treatment was applied to 15 2-yr-old Valencia orange trees. Pyroxychlor (formulation M-4113, 60 g/liter) was applied as a soil drench (~ 75 liters/tree) in basins formed around each tree.

[y]Each treatment was applied to six 2-yr-old nucellar red grapefruit. Pyroxychlor (formulation M-4116, 240 g/liter) was applied to trunks without dilution or additives.

[z]Means significantly different from the corresponding controls according to Duncan's multiple range test ($P = 0.05$).

Application of pyroxychlor in combination with Estab caused a superficial necrosis on some trunks, especially where it came in contact with nonsuberized tissues.

DISCUSSION

Some fungicides applied at high concentrations as trunk paints had long-term activity against Phytophthora zoospores. Captafol was inhibitory to *Phytophthora* at much lower concentrations than captan or the copper fungicides, but some copper fungicides performed as well as captafol in long-term tests. Duration of activity of copper fungicides varied greatly. When applied at concentrations containing the same metallic copper content, liquid copper formulations performed better than did the wettable powders. Materials with a high proportion of large particle sizes such as basic copper sulfate were quickly washed off by rainfall and had short residual activity. Wrapping of trees with polyurethane mats extended the duration of fungicidal activity on the trunks. The presently used standard trunk paint at the lowest recommended rate was short lived, but when applied at a higher concentration on wrapped trees, it provided longer residual activity. Captafol or some copper fungicides applied at sufficiently high concentrations provide much more effective long-term protection against Phytophthora infection than the presently used material.

Surgical treatment of foot rot lesions has been evaluated previously and found to be moderately (1, 6) to marginally effective (2). In the present study, treatment was effective in reducing the expansion of the area affected by foot rot lesions on mild to moderately affected trees. Tree survival was not appreciably increased by treatment, and any benefit obtained was probably not sufficient to offset the cost of the operation.

Pyroxychlor applied as a trunk paint was relatively short-lived with no detectable activity 13 days after application. In pyroxychlor-treated trees, no activity was detectable outside of the treated area, but activity was detectable on the cambial surface of the bark in the treated area. Since growth of *Phytophthora* is confined to the bark and cambial tissues, limited movement of the fungicide should, theoretically, reduce growth of *Phytophthora* in infected trees and aid in recovery. When pyroxychlor was applied to trees infected by *Phytophthora* as a trunk paint, some curative action was noted, but when applied as a soil drench, it was not effective in reducing lesion expansion on infected trees.

Control of foot rot depends largely on the use of preventative measures. By the time foot rot symptoms appear, sufficient bark death and girdling has occurred to cause permanent damage to the tree. However, if infections are detected early, surgical treatment or application of a systemic fungicide such as pyroxychlor may be useful in preventing lesion enlargement and reducing further damage which may result in tree loss.

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