Persistence of Systemic Activity for Fungicides Applied to Citrus Trunks to Control Phytophthora Gummosis

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ABSTRACT

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The systemic activity of metalaxyl, fosetyl-Al, phosphorous acid (H_3PO_3), oxadixyl, propamocarb, benalaxyl, and ethazol was examined for control of Phytophthora gummosis on citrus. Sections of tree trunks in a 15-yr-old tangelo block were painted with one of the test fungicides. Strips of bark were then removed periodically from within as well as below the treated area and inoculated on the cambium with *Phytophthora parasitica* and *P. citrophthora*. For at least 117 days after application, bark tissues treated with metalaxyl, fosetyl-Al, H_3PO_3, or oxadixyl were inhibitory to growth of both fungi. Benalaxyl significantly suppressed the growth of *P. parasitica* but had no apparent effect on *P. citrophthora*. Propamocarb and ethazol did not inhibit the growth of either *Phytophthora* sp. Significant basipetal translocation of fungicide activity was observed at least 15 cm from bark treated with metalaxyl, fosetyl-Al, and H_3PO_3. A trunk paint application of fosetyl-Al to 5-yr-old lemon trees was more inhibitory than a foliar application to growth of *P. parasitica* and *P. citrophthora* on bark tissue. One application of metalaxyl, fosetyl-Al, or H_3PO_3 to the trunk significantly reduced canker development on tangelo trees subsequently inoculated with the two *Phytophthora* spp. Application of fungicides to the tree trunk appears to be an effective procedure for control of Phytophthora gummosis of citrus.

Phytophthora gummosis is a serious and widespread disease problem in citrus groves of Arizona. Disease incidence is especially high in groves established with the graft union at or below the soil surface, exposing susceptible scion tissue to *Phytophthora citropothora* (R. E. Smith & E. H. Smith) Leonian and *P. parasitica* Dastur. Severe losses can also occur in groves subjected to flood-irrigation as well as in plantings on susceptible rootstocks.

The introduction of the systemic fungicides metalaxyl and fosetyl-Al has facilitated control of Phytophthora gummosis of citrus (4–7,10,12,14,15). Methods of fungicide application include foliar spray or trunk injection with fosetyl-Al (3–5,10,15), trunk paint with metalaxyl or fosetyl-Al (4–6,14,15), and soil drench with both materials (4–6,12,14,15). Additionally, reports suggest that phosphorous acid (H_3PO_3) has high efficacy against diseases caused by several *Phytophthora* spp. (2,8,9,13). In plant tissue, fosetyl-Al is degraded to H_3PO_3 (16).

Because of the seriousness of Phytophthora gummosis in Arizona, experiments were begun to test the effectiveness of direct trunk application of metalaxyl, fosetyl-Al, and H_3PO_3 for disease control. Other fungicides active against plant pathogens in the order Peronosporales, i.e., benalaxyl, oxadixyl, propamocarb, and ethazol, were included in the study, and the basipetal translocation of these materials was also investigated. A partial account of this work was reported earlier (11).

MATERIALS AND METHODS

Efficacy of trunk application of fungicides. Initial experiments were conducted to compare the relative ability of several fungicides to impede subsequent growth of *P. citrophthora* and *P. parasitica* on treated bark tissue. In a 15-yr-old high-density planting of

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tangelo trees (*Citrus reticulata* Blanco × *C. paradisi* Macf. 'Orlando'), a 40-cm section of trunk on each of 10 trees was painted with metalaxyl (Ridomil 2E at 60 g a.i./L), fosetyl-Al (Aliette 80WP at 300 g a.i./L), H_3PO_3 (200 g/L), oxadixyl (SDS 59891 25WP at 60 g a.i./L), propamocarb (Banol 6S at 120 g a.i./L), benalaxyl (Galben 2E at 120 g a.i./L), or ethazol (Truban 25EC at 60 g a.i./L). Forty-five days after treatment, a vertical strip of bark (10 cm long × 1.5 cm wide) was removed from the initially treated area of each trunk and inoculated in the center by placing a 6-mm-diameter disk of V-8 juice agar with either *P. citrophthora* or *P. parasitica* on the cambium. Inoculated bark strips were incubated for 4 days at 24 C in moist chambers, after which the length of resulting lesions was recorded.

Persistence of fungicide activity and detection of basipetal translocation within the bark. Again using a 15-yr-old planting of tangelo trees, a 20-cm section of trunk on each of 10 trees was painted with the 2E formulation of metalaxyl (60 g a.i./L), the 80WP formulation of fosetyl-Al (300 a.i./L), the 10% calcium formulation of fosetyl-Al (100 g a.i./L), H_3PO_3 (200 g/L), or the 25WP formulation of oxadixyl (60 g a.i./L), all of which had shown high activity in previous tests. In a second experiment, 10 5-yr-old lemon trees (*C. limon* (L.) Burm. 'Lisbon') were treated either by making a foliar application of fosetyl-Al (80WP formulation at 5 g a.i./L) or by painting a 20-cm section of trunk with fosetyl-Al (80WP formulation at 300 g a.i./L), metalaxyl (2E formulation at 16 g a.i./L), or H_3PO_3 (200 g/L). At various times after treatment, vertical bark strips were collected from within the treated section of trunk as well as 15 cm below the treated zone on tangelo and lemon trees. Bark samples were inoculated and incubated, and lengths of resultant lesions were recorded as described earlier.

When fungicides were applied to sections of tangelo and lemon trunks, care was taken to ensure that no fungicide was applied to the bark below the treatment zone. Sparse rainfall during these studies was assumed not to wash fungicide from the treated zone to the untreated bark. No rainfall occurred until 109 and 73 days after initiation of experiments with tangelo and lemon trees, respectively. Total precipitation for the duration of these tests was 11 mm. Rainfall occurred on 8 days and ranged from 0.5 to 2.8 mm.

Trunk inoculation experiments. Trunks of Orlando tangelo trees were painted with one of several fungicides, applied to a 40-cm section of trunk on each of 10 trees. Fungicide treatments included metalaxyl (2E formulation at 60 g a.i./L), fosetyl-Al (80WP formulation at 300 g a.i./L or 10% calcium formulation at 100 g a.i./L), oxadixyl (25WP formulation at 60 g a.i./L), propamocarb (6S formulation at 120 g a.i./L), and benalaxyl (2E formulation at 120 g a.i./L). Trees were then inoculated by placing a 6-mm-diameter agar disk of V-8 juice agar with *P. citrophthora* or *P. parasitica* into small wounds in the bark. Trees were inoculated both in the treated zone and 35 cm below the treated zone. Wounds were wrapped with black plastic electrical tape. After 15 wk, the length of resultant lesions was recorded.

All experiments were established using single tree replicates in a randomized complete block design and performed at least twice. Data from representative experiments are presented.

Table 1. Effect of fungicides applied as trunk paint to 15-yr-old tangelo trees on subsequent growth of *Phytophthora parasitica* or *P. citrophthora* in treated bark tissue

Test pathogen and fungicide[x]	Rate (g a.i./L)	Percent inhibition of canker development 45 days after treatment[y]
P. parasitica		
No fungicide	...	0 a
Metalaxyl	60	83 de
Fosetyl-Al	300	100 e
H_3PO_3[z]	200	100 e
Oxadixyl	60	50 cd
Propamocarb	120	17 ab
Benalaxyl	120	33 bc
Ethazol	60	0 a
P. citrophthora		
No fungicide	...	0 a
Metalaxyl	60	60 bc
Fosetyl-Al	300	100 c
H_3PO_3[z]	200	100 c
Oxadixyl	60	0 a
Propamocarb	120	20 ab
Benalaxyl	120	0 a
Ethazol	60	0 a

[x] Each fungicide was applied to a 40-cm section of trunk on each of 10 tangelo trees. Average volume of each fungicide treatment applied per tree ranged from 8 to 13 ml.

[y] Average percent inhibition of canker development compared with untreated control 4 days after inoculation of excised bark cambium tissue. Average length of lesions on control cambium tissue was 12 and 20 mm with *P. parasitica* and *P. citrophthora*, respectively. For each pathogen, numbers with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

[z] KOH was added to the H_3PO_3 solution until a pH of 6.1 was attained. Application of H_3PO_3 alone (pH 1.8) usually damaged treated bark tissue.

Table 2. Inhibition of canker development in tangelo tree bark after trunk paint application of fungicides

Test pathogen and fungicide[x]	Rate (g a.i./L)	Percent inhibition of canker development after:[y] 12 days	43 days	71 days	117 days	160 days
Phytophthora parasitica						
No fungicide	...	0 a	0 a	0 a	0 a	0 a
Fosetyl-Al WP	300	96 c	95 b	87 c	37 ab	0 a
Fosetyl-Al Ca	100	99 c	95 b	86 c	60 bc	52 bc
Metalaxyl	60	100 c	91 b	100 c	80 c	73 c
H_3PO_3[z]	200	100 c	100 b	100 c	55 bc	53 bc
Oxadixyl	60	59 b	22 a	48 b	43 bc	29 ab
P. citrophthora						
No fungicide	...	0 a	0 a	0 a	0 a	0 a
Fosetyl-Al WP	300	97 c	99 b	99 d	53 b	24 a
Fosetyl-Al Ca	100	99 c	100 b	97 d	84 b	71 b
Metalaxyl	60	81 c	91 b	70 c	62 b	64 b
H_3PO_3[z]	200	100 c	100 d	100 d	74 b	72 b
Oxadixyl	60	34 b	15 a	38 b	47 b	6 a

[x] Each fungicide was applied to a 20-cm section of trunk on each of 10 tangelo trees. Average volume of each fungicide treatment applied per tree ranged from 4 to 6 ml.

[y] Average percent inhibition of canker development compared with untreated control 4 days after inoculation of excised bark cambium tissue. Average length of lesions on control cambium tissue was 12 and 20 mm with *P. parasitica* and *P. citrophthora*, respectively. For each pathogen, numbers in each column with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

[z] KOH was added to the H_3PO_3 solution until a pH of 6.1 was attained. Application of H_3PO_3 alone (pH 1.8) usually damaged treated bark tissue.

RESULTS

Efficacy of trunk application of fungicides. A single application of fosetyl-Al, metalaxyl, or H_3PO_3 was highly inhibitory to subsequent growth of *P. parasitica* or *P. citrophthora* on cambium tissue of treated bark (Table 1). Trunk application of oxadixyl or benalaxyl significantly suppressed growth of *P. parasitica* but had no apparent effect on *P. citrophthora*. Propamocarb and ethazol did not inhibit growth of either *Phytophthora* sp.

Persistence of fungicide activity. Cambium tissue from tangelo bark treated once with fosetyl-Al (calcium formulation), metalaxyl, or H_3PO_3 was suppressive to subsequent growth of *P. parasitica* and *P. citrophthora*, even after 160 days (Table 2). The calcium formulation of fosetyl-Al remained active for at least 160 days, whereas the activity of the wettable powder formulation of this material decreased more rapidly (Table 2). Variable fungicidal activity was observed on bark treated with oxadixyl. Growth of *P. parasitica* and *P. citrophthora* was significantly reduced on bark tested 12, 71, and 117 days after treatment with oxadixyl but not on bark tested 43 days after treatment. Generally, the degree of inhibition with oxadixyl was lower than values achieved with fosetyl-Al, metalaxyl, or H_3PO_3.

The persistence of fungicidal activity observed with treated bark from lemon trees is summarized in Table 3. As observed with bark from tangelo trees, a trunk treatment with fosetyl-Al, metalaxyl, or H_3PO_3 arrested subsequent growth of *P. parasitica* and *P. citrophthora*, even 138 days after application of materials (Table 3). A foliar application of fosetyl-Al did not restrict growth of the test *Phytophthora* spp. on trunk bark tissue as well as a trunk paint application of the same fungicide (Table 3).

Table 3. Inhibition of canker development in lemon tree bark after fungicide treatment

Test pathogen and fungicide[w]	Treatment	Rate (g a.i./L)	Percent inhibition of canker development after:[x] 10 days	41 days	90 days	138 days
Phytophthora parasitica						
No fungicide	...	...	0 a	0 a	0 a	0 a
Metalaxyl	Trunk paint	16	79 c	89 c	61 b	38 b
H_3PO_3[y]	Trunk paint	200	94 c	96 c	76 b	68 c
Fosetyl-Al	Trunk paint	300[z]	93 c	95 c	82 b	74 c
Fosetyl-Al	Foliar spray	5[z]	41 b	49 b	22 a	6 a
P. citrophthora						
No fungicide	...	...	0 a	0 a	0 a	0 a
Metalaxyl	Trunk paint	16	72 c	86 c	39 b	31 b
H_3PO_3[y]	Trunk paint	200	82 c	99 c	90 c	73 c
Fosetyl-Al	Trunk paint	300[z]	99 c	97 c	78 c	82 c
Fosetyl-Al	Foliar spray	5[z]	65 b	48 b	30 b	1 ab

[w] Each trunk paint treatment was applied to a 20-cm section of trunk on each of 10 lemon trees. Average volume of each fungicide applied per tree ranged from 4 to 8 ml.
[x] Average percent inhibition of canker development compared with untreated control 4 days after inoculation of excised bark cambium tissue. Average length of lesions on control cambium tissue was 17 and 16 mm with *P. parasitica* and *P. citrophthora*, respectively. For each pathogen, numbers in each column with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.
[y] KOH was added to the H_3PO_3 solution until a pH of 6.1 was attained. Application of H_3PO_3 alone (pH 1.8) usually damaged treated bark tissue.
[z] Average amount of fosetyl-Al applied per tree was 0.9 g as a trunk paint and 10.9 g as a foliar spray, using 4 ml and 2,000 ml of fungicide solution for each treatment, respectively.

Table 4. Inhibition of canker development in tangelo tree bark collected 15 cm below area painted with fungicides

Test pathogen and fungicide[y]	Rate (g a.i./L)	Percent inhibition of canker development after:[z] 12 days	43 days	71 days	117 days	160 days
Phytophthora parasitica						
No fungicide	...	0 a	0 a	0 a	0 a	0 a
Fosetyl-Al WP	300	44 b	30 ab	51 b	46 b	12 ab
Fosetyl-Al Ca	100	35 b	36 b	56 b	21 ab	19 ab
Metalaxyl	60	37 b	40 b	52 b	28 ab	47 b
H_3PO_3	200	34 b	10 ab	48 b	46 b	15 ab
Oxadixyl	60	2 a	0 a	29 ab	7 ab	32 ab
P. citrophthora						
No fungicide	...	0 a	0 a	0 a	0 a	0 a
Fosetyl-Al WP	300	74 b	51 c	50 b	62 c	36 bc
Fosetyl-Al Ca	100	55 b	55 c	47 b	51 c	18 ab
Metalaxyl	60	5 a	17 ab	39 b	45 bc	32 abc
H_3PO_3	200	44 b	44 bc	50 b	66 c	54 c
Oxadixyl	60	11 a	0 a	4 a	18 ab	9 ab

[y] Systemic activity resulting from basipetal translocation of fungicide within tangelo bark tissue.
[z] Average percent inhibition of canker development compared with untreated control 4 days after inoculation of excised bark cambium tissue. Average length of lesions on control cambium tissue was 12 and 20 mm with *P. parasitica* and *P. citrophthora*, respectively. For each pathogen, numbers in each column with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

Detection of basipetal translocation of systemic activity within bark tissue. Variable inhibition of *P. parasitica* and *P. citrophthora* was observed on bark strips removed 15 cm below the section of tangelo trunks treated with fosetyl-Al, metalaxyl, or H_3PO_3 (Table 4). When inoculated with *P. parasitica*, consistent inhibition of pathogen growth was observed on tangelo cambium tissue up to 71 days after treatment with the calcium formulation of fosetyl-Al or metalaxyl. Canker formation was consistently inhibited on tangelo cambium tissue inoculated with *P. citrophthora* up to 117 days after treatment with H_3PO_3 and the wettable powder or calcium formulation of fosetyl-Al (Table 4). Detection of basipetal movement of fungicide activity was inconsistent with the wettable powder formulation of fosetyl-Al and H_3PO_3 when cambium tissue was inoculated with *P. parasitica* and with the metalaxyl treatment when tissue was inoculated with *P. citrophthora*. No significant basipetal translocation of oxadixyl was detected in tangelo bark tissue (Table 4).

Basipetal movement of fungicide activity against *P. parasitica* was detected 10 days after treatment of lemon trees with metalaxyl and up to 138 days after treatment with H_3PO_3 (Table 5). Fosetyl-Al activity against *P. parasitica* was variable during the experimental period. Significant activity against *P. citrophthora* was observed up to 138 days after treatment of lemon trees with H_3PO_3 or fosetyl-Al (Table 5). Results from lemon trees treated with metalaxyl were inconsistent.

Trunk inoculation. Canker development on sections of tangelo tree trunks was highly restricted on trees treated with metalaxyl, fosetyl-Al, or H_3PO_3 and inoculated with *P. parasitica* or *P. citrophthora* (Table 6). Significant inhibition of *P. parasitica* was also observed on bark treated with oxadixyl or propamocarb. Canker development on sites 35 cm below treated sections of trunks was retarded significantly by H_3PO_3 and to a lesser extent by fosetyl-Al and metalaxyl (Table 6).

DISCUSSION

Results of this study agree with those of previous work (4–6,14) showing that metalaxyl applied as a trunk paint is highly effective for controlling *Phytophthora* infection on citrus trunks. The inconsistent but detected basipetal movement of metalaxyl has been observed by Timmer and Castle (15), even though the primary movement within the plant is acropetal (4–6,14).

Trunk application of metalaxyl for control of Phytophthora gummosis of citrus is superior to soil drench treatments. Previous investigations showed that no fungitoxic activity was detected in trunk bark after soil drench application of metalaxyl (4,5,15). Trunk application also circumvents the observed biodegradation of metalaxyl in soil (1).

In our studies, fosetyl-Al or H_3PO_3 applied to the trunk was as effective as metalaxyl for control of *Phytophthora* infection on tangelo and lemon trees. Other workers (4,5,15) have reported inconsistent results with fosetyl-Al on citrus trees. The variability in detectable fungitoxic activity could be explained by several factors, including the method used to detect activity of fosetyl-Al, variable uptake by different tissues of the same *Citrus* sp., or differences in uptake between *Citrus* spp.

The equivalent activity of fosetyl-Al and H_3PO_3 is consistent with work suggesting that fosetyl-Al is degraded to H_3PO_3 in plant tissue (16). We observed differences in persistence and magnitude of activity between H_3PO_3, fosetyl-Al formulated as a wettable powder (fosetyl-Al WP), and fosetyl-Al in an aqueous solution containing calcium (fosetyl-Al Ca). At 160 days after treatment of tangelo trunks, H_3PO_3 and fosetyl-Al Ca showed significantly higher activity than fosetyl-Al WP using the bark strip assay, but results from tangelo trunk inoculations suggest that fosetyl-Al Ca, fosetyl-Al WP, and H_3PO_3 were all effective in suppressing lesion development on treated bark. Differences between testing techniques could account for this variability.

Foliar application of fosetyl-Al to lemon trees provided significantly less protection against *Phytophthora* infection on trunk bark tissue than a direct application of the fungicide to the bark. Davis (5) also found that a foliar spray of fosetyl-Al did not significantly inhibit *P. parasitica* colonization of trunk bark tissues in 5-yr-old grapefruit trees. On the other hand, Laville and Chalandon (10) reported that foliar sprays of fosetyl-Al were highly effective for controlling Phytophthora gummosis of citrus. Differences in age of trees, the species of citrus, time of application (during or between a growth flush), as well as different methodologies for measurement of the efficacy of chemicals could account for these discrepancies.

Application of unbuffered H_3PO_3 (pH 1.8) to trunk bark of tangelo and lemon trees resulted in severe necrosis of tissue, followed by profuse gumming. Treatment of bark with the mixture of H_3PO_3 + KOH (pH 6.1) did not cause damage to bark tissue.

Oxadixyl, benalaxyl, and propamocarb gave inconsistent results in the bark strip assay as well as the trunk inoculation tests. Perhaps different formulations of these compounds could result in higher and more consistent fungitoxic activity. Ethazol showed no systemic activity in our tests.

The bark strip assay used in these studies is a rapid and reliable technique for testing efficacy of fungicides for control of Phytophthora gummosis of citrus. Results from this assay are comparable to data obtained from trunk inoculation experiments. Results are known in 4 days instead of several weeks or months, however. The bark strip assay also allows periodic testing to monitor changes in fungitoxic activity.

Table 5. Inhibition of canker development in lemon tree bark collected 15 cm below area painted with fungicides

Test pathogen and fungicide[y]	Rate (g a.i./L)	Percent inhibition of canker development after:[z] 10 days	41 days	90 days	138 days
Phytophthora parasitica					
No fungicide	···	0 a	0 a	0 a	0 a
Metalaxyl	16	32 b	19 a	11 a	17 a
H_3PO_3	200	66 c	65 b	37 b	60 b
Fosetyl-Al	300	24 ab	49 b	24 ab	63 b
P. citrophthora					
No fungicide	···	0 a	0 a	0 a	0 a
Metalaxyl	16	26 a	16 ab	0 a	38 b
H_3PO_3	200	71 b	71 c	46 b	74 c
Fosetyl-Al	300	65 b	43 bc	28 b	77 c

[y] Systemic activity resulting from basipetal translocation of fungicide within lemon bark tissue.
[z] Average percent inhibition of canker development compared with untreated control 4 days after inoculation of excised bark cambium tissue. Average length of lesions on control cambium tissue was 17 and 16 mm with *P. parasitica* and *P. citrophthora*, respectively. For each pathogen, numbers in each column with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.

Table 6. Effect of fungicide treatment on development of trunk lesions in treated bark as well as in bark 35 cm below treated bark zone on 15-yr-old tangelo trees

Test pathogen and fungicide	Rate (g a.i./L)	Lesion length[x] (mm) in: Treated bark[y]	Bark 35 cm below treated bark[z]
Phytophthora parasitica			
No fungicide	···	18 a	22 ab
Metalaxyl	60	3 de	16 bcd
Fosetyl-Al WP	300	4 de	14 cd
Fosetyl-Al Ca	100	1 e	20 abc
H_3PO_3	200	6 cd	9 d
Oxadixyl	60	12 b	22 ab
Propamocarb	120	11 bc	20 abc
Benalaxyl	120	18 a	24 a
P. citrophthora			
No fungicide	···	23 ab	32 ab
Metalaxyl	60	10 c	29 ab
Fosetyl-Al WP	300	6 cd	20 bc
Fosetyl-Al Ca	100	1 d	23 bc
H_3PO_3	200	9 cd	13 c
Oxadixyl	60	22 ab	36 a
Propamocarb	120	20 ab	31 ab
Benalaxyl	120	25 a	36 a

[x] Determined 15 wk after inoculation. Average of 10 replicates per treatment. For each pathogen, numbers in each column with the same letter do not differ ($P = 0.05$) according to Duncan's multiple range test.
[y] Each fungicide applied to a 40-cm section of trunk on each of 10 tangelo trees. Average volume of each fungicide treatment applied per tree ranged from 8 to 13 ml.
[z] Systemic activity resulting from basipetal translocation of fungicide within tangelo bark tissue.

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