

## **Preliminary Evaluation of Nine Fungicides for Control of *Phytophthora cactorum* and *P. citrophthora* Associated with Crown Rot in Peach Trees**

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Excised twig assay and excised stem inoculation were used to evaluate nine fungicides (metalaxyl, fosetyl-Al, copper hydroxide, copper sulfate, copper oxychloride, captan, quin-tozene, propamocarb and chlorothalonil) against *Phytophthora cactorum* and *P. citrophthora* associated with crown rot in peach trees. Segments were soaked in fungicide solutions at different concentrations and then inserted vertically into *P. cactorum* or *P. citrophthora* cultures growing on cornmeal agar plus antibiotics, or inoculated by inserting a mycelium-bearing agar plug directly into the cambium. Following incubation, the bark was scraped off and length of necrosis was measured. Metalaxyl was the only fungicide that inhibited canker development on segments at the manufacturer-recommended concentration. Fosetyl-Al, captan, copper hydroxide and copper sulfate inhibited canker development at 3, 4, 4 and 8 g l<sup>-1</sup>, respectively. The other fungicides did not affect canker length significantly compared with non-treated twigs, with the exception of propamocarb, which reduced the development of *P. cactorum* on excised stems. The tested methods enabled rapid and effective evaluation of a large number of chemicals to prevent crown rot diseases caused by *Phytophthora* in the laboratory.

KEY WORDS: Crown rot; fungicides; chemical evaluation methods; peach tree; *Phytophthora cactorum*; *Phytophthora citrophthora*.

### INTRODUCTION

An excised twig assay developed by Borecki and Millikan (2) and amended by Jeffers *et al.* (11) was used to determine the pathogenicity of different species of Pythiaceae fungi such as *Phytophthora cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. cryptogea* and *P. megasperma* to compare relative virulence of isolates within a species, and possible relative resistance of different apple scion and rootstock cultivars (8,12,13,23,24). Also, it was used by Scott *et al.* (20) for screening micropropagated shoots from almond rootstocks *in vitro* for their response to *P. cambivora*. This method was used to determine the seasonal variation in colonization of apple rootstocks by *Phytophthora* species associated with crown and root rot in apple trees (10,25). Browne and Mircetich (4) investigated the relationship between temporal susceptibility and severity of crown rot development in an orchard to expand basic knowledge on development of *Phytophthora* crown rot of apple in excised shoots *in vitro*. It is one of the least laborious assays and also allows ample

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replications. Because of the uniform inoculation of the bases of the twig segments, lesions developed only upward rather than in several directions (11). Therefore, twig segments do not need identical wounding, because measurement of necrosis begins at the agar surface.

The excised stem inoculation method has also been employed to determine the pathogenicity of *P. cactorum* and *P. cambivora* (19), and for evaluating seasonal changes in susceptibility of citrus rootstocks to *P. citrophthora* and *P. parasitica* (17). Matheron and Matejka (16) tested the effects of temperature on growth of *P. citrophthora* and *P. parasitica* using this method.

The use of amended agar is one of the most common methods to evaluate chemicals. The technique involves cultivation of the test pathogen on an agar medium containing the test chemical, and then measuring fungal growth (5).

The possibility of using the excised twig assay and excised stem inoculation method to evaluate chemicals against *Phytophthora* species associated with crown and root rot in peach trees was not tested previously. These methods are described here for the first time for evaluating the fungicidal activity of nine chemicals: metalaxyl + mancozeb (Ridomil MZ 63.5), fosetyl-Al (Aliette 80WP), copper hydroxide (Kocide 80WG), quintozene (Terraclor), copper sulfate (Bordeaux mixture), copper oxychloride (copper oxychloride 40FL), captan (Captan 50), chlorothalonil (Teren 75 WP), propamocarb (Promess 72.2 SL), in preventing crown rot of peach trees caused by *P. cactorum* and *P. citrophthora*. In addition, the *in vitro* effects of fungicides on mycelial growth of *P. cactorum* and *P. citrophthora* are reported.

## MATERIALS AND METHODS

One isolate each of *P. cactorum* and *P. citrophthora* was used in all experiments. Both isolates were used in previous work (21) and found to be highly pathogenic to peach trees. *P. cactorum* was isolated from almond grafted on GF677 rootstock and *P. citrophthora* from citrus grafted on bitter orange in 1998. Fungi were maintained on cornmeal agar (CMA) at 22°C. To prepare fresh cultures, agar plugs bearing mycelium of *P. cactorum* or *P. citrophthora* were transferred onto plates containing cornmeal agar. These plates were then incubated at 21°C for 7 days.

**Exp. 1 – Excised twig assay** Cornmeal agar amended with antibiotics (pimaricin 10 mg l<sup>-1</sup>, ampicillin 250 mg l<sup>-1</sup>, rifampicin 10 mg l<sup>-1</sup>) to avoid contamination was added to Pyrex jars (12 cm high, 9 cm diam) to form an agar depth of ~ 1 cm. An agar plug with mycelium of *P. cactorum* or *P. citrophthora* from a 7-day-old culture, was transferred into each jar. The jars were then sealed with Parafilm and placed in incubators at 23°C in the dark until colony growth covered the agar surface.

One-year-old dormant shoots ~ 40 cm in length were collected from 3-year-old 'KID I' peach rootstocks. Trees did not show any symptoms of disease or signs of injury at this time. Segments 70 mm in length and ~ 10 mm in diameter were cut from the central portion of the shoots. The basal end of each twig segment was then cut tangentially with a sterile scalpel, ~ 10 mm from the edge and 1 mm deep, on opposite sides, to expose the phloem and cambium to infection by a pathogen.

Suspensions of fungicides were prepared by mixing fungicide in sterile distilled water at four different concentrations, based on active ingredients (one being the manufacturer-recommended concentration) (Table 1). Ten of the pared segments were soaked in each of the fungicide suspensions for 1 min and then placed on a soft paper towel until dry. Twigs of

each fungicide were inserted vertically, distal end up, at the periphery of the fungal colony. The jars were then resealed with Parafilm and returned to the incubator (23 °C in the dark). After 4 days, twigs were removed from agar and the epidermis was stripped off to reveal the extent of internal discoloration. The results were obtained by subtracting depth of agar from the total length of necrosis. There were ten jars for each fungicide concentration, five jars for each species.

**Exp. 2 – Excised stem inoculation** Two-year-old dormant shoots ~ 50 cm in length were collected from 3-year-old KID I peach rootstocks; segments 10 cm in length and ~ 2 cm in diameter were cut from the central part. Trees did not show any symptoms of disease or signs of injury at this time. The segments were wounded with a sharp knife by removing 6 mm of bark from the central part. Segments were soaked in fungicide suspension for 1 min and then dried on a soft paper towel. Suspensions were prepared by mixing a fungicide in sterile distilled water at four different concentrations based on active ingredients (one being the manufacturer-recommended concentration) (Table 2).

Inoculations were made by inserting an agar plug (6 mm diam) bearing mycelium of *P. cactorum* or *P. citrophthora* directly onto the cambium. Wounds were covered with adhesive tape to avoid desiccation and segments were incubated at 23 °C for 5 days. There were 40 segments for each fungicide concentration, 20 for each species. The data were obtained by measuring the length of developed cankers.

**Exp. 3 – Linear growth** The effect of the fungicides on mycelial growth of *P. cactorum* and *P. citrophthora* was determined by growing fungi on CMA amended with various concentrations of fungicides. Stock suspensions of fungicides were prepared by mixing the fungicide in sterile distilled water at appropriate concentrations; it was added to CMA after autoclaving to give final concentrations of 10, 100, 500, 1000 mg l<sup>-1</sup>. An agar plug (6 mm diam) taken from the margin of an actively growing colony of *P. cactorum* or *P. citrophthora* was placed in the center of agar plates and incubated at 23 °C. Twenty replicate plates were used for each treatment, ten for each species. Colony diameter measurements of mycelial growth were taken 4 days after initial transfer. Growth on unamended agar was used to determine the degree of inhibition.

**Statistical analysis** The experiments were established using a randomized design. Data were analyzed by one-way analysis of variance. To separate groups of treatment means which were not significantly different, Duncan's Multiple Range Test ( $P = 0.05$ ) was used. Each experiment was conducted three times; results were similar (Bartlett's test,  $P < 0.05$ ) and the data were therefore combined. Data of Exp. 3 were also expressed as percentage disease control relative to non-fungicide (Abbott's formula) and the mean effective concentration (ED<sub>50</sub>), and its 95% confidence limits were calculated by probit analysis. The significance of the probit regression lines was tested by calculating the correlation coefficient,  $r$ . The confidence limits were used to compare the ED<sub>50</sub> of the different fungicides.

## RESULTS

**Exp. 1 – Excised twig assay** Metalaxyl was the only fungicide tested that inhibited canker development completely at the manufacturer-recommended concentration. No significant difference between metalaxyl and copper hydroxide was observed when mycelial growth of *P. cactorum* was evaluated. Stronger development of *P. citrophthora*

was found on twigs treated with copper hydroxide than on those treated with metalaxyl. Copper hydroxide reduced the development of *P. cactorum* significantly more than any other fungicide tested. The growth of *P. citrophthora* on twigs treated with copper hydroxide did not differ from on those treated with fosetyl-Al. Copper hydroxide was more effective than all other tested fungicides against both fungi. Development of *P. cactorum* and *P. citrophthora* on twigs treated with fosetyl-Al or copper sulfate was inhibited moderately compared with non-treated twigs. Significant differences were observed in canker length between fosetyl-Al and copper sulfate treatments. Both of these fungicides were significantly more effective against *P. cactorum* and *P. citrophthora* than were chlorothalonil, captan, copper oxychloride, propamocarb and quinterozone. Growth of *P. cactorum* and *P. citrophthora* on non-treated twigs did not differ from that on twigs treated with captan, chlorothalonil, copper oxychloride, propamocarb, or quinterozone at the manufacturer-recommended concentration (Table 1). Fosetyl-Al and copper hydroxide inhibited the development of both fungi at 3 and 4 g l<sup>-1</sup>, respectively. Copper sulfate reduced significantly the growth of both fungi at 6 g l<sup>-1</sup> and was inhibitory at 8 g l<sup>-1</sup>. Also, canker development caused by *P. cactorum* or *P. citrophthora* was inhibited on twigs treated with captan at 4 g l<sup>-1</sup>. In contrast, no significant difference existed between twigs treated with quinterozone, copper oxychloride, chlorothalonil or propamocarb, and non-treated twigs, at any of the concentrations tested (Table 1).

**Exp. 2 – Excised stem inoculation** Generally, the results generated with excised stems agree with those obtained in the excised twig assay. At the manufacturer-recommended concentration, both metalaxyl and fosetyl-Al inhibited the development of *P. cactorum* and *P. citrophthora* on segments almost completely. Copper hydroxide reduced canker development significantly, but significantly less than metalaxyl and fosetyl-Al. There were no significant differences among copper sulfate, propamocarb and quinterozone in the development of *P. cactorum*. Copper sulfate was more effective against *P. cactorum* and *P. citrophthora* than chlorothalonil, copper oxychloride and captan, but less effective than copper hydroxide. Also, it reduced the development of *P. citrophthora* significantly more than did propamocarb and quinterozone. There were no significant differences in growth of *P. cactorum* among propamocarb, copper oxychloride, chlorothalonil and quinterozone at the manufacturer-recommended concentrations. Development of *P. cactorum* on segments treated with chlorothalonil, captan, quinterozone or copper oxychloride did not differ compared with non-treated segments. Growth of *P. citrophthora* on twigs treated with propamocarb, quinterozone, chlorothalonil, copper oxychloride or captan at the manufacturer-recommended concentrations did not differ significantly from growth on untreated twigs (Table 2).

Copper hydroxide could inhibit the development of both fungi on segments at 4 g l<sup>-1</sup>; copper sulfate reduced the growth of both fungi significantly at 6 g l<sup>-1</sup>, and was inhibitory at 8 g l<sup>-1</sup>. Captan was effective in inhibiting canker development at 4 g l<sup>-1</sup>. Propamocarb was able to reduce the mycelial growth of *P. cactorum* on segments at 3 g l<sup>-1</sup>, but could not inhibit the fungal growth at a concentration as high as 5 g l<sup>-1</sup>. Propamocarb had no effect on the growth of *P. citrophthora* at any of the concentrations tested. The development of fungi on segments treated with quinterozone, copper oxychloride or chlorothalonil did not differ significantly from that on non-treated segments at any of the concentrations tested.

TABLE 1. Canker development (in cm) on excised peach twigs after treatment with fungicides at four different concentrations (a.i., g l<sup>-1</sup>)

Fungicides	Concn. 1 <sup>z</sup>		Concn. 2		Concn. 3		Concn. 4	
	P. cac. <sup>y</sup>	P. cit. <sup>y</sup>	P. cac.	P. cit.	P. cac.	P. cit.	P. cac.	P. cit.
None	-	2.15 <sup>a,w</sup>	1.95 <sup>a</sup>	1.56 <sup>a</sup>	-	2.26 <sup>a</sup>	1.88 <sup>a</sup>	1.56 <sup>a</sup>
Quintozene	0.16	2.16 <sup>a</sup>	1.93 <sup>a</sup>	1.45 <sup>a</sup>	0.3	2.25 <sup>a</sup>	1.81 <sup>a</sup>	1.50 <sup>a</sup>
Copper oxychloride	5	2.14 <sup>a</sup>	1.90 <sup>a</sup>	1.54 <sup>a</sup>	9	2.14 <sup>a</sup>	1.92 <sup>a</sup>	1.62 <sup>a</sup>
Propamocarb	2.1	2.13 <sup>a</sup>	1.78 <sup>a</sup>	1.51 <sup>a</sup>	4	2.20 <sup>a</sup>	1.86 <sup>a</sup>	1.59 <sup>a</sup>
Chlorothalonil	1.5	2.09 <sup>a</sup>	1.82 <sup>a</sup>	1.42 <sup>a</sup>	3	2.19 <sup>a</sup>	1.90 <sup>a</sup>	1.61 <sup>a</sup>
Captan	1.45	2.01 <sup>a</sup>	1.88 <sup>a</sup>	1.53 <sup>a</sup>	3	2.19 <sup>a</sup>	1.97 <sup>a</sup>	0b
Copper sulfate	4.5	1.28 <sup>b</sup>	0.42 <sup>b</sup>	0.51 <sup>b</sup>	8	0d	0b	0b
Fosetyl-Al	2.2	0.86 <sup>c</sup>	0c	0c	3	0e	0b	0b
Copper hydroxide	2.8	0.22 <sup>d</sup>	0c	0c	4	0f	0b	0b
Metalaxyl	0.23	0d	0c	0c	0.23	0f	0b	0b

<sup>z</sup>Concentration recommended by manufacturer (active ingredients, in g l<sup>-1</sup>).

<sup>y</sup>P. cac. = *Phytophthora cactorum*; P. cit. = *Phytophthora citrophthora*.

<sup>x</sup>Values are means of three experiments, each with 50 replicates.

<sup>w</sup>Within columns, values followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Duncan's Multiple Range Test.

TABLE 2. Canker development (in cm) on excised peach stems after treatment with fungicides at four different concentrations (a.i., g l<sup>-1</sup>)

Fungicides	Concn. 1 <sup>z</sup>		Concn. 2		Concn. 3		Concn. 4	
	P. cac. <sup>y</sup>	P. cit. <sup>y</sup>	P. cac.	P. cit.	P. cac.	P. cit.	P. cac.	P. cit.
None	-	2.30 <sup>ab<sup>x,w</sup></sup>	2.90 <sup>a</sup>	2.50 <sup>a</sup>	-	3.40 <sup>a</sup>	3.00 <sup>a</sup>	2.30 <sup>a</sup>
Captan	1.45 <sup>w</sup>	2.70 <sup>a</sup>	3.00 <sup>a</sup>	2.40 <sup>a</sup>	3	3.40 <sup>a</sup>	0b	0b
Copper oxychloride	5	2.10 <sup>bc</sup>	3.10 <sup>a</sup>	2.70 <sup>a</sup>	9	3.60 <sup>a</sup>	3.00 <sup>a</sup>	2.30 <sup>a</sup>
Chlorothalonil	1.5	2.10 <sup>bc</sup>	3.00 <sup>a</sup>	2.70 <sup>a</sup>	3	3.40 <sup>a</sup>	3.00 <sup>a</sup>	2.20 <sup>a</sup>
Quintozene	0.16	1.80 <sup>bcd</sup>	3.00 <sup>a</sup>	2.70 <sup>a</sup>	0.3	3.50 <sup>a</sup>	3.00 <sup>a</sup>	2.20 <sup>a</sup>
Propamocarb	2.1	1.60 <sup>cd</sup>	1.90 <sup>b</sup>	2.40 <sup>a</sup>	4	1.90 <sup>b</sup>	2.00 <sup>b</sup>	2.30 <sup>a</sup>
Copper sulfate	4.5	1.40 <sup>d</sup>	0.80 <sup>c</sup>	0.90 <sup>b</sup>	8	0.10 <sup>c</sup>	0c	0b
Copper hydroxide	2.8	0.70 <sup>e</sup>	0d	0c	4	0c	0c	0b
Fosetyl-Al	2.2	0.10 <sup>f</sup>	0d	0c	3	0c	0c	0b
Metalaxyl	0.23	0f	0d	0c	0.23	0c	0c	0b

<sup>z</sup>Concentration recommended by manufacturer (active ingredients, in g l<sup>-1</sup>).

<sup>y</sup>P. cac. = *Phytophthora cactorum*; P. cit. = *Phytophthora citrophthora*.

<sup>x</sup>Values are means of three experiments, each with 50 replicates.

<sup>w</sup>Within columns, values followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Duncan's Multiple Range Test.

TABLE 3. Effect of nine fungicides at four different concentrations on mycelial growth of *Phytophthora cactorum* and *P. citrophthora* in vitro

Concn. (mg l <sup>-1</sup> )	None	Chlorothalomid	Propamocarb	Fosetyl-AI	Quintozene	Captan	Copper oxychloride	Copper sulfate	Copper hydroxide	Metalaxyl
0	6.1a <sup>z,y</sup>	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a	6.1a
10	6.1A	6.33aA	3.18bD	5.18bB	4.13bC	5.2bB	4.18bC	6.13aA	1.47bE	6.1a
100	6.1A	5.38bB	2.88bF	4.85bC	3.65bE	4.33cD	1.5cG	3.75bE	0cH	0.78bF
500	6.1A	3.83cB	2.83 bC	1.3cD	0.98cE	1.3dD	0dG	0dG	0cG	0cG
1000	6.1A	3.43cB	1.88cC	0.48dD	0dE	0eE	0dE	0cE	0cE	0cE
Mycelial growth of <i>P. citrophthora</i> (cm)										
0	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
10	8A	5.88bC	5.4bD	6.35bB	6.5bB	6.4bB	5.55bD	6.68bB	0.43bE	0.2bE
100	8A	6bB	2.88cE	5.3cC	5.95bB	3.5cD	2.83cE	2.43cF	0cG	0bG
500	8A	2.4cC	2.2cC	1.73dD	2.7cB	1.88dD	0dE	0dE	0cE	0bE
1000	8A	2.75dB	1.3dC	0.6eD	0dD	0eD	0dD	0eD	0cD	0bD

<sup>z</sup> Values are means of three experiments, each with ten replicates.

<sup>y</sup> Significant differences ( $P = 0.05$ ) indicated within columns (concentrations) by different lowercase letters, and within rows (fungicides) by capital letters, according to Duncan's Multiple Range Test.

TABLE 4. Fungicide median effective concentration, ED<sub>50</sub> after probit analysis<sup>z</sup>

Fungicides	ED <sub>50</sub> (mg l <sup>-1</sup> )	95% Confid. limits		<i>r</i> of % control on log (concn.)	<i>P</i> sign.	ED <sub>50</sub> (mg l <sup>-1</sup> )	95% Confid. limits		<i>r</i> of % control on log (concn.)	<i>P</i> sign.
		Lower	Upper				Lower	Upper		
Chlorothalomid	1125.4d	951.8	1372.0	0.969	0.000	212.7e	125.5	379.5	0.810	0.000
Propamocarb	29.3b	9.8	57.8	0.912	0.000	41.9bgd	32.4	52.6	0.984	0.000
Fosetyl-AI	145.2g	96.9	213.3	0.912	0.002	100.6de	71.5	138.1	0.948	0.000
Quintozene	53.4bg	30.0	85.4	0.912	0.000	130.4de	77.3	212.5	0.899	0.000
Captan	112.9g	76.9	161.6	0.943	0.000	63.4gde	45.6	85.1	0.989	0.000
Copper oxychloride	0.2a	0.0	4.5	0.717	0.000	29.7bgd	21.4	39.6	0.989	0.000
Copper sulfate	110.2g	106.1	161.1	0.566	0.028	39.8bg	34.0	46.3	0.985	0.000
Copper hydroxide	0.8a	0.1	3.4	0.826	0.000	1.2a	0.1	2.9	0.858	0.000
Metalaxyl	1.9a	0.1	7.3	0.717	0.020	1.2a	*		0.488	0.153

\* No possible confidence limits because of linear dependency.

<sup>z</sup> Fungicides with an ED<sub>50</sub> followed by a common letter are arranged in the same group of response ( $P = 0.05$ ).

**Exp. 3 – Mycelial growth** All tested fungicides reduced mycelial growth of *P. cactorum* and *P. citrophthora* at 10 mg l<sup>-1</sup>. At this concentration, metalaxyl and copper hydroxide were the most effective fungicides and only chlorothalonil and copper sulfate had no effect on the growth of *P. cactorum*. Copper oxychloride and propamocarb reduced the mycelial growth of both pathogens significantly more than did copper sulfate, captan, chlorothalonil or fosetyl-Al at 10 mg l<sup>-1</sup>. Quintozenone caused a similar reduction in mycelial growth of *P. cactorum* to copper oxychloride at 10 mg l<sup>-1</sup>, but was less effective than copper oxychloride against *P. citrophthora* at the same concentration. Metalaxyl and copper hydroxide at 100 mg l<sup>-1</sup> suppressed mycelial growth of *P. cactorum* and *P. citrophthora* completely. Copper oxychloride, captan, copper sulfate and chlorothalonil reduced growth of *P. cactorum* significantly; there was no significant difference in mycelial growth of *P. cactorum* cultured on agar amended with propamocarb, fosetyl-Al or quintozenone at 100 as compared with 10 mg l<sup>-1</sup>. There was significantly less mycelial growth of *P. citrophthora* on agar grown with copper sulfate, copper oxychloride, captan, fosetyl-Al and propamocarb at 100 as compared with 10 mg l<sup>-1</sup>; quintozenone and chlorothalonil did not affect growth of *P. citrophthora* at 100 mg l<sup>-1</sup>. At 100 mg l<sup>-1</sup>, copper sulfate, copper oxychloride and propamocarb reduced the radial growth of both *Phytophthora* species significantly more than did captan, fosetyl-Al or chlorothalonil. No significant difference was observed in radial growth of *P. cactorum* cultured on cornmeal agar amended with quintozenone or copper sulfate at 100 mg l<sup>-1</sup>. In contrast, at the same concentration, copper sulfate was more effective than quintozenone in reduction of the mycelial growth of *P. citrophthora*. Both copper oxychloride and copper sulfate at 500 mg l<sup>-1</sup> inhibited growth of *P. cactorum* and *P. citrophthora*. Growth of both fungi was reduced significantly by chlorothalonil, fosetyl-Al, quintozenone and captan at 500 mg l<sup>-1</sup>. Only propamocarb did not influence mycelial growth at 500 mg l<sup>-1</sup>. Captan and fosetyl-Al were more effective than propamocarb and chlorothalonil at 500 mg l<sup>-1</sup>. At 500 mg l<sup>-1</sup>, quintozenone was more effective against *P. cactorum* than captan and fosetyl-Al but less effective against *P. citrophthora* than captan and fosetyl-Al. At 1000 mg l<sup>-1</sup>, captan and quintozenone suppressed growth of *P. cactorum* and *P. citrophthora* completely, and fosetyl-Al and propamocarb reduced growth of both fungi significantly; chlorothalonil did not affect growth at all (Table 3).

Based on the probit analysis (Table 4), it is seen that copper oxychloride, copper hydroxide and metalaxyl are the most effective fungicides at the lowest concentrations against *P. cactorum*, followed by propamocarb and quintozenone. The ED<sub>50</sub> of chlorothalonil was beyond the limits of the experimental data, suggesting the need to test higher concentrations if accurate results and comparisons are wanted. Copper hydroxide and metalaxyl were the most effective fungicides for controlling *P. citrophthora*, followed by copper oxychloride, copper sulfate and propamocarb, and last by chlorothalonil – which required the highest concentration among all fungicides tested.

Copper hydroxide and metalaxyl were the most effective fungicides for controlling both fungi. Copper oxychloride, on the other hand, which was one of the most effective fungicides for controlling *P. cactorum*, was less effective than copper hydroxide and metalaxyl in controlling *P. citrophthora*. Captan and copper sulfate were more effective in controlling *P. citrophthora* than *P. cactorum*, whereas the inverse was true for quintozenone. Propamocarb was quite effective in controlling both *P. cactorum* and *P. citrophthora*, whereas fosetyl-Al and chlorothalonil required the highest concentration to achieve the ED<sub>50</sub> response level.

## DISCUSSION

Results of this study support those of previous works showing that both metalaxyl and fosetyl-Al provide effective control of diseases caused by *Phytophthora* species (6,9,14,15,18,26). However, fosetyl-Al had the ability to inhibit completely the development of both fungi only at a concentration higher than that recommended by the manufacturer.

In the present study, also copper hydroxide was an effective fungicide against *P. cactorum* and *P. citrophthora*. Jeffers (9) reported that copper hydroxide was effective as a preplant root treatment to reduce the incidence of *Phytophthora* on dormant apple rootstocks, but less effective than metalaxyl. Copper sulfate applied as trunk paint is widely used for preventing infections of fruit trees by *Phytophthora* species in Greece. It was found by us to reduce canker development at  $6 \text{ g l}^{-1}$  and to inhibit the growth of both fungi at  $8 \text{ g l}^{-1}$ . It was less effective than fosetyl-Al, metalaxyl and copper hydroxide at the manufacturer-recommended concentration. Brown and Hendrix (3) found that copper sulfate was inadequate for controlling *P. cactorum* collar rot of apple. Copper oxychloride was ineffective *in vivo* (Tables 1 and 2). It has been reported that on citrus trees infected by *Phytophthora*, excision of affected tissue and painting with any copper fungicide improved tree recovery (22).

Captan was also an effective fungicide against both fungi at  $4 \text{ g l}^{-1}$ . Similarly, Brown and Hendrix (3) found that captan, at  $100 \text{ mg l}^{-1}$ , significantly reduced growth of *P. cactorum* *in vitro*. Timmer (22) reported that  $60 \text{ mg l}^{-1}$  of captan was required to achieve 100% inhibition of *Phytophthora* in a bioassay. Chlorothalonil and quintozone were ineffective in controlling canker development caused by *Phytophthora* *in vivo* (Tables 1 and 2). Terrachlor (quintozone) was effective in controlling root rot of azalea caused by *P. cinnamomi* when applied at least four times in a 28-week period (1). Propamocarb, in our excised stem experiments, reduced the growth of *P. cactorum* at the manufacturer-recommended concentration, but its effectiveness could not be improved by increasing the concentration. In addition, this fungicide could not influence the development of *P. citrophthora* at any of the concentrations tested. It is possible that it has selective activity depending on the *Phytophthora* species. Erwin and Ribeiro (7) reported that propamocarb gave good control of *Phytophthora* diseases. However, Matheron and Matejka (14) found that propamocarb and etridiazole did not inhibit growth of *P. parasitica* and *P. citrophthora* *in situ*.

Some differences were observed between *in vivo* and *in vitro* experiments. Whereas propamocarb, copper oxychloride and quintozone were ineffective against *P. cactorum* and *P. citrophthora* *in vivo*, these fungicides showed some activity against both pathogens *in vitro*. It is possible that the fungicidal activity of these fungicides was influenced when the material was applied to plant tissues (stems and twigs). However, the factors responsible for a reduction in activity of fungicides after application on stems and twigs have not been identified.

Both the excised twig and excised stem inoculation assay used in this study are rapid and reliable techniques for testing the efficacy of fungicides in the prevention of *P. cactorum* and *P. citrophthora* associated with crown rot on peach trees. Results can be generated in a few days instead of the several weeks or months needed with previous methods. The methods described in this paper are quite simple and allow ample replications.

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