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EFFECT OF METALAXYL, FOSETYL-AL, DIMETHOMORPH, CYMOXANIL ON DEVELOPMENT AND CONTROL *PHYTOPHTHORA* ON PEACH TREE *IN VITRO*

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Four fungicides were evaluated for their effectiveness against *Phytophthora* diseases of peach trees and their ability to absorbed and translocated by roots of peach tree in laboratory. Metalaxyl suppressed the development of *P. cactorum* and *P. citrophthora* on segments. Fosetyl-Al showed variable fungicidal activity against *P. cactorum* and *P. citrophthora*. Both dimethomorph and cymoxanil were not effective to inhibit the growth of fungi on segments. Section of tree trunks in 2-yr-old GF 677 trees were painted with one of the test fungicides. Strips of bark were removed 10 and 20 days after painting within treated area and inoculated with *P. cactorum* and *P. citrophthora*. Generally, the results agree with them obtained with excised stem and excised twig methods. Exception is the dimethomorph that reduced the development of *P. cactorum* and *P. citrophthora*. This study indicated that application with metalaxyl to the peach tree appears to be an effective procedure to control *Phytophthora* diseases on peach trees.

Keywords: Application; Crown rot; Fungicides; Peach trees; *Phytophthora* translocation

INTRODUCTION

Phytophthora is one of the most damaging soilborne disease-causing organism affecting the crown and roots of fruit trees. Various species of

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Phytophthora are associated with this disease worldwide including *P. cactorum, P. citrophthora, P. citricola, P. megasperma, P. cambivora, P. cinnamomi, P. cryptogea, P. syringae, P. drechsleri* (Chitzanidis and Stylianides, 1987; Flores and Hindal, 1983; Jeffers and Aldwinckle, 1988; Jeffers *et al.*, 1982; Kim *et al.*, 1985; Kouyeas, 1977; Matheron *et al.*, 1988; Matheron and Matejka, 1990; Matheron *et al.*, 1990; Mircetich and Matheron, 1976; Stylianides *et al.*, 1985; Wilcox and Ellis, 1989). Outbreaks of crown rot are random and depend on excess soil water, suitable temperatures, and host susceptibility (Jacobs and Johnson, 1996; Matheron and Matejka, 1992; Matheron and Porchas, 1996; Utkhede and Smith, 1993, 1994; Wilcox, 1993; Wilcox and Mircetich, 1985).

Control of crown rot disease is very difficult because *Phytophthora* is a polycyclic pathogen (Ristaino, 1991). The systemic fungicides, metalaxyl and fosetyl-Al, have been found to be effective to control several diseases caused by oomycetes (Coffey and Joseph, 1985; El-Hamalawi *et al.*, 1995; Ellis *et al.*, 1982; Matheron and Matejka, 1988; Matheron and Matejka, 1991; Matheron and Mircetich, 1985; Papavizas and Bowers, 1981; Rana and Gupta, 1984; Wicks and Hall, 1988).

Dimethomorph is a relative new fungicide that seems to be active against oomycete fungal pathogens regardless of their sensitivity to phenylamide fungicide (Cohen *et al.*, 1995). Cymoxani exhibits local systemic activity against oomycetes (Erwin and Ribeiro, 1996). Both dimethomorph and cymoxanil have not evaluated for their ability to control *Phytophthora* crown rot of peach trees.

This paper reports laboratory studies on evaluation of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil for the control of *P. cactorum* and *P. citrophthora* on peach trees. Also, this study investigated the ability of fungicides to be absorbed and systemically translocated by the roots of peach trees.

MATERIAL AND METHODS

One isolate of *P. cactorum* and one isolate of *P. citrophthora* were used in this study. *P. cactorum* was an almond isolate and *P. citrophthora* was recovered from citrus. Both isolates were maintained with paraffin oil at 22°C. Fresh cultures were prepared with transferring agar plugs bearing mycelium of

fungus on plates containing cornmeal agar. The plates were then placed in an incubator at 23°C for six days.

GF 677 plants were bought from a commercial tissue culture station (Vitro Hellas), and planted in the experimental field of the Pomology Institute, Naoussa. Fungicides were applied to 2-yr-old tree as soil drenches of 500 ml fungicide solution per tree using metalaxyl (Ridomil 2E, 2 g/tree), fosetyl-Al (Aliette WP, 2 g/tree), dimethomorph (dimethomorph + propineb, 3 g/tree) and cymoxanil (Diametan 3 g/tree). Ten trees (replicates) were used for each fungicide.

Experiment 1 Ten, twenty, and thirty days after drenching, stem pieces 10-cm-long about 2 cm diameter were cut from the central part of wooded shoots. The excised stems were inoculated with removing a 6 mm bark to expose the cambium and inserted a 6 mm agar plug bearing mycelium of *P. cactorum* or *P. citrophthora* direct on cambium. Wound was covered with petroleum jelly and wrapped with adhesive tape for avoiding desiccation. Segments were then placed in wet chamber at 23°C for six days. There were eighty segments for each *Phytophthora* species, twenty segments for each fungicide. Segments taken from untreated trees were used as control. Results were collected with measuring the vertical distance of developed canker.

Experiment 2 Wooded shoots were collected from non-drenched GF 677 trees. Segments of 10-cm-long and about 2-cm-diameter were cut from the central part of shoots. Fungicide solutions were prepared with mixing fungicide in sterile water (2 g/l for metalaxyl and fosetyl-Al and 3 g/l for dimethomorph and cymoxanil). Segments were soaked in solution for 3 mm, and then, dried on towel paper. Eighty segments were used for each *Phytophthora* species, twenty for each fungicide. Segments soaked in sterile water were used as control. Inoculations and collections of results were made as described in Experiment 1.

Experiment 3 Inocula were prepared with dispensing cornmeal agar amended with antibiotics (CMA+A; pimaricin 10 mg, ampicillin 250 mg, rifampicin 10 mg) in Pyrex jars to give an agar depth about 1 cm. Two agar plugs bearing mycelium of *P. cactorum* or *P. citrophthora* were transferred in each jar. Jars were sealed with parafilm and placed in an incubator in the dark at 23°C until mycelium covered agar surface.

Wooded shoots were collected from trees drenched with the tested fungicides ten, twenty and thirty days after application. Twigs of 70 mm long and

about 1 cm diameter were cut from the central part. The bark was removed from the base of each twig to expose cambium. Fifteen twigs were placed upright in the agar medium and the jars were resealed for returning to the incubator for five days. At the end of the incubation period the bark was removed to expose the cambium and length lesion was measured.

There were eight jars for each *Phytophthora* species, two jars for each fungicide. Also, two jars without inoculum were used as control.

Experiment 4 Preparing of inocula and collection of results were made as described in experiment 3. Again, there were eight jars for each *Phytophthora* species, two for each fungicide. Also, two jars without inoculum were used as control. Twigs were cut from wooded shoots collected from non-treated GF 677 trees. Base bark of twigs was removed with a sharp knife to expose cambium and thirty of pared twigs were soaked in each fungicide solution (2 g/l for metalaxyl and fosetyl-Al, 3 g/l for dimethomorph and cymoxanil) for 3 mm and then, were dried on towel paper. Fifteen twigs were inserted vertically into the agar medium and the jars were resealed and returned in incubators for five days at 23°C in the dark.

Experiment 5 All inoculations were performed in laboratory using the bark strip assay described by Matheron and Matejka (1988). In a 2-year-old GF 677 plants, a 20 cm section of trunk on each of twenty plants was painted with metalaxyl (150 g/l), fosetyl-Al (150 g/l), dimethomorph (150 g/l), cymoxanil (150 g/1) and tap water as control. Ten and twenty days after treatments two vertical strips of bark (10 cm long × 1,5 cm wide) were removed from the initially treated of each trunk and inoculated in the center by placing a 6-mm-diameter plug of cornmeal agar with *P. cactorum* or *P. citrophthora* on the cambium. Inoculated bark strips were incubated for four days at 23°C in moist chambers, after which the vertical length of necrosis was measured.

Eighty bark strips were used for each *Phytophthora* species, twenty strips for each fungicide.

Statistic Analysis

The experimental design used throughout the laboratory experiments was completely randomized. Data obtained from each experiment were analyzed by analysis of variance using the Duncan's multiple range test ($P = 0.05$). All experiments were performed three times and values represented the mean of experiments.

RESULTS

Experiment 1 When bark was removed, developed cankers were detected to extent mainly upward and downward. Segments obtained from trees treated with metalaxyl appeared healthy. Metalaxyl nearly suppressed the development of *P. cactorum* and *P. citrophthora* for at least thirty days. No significant difference was observed in the growth of *P. cactorum* on twigs collected twenty days after drenching with fosetyl-Al compared with non-treated twigs. However, fosetyl-Al reduced significantly the development of both fungi ten and thirty days after drenching. Also, fosetyl-Al was effective against *P. citrophthora* on segments collected twenty days after application. Both cymoxanil and dimethomorph were ineffective fungicides against *P. cactorum* and *P. citrophthora* (Tab. I).

Experiment 2 Segments treated with dimethomorph or cymoxanil and untreated segments showed the symptom of gummosis. *P. cactorum* and *P. citrophthora* did not colonize the segments treated with metalaxyl. Fosetyl-Al reduced significantly the growth of *P. cactorum* compared with cymoxanil and dimethomorph but it was less effective than metalaxyl. No significant difference was observed between fosetyl-Al and metalaxyl

TABLE I Effect of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil applied as soil drench to 2-yr-old GF 677 trees on subsequent growth of *P. cactorum* and *P. citrophthora* in excised stem segments

	Length lesion[y]		
Fungicides[x]	10 *days*	20 *days*	30 *days*
P. cactorum			
Control	2,64 a[z]	2,67 a	2,71 Ab
Cymoxanil	2,87 a	2,87 a	3,09 A
Dimethomorph	2,86 a	2,87 a	2,83 Bc
Fosetyl-Al	1,93 b	2,69 a	2,44 C
Metalaxyl	0,69 c	0 b	0 D
P. citrophthora			
Control	2,08 a	2,11 a	2,52 A
Cymoxanil	2 a	1,98 a	2,56 A
Dimethomorph	2,1 a	1,89 a	2,49 A
Fosetyl-Al	1,23 c	0,88 b	1,75 B
Metalaxyl	0,21 d	0 c	0,2 C

[x]Fungicide solutions were prepared by mixing appropriate concentration of fungicide with tap water.
[y]Values are the means of three experiments, each with twenty replicates.
[z]Numbers within a column with the same latter do not differ each other according to Duncan's multiple range test, $P = 0.05$.

against *P. citrophthora*. In contrast, developed cankers were observed on segments treated with dimethomorph or cymoxanil the size of which did not differ from untreated segments (Tab. II).

Experiment 3 Metalaxyl was very effective against *P. cactorum* and *P. citrophthora*. Twigs collected from trees drenched with metalaxyl were little colonized. In contrast, developed canker was observed on twigs treated with fosetyl-Al, dimethomorph, and cymoxanil. There was no significant difference in the development of *P. cactorum* and *P. citrophthora* on twigs treated with fosetyl-Al, dimethomorph, cymoxanil (Tab. III).

Experiment 4 Again metalaxyl inhibited the development of *P. cactorum* and *P. citrophthora*. The growth of *P. cactorum* on twigs treated with fosetyl-Al was significantly reduced but fosetyl-Al was less effective compared with metalaxyl. No significant difference was observed in the growth of *P. citrophthora* between fosetyl-Al and metalaxyl. Developed canker was appeared on twigs treated with dimethomorph or cymoxanil. Colonization of twigs treated with dimethomorph or cymoxanil did not differ significant compared with non-treated twigs (Tab. IV).

Experiment 5 Variable inhibition of *Phytophthora* was observed on bark strips removed from within the section of GF677 tree trunks treated with metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil. *P. cactorum* and *P. citrophthora* were grown least on strips treated with metalaxyl collected one and two weeks after application. Colonization of strips treated with fosetyl-Al was significantly less compared with non-treated and cymoxanil but, it was less effective compared with metalaxyl. There was not significant

TABLE II Effect of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil on excised stem soaked in fungicide solution on the development of cankers caused by *Phytophthora cactorum* and *P. citrophthora*

	Length lesion[y]	
Fungicides[x]	*P. cactorum*	*P. citrophthora*
Control	2,13 a[y]	1,98 a[z]
Cymoxanil	2,16 a	1,8 A
Dimethomorph	2,2 a	1,82 a
Fosetyl-Al	0,84 b	0,23 b
Metalaxyl	0 c	0,1 b

[x]Fungicide solutions were prepared by mixing appropriate concentration of fungicide with tap water.
[y]Values are the means of three experiments, each with twenty replicates.
[z]Numbers within a column with the same latter do not differ each other according to Duncan's multiple range test, $P=0.05$.

TABLE III Effect of of metalaxyl, fosetyl-Al, dimethomorph. and cymoxanil applied as soil drench to 2-yr-old GF 677 trees subsequent growth of *P. cactorum* and *P. cirtrophthora* in excised twigs

	Length lesion[y]		
Fungicides[x]	10 *days*	20 *days*	30 *days*
P. cactorum			
Control	2,52 a[z]	2,52 a	2,56 a
Cymoxanil	2,51 a	2,43 a	2,6 a
Dimethomorph	2,69 a	2,59 a	2,58 a
Fosetyl-Al	2,44 a	2,23 a	2,42 a
Metalaxyl	0,11 b	0,53 b	0,11 b
P. citrophthora			
Control	2,12 a	2,12 a	2,12 a
Cymoxanil	1,98 a	2,21 a	2,15 a
Dimethomorph	2,01 a	2,31 a	2,11 a
Fosetyl-Al	1,88 a	2,19 a	2,2 a
Metalaxyl	0 b	0,12 b	0,65 b

[x]Fungicide solutions were prepared by mixing appropriate concentration of fungicide with tap water.
[y]Values are the means of three experiments, each with thirty replicates.
[z]Numbers within a column with the same latter do not differ each other according to Duncan's multiple range test, $P=0.05$.

TABLE IV Effect of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil on excised twigs, soaked in fungicide solution, on the development of cankers caused by *P. cactorum* and *P. citrophthora*

	Length lesion[y]	
Fungicides[x]	*P. cactorum*	*P. citrophthora*
Control	2,14 a[z]	1,66 a
Cymoxanil	2,2 a	1,59 a
Dimethomorph	2,16 a	1,46 a
Fosetyl-Al	0,44 b	0,21 b
Metalaxyl	0 c	0,1 b

[x]Fungicide solutions were prepared by mixing appropriate concentration of fungicide with tap water.
[y]Values are the means of three experiments, each with thirty replicates.
[z]Numbers within a column with the same latter do not differ each other according to Duncan's multiple range test, $P=0.05$.

difference in the growth of *P. cactorum* on strips between fosetyl-Al and dimethomorph ten days after painting but, fosetyl-Al was significant more effective twenty days after. Also, the growth of *P. citrophthora* on strips treated with fosetyl-Al was significantly reduced compared with dimethomorph. Dimethomorph reduced significant the development of fungus on strips significantly compared with cymoxanil and untreated, but it was less effective than metalaxyl. Treated strips with cymoxanil did not influence the development of *P. cactorum* and *P. citrophthora* (Tab. V).

TABLE V Inhibition of canker development in GF 677 tree bark after trunk paint application of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil

Fungicides[x]	*Length lesion*[y]	
	10 *days*	20 *days*
P. cactorum		
Control	6,2 a[z]	6,6 a
Cymoxanil	5,9 a	6.6 a
Dimethomorph	5,3 b	4,8 b
Fosetyl-Al	4,8 b	3,6 c
Metalaxyl	1 c	1,2 d
P. citrophthora		
Control	5,8 a	5,6 a
Cymoxanil	5,6 a	5,8 a
Dimethomorph	5,65 b	4,3 b
Fosetyl-Al	3,2 c	2,1 c
Metalaxyl	0,3 d	0,2 d

[x]Fungicide solutions were prepared by mixing appropriate concentration of fungicide with tap water.
[y]Values are the means of three experiments, each with ten replicates.
[z]Numbers within a column with the same latter do not differ each other according to Duncan's multiple range test, $P=0.05$.

DISCUSSION

Excised twig assay and excised stem inoculation method are reliable and effective to evaluate the effectiveness of different chemicals against *Phytophthora* diseases in laboratory (Thomidis, unpublished). Furthermore, chemicals can be screened quickly at any time of year under controlled environment using ample replications. Also, with the excised twig assay and excised stem inoculation method can test the ability of chemicals to be absorbed and translocated through the roots of plants.

In this study, excised twig assay and excised stem inoculation method were used to evaluate the fungicidal activity of four chemicals against *P. cactorum* and *P. citrophthora* and their systemical ability. Metalaxyl suppressed the development of fungi in all experiments. It had the ability to be absorbed and translocated by the roots of plants. The results agree with them obtained by other researchers (Jeffers, 1992; Matheron and Matejka, 1988; Matheron and Mircetich, 1985; Utkhede, 1987). Fosetyl-Al was also an effective fungicide when segments were soaked in fungicide solution at appropriate concentration, but it was less effective than metalaxyl. In contrast, both *P. cactorum* and *P. citrophthora* developed extensive necrosis on

segments obtained from trees drenched with fosetyl-Al. It is possible that fosetyl-Al has reduced ability to be absorbed and translocated by the roots of tree. Fosetyl-Al has been found to be a very effective fungicide to control *Phytophthora* crown and root rots applied as foliar sprays (Wicks and Hall, 1988). Both dimethomorph and cymoxanil were not effective fungicides to control *P. cactorum* and *P. citrophthora*. However, the growth of *P. cactorum* grown on agar amended with dimethomorph was inhibited as low as concentration at 100 ppm (Thomidis and Elena, In press).

In the bark strip assay, metalaxyl was the most effective fungicide. It inhibited the development of both *P. cactorum* and *P. citrophthora* on bark strips. Fosetyl-Al was also an effective fungicide but it was less effective than metalaxyl. Dimethomorph seemed to reduce colonization of strips by *P. cactorum* and *P. citrophthora*. It suggests more investigation to be conducted to evaluate the fungicidal activity of dimethomorph against *Phytophthora* diseases. Cymoxanil was an ineffective fungicide against *P. cactorum* and *P. citrophthora*.

In this study found evidence about the effectiveness of these chemicals against crown and root rots caused by *Phytophthora*. Application with metalaxyl to the peach tree appears to be an effective procedure to control *Phytophthora* diseases on peach trees.

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