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Effectiveness of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil against *Phytophthora cactorum* and *P. citrophthora* of peach tree

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Summary. Metalaxyl, fosetyl-Al, dimethomorph and cymoxanil were applied as a soil drench to evaluate their ability to control *in vitro* and *in vivo Phytophthora cactorum* and *P. citrophthora,* agents of crown rot of peach tree. In the *in vivo* assays, metalaxyl, applied as soil drench at 2 g/tree, was the most effective, reducing growth of *P. cactorum* and *P. citrophthora*. Fosetyl-Al was less effective against both pathogens, while cymoxanil and dimethomorph did not inhibit growth. When scraped stem cankers were painted with metalaxyl, fosetyl-Al or dimethomorpth at 150 g/l *Phytophthora* colonization was inhibited, but cymoxanil applied in the same way was ineffective. In the *in vitro* tests, metalaxyl and dimethomorph at concentrations as low as 100 mg l^{-1} completely inhibited growth of *P. cactorum* and *P. citrophthora*. Fosetyl-Al only did so at concentration of 1500 mg l^{-1} and over. Cymoxanil was less effective since even at 2000 mg l^{-1} it did not prevent mycelial growth of *P. cactorum* and *P. citrophthora*. This study demonstrated that *Phytophthora* diseases of peach tree can be controlled by metalaxyl applied as a soil drench and by metalaxyl, fosetyl-Al and dimethomorph painted on scraped stem cankers.

Key words: crown rot, fungicides, peach tree, *Phytophthora*.

Introduction

Phytophthora is a microbial genus that includes species causing many diseases (Erwin and Ribeiro, 1996). These species infect and kill nearly all species of deciduous fruit trees. *Phytophthora* crown and root rots, which are caused mainly by *P. cactorum*, *P. megasperma, P. citrophthora, P. cinnamomi, P. cambivora, P. syringae* and *P. cryptogea* (Sarejanni, 1935; Kouyeas, 1971; Kouyeas, 1977; Flores and Hidal, 1983; Kim *et al.,* 1985; Stylianides *et al.*, 1985; Chitzanidis and Stylianides, 1987; Wilcox and Ellis, 1989) are some of the most damaging diseases of peach orchards. In Greece two types of apoplexy relating to crown and root rots of peach have been reported. In the first type, infected trees show symptoms during the hot summer period. Both *P. cactorum* and *P. citrophthora* are associated with this type of apoplexy. The second type is caused by *P. syringae* and produces symptoms in late winter or early spring (Kouyeas, 1977). Disease incidence is greater in heavy and poorly drained soils (Harris and Tobutt, 1986; Wilcox and Mircetich, 1985; Jacobs and Jonson, 1996), but although partial control may be achieved by improving soil conditions, this is often difficult and costly in established orchards. Several peach root rootstocks have been selected for their resistance to *Phytophthora* rot, but complete immunity has not been achieved (Kouyeas, 1977; Stylianidis *et al.,* 1985; Chitzanidis and Stylianidis,

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1987) and rootstock susceptibility varies from one region to another (Utkhede and Smith, 1994). For the time being the use of chemicals, therefore, remains essential for preventing and curing crown rot disease of *Phytophthora* spp.

Although some chemical compounds (captan, captafol, ethazole, copper hydroxide) act against *Phytophthora,* none of them controls the rots caused by *Phytophthora* spp. (Timmer, 1977; Benson, 1979; Brown and Hendrix, 1980; Papavizas and Bowers, 1981). The effectiveness of systemic fungicides such as the acylalanine and phosphonate to control *Phytophthora* has been demonstrated. Of the acylalanine fungicides, the most important is metalaxyl (Ellis *et al.,* 1982). It is effective against oomycetes, especially *Pythium* and *Phytophthora*, and against downy mildews. The Phosphonate fungicides include fosetyl-Al and its breakdown product, phosphorous acid (Matheron and Matejka, 1988). Both fosetyl-Al and metalaxyl give good control of *Phytophthora* rots after they become established (Erwin and Ribeiro, 1996).

Recently, two new fungicides, dimethomorph and cymoxanil, have been developed that are effective for the control of plant pathogens in the order *Peronosporales* (Erwin and Ribeiro, 1996). However, more investigation is needed on these agents to evaluate how well they impede growth of *Phytophthora* in fruit orchards.

The aim of this study was to examine the effectiveness of metalaxyl (Ridomil 2E), fosetyl-Al (Aliette WP), dimethomorph (Acrobat), and cymoxanil (Diametan), applied as soil drenches or by painting on pre-existing stem cankers, in suppressing canker development on peach tree inoculated with *P. cactorum* or *P. citrophthora*.

Materials and methods

Isolation

One isolate of *P. cactorum* from peach tree and one of *P. citrophthora* from citrus were used in all experiments. Both had been shown in previous studies to be pathogenic to peach trees (Thomidis, 2000). The fungi were isolated on cornmeal agar (CMA) amended with antibiotics (mycostatin 100 mg, polymyxin 50 mg and penicillin 20 mg). They were maintained on CMA at 22°C in the collection of the Benaki Phytopathological Institute. Fresh cultures were prepared by transferring agar disks with mycelium of *P. cactorum* or *P. citrophthora* to cornmeal agar plates. The plates were then transferred to an incubator at 23°C until the mycelium covered the agar surface.

Laboratory experiment

Linear growth. This experiment was based on that described by Wicks and Hall (1988). Stock solutions of fungicide were prepared by mixing metalaxyl (Ridomil 2E), fosetyl-Al (Aliette WP), dimethomorph (Acrobat), and cymoxanil (Diametan) with distilled water at the appropriate concentrations and adding the mixture to CMA after autoclaving.

Four-mm-diameter agar disks colonized by *P. cactorum* or *P. citrophthora* and taken from the margin of 7-day-old cultures were used in all studies. Fifteen-ml aliquots of medium containing 0, 10, 100, 250, 500, 1000, 1500 and 2000 mg l^{-1} of each fungicide were added to twelve Petri dishes per fungicide concentration, six dishes per species. One colonized agar disk was placed in the center of each plate. Plates were incubated at 24°C and the radial growth of the fungus was measured after four days.

Field experiment

Field experiments were performed at the Pomology Institute Naoussa. KID I peach seedlings were grown at a tissue culture station (Vitro Hellas) in 1997. The plants were planted in experimental fields at the Pomology Institute.

Fungi were recovered using the selective medium developed by Jeffers and Martin (1986).

Soil-drench experiment. Two-year-old plants were inoculated 15 cm above the soil surface. A small disk of bark was removed from the trunk and a 4-mm-diam disk of agar culture of *P. cactorum* or *P. citrophthora* was inserted in the wound. The bark was replaced on the agar disk and the wound covered with cotton and tape. Cotton was moistened before wrapping in order to prevent drying.

One, two and three wk after inoculation, the 180 plants for each fungicide were each drenched with 500 ml suspension (60 plants per fungicide concentration, 3 concentrations: 1 g/tree, 1.5 g/tree, 2 g/tree). As a control seedlings were drenched with only water. Two wk after drenching, disease severity was determined by removing the wrapping material and bark and measuring the length of the

cankers that had developed at the inoculation sites.

Sixty plants were inoculated with each fungicide concentration, twenty plants at each inoculation date. Of these, ten were inoculated with *P. cactorum* and ten with *P. citrophthora*.

Painting stem cankers. Two-year-old KID I peach plants were inoculated by placing a 4-mm-diameter disk of CMA with *P. cactorum* or *P. citrophthora* on the cambium. Ten days after inoculation, the resulting stem cankers were prepared for fungicide application by removing the cuticle, epidermis and outer layer of bark around the stem. The exposed area plus a 2-cm-wide margin of healthy tissue was scraped.

The scraped cankered areas were painted with a high concentration (150 g l^{-1}) of one of metalaxyl, fosetyl-Al, dimethomorph or cymoxanil, covering the cankers and the surrounding area approximately 2–3 cm above and below the canker margins. Plants painted with tap water were used as controls. Twenty plants were treated with each fungicide, ten with *P. cactorum* and ten with *P. citrophthora* cankers.

Statistical analysis

The experiments were carried out using a complete randomized design. Data from field and laboratory experiments were analyzed by one-way analysis of variance and treatment means were separated by Duncan's Multiple Range Test (P=0.05). All experiments were performed twice. Since results from both experiments were similar according to Bartlett's statistical test, the data from them were combined.

Results

Laboratory experiment

Linear growth All four fungicides tested *in vitro* significantly reduced mycelial growth of *P. cactorum* and *P. citrophthora* at all concentrations (Tables 1 and 2). The most effective were metalaxyl and dimethomorph, which gave more than 90% inhibition at the lowest concentration of 10 mg l^{-1}. At all the higher concentrations (100–2000 mg l^{-1}), inhibition was complete. With fosetyl-Al, percent inhibition increased more gradually with increasing concentration, from 20% for *P. cactorum* and 32% for *P. citrophthora* at 10 mg l^{-1} to about 97% inhibition at 1000 mg l^{-1} for both fungi; here complete inhibition was obtained only with concentrations of 1500 and 2000 mg l^{-1}. The effect of cymoxanil was basically similar to that of fosetyl-Al, but it failed to achieve complete inhibition of either fungus even at the highest concentration of 2000 mg l^{-1}.

Field experiment

Soil drench experiment. Metalaxyl applied at 2 g/tree 1 or 3 weeks after inoculation with *P. cactorum* or *P. citrophthora* was most effective in inhibiting cankers (Table 3, 4). The effect was so marked

Table 1. Effect of metalaxyl, fosetyl-Al, dimethomorph and cymoxanil at various concentrations on radial growth of *Phytophthora cactorum in vitro.*

Concentrations (mg/ml)	Radial growth of *P. cactorum* (cm)[a]			
	Metalaxyl[b]	Fosetyl-Al	Dimethomorph	Cymoxanil
0	7.61 a[c]	7.61 a	7.61 a	7.61 a
10	0.63 b	6.11 b	0.34 b	6.20 b
100	0.00 c	5.93 b	0.00 c	5.78 b
250	0.00 c	2.94 c	0.00 c	2.89 c
500	0.00 c	2.14 c	0.00 c	2.14 c
1000	0.00 c	0.20 d	0.00 c	1.79 cd
1500	0.00 c	0.00 d	0.00 c	0.84 de
2000	0.00 c	0.00 d	0.00 c	0.49 e

[a] Each value is the mean of two experiments, each with six replicates.
[b] Chemicals were added to cooled cornmeal agar, and data were taken 4 days after transferring.
[c] Values followed by different letters are significantly different (P=0.05) according to Duncan's multiple range test.

Table 2. Effect of metalaxyl, fosetyl-Al, dimethomorph and cymoxanil at various concentrations on radial growth of *Phytophthora citrophthora in vitro*.

Concentrations (mg/ml)	Radial growth of *P. citrophthora* (cm)[a]			
	Metalaxyl[b]	Fosetyl-Al	Dimethomorph	Cymoxanil
0	8.00 a[c]	8.00 a	8.00 a	8.00 a
10	0.31 b	5.50 b	1.40 b	6.70 b
100	0.00 c	5.20 b	0.00 c	6.00 b
250	0.00 c	1.34 c	0.00 c	4.30 c
500	0.00 c	1.00 c	0.00 c	3.90 c
1000	0.00 c	0.35 d	0.00 c	3.20 cd
1500	0.00 c	0.00 d	0.00 c	2.00 d
2000	0.00 c	0.00 d	0.00 c	2.10 d

[a], [b], [c], See Table 1.

Table 3. Canker length (cm) of 2-year-old KID I peach seedlings after inoculation with *Phytophthora cactorum*. Inoculated plants were soil-drenched with metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil one, two and three weeks after the inoculations.

Fungicide[a]	Rates (g/tree)	Canker length (cm)[b]		
		1	2	3
Control	-	18.20 a[c]	21.50 a	29.40 a
Cymoxanil	1	19.20 a	22.50 a	29.20 a
Metalaxyl	1	19.00 a	21.90 a	29.10 a
Fosetyl-Al	1	18.70 a	22.30 a	30.50 a
Dimethomorph	1	18.00 a	21.80 a	30.10 a
Control	-	18.60 a	22.30 a	29.40 a
Fosetyl-Al	1.5	19.10 a	21.80 a	30.70 a
Cymoxanil	1.5	18.50 a	22.10 a	29.10 a
Metalaxyl	1.5	18.00 a	20.90 a	30.00 a
Dimethomorph	1.5	17.90 a	21.10 a	30.20 a
Control	-	18.60 ab	21.80 a	29.40 a
Dimethomorph	2	20.80 a[z]	22.00 a	28.60 a
Cymoxanil	2	20.00 ab	21.20 a	29.00 a
Fosetyl-Al	2	16.20 b	19.50 ab	24.60 b
Metalaxyl	2	10.40 c	17.60 b	19.60 c

[a] Fungicides were applied as drench of 500 ml per plant.
[b] Values are the means of two experiments, each with ten replicates.
[c] Values followed by different letters are significantly different (P=0.05) according to Duncan's Multiple Range Test.

that plants in this treatment group appeared healthy. The effectiveness of metalaxyl was slightly less when it was applied 2 weeks after *Phytophthora* inoculation. Of the other fungicides, only fosetyl-Al gave some control of necrosis, especially when applied at 2 g l^{-1}, 1 and 3 weeks after pathogen inoculation. All fungicides reduced canker development when applied at 1 and 1.5 g/tree. *P. cactorum* and *P. citrophthora* were isolated from peach tree without fungicide treatment and from two trees treated with cymoxanil and dimethomorph, but not from trees treated with metalaxyl or fosetyl-Al.

Table 4. Canker length (cm) of 2-year-old KID I peach seedlings after inoculation with *Phytophthora citrophthora*. Inoculated plants were soil-drenched with metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil one, two and three weeks after the inoculations.

Fungicide[a]	Rates (g/tree)	Canker length (cm)[b]		
		1	2	3
Dimethomorph	1	17.10 a[c]	19.00 a	24.00 a
Cymoxanil	1	16.80 a	20.10 a	22.90 a
Control	-	16.20 a	19.40 a	23.50 a
Metalaxyl	1	16.00 a	19.60 a	23.10 a
Fosetyl-Al	1	15.90 a	20.00 a	22.70 a
Fosetyl-Al	1.5	17.10 a	19.30 a	22.90 a
Dimethomorph	1.5	16.80 a	19.00 a	24.00 a
Cymoxanil	1.5	16.60 a	18.80 a	24.10 a
Control	1.5	16.20 a	20.00 a	23.50 a
Metalaxyl	1.5	15.90 a	19.10 a	22.60 a
Cymoxanil	2.25	16.20 a	18.80 a	23.00 a
Control	-	16.20 a	19.40 a	23.50 a
Dimethomorph	2.25	15.60 a	19.40 a	20.20 a
Fosetyl-Al	2.25	11.50 b	18.40 a	20.20 b
Metalaxyl	2.25	7.00 c	132.00 b	16.00 c

[a], [b], [c], See Table 3.

Painting stem cankers. Metalaxyl, fosetyl-Al and, to a less extent, dimethomorph painted on scraped active stem cankers reduced futher development of these cankers. Three weeks after application of either metalaxyl or fosetyl-Al to the stem cankers on KID I trees, the cankers had nearly ceased to expand and *P. cactorum* and *P. citrophthora* were not recovered from infected tissues. Painting with dimethomorph significantly reduced development of *P. cactorum* compared with untreated plants, but the reduction did not differ significantly from that caused by cymoxanil, which was least effective (Table 5). *P. cactorum* and *P. citrophthora* were recovered only from two plants painted with cymoxanil and two untreated plants.

Table 5. Canker length of metalaxyl, fosetyl-Al, dimethomorph, and cymoxanil painted on scraped stem cankers of *Phytophthora cactorum* and *P. citrophthora* on KID I peach seedlings.

Fungicide	Rates (g l^{-1})	Canker length (cm)[a]	
		P. cactorum	*P. citrophthora*
Control	-	7.70 a[b]	6.24 a
Cymoxanil	150	5.40 ab	5.75 a
Dimethomorph	150	4.20 bc	3.12 b
Fosetyl-Al	150	2.16 c	1.50 c
Metalaxyl	150	1.40 c	0.34 c

[a] Values are the mean of two experiments, each with ten replicates.
[b] Values followed by different letters are significantly different (P=0.05) according to Duncan's Multiple Range Test.

Discussion

The experiment *in vitro* showed that all four fungicides effectively reduced mycelial growth of one isolate of *P. cactorum* and one of *P. citrophthora* on KID I peach seedlings. Metalaxyl and fosetyl-Al were more effective than cymoxanil and dimethomorph. The experiment *in vivo,* on the other hand, carried out on two-year-old peach trees with stem cankers artificially induced 15 cm from the ground 1–3 wk prior to fungicide application, gave less clear-cut results, with only one fungicide, metalaxyl applied as a soil drench of 2 g per 500 ml per tree, providing effective control of the artificial stem cankers, the fungicides being either completely ineffective or only moderately effective. Metalaxyl-treated trees, had smaller cankers at the inoculation site than the corresponding control trees and these cankers slowly regressed, leaving the tree apparently healthy and free from the pathogen. No fungus was re-isolated from these cankers. The only other fungicide with some inhibitory effect was fosetyl-Al, but it was not as effective as metalaxyl. In the other *in vivo* tests, in which scraped stem cankers were painted with fungicide, metalaxyl and to a somewhat less extent fosetyl-Al again effectively controlled both rot fungi.

The fungicides used in this study have been tested also in quite recent years against fungi that cause many serious diseases. It has been found that metalaxyl as soil drench effectively controls apple collar rot caused by *Phytophthora cactorum* (Ellis *et al.,* 1982), while fosetyl-Al applied to the tree trunks controls *Phytophthora* gummosis of citrus (Matheron and Matejka, 1988). Both these fungicides inhibit sporulation and suppress the development of *Phytophthora* (Ellis *et al.,* 1982; Rana and Gupta, 1984; Matheron and Mircetich, 1985; Coffey and Joseph, 1985; Utkhede, 1987; Matheron and Matejka, 1988; Wicks and Hall, 1988; Matheron and Matejka, 1991; Jeffers, 1992; El-Hamalawi *et al.,* 1995).

Dimethomorph is a relatively new systemic fungicide that seems promising for the control of oomycete disease in the field, especially in areas where phenylalanine-resistant fungal populations prevail (Cohen *et al.,* 1995). Matheron and Porchas (2000) suggested that dimethomorph acted against *Phytophthora* spp. in a way comparable to that of the established fungicides fosetyl-Al and metalaxyl.

Cymoxanil has been used in fungicide mixtures to prevent the buildup of resistance to phenylamide fungicides in *Phytophthora* (Samoucha and Gisi, 1987). Dimethomorph and cymoxanil have not been tested for control of *Phytophthora* crown rot of fruit trees.

Fosetyl-Al applied as a foliar spray has been reported to be effective against *Phytophthora* disease (Wicks and Hall, 1988). Both metalaxyl and fosetyl-Al prevented growth of *P. cactorum* and *P. citrophthora* on peach trees when applied as stem-trunk paint (Thomidis and Elena, 2001). Cymoxanil and dimethomorph when applied as a soil drench failed to control already existing cankers caused by *P. cactorum* and *P. citrophthora* on peach trees and also failed to suppress the growth of *P. cactorum* when applied either as a stem-trunk paint or as a pre-inoculation soil drench (Thomidis and Elena, 2001).

Similarly, El-Hamalawi *et al.,* (1995), reported that fosetyl-Al painted on scraped cankered bark controlled avocado stem canker caused by *P. citricola*. In contrast, cymoxanil did not show any effect against active cankers caused by *P. cactorum* or *P. citrophthora* on peach trees.

On the whole the study confirmed the findings of other that these fungicides are effective against *Phytophthora* spp., and especially that metalaxyl is effective against *P. cactorum* and *P. citrophthora* on peach.

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