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Persistence of the systemic activity of metalaxyl and fosetyl-Al applied as a soil drench or foliar spray to control *Phytophthora* crown rot of peach

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Summary. The persistence of the fungicides metalaxyl and fosetyl-Al, when applied by soil drenching or as foliar sprays against crown rot of peach by *Phytophthora cactorum* or *P. citrophthora* was examined. Metalaxyl and fosetyl-Al applied by soil drenching inhibited both *Phytophthora* species for 40 days, but inhibition was reduced after 60 days. Foliar application with fosetyl-Al inhibited both pathogens for at least 20 days, but metalaxyl was ineffective as a foliar spray. The data suggest that when applied as a soil drench both metalaxyl and fosetyl-Al provide effective control of *Phytophthora* crown rot of peach trees.

Key words: crown rot, fungicides, *Phytophthora*, systemic activity.

Introduction

Crown and root disorders are responsible for considerable annual losses in commercial peach orchards in northern Greece (Thomidis, 2000a, 2000b). *Phytophthora* spp. are common causal agents of crown rot in some of these orchards. A number of species including *P. megasperma, P. citrophthora, P. cambivora, P. cinnamomi*, *P. cryptogea* and *P. syringae* have been associated with *Phytophthora* diseases of peach trees, although *P. cactorum* is the most important (Mircetich and Keil, 1970; Flores and Hindal, 1983; Kim *et al.,* 1985; Wilcox and Ellis, 1989).

As no peach rootstock is resistant to all *Phytophthora* species (Thomidis, 2000a, 2000b), a chemical control strategy is required. The efficacy of the systemic fungicides fosetyl-Al (Aliette, Rhone Poulenc) and metalaxyl (Ridomil, Ciba-Geigy) has been demonstrated (Rana and Gupta, 1984; Matheron and Matejka, 1991). Fungicide application can be by foliar spraying, trunk injection with fosetyl-Al, trunk paint or soil drenching (Matheron and Matejka, 1988; El-Hamalawi *et al.,* 1995).

The objective of this study was to evaluate the persistence of systemic activity of metalaxyl and fosetyl-Al in peach tree after soil drenching or foliar spray application of these agents.

Materials and methods

Fungal isolates and plant material

In all experiments, one isolate of *P. cactorum* and one of *P. citrophthora* was used. These isolates had proved highly pathogenic on peach trees in

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previous works (Thomidis, 2000a, 2000b). Fresh cultures were prepared by transferring agar plugs with mycelium to plates containing fresh cornmeal agar. Plates were then incubated at 23 °C until mycelium covered the agar surface. Cornmeal plates were utilized when needed for inoculum preparation. In all the experiments performed two-year-old PR204 peach trees were used

Laboratory experiments

Excised twig assay.

Inoculum was prepared by placing an agar plug with mycelium from each *Phytophthora* isolate in sterile pyrex jars (9 cm diam., 12 cm high) containing cornmeal agar amended with 10 mg pimaricin (one isolate per jar). Jars were then sealed with Parafilm and incubated at 23 °C until mycelium growth covered the agar surface.

Peach trees were drenched with metalaxyl or fosetyl-Al (2 g/tree; as recommended by manufacturer) 2, 20, 40 and 60 days before inoculation. For inoculation, twigs about 10 mm in diameter and 70 mm long were cut from the central part of 2-yr-old shoots. They were disinfected in 10% domestic chloride (sodium hypochloride, 4.89%) for 3 min and then washed in sterile distilled water three times. Twigs were dried on soft paper and the basal end of each segment was pared by making tangential cuts 10 mm long and 1-2 mm deep on opposite sides with a sterile scalpel to expose the phloem-cambium region.

Ten of these twigs per fungicide were placed vertically in the agar medium of each jar at the periphery of the fungal colony. Jars were sealed with Parafilm to avoid contamination and desiccation and incubated at 23 °C in the dark for four days. Sixteen jars were used for each fungicide, four jars for each treatment; two jars were inoculated with *P. cactorum* and two with *P. citrophthora*. Twigs from untreated trees were used as control.

After incubation, the bark was scraped to expose any necrosis. The results were recorded by measuring the length of the necrosis.

Excised segment assay

Peach trees were drenched with metalaxyl (Ridomil 2E, 2 g/tree) or fosetyl-Al (Aliette WP, 2 g/tree) 2, 20, 40 and 60 days before inoculation. For inoculation, segments about 10 cm long and 2 cm in diameter were collected from each tree. Segments were wounded by removing a 6-mm strip of bark to expose the cambium using a sharp knife and inoculated in the centre by placing a 6-mm-diameter disk of cornmeal agar with *P. cactorum* or *P. citrophthora* in the centre of the cambium. Wounds were covered with adhesive tape to avoid desiccation and incubated for four days at 23 °C in moist chambers, after which the length of the necrosis was measured.

There were 160 segments per fungicide, 40 segments per treatment, half of which were inoculated with *P. cactorum* and the rest with *P. citrophthora*. Twenty segments from untreated trees were used as control.

Glasshouse experiment

Peach trees were grown in 1-l plastic pots in the glasshouse. The mean trunk diameter of the trees used in this experiment was 2–3 cm. Trees were drenched with 500 ml of one of the fungicide solution (Ridomil 2E at 2g/tree; Aliette WP at 2 g/tree; as recommended by manufacturer) 2, 20, 40 and 60 days before inoculation.

Using a flamed sharp knife, a 6 mm strip of bark was removed from the trunk about 10 cm above the soil surface. Inoculations were done by transferring an agar plug with mycelium of a pathogen to the wound. Wounds were then covered with petroleum jelly and sealed with adhesive tape. Results were expressed as the length of necrosis (cm) produced.

There were 80 pots per fungicide, 20 pots per treatment, ten of which were inoculated with *P. cactorum* and ten with *P. citrophthora*. Ten untreated trees were used as control.

Field experiment

Soil drench experiments

Peach trees were drenched with metalaxyl or fosetyl-Al 2, 20, 40 and 60 days before inoculation. Drench solutions were prepared by mixing 2 g fungicide and 500 ml tap water for each tree. Trees were inoculated on the trunk 10 cm above the soil surface by removing a 6-mm strip of bark to expose the cambium. Agar plug, taken from the periphery of a culture, was placed directly on the cambium. Wounds were covered with petroleum jelly and sealed with adhesive tape. There were 48 trees per fungicide, six trees per treatment, half of which were inoculated with *P. cactorum* and half with *P.*

citrophthora. Six untreated trees were used as control. Results were recorded 15 days after inoculation by measuring the length of the necrosis. Recovery of pathogens was on the selective medium of Jeffers and Martin (1986).

Foliar spray experiments

Peach trees were sprayed with metalaxyl (Ridomil 2E at 1g l^{-1}) or fosetyl-Al (Aliette WP at 1 g l^{-1}) 2, 20, 40 and 60 days before inoculation. Inoculations were as in the soil drench experiment, on 48 trees per fungicide, six trees per treatment, half of which were inoculated with *P. cactorum*, and half with *P. citrophthora*. Six untreated trees were used as control. Results were recorded 15 days after inoculation by measuring the length of necrosis. Recovery of fungus was on the selective medium of Jeffers and Martin (1986).

Statistical analysis

A completely randomised experimental design was used throughout the experiments. Data were analysed by one-way analysis of variance (ANOVA). To combine experiments, Bartlett's test of homogeneity of variance was used and treatment means were separated by Duncan's Multiple Range Test (P=0.05). All experiments were repeated twice.

Results and discussion

Results from different experiments were in good agreement. They showed that soil drenches with metalaxyl and fosetyl-Al were effective against *Phytophthora* crown rot of peach trees (Table 1 and 2). Many other researchers have successfully used metalaxyl and fosetyl-Al to control diseases caused by fungi of the genus *Phytophthora* (Ellis *et al.,* 1982; Matheron and Mircetich, 1985; Utkhede, 1987; Matheron and Matejka, 1988; Wicks and Hall, 1988; Jeffers, 1992; Guest *et al.,* 1994). It has also been found that both these fungicides reduce sporulation of *Phytophthora* (Rana and Gupta, 1984; Coffey and Joseph, 1985; Hanson and Shattock, 1998). El-Hamalawi *et al.* (1995) reported that fosetyl-Al applied as a soil drench or foliar spray was effective in controlling stem canker diseases.

In the field and glasshouse experiments of this study, both fosetyl-Al and metalaxyl completely inhibited *P. cactorum* and *P. citrophthora* for at least 40 days when they were applied as a soil drench (Table 1). These fungicides still reduced development of both pathogens significantly when applied 60 days before inoculation. Matheron and Mircetich (1985), found that both metalaxyl and fosetyl-Al were active in the stems of walnut for at

Table 1. Inhibition of canker development on PR204 peach trees inoculated with *Phytophthora cactorum* or *P. citrophthora* 2, 20, 40 and 60 days after soil drenching with two fungicides in the glasshouse and in the field.

Days between fungicide application and inoculation	Length of necrosis (cm)[a]			
	Glasshouse		Field	
	Metalaxyl	Fosetyl-Al	Metalaxyl	Fosetyl-Al
P. cactorum				
Control	8.2 a[b]	8.2 a	6.7 a	6.7 a
60 days	6.8 b	7.1 b	2.9 b	1.2 b
40 days	0 c	0 c	0 c	0 c
20 days	0 c	0 c	0 c	0 c
2 days	0 c	0 c	0 c	0 c
P. citrophthora				
Control	7.6 a	7.6 a	5.3 a	5.4 a
60 days	5.8 b	6.1 b	1.9 b	2 b
40 days	0 c	0 c	0 c	0 c
20 days	0 c	0 c	0 c	0 c
2 days	0 c	0 c	0 c	0 c

[a] Values are the means of two experiments with ten replicates for each treatment.
[b] Numbers in each column with the same letter do not differ (P=0.05) according to Duncan's multiple range test.

Table 2. Inhibition of canker development on twigs and segments of PR204 peach trees inoculated with *Phytophthora cactorum* or *P. citrophthora* 2, 20, 40 and 60 days after soil drenching with two fungicides in the laboratory.

Days between fungicide application and inoculation	Length of necrosis (cm)[a]			
	Twigs		Segments	
	Metalaxyl	Fosetyl-Al	Metalaxyl	Fosetyl-Al
P. cactorum				
Control	2.7 a[b]	2.7 a	3.2 a	3.2 a
60 days	2.7 a	2 a	3.9 a	3.4 a
40 days	2.6 a	2 a	3.1 a	3 a
20 days	0 b	0.1 b	0 b	0 b
2 days	0 b	0.2 b	0 b	0 b
P. citrophthora				
Control	2.4 a	2.4 a	2.9 a	2.9 a
60 days	2.7 a	2.6 a	2.7 a	2.9 a
40 days	2.3 a	2.5 a	2.7 a	3 a
20 days	0.2 b	0.7 b	0 c	0 b
2 days	0.1 b	0.7 b	0 c	0 b

[a] Values are the means of two experiments with twenty replicates for each treatment.
[b] Numbers in each column with the same letter do not differ (P=0.05) according to Duncan's multiple range test.

least 54 days after application. Later, they reported that both fungicides were still active even after 160 days (Matheron and Matejka, 1988). It has been reported that metalaxyl and fosetyl-Al are absorbed through the roots and are then translocated upward by the plant through the xylem (Gupta *et al.,* 1985; El-Hamalawi *et al.,* 1995). Similarly, the results from laboratory experiments showed that both metalaxyl and fosetyl-Al moved basipetally in the tree. The absorption of metalaxyl and fosetyl-Al by peach roots may be essential for these fungicides to be effective under orchard conditions.

In the laboratory experiments, both fungicides lost their fungicidal activity earlier than in the field and glasshouse experiments (Table 2). It is possible that the activity of metalaxyl and fosetyl-Al is reduced as they move in a basipetal direction.

In this study, it was also found that foliar application with fosetyl-Al inhibited both pathogens for at least 20 days (Table 3). Erwin and Ribeiro (1996) reported that the active ingredient of fosetyl-Al (phosphonate) was a unique phloem-mobile xenobiotic with remarkable antifungal activity against some important oomycete plant pathogens. However, its persistence in tissues as a leaf spray

Table 3. Inhibition of canker development on PR204 peach trees inoculated with *Phytophthora cactorum* or *P. citrophthora* 2, 20, 40 and 60 days after foliar spray with two fungicides in the field.

Days between fungicide application and inoculation	Length of necrosis (cm)[a]	
	Metalaxyl	Fosetyl-Al
P. cactorum		
Control	7.7 a[b]	7.7 a
60 days	7.7 a	8 a
40 days	7.9 a	7.6 a
20 days	8 a	0 c
2 days	7.8 a	0 c
P. citrophthora		
Control	6.6 a	6.6 a
60 days	6.8 a	6.4 a
40 days	6.7 a	6.5 a
20 days	6.9 a	0 b
2 days	7 a	0 b

[a] Values are the means of two experiments with six replicates for each treatment.
[b] Numbers in each column with the same letter do not differ (P=0.05) according to Duncan's multiple range test.

was shorter than when applied as a soil drench. Metalaxyl did not affect the development of *P. cactorum* and *P. citrophthora* on PR204 peach trees when it was applied as foliar spray. This is probably because metalaxyl does not have the ability to move downwards.

Generally, soil drenching with metalaxyl or fosetyl-Al is an effective means to protect peach trees against *Phytophthora* if these fungicides are applied in this way a month before peak colonization.

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