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Activity of Metalaxyl-M+mancozeb, Fosetyl-Al, and Phosphorous Acid against Phytophthora Crown and Root Rot of Apricot and Cherry Caused by *Phytophthora palmivora*

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Abstract

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Crown and root rot, caused by the Oomycete pathogen *Phytophthora palmivora*, has become a destructive disease of apricot and cherry in eastern Turkey. There are no currently registered fungicides labeled for its control. In greenhouse experiments conducted in 2012 and 2013, 1-year-old potted apricot rootstock Zerdali and cherry rootstock Mahaleb plants were treated either with foliar spray of fosetyl-Al (140, 160, and 180 g a.i./100 l) or phosphorous acid (187.5, 200, and 215 g a.i./100 l) or soil drench of 100 ml of metalaxyl-M (= mefenoxam)+mancozeb (12+192, 16+256, and 20+320 g a.i./100 l) one day after wound inoculation of crowns and roots. In both years, phosphorous acid at 200 and 215 g a.i./100 l, fosetyl-Al at 160 and 180 g a.i./100 l, and metalaxyl-M+mancozeb at 20+320 g a.i./100 l significantly reduced the root rot severity on Zerdali by 70.68–80.00% and crown rot severity on both Zerdali and Mahaleb, by 68.32–91.96 and 74.21–82.60%, respectively, compared with phosphorous acid at 187.5 g a.i./100 l, fosetyl-Al at 140 g a.i./100 l, metalaxyl-M+mancozeb at 12+192 and 16+256 g a.i./100 l and control. Moreover, fosetyl-Al at 180 g a.i./100 l and metalaxyl-M+mancozeb at 20+320 g a.i./100 l significantly reduced the root rot severity on Mahaleb compared to fosetyl-Al at 140 and 160 g a.i./100 l, metalaxyl-M+mancozeb at 12+192 and 16+256 g a.i./100 l, phosphorous acid treatments and control in 2012, providing the best control of the disease by 88.00–90.68%. Two/three phosphorous acid foliar applications at 200 g a.i./100 l suppressed symptom development when field applications were made on a curative basis in 2014 and 2015.

Keywords: *Prunus armeniaca*; *Prunus avium*; rootstock; agrochemicals; greenhouse; field

The climatic conditions of Turkey are favourable for fruit production. Annual production of stone fruits is approximately 2 045 000 tonnes (http://tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul). Turkey is a leading producer country in the world of both apricots (*Prunus armeniaca* L.) and cherries (*Prunus avium* L.), members of stone fruits, with production of 780 138 and 494 325 t (http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567), respectively. The impact of diseases in both *Prunus* genera can therefore be significant. Since 2012 considerable losses of apricot and cherry trees have been observed in eastern Turkey due to Phytophthora crown and root rot caused by *Phytophthora palmivora* (E.J. Butler) E. J. Butler 1919 [1918] (Peronosporales) (Türkölmez *et al.* 2015a, b). Because of the destructive and widespread crown and root rot disease of apricot and cherry trees in Malatya, Elazığ, and Mardin provinces in eastern Turkey, many

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farmers, dependent on these fruits for subsistence, lost their crops and the diseases became the biggest constraint to their production. The severe crown and root rot caused by *P. palmivora* and high incidence recorded in the infected orchards suggests that this pathogen poses a potential threat to all fruit orchards in this valuable fruit production region of Turkey. Currently, there are no commercially available apricot and cherry cultivars with genetic resistance to crown and root rot. Due to the low efficiency of the available cultural and genetic control measures, application of fungicides is required for economic management of the disease. Without proper management, crown and root rot can cause complete crop loss.

Fosetyl-Al (aluminium *tris-O*-ethyl phosphonate) and metalaxyl-M+mancozeb have been registered to control many diseases caused by *Phytophthora* spp. on agricultural and horticultural crops. Within the last 40 years, chemical control of oomycetes has undergone dramatic changes with the detection and introduction of single-site fungicides such as phenylamides and fosetyl-Al (Gisi & Sierotzki 2008). Phenylamides (FRAC Group 4; Mode of Action Sub-Group A1; http://www.frac.info/frac/index.htm) such as mefenoxam (metalaxyl-M) are systemic fungicides specifically used to control diseases caused by oomycetes including damping off and root and lower stem rots caused *Pythium* and *Phytophthora*. They inhibit ribosomal RNA synthesis, specifically polymerization of r-RNA (Davidse 1995). Metalaxyl-M is one of the two enantiomers of metalaxyl. The marketing of pre-mixes of metalaxyl-M with non-related preventive fungicides ensures compliance with a resistance management strategy. For instance, the manufacturer of metalaxyl-M markets premixes with mancozeb (a dithiocarbamate, multi-site fungicide), and sells it under the trade name Ridomil Gold. Within the chemical class of phosphonates, fosetyl-Al, an aluminium salt of the diethyl ester of phosphorous acid, was registered by the EPA in 1983 (EPA 1991), and is sold under the trade name Aliette®. It is a systemic fungicide often used to treat plants infected by oomycetes causing root rots because it is transferred to the roots (Cohen & Coffey 1986). Inside the plant, fosetyl-Al ionizes into phosphonate, and therefore fosetyl-Al belongs to the group of phosphorous acid compounds (Cohen & Coffey 1986; McGrath 2004). Phosphorous acid, the conjugate acid of the phosphite anion, and an anionic metabolite or a breakdown product of the systemic fungicide fosetyl-Al, is also translocated from the shoots to the roots. It is composed of mono- and dibasic sodium, potassium, and ammonium salts and has both direct and indirect effects on oomycetes (McGrath 2004). It directly inhibits a particular process (oxidative phosphorylation) in the metabolism of oomycetes (McGrath 2004). An indirect effect is the stimulation of the plant's natural defence response against the pathogen attack (Smillie *et al*. 1989). Resistant isolates in field populations have never been detected for either fosetyl-Al or phosphorous acid.

Currently, there is no registered product to control Phytophthora crown and root rot of apricot and cherry caused by *P. palmivora* in Turkey. Fosetyl-Al and phosphorous acid (https://bkubeta.tarim.gov.tr/AktifMadde/Details/897) are registered for use against a root rot disease, Phytophthora root rot of citrus caused by *P. citrophthora*. However, their efficiency has not been tested against Phytophthora crown and root rot of apricot and cherry caused by *P. palmivora* yet.

This paper reports the results of two greenhouse experiments conducted in 2012 and 2013 which evaluated the efficacies of three concentrations of fosetyl-Al, metalaxyl-M+mancozeb, and phosphorous acid on Phytophthora crown and root rot on apricot and cherry rootstocks. These chemicals were chosen based on their potential for the control of oomycetes and availability in Turkey. Field experiments further assessed the efficacy of phosphorous acid against Phytophthora crown and root rot on apricot cvs Kabaaşı and Hacıhaliloğlu and sweet cherry cv. Ziraat 0900, commonly grown cultivars in eastern Turkey.

MATERIAL AND METHODS

Greenhouse experiments

Plants. One-year-old seedlings of apricot (*Prunus armeniaca* L.) Zerdali and cherry (*Prunus mahaleb* L.) Mahaleb rootstocks grown in 5-l pots were obtained from the nurseries of Apricot Research Station (Yeşilyurt, Malatya, Turkey) and Horticulture Department at Dicle University (Sur, Diyarbakır, Turkey), respectively. Inoculations were carried out at the Diyarbakır Plant Protection Research Station (Yenişehir, Diyarbakır, Turkey). After inoculations, all plants were transferred to and incubated for 3 months in a greenhouse where air temperatures ranged from 25°C to 32°C during the day, above 20°C at night in July–September. Fungicide spray or drench applications were done one day after inoculations.

Phytophthora palmivora isolates, inoculum production and inoculation. *P. palmivora* isolates

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obtained from the roots of *P. armeniaca* (Ph1 isolate, GenBank Accession No. KF723832) (Türkölmez *et al*. 2015a) and *P. avium* (Ph3 isolate, GenBank Accession No. KF831195) (Türkölmez *et al*. 2015b) were selected for root inoculations of Zerdali and Mahaleb plants, respectively. Both isolates previously produced the most severe symptoms on their own hosts (Türkölmez *et al*. 2015a, b). Primary roots and crowns (on the soil line) were underbark inoculated (Türkölmez *et al*. 2015a) using 5-mm-diameter grated apple corn meal agar (GACMA) (Türkölmez *et al*. 2015a) plugs colonised with *P. palmivora* (2 plugs for the crown, 8 for roots, 10 per plant). Small incisions were made with a sterile scalpel in an upward direction through the periderm to the phloem of crowns and roots. The incisions were then sealed with Parafilm to prevent inoculum desiccation. Incisions in the primary roots were not wrapped with Parafilm. The negative control plants were inoculated with sterile GACMA plugs. Root parts were then covered with soil and the soil was kept saturated by watering plants daily for the duration of the experiments.

Fungicide application. One day post-inoculation, plants were sprayed using a low-volume hand-held sprayer or soil drenched in greenhouse experiments. The chemical treatments included Ridomil Gold MZ 68 WP (active ingredients 4% metalaxyl-M, 64% mancozeb, water dispersible granule formulation, Syngenta) applied at 12+192, 16+256, and 20+320 g a.i./100 l, Aliette 800 WG (a.i. 80% fosetyl-Al, Bayer) applied at 140, 160, and 180 g a.i./100 l, Kingon fosforoz asid (the trade name was previously Masterfos, a.i. 50% phosphorous acid, soluble concentrate formulation; Akin Agro Gübre, Turkey) applied at 187.5, 200, and 215 g a.i./100 l of water. Fosetyl-Al and phosphorous acid were applied as foliar sprays (by foliar spraying each seedling with 100 ml of the suspension), and metalaxyl-M+mancozeb were applied by soil drenching (by pouring 100 ml of the fungicide suspension into the soil of each pot). Single applications of these treatments were applied to the respective pots on June 26, 2012 and June 27, 2013. Negative and positive control plants were only sprayed with foliar spray or soil drench treatments with water were used.

Experimental design. Fungicide efficacy trials were conducted in 2012 and 2013. The experimental design was a completely randomized block design with 9 treatments in which three concentrations of three products were applied per block and 5 replications (3 plants, each grown in separate pots, per replication). Each treatment included 5 inoculated (positive) control plants; therefore each replication consisted of 9 positive control plants. Nine individuals received blank inoculations and treatments (negative control) for each host species. In total, 135 inoculated plants applied fungicides + 45 positive control plants (inoculated but not treated with fungicides) + 9 non-inoculated negative control plants = 189 plants for each host species (plants of Zerdali and Mahaleb used as rootstocks for *Prunus armeniaca* L. and *Prunus avium* L., respectively) and 378 plants for either year of the experiment were used.

Disease assessments. The roots were washed with tap water and symptoms were rated according to an empirical scale of 0–5 (Türkölmez *et al*. 2015a) on the basis of root rot severity three months after inoculation. Crown rot severity was rated according to the measured lesion length above and below the inoculation point three months after inoculation. The visible crown lesion lengths were measured and recorded for every single plant. The presence of the pathogen at or near the border of measured crown lesions and in the root lesions incited by *P. palmivora* was confirmed by plating crown sections onto carrot corn meal agar (GCCMA) (Türkölmez *et al*. 2015a) amended with P_5ARP (5 mg of pimaricin, 250 mg of ampicillin, 10 mg of rifampicin, 100 mg of pentachloronitrobenzene, and 50 mg of hymexazol in 1 l of water) selective for *Phytophthora* and incubated in darkness at 27°C for 7–12 days. The morphological characteristics of sporangia (shape, papillation, and caducity) and chlamydospores (incidence and shape) were examined. Colonies consistent with the morphological characteristics of *P. palmivora* (Erwin & Ribeiro 1996) were scored positive. Disease (root rot) severity index (DSI%) was calculated using the Townsend-Heuberger formula: $DSI = \Sigma(n_v)/V_{max} N \times 100$, where: v – degree of root infection according to the scale of 0–5 infection classes; *n* – number of plants (roots) in each class; V_{max} – highest infection class; *N* – total number of plants screened. The efficacy of treatments on root rot was calculated based on the disease severity index (DSI) using Abbott's formula.

$$\text{Efficacy (\%)} = \{(DSI_{\text{positive control}} - DSI_{\text{treatment}})/DSI_{\text{positive control}}\} \times 100$$

The efficacy of treatments on crown rot was calculated based on the crown rot severity (the visible crown lesion length) using Abbott's formula.

$$\text{Efficacy (\%)} = \{(CRS_{\text{positive control}} - CRS_{\text{treatment}})/CRS_{\text{positive control}}\} \times 100$$

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Field experiments

The efficacy of phosphorous acid was further assessed in field experiments in commercial orchards at six locations in 2014 and 2015. Experimental sites in field experiments were chosen from orchards with high disease incidence where *P. palmivora* was isolated (Türkölmez *et al.* 2015a, b). Phosphorous acid (Kingon fosforoz asid) at 200 g a.i./100 l (at the concentration recommended by the manufacturer) was applied in six commercial orchards (three apricot and three sweet cherry orchards) at the following locations and on the following application dates in both years: (*i*) 15-year-old apricot orchard, cvs Hacıhaliloğlu and Kabaaşı grafted on Zerdali rootstock at Kale in Malatya Province; phosphorous acid was applied on May 7 and 28, and June 18; (*ii*) 11-year-old apricot orchard, cv. Kabaaşı grafted on Zerdali rootstock at Doğanşehir in Malatya Province; phosphorous acid was applied on May, 7 and 29; (*iii*) 8-year-old apricot orchard, cv. Kabaaşı grafted on Zerdali rootstock at Baskil in Elazığ Province; phosphorous acid was applied on May 10, June 8 and 29; (*iv*) 12-year-old sweet cherry orchard, cv. Ziraat 0900 grafted on Mahaleb rootstock at Mazıdağı in Mardin Province; phosphorous acid was applied on May 3 and 23; (*v*) 8-year-old sweet cherry orchard, cv. Ziraat 0900 grafted on Mahaleb rootstock at Yeşilli in Mardin Province; phosphorous acid was applied on May 3 and 23, and June 13; (*vi*) 8-year-old sweet cherry orchard, cv. Ziraat 0900 grafted on Mahaleb rootstock at Harput in Elazığ Province; phosphorous acid was applied on May 12, June 2, and July 2. All six orchards were drip irrigated. In all experiments phosphorous acid was applied to individual trees using a garden sprayer with a sprayer gun, a 15 m hose, and a 100-l capacity tank with 80 l/min membrane pump (Lusna, Turkey) powered by a tractor as foliar sprays. Spray volumes varied according to the size and canopy density of the tree, but all trees were sprayed to runoff (~3–5 l/tree). Untreated control plots were included for comparison in all field experiments. Treated and untreated plots included 250–900 and 20–100 trees, respectively, with regard to the number of symptomatic and asymptomatic trees in each orchard. The trials were inspected at the beginning (at 1st application date) and at the end of the experiments (in August) to monitor the trees exhibiting foliar symptoms of root and crown rot. The number of symptomatic trees and healthy trees with vigorous foliage was recorded both for phosphorous acid treated and untreated control plots in all six orchards. Disease incidence (%) was calculated using the proportion of apricot/sweet cherry trees showing any one of the foliar symptoms of Phytophthora crown and root rot (poor growth with sparse off-colour foliage, wilt, yellowing, crown dieback or collapse) relative to the total number of apricot/sweet cherry trees in each orchard/plot. The efficacy of the phosphorous acid treatment was calculated based on the disease incidence (DI) (in %) using the following formula:

$$\text{Efficacy (\%)} = \left\{\left(DI_{\text{at 1st application date}} - DI_{\text{at final evaluation date}}\right) / DI_{\text{at 1st application date}}\right\} \times 100$$

Statistical analysis

The independent variables for greenhouse experiments were three fosetyl-Al (140, 160, and 180 g a.i./100 l), three metalaxyl-M+mancozeb (12+192, 16+256, and 20+320 g a.i./100 l) and three phosphorous acid (187.5, 200, and 215 g a.i./100 l) concentrations; the dependent variables were visible root rot and crown rot severity indices at the end of experiments. To evaluate differences between the treatments more clearly, we did not include any disease severity indices of negative controls in statistical analyses. All statistical analyses were conducted using SPSS v21. The data on root rot and crown rot severity on Zerdali apricot rootstock and Mahaleb cherry rootstock were analysed using a one-way ANOVA and the means were separated by Duncan's multiple range test ($P < 0.05$).

The data on the disease incidence for initial and final foliar evaluation dates in the field experiments and efficacy of phosphorous acid applications on Phytophthora crown and root rot incidence were expressed as percentages. Incidence data was also analysed using a one-way ANOVA and the means of treatments and control plots and the means of six orchards were compared for significance using Duncan's multiple range test ($P < 0.05$).

RESULTS

Greenhouse experiments. During the experiments conducted both in 2012 and 2013, symptoms of *Phytophthora* root rot were apparent 20 days after inoculation in positive control seedlings and the disease continued to develop as the incubation period progressed.

During the first experiments conducted in 2012, results from the assessments of disease severity revealed that fosetyl-Al (at 160 and 180 g a.i./100 l),

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metalaxyl-M+mancozeb (at 20+320 g a.i./100 l), and phosphorous acid (at 200 and 215 g a.i./100 l) significantly ($P < 0.05$) reduced the severity of damage to the roots on Zerdali rootstock with at least 70% control efficacy of root rot compared to the other treatments and the untreated control (Table 1). The assessment conducted on Mahaleb showed that fosetyl-Al at 180 g a.i./100 l and metalaxyl-M+mancozeb at 20+320 g a.i./100 l significantly ($P < 0.05$) reduced the disease severity decreasing the root rot by 90.68 and 88.00%, respectively, compared to the other treatments and the untreated control during the 2012 experiments. The disease severity in plants treated with fosetyl-Al at 160 g a.i./100 l was not significantly different ($P < 0.05$) from that in plants treated with phosphorous acid at 200 and 215 g a.i./100 l; and these treatments yielded more reduction in disease severity (70.68–90.68%) than the other treatments (Table 1).

Results from the second greenhouse experiment conducted in 2013 showed that the roots of Zerdali treated with fosetyl-Al (at 160 and 180 g a.i./100 l), metalaxyl-M+mancozeb (at 20+320 g a.i./100 l), and phosphorous acid (at 200 and 215 g a.i./100 l) had significantly ($P < 0.05$) less root rot by providing 77.28–80.00% root rot control than the other treatments like in the previous experiment. Fosetyl-Al at 140 g a.i./100 l provided more reduction ($P < 0.05$) in root rot severity than phosphorous acid at 187.5 g a.i./100 l and metalaxyl-M+mancozeb at 16+256 and 12+192 g a.i./100 l (Table 1). No differences were observed between the metalaxyl-M+mancozeb (at 20+320 g a.i./100 l), fosetyl-Al (at 160 and 180 g a.i./100 l), and phosphorous acid (at 200 and 215 g a.i./100 l) treatments on cv. Mahaleb and all these treatments significantly reduced the percentage of the infected root area, providing 74.60–82.60% control of the root rot compared to other treatments (Table 1). All these treatments significantly ($P < 0.05$) reduced the root rot severity on both Zerdali and Mahaleb compared to the positive control without treatments in both years.

Efficiency when averaged over two experimental years under greenhouse conditions, higher rates of fosetyl-Al applied as a foliar spray at 160 and 180 g a.i./100 l, metalaxyl-M+mancozeb applied at a rate of 20+320 g a.i./100 l as a soil drench, and phosphorous acid applied as a foliar spray at 200 and 215 g a.i./100 l provided 74.68–77.34 and 72.68–84.68% control of the root rot on cvs Zerdali and Mahaleb, respectively, whereas lower rates of fosetyl-Al at 140 g a.i./100 l, metalaxyl-M+mancozeb at 16+256 g a.i./100 l, and phosphorous acid at 187.5 g a.i./100 l resulted in 47.34–61.98% and 48.68–60.00% root rot control efficacy on Zerdali and Mahaleb, respectively (Table 1).

The most effective compounds ($P < 0.05$) for the inhibition of canker lesion development at the crown were fosetyl-Al (at 160 and 180 g a.i./100 l), metalaxyl-M+mancozeb (at 20+320 g a.i./100 l), and phosphorous acid (at 200 and 215 g a.i./100 l), resulting in average measurements of less than or equal to 2.0 cm (0.5–2.0 cm) crown lesions on both cvs Zerdali and Mahaleb (Table 1). Efficiencies of these rates of fungicides when averaged over two experimental years were 75.98–88.45 and 75.26–83.43% on Zerdali and Mahaleb, respectively. Lesion development was effectively restricted to the inoculation point (0.5 cm) on most plants when the highest rates of fungicides were applied. The inhibition of lesion development on the plants treated with lower rates of fungicides was lower (2.7–3.7 cm) than that of the above-mentioned concentrations. No lesions developed when the negative control plants were inoculated with sterile GACMA plugs. However, the crown lesion length increased on inoculated but untreated positive control plants with 6.08 and 6.80 cm average of both years on cvs Zerdali and Mahaleb, respectively. Stem girdling was observed only in several control plants. In the majority of the positive control plants, lesion extension below the inoculation point reached near the stem base. All treatments also reduced ($P < 0.05$) the crown rot severity on both cvs Zerdali and Mahaleb compared to the positive control without treatments in both years. Crown and root infections were confirmed by the pathogen re-isolation from the roots and by the measured areas of crown lesions on inoculated plants. *P. palmivora* was re-isolated from 94.5% of positive control seedlings exhibiting disease symptoms, confirming this as the causal agent.

Field experiments. In the field experiments, the disease incidence was significantly lower ($P < 0.05$) in the phosphorous acid-treated plots than in the control plots without phosphorous acid in all apricot and cherry orchards (trials) (Table 2). Since significant differences were found in the results between treated and control plots for disease incidence in all orchards, incidence data from treated and control plots from different orchards were also analysed separately and the statistics of incidence data among orchards are also presented in Table 2. In all Trials, the Phytophthora crown and root rot incidence by mid-May 2014 was 80, 100, 100, 50, 100, and 100% in untreated plots and 89.23, 97.67, 90.00, 80.00, 92.00, and 76.67% in plots

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Table 1. Efficacy (%) of different application rates of various fungicides (metalaxyl-M+mancozeb, fosetyl-Al, and phosphorous acid) application on the Phytophthora root and crown rot severity on Zerdali apricot rootstock and Mahaleb cherry rootstock, 3 months after wound-inoculation with *P. palmivora* isolates in a screenhouse experiment established in June 2012 and 2013

Treatment	Application rate (g a.i. 100/l)	Root rot severity[1,3] (RRS) (0–5) and efficacy[4,5] (E) (%) of different rates of the fungicides					Crown rot severity[2,3] (CRS) (cm) and efficacy[4,5] (E) (%) of different rates of the fungicides				
		2012		2013		mean	2012		2013		mean
		RRS	E	RRS	E	E	CRS	E	CRS	E	E
Apricot rootstock Zerdali											
Fosetyl-Al	140	1.87[bc 1,3]	62.64[4]	1.93[b 1,3]	61.32[4]	61.98[5]	2.71[b 2,3]	50.09[4]	3.52[c 2,3]	47.62[4]	48.86[5]
	160	1.47[ab]	70.68	1.07[a]	78.68	74.68	1.72[ab]	68.32	1.10[ab]	83.63	75.98
	180	1.27[a]	74.68	1.00[a]	80.00	77.34	1.24[a]	77.16	1.05[ab]	84.38	80.77
Metalaxyl-M+ mancozeb	12+192	3.33[d]	33.36	3.33[d]	33.36	33.36	2.83[b]	47.88	3.73[c]	44.49	46.19
	16+256	2.86[d]	42.72	2.40[c]	51.96	47.34	2.74[b]	49.54	1.44[b]	78.57	64.06
	20+320	1.27[a]	74.64	1.13[a]	77.28	75.96	0.93[a]	82.87	0.54[a]	91.96	87.42
Phosphorous acid	187.5	2.27[c]	54.68	2.47[c]	50.64	52.66	2.78[b]	48.80	3.54[c]	47.32	48.06
	200	1.33[a]	73.32	1.13[a]	77.36	75.34	1.06[a]	80.48	0.93[ab]	86.16	83.32
	215	1.27[a]	74.68	1.07[a]	78.68	76.68	0.81[a]	85.08	0.55[a]	91.82	88.45
Positive control	–	5.00[e]	–	5.00[e]	–	–	5.43[c]	–	6.72[d]	–	–
Negative control	–	0.00	–	0.00	–	–	0.50	–	0.50	–	–
Cherry rootstock Mahaleb											
Fosetyl-Al	140	2.07[c 1,3]	58.64[4]	1.93[b 1,3]	61.36[4]	60.00[5]	3.54[b 2,3]	3.61[4]	3.61[b 2,3]	50.34[4]	47.17[5]
	160	1.47[b]	70.68	1.07[a]	78.68	74.68	1.63[a]	1.52	1.52[a]	79.09	76.65
	180	0.47[a]	90.68	1.07[a]	78.68	84.68	1.44[a]	1.15	1.15[a]	84.18	80.70
Metalaxyl-M+ mancozeb	12+192	3.33[e]	33.36	3.40[c]	32.00	32.68	3.68[b]	3.72	3.72[b]	48.83	45.30
	16+256	2.80[d]	44.04	2.20[b]	55.96	50.00	3.43[b]	3.47	3.47[b]	52.27	49.00
	20+320	0.60[a]	88.00	1.20[a]	76.00	82.00	1.30[a]	1.13	1.13[a]	84.46	81.94
Phosphorous acid	187.5	3.00[de]	40.04	2.13[b]	57.32	48.68	3.02[b]	3.42	3.42[b]	52.96	52.59
	200	1.47[b]	70.68	1.27[a]	74.68	72.68	1.98[a]	1.32	1.32[a]	81.84	75.26
	215	1.13[b]	77.32	0.87[a]	82.64	79.98	1.33[a]	0.88	0.88[a]	87.90	83.43
Positive control	–	5.00[f]	–	5.00[d]	–	–	6.32[c]	7.27	7.27[c]	–	–
Negative control	–	0.00	–	0.00	–	–	0.50	0.50	0.50	–	–

[1]treatment means are a scaled index of root rot severity where 0 = zero necrotic root (no disease); 1 = damage (lesions or rots) on a few roots (up to 20% of roots), 2 = 21–40% of root area affected (loss of some lateral roots), 3 = 41–60% of root area affected (loss of most lateral roots), 4 = 61–80% of root area affected (loss of most lateral roots and some cortex loss on taproots), 5 = damage on 81–100% of roots (loss or death of all lateral roots and loss of cortex tissue on taproots); [2]crown rot severity was estimated by measuring the crown lesion length (cm); [3]means within a column not followed by the same letter are significantly different ($P < 0.05$) by Duncan's multiple range test; [4]efficacy of treatments on root rot was calculated based on the disease severity index (DSI) using Abbott's formula; [5]treatment efficacies, when averaged over two experimental years

that had been treated with phosphorous acid (200 g a.i./100 l) at that time (Table 2). The disease incidence was lowest ($P < 0.05$) in orchards that received three 20/21-day interval applications of phosphorous acid in Trials 1, 3, 5, and 6 at final evaluation dates in August. Three applications of phosphorous acid almost completely suppressed the disease (0.0% incidence in three orchards and 0.56% incidence in one orchard)

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Table 2. The percentage of apricot and cherry trees exhibiting Phytophthora root and crown rot foliar symptoms in six orchards, and mean incidence and efficacy after two/three foliar sprays (20/21-day interval) with phosphorous acid with indication of locations, application dates, tree age etc.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Location/Province	Kale/ Malatya	Doğanşehir/ Malatya	Baskil/ Elazığ	Mazıdağı/ Mardin	Yeşilli/ Mardin	Harput/ Elazığ
Host species		*Prunus armeniaca*			*Prunus avium*	
Cultivar	Hacıhaliloğlu and Kabaaşı	Kabaaşı	Kabaaşı		Ziraat 0900	
Rootstock		Zerdali			Mahaleb	
Tree age (year)	15	11	8	12	8	8
No of plants[1]/treatment	650	430	300	250	250	900
No of plants/control	50	20	30	50	30	100
1st application date	07 May	07 May	10 May	03 May	03 May	12 May
2nd application date	28 May	29 May	8 June	23 May	23 May	2 June
3rd application date	18 June	–	29 June	-	13 June	2 July
Disease incidence at 1st application (%)						
date[1,3]/treatment	89.23[b]	97.67[a]	90.00[b]	80.00[c]	92.00[b]	76.67[c]
date[1,3]/control	80[b]	100[a]	100[a]	50[c]	100[a]	100[a]
Evaluation date	08 Aug	08 Aug	14 Aug	03 Aug	03 Aug	13 Aug
Final disease incidence[1,3,4]/treatment (%)	0.00[aA]	4.65[bA]	0.00[aA]	10.0[cA]	0.00[aA]	0.56[aA]
Final disease incidence[1,3,4]/control (%)	100[aB]	100[aB]	100[aB]	100[aB]	100[aB]	100[aB]
Average efficacy[2,3] (%)	100[a]	95.24[ab]	100[a]	87.50[b]	100[a]	99.28[a]

[1]mean disease incidence (%) was calculated as the number of trees showing foliar symptoms of Phytophthora root and crown rot relative to the number of trees per treatment; [2]efficacy of the treatment was calculated based on the disease incidence (%) (DI) using the following formula: Efficacy (%) = $\{(DI_{at\ 1^{st}\ application\ date} - DI_{at\ final\ evaluation\ date})/DI_{at\ 1^{st}\ application\ date}\} \times 100$; [3]means within a row not followed by the same lower case letter are significantly different ($P < 0.05$) by Duncan's multiple range test; [4]means within a column not followed by the same capital letter are significantly different ($P < 0.05$) by Duncan's multiple range test

when applied at a 20/21-day interval from early May to early August (Table 2). Two applications of phosphorous acid when applied at a 20/21-day interval from early May to late May also resulted in lower disease incidence by 4.65 and 10.00% in Trials 2 and 4, respectively, compared to untreated controls (Table 2). However, a significant decrease in disease incidence ($P < 0.05$) was monitored in the plots that received three applications of phosphorous acid as compared to the plots that received two applications of phosphorous acid (Table 2). By mid-August 2014, foliar symptoms of crown and root rot incidence were 100% in all untreated control plots and tree mortality was highly prevalent in these control plots. For instance, the disease incidence in untreated plot 4 in Mazıdağı/Mardin increased from 50% to 100% between May 3 and August 3, 2014 (Table 2). Phosphorous acid treatment (applied at 200 g a.i./100 l) used alone (when applied two or three times) provided similar control ($P < 0.05$) of the disease (95.24–100%) on apricot and cherry in all experimental sites, whereas two applications of phosphorous acid to cherry in Mazıdağı/Mardin (Trial 4) resulted in lower ($P < 0.05$) efficacy (87.5%) compared with those in other sites (Table 2). In August 2015, all apricot and cherry trees in untreated plots were totally killed by this oomycete. With only a few exceptions, no diseased tree was found in any phosphorous acid treated plots in all sites in August 2015; and thus phosphorous acid provided nearly 100% control in 2 years. Since these results of 2015 applications were so steady or homogeneous, the results of 2015 trials were not presented in Table 2.

DISCUSSION

Phytophthora palmivora causes a devastating disease on many plant species in Turkey (Dervis *et al.*

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2011; Türkölmez *et al.* 2015a, b, c, 2016a, b; Çiftçi *et al.* 2016). The pathogen already caused severe epidemics of apricot and cherry tree decline and death in the Eastern Anatolia Region (Türkölmez *et al* 2015a, b). *P. palmivora* also poses one of the greatest risks on other potential hosts and should not be ignored in other regions of Turkey. Since the pathogen was newly reported on apricot and cherry worldwide, effective chemicals against the disease are not known yet. Since there are few reports in the scientific literature devoted to the Phytophthora root and crown rot control in *Prunus* spp., effective fungicides against *Phytophthora* spp. causing crown and root rot diseases on different plant species (Fenn & Coffey 1984; Matheron & Mircetich 1985; Bielenin & Jones 1988; Utkhede & Smith 1991, 1995; Ferrin & Rohde 1992; Wong & Wong 1996; Thomidis & Elena 2001) were chosen based on their potential to control oomycetes. The effectiveness of metalaxyl-based or mix fungicides in suppressing oomycete pathogens has been demonstrated in many studies (Erwin & Ribeiro 1996; Cooke & Little 2002; Johnson *et al.* 2004; Mayton *et al.* 2008). However, due to the high cost of metalaxyl-based or mix fungicides and resistance of oomycete pathogens to metalaxyl, their use became uneconomic in many situations and phosphorous acid has been registered in the last decade and is currently the most common foliar fungicide for the management of oomycete diseases (Gómez-Merino & Trejo-Téllez 2015). Phosphorous acid was effective when applied as a root drench against *P. cinnamomi*, *P. nicotianae*, *P. palmivora*, and *P. capsici* in lupin, tobacco, papaya, and pepper, respectively (Smillie *et al.* 1989; Forster *et al.* 1998). Trunk injections of phosphite controlled the severe root rot (*P. cinnamomi*) of avocado trees, trunk rot (*P. cactorum*) of peach trees (De Boer *et al.* 1990). Foliar sprays of phosphite controlled root rot (*P. nicotianae* var. *parasitica*) and trunk canker (*P. citrophthora*) of mandarin trees, and root and heart rot (*P. cinnamomi*) of pineapples, and reduced the severity of root rot (*P. clandestina*) of subterranean clover and root rot (*P. nicotianae* var. *nicotianae*) of tomatoes (De Boer *et al.* 1990). It is also effective against Phytophthora root and crown rot on tomato and green pepper in a hydroponic culture (Forster *et al.* 1998). Phosphorous acid-based products have also been successfully used in forest settings to control Phytophthora root rot caused by *P. cactorum* in field-grown *Pinus radiata* nurseries (Reglinski *et al.* 2009), sudden oak death epidemics caused by *Phytophthora ramorum* (Kanaskie *et al.* 2011), and *P. cinnamomi* root rot in mature *Banksia* spp. and *Eucalyptus marginata* trees (Shearer & Fairman 2007) and in glasshouse-grown radiata pine (Ali *et al.* 2000).

In the present work the effects of two downwardly mobile systemic chemicals, fosetyl-Al (foliar spray) and phosphorous acid (foliar spray) and a combination of metalaxyl-M+mancozeb products (soil drench), on the severity of crown and root rots of apricot Zerdali and cherry Mahaleb were investigated. They were compared and evaluated by rating the root rot and measuring the crown lesion length at the conclusion of the experiment. Phosphorous acid, found to be equally effective as fosetyl-Al and metalaxyl-M+mancozeb in controlling the disease in greenhouse experiments, was selected to determine its efficacy in preventing the decline and mortality of apricot and cherry trees in the field. In seedling assays, all fungicides and doses were effective in reducing disease infections when applied 24 h after inoculation. However, the disease protection increased with increasing concentrations of metalaxyl-M+mancozeb, fosetyl-Al, and phosphorous acid in both apricot and cherry rootstocks. A field study was carried out in Malatya, Elazığ, and Mardin provinces. In these provinces, approximately nine thousand tons of cherry fruits are produced from 355 thousand sweet cherry and 135 thousand sour cherry trees (http://tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul). With 407 thousand tons of apricot production, Malatya and Elazığ provinces are the centre of the apricot industry in the world (http://tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul). These two provinces supply 90% of dried and 62% of fresh apricot production of Turkey. Results obtained in the field showed that a dose of 200 g a.i./100 l of phosphorous acid applied twice or three times a year was efficient in controlling the disease even in orchards seriously affected by the pathogen. In all sites of field experiments, the disease incidence on trees increased in plots with trees treated with no phosphorus acid. Scattered or clustered mortality occurred in these plots. Most of the plants treated with phosphorous acid, however, gained the totally healthy appearance three months after the commencement of treatments; and significant reductions in the cumulative incidence of the disease symptoms without any tree mortality were seen in plots treated with phosphorous acid. Field experiments by site, host species or cultivar, and tree age showed no correlation between the susceptibility

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level and efficacy of phosphorous acid. Overall, the Phytophthora root and crown rot control by phosphorous acid appeared relatively stable in field experiments across locations. Because phosphorous acid provided nearly 100% control in the duration of the study, it is probable that phosphorous acid would provide protection for longer periods. Thus, phosphorous acid applications would not only protect plants that were not infected yet but also completely suppress symptom development when field applications were made on a curative basis and contribute to the formation of asymptomatic plants. Interestingly, lower efficacy (87.5%) was found only in Mazıdağı/Mardin (Trial 4). In this orchard, cherry trees were grown on poorly drained soils with clay loam. Heavy, wet soils that remain saturated for extended periods which are required conditions for the disease development might have led to stimulate the development of *P. palmivora* and resulted in a severe disease. Diverse factors affecting the relationship between the disease and the epidemiological factors such as high soil inoculum potential, irrigation and fertilization regimes, tillage, geographical provenance, and climatic data might also have contributed to the lower efficacy in this orchard. Indeed, it would be inadvisable to rely solely on phosphorous acid for the control of this pathogen and integrated strategies combining the chemical control with biological and cultural control options should be implemented.

These investigations demonstrate the potential benefits of phosphorous acid as a management tool for the control of Phytophthora crown and root rot of apricot and cherry. Similar results of post-infection applications have been observed in other numerous pathosystems. Influences of apricot and cherry cultivars/rootstocks, geographical location, application rate, and timing of applications of phosphorous acid on the control of Phytophthora root and crown rot warrant further investigations.

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