Effects of Fosetyl-Al, Metalaxyl, and *Enterobacter aerogenes* on Crown and Root Rot of Apple Trees Caused by *Phytophthora cactorum* in British Columbia

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ABSTRACT

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Fosetyl-Al applied as a foliar spray and a drench, metalaxyl as a granular broadcast and drench, and *Enterobacter aerogenes* as a drench were evaluated for 3 yr at three sites for control of crown and root rot, caused by *Phytophthora cactorum*, in naturally infected apple trees. All chemical and biological treatments reduced crown and root rot disease ratings of apple trees. This indicates that these treatments have the ability to arrest further symptom development in naturally infected trees if they are treated as soon as symptoms appear. Fosetyl-Al as a drench and metalaxyl as granular broadcast significantly reduced death of trees infected with crown and root rot in orchard 1. Fosetyl-Al applied as a foliar spray, metalaxyl as a granular broadcast or drench, and *E. aerogenes* (strain B8) as drench applications significantly increased fruit yield and reduced crown and root rot disease ratings. The biological treatment with *E. aerogenes* has as much ability as chemical treatments to control crown and root rot of apple trees.

Phytophthora cactorum (Lebert & Cohn) J. Schröt. is the major causal agent of crown and root rot disease of apple trees (*Malus pumila* (L.) Mill.) in British Columbia (18,30). Several other species of *Phytophthora* such as *P. cambivora* (Petri) Buisman (13,15,17,24), *P. citricola* Sawada (12), *P. cinnamomi* Rands (15), *P. cryptogea* Pethybr. & Lafferty (13), *P. drechsleri* Tucker (15,17), *P. megasperma* Drechsler (14,15,23), *P. syringae* (Kleb.) Kleb. (12), and *P. parasitica* Dastur (17) have been implicated in crown and root rot of apple trees in various parts of the world.

Several apple rootstocks, breeding lines, and germ plasm collections have been screened for resistance to crown and root rot (3,20,29,32), but complete immunity to the disease has never been discovered and rootstock susceptibility varies from one region to another (18).

Recommended methods for control of crown and root rot in British Columbia include using tolerant rootstocks; inarching partially girdled mature trees with resistant seedlings; improving drainage; eliminating weeds around the base of trees; reducing nitrogen fertilizer applications; and using preventive chemical treatments with maneb, mancozeb, and ferbam. These chemical sprays are not now in general use.

The likelihood of controlling crown and root rot with fungicides was enhanced by the discovery of metalaxyl and fosetyl-Al. These systemic fungicides are active against several *Phytophthora* spp. (5), including *P. cactorum* on apple (7,8,10,21,28,31). Fosetyl-Al as a foliar spray and metalaxyl (granular) as a broadcast application have not been tested for control of crown and root rot of apple trees in the Okanagan and Similkameen valleys of British Columbia. Biological control of apple crown and root rot has been achieved by the application of *Enterobacter aerogenes* Hormaeche and Edwards (strain B8) as a soil and trunk drench under field conditions at two sites in the Okanagan and Similkameen valleys (30). The efficacy of this biological control agent of crown and root rot of apple trees needs to be tested further.

The purpose of this study was to evaluate the efficacy of spray applications of fosetyl-Al, broadcast applications of metalaxyl (granules), and drench applications of *E. aerogenes* for control of apple crown and root rot over 3 yr at three sites in the Okanagan and Similkameen valleys of British Columbia. The effects of these treatments on shoot growth, trunk diameter, disease rating, and fruit yield were also studied.

MATERIALS AND METHODS

The fungicides evaluated were metalaxyl (Ridomil, Ciba-Geigy Canada Ltd.), fosetyl-Al (Aliette, Rhone-Poulenc Sanitaire, Lyon, France), and biological agent *E. aerogenes* (strain B8, Agriculture Canada, Research Station, Summerland, British Columbia).

Effect of fungicide drenches on the control of crown rot. Studies were conducted in three commercial orchards in British Columbia with a history of crown rot. Orchard 1, located near Kelowna, contained 25-yr-old McIntosh trees on MM.104 rootstock; orchard 2, located near Keremeos, contained 7-yr-old McIntosh trees on M.7; and orchard 3, located near Vernon, contained 10-yr-old McIntosh trees on M.7. At each location, 120 trees, including both those with crown rot symptoms and apparently healthy trees, were selected at random from the center of the orchards. They were subdivided into blocks of five trees; each tree within a block received the same treatment or remained untreated. The chemical and biological treatments were evaluated as soil and trunk drenches, granular broadcast applications, or foliar sprays in a randomized complete block design with four replications.

The treatment application rates are given in Tables 1-5. Control trees were not treated with any biological or chemical agents. The drench applications were made on and around the base of the trunk with each tree receiving 5 L of spray. The trunks were sprayed for drench and trunk applications to a height of about 60 cm above ground level. Granular metalaxyl was applied to a 1 × 1 m square area at the base of each tree. Treatments of fosetyl-Al (drench), metalaxyl (drench or broadcast granules), and *E. aerogenes* strain B8 were applied to the three orchards during May or June and October 1986, May and September 1987, and May 1988. Treatments of fosetyl-Al as foliar sprays were applied to the three orchards during May or June, July and October 1986; May, July, and September 1987; and May and July 1988. Fall applications of all treatments were made after the harvest of fruits.

Suspensions of *E. aerogenes* (B8) were prepared as described previously (30). Bacterial suspensions were prepared by scraping the growth from each culture plate into 100 ml of sterilized water. Approximately 1×10^{10} colony-forming units (cfu) of bacteria were applied to each tree. The chemical and biological treatments were applied with a plot sprayer discharging 10 L/min.

Just before treatment, about 15 cm of soil was removed from the base of trees to assess the extent of crown and root rot infection as follows: initial—less than one-fourth of the bark/roots at the crown region infected by *P. cactorum*;

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intermediate—about one-fourth to one-half of the bark/roots infected; terminal—more than half of the bark/roots infected.

The trunk diameter of the selected trees in all three orchards was measured on the day the first treatments were applied. Trunk diameter of each tree and the shoot length (current season) of each of five randomly selected branches per tree were measured and a disease rating (1 = healthy, 5 = dead) was assigned to each tree after the harvest of fruits. Fruit yield was measured in orchards 2 and 3 only. Bark and root samples were collected at the beginning of the experiment and before each application of the treatments for all trees except those that never showed any sign of disease. The presence or absence of *P. cactorum* in bark of infected trees was confirmed on 10 plates per sample by isolation on a selective medium (19). This medium contained 1 g of potassium dihydrogen phosphate, 0.1 g of magnesium sulfate heptahydrate, 1 g of DL-threonine, 0.02 g of thiamine hydrochloride, 0.2 g of sodium taurocholate, 20 g of sucrose, 20 g of agar, and 1,000 ml of distilled water. Benomyl dissolved in dimethyl sulfoxide (DMSO) was added at a concentration of 20 μg of benomyl and 5 μl of DMSO per milliliter before the plates were poured. Infected bark (10 g) from each sample was added to 100 ml of distilled water, thoroughly mixed, and 1-ml aliquots were dispensed on the selected medium. Plates with bark suspensions were incubated at 18 C. Typical colonies of *P. cactorum* were recognized at ×40 magnification. Infected roots were plated on P10VP selective medium (27), 10 plates per sample, and colonies typical of *P. cactorum* were examined under a microscope to confirm root infection by the pathogen. Fertilization and insect, weed, and foliar disease control measures were carried out by the grower, as prescribed for apples in British Columbia (4).

Increase in trunk diameter was determined by subtracting the value for spring 1986 from fall 1986 (1986), by subtracting the value for fall 1986 from fall 1987 (1987), and by subtracting the value for fall 1987 from fall 1988 (1988). The mean annual increase in trunk diameter for 1986–1988 was calculated. Data for all treatments except for trees that had died were subjected to statistical analysis of variance for F statistics with a general linear models procedure (SAS Institute Inc., Cary, NC). The Duncan's new multiple range comparison test was used to compare treatments. For comparison, trees that died in various treatments during a 3-yr period were compared by Fisher's exact test for two proportions (6).

RESULTS

Bark and root samples taken in spring 1986 from all trees with typical symptoms of crown and root rot contained viable *P. cactorum*. *P. cactorum* was not isolated from trees in which infection had apparently been eradicated by fungicide or biological treatments. Metalaxyl (granular) applied as a broadcast treatment prevented death of all trees showing initial, intermediate, or terminal symptoms of crown and root rot in orchard 1 (Table 1). Fosetyl-Al applied as soil and trunk drenches prevented death of all trees showing initial symptoms of crown rot. Total number of trees dead over a 3-yr period in metalaxyl applied as a broadcast and fosetyl-Al applied as a drench were significantly less than the control in orchard 1 (Table 1). No significant differences were observed between treatments and the control for total number of trees dead during the 3-yr period in orchards 2 or 3 (*data not shown*).

Analysis of variance for shoot growth, trunk diameter, disease severity, and yield indicated that the effects of year, site, and their interaction were significant at 5% level of significance for all four measurements (*data not shown*). Therefore, comparisons were made among treatments for individual years and orchards. The mean values for orchards 1, 2, and 3 for four measurements are presented in Tables 2–5. Shoot length was not affected by any of the treatments in orchards 1 and 3 (Table 2). In orchard 2, fosetyl-Al applied as a foliar spray and metalaxyl as a granular broadcast or drench application significantly increased shoot growth. When the data were pooled for all 3 yr and three sites, fosetyl-Al applied as foliar and drench treatments and metalaxyl as a granular broadcast and drench significantly increased the annual shoot growth.

Table 1. Effect of treatments applied during 1986–1988 as soil and trunk drench, broadcast, or foliar application on the number of apple trees dead in orchard 1, Kelowna, British Columbia

			Number of trees[a]						
Treatment	Mode of application	Rate g a.i./tree	Initial symptoms 1986	Dead by 1988	Intermediate symptoms 1986	Dead by 1988	Terminal symptoms 1986	Dead by 1988	Total dead trees[b]
Fosetyl-Al	Foliar	5	11	1	6	1	3	3	5
Fosetyl-Al	Drench	10	11	0	8	1	1	1	2**
Metalaxyl (granular)	Broadcast	1	9	0	10	0	1	0	0**
Metalaxyl	Drench	1	6	0	10	1	4	4	5
Enterobacter aerogenes (B8)	Drench	1×10^{10}[c]	9	1	9	2	2	2	5
Control		...	5	3	12	6	3	3	12

[a] Number of trees, out of a total of 20 per treatment, showing various stages of crown and root rot symptoms.
[b] ** = Significantly different from the control at 1% level of significance according to Fisher's exact test for two proportions.
[c] In colony-forming units.

Table 2. Effect of chemical and biological treatments applied as foliar, drench, or broadcast on annual mean shoot growth in 3 yr in three orchards in British Columbia

			Mean shoot growth (cm)			
Treatment	Mode of application	Rate g a.i./tree	Orchard 1	Orchard 2	Orchard 3	Grand mean
Fosetyl-Al	Foliar	5	14.9 a[y]	27.7 a	39.7 a	27.8 a
Fosetyl-Al	Drench	10	16.2 a	22.4 abc	37.6 a	25.5 ab
Metalaxyl (granular)	Broadcast	1	13.7 a	26.5 ab	39.3 a	26.5 a
Metalaxyl	Drench	1	13.0 a	27.8 a	37.8 a	26.8 a
Enterobacter aerogenes (B8)	Drench	1×10^{10}[z]	13.0 a	19.6 bc	38.6 a	23.2 bc
Control	...	...	15.1 a	15.4 c	36.1 a	22.7 c
SE			1.5	2.0	2.2	0.8

[y] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] In colony-forming units.

Table 3. Effect of chemical and biological treatments applied as foliar, drench, or broadcast on increased mean trunk diameter in 3 yr in three orchards in British Columbia

Treatment	Mode of application	Rate g a.i./tree	Trunk diameter cumulative mean (mm)			Grand mean
			Orchard 1	Orchard 2	Orchard 3	
Fosetyl-Al	Foliar	5	18.9 ab[y]	24.9 ab	26.7 a	23.5 a
Fosetyl-Al	Drench	10	16.5 ab	21.8 abc	21.5 a	19.9 a
Metalaxyl (granular)	Broadcast	1	12.7 b	24.9 ab	24.2 a	20.8 a
Metalaxyl	Drench	1	15.3 b	26.7 a	26.6 a	22.9 a
Enterobacter aerogenes (B8)	Drench	1×10^{10}[z]	16.4 b	21.9 abc	27.7 a	22.0 a
Control	...	...	25.6 a	14.9 c	21.7 a	20.7 a
SE			2.8	1.9	1.9	1.3

[y] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] In colony-forming units.

Table 4. Effect of chemical and biological treatments applied as foliar, drench, or broadcast on mean disease rating[x] of 3 yr in three orchards in British Columbia

Treatment	Mode of application	Rate g a.i./tree	Mean disease rating			Grand mean
			Orchard 1	Orchard 2	Orchard 3	
Fosetyl-Al	Foliar	5	1.9 ab[y]	1.4 c	1.1 a	1.5 bc
Fosetyl-Al	Drench	10	1.5 bc	2.0 ab	1.2 a	1.6 bc
Metalaxyl (granular)	Broadcast	1	1.4 c	1.7 bc	1.1 a	1.4 c
Metalaxyl	Drench	1	2.1 a	1.4 c	1.1 a	1.5 bc
Enterobacter aerogenes (B8)	Drench	1×10^{10}[z]	2.0 ab	1.9 b	1.1 a	1.7 b
Control	...	...	2.4 a	2.5 a	1.2 a	2.0 a
SE			0.4	0.1	0.04	0.06

[x] 1 = healthy, 5 = dead.
[y] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] In colony-forming units.

Table 5. Effect of chemical and biological treatments applied as foliar, drench, or broadcast on mean fruit yield in 3 yr in three orchards in British Columbia

Treatment	Mode of application	Rate g a.i./tree	Mean fruit yield (kg)		Grand mean
			Orchard 2	Orchard 3	
Fosetyl-Al	Foliar	5	13.9 a[y]	34.0 ab	24.8 a
Fosetyl-Al	Drench	10	9.7 ab	30.2 b	19.9 b
Metalaxyl (granular)	Broadcast	1	13.3 a	34.4 ab	24.0 a
Metalaxyl	Drench	1	13.2 a	36.6 ab	24.9 a
Enterobacter aerogenes (B8)	Drench	1×10^{10}[z]	12.8 a	39.6 a	26.2 a
Control	...	...	6.8 b	30.8 b	18.8 b
SE			1.5	2.2	1.1

[y] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] In colony-forming units.

Metalaxyl as a granular broadcast or drench application and *E. aerogenes* applied as a drench significantly reduced trunk growth when data for all 3 yr were pooled together in orchard 1 (Table 3). In orchard 2, fosetyl-Al applied as a foliar spray and metalaxyl applied as a granular broadcast or drench increased trunk diameter over the control. For orchard 3, no significant increase in trunk diameter was observed in any of the treatments. No differences in increased trunk diameter were seen with any treatment in the grand mean for all 3 yr and three sites.

In orchard 1, fosetyl-Al applied as a drench and metalaxyl applied as a granular broadcast decreased disease rating (Table 4). Fosetyl-Al applied as a foliar spray, metalaxyl applied as a granular broadcast or drench, and *E. aerogenes* applied as a drench significantly reduced the mean disease rating for 3 yr in orchard 2. In orchard 3, no differences were observed between treatments and the control. However, all treatments reduced disease rating as compared with the control in the grand mean for all 3 yr and three sites.

All treatments except fosetyl-Al applied as a drench increased yield of apples in orchard 2 (Table 5). The biological treatment with *E. aerogenes* significantly increased yield of apples in orchard 3. In the grand mean for all 3 yr and two sites, fosetyl-Al applied as a foliar spray, metalaxyl applied as a granular broadcast or drench, and *E. aerogenes* strain B8 applied as a drench increased fruit yield. *E. aerogenes* B8 increased fruit yield in the mean for all 3 yr in both orchards.

DISCUSSION

In the absence of a proven, economical method of preventing crown and root rot, it is probable that early diagnosis and timely curative treatment will remain the most practical and economic ways of dealing with this disease. However, soil application of maneb, mancozeb, or ferbam is still the recommended procedure for bearing fruit trees in British Columbia (4). This procedure is tedious and only partially successful. Simpler and more effective procedures seem possible because of the discovery of new, systemic fungicides such as metalaxyl and fosetyl-Al.

As reported for apple (21), foliar sprays with fosetyl-Al are highly effective in preventing cankers caused by *P. cactorum*. Our results indicate that foliar sprays of fosetyl-Al helped to increase shoot growth and fruit yield and reduce disease rating. All treatments were effective in reducing the disease index (Table 4). This is in agreement with our earlier observations (30,31) in which it was shown that *E. aerogenes*, metalaxyl, and fosetyl-Al applied as a drench can arrest further symptom development in trees naturally infected with *P. cactorum* if the trees are treated at an early stage of the disease. In the present trials, fosetyl-Al applied as a drench prevented the death of the total number of trees in orchard 1 (Table 1). That metalaxyl (granular) applied as broadcast prevented the death of all trees showing initial, intermediate, or terminal symptoms of crown and root rot in the Kelowna orchard is a very significant observation (Table 1). This effect may be attributable to slow release of metalaxyl over a long period of time by granular formulations.

A number of application methods, such as soil drench, broadcast, trunk paint, trunk injection, and foliar application of fungicides, have been tried on various crops (1,7,8,16,25,26,28,30,31). Application of metalaxyl as a soil and trunk drench at the base of the tree has appeared to be an effective method (9, 22,30). Application of metalaxyl in granular formulations seems to be more effective than application as a drench for control of apple crown and root rot disease.

The mechanism of action of fosetyl-Al is not completely understood. Research indicates that it gradually breaks down to phosphonic acid, which protects against *Phytophthora* diseases by stimulating a host defense response (2,5,22). However, Fenn and Coffey (11) demonstrated that the increase in antifungal compounds could be a secondary host response to a direct effect of phosphonic acid on the pathogen. In the present study, it was demonstrated that foliar application of fosetyl-Al increased shoot growth and fruit yield and reduced disease ratings of apple trees. Moreover, from the grower's point of view, the application of a foliar spray is more easily accomplished than a drench application.

The application of biological treatment *E. aerogenes* strain B8 reduced disease rating and increased fruit yield, which are important characteristics to measure the effectiveness of any treatment. This confirms our earlier observation (30) that *E. aerogenes* has potential for practical field control of crown and root rot of apple trees in British Columbia. A patent on the use of this bacterium for control of apple crown and root rot is pending in Canada and the United States. Apple growers in the Okanagan and Similkameen valleys generally experience small annual tree loss from crown and root rot. The replacement costs of scattered trees infected with crown and root rot are increasing and becoming prohibitive. Moreover, new trees planted on old sites are likely to suffer from apple replant disease. Metalaxyl and fosetyl-Al are not registered in Canada for control of crown and root rot of bearing apple trees. Considering the environmental problems with chemical control, biological control of crown and root rot of apple trees with *E. aerogenes* (B8) could become an alternative treatment.

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