Effects of Metalaxyl on *Phytophthora cactorum* and Collar Rot of Apple

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ABSTRACT

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Metalaxyl at low concentrations was highly inhibitory to mycelial growth, sporangia production, and zoospore germination of *Phytophthora cactorum*. Following soil drenches in the greenhouse, metalaxyl was taken up by apple roots and translocated upward in sufficient quantity to prevent growth of *P. cactorum* and *Pythium ultimum* on sections of wood taken from 15 cm above the soil line. Soil drenches prevented infection of apple trees inoculated with *P. cactorum* in the greenhouse. Soil drenches around naturally infected trees in the field resulted in remission of typical collar rot symptoms. The use of metalaxyl as a soil drench may provide an effective new method of controlling apple collar rot.

Collar or crown rot of apple, which is caused by *Phytophthora cactorum* (Leb & Cohn.) Schroet., is a serious problem in many Ohio apple orchards. Disease incidence is greater in heavy and poorly drained soils (3,12). At present, there are no effective control measures for this disease (10,12). Differences in susceptibility of various apple rootstocks have been reported (14); however, few apple cultivars or commercially used clonal rootstocks are considered highly resistant (10,12), and the reports are inconsistent. Fungicide drenches or sprays applied to soil or infected portions of the tree have not adequately controlled collar rot (9).

The systemic fungicide metalaxyl (Ridomil®, Subdue®; CIBA-GEIGY Corp., Greensboro, NC 27419) has effectively controlled several diseases caused by fungi in the Oomycetes (1,2,5–7, 9,11,19,20,22). Farih et al (8) reported that metalaxyl at low concentrations was highly inhibitory to mycelial growth and formation of sporangia, chlamydospores, and oospores of *P. parasitica* and/or *P. citrophthora*. Chlamydospore germination was sensitive to low concentrations, but zoospore germination was not greatly reduced. They suggested that metalaxyl controls diseases by affecting the pathogens at all stages of their life cycle (4). In addition to being highly efficacious against Oomycetes, uptake by roots and acropetal translocation have been reported in several plant species following soil application (1,6,16,17,21). Soil drenches and stem paints containing metalaxyl have been reported to control Phytophthora gummosis of sweet orange seedlings (7).

This paper reports the in vitro effects of metalaxyl on mycelial growth, sporangia production, and zoospore germination of *P. cactorum*; upward movement of the fungicide in apple trees following soil drenches; and efficacy of the fungicide for postinfection control of apple collar rot.

MATERIALS AND METHODS

Fungus. The isolate of *P. cactorum* used in all studies was obtained from an infected apple tree on MM106 rootstock. Stock cultures were maintained on dilute V-8 juice agar (13) in 3.5-ml medicine bottles.

Effect of metalaxyl on mycelial growth. Stock solutions of fungicide were prepared by mixing metalaxyl formulation 2EC (240 g a.i./L) in sterile distilled water at appropriate concentrations and then adding it to lima bean agar (LBA) (18) after autoclaving. Five-millimeter-diameter LBA disks colonized by *P. cactorum* taken from the margin of 5-day-old cultures were used in all studies unless otherwise indicated. Fifteen milliliters of media containing 0, 1, 10, 50, 100, 250, 500, or 1,000 μg of metalaxyl per milliliter were added to each of 10 petri dishes. One colonized LBA disk was placed in the center of each plate. Plates were incubated at 24 C and fungus growth was measured daily.

To determine if mycelia were still alive after 7 days, all disks were transferred to LBA without fungicide. Presence or absence of growth was recorded 2 wk after transfer. The experiment was repeated once.

Effect of metalaxyl on sporangia formation. Three disks were placed in each of three 52-mm-diameter petri dishes containing 5 ml of solution with 0, 10, 100, 250, 500, or 1,000 μg of metalaxyl per milliliter in sterile distilled water and incubated in the light for 3 days. Sporangia present in four different microscope fields (×160) on each agar disk were counted and recorded daily. The experiment was repeated once.

Effect of metalaxyl on zoospore germination. Zoospores were produced and their concentration standardized as previously described (15).

The effect of metalaxyl on zoospore viability was measured by pipetting 0.5 ml of a suspension containing 10^5 zoospores per milliliter into 4.5 ml of solution containing 0, 10, 100, 250, 500, or 1,000 μg of metalaxyl per milliliter in sterile distilled water. There were three replicate plates per concentration. After 2 and 4 hr, 200 zoospores in each plate were examined under the microscope at ×160. Zoospores were recorded as either germinated, ungerminated, or lysed. The experiment was repeated once.

Root uptake and translocation of metalaxyl in apple trees. Four-year-old Delicious trees on MM106 rootstock were grown in 8-L plastic pots in the greenhouse. The mean trunk diameter for all trees used in greenhouse studies was 3.1 cm at 15 cm above the soil line. On 2 March 1981, trees were drenched with 500 ml of solution containing 0, 50, 100, 250, or 500 μg of metalaxyl per milliliter in distilled water. Care was taken to avoid direct contact of the tree trunk with the fungicide. This was done by slowly adding the solution to the soil to avoid flooding the pot. There were three trees (replications) per concentration arranged on the greenhouse bench in a randomized block design.

At 72 hr after drenching, wedge-shaped sections were cut from each tree at 5, 10, and 15 cm above the crown (soil line). Wedges (each 1 cm wide, 7 mm long, and 5 mm deep) were cut transversely with the xylem and contained phloem (bark) and secondary xylem. These were then placed phloem-side down on pentachloronitrobenzene-benomyl-neomycin-chloramphenicol (PBNC) medium (13) and a colonized agar disk was placed on the upper surface of each wedge. All disks were in contact with secondary xylem. Observations for

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fungal growth on the agar plug and wood surrounding the plug were made daily. The experiment was repeated once with both *P. cactorum* and *Pythium ultimum* used as test fungi.

Effect of metalaxyl drenches on recovery of *P. cactorum* from inoculated apple trees. Four-year-old Delicious trees on MM106 rootstock were grown in the greenhouse as previously described. On 9 March 1981, holes (4 mm wide × 1 cm deep) were drilled into the trees at 2 and 15 cm above the crown (soil line). Two 4-mm-diameter agar (PBNC) plugs from 5-day-old cultures of *P. cactorum* were placed in each hole and pushed to the back of the hole by using a cork borer with a metal rod down the center. Holes were then plugged with Vaseline petroleum jelly. After 48 hr, three trees (replications) were drenched with 500 ml of a 0, 50, 100, 250, or 500 μg/ml solution of metalaxyl in distilled water. Care was taken not to allow fungicide to directly contact the trunk. Treatments were arranged in a randomized block design.

At 21 days after the drenching treatment, the trees were removed from the pots and the periderm (bark) was removed. Notes on wood discoloration (lesion formation) were made and each tree was cut into sections approximately 5 mm long from the soil line to 20 cm up the trunk. Each section from bottom to top was numbered and its location in relation to inoculation holes was recorded. The sections were surface disinfested in a 0.25% solution of sodium hypochlorite for 2 min and washed in sterile distilled water for 1 min. Sections were plated on PBNC medium and incubated at 24 C. All sections were examined and the presence or absence of *P. cactorum* was recorded after 7 days. The experiment was repeated once.

Field study. A 5-yr-old commercial apple orchard of cultivar Jonathan on MM111 rootstock near Frederictown, OH, developed severe collar rot in 1979. Infection appeared to be very uniform across the orchard. Most trees had the following symptoms: yellowing or chlorosis of leaves in the spring (in the fall this discoloration often changed to a purplish or bronze color); reduced terminal growth; reduced fruit size and yield; and (on most trees) a visible canker or discolored, rotted bark was visible at and below the soil line or crown of the tree. Forty-five trees with the above symptoms were selected for treatment. The mean trunk diameter for all trees used in the field study was 5.2 cm at 15 cm above the soil line. Bark and wood samples were cut from the margin of discolored tissue on each tree, surface disinfested as previously described, and plated on PBNC prior to treatment. Fifteen trees (replications) were treated with water as checks. Fifteen trees each were drenched with 1 L of a 250 or a 500 μg/ml solution of metalaxyl in water around the base of the trunk on 5 September 1980, 4 May 1981, and 7 August 1981.

On 14 August 1981 (approximately 1 yr after initial treatment), terminal growth (current season) was measured on each of 10 randomly selected branches per tree. Differences in leaf color and reflectance were also measured by using a Gardner automatic color difference meter (Gardner Laboratory, Inc., P.O. Box 5728, Bethesda, MD 20014) standardized with the white plate. Measurements were taken in the midsection of five randomly selected leaves from each tree. Leaves were placed so that the midrib bisected the 2.54-cm (1-inch) orifice of the meter. Isolations were made as previously described from bark and wood samples cut from the same region where previous samples were taken.

TABLE 1. Effect of various concentrations of metalaxyl on mycelial growth of *Phytophthora cactorum* in vitro[x]

Metalaxyl (μg/ml)	Growth (cm)	
	7 days	14 days[y]
0	4	4.0 a[z]
1	0	4.0 a
10	0	4.0 a
50	0	3.1 b
100	0	2.2 c
250	0	1.0 d
500	0	0.8 d
1,000	0	0.3 e

[x]All tests were conducted on lima bean agar. Metalaxyl was incorporated after autoclaving. Five-millimeter-diameter agar disks of *P. cactorum* were placed in the center of petri dishes; 4 = growth to edge of plate.
[y]After 7 days all 5-mm-diameter disks were transferred to lima bean agar without metalaxyl. Growth was recorded after 2 wk.
[z]Numbers followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

RESULTS AND DISCUSSION

No mycelial growth was observed on any plate containing agar media amended with metalaxyl (Table 1). Metalaxyl was highly inhibitory to mycelial growth of *P. cactorum* at concentrations as low as 1 μg/ml. At 2 wk after transfer to nonamended media, growth was observed on all plugs, but growth rate after exposure to metalaxyl was reduced with exposure to increased concentrations of the compound, and only slight growth was observed from agar plugs exposed to 1,000 μg/ml for 7 days (Table 1). Metalaxyl appeared to be fungistatic and not fungicidal to mycelium of *P. cactorum*.

Although metalaxyl was fungistatic to mycelium of *P. cactorum*, it appeared to be fungicidal to zoospores. After 2 hr in a 100 μg/ml solution, 83% of all zoospores observed had lysed compared with 4% in the distilled water control (Table 2). At 10 μg/ml, the fungicidal effect, although greatly reduced, was still present. At concentrations of 100 μg/ml and above very few zoospores germinated. Those that did germinate formed small germ tubes that did not elongate.

Sporangia production of *P. cactorum* was significantly reduced at concentrations of 1 μg/ml (Table 3). Sporangia that were produced at concentrations of 100 μg/ml and higher were lysed within 72 hr. The effects of metalaxyl on mycelial growth, sporangia production, and zoospore germination of *P. cactorum* observed in this study were very similar to those reported for *P. parasitica* and *P. citrophthora* (8), except that metalaxyl appears to be much more fungitoxic to zoospores of *P. cactorum* than to those of *P. parasitica* and *P. citrophthora*. These results suggest that metalaxyl could be highly efficacious in controlling apple collar rot since it affects *P. cactorum* in at least three important stages of its

TABLE 2. Effect of various concentrations of metalaxyl on zoospore germination of *Phytophthora cactorum*

Metalaxyl (μg/ml)	Mean number of zoospores[x]: after 2 hr			after 4 hr		
	Germinated	Non-germinated	Lysed[y]	Germinated	Non-germinated	Lysed
0	139 a[z]	53 a	8 a	163 a	19 b	18 a
10	77 b	75 b	48 b	73 b	40 a	87 b
100	13 c	20 c	167 c	12 c	13 b	175 c
250	10 c	7 b	183 d	8 c	0 c	192 d
500	2 d	1 e	197 e	1 d	0 c	199 d
1,000	1 d	0 e	199 e	0 d	0 c	200 d

[x]Based on counting 200 zoospores per each of three dishes (replications) per concentration under a microscope at ×160.
[y]Lysed = cell wall broken or internal contents disorganized.
[z]Numbers followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

TABLE 3. Effect of various concentrations of metalaxyl on sporangia production of *Phytophthora cactorum* in vitro[x]

Metalaxyl (μg/ml)	Mean number of sporangia after[y]: 24 hr	48 hr	72 hr
0	32.1 a[z]	65.6 a	119.1 a
1	6.5 b	12.3 b	19.4 b
10	1.5 c	2.8 c	4.9 c
100	1.2 c	1.2 d	3.0 d
250	1.2 c	1.1 d	1.2 e
500	0.7 c	0.6 d	0.6 e
1,000	0.6 c	0.2 d	0.2 e

[x] Lima bean agar disks colonized by *P. cactorum* were placed in 5 ml of each concentration of metalaxyl in distilled water.
[y] Based on the mean of all sporangia in four microscope fields (×160) on three 5-mm-diameter lima bean agar disks in each of three petri dishes (replications) per concentration.
[z] Numbers followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

TABLE 4. Effect of soil drenches with metalaxyl on leaf color measurements and terminal growth of Jonathan apple trees infected with collar rot[w]

Metalaxyl (μg/ml)	Mean color values[x] L	A−	B+	Terminal growth[y] (cm)
0	32.3 a[z]	3.2 a	7.4 a	8.8 a
250	23.2 b	12.0 b	5.6 a	50.5 b
500	23.0 b	11.9 b	6.0 a	51.3 b

[w] All trees developed typical collar rot symptoms in 1979. Trees received fungicide drenches on 5 September 1980 and 4 May 1981. Color measurements were taken on 15 July 1981 and terminal growth was measured on 14 August 1981.
[x] L = reflectance; A− = greenness; B+ = yellowness. Based on readings from five terminal leaves per tree.
[y] Based on terminal growth of 10 randomly selected branches per tree.
[z] Numbers followed by letters in the same column are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

life cycle.

At concentrations tested on 4-yr-old trees in the greenhouse, metalaxyl was taken up by apple roots within 42 hr and translocated upward in sufficient concentration to completely inhibit the growth of *P. cactorum* and *P. ultimum* on wood cut from 15 cm above the soil line. Three days after placing colonized agar disks on wedges, both fungi had grown from agar disks, colonized the wood, and grown out onto the surrounding media on all wedges cut from nontreated trees. No growth was observed on agar disks on any of the wedges cut from metalaxyl-treated trees. Root uptake of metalaxyl and translocation through the crown region following a soil drench, combined with the highly efficacious nature of metalaxyl against *P. cactorum*, further suggests that it might be beneficial in controlling apple collar rot.

P. cactorum was isolated from all inoculated, nontreated trees in the greenhouse. The fungus was recovered from trunk cross sections up to 3 cm above and below each inoculation hole. In addition, inoculated, nontreated trees had a brown discoloration in the wood extending for up to 4 cm above and below each hole. *P. cactorum* was not recovered from any inoculated trees that were treated with metalaxyl, and no discoloration was observed in wood around the inoculation holes. At the concentrations tested, metalaxyl was taken up by roots and translocated upward in sufficient concentrations to control *P. cactorum* infection in inoculated trees up to 15 cm above the soil line.

P. cactorum was isolated from 25 of the 45 trees in the field trial before treatment began. By June 1981 (after two treatments) marked differences were observed between treated and nontreated trees. Four of the nontreated trees died during the summer of 1981. There was no visible differences between trees treated with 250 or 500 μg of metalaxyl per milliliter. All treated trees developed green (apparently normal) leaves. Leaves from treated trees were significantly greener than those from nontreated trees (Table 4). There were no significant differences in greenness between trees treated with 250 or 500 μg of metalaxyl per milliliter. There were no significant differences in yellowness of leaves between any treatments. Leaves from nontreated trees had significantly greater reflectance than leaves from treated trees. The reason for this is not known, but it is probably related to differences in wax or cutin deposition on the leaf surface.

Terminal growth was significantly greater on treated than on nontreated trees (Table 4), but there were no significant differences between trees treated with 250 or 500 μg of metalaxyl per milliliter. In addition to increased terminal growth and increased green color of leaves, all visible cankers on treated trees developed apparently healthy callus tissue at the margin. *P. cactorum* was not recovered from any treated trees, but was recovered from seven of 11 surviving nontreated trees. There were no changes in visible cankers on the nontreated trees.

The results of this study indicate that metalaxyl is highly efficacious against *P. cactorum* and is readily taken up by apple roots and translocated upward following application as a soil drench. Metalaxyl controlled *P. cactorum* in inoculated trees in the greenhouse and apparently provided postinfection curative activity on infected trees in the field. Up to the present, there have been no satisfactory chemical control recommendations for apple collar rot. Soil drenches with metalaxyl may provide a new and effective method of controlling this disease.

LITERATURE CITED

1. Benson, D. M. 1979. Efficacy and in vitro activity of two systemic acylalanines and ethazole for control of *Phytophthora cinnamomi* root rot of azalea. Phytopathology 69:174-178.
2. Bertrand, A., Ducret, J., Debourge, D. C., and Horriere, D. 1977. Études des proprietes d'une nouvelle famillie de fongicides: Les monoethyl phosphites metalliques. Caracteristiques physicochimiques et proprietes biologiques. Phytiatr.-Phytopharm. 26:3-17.
3. Braun, H., and Wilcke, D. E. 1963. Untersuchungen über Bodenverdichtungen und ihre Beziehungen zum Auftreten der Kragenfäule. Phytopathol. Z. 46:71-86.
4. Bruck, R. I., Fry, W. E., and Apple, A. E. 1980. Effect of metalaxyl, an acylalanine fungicide, on developmental stages of *Phytophthora infestans*. Phytopathology 70:597-601.
5. Clerjeau, M., and Beyries, A. 1977. Étude comparée de l'action preventive et du pouvoir systemique de quelques fongicides nouveaux (phosphites-prothiocarbe-pyroxychlore) sur poivron vis à vis de *Phytophthora capsici*. Phytiatr.-Phytopharm. 26:73-83.
6. Cohen, Y., Reuveni, M., and Eyal, H. 1979. The systemic antifungal activity of Ridomil against *Phytophthora infestans* on tomato plants. Phytopathology 69:645-649.
7. Farih, A., Menge, J. A., Tsao, P. H., and Ohr, H. D. 1981. Metalaxyl and efosite aluminum for control of Phytophthora gummosis and root rot on citrus. Plant Dis. 65:654-657.
8. Farih, A., Tsao, P. H., and Menge, J. A. 1981. *In vitro* effects of metalaxyl on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. Plant Dis. 65:651-654.
9. Frossard, P., Haury, A., and LaVille, E. 1977. Resultats preliminaires concernant l'activité de l'ethyl phosphite d'aluminum (LS74-783) sur les maladies a Phytophthora des agrumes, de l'avocatier et de l'ananas. Phytiatr.-Phytopharm. 26:55-61.
10. McIntosh, D. L. 1975. Proceedings of the 1974 APDW workshop on crown rot of apple trees. Can. Plant Dis. Surv. 55:109-116.
11. McIntyre, J. L., and Walton, G. S. 1981. Control of strawberry red stele caused by *Phytophthora fragariae*. Plant Dis. 65:835-836.
12. Palm, E. W., and Millikan, D. F. 1967. Collar rot of apples. Univ. of Missouri Extension Division, Science and Technology Guides 2:6-23-24.
13. Schmitthenner, A. F. 1973. Isolation and identification methods for *Phytophthora* and *Pythium*. Proc. of the First Woody Ornamental Disease Workshop. University of Missouri, Columbia. 128 pp.
14. Sewell, G. W. F., and Wilson, J. F. 1959. Resistance trials of some apple rootstock varieties to *Phytophthora cactorum* (Leb & Cohn.) Schroet. J. Hortic. Sci. 34:51-58.
15. Spring, D. E., Ellis, M. A., Spotts, R. A., Hoitink, H. A. J., and Schmitthenner, A. F. 1980. Suppression of the apple collar rot pathogen in composted hardwood bark. Phytopathology 70:1209-1212.
16. Staub, T., Dahmen, H., and Schwinn, F. J. 1978. Biological characterization of uptake and translocation of fungicidal acylalanines in grape and tomato plants. Z. Pflanzenkr. Pflanzenschutz. 85:162-168.

17. Timmer, L. W. 1979. Preventive and systemic activity of experimental fungicides against *Phytophthora parasitica* on citrus. Plant Dis. Rep. 63:324-327.
18. Tuite, J. 1969. Plant pathological methods, fungi and bacteria. Burgess Publ. Co., Minneapolis, MN. 329 pp.
19. Williams, D. J., Beach, B. G. W., Horriere, D., and Marechal, G. 1977. LS74-783, a new systemic fungicide with activity against phytocomycete diseases. Pages 565–573 in: Proc. Br. Crop Prot. Conf. Pest and Diseases, 1977. 1045 pp.
20. Young, T. R., Seifried, E. B., and Biehn, W. L. 1977. Acylalanines: A new class of systemic fungicides. Proc. Fla. State Hortic. Soc. 90:327-329.
21. Zaki, A. I., Zentmyer, G. A., and LeBaron, H. M. 1981. Systemic translocation of C-labeled metalaxyl in tomato, avocado, and *Persea indica*. Phytopathology 71:509-514.
22. Zentmyer, G. A. 1978. New organic fungicides for control of Phytophthora root rots. (Abstr.) Page 362 in: Abstracts of Papers, 3rd Int. Congr. Plant Pathol., München, Germany.