Efficacy and In Vitro Activity of Selected Fungicides for Control of *Phytophthora cactorum* Collar Rot of Apple

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ABSTRACT

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Phytophthora cactorum was consistently recovered from the root systems of nursery transplants and was the primary inoculum source in collar rot areas in Georgia. Captafol as a root dip was more effective in reducing the recovery of *P. cactorum* from MM106 and M26 apple stocks than ethazole, benomyl, maneb, PCNB, and copper sulfate. Captan, PCNB, ethazole, benomyl, and captafol at 100 ppm significantly reduced the growth of *P. cactorum* in vitro. Fungicide trunk sprays and drenches did not significantly reduce the incidence of collar rot in the field over a 3-yr period. Copper sulfate was inadequate for control because of phytotoxicity in the root dip treatments and burr knot formation on trees drenched in the field. Incidence of collar rot was more severe on trees under stress.

Phytophthora cactorum (Leb. & Cohn) Schroet. has been reported as possibly the most common and damaging soilborne disease-causing organism on apple (*Malus domestica* Borkh.) (8). Plants infected with *P. cactorum* show various degrees of injury near the soil line and have been described as having collar rot (3,4) or crown rot (12). Crown rot is initiated where the roots join the trunk of the rootstock, and collar rot may affect the scion variety (13). Symptoms first expressed by apple trees are reduced vigor and chlorotic foliage (8) that sometimes becomes reddish or purple (15). There may be discoloration of the bark and girdling extending from the soil line or below to 25 cm above ground level (16).

Incidence and severity of collar rot of apple caused by *P. cactorum* have been correlated, either experimentally or under field conditions, with excess moisture or rainfall, temperature, and heavy, poorly drained soils (5); apple cultivars or rootstocks (1,9); the isolate of the organism involved (2); growth regulators (6); cultural practices (13); and nursery stock quality (11).

Isolation of *P. cactorum* from soil or tissue is difficult and often not repeatable (14,17). Factors that affect isolations are vigor of the organism, competition of other organisms in the soil, and age or vigor of the infected tree tissue (13).

The objectives of our study were to monitor the behavior and development in diseased orchards and explore the possibility of inoculum dispersal by infected transplants from other states.

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METHODS AND RESULTS

Bioassay. The *P. cactorum* isolate used was from a diseased apple tree in McDuffie County, Georgia. Inoculum was grown in petri dishes on hempseed agar (HSA) for 7 days at about 21 C. Uniform agar plugs of mycelium were removed from the advancing margins with a No. 2 cork borer (5 mm diam.). Four agar plugs were placed on each petri dish and five petri dishes were used for each rate of chemical. Chemicals were added to cooled HSA to obtain the desired concentration.

Fungicide treatments in 1976 were: 1) ethazole (Truban 24 EC), 2) captafol (Difolatan 39 Flowable), 3) benomyl (Benlate 50 WP), and 4) captan (Captan 50 WP). Rates of 1,000, 500, 250, 100, 50, and 0 ppm were studied for each chemical.

Chemicals used in 1979 were: 1) benomyl, 2) captafol, 3) pentachloronitrobenzene (PCNB, Terraclor 75 WP), and 4) captan. Rates of 700, 600, 500, 400, 300, 200, 100, and 0 ppm were studied for each chemical. All treatments were placed under fluorescent lights on a laboratory bench at about 21 C. Measurements on growth were taken 4 days after initial transfer.

Growth of *P. cactorum* was significantly reduced in vitro by all chemicals at the rate of 50 ppm in 1976. Benomyl reduced growth 100% at 500 ppm and ethazole, at 1,000 ppm. Results of the 1979 bioassay were similar to those of 1976 (Table 1). Captafol did not significantly reduce the growth of *P. cactorum* at rates above 100 ppm, although it did significantly reduce mycelial growth at 100 ppm. This reduction at 100 ppm was greater than that of any other chemical tested. Benomyl and captan at 100 ppm significantly reduced the growth of *P. cactorum* over the control. No differences were observed in those rates after 200 ppm for both benomyl and captan. Benomyl inhibited growth 100% when incorporated at 400 ppm and at all higher rates. PCNB reduced growth of *P. cactorum* gradually with each increase in

rate but did not completely inhibit growth at 700 ppm.

Field studies. Field studies were conducted for three consecutive years in three established apple orchards with a history of collar rot. Tissue and soil samples were taken in all orchards before treatment and each fall thereafter until spring 1979. The Campbell apple technique was used for soil and tissue isolations (7). Five apples, each with two holes, were used per soil sample for a total of 10 subsamples. Transfers from rotting apples were made to HSA.

Fungicide treatments were either applied to the trunk from scaffold limbs to the ground to run off (trunk sprays) or applied to the ground at the base of the tree (drench treatments). Amounts of total spray used per established tree were about 3.79 L for the trunk sprays and about 7.60 L for the drench treatments. Fungicide treatments and rates per liter were: 1) copper sulfate (7.2 g) as a drench plus captafol (12.6 ml) as a trunk spray; 2) copper sulfate (7.2 g) as a drench plus benomyl (1.2 g) as a trunk spray; 3) copper sulfate (7.2 g) as a drench plus PCNB (7.2 g) as a drench and benomyl (1.2 g) as a trunk spray; 4) copper sulfate (7.2 g) as a drench; 5) captafol (12.6 ml) as a drench and trunk spray; 6) benomyl (1.2 g) as a drench and trunk spray; 7) copper sulfate (7.2 g) as a drench plus PCNB (7.2 g) as a drench and captafol (12.6 ml) as a trunk spray; and 8) PCNB (7.2 g) as a drench plus benomyl (1.2 g) as a trunk spray. The drenches were applied in the spring and the trunk sprays were applied in both spring and fall. Treatments were randomized with five trees per treatment with four replicates in Burke County and Fannin County orchards and one-tree treatments with 12 replicates in McDuffie County. Untreated control trees were included. Apple trees were in the fourth leaf in Burke County, in the sixth leaf in McDuffie County, and in the first leaf in Fannin County.

P. cactorum was isolated most often during fall 1976 in the Burke County and McDuffie County orchards. In both orchards, the number of positive isolations decreased in most treatments during fall 1977 and fall 1978. The number of isolations from the control plots in Burke and McDuffie counties were consistently higher in most cases than other treatments, but this difference was only significant in the fall 1978 isolations in McDuffie County.

P. cactorum was isolated less often during fall 1978 than either fall 1976 or fall 1977 in Fannin County. Positive isolations were higher and more consistent during fall 1976 and fall 1977 than the corresponding isolations from Burke and McDuffie counties. No treatments consistently reduced the recovery of *P. cactorum* over the 3-yr period. Burr knot was consistently observed on apple trees treated with copper sulfate.

Greenhouse studies. Bioassay and field studies were supplemented with greenhouse studies in 1978 and 1979. The lack of natural distribution of *P. cactorum* in Georgia soils (10) prompted us to qualitatively assay apple trees for *P. cactorum* before planting. Trees from six different nurseries were assayed. Apple trees were removed from the shipping container and the roots of 50 randomly selected trees were washed in 3.79 L of sterile distilled water. Large particles and debris were removed by pouring the solution through three layers of cheesecloth, then sieving it through 100-mesh Tyler screen. The total volume of liquid was reduced by filtering the solution through medium porous Whatman No. 1 filter paper that retained 98% of 11-μm particles. All but about 100 ml of the solution was allowed to pass through the funnel. This amount was used to wash the funnel out into a 200-ml beaker. An additional 25 ml of sterile distilled water was used to further wash out the funnel, which was not allowed to dry. The filtrate was discarded, and the material collected from the funnel was placed in apple fruit. A sterile 50-ml syringe and cannula was used to inject 5 ml of the concentrated solution into the apple fruit. Isolations were made from rotting apples after six days and plated on HSA.

The repeated successful isolations of *P. cactorum* from apple transplants prompted us to conduct a final test with broad-spectrum and selective chemicals to determine the effectiveness and practicality of root dips for chemical control. Rootstocks of MM106 and M26 were used to determine any differences in rootstock susceptibility and phytotoxicity. Treatments and rates per liter of sterile distilled water were: 1) captafol, 31.28 ml; 2) copper sulfate, 17.94 g; 3) PCNB, 17.94 g; 4) ethazole (Terrazole 35 WP), 1.87 g; 5) maneb (Dithane M45 80 WP), 4.49 g; and 6) benomyl, 2.99 g. Appropriate check plants were treated with sterile distilled water. The rootstocks were assayed for *P. cactorum* as previously described. Roots showing lesions were also plated on HSA. *P. cactorum* was found to be associated with the root systems of both rootstocks. The trees were dipped in the appropriate chemical for 30 sec, then potted in a 20-cm diameter black polyethylene pot using fumigated (methyl bromide 454 g/m^3) clay loam top soil-sand (1:1,v/v). Each replicate consisted of five trees of each cultivar dipped in each chemical treatment. Six replicates were arranged in a randomized split plot design on the greenhouse bench. Data on growth and phytotoxicity were taken periodically, and soil samples were assayed every 3 mo for *P. cactorum*. After 324 days, root weights were determined and soil and root isolations were made.

Seven lots of trees from six apple nurseries were assayed, and 87% had *P. cactorum* associated with their root systems. *P. cactorum* was also associated with the roots of the apple trees used in the root dip study. Small lesions were observed on feeder roots, and *P. cactorum* was consistently isolated from these lesions.

Mortality of the apple rootstocks varied considerably depending on the chemical treatment in the dip study (Table 2). Copper sulfate caused high mortality of both rootstocks, killing 90 and 46% of the MM106 and M26 apple trees, respectively. Isolations of *P. cactorum* were consistently lower in the captafol-treated trees in both rootstocks over the entire 324-day period than in all the other treatments.

Regardless of root stock, apple trees had significantly greater fresh and dry root weights when treated with water than with most other treatments except PCNB. Trees treated with copper sulfate were smaller and had lower root weights than those receiving any other treatment. A rootstock root dip interaction occurred, and MM106 trees were generally taller than M26 plants except those treated with PCNB and copper sulfate. Fresh root weights from M26

Table 1. Effect of five chemical treatments at seven rates on growth of *Phytophthora cactorum* in vitro[w]

Rate (ppm)	Growth (mm)[x]				
	Captan	PCNB	Benomyl	Captafol	Ethazole[y]
0	21.40 a[z]	22.30 a	21.80 a	22.15 a	19.7 a
100	10.30 b	14.35 b	5.20 b	3.85 b	10.5 b
200	0.85 c	11.20 c	1.00 c	3.40 b	···
300	0.70 c	7.35 d	0.60 c	3.25 b	···
400	0.65 c	5.40 e	0.00 c	3.00 b	···
500	0.40 c	4.30 ef	0.00 c	3.05 b	3.70 c
600	0.35 c	2.70 fg	0.00 c	3.00 b	···
700	0.15 c	2.05 g	0.00 c	3.00 b	···

[w] Chemicals were added to cooled hempseed agar, and data were taken 4 days after inoculum transfer.
[x] Each value is the mean of five replicates.
[y] At 1,000 ppm, 0 growth.
[z] Values followed by different letters are significantly different ($P = 0.01$) according to Duncan's multiple range test.

Table 2. Effect of six chemical root dips on growth of two apple rootstocks and recovery of *Phytophthora cactorum*[w]

Treatment[x]	MM106 Height (cm)	MM106 Mortality (%)	MM106 *P. cactorum* recovery (%)[y] 90	MM106 *P. cactorum* recovery (%)[y] 324	M26 Height (cm)	M26 Mortality (%)	M26 *P. cactorum* recovery (%)[y] 90	M26 *P. cactorum* recovery (%)[y] 324
Water (control)	65.58 a[z]	3.3	65 b	68 a	63.39 a	0.0	63 b	60 a
Ethazole	70.63 a	3.3	51 c	48 b	52.23 a	6.7	53 c	60 a
Captafol	69.30 a	0.0	6 e	0 c	46.97 a	3.3	10 e	37 c
Benomyl	67.13 a	3.3	62 b	68 a	54.20 a	10.0	51 c	48 b
Maneb	63.77 a	6.7	60 b	64 a	56.95 a	3.3	65 b	62 a
PCNB	57.47 a	13.3	26 d	42 b	59.21 a	3.3	27 d	65 a
Copper sulfate	7.30 b	90.0	100 a	0 c	32.76 a	46.7	78 a	50 b

[w] Data taken from six replicates each containing five plants in 20-cm pots per treatment arranged in a randomized split plot design on greenhouse benches.

[x] Formulations of chemicals and rate per liter were: benomyl (Benlate), 2.99 g: maneb (Dithane M45), 4.49 g; ethazole (Terrazole), 1.87 g; PCNB (Terraclor), 17.94 g; copper sulfate, 17.94 g; captafol (Difolatan), 31.28 ml.

[y] Percentage of recovery of *P. cactorum* at 90 and 324 days after root treatment by the Campbell apple baiting technique.

[z] Values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

trees were significantly more ($P = 0.05$) than those from MM106 trees.

DISCUSSION

These data show that *P. cactorum*-infected apple trees are being brought into Georgia for transplanting. Julis et al (11) recently reported similar findings in North Carolina. The lack of distribution of *P. cactorum* in Georgia soils indicates that our primary inoculum is being brought into the orchards in this manner.

The most effective treatment for the reduction of *P. cactorum* by root dip was captafol, and no phytotoxic symptoms were observed throughout the 324-day period. Although there was a significant reduction in fresh and dry root weights over the controls, there was no significant difference in top growth with this treatment. The minor problem of reduced fresh root weight loss is outweighed by the ability of captafol to control *P. cactorum*.

Control of *P. cactorum* with any of the chemicals used in these tests on established apple orchard trees was not sufficient to consistently reduce the incidence of collar rot. Poor control may be attributed to inability of the chemicals to penetrate the infected tissue. The trees in Burke and McDuffie counties were under stress from poor drainage, and no attempts were made to eliminate this problem. Trees on the other sites that were received in the same lot as those in the collar rot stress area did not show decline symptoms, but in some cases *P. cactorum* was isolated from the tissue. The trees in the Fannin County orchard were grown on the southern face of an approximately 30° slope. Although this apparently would allow good drainage, the soil was a heavy clay, resulting in poor percolation and putting the trees in a stress condition. These physical factors have been reported to increase the incidence of collar rot (13). The decrease in the amount of *P. cactorum* recovered over the 3-yr period may be attributed to reduction in the amount of seasonal rainfall during 1977 and 1978. Every effort should be made to insure that the site is well suited for the transplants, and proper cultural practices are mandatory.

LITERATURE CITED

1. ALDWINCKLE, H. S., and F. J. POLACH. 1974. Differential interaction between apple cultivars and *Phytophthora cactorum* isolates. (Abstr.) Proc. Am. Phytopathol. Soc. 1:132-133.
2. ALDWINCKLE, H. S., F. J. POLACH, W. T. MOLIN, and R. C. PEARSON. 1975. Pathogenicity of collar rot *Phytophthora cactorum* isolates from New York apple trees and other sources. Phytopathology 65:989-994.
3. BAINES, R. C. 1939. Phytophthora trunk canker or collar rot of apple trees. J. Agric. Res. 59:159-184.
4. BALLARD, J. 1973. Collar rot in apples: Causes and controls. Mt. Grow. 329:6-7.
5. BANIHASHEMI, Z., and J. E. MITCHELL. 1976. Factors affecting oospore germination in *Phytophthora cactorum*, the incitant of apple collar rot. Phytopathology 66:443-448.
6. BORECKI, Z., and D. F. MILLIKAN. 1968. The role of IAA in host reaction to different isolates of *Phytophthora cactorum*. (Abstr.) Phytopathology 58:1044.
7. CAMPBELL, W. A. 1949. A method of isolating *Phytophthora cinnamomi* directly from soil. Plant Dis. Rep. 33:134-135.
8. CLAYTON, C. N., A. J. JULIS, and T. B. SUTTON. 1976. Root rot diseases of apple in North Carolina. N.C. State Univ. Bull. 455. 11 pp.
9. DUBIN, H. J., and R. C. McCRUM. 1975. *Phytophthora cactorum* associated with crown rot of apples in Maine. Plant Dis. Rep. 59:365-366.
10. HENDRIX, F. F., and W. A. CAMPBELL. 1970. Distribution of *Phytophthora* and *Pythium* species in soils in the Continental United States. Can. J. Bot. 48:377-384.
11. JULIS, A. J., C. N. CLAYTON, and T. B. SUTTON. 1978. Detection and distribution of *Phytophthora cactorum* and *Phytophthora cambivora* on apple root stocks. Plant Dis. Rep. 62:516-520.
12. McINTOSH, D. L. 1969. Suitability of several fungicides for treating soil infested with *Phytophthora cactorum* to prevent crown rot disease of apple trees. Plant Dis. Rep. 53:182-185.
13. McINTOSH, D. L. 1975. Proceedings of 1974 APDW (Apple and Pear Disease Workers) workshop on crown rot (*Phytophthora cactorum*) of apple trees. Can. Plant Dis. Surv. 55:109-116.
14. McINTOSH, D. L. 1977. *Phytophthora cactorum* propagule density levels in orchard soil. Plant Dis. Rep. 61:528-532.
15. McINTOSH, D. L., and I. C. MacSWAN. 1966. The occurrence of collar rot caused by *Phytophthora cactorum* in a planting of apple trees aged 1 to 7 years. Plant Dis. Rep. 50:267-270.
16. MILLER, P. M. 1974. Collar rot *Phytophthora cactorum* of apples. Pages 10-11 in: Rep. Conn. Pomol. Soc. Proc. 1973. 30 pp.
17. ROSS, R. G., and C. O. GOURLEY. 1973. Crown rot of apple trees in Nova Scotia. Can. Plant Dis. Surv. 53:1-4.