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Cherry tree decline in Michigan: Associated pythiaceous species, pathogenicity and control

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CHERRY TREE DECLINE IN MICHIGAN: ASSOCIATED PYTHIACEOUS SPECIES, PATHOGENICITY AND CONTROL

By

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ABSTRACT

CHERRY TREE DECLINE IN MICHIGAN; ASSOCIATED PYTHIACEOUS SPECIES, PATHOGENICITY, AND CONTROL

By

Margaret Lydia Smither

Montmorency sour cherry trees in Michigan, especially those planted on heavy soils, exhibit decline symptoms of poor growth and discolored and diminished root systems. The problem is most severe in orchards with trees grafted onto Mahaleb rootstocks.

Isolations for *Pythium* species were made from roots and soil. *Pythium* was commonly associated with roots of trees from orchards with heavy clay soils but not with roots of trees from orchards with light sandy soils, and populations in heavy soils were high compared to populations in light soils. *P. irregulare* was isolated most frequently from discolored and necrotic cherry roots and from cherry orchard soils. Other species isolated included: *P. sylvaticum*, *P. ultimum*, *P. rostratum*, and several infrequently isolated *Pythium* spp.. In greenhouse trials root discoloration and necrosis and a reduction in root and shoot growth occurred on Mahaleb seedlings inoculated with *P. irregulare*. Growth reductions and necrosis were greater when seedlings were flooded, but unflooded seedlings exhibited symptoms as well. None of the seedlings inoculated with *P. irregulare* died after 3 months. *P. irregulare* may contribute to a

reduction in growth of cherry trees planted on heavy soils.

Orchards and woodlands in Michigan were found to differ in composition of *Pythium* species. *P. irregulare*, *P. sylvaticum*, and *P. ultimum* were frequently isolated from orchard soils but were not isolated from woodland soils. *Pythium* group 'HS' was isolated most frequently from woodland soils, followed by *Pythium* group 'X', *P. rostratum*, and *Pythium* group 'Y'. Populations of *P. irregulare*, *P. sylvaticum*, and *P. ultimum* in two orchards sampled over 2 yr and one orchard over 1 year, varied independently.

Seven pythiaceous species associated with the roots of sour cherry in Michigan were tested for their sensitivity to metalaxyl and phosphorous acid in culture. All fungi were inhibited by both fungicides and sensitivity varied between species. Mahaleb seedlings exhibited phytotoxic symptoms at 400 and 800 μg/ml metalaxyl. Metalaxyl was more effective than fosetyl-Al in preventing fungus induced root necrosis and growth reduction to 9-wk-old Mahaleb seedlings growing in soil infested with *P. irregulare*, *Ph. megasperma*, and *Ph. cactorum*.

To my father

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INTRODUCTION AND GENERAL LITERATURE REVIEW

Michigan is the leading state in sour cherry production accounting for about two thirds of the total production in the United States (27). The economic value of the crop for 1981 was set at over 40 million dollars (26). The cherry producing areas are in the west along the shores of Lake Michigan. Concentrations of production occur in the northwest around Traverse City, in the west central region around Hart and Shelby, and in the south west around Benton Harbor. High prices of cherries in the last decade resulted in a 14% increase of land under cherry production with a total of 4.5 million trees planted by 1982 (27). However as cherry production increased so did instances of poor tree vigor and decreased longevity.

Tree decline is a comprehensive term encompassing short life and replant problems (47,55). Tree decline problems have been identified throughout the world and recognised for over two hundred years (42). The main symptom is reduced vigor with a possible reduction in life span. Root systems are weak and poorly developed or necrotic. Cankers may develop on the trunk. Often there is no clearly defined causal agent but rather a combination of several biotic and abiotic factors (22,23,47,55). These may include;

environmental factors such as cold injury, stresses due to drought or oxygen defeciency caused by waterlogging, and injury caused by damaging cultural practices; pathogenic factors of infection by bacteria, fungi, viruses, mycoplasma-like-organisms, and nematodes; injury from insect infestation; and physiological factors such as toxicity from plant and spray residues, and biochemical and hormonal imbalances (55). Savory distinguished between specific replant disorders which occurred when one species followed the same or a closely related species and nonspecific replant disorders affecting all tree crops (42). This distinction has also been used to classify other types of tree decline problems (47). The literature pertaining to tree decline has been reviewed in several articles (42,47,55).

Stone Fruit Decline was the subject of a conference at Michigan State University in 1982 (46). Areas of research which were selected as most critical included study of pplant pathogenic viruses, root rots, rootstock selection and improvement, mechanical harvesting, and trunk and tree structure. A grant was awarded to study root rot of cherry in Michigan through the Stone Fruit Decline Project.

Root rot of sour cherry in Michigan caused by Armillaria occurs on sandy sites from the Hart/Shelby area north to the Traverse City area. Three species of Armillaria have been isolated from these orchard sites which have been cleared from woodlands of oak and pine on which the Armillaria species were endemic (39,51). Root rot caused by *Phytophthora* occurs throughout the cherry production area, although symptoms are most severe on poorly drained soils. The symptoms include extensive lateral root necrosis and development of a canker on the lower trunk below the graft union (6). A preliminary survey associated *Pythium* species with decline only on heavy poorly drained soils. The root rot symptoms include extensive necrosis and eventual absence of lateral branchlet roots. *Pythium* and *Phytophthora* are closely related genera (13) and may be expected to be present under similar conditions. Symptoms for the three pathogens are most severe when the trees are planted on Mahaleb (*Prunus mahaleb* L.) rootstocks although orchards on Mazzard (*P. avium* L.) rootstocks are also affected.

The genus *Pythium* contains a large number of species which have a world wide distribution and can be isolated from diverse habitats. Many are soil-bourne plant pathogens and under suitable conditions can cause severe economic losses on a wide range of crops, including beans (1,21,37), wheat (9), strawberry (35,52), and grape (48). Unlike the closely related genus *Phytophthora* not all species are pathogens. *Pythium* species survive in soil by saprophytic growth and production of resistant resting structures (13,50). They tend to be more successful under conditions of high soil moisture (29,49), but are poor competitors with a well established soil microflora (4,13,49). *Pythium* species have the ability to colonise host tissue (13,28,50), and produce a variety of cellulytic and pectolytic enzymes (10,50). The literature about the genus *Pythium* has been reviewed (13,50).

The objectives of this study were to isolate, identify and examine the pathogenicity of *Pythum* species found associated with cherry trees showing poor growth and decline on heavy sites; to investigate the ecology of *Pythium* species in orchard and woodland soils; and to examine possible control measures of rootstock selection and chemical control. The literature on root and collar rot of cherry and other deciduous fruit tree crops caused by pythiaceous species will be reviewed in the remainder of the introduction.

Environmental conditions particularly soil moisture levels influence the development of disease caused by pythiaceous species. Populations of *Pythium* species are higher under conditions of high soil moisture (4,29,49). Tree loss due to *Phytophthora* infection is typically greater in poorly drained areas (25,33). Planting trees on mounds, thus improving drainage, was reported as beneficial for reducing losses caused by *Ph. cactorum* on apple (25). Subsoiling before planting improved peach tree growth and reduced losses due to peach tree short life (41). Improvement was believed due to greater stability in soil moisture levels. Any practice which reduces stress and improves tree vigor will tend to ameliorate disease caused by *Pythium* species.

Pythium species isolated from roots of poorly growing cherry trees have been reported as pathogenic to Mahaleb seedlings (12). _Pythium_ species isolated from roots of diseased trees were pathogenic to peach seedlings (14,15,28,38). However, the importance of _Pythium_ species to peach tree decline has been questioned by some research workers, as disease symptoms were not reproduced in pathogenicity tests. Unfortunately the temperatures at which the experiments were run was not reported (20,29,30,36), and pathogenicity of the _Pythium_ species been shown to be dependent on temperature (7). In addition total _Pythium_ populations were not correlated with decline symptoms (29).

Pythium species cause decline and replant problems of apple. Several _Pythium_ species including _P. irregulare_ Buisman and _P. sylvaticum_ Campbell and Hendrix isolated from roots of declining trees or soil from replant sites were pathogenic to roots of apple seedlings and thought to contribute to apple replant problems in New York (16) and Europe (34,43). _Pythium_ species isolated from apple trees with collar rot in New York were pathogenic to apple seedlings (17). _Pythium ultimum_ from apple trees in Poland was found to cause a trunk canker (5). _Pythium_ species also caused a retardation of tree growth in an apple orchard in Australia (45).

Phytophthora species have been implicated as the primary causal agents of root and crown rots of fruit trees. Root and collar rot of sweet cherry on wet sites in California was first shown to be caused by Phytophthora in 1976 (32). The following species have since been found to be pathogenic to Mahaleb; Ph. cambivora (Petri) Buisman (32,54), Ph. cryptogaea Pethyb. and Laff. (6,53,54), Ph. drechsleri Tucker (32,54), and Ph. megasperma Drechsler (6,32,53,54), Ph. cactorum (Leb. and Cohn)Schroet (6,24), and Ph. syringae (Kleb.)Kleb. (19). Ph. cambivora, Ph. cryptogaea, and Ph. megasperma have been isolated from soils in South Australian cherry orchards (8). Mahaleb rootstocks have been reported as more susceptible to Phytophthora infection than Mazzard and other cherry rootstocks (24,33,54).

A root and collar rot of peach was caused by Phytophthora cinnamomi (32), Ph. cactorum (11,28) and Ph. syringae (19,56).

Apple collar and root rot is caused by Ph. cactorum and the disease has a world wide distribution (2,3,8,25,44). Ph. cambivora (18), Ph. citricola (44), Ph. megasperma (17,40), and Ph. syringae (44) have also been reported to cause collar rot of apple.

The role of Phytophthora species in rapid decline of stone fruit trees has recieved more attention than that of Pythium species because of the greater relative virulence of Phytophthora. In stone fruit growing regions with slow tree declines a more thorough investigation of the role of Pythium species should be undertaken.

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PART I

PYTHIUM SPECIES ASSOCIATED WITH THE ROOTS OF SOUR CHERRY AND THE EFFECT OF *P. IRREGULARE* ON THE GROWTH OF MAHALEB CHERRY

ABSTRACT

Isolations for *Pythium* species were made during the early summer of 1984-1985 from 73 sour cherry trees in 12 Michigan orchards, from Mahaleb seedlings in a commercial nursery, and from soil samples from nine orchards. *Pythium* was commonly associated with roots of trees from orchards with heavy soil but not with roots of trees from orchards with light soil, and populations in heavy soils were high compared to populations in light soils. *P. irregulare* was isolated most frequently from discolored and necrotic cherry roots and from cherry orchard soils. Other species isolated included: *P. sylvaticum*, *P. ultimum*, *P. rostratum*, and several infrequently isolated *Pythium* spp.. In greenhouse trials, a reduction in root and shoot growth and root discoloration and necrosis were obtained on Mahaleb seedlings inoculated with *P. irregulare*. Growth reductions and necrosis were greater when seedlings were flooded, but unflooded seedlings exhibited symptoms as well. None of the seedlings inoculated with *P. irregulare* died after 3 months. This suggests that *P. irregulare* is not a primary cause in the decline and death of cherry trees in Michigan orchards, but it may contribute to a reduction in growth of cherry trees planted on heavy soils. No interaction between *Pythium* and *Phytophthora* was detected on Mahaleb seedlings when *P. irregulare* was inoculated with *Ph. cactorum* and *Ph. megasperma*.

INTRODUCTION

Root rot is an important problem on Montmorency sour cherry trees (*Prunus cerasus* L.) grafted on *Prunus mahaleb* L. (Mahaleb) seedling rootstocks in Michigan. Three species of *Armillaria* reported to be primary causal agents for root rot on sites with light well-drained soils (11). Five species of *Phytophthora* were reported to be primary causal agents for root rot on sites with heavy poorly-drained soils (1). Populations of *Pythium* were high in many soils where Phytophthora root and crown rot was identified as the cause of tree death (M. L. Smither, unpublished). Although *Pythium* has been reported (2) to cause a damping-off of young Mahaleb seedlings grown in naturally infested orchard soils, it is not considered a pathogen of sour cherry trees and the presence of *Pythium* is usually ignored when making isolations for *Phytophthora*. Recent reports suggest *Pythium* may be involved in root rot problems on apple (6,12), particularly in replant sites. The role of *Pythium* has not been defined and therefore warrants further study.

The overall objective of this study was to determine if *Pythium*, in addition to *Phytophthora*, was involved in root rot of sour cherry on sites with poorly drained soils. Subobjectives were to isolate and identify the *Pythium* species associated with sour cherry trees and orchard soils, to test the effect of the predominant *Pythium* species *P. irregulare* on the growth of Mahaleb cherry, and to investigate the effect of *P. irregulare* and two *Phytophthora* species combined on the growth of Mahaleb cherry.

MATERIALS AND METHODS

Isolation of *Pythium* from cherry roots. Root pieces were collected from sour cherry trees with reduced growth of terminal shoots. The orchards were located in Berrien, Kent, Mason, Oceana, and Van Buren counties. Four to six trees were sampled per orchard. A total of 73 trees from 12 orchards were sampled during the early summer of 1984 and 1985. In addition, 22 Mahaleb seedlings received by a local nursery from a supplier on the West Coast were sampled prior to planting in Michigan.

The samples were transported to the laboratory in plastic bags in an ice chest, soaked overnight in tap water, then thoroughly washed to remove soil particles, dried on paper towels, and rinsed in sterile distilled water. Rootlet pieces 5 mm in length were pressed into corn meal agar (CMA) (Difco) amended with 10 mg pimaricin (0.4 ml of a 2.5% suspension), 250 mg ampicillin, 10 mg rifampicin, and 50 mg pentachloronitrobenzene (Sigma Chemical Co., Saint Louis, MO)(6). Twenty-40 plates of amended corn meal agar (CMA-PARP), each with 10 root pieces, were prepared from root samples collected from each tree. The plates were incubated at 21 C in darkness and examined regularly with a binocular microscope for fungal growth. Hyphal tips were transferred from all *Pythium* colonies to fresh CMA-PARP plates and then to CMA prior to identification.

Isolation of *Pythium* from orchard soil. The population of *Pythium* in soil from sour cherry orchards was estimated by surface soil dilution plates (9). Four cores of soil were taken between the drip line and the trunk from each of 10 trees per orchard. The cores were taken to a depth of 15 cm with a Hoffer soil sampler and bulked for each tree in a plastic bag. Samples were transported to the laboratory in an ice chest and stored at 4 C until processed. Soil samples were air dried and then passed sequentially through 5- and 1.68-mm-mesh sieves. A 1 g subsample was taken from each bulk sample, suspended in 0.2% water agar, and dilution plated at 1:10, 1:50, and 1:100 on fresh CMA-PARP selective medium. Diluted samples (1 ml) were distributed over the surface of the agar with a sterile glass rod. Three replicate plates were made per dilution. After 42 hr incubation in darkness, the soil was removed by washing each plate under running tap water and the *Pythium* colonies counted. Hyphal tips were transferred from the edge of 5 to 15 colonies per sample to CMA-PARP plates and then after incubation for 48 hr at 21 C to CMA.

Identification of *Pythium* species. Fungal structures were examined after 1-2 wk and identifications were made based on colony morphology, growth rates, and characteristics of sporangia, hyphal swellings, oogonia, antheridia, and oospores using the key of Van der Plaats-Niterink (13). The identification of the heterothallic species P. *sylvaticum* Campbell and Hendrix was confirmed by crossing each suspect isolate with strains ATCC 18195 and ATCC 18196 of P. *sylvaticum* on potato-carrot agar (13).

Effect on the growth of Mahaleb cherry seedlings. The virulence of nine isolates of P. *irregulare* isolated from roots and soil in five orchards were compared. The test was conducted in a growth chamber with 6-wk-old Mahaleb seedlings. The basic procedure was as described by Mircetich and Matheron (10). Inoculum of P. *irregulare* was prepared by growing each isolate at 21 C for 8 days on 200 ml vermiculite and 20 g oat kernels moistened with 100 ml V-8 juice solution (200 ml V-8 juice (Campbell Soup Co. Camden, NJ), 2 g $CaCO_3$, and 800 ml distilled water) sterilized in 500 ml Erlenmeyer flasks. The inoculum was washed with distilled water over cheesecloth in a Buchner funnel to remove unassimilated nutrients, then mixed at the rate of 20 cc per 1000 cc of sterilized soil/sand/peat (1/1/1 by volume). Seedlings were planted in 4 L plastic pots with six replicates. The controls received vermiculite/oat mixture without fungus. Maximum daytime temperatures were 21 C with 14 hr of light. The nighttime minimum was 18 C. The seedlings were fertilized biweekly (Peters Professional, 20:20:20, Peters Fertilizer Products, W. R. Grace and Co., Fogelsville, PA). The experiment was terminated after 18 wk. Isolations from root tissue were made on CMA-PARP selective medium. Dry weights of the roots and shoots were recorded. The experiment was repeated. Data were analyzed by an analysis of variance for a completely randomized experimental design.

Interaction of *Pythium irregulare* and *Phytophthora* spp.. Four isolates of *P. irregulare* (isolates B306, C6, J210, and L215) were used in single and combined treatments with *Ph. cactorum* (B2) and two isolates of *Ph. megasperma* (M224 and M333). All isolates were from cherry orchards in Michigan. *Ph. megasperma* M224 was avirulent and M333 was virulent to Mahaleb cherry in a previous pathogenicity test (1). Inoculum of *P. irregulare* and of *Phytophthora* spp. was prepared as described in the preceding section except oat kernels were omitted from the mixture and the isolates were grown for 5 wk. Inoculum was mixed per 1000 cc of sterilized soil/sand/peat as follows: control treatments, 40 cc of vermiculite without fungus; single species treatments, 20 cc fungal inoculum + 20 cc vermiculite without fungus; and combined species treatments, 40 cc combined fungal inoculum. The seedlings were planted in 1 L containers and arranged in eight blocks on a greenhouse bench. All treatments in one half of each block were flooded for 48 hr every 2 wk by immersing the pots in water. The experiment was conducted during the winter, the temperature was maintained at 21 C, with 16 hr of light. The plants were fertilized as previously described. The experiment was terminated after 15 wk. Isolations from root tissue were made on CMA-PARP selective medium and on a _Phytophthora_ selective medium (3). _P. irregulare_ populations in soil were monitored with a soil assay on CMA-PARP selective medium. Dry weights of the shoots and roots were recorded. The experiment was repeated. Data were analyzed for a 2x8 split-plot factorial design with randomized complete blocks. The main factor of flooding versus nonflooding was split across four treatments involving single isolates, three treatments involving one isolate of _P. irregulare_ plus one isolate of _Phytophthora_, plus an uninoculated control.

RESULTS

Pythium species isolated from roots. _P. irregulare_, _P. sylvaticum_, _P. ultimum_ Trow., _P. rostratum_ Butler, and _Pythium_ spp. were isolated from roots of sour cherry trees and from the roots of Mahaleb liners (Table 1). _P. irregulare_ was isolated most frequently. It was isolated from trees in all orchards except N and U. These latter two orchards were located on sites with sandy soil. All other orchards except orchard RW where the trees were declining due to X-disease, were located on sites with heavy soils.

Pythium species isolated from soil. The six sour cherry orchards on clay soils had _Pythium_ populations estimated at 54.3 to 746.4 propagules per gram of soil (Table 2). The

Table 1. Species of Pythium isolated from the roots of sour cherry trees with reduced terminal growth or from the roots of Mahaleb seedlings

		Pythium species isolated[a]									
		P. irregulare		P. sylvaticum		P. ultimum		P. rostratum		Pythium spp.[c]	
Year	Orchard code	Fraction of trees	%[b]	Fraction of trees	%	Fraction of trees	%	Fraction of trees	%	Fraction of trees	%
1984	B	3/4	13	1/4	1	0/4	0	1/4	1	0/4	0
	E	4/5	-	0/5	-	0/5	-	0/5	-	1/5	-
	J	3/4	13	2/4	4	0/4	0	0/4	0	2/4	1
	L	6/6	22	2/6	4	1/6	1	1/6	2	0/6	0
	N	5/5	-	1/5	-	2/5	-	1/5	-	0/5	-
	N[d]	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-
	U[d]	0/5	-	0/5	-	0/5	-	0/5	-	0/5	-
1985	B	6/6	38	0/6	0	0/6	0	1/6	2	2/6	2
	D	5/5	24	0/5	0	0/5	0	0/5	0	0/5	0
	F	4/5	20	2/5	9	1/5	7	0/5	0	1/5	0
	J	3/4	15	4/4	8	2/4	10	0/4	0	0/4	0
	JC	5/5	19	2/5	7	0/5	0	2/5	2	4/5	3
	L	5/5	72	2/5	19	2/5	7	0/5	0	0/5	0
	P	4/4	79	0/4	0	1/4	15	1/4	2	1/4	2
	RW[d]	4/5	19	0/5	0	0/5	0	0/5	0	0/5	0
	X[e]	11/12	-	10/12	-	11/12	-	0/12	-	2/12	-
	Y[e]	10/10	-	8/10	-	0/10	-	2/10	-	0/10	-
	Totals	78/95		34/95		20/95		10/95		13/95	

[a] Samples were collected in early summer. Twenty to 40 plates of CMA-PARP medium (6) containing ten rootlet or bark pieces were prepared from each tree. Two or more fungal species were often isolated from the same tree.
[b] Values for Pythium incidence are expressed as a percentage of the total number of plated tissue pieces.
[c] Combined total of remaining Pythium spp.
[d] Orchards were located on sites with sandy soil.
[e] Isolations were from Mahaleb liners from the West Coast.

Table 2. Populations of *Pythium* species as estimated by dilution-plate counts from soil taken from sour cherry orchards

	Pythium population by species (propagules per gram (ppg))[a]										
Year:	1984							1985			
Orchard code:	B	E	J	L	N	N[b]	U[b]	J	L	P	RW[b]
P. irregulare	52.5	56.0	52.5	32.6	74.3	1.0	0.0	52.1	279.2	64.0	1.5
P. sylvaticum	6.1	5.4	122.6	12.5	157.4	0.0	1.0	25.2	17.2	0.0	0.0
P. ultimum	9.9	6.9	19.8	3.2	52.0	0.0	0.0	23.1	259.2	11.3	1.8
P. rostratum	6.8	2.3	13.1	6.0	52.4	0.3	1.0	1.9	18.0	4.2	0.3
Pythium spp.[c]	0.8	6.1	10.9	0.0	100.5	1.7	0.6	16.1	0.0	0.0	0.0
Total	76.1	76.4	219.0	54.3	437.0	3.0	2.6	119.3	746.4	79.5	3.5

[a]Samples were collected once in early summer. Each value is the mean of ten soil samples per orchard. Each soil sample is the composite of four sub samples collected around one tree.
[b]Orchards were located on sites with sandy soil.
[c]Combined total of remaining *Pythium* spp.

three orchards on sandy soil (orchards N, U, and RW) had very low *Pythium* populations estimated at 2.6 to 3.5 propagules per gram of soil. *P. irregulare* was the *Pythium* species isolated most consistently (50% of all propagules) from clay soils in both years, followed by *P. sylvaticum* (19%) and by *P. ultimum* (15%). Estimates for *P. sylvaticum* were high in soils from orchards J and N in 1984, and for *P. ultimum* in soils from orchard N in 1984 and orchard L in 1985. *P. rostratum* (6%) was found primarily in orchard N in 1984.

Effect of *Pythium irregulare* on growth of Mahaleb seedlings. The dry weights of shoots and roots of Mahaleb seedlings in soil inoculated with *P. irregulare* were significantly (*P*=0.05) reduced over the dry weights of the noninoculated seedlings (Table 3, Figure 1). Similar results were obtained in both experiments, although dry weights were lower for all plants in experiment 2. Seedlings inoculated with *P. irregulare* exhibited many discolored rootlets and diminished root systems while noninoculated seedlings exhibited healthy roots and a greater density of rootlets. Although root and shoot growth were reduced, none of the seedlings were killed. There was no consistent difference in virulence between isolates. *P. irregulare* was reisolated from the roots of plants grown in infested soil but not from roots of plants grown in noninfested soil.

Interaction of *Pythium irregulare* and *Phytophthora*. In an initial analysis of variance, no significant differences

Table 3. Effect of nine isolates of *Pythium irregulare* on the growth of Mahaleb seedlings growing in unflooded soil

	Experiment 1				Experiment 2			
	Shoots[a]		Roots[a]		Shoots[a]		Roots[a]	
Isolate number[b]	Dry weight (g)	Relative growth (%)	Dry weight (g)	Relative growth (%)	Dry weight (g)	Relative growth (%)	Dry weight (g)	Relative growth (%)
B17	10.4 ab[c]	82	3.0 bc	73	2.7 b	45	1.4 b	48
B306	9.0 bc	71	2.9 bc	71	2.6 b	43	1.5 b	52
C6	6.1 c	48	2.6 bc	63	2.5 b	42	1.4 b	48
C304	6.1 c	48	2.7 bc	66	3.3 b	55	1.6 b	56
J210	6.0 c	47	2.6 bc	63	2.7 b	45	1.2 b	41
J301	6.0 c	47	2.2 c	54	2.3 b	39	1.5 b	52
L215	7.8 bc	61	2.7 bc	66	2.6 b	43	1.2 b	41
L304	8.3 bc	65	2.9 bc	71	4.9 ab	82	2.3 ab	79
P309	8.5 bc	67	3.5 ab	85	3.8 ab	63	1.9 ab	66
Control	12.7 a	100	4.1 a	100	6.0 a	100	2.9 a	100

[a]Six-wk-old Mahaleb seedlings were transplanted into soil infested with 20 cc of inoculum per 1000 cc of soil. The experiment was conducted in a growth chamber for 3 mo. There were six replicates.
[b]The letter preceding each isolate number codes for the orchard of origin.
[c]Values followed by the same letter do not differ significantly (*P*=0.05) using Duncan's multiple range test.

Figure 1. Roots of representative Mahaleb seedlings grown in a growth chamber for 18 wk in a soil/sand/peat mixture artificially infested with <u>Pythium irregulare</u>. Soil in the pots was (a) uninfested; or infested with <u>P</u>. <u>irregulare</u> isolated from orchard B, (b) root and (c) soil; orchard C, (d) root and (e) soil; orchard J, (f) root and (g) soil; orchard L, (h) root and (i) soil; and orchard P, (k) soil. The treatments were not flooded.

were detected in the dry weights of Mahaleb seedlings inoculated with the four isolates of *P. irregulare*. Therefore, only the results obtained with isolate C6 of *P. irregulare* were presented in Tables 4-5 to illustrate the results obtained with all *Pythium* isolates.

Dry weights of Mahaleb seedlings were significantly (*P*=0.01) reduced in both experiments by flooding for 48 hr every 2 wk (Table 4). In the uninoculated controls flooding reduced shoot dry weights by 43.1-46.7% and root dry weights by 20.7-40.7% (Table 5, Figure 2). Significant differences (*P*=0.01) in dry weights among the inoculation treatments were detected in both experiments, but no significant interaction between any of the inoculation treatments and the flooding treatments was detected by the analysis of variance (Table 4). *P. irregulare*, *Ph. megasperma*, and *Ph. cactorum* significantly reduced shoot and root dry weights under flooded and unflooded conditions. Dry weights of Mahaleb seedlings inoculated with *P. irregulare* and a *Phytophthora* sp. were not significantly different from the dry weights of seedlings inoculated with either fungus alone, except *Ph. megasperma* M224 in experiment 1 did not reduce the dry weights of shoots as much as the other treatments and it failed to reduce root dry weights significantly from the control (Table 5). The reduced growth from roots and shoots from inoculation is evident in Figures 2-3. No seedlings inoculated with *P. irregulare* were killed (Table 5). In inoculation treatments involving

Table 4. Analysis of variance for shoot and root dry weights of Mahaleb seedlings growing in soil infested with Pythium irregulare and two Phytophthora species in single and combined treatments under flooded and unflooded conditions

		Shoot dry weight		Root dry weight	
Source of variation	Degrees of freedom	Mean square	F value	Mean square	F value
Experiment 1					
Replication	7	1.424	0.83	0.432	0.79
Flooding	1	131.828	76.95**[a]	77.969	142.95**
Error	7	1.713		0.545	
Treatments	7	14.933	15.97**	2.244	11.16**
Treatment x flooding	7	0.798	0.85	0.411	2.04
Error	98	0.935		0.201	
Experiment 2					
Replication	7	3.526	1.28	1.443	2.15
Flooding	1	124.228	45.04**	37.303	55.50**
Error	7	2.758		0.672	
Treatments	7	10.735	9.51**	1.940	3.70**
Treatment x flooding	7	1.196	1.06	0.362	0.69
Error	98	1.129		0.524	

[a]** indicates that values were significant (P=0.01).

Table 5. Effect of Pythium irregulare (P.) and two Phytophthora (Ph.) species on the growth of Mahaleb seedlings in single and combined treatments under flooded and unflooded conditions

Fungus and isolate number	Shoots: Unflooded Dry weight (g)	Shoots: Unflooded Relative growth[b] (%)	Shoots: Flooded Dry weight (g)	Shoots: Flooded Relative growth[b] (%)	Shoots: Mean dry weight	Roots: Unflooded Dry weight (g)	Roots: Unflooded Relative growth[b] (%)	Roots: Flooded Dry weight (g)	Roots: Flooded Relative growth[b] (%)	Roots: Mean dry weight	Dead
Experiment 1											
P. irregulare C6	3.8	65	1.4	42	2.5 c[c]	2.1	77	1.1	33	1.4 b[c]	0
Ph. megasperma M224	4.6	79	2.8	85	3.7 b	2.7	101	1.3	81	2.0 a	0
Isolates C6 + M224	3.0	52	1.3	39	2.1 c	2.1	93	0.6	37	1.3 b	0
Ph. megasperma M333	3.0	52	1.2	36	2.1 c	2.3	85	0.4	25	1.4 b	1
Isolates C6 + M333	3.5	60	1.0	30	2.2 c	2.5	92	0.3	19	1.4 b	3
Ph. cactorum B2	2.8	48	1.2	36	1.9 c	1.9	70	0.5	31	1.1 b	1
Isolates C6 + B2	2.7	47	1.0	30	1.8 c	2.0	71	0.4	25	1.2 b	2
Control	5.8	100	3.3	100	4.5 a	2.7	100	1.6	100	2.2 a	0
Flooded means	3.6		1.6			2.3		0.7			
Experiment 2											
P. irregulare C6	3.3	53	1.4	42	2.4 b	2.5	86	1.1	48	1.8 b	0
Ph. megasperma M224	2.9	47	1.8	54	2.4 b	2.0	69	1.4	61	1.7 b	0
Isolates C6 + M224	3.4	55	1.8	54	2.6 b	2.3	79	1.1	48	1.7 b	0
Ph. megasperma M333	3.7	60	1.7	51	2.7 b	2.4	83	1.2	52	1.8 b	3
Isolates C6 + M333	3.0	48	1.1	33	2.1 b	1.9	65	0.9	39	1.4 b	1
Ph. cactorum B2	3.9	63	1.8	54	2.8 b	2.3	79	1.1	48	1.7 b	2
Isolates C6 + B2	3.7	60	1.3	39	2.5 b	2.2	76	1.0	43	1.6 b	1
Control	6.2	100	3.3	100	4.7 a	2.9	100	2.3	100	2.6 a	0
Flooded means	3.8		1.8			2.3		1.2			

[a] Six-wk-old Mahaleb seedlings were transplanted in soil infested with 20 cc of inoculum per 1000 cc of soil, flooding was for 48 hr every 2 wk. The experiment was conducted in a greenhouse for 15 wk. There were eight replicates.
[b] Values are expressed as a percentage of control.
[c] Values followed by the same letter do not differ significantly (P=0.05) using Duncans Multiple Range test.

Figure 2. Shoots (A) and roots (B) of representative Mahaleb seedlings grown in a greenhouse for 3 mo in a soil/sand/peat mixture. Soil in the pots was (a) uninfested and non-flooded, (b) uninfested and flooded, or flooded and infested with (c) Pythium. irregulare C6, (d) Phytophthora. megasperma, avirulent isolate M224, (e) P. irregulare C6/Ph. megasperma, avirulent M224 (f) Ph. megasperma, virulent M333 (g) P. irregulare C6/Ph. megasperma, virulent M333

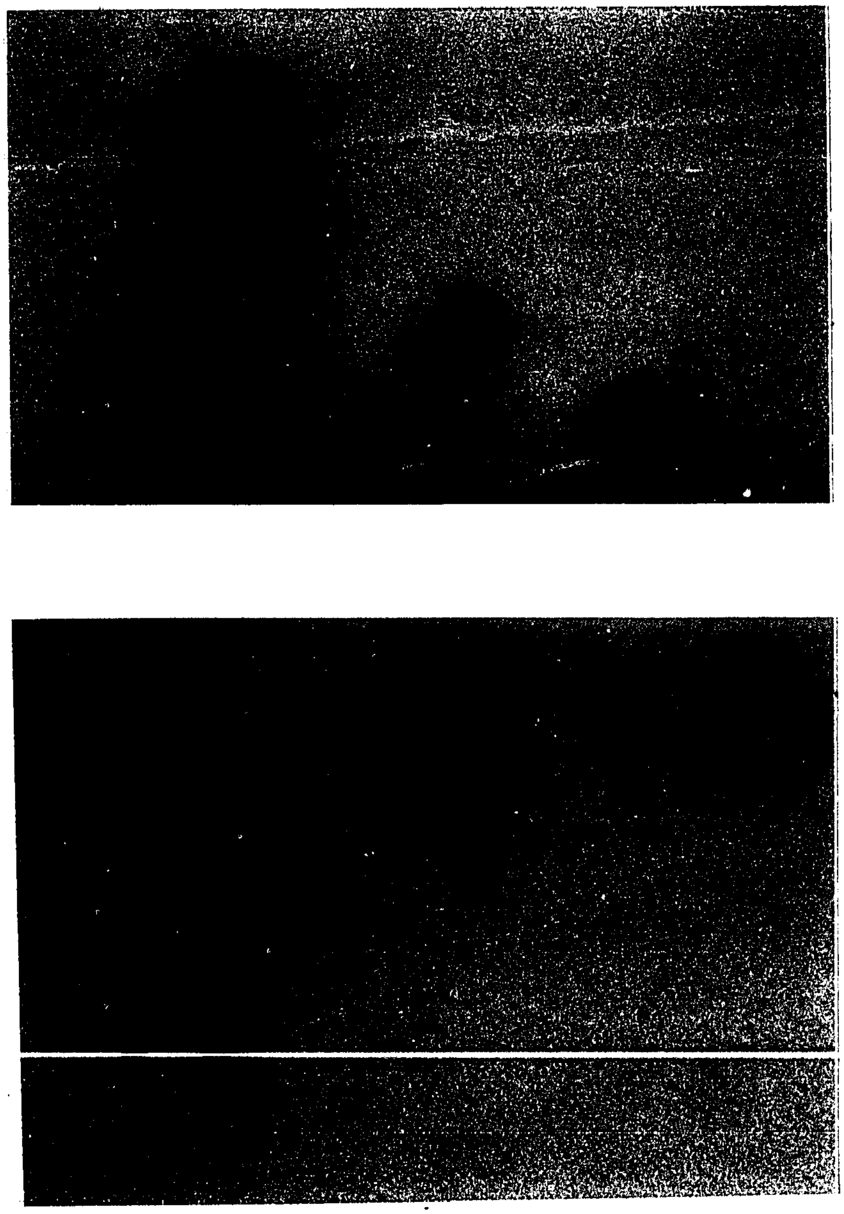

Figure 3. Shoots (A) and roots (B) of representative Mahaleb seedlings grown in a greenhouse for 3 mo in a soil/sand/peat mixture. Soil in the pots was (a) uninfested and flooded, or flooded and infested with (b) *Pythium*. *irregulare* C6, (c) *Phytophthora*. *cactorum* B2, (d) *P*. *irregulare* C6/*Ph*. *cactorum* B2.

a *Phytophthora* sp., 1-3 seedlings were dead at the end of the experiment, except no seedlings were killed in treatments involving *Ph*. *megasperma* M224 (Table 5).

Soil populations of *P*. *irregulare* J210 averaged 250 propagules per gram of soil for the unflooded treatment and 270 propagules per gram of soil for the flooded treatment. *P*. *irregulare* was not reisolated from soil taken from the uninoculated treatment, but *P*. *irregulare*, *Ph*. *megasperma*, and *Ph*. *cactorum* were reisolated from the single and combined treatments (Table 6).

DISCUSSION

There was an universal association of *Pythium* species and particularly of *P*. *irregulare* with sour cherry trees located on heavy soils but not with trees located on sandy soils, and *Pythium* populations in heavy soils were high compared to populations in sandy soils. *Pythium* species have been associated with peach tree decline in the southern United States (4,8). One study correlated *Pythium* populations with decline symptoms (4), while another using total *Pythium* found no relationship (8). *P*. *irregulare* failed to kill Mahaleb cherry seedlings in greenhouse and growth chamber trials suggesting that *Pythium* is not the primary cause of death of cherry trees in Michigan. However *P*. *irregulare* consistently caused reductions in shoot and root growth in these trials indicating that *P*. *irregulare* is a pathogen of cherry and may contribute to the reduction in growth of

Table 6. Reisolation of *Pythium irregulare* and two *Phytophthora* species from Mahaleb seedlings (experiment 2) grown for 15 weeks with periodic flooding

Fungus and isolate number		Reisolations[a] *P. irregulare*[b]	Reisolations[a] *Phytophthora*[c]
P. irregulare	J210	8/8	0/8
Ph. megasperma	M224	0/8	5/8
Ph. megasperma / *P. irregulare*	M224/ J210	7/7	3/7
Ph. megasperma	M333	0/5	5/5
Ph. megasperma / *P. irregulare*	M333/ J210	6/6	4/6
Ph. cactorum	B2	0/6	6/6
Ph. cactorum / *P. irregulare*	B2/ J210	6/6	6/6
Control		1/8	0/8

[a]Fraction of Mahaleb seedlings from which *P. irregulare* or *Phytophthora* were reisolated.
[b]Reisolations were made on CMA-PARP selective medium.
[c]Reisolations were made on *Phytophthora* selective medium.

cherry trees.

Recently Bielenin and Jones (1) identified _Ph. megasperma_, _Ph. cactorum_, _Ph. cambivora_ (Petri) Busiman, _Ph. cryptogea_ Pethyb. and Laff., and _Ph. syringae_ (Kleb.) Kleb. as causal agents in the decline and death of sour cherry trees in Michigan located on sites with poorly drained soils. In the present study, infection by _P. irregulare_ in combination with _Ph. megasperma_ and _Ph. cactorum_ did not increase the death of seedlings over _Phytophthora_ alone. In single and combined treatments reductions in dry weight were similar for _P. irregulare_ and _Phytophthora_ indicating that competition for infection sites may have occurred between the genera in the combined treatments. In mature trees _P. irregulare_ appears to be confined to the lateral roots (7), while _Ph. cactorum_ is associated with the crown (1,14) and _Ph. megasperma_ with the lateral and main roots (1,11,14).

Pythium has been implicated in the apple replant disease (5,12) and has been repeatedly shown to cause discoloration and necrosis of lateral roots, and inhibition of root development of apple seedlings in greenhouse inoculation trials. In the present study, necrosis of roots and a statistically significant reduction in growth were demonstrated in inoculation trials with Mahaleb cherry. Root necrosis and reduced weights were obtained even when seedlings were inoculated but not flooded, although the reduction in growth and deterioration of young roots were greater with flooding. However, there was no indication of a replant problem in the orchards studied here, as growth of the young trees was good for the first few years. Stunting, decline and death of the trees developed when the trees came into bearing.

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PART II

SPECIES AND POPULATIONS OF *PYTHIUM* ISOLATED FROM SOUR CHERRY ORCHARD AND WOODLAND SOILS IN MICHIGAN

ABSTRACT

Pythium populations were high (54.3 to 746.4 viable propagules per gram of soil) in eight orchards on clay soil and low (1.2 to 3.5 propagules per gram of soil) in four orchards on sandy soil sampled in western Michigan in 1984-1985. Populations were also high in two woodlands adjacent to orchards in 1986, and in six woodlands in western and central Michigan in 1987. Orchards and woodlands in Michigan were found to differ in composition of _Pythium_ species. In soil from orchards, _P_. _irregulare_ was isolated most frequently (47% of _Pythium_ propagules), followed by _P_. _sylvaticum_ (26%), _P_. _ultimum_ (13%), _P_. _rostratum_ (6%), and _Pythium_ group 'HS' (8%). In soil from woodlands, _Pythium_ group 'HS' was isolated most frequently (60%) followed by _Pythium_ group 'X' (29%), _P_. _rostratum_ (2%), and _Pythium_ group 'Y' (9%). The first two species were isolated from soils of all woodlands, while the latter two species were isolated from two woodland sites. No _P_. _irregulare_, _P_. _sylvaticum_, or _P_. _ultimum_ were isolated from woodland soils. Populations of _P_. _irregulare_, _P_. _sylvaticum_, and _P_. _ultimum_ in two orchards sampled over 2 yr and one orchard over 1 yr, varied independently.

INTRODUCTION

Sour cherry (*Prunus cerasus* L. Montmorency) orchards in southwestern Michigan are planted on sites that formerly were beech-maple woodlands. It was observed that trees in orchards planted on heavy clay soils frequently exhibited decline symptoms of poor growth and decreased longevity and had root systems that exhibited extensive discoloration and decay of lateral roots. Trees in orchards on sandy soils grew well and had root systems with little discoloration of lateral roots. *Pythium irregulare* (Buisman) was frequently isolated from roots of trees with discolored and necrotic lateral roots, and isolates of *P. irregulare* reduced the growth of Mahaleb seedlings in pathogenicity trials conducted in the greenhouse and growth chamber (17). Seedlings of *Prunus mahaleb* L. are the most common rootstock used for propagation of cherry trees.

Although *Pythium* species are soilborne pathogens of a wide range of crops (6,19), they are poor competitors among an established soil microflora (2,6). Species common in cultivated soils are infrequently present in soils from undisturbed sites (18). *Pythium* species have been associated with peach tree decline (7,12). One study correlated *Pythium* populations with decline symptoms (7), while another using total *Pythium* found no relationship (11). *Pythium* species isolated from peach decline orchards in Georgia were also isolated from forest soils in the southeastern United States (4). There have been no reports of studies on the composition of populations of *Pythium* species in cherry orchard soils, nor have there been reports of comparisons in *Pythium* populations between cherry orchard soils and woodland soils.

The objectives of this study were to investigate the species and populations of *Pythium* in clay and sandy soils from cherry orchard sites and in soil from woodlands adjacent to orchards.

MATERIALS AND METHODS

Sampling sites. Eight commercial sour cherry orchards on clay soil exhibiting poor growth and two on sandy soil with healthy trees were sampled in 1984 and four orchards on clay soil and two on sandy soil were sampled in 1985. Each orchard was sampled once in early summer. In addition, two of the orchards on clay soil (orchards J and L) were sampled four times both in 1984 and in 1985, and a third orchard on clay soil (orchard P) was sampled four times in 1985. Two sour cherry orchards and adjacent woodlands were sampled once in early summer in 1986 and six woodlands were sampled once in October in 1987. The woodlands were composed primarily of beech (*Fagus grandifolia* Ehrh)., red maple (*Acer rubrum* L.), silver maple (*A. saccharinum* L.) and hard maple (*A. saccharum* Marsh). All sites were in Berrien and Van Buren counties of western Michigan except woodland sites

MSU F and MSU G were located in East Lansing (Ingham county) in central Michigan.

Isolation of Pythium species from soil. The populations of Pythium species were estimated by surface soil dilution plates (13). The isolation medium used was corn meal agar (CMA) (Difco) amended with 10 mg pimaricin, 250 mg ampicillin, 10 mg rifampicin, and 50 mg pentachloronitrobenzene (CMA-PARP) per liter (9). Four cores of soil were taken between the drip line and the trunk from each of 10 trees per orchard. Woodland sites were sampled like orchard sites, except 5 rather than 10 trees per site were sampled in 1987. The cores were taken to a depth of 15 cm with a Hoffer soil sampler and mixed for each tree in a plastic bag. The samples were transported to the laboratory in an ice chest and stored at 4 C until processed. Soil samples were air dried and then passed sequentially through 5- and 1.68-mm-mesh sieves. A 1 g subsample from each bulk sample was suspended in 0.2% water agar and dilution plated at 1:10, 1:50, and 1:100 on fresh CMA-PARP selective medium. Diluted samples (1 ml) were distributed over the surface with a sterile glass rod. Three replicate plates were made per subsample and per dilution (nine plates per tree). After 42 hr incubation in darkness at 21 C, the soil was removed by washing each plate under running tap water and the number of Pythium colonies counted.

Identification of *Pythium* species. Hyphal tips from 5 to 15 colonies per sample were transferred to CMA-PARP plates and then, after incubation for 48 hr at 21 C, to CMA. Growth rates were recorded after 24 hr incubation on CMA at 25 C. Fungal structures were examined after the cultures had grown for 1-2 wk. Identifications of the various *Pythium* species were made with the aid of the key of Van der Plaats-Niterink (19). Identification of the heterothallic species *P. sylvaticum* was confirmed by crossing each suspect isolate with strains ATCC 18195 and ATCC 18196 of *P. sylvaticum* on potato-carrot agar (19).

RESULTS

Pythium species identified. Identifications were based on colony morphology, growth rates, and charachteristics of sporangia, hyphal swellings, oogonia, antheridia, and oospores. *P. irregulare*, *P. sylvaticum* Campbell and Hendrix, *P. ultimum* Trow., *P. rostratum* Butler, and *Pythium* group 'HS' were as described by Van der Plaats-Niterink (19). Two groups of *Pythium* isolates could not be identified, as oogonia were not produced, even when crosses were made on potato-carrot agar. These were designated *Pythium* group 'X' and *Pythium* group 'Y'. Colony morphology was radiate on CMA although *Pythium* group 'X' had a variable rosette pattern. Growth rates of *Pythium* group 'X' and *Pythium* group 'Y' were 20 and 13 mm, respectively, in 24 hr at 25 C on CMA. *Pythium* group 'X' produced numerous terminal and intercalary hyphal swellings 20-25 m in diameter on CMA and in water. *Pythium* group 'Y' produced sparse, ovoid intercalary hyphal swellings on CMA.

Pythium populations in soil. Samples of soil from eight sour cherry orchards on clay soils had *Pythium* populations estimated at 54.3 to 746.4 propagules per gram of soil (Table 1). Samples from four orchards on sandy soil had very low *Pythium* populations estimated at 1.2 to 3.5 propagules per gram of soil. *P. irregulare* was isolated most consistently (47% of all propagules) from clay soils in both years, followed by *P. sylvaticum* (26%) and *P. ultimum* (13%). Estimates for these latter species were high in soils of some orchards but not in both years. *P. rostratum* (6%) and *Pythium* group 'HS' (8%) were found primarily in orchard N in 1984, and in orchard C in 1985.

The seven woodland sites had *Pythium* populations estimated at 180 to 1153 propagules per gram of soil (Table 2). *Pythium* group 'HS' and *Pythium* group 'X' were isolated from soil taken from each of the sites. *Pythium* group 'HS' was isolated most frequently (60%), followed by *Pythium* group 'X' (29%); *P. rostratum* (2%) was isolated from woodland J in 1986 and MSU F in 1987; and *Pythium* group 'Y' (9%) was isolated from woodland sites C and J in 1986 and site B in 1987.

Sequential isolations from soil taken from two orchards over two growing seasons and one orchard over one growing season showed that populations of *Pythium* species varied

Table 1. Populations of *Pythium* species as estimated by dilution-plate counts from soil taken from sour cherry orchards planted on two soil types

		Pythium population by species (propagules per gram (ppg))[a]						
Orchard code	Soil type	*P. irregulare*	*P. sylvaticum*	*P. ultimum*	*P. rostratum*	*P.* 'HS'	*P.* spp.	Total (ppg)
1984 growing season								
B	Clay	52.5	6.1	9.9	6.8	0.0	0.8	76.1
C	Clay	26.2	26.7	0.0	0.0	2.8	0.0	55.7
G	Clay	56.0	5.4	6.9	2.3	6.1	0.0	76.4
H	Clay	52.3	17.4	26.2	5.0	23.7	0.0	124.6
J	Clay	52.5	122.6	19.8	13.1	10.9	0.0	219.0
L	Clay	32.6	12.5	3.2	6.0	0.0	0.0	54.3
P	Clay	47.8	0.8	16.4	8.2	0.0	1.5	74.7
N	Clay	74.3	157.4	52.4	52.4	100.5	0.0	437.0
N	Sand	1.0	0.0	0.0	0.3	1.7	0.0	3.0
U	Sand	0.0	1.0	0.0	1.0	0.6	0.0	2.6
1985 growing season								
J	Clay	52.1	25.2	23.1	1.9	16.1	0.0	119.3
L	Clay	279.2	17.2	259.2	18.0	0.0	0.0	746.4
P	Clay	64.0	0.0	11.3	4.2	0.0	0.0	79.5
C	Clay	67.1	189.5	0.0	26.1	160.5	0.0	443.2
C	Sand	0.6	0.6	0.0	0.0	0.0	0.0	1.2
RW	Sand	1.5	0.0	1.8	0.3	0.0	0.0	3.5

[a]Each value is the mean of ten soil samples per orchard. Each soil sample is the composite of four sub-samples collected around one tree. Sites were sampled on one occasion.

Table 2. Populations of *Pythium* species as estimated by dilution-plate counts from soil taken from sour cherry orchards and woodlands

Site	Soil type	*Pythium* population by species (propagules per gram (ppg))[a]							
		P. irregulare	*P. sylvaticum*	*P. ultimum*	*P. rostratum*	*P.* 'HS'	*P.* 'X'	*P.* 'Y'	Total (ppg)
May 1986									
C orchard	Clay	46	50	0	11	123	0	0	240
C woodland	Clay	0	0	0	0	768	238	147	1153
J orchard	Clay	72	47	30	0	26	0	6	181
J woodland	Clay	0	0	0	51	426	165	258	900
October 1987 Woodland sites									
B[b]	Sand	0	0	0	0	204	34	102	340
C[b]	Clay	0	0	0	0	262	168	0	420
H[b]	Sand	0	0	0	0	130	130	0	260
L[c]	Clay	0	0	0	0	88	132	0	260
MSU F[b]	Sandy loam	0	0	0	18	126	36	0	180
MSU G[b]	Sandy loam	0	0	0	0	504	56	0	560

[a]Each value is the mean of ten soil samples taken in 1986 and five soil samples in 1987. Each soil sample is the composite of four subsamples collected around one tree.
[b]Sites were adjacent to sour cherry orchards.
[c]Site was not close to a cherry orchard.

independently of each other (Figure 1-3). Estimates of total *Pythium* populations in soil from orchard J fluctuated from a low of 107.0 propagules per gram in July 1984 to a high of 313.2 propagules per gram in October 1985 (Figure 1); from orchard L, from 54.3 propagules per gram in May 1984 to 1009.0 propagules per gram in October 1985 (Figure 2); and from orchard P, from 79.5 propagules per gram in May 1985 to 351.1 propagules per gram in August 1985 (Figure 3).

DISCUSSION

Soils from orchards and from woodlands in Michigan were found to differ in their composition of *Pythium* species. *P. rostratum* and *Pythium* group 'HS' were isolated from both habitats, indicating that these species were competitive in both cultivated and woodland soils. *Pythium* group 'HS' was the most frequent isolate from woodland, parkland, and farmland soils in England (1,5), however its pathogenicity to crop plants has not been investigated. *P. rostratum* has been isolated from a variety of habitats (1,5,18), has a worldwide distribution, and has been found to be non-pathogenic or weakly pathogenic on a wide variety of hosts (19). *P. irregulare*, *P. sylvaticum*, and *P. ultimum* were isolated from soil from orchards but were not detected in soil from woodlands. All three species have worldwide distributions and have been isolated from a wide variety of

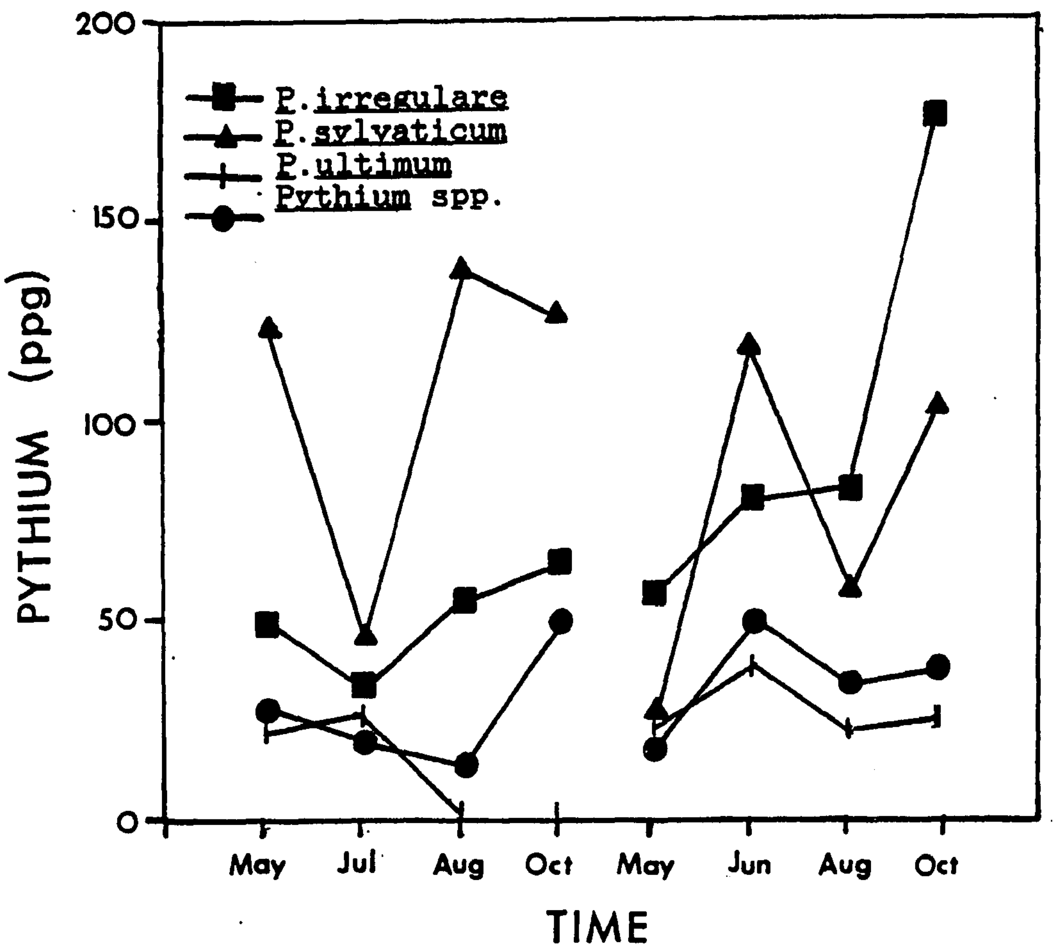

Figure 1. Fluctuations in population (propagules per gram of soil) of *Pythium* species as estimated by dilution-plate counts for soil samples taken from sour cherry orchard J in southwest Michigan during 1984 and 1985. Species identified were *P. irregulare*, *P. sylvaticum*, *P. ultimum*, and *Pythium* spp..

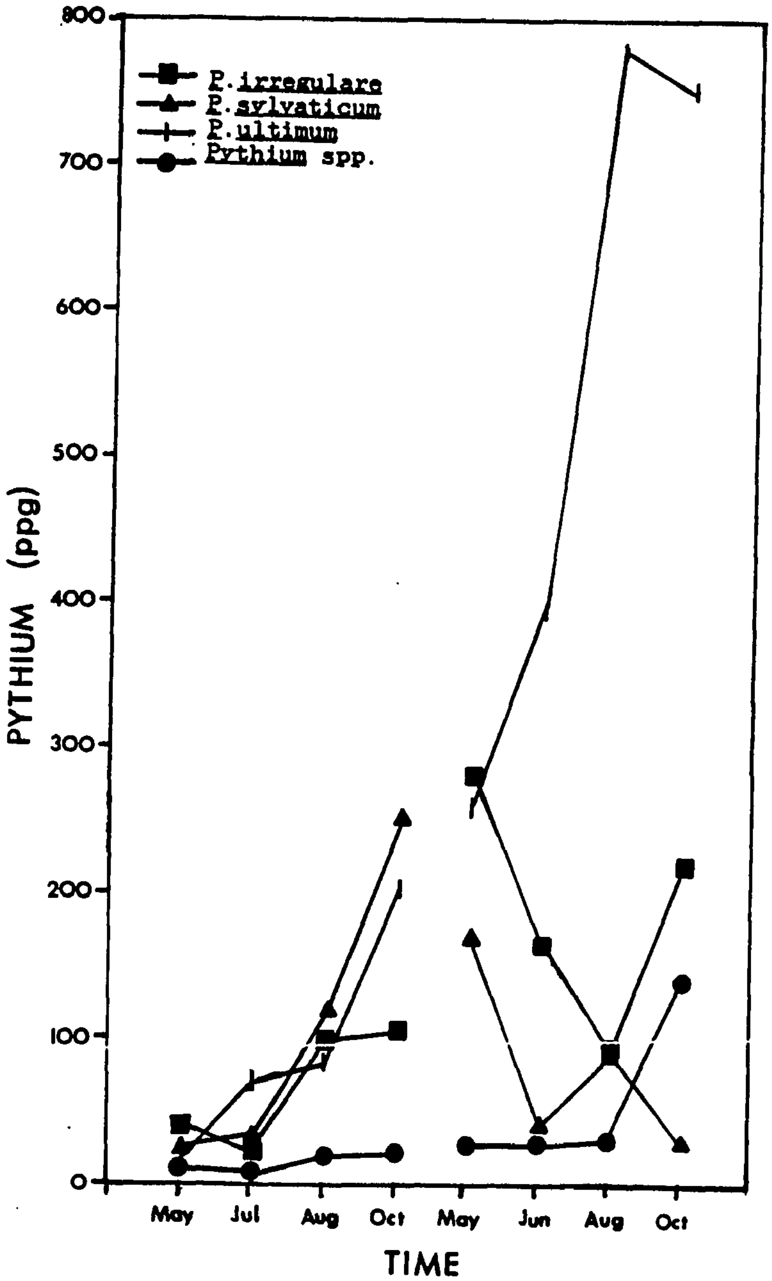

Figure 2. Fluctuations in population (propagules per gram of soil) of <u>Pythium</u> species as estimated by dilution-plate counts for soil samples taken from sour cherry orchard L in southwest Michigan during 1984 and 1985. Species identified were <u>P</u>. <u>irregulare</u>, <u>P</u>. <u>sylvaticum</u>, <u>P</u>. <u>ultimum</u>, and <u>Pythium</u> spp..

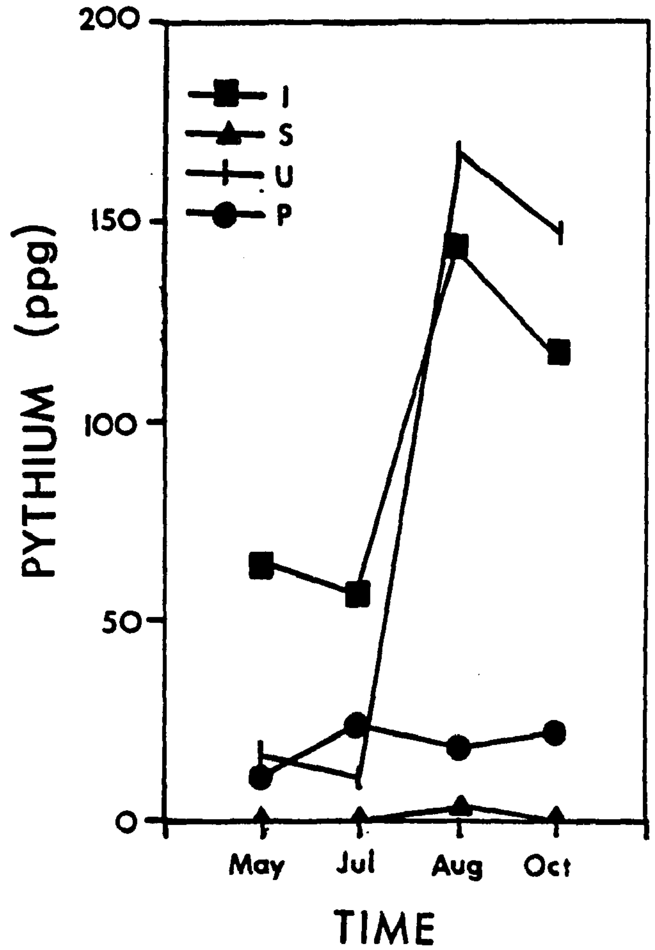

Figure 3. Fluctuations in population (propagules per gram of soil) of *Pythium* species as estimated by dilution-plate counts for soil samples taken from sour cherry orchard P in southwest Michigan during 1985. Species identified were I) *P. irregulare*, S) *P. sylvaticum*, U) *P. ultimum*, and P) *Pythium* spp..

hosts (19). Among deciduous orchard crops, *P. irregulare* is reported as pathogenic to apple (8,16), to peach (7,12), and to cherry (17); *P. sylvaticum* to apple (16); and *P. ultimum* to apple (3) and to peach (7,12).

The *Pythium* species found in soils from seven woodland sites were similar regardless of soil type, proximity to orchard sites, and location in Michigan. The predominant species of trees in all woodland sites were beech and maple, indicating that the areas had previously belonged to the beech-maple climax community, which was common over southern Michigan. The trees in the sites had obviously previously been clean cut or selectively felled, and the sites showed varying degrees of disturbance, but had never been used as orchard sites. There appears to be a stable association between the isolated *Pythium* species and the beech/maple community as indicated by the similarity between the *Pythium* species isolated from diverse counties in Michigan.

Populations of *Pythium* species in soils taken from sour cherry orchards were high in clay soils and low in sandy soils. The physical and chemical properties of soil affect *Pythium* populations. Pythium populations were reported to be higher under conditions of high soil moisture (1, 11, 18) and high soil organic content (10), and lower under acid conditions (5). Clay soils favor retention of moisture, and soil organic matter in the soils of orchards was increased due to mechanical cultivation.

Populations of *Pythium* species from orchards on clay soils varied over time. Populations of Pythium species in bean field soils were reported to vary independently in response to temperature (10,14), and incorporation of organic matter (10). The predominant species in woodland, parkland, and farmland soils in England were reported to show seasonal fluctuations in population (1).

This study has practical implications because P. irregulare was found to reduce the growth of Mahaleb seedlings in greenhouse and growth chamber studies. P. irregulare appears at 10 fold greater population densities in soil from orchards on clay soils than on sandy soils (17). It may limit the growth of cherry trees planted on sites with clay soil. One method for avoiding this possibility would be to establish new cherry orchards on sandy, well drained sites. Additionally the pathogenic Pythium species were not detected in woodland soils even on clay soils, so on newly cleared sites on clay soils it may be possible to prevent the introduction of these Pythium species. However it is possible that these species are present in woodlands at undetectable levels and could increase under cultivation. This is in contrast to root rot caused by Armillaria species which are endemic on pine and oak and cause disease in orchards planted on sites cleared from these woodlands in Michigan (12).

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PART III

EFFECTS OF METALAXYL AND FOSETYL-AL ON GROWTH OF *PHYTOPHTHORA* AND *PYTHIUM* SPECIES 'IN VITRO' AND ON THEIR PATHOGENICITY TO MAHALEB CHERRY

ABSTRACT

Seven pythiaceous species associated with roots of sour cherry in Michigan were tested for their sensitivity to metalaxyl and phosphorus acid in culture. All fungi were inhibited by both fungicides and sensitivity varied between species. The ED_{50} values for metalaxyl and phosphorus acid, respectively were: *Phytophthora cactorum*, 0.06 and 7.5; *Ph. citricola*, 0.17 and <7.5; *Ph. drechsleri*, 0.26 and 27.0; *Ph. megasperma*, 1.52 and 49.5; *Pythium irregulare*, 0.49 and 80.0; *P. sylvaticum*, 0.5 and 78.1; and *P. ultimum* 0.06 and 78.7 μg/ml. Phytotoxic symptoms were observed on 1-yr-old Mahaleb seedlings at 400 and 800 μg/ml metalaxyl. Significant (*P*=0.05) reductions in shoot and root dry weight occurred with 9-wk-old Mahaleb seedlings growing in soil infested with *P. irregulare*, *Ph.megasperma*, and *Ph. cactorum* in a growth chamber. Metalaxyl was more effective than fosetyl-Al in preventing fungus induced necrosis and growth reduction.

INTRODUCTION

Root rot of sour cherry trees occurs in orchards planted on poorly drained soils in Michigan. *Phytophthora* species are considered to be the primary causal agents of cherry root and crown rot in Michigan (2), California (22), and New York (29). *Pythium* species, and *P. irregulare* in particular, were found to be associated with lateral root necrosis of cherry trees, and caused reductions in growth of Mahaleb seedlings in greenhouse trials (M. L. Smither, unpublished).

Metalaxyl is an acylalanine fungicide and it has been used commercially and experimentally to control diseases induced by Phytophthora (1,5,11,13,15,28) and damping-off and blight induced by Pythium (23,26). Variations in sensitivity to metalaxyl occur between species (6,8,17,21), and thus variations in the degree of control exerted by metalaxyl can be expected on the Phytophthora and Pythium species associated with cherry decline. The development of resistance to metalaxyl has occurred (4,10).

Fosetyl-Al (aluminium tris(O-ethyl phosphonate)) has been used effectively to control diseases induced by Phytophthora (11,13,16,24). High concentrations of fosetyl-Al are required to inhibit Phytophthora 'in vitro'. Fenn and Coffey convincingly demonstrated that the effective fungicidal agent is the breakdown product phosphorous acid (18,19). As with metalaxyl variations in sensitivity occur between species (7,8,18). The development of resistance to phosphorus acid has been demonstrated experimentally (3).

The objectives of this study were to test the sensitivity of pythiaceous species isolated from the roots of cherry to metalaxyl and phosphorus acid in culture, and to determine the effectiveness of metalaxyl and fosetyl-Al for the control of root rot of Mahaleb cherry seedlings caused by various pythiaceous species.

MATERIALS AND METHODS

Sensitivity of species to metalaxyl and fosetyl-Al in culture. Single isolates of *Phytophthora cactorum* (B1) (Leb. & Cohn) Schroet., *Ph. citricola* (H1) Sawada, *Ph. drechsleri* (B1) Tucker, *Ph. megasperma* (B1) Drechsler, *Pythium irregulare* (L215) Buisman, *P. sylvaticum* (C201) Campbell and Hendrix, and *P. ultimum* (B302) Trow. isolated from cherry orchards in Michigan were tested for their sensitivity to phosphorous acid and metalaxyl in culture. The fungal isolates were grown for 5 days on corn meal agar (CMA). Disks 5 mm in diameter were taken from the colony margin and placed in the center of six replicate plates amended with metalaxyl or phosphorous acid. Concentrations of metalaxyl were 0, 0.01, 0.1, 1, 10, and 100 μg/ml. The experiment was repeated. Concentrations of phosphorous acid were 0, 10, 50, 100, 250, and 500 μg/ml in an initial experiment. The experiment was repeated twice at rates of 0, 10, 25, 50, 75, and 100 μg/ml. The plates were stored in darkness at 21 C and radial colony growth was measured daily for 7 days. ED_{50} values were calculated from linear regression lines obtained by plotting the mean percent inhibition of mycelial growth against the concentration of metalaxyl and phosphorus acid.

Fungicide trials. Fungal inoculum for the three fungicide trials was prepared by growing the isolates at 21 C for 5 wk on 200 cc vermiculite moistened with 100 ml V-8 juice solution (200 ml V-8 juice, 2 g $CaCO_3$, and 800 ml distilled water) sterilized in 500 ml Erlenmeyer flasks (22). The inoculum was washed with distilled water over cheesecloth in a Buchner funnel to remove unassimilated nutrients and mixed at the rate of 10 cc/1000 cc with sterilized soil/sand/peat (1/1/1). Controls recieved the vermiculite mixture but no fungus.

Experiment one was conducted in a greenhouse with dormant 1-yr-old Mahaleb seedlings planted directly into soil infested with isolates of *Ph. cactorum* (B1), *Ph. citricola* (H1), *Ph. megasperma* (CA), *P. irregulare* (C6), and *P. ultimum* (G7). The experimental units consisted of three seedlings in 4 L containers, which were arranged as randomised complete blocks with six replications. The maximum daytime temperatures were 24 C, nighttime minimum was 18 C, with 14 hr of light. Fungicide solutions were applied to the soil after 8 days at rates of 0, 10, 25, 100, 400, and 800 μg/ml metalaxyl in 250 ml distilled water. The experiment was terminated after 3 mo. Dry weights of roots and shoots were recorded.

Experiment two was conducted outside, shaded by lath, with dormant 1-yr-old Mahaleb seedlings planted directly into soil infested with isolates of *Ph. cactorum* (B1), *Ph. citricola* (H1), *Ph. megasperma* (CA), *P. irregulare* (L215), and *P. sylvaticum* (C201). The experiment was set up as for experiment one. Fungicide treatments were applied 6 wk after planting. Metalaxyl was applied at 100μg/ml in 250 ml distilled water as a soil drench. Fosetyl-Al was sprayed onto the foliage to run-off with a hand sprayer, at rates of 1.5 and 3.0 g a.i. L. The experiment was terminated after 3 mo. Dry weights of roots and shoots were recorded.

Experiment three was conducted in a growth chamber using 9-wk-old Mahaleb seedlings, and isolates of P. irregulare (C6), Ph. cactorum (B2) and Ph. megasperma (B1). Combined Phytophthora and P. irregulare treatments used 10 cc of fungal inoculum from each isolate. Seedlings were planted in 1 L pots with eight replicates. Metalaxyl was applied at the rate of 70 μg a.i./ml in 50 ml distilled water, as a soil drench after the seedlings had been transplanted. Fosetyl-Al was sprayed onto the foliage to run-off with a hand sprayer at a rate of 4 g a.i./L. Fungicides were reapplied at monthly intervals. Maximum daytime temperatures were 21 C with 14 hr of light. The nighttime minimum was 18 C. The seedlings were flooded for 48 hr every 2 wk and fertilized biweekly. Dry weights of the roots and shoots were recorded after 10 wk. This experiment was repeated in a greenhouse.

RESULTS

Sensitivity of species to metalaxyl and phosphorous acid in culture. Rates of fungal growth were reduced with increasing metalaxyl concentration (Figure 1). Growth of Ph. cactorum and P. ultimum was completely inhibited above 1 μg/ml. Growth of P. irregulare and P. sylvaticum at 10 and 100 μg/ml only occurred during the initial 24 hours. Slow growth of Ph. drechsleri and Ph. megasperma continued at 100 μg/ml. The ED_{50} values were lowest, 0.06 μg/ml, for Ph. cactorum and P. ultimum (Table 1). Ph. megasperma had the highest ED_{50} value, 1.52 μg/ml, of the fungi tested.

Rates of fungal growth were reduced with increasing phosphorous acid concentration (Figure 2) Growth of all isolates was prevented with 500 μg/ml. Ph. citricola was the most sensitive of the species tested, with an ED_{50} of <10 μg/ml. Ph. megasperma had the highest ED_{50} value, 49.5 μg/ml of the Phytophthora species tested. The three Pythium species were least sensitive with ED_{50} values between 78.1 and 80 μg/ml (Table 1). The experiments were repeated with similar results.

Fungicide trials. In experiment one, dry weights of Mahaleb seedlings for all fungal treatments were highest with metalaxyl concentrations from 10-100 μg/ml (Table 2). Phytotoxic symptoms of leaf margin necrosis occured at 400 and 800 μg/ml (Figure 3). A factorial analysis showed that

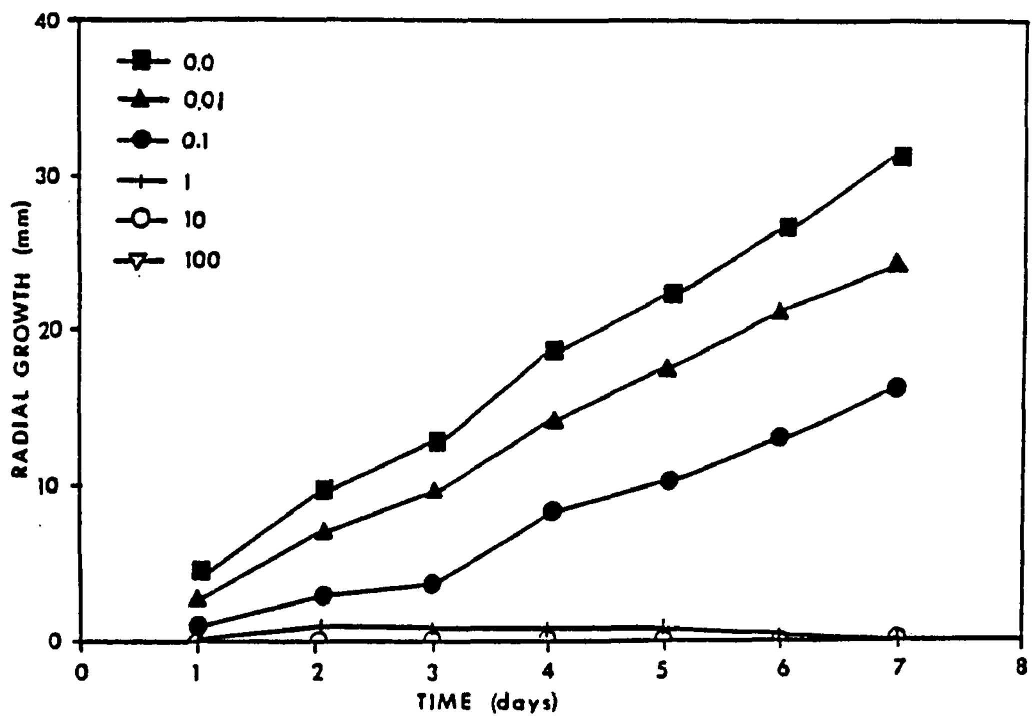

Figure 1a. Radial growth of Phytophthora cactorum in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

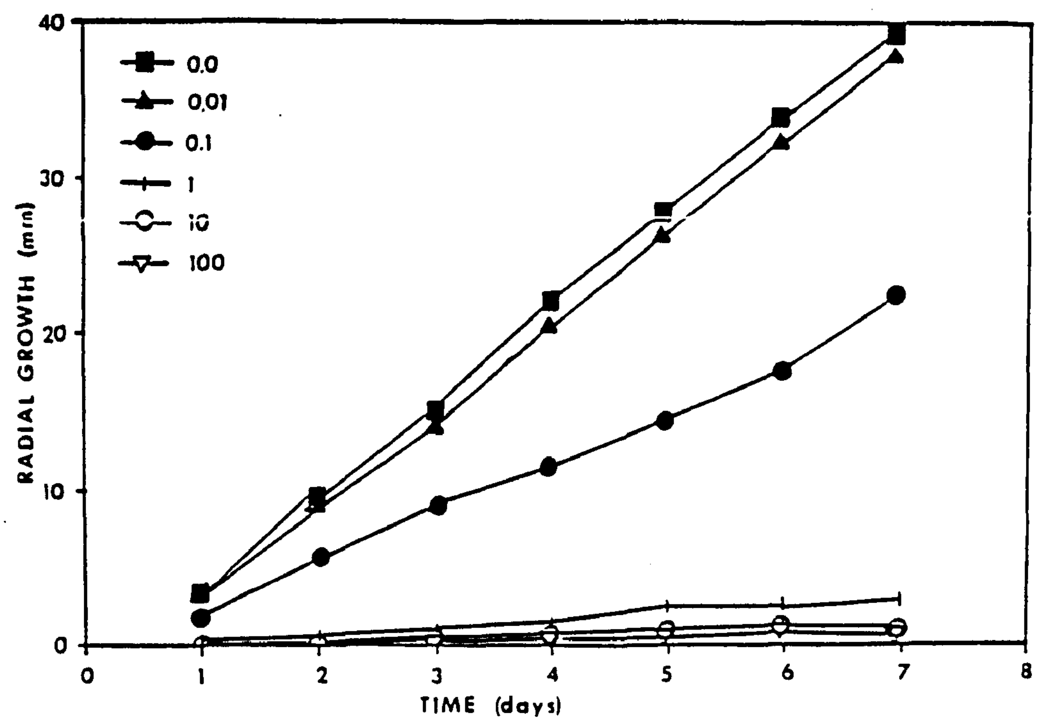

Figure 1b. Radial growth of Phytophthora citricola in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

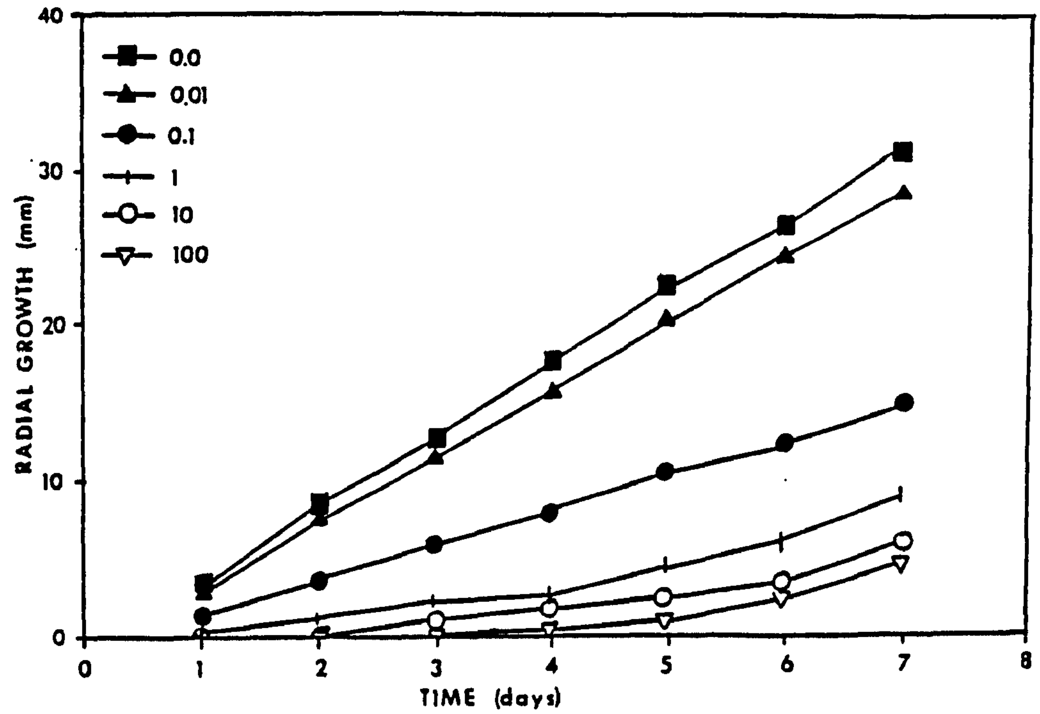

Figure 1c. Radial growth of <u>Phytophthora drechsleri</u> in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

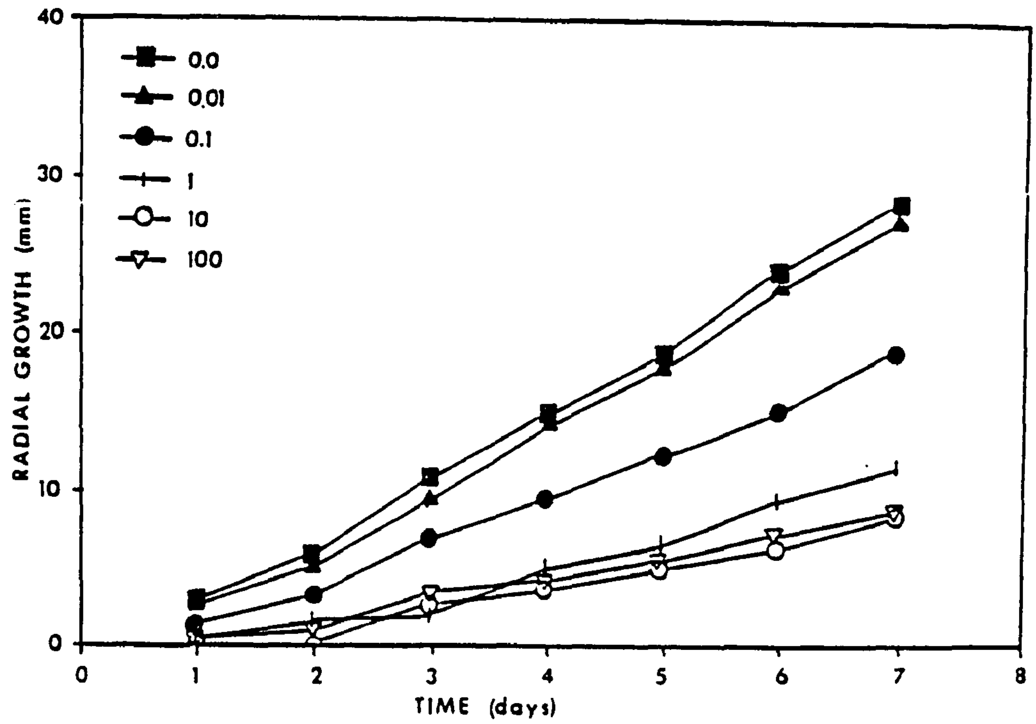

Figure 1d. Radial growth of <u>Phytophthora megasperma</u> in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

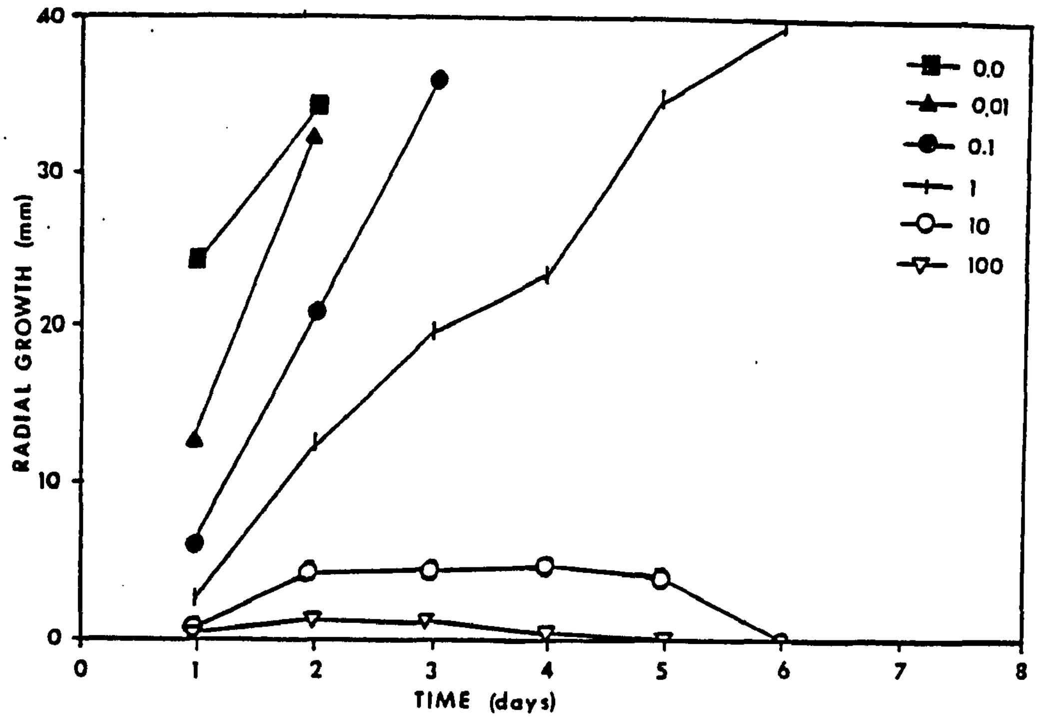

Figure 1e. Radial growth of <u>Pythium irregulare</u> in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

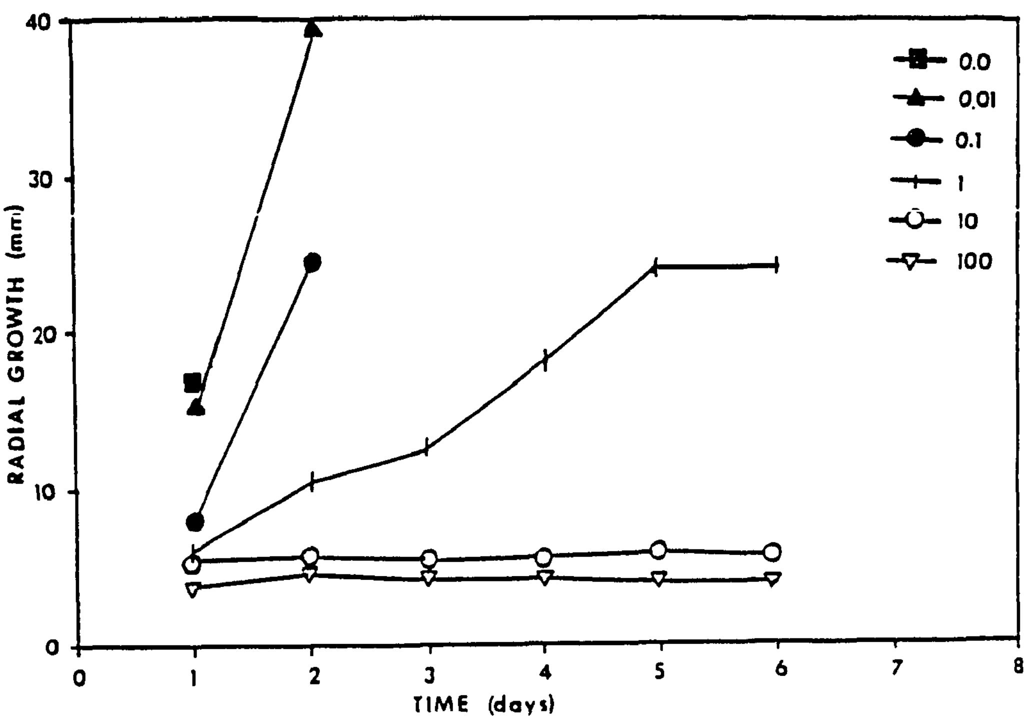

Figure 1f. Radial growth of <u>Pythium sylvaticum</u> in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

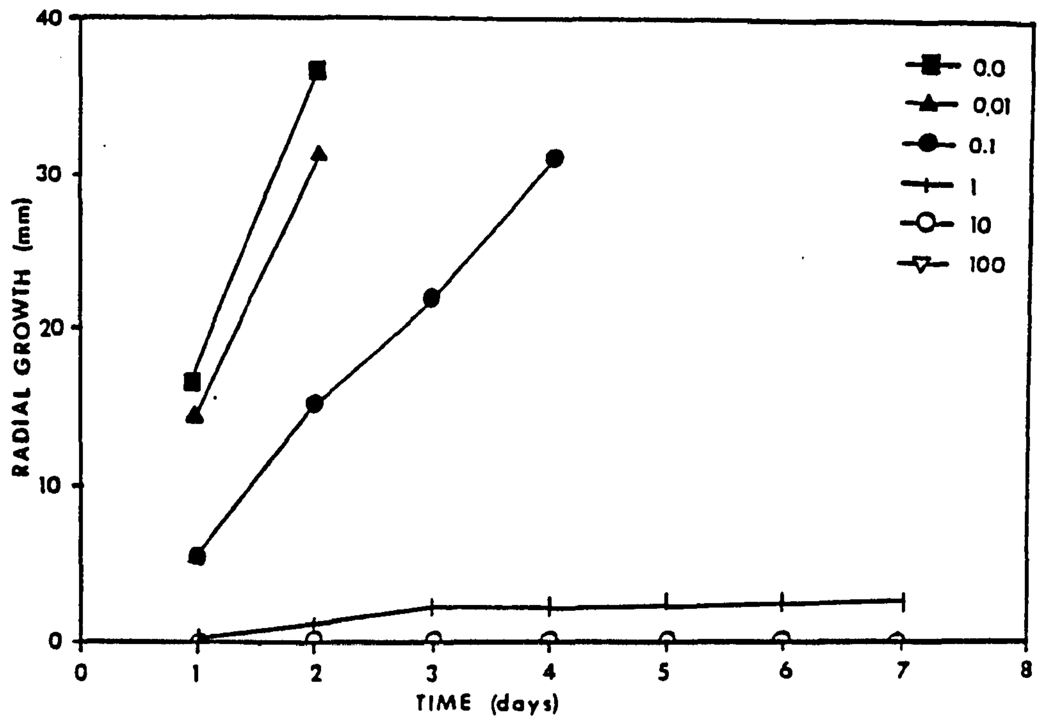

Figure 1g. Radial growth of <u>Pythium</u> <u>ultimum</u> in culture amended with metalaxyl at rates of 0.0, 0.01, 0.1, 1, 10, and 100 μg/ml.

Table 1. ED_{50} values for *Phytophthora* (*Ph.*) and *Pythium* (*P.*) species to metalaxyl and phosphorus acid in culture

	ED_{50} values	
Fungus	Metalaxyl $\mu g/ml$	Phosphorus acid $\mu g/ml$
Ph. cactorum	0.06[x]	7.5
Ph. citricola	0.17	<10[y]
Ph. drechsleri	0.26	27.0
Ph. megasperma	1.52	49.5
P. irregulare	0.49	80.0
P. sylvaticum	0.50	78.1
P. ultimum	0.06	78.7

[x]Values were calculated from means of six replicate plates. The experiment was repeated with similar results.

[y]The value was too low to be calculated by this concentration range.

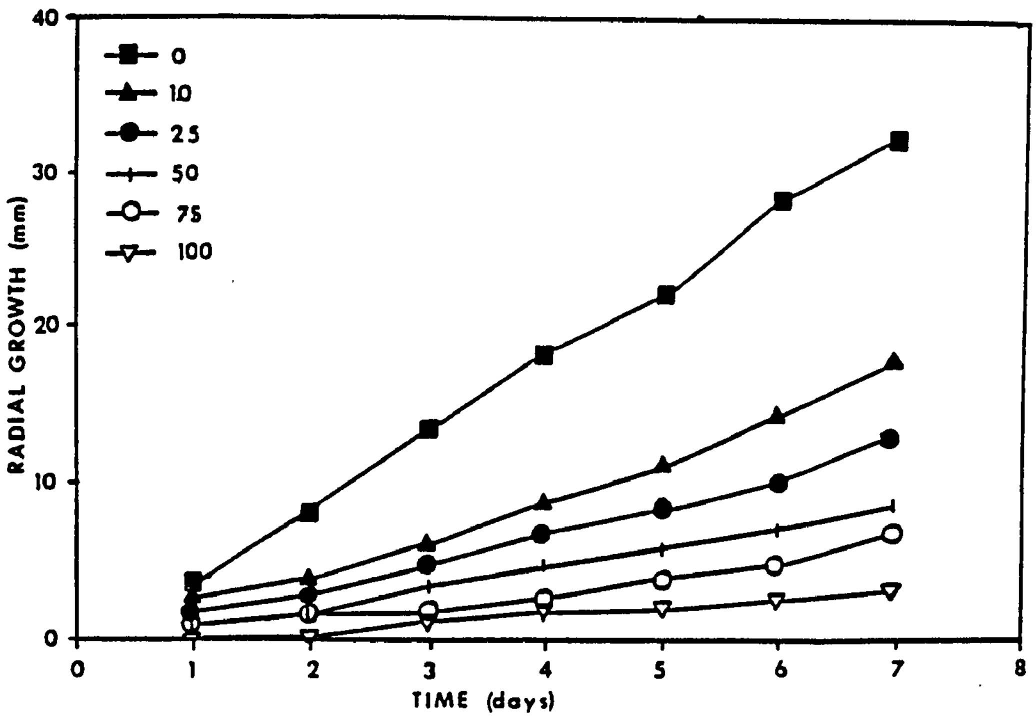

Figure 2a. Radial growth of <u>Phytophthora</u> <u>cactorum</u> in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 µg/ml.

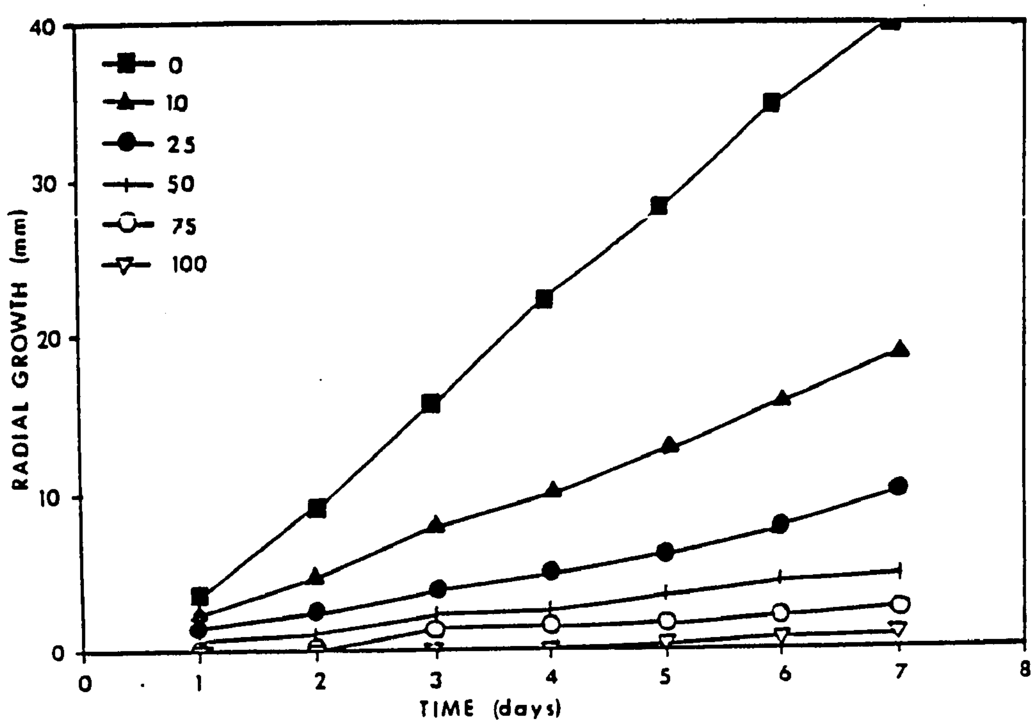

Figure 2b. Radial growth of <u>Phytophthora</u> <u>citricola</u> in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 µg/ml.

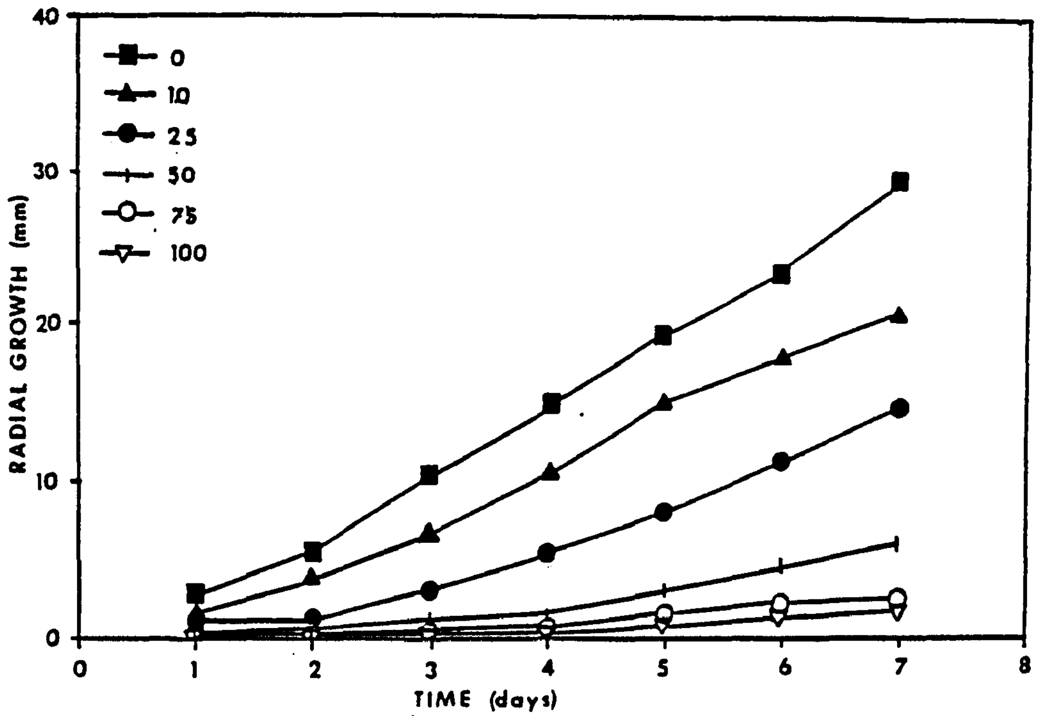

Figure 2c. Radial growth of Phytophthora drechsleri in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 $\mu g/ml$.

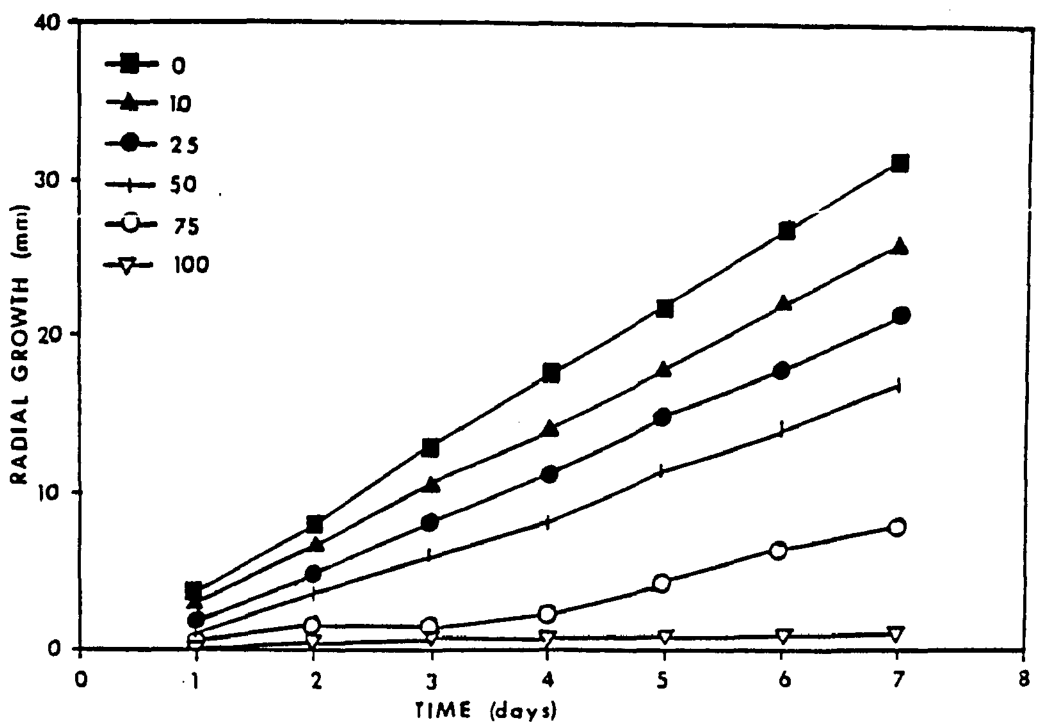

Figure 2d. Radial growth of Phytophthora megasperma in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 $\mu g/ml$.

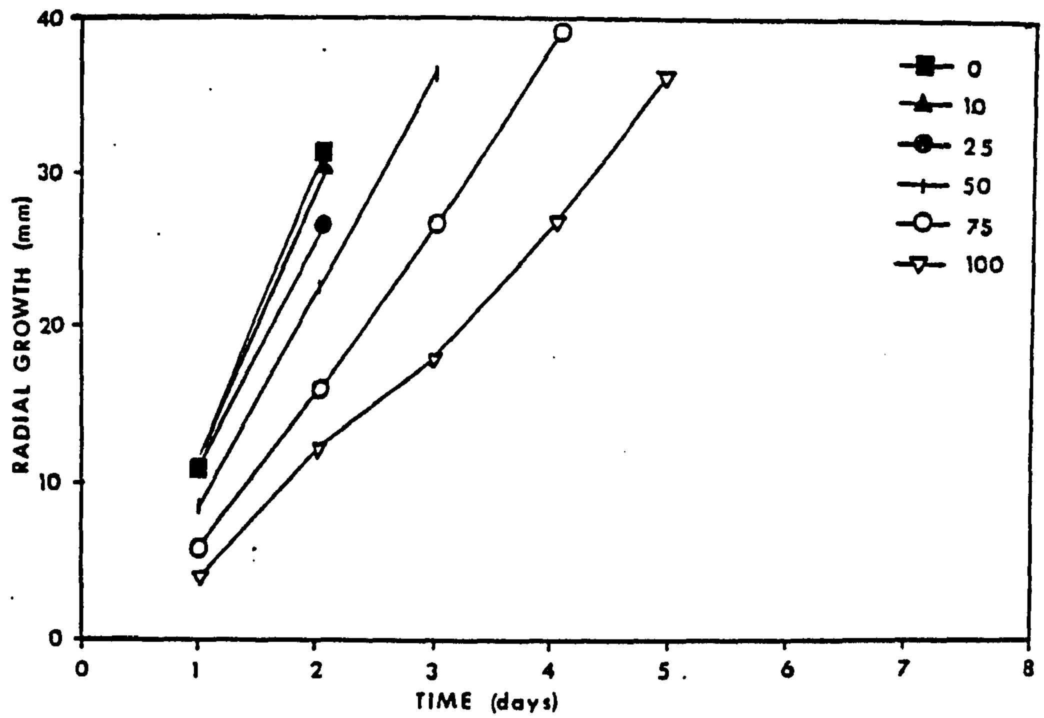

Figure 2e. Radial growth of Pythium irregulare in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 μg/ml.

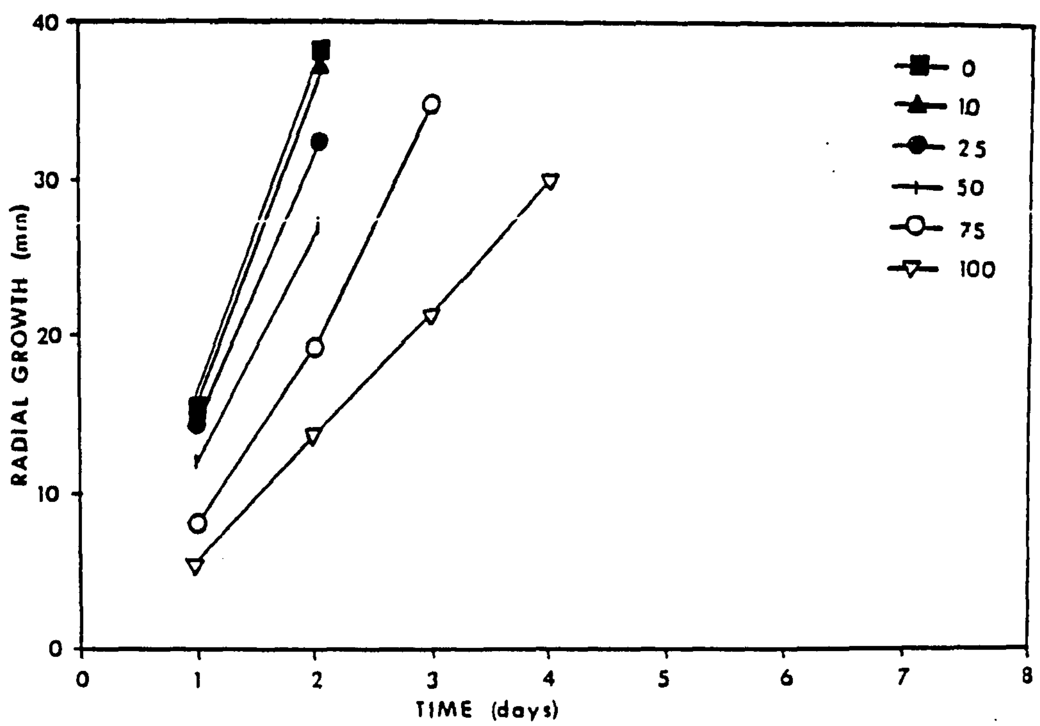

Figure 2f. Radial growth of Pythium sylvaticum in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 μg/ml.

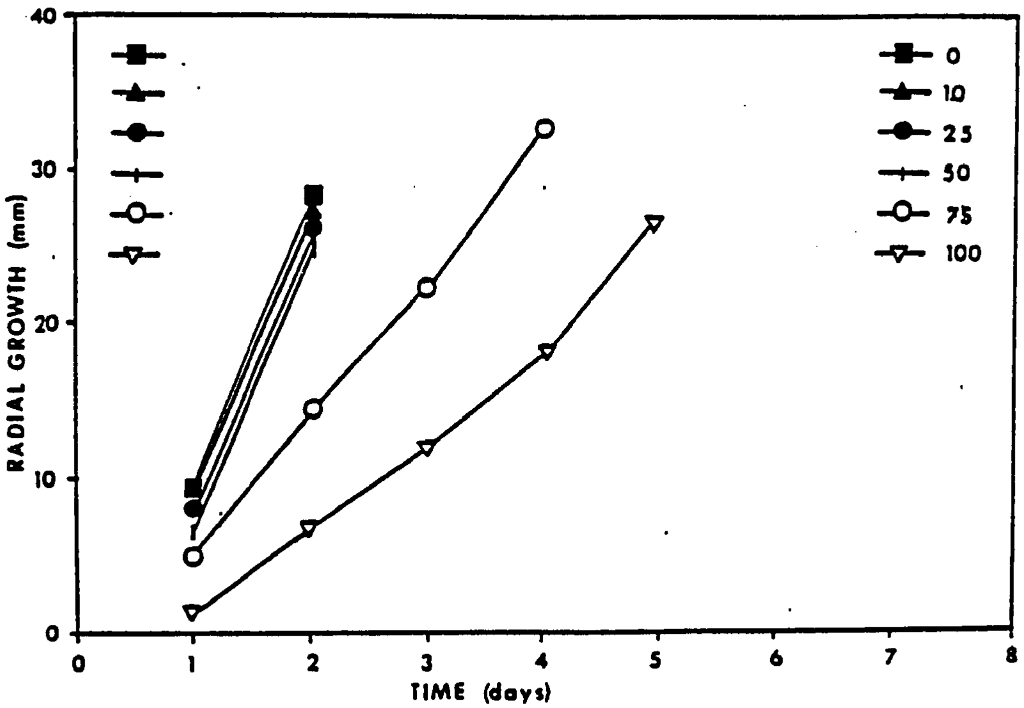

Figure 2g. Radial growth of <u>Pythium ultimum</u> in culture amended with phosphorous acid at rates of 0, 10, 25, 50, 75, and 100 μg/ml.

Table 2. Effect of various concentrations of metalaxyl on growth of 1-yr-old Mahaleb seedlings in *Phytophthora* and *Pythium* infested soil in the greenhouse

Concentration of metalaxyl[b] (µg/ml)	Shoot dry weight (g)[a]						Root dry weight (g)[a]					
	Control	*Ph. cactorum*	*Ph. citricola*	*Ph. megasperma*	*P. irregulare*	*P. ultimum*	Control	*Ph. cactorum*	*Ph. citricola*	*Ph. megasperma*	*P. irregulare*	*P. ultimum*
0	9.7 ab[c]	7.2 ab	6.8 b	13.2 a	7.7 ab	12.9 a	2.9 abc	1.3 c	2.1 a	3.4 ab	2.6 ab	3.8 a
10	11.0 ab	10.5 a	10.9 ab	11.9 a	9.8 ab	13.1 a	3.3 abc	3.1 ab	2.5 a	4.1 a	2.6 ab	3.8 ab
25	13.9 a	11.6 a	13.7 a	9.9 a	12.0 a	12.4 a	3.5 ab	3.7 a	3.3 a	2.0 a	3.6 ab	3.6 abc
100	13.9 a	8.9 a	10.7 ab	11.5 a	11.7 a	9.7 a	4.9 a	2.2 bc	2.1 a	3.0 a	4.1 a	2.2 abc
400	9.8 ab	8.8 a	9.3 ab	12.6 a	8.6 ab	8.9 b	2.3 bc	1.4 c	1.6 a	2.7 a	2.7 ab	1.6 c
800	8.0 b	7.9 a	7.1 b	9.7 a	6.4 a	8.7 ab	1.4 c	1.2 c	1.5 a	2.4 a	1.6 b	1.7 bc

[a]Dormant 1-yr-old Mahaleb seedlings were transplanted in soil infested with 10 cc of inoculum per 1000 cc of soil. The experiment was conducted in a greenhouse for 3mo. There were six replicates.

[b]Metalaxyl(250 ml per container) was applied as a soil drench

[c]Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test (P=0.05).

Figure 3. Phytotoxic symptoms caused by metalaxyl at rates of (a) 0, (b) 100, and (c) 400 $\mu g/ml$ on leaves of Mahaleb cherry.

there were no significant variations in shoot and root dry weights due to soil infesting fungi or metalaxyl, as there was a high degree of variability between experimental units. Analyses of variance calculated separately for each of the soil infesting fungi indicated that metalaxyl significantly (P=0.05) increased root dry weight of Mahaleb seedlings planted in soil infested with *Ph. cactorum*.

In experiment two, shoot and root dry weights of Mahaleb seedlings tended to be lower when grown in infested soil or treated with fosetyl-Al (Table 3). There were no significant variations in dry weight due to soil infesting fungi or the fungicides metalaxyl and fosetyl-Al as estimated by a factorial analysis.

In experiment three, *Ph. cactorum*, *Ph. megasperma*, and *P. irregulare*, alone or in combination all caused significant reductions in shoot and root dry weights (Table 4, Figure 4) A factorial analysis also found significant (P=0.05) differences between fungicide treatments. There were no significant interaction effects. Metalaxyl controlled the soil infesting fungi as shoot and root dry weights of metalaxyl treated seedlings grown in infested soil were not significantly reduced from the metalaxyl treated control. Fosetyl-Al reduced shoot and root dry weights of seedlings growing in uninfested soil. Dry weights of fosetyl-Al treated seedlings grown in infested soil were not significantly different from the treated control. The experiment was repeated with similar results.

Table 3. Effect of fosetyl-Al and metalaxyl on growth of 1-yr-old Mahaleb seedlings in *Phytophthora* and *Pythium* infested soil under lath

Treatment and rate	Shoot dry weight (g)[a]						Root dry weight (g)[a]					
	Control	*Ph. cactorum*	*Ph. citricola*	*Ph. megasperma*	*P. irregulare*	*P. sylvaticum*	Control	*Ph. cactorum*	*Ph. citricola*	*Ph. megasperma*	*P. irregulare*	*P. sylvaticum*
Control	7.1[b]	5.2	4.5	5.4	6.7	6.4	3.0	1.8	2.5	2.0	2.5	2.4
Fosetyl-Al[c] 1.5 g a.i./L	5.2	2.5	3.9	4.1	4.7	4.2	2.8	0.5	1.4	1.7	2.2	2.8
Fosetyl-Al[c] 3.0 g a.i./L	6.4	2.7	4.5	4.1	4.8	4.8	3.0	1.0	1.7	2.1	2.3	1.8
Metalaxyl[d] 100 µg/ml	6.6	5.1	2.9	4.1	5.6	5.5	2.6	2.0	1.0	1.6	3.0	2.6

[a] Dormant 1-yr-old Mahaleb seedlings were transplanted in soil infested with 10 cc of inoculum per 1000 cc of soil. The experiment was conducted outside, shaded by lath, for 3mo. There were six replicates.

[b] Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test ($P=0.05$).

[c] Fosetyl-Al was sprayed onto the foliage to run-off with a hand sprayer at a

[d] Metalaxyl (250 ml per container) was applied as a soil drench.

Table 4. Effect of fungicides metalaxyl and fosetyl-Al on growth of Mahaleb seedlings growing in soil infested with *Phytophthora* species and *Pythium irregulare*

Fungicides Treatment[a]	Dry weight (g) Control Shoot	Control Root	Metalaxyl[b] Shoot	Metalaxyl[b] Root	Fosetyl-Al[c] Shoot	Fosetyl-Al[c] Root
Control	2.7 a x[d]	1.4 a X	2.6 a x	1.2 a X	2.0 a x	1.0 a X
Ph. cactorum	1.0 b x	0.4 b X	1.7 a x	1.0 a X	1.3 a x	0.6 a X
Ph. megasperma	1.5 b x	0.8 b XY	1.7 a x	1.2 a X	0.8 a x	0.3 a Y
P. irregulare	1.0 b y	0.4 b Y	2.2 a x	1.2 a X	1.4 a y	0.7 a Y
Ph. cactorum/ *P. irregulare*	1.1 b y	0.5 b Y	2.8 a x	1.4 a X	1.3 a y	0.7 a Y
Ph. megasperma/ *P. irregulare*	1.1 b y	0.3 b Y	2.6 a x	1.6 a X	1.3 a y	0.5 a Y

[a]Six-wk-old Mahaleb seedlings were transplanted in soil infested with 20 cc of inoculum per 1000 cc of soil. The experiment was conducted in a greenhouse for 2 mo. There were eight replicates. Plants were flooded for 48 hr every 2 wk.

[b]Metalaxyl was applied at the rate of 70 μg a.i./ml in 50 ml distilled water, as a soil drench after the seedlings had been transplanted.

[c]Fosetyl-Al was sprayed onto the foliage to run-off with a hand sprayer at a rate of 4 g a.i./L.

[d]Values in columns (a,b) and rows (x,y and X,Y) followed by the same letter do not differ significantly according to Duncan's multiple range test (*P*=0.05). There was no interaction between the main effects according to a factorial analysis.

Figure 4. Roots of representative Mahaleb seedlings grown in a) uninfested soil, or soil infested with b) Phytophthora cactorum, c) Ph. megasperma and d) Pythium irregulare. Roots in columns were x) untreated, or treated with y) metalaxyl, and z) fosetyl-Al.

DISCUSSION

The fungal species associated with root rot of sour cherries in Michigan differed in their sensitivity to metalaxyl and phosphorous acid in culture. Of the fungi tested *Ph. cactorum* was the most sensitive to both fungicides. The high sensitivity of *Ph. citricola* to phosphorous acid confirms another report (7). The three *Pythium* species showed a similar low sensitivity to phosphorous acid. *P. ultimum* had a high sensitivity to metalaxyl, while *P. irregulare* and *P. sylvaticum* were less sensitive.

The fungicide trials showed that metalaxyl was more effective than fosetyl-Al in increasing shoot and root dry weights of plants growing in soil infested with *Phytophthora* species and *P. irregulare*. However at high concentrations of metalaxyl phytotoxic symptoms ocurred with reduced growth. Reductions of dry weights of plants growing in uninfested soil when treated with fosetyl-Al was probably due to stress caused by the chemical. The inconclusive results obtained from the first two experiments were probably due to uncontrolled temperatures during the course of the experiments, and previous contamination of the 1-yr-old Mahaleb seedlings with *Phytophthora* and *Pythium* species.

Effective disease control requires that a fungicide is present at the site of infection at a high enough concentration to inhibit or kill the invading organism. Diseases successfully controlled by metalaxyl include collar rot of apple (15) and foot rot of citrus (13,16,28,), where a canker is produced on the trunk and control is effected by concentration of the fungicide at the infection site. Metalaxyl has controlled root rot of woody plants when applied as a soil drench with nursery or container grown plants (1,5).

Control of the lateral root necrosis associated with cherry decline requires that the fungicide is present at an inhibitory concentration in the roots. Metalaxyl is strongly adsorbed to soil organic matter and in loam soils was found to stay in the top 7.5 cm (27). Metalaxyl is applied as a soil drench as no basipetal translocation occurs (14,30), and it is questionable if on the heavy soils of symptomatic orchards metalaxyl would reach all the feeder roots. Fosetyl-Al is applied as a foliar spray as basipetal translocation occurs (14,24), however it is not known if the concentrations reaching the feeder roots are high enough to prevent infection.

The longterm prospects for control of pythiaceous species with these chemicals are not good as there are several reasons for doubting the long term effectiveness of these chemicals. Species differ in their sensitivity to the fungicides, and as more than one species are commonly found together selection of the more tolerant species will occur. Resistance to metalaxyl has already occured, particularly with foliar pathogens (4,10), and resistance to fosetyl-Al has been demonstrated experimentally (3). There are reports of the failure of metalaxyl to control diseases induced by _Ph_. _megasperma_ and _Ph_. _drechsleri_ (20), and _Pythium_ species (12,25).

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APPENDICES

APPENDIX A

DESCRIPTIONS OF *PYTHIUM* SPECIES ASSOCIATED WITH CHERRY TREES

Colony morphology and growth rate. Colonies of Pythium irregulare Buisman, P. sylvaticum Campbell and P. ultimum Trow. were radiate on CMA and produced aerial mycelium. P. irregulare had a finer growth pattern with a maximum hyphal diameter of 6 μm. P. sylvaticum and P. ultimum had a maximum hyphal diameter of 11 μm. Growth rates on CMA at 21 C for P. irregulare, P. sylvaticum, and P. ultimum were 17, 20, and 18 mm in 24 hr, respectively. Colonies of P. rostratum Butler lacked aerial mycelium and grew in a chrysanthemum pattern at a rate of 8 mm in 24 hr. Colonies of Pythium group 'HS' were fine and radiate with no aerial mycelium and grew 12 mm in 24 hr.

Sporangia and hyphal swellings. The structures described here were produced on solid media and/or in a 1:1 mixture of sterile pond and distilled water. Observations and measurements were made on structures stained with cotton blue in lactophenol. Sporangia of P. irregulare were produced terminally or intercalary, varied in size from 12-25 μm in diameter, and were globose to ellipsoid, limoniform to barrel shaped (Figure 1a). Hyphal swellings of P. sylvaticum less than 30 μm in diameter, and intercalary (Figure 2a). Hyphal swellings of P. ultimum were globose, intercalery and less than 30 μm in diameter. Sporangia of P. rostratum were produced only in water and were intercalary. Hyphal swellings of Pythium group 'HS' were intercalary, numerous on CMA, and less than 30 μm in diameter. All structures germinated directly with one or more germ tubes or produced zoospores.

Characteristics of oogonia, antheridia, and oospores. P. irregulare, P. ultimum, and P. rostratum were homothallic. P. sylvaticum was heterothallic although some strains were self-compatible. Pythium group 'HS' produced no oogonia. Oogonia of P. irregulare were irregular with a variable number of blunt conical projections (Figure 1b). Oogonia of P. sylvaticum and P. ultimum were globose (Figure 2a, 3a, 3b). The number of antheridia per oogonium for P. irregulare, P. sylvaticum, and P. ultimum were 1-2, 2-4, and 1-3, respectively, and all were paragynous. Antheridia of P. sylvaticum were branched while those of P. ultimum were typically attached close to the oogonial stalk. Antheridia of P. rostratum were hypogynous and paragynous with 1-2 antheridia per oogonium. Oospores of P. irregulare, P. sylvaticum, and P. ultimum were aplerotic. Oospores of P. rostratum were plerotic.

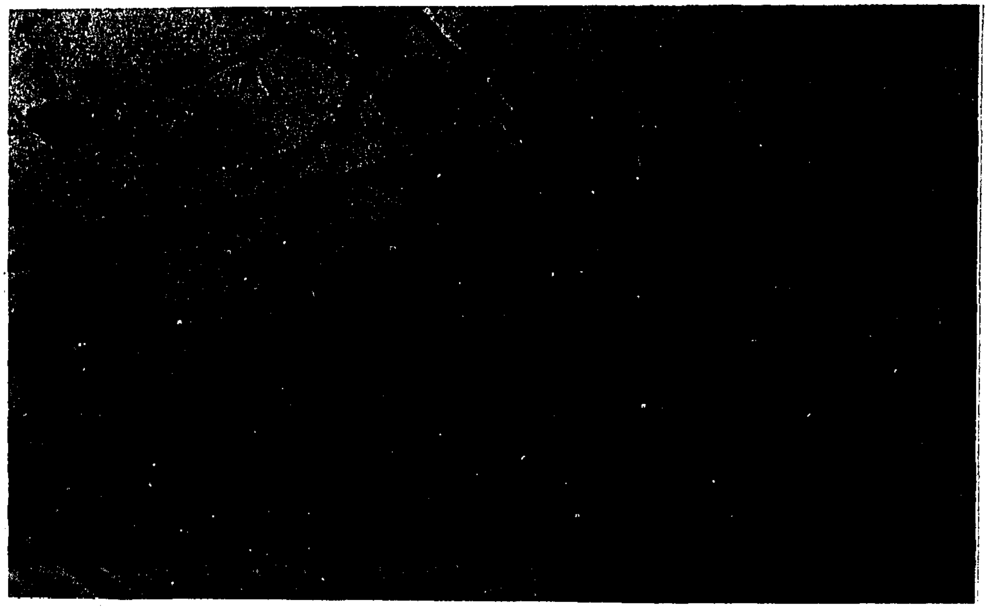

Figure 1a. Pythium irregulare sporangia.

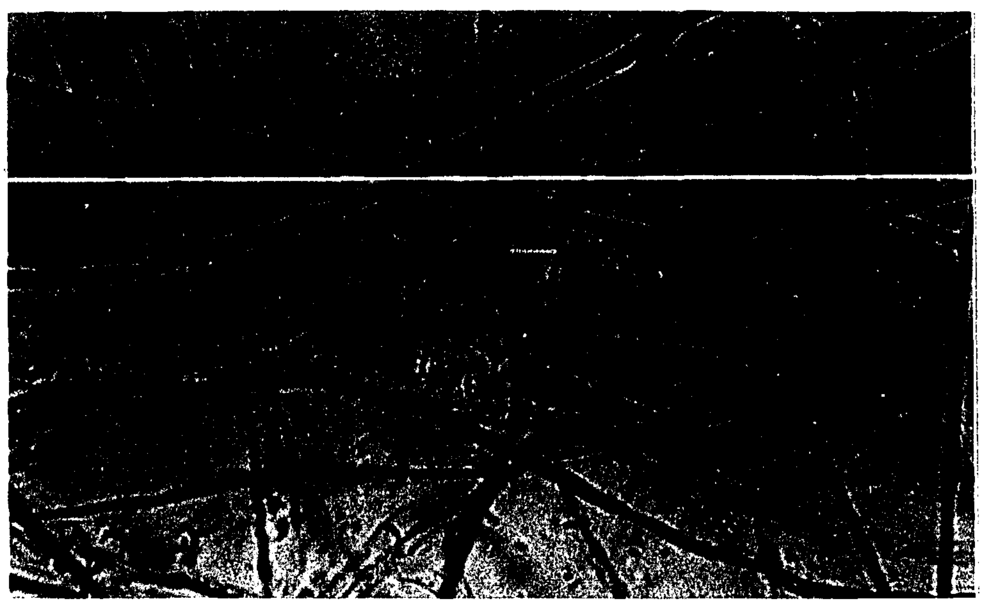

Figure 1b. Pythium irregulare irregularly shaped oogonium with aplerotic oospore.

Figure 2a. *Pythium sylvaticum* hyphal swelling.

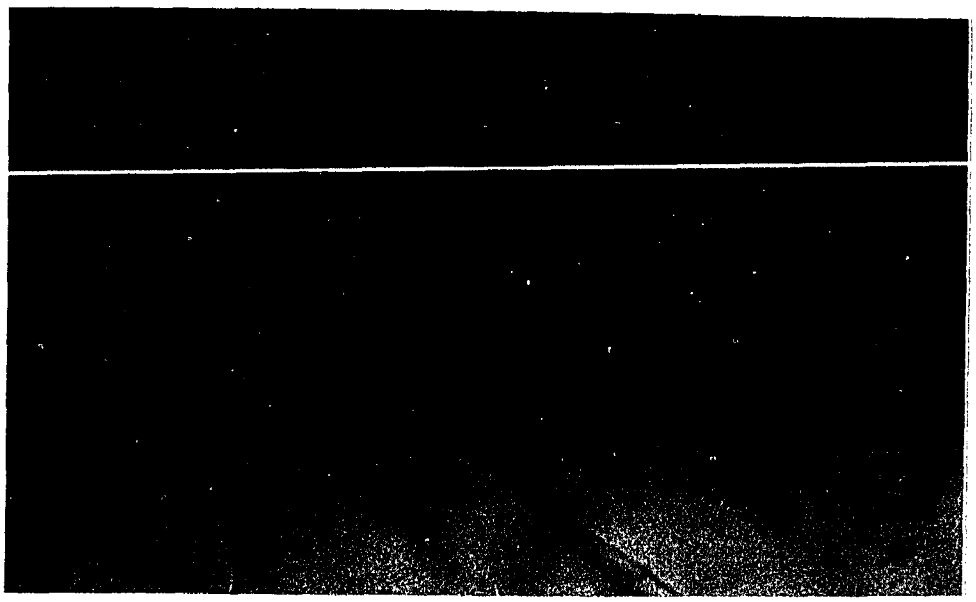

Figure 2b. *Pythium sylvaticum* oogonium surrounded by branched antheridial cells.

Figure 3a. *Pythium ultimum* oogonium with paragynous antheridium attached close to the oogonial stalk.

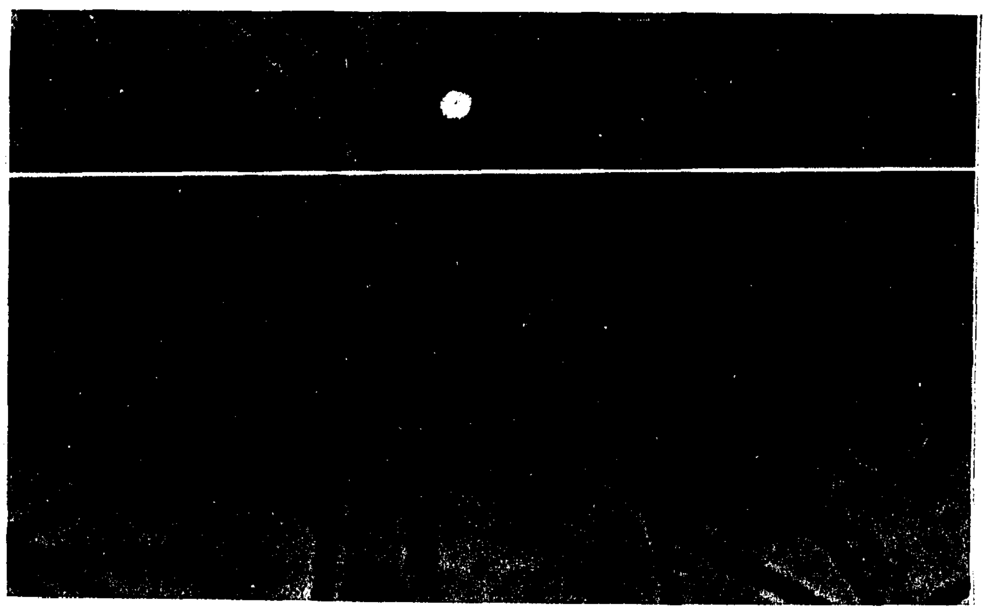

Figure 3b. *Pythium ultimum* oogonium with paragynous antheridia and an aplerotic oospore.

APPENDIX B

EFFECT OF *PYTHIUM* AND *PHYTOPHTHORA* ISOLATED IN MICHIGAN ON GROWTH OF MAHALEB CHERRY SEEDLINGS

INTRODUCTION

Root rot of sour cherry trees (*Prunus cerasus* L.) occurs in orchards planted on poorly-drained soils in Michigan. *Phytophthora* species are considered to be primary causal agents of cherry root rot in California (5,9), and New York (8). Recently, Bielenin and Jones reported isolation of *Ph. megasperma* Drechsler, *Ph. cactorum* (Leb. & Cohn) Schroet., *Ph. cryptogea* Pethyb. & Laff., *Ph. syringae* (Kleb.) Kleb., and *Ph. cambivora* (Petri) Buisman from cherry orchards in Michigan (1). *Pythium* species, particularly *P. irregulare* Buisman, were found to be associated with lateral necrosis of cherry trees, and were pathogenic to Mahaleb cherry (M. L. Smither, unpublished).

The objective of this study was to determine the effect of *Pythium* and *Phytophthora* species isolated in Michigan on the growth of Mahaleb cherry seedlings.

MATERIALS AND METHODS

Isolation and identification of species *P. irregulare* was isolated from roots, and *P. sylvaticum* Campbell and Hendrix, and *P. ultimum* Trow. from soil as previously described (M. L. Smither, unpublished). *Ph. cactorum* and *Ph. citricola* Sawada were isolated from soil using apple cotyledons as bait (4). Soil samples were sieved through 5 and 2 mm mesh sieves, and a 30 ml sub-sample was placed in a 250 ml beaker to air dry. After 3 days the soil in each beaker was moistened by adding 5 ml of sterile distilled water. The top of each beaker was covered with clear plastic held in place with a rubber band. After 3 days in a growth chamber at 21 C, the soil in each beaker was flooded with distilled water to a depth of 2 cm above the soil surface. Apple cotyledons (variety McIntosh) were floated on the surface of the water. Starting daily after the third day, any plant tissue with necrosis was removed and examined with a binocular microscope for Phytophthora. Isolations were made from infected tissue using CMA-PARP selective medium (3). Identifications were made based on the keys of Ribeiro (6), and Waterhouse (7). Isolates of Ph. megasperma, and Ph. drechsleri Tucker from cherry roots were supplied by G. C. Adams.

Pathogenicity test. The test was conducted in a growth chamber using 6-wk-old Mahaleb seedlings. The basic procedure was as described by Mircetich and Matheron (5). Inoculum of Pythium and Phytophthora species was prepared by growing each isolate at 21 C for 5 wk on 200 cc vermiculite and 20 g oat kernels moistened with 100 ml V-8 juice solution (200 ml V-8 juice, 2 g $CaCO_3$, and 800 ml distilled water) sterilized in 500 ml Erlenmeyer flasks. The inoculum was washed with distilled water over cheesecloth in a Buchner funnel to remove unassimilated nutrients, then mixed at the rate of 20 cc per 1000 cc of sterilized soil/sand/peat (1/1/1 by volume). Seedlings were planted in 4 L plastic pots with six replicates. The controls received vermiculite/oat mixture but no fungus. Maximum daytime temperatures were 21 C with 14 hr of light. The nighttime minimum was 18 C. The seedlings, apart from one control treatment, were flooded for 48 hr every 2 wk. All were fertilized biweekly. Dry weights of the roots and shoots were recorded after 18 wk. Reisolations were made on CMA-PARP selective medium. This experiment was repeated twice in the greenhouse and twice in the growth chamber with other isolates of these fungi.

RESULTS

Pathogenicity test. P. *irregulare*, P. *ultimum*, Ph. *cactorum*, Ph. *citricola*, Ph. *megasperma* and one isolate of Ph. *drechsleri* significantly reduced dry weights of roots and shoots of Mahaleb seedlings grown in a growth chamber (Table 1, Figure 1). P. *irregulare*, Ph. *cactorum*, and Ph. *citricola* caused the greatest reduction and P. *sylvaticum* the least reduction in shoot and root dry weights. One isolate of Ph. *drechsleri*, and P. *sylvaticum* isolated from soil did not significantly reduce dry weights of roots and shoots. All flooded treatments had significantly reduced growth as compared to the non-flooded control. Infections by P. *irregulare* resulted in extensive root discoloration and decay, similar to symptoms observed in the orchards. *Phytophthora* infections caused cankers on the collar region and main tap root. *Pythium* and *Phytophthora* species were

Table 1. Virulence of Pythium and Phytophthora species isolated from Michigan sour cherry orchards to Mahaleb seedlings

Fungus and isolate number	Shoots[x]		Roots[x]	
	Dry weight (g)	Relative growth[y] (%)	Dry weight (g)	Relative growth[y] (%)
Pythium irregulare				
L215	2.0 cd[z]	41	1.0 de	42
Pythium sylvaticum				
J310	3.2 bc	65	1.9 bc	79
Pythium ultimum				
B302	2.0 cd	41	1.4 cde	58
Phytophthora cactorum				
B1	0.7 d	14	0.6 e	25
Phytophthora citricola				
H2	1.5 cd	31	0.6 e	25
Phytophthora drechsleri				
B1	3.3 bc	67	1.6 cd	66
JC16	2.6 cd	53	1.2 cde	50
Phytophthora megasperma				
B1	2.4 cd	49	1.3 cde	54
Control				
noninoculated flooded	4.9 b	100	2.4 ab	100
noninoculated nonflooded	7.3 a	149	2.8 a	116

[x]Six-wk-old Mahaleb seedlings were transplanted in soil infested with 20 cc of inoculum per 1000 cc of soil. The seedlings apart from the non-flooded control were flooded for 48 hr every 2 wk. The experiment was conducted in a growth chamber for 3 mo. There were six replicates.
[y]Values are expressed as a percentage of flooded control.
[z]Values followed by the same letter do not differ significantly (P=0.05) using Duncans Multiple Range test.

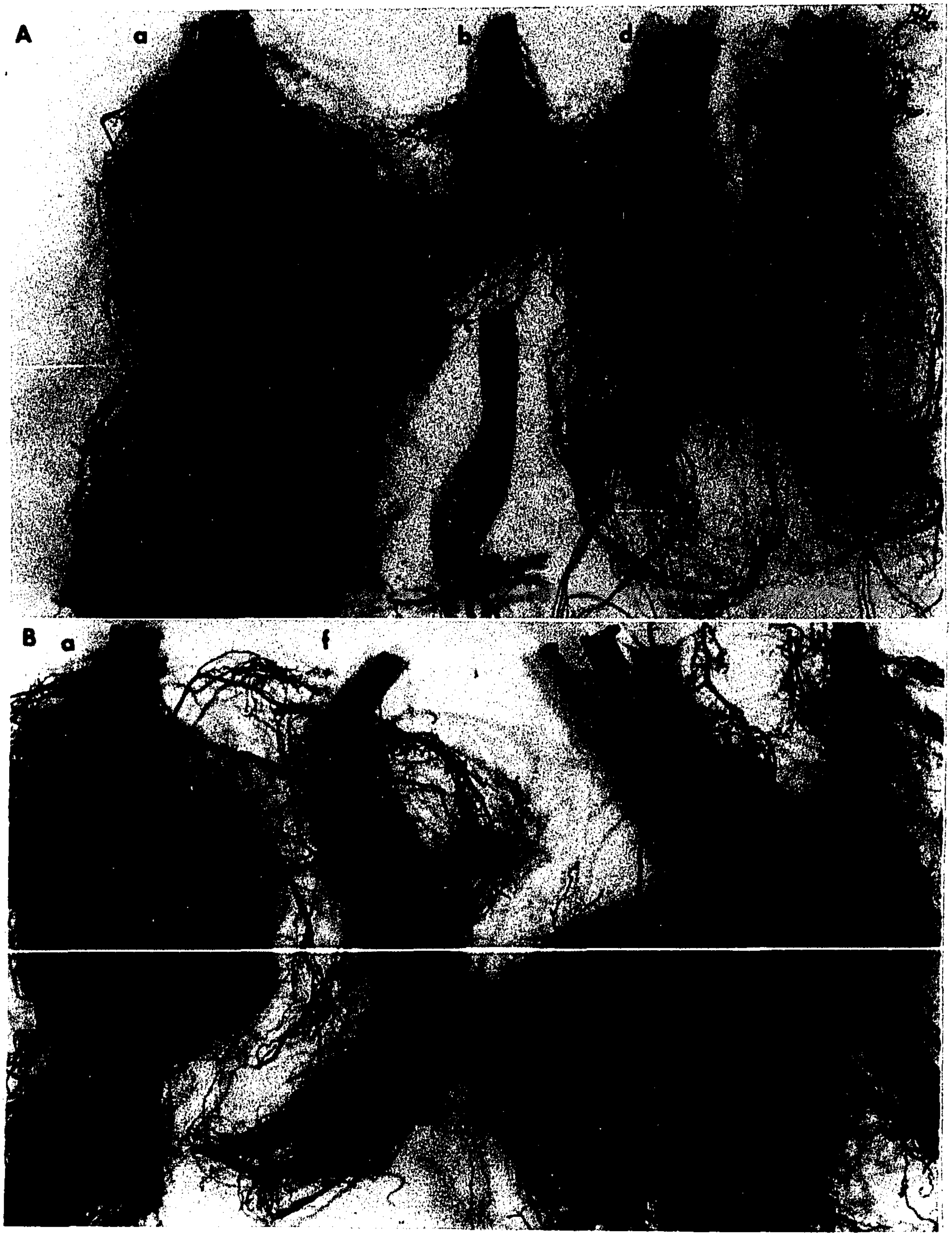

Figure 1. Roots of representative Mahaleb seedlings grown in a growth chamber for 18 wk in a soil/sand/peat mixture infested with (A) Phytophthora species and (B) Pythium species. Soil in the pots was (a) uninfested and non-flooded, or flooded and infested with (b) Ph. cactorum, (c) Ph. citricola, (d) Ph. drechsleri, (e) Ph. megasperma, (f) P. irregulare, (g) P. sylvaticum, and (h) P. ultimum.

routinely reisolated from seedlings growing in infested soil but not from control plants. These results were similar to those from two experiments conducted in a greenhouse and two experiments conducted in a growth chamber.

DISCUSSION

All the species tested caused reductions in dry weight of Mahaleb seedlings. Although *Phytophthora* species are considered to be the primary causal agents of cherry root and crown rot (1,5,8,9), the consistent results obtained with *Pythium* species, over a series of experiments, indicates that these fungi should also be considered pathogens of cherry.

The large number of *Phytophthora* and *Pythium* species isolated from cherry orchards in Michigan may retard the development of a suitable control stratagy using fungicides or host resistance. A suitable method of control must be active against all the species which may be present. Phytophthora root and crown rot is associated with poorly-drained soils, and is intensified by flooding (10). *Pythium* species are also associated with poorly-drained soils (M. L. Smither, unpublished). Therefore the most effective means of control maybe to improve soil conditions by selecting sites with well-drained soils, and using drainage systems.

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APPENDIX C

THE EFFECTS OF INCREASED SOIL COMPACTION LEVELS WITH *PYTHIUM IRREGULARE* AND *PHYTOPHTHORA MEGASPERMA* ON GROWTH OF MAHALEB SEEDLINGS AND ETHYLENE LEVELS IN SOIL

ABSTRACT

Sectioned pipes were used to study the effects of soil compaction levels of 1.0, 1.3, and 1.7 g/cc, and *Phytophthora megasperma* and *Pythium irregulare* on growth of Mahaleb seedlings. There were significant reductions (P=0.05)in dry weights of Mahaleb seedlings with increased bulk density levels. *P. irregulare* caused significant reductions in dry weight in one of two experiments. *P. irregulare* populations were an average of 104.3 (propagules per gram) ppg with no Mahaleb seedlings and 248.7 ppg with Mahaleb seedlings. *P. irregulare* populations were higher with increasing bulk densities. Ethylene levels in the basal sections of the pipe was measured using a Varian aerograph 1400 gas chromatograph. Ethylene levels over 1 ppm were detected in treatments with Mahaleb seedlings at 1.7 g/cc soil bulk density.

INTRODUCTION

Cherry trees planted on clay soils in Michigan tend to exhibit poor growth and survival. *Phytophthora* species pathogenic to cherry have been isolated from trees and soil (3,12). *Pythium irregulare* has been found to be pathogenic to cherry, and high populations of *Pythium* species are associated with heavy clay soils (M. L. Smither, unpublished).

Soil compaction occurs frequently on clay soils. The compacted zones impede root growth resulting in a shallow, restricted root system (5). Reduced diffusion of gases and reduced water movement cause waterlogging and sites of anaerobiosis which may predispose trees to infection (17). Phytophthora species which infect by motile zoospores and Pythium species which infect by zoospores or directly germinating sporangia are stimulated by fluctuations in matric potentials which occur near the surface (6).

Ethylene is a growth regulator produced by plants and microorganisms including fungi (8,18). Ethylene concentrations increase in soils under anaerobic conditions (9,15), and may directly affect plants causing reductions in root extension (16) and leaf chlorosis and premature senescence (7). It is not known if ethylene predisposes the host to infection (1,2,13,14), although increased ethylene concentrations often occur after infection (1,4).

The objectives were to study the effects of soil compaction levels and the pathogens, Phytophthora megasperma and Pythium irregulare, on growth of Mahaleb seedlings, and to investigate variations in soil ethylene concentrations caused by these factors.

MATERIALS AND METHODS

Pasteurized sandy loam orchard soil was sieved through 10 and 30 mesh sieves to retain soil particles between 0.05 and

2 mm in diameter. PVC pipe of 7.5 cm diameter was cut into 2.5, 5.0, and 7.5 cm sections. A 5 mm hole drilled 4.0 cm from the base of the 7.5 cm section was closed with a rubber septum. The 7.5 cm sections were covered at the base with a piece of Whatman No 1 filter paper under two layers of cheesecloth held in place with a rubber band. The sections were filled with soil and placed in aluminum trays which were half filled with water. More soil was added to maintain the level with the top of the sections as the soil subsided upon wetting. Soil densities of 1.0, 1.3 and 1.7 g/cc were obtained by placing 135.5, 176.2, and 230.4 g of soil at 18% moisture in the 2.5 cm section and compressing the higher densities with a flat weight. The 2.5 cm section was taped over the saturated soil, and the 5.0 cm section was taped in place over the compressed section and filled to the surface with soil. The completed cores were left until water had risen to the surface through the compacted layer.

Roots of four-wk-old Mahaleb seedlings were washed free from soil and cut back to 1.5 cm before planting in the top section.

Inoculum of Pythium and Phytophthora species was prepared by growing each isolate at 21 C for 5 wk on 200 cc vermiculite moistened with 100 ml V-8 juice solution (200 ml V-8 juice, 2 g $CaCO_3$, and 800 ml distilled water) sterilized in 500 ml Erlenmeyer flasks (12). The inoculum was washed with distilled water over cheesecloth in a

Buchner funnel to remove unassimilated nutrients. Inoculum (2cc) was placed in a 1 cm furrow in the soil around the periphery of the core.

The cores were placed in individual aluminum foil pie pans and kept in a growth chamber at 65 C with 14 hr of light. The cores were watered by applying 50 ml of distilled water to the top of the cores as required.

Ethylene samples (1 cc) were taken each week from the basal portion of the cores through the rubber septum. The samples were tested for ethylene concentration (ppm) with a Varian aerograph 1400 gas chromatograph.

After 8 wk the experiment was terminated. Measurements were made of root and shoot dry weights. The population of Pythium propagules in soil was determined with a surface soil dilution plate assay (11). Samples were collected with a 5 mm diameter cork borer. Three sub samples were taken from the top and bottom sections of each core. From each soil sample, dilutions of 1:10, 1:50, and 1:100 were prepared in molten sterile 0.2% water agar in test tubes. Diluted samples (1 ml) were distributed over the surface of fresh corn meal agar (Difco) amended with 10 mg pimaricin (0.4 ml of a 2.5% suspension), 250 mg ampicillin, 10 mg rifampicin, and 50 mg pentachloronitrobenzene, (Sigma Chemical Co., Saint Louis, MO) (CMA-PARP) (10) with a sterile glass rod. There were three replicate plates for each dilution. The plates were incubated inverted in plastic bags in the dark at 21 C. After 42 hr, the soil was removed by washing each plate under running tap water. A colony count was made and the number of propagules per gram of dried soil was determined.

An initial experiment was set up as a 3x2x2 factorial with 3 levels of compacted soil; no plant or 3-wk-old Mahaleb seedlings; and no fungus or *Pythium irregulare*. There were five replications for each treatment. A second experiment was conducted as a 3x2x3 factorial with *Phytophthora megasperma* as the additional treatment. The procedures followed were those previously described.

RESULTS

Increasing bulk density caused significant reductions (*P*=0.05)in root dry weight of Mahaleb seedlings in the initial experiment (Table 1). *P. irregulare* caused significant reductions in dry weight. Interaction effects were not significant. *P. irregulare* populations in the top sections at 1.0, 1.3, and 1.7 g/cc bulk density were 93, 250 and 217 ppg respectively. The population was 3 ppg in the basal section at 1.0 g/cc but *P. irregulare* was not detected in the basal sections from higher bulk densities. Populations were not determined in treatments with Mahaleb seedlings.

Increasing bulk density caused significant reductions in dry weight of Mahaleb seedlings in the second experiment (Table 2). In this experiment neither *Ph. megasperma* nor *P. irregulare* caused reductions significantly greater than

Table 1. Effects of soil bulk density and *Pythium irregulare* on growth of Mahaleb seedlings

Treatment	Bulk density (g/cc)	Shoot[x] Dry weight (g)	Shoot[x] Dry weight reduction (%)	Root Dry weight (g)	Root Dry weight reduction (%)
	1.0	0.31[z]		0.51	
Control	1.3	0.24	23	0.38	25
	1.7	0.22	29	0.20	61
	Mean	0.26		0.36	
	1.0	0.22	29	0.39	24
P. irregulare[y]	1.3	0.23	25	0.28	45
	1.7	0.18	42	0.19	63
	Mean	0.21		0.29	
	1.0	0.26		0.45	
Mean	1.3	0.24		0.36	
	1.7	0.20		0.20	
	LSD (*P*=0.05)	0.06		0.02	

[x]Four-wk-old Mahaleb seedlings were transplanted into pasteurized soil in 7.5 cm diameter PVC pipe. The experiment was conducted in a growth chamber for 8 weeks at 21 C.
[y]Inoculum of *P. irregulare* was applied in a 1 cm furrow around the periphery of the core.
[z]A factorial analysis showed significant differences (*P*=0.05) between the main effects and no significant interaction.

Table 2. Effects of soil bulk density, *Pythium irregulare* and *Phytophthora megasperma* on growth of Mahaleb seedlings

Treatment	Bulk density (g/cc)	Shoot[x] Dry weight (g)	Shoot[x] Dry weight reduction (%)	Root Dry weight (g)	Root Dry weight reduction (%)
	1.0	1.5[z]		1.0	
Control	1.3	1.2	20	0.7	30
	1.7	1.3	13	0.3	70
	Mean	1.3		0.7	
	1.0	1.5	0	0.8	20
P. irregulare[Y]	1.3	1.2	20	0.6	40
	1.7	1.2	20	0.3	70
	Mean	1.3		0.6	
	1.0	1.2	20	0.7	30
Ph. megasperma[Y]	1.3	0.8	47	0.7	30
	1.7	1.1	27	0.4	60
	Mean	1.0		0.6	
	1.0	1.4		0.8	
Mean	1.3	1.1		0.7	
	1.7	1.0		0.3	
	LSD (*P*=0.05)	0.3		0.2	

[x]Four-wk-old Mahaleb seedlings were transplanted into pasteurized soil in 7.5 cm diameter PVC pipe. The experiment was conducted in a growth chamber for 8 weeks at 21 C.

[Y]Inoculum of *P. irregulare* and *Ph. megasperma* were applied in a 1 cm furrow around the periphery of the core.

[z]A factorial analysis showed significant differences (*P*=0.05) with bulk density for both shoot and root dry weights. Fungus treatments did not cause significant differences, nor were there interaction effects.

those without the pathogens. There were no interaction effects. *P. irregulare* populations in the top sections at 1.0, 1.3, and 1.7 g/cc with no Mahaleb seedlings were 100, 87, and 126 ppg; and with Mahaleb seedlings were 183, 196, and 367 ppg. *P. irregulare* at 26 ppg was detected in the basal section of the non- seedling treatment at 1.0 g/cc. *P. irregulare* was not detected in the other treatments.

Ethylene was not detected at 1.0 g/cc bulk density; concentrations were <0.01 ppm in the Mahaleb seedling treatments at 1.3 g/cc bulk density; and at 1.7 g/cc bulk density were <0.01 ppm, control; 0.04 ppm ,*Pythium* control; 2.02 ppm, Mahaleb control; 1.13 ppm, Mahaleb and *Pythium*; and 1.1 ppm Mahaleb and *Phytophthora*.

DISCUSSION

Increased soil compaction levels were shown to reduce dry weights of Mahaleb seedlings. There was no interaction effect between soil compaction and reduction in dry weight caused by *P. irregulare*. Populations of *P. irregulare* increased with soil compaction levels. *P. irregulare* significantly reduced dry weights in the first experiment, but neither *P. irregulare* nor *Ph. megasperma* caused significant reductions in dry weight in the second experiment. The reason for this is not clear, although *Ph. megasperma* requires periodic flooding for pathogenicity (19), and none of the seedlings grew well.

These findings can be correlated with instances of poor growth in orchards with high soil compaction levels. Several factors may be involved in the poor root growth which occurs under such conditions. Root penetrance is reduced through zones of compacted soil (5). Anaerobiosis may predispose plants to infection (17,19). Root growth is decreased upon exposure to increased ethylene concentrations (7,16). However there is no clear evidence that increased ethylene concentrations predispose plants to infection (1,2,13,14).

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APPENDIX D

VARIATION IN SUSCEPTIBILITY OF CHERRY ROOTSTOCK CULTIVARS TO INFECTION BY *PHYTOPHTHORA* SPECIES AND *PYTHIUM IRREGULARE*

ABSTRACT

A dormant excised twig assay was used to determine the relative resistance of cherry rootstock cultivars to infection by _Phytophthora_ species and _Pythium irregulare_. Twigs were placed base down in previously inoculated agar. The resulting necrosis above the agar surface was recorded after 10 days. During 1983 dormant shoots of Mahaleb clones 163091, 193688, 193693, 194096, Mazzard, MxM #2, MxM #14, MxM #39, MxM #60, MxM #97, Meteor, and Vladimir were tested with _Ph_. _cactorum_, _Ph_. _drechsleri_, _Ph_. _megasperma_ and _P_. _irregulare_. During 1984 dormant shoots of Mahaleb seedlings and clone 163091, Mazzard, MxM #2, MxM #14, MxM #39, MxM #60, MxM #97, Vladimir, and Colt were tested with _Ph_. _cactorum_, _Ph_. _citricola_, _Ph_. _drechsleri_, _Ph_. _megasperma_ and _P_. _irregulare_. The assays showed that rootstocks differed in relative resistance to infection by _Phytophthora_ species and _P_. _irregulare_. Dry weights of Mahaleb and MxM #60 were significantly reduced when grown in soil infested with _Ph_. _cactorum_, _Ph_. _drechsleri_, _Ph_. _megasperma_ and _P_. _irregulare_, but dry weights of MxM #2, MxM #39, and MxM #46 were not significantly reduced. There were significant (_P_=0.05) correlations of 0.463 and 0.521 between the rootsock trial and twigs assays of 1983 and 1984.

INTRODUCTION

Sour cherry production is of major economic importance to Michigan. In orchards planted on heavy soils losses from root and collar rot are often severe. *Phytophthora* species cause root and collar rot of cherry in Michigan (4), California (10,17), and New York (16). *Pythium irregulare* Buisman has been associated with poorly growing cherry trees in Michigan (M. L. Smither, unpublished).

Mahaleb (*Prunus mahaleb* L.) is the rootstock most frequently used with Montmorency sour cherry (*P. cerasus* L.). Mahaleb is preferred to Mazzard (*P. avium* L.) as it is more resistant to crown gall and root lesion nematodes (7,12). However Mahaleb grows poorly on heavy soils (7), and is more susceptible than Mazzard to diseases induced by *Phytophthora* species (10,11,17). Mahaleb and Mazzard are both seedling rootstocks and therefore differences in resistance to infection can be expected between individuals. Several other rootstocks have been developed and their resistance to diseases induced by *Phytophthora* and *Pythium* needs to be evaluated.

Excised twig assays have been used as a rapid method to examine variations in pathogenicity and virulence of fungal isolates (2,9,13), to investigate variations in resistance to infection associated with host genotype (1,14), and seasonal differences (3). In one assay bark discs were removed from the mid-way point of excised twigs, fungal inoculum was applied, and the resulting necrotic area was measured after several days (6). A second assay method placed twigs with the bark stripped from the base in agar inoculated with a fungal pathogen. The resulting necrosis length above the agar was measured after several days (8). The second assay method was used in this work.

The objectives were to examine the relative resistance of cherry rootstock cultivars to _Phytophthora_ species and _P. irregulare_ infection with an excised twig assay and rootstock trial.

MATERIALS AND METHODS

Excised dormant twig assay Dormant shoots of cherry rootstock cultivars were tested for relative resistance to infection by _Pythophthora_ species and _P. irregulare_ using an excised twig assay (8). Fungi were grown on 30 ml corn meal agar (CMA) plus agar (17 g CMA, 5 g Bacto agar, and 1 L distilled water amended with 10 mg pimaricin [Sigma Chemical Co., Saint Louis, MO]) in sterilized 250 ml beakers. Control beakers contained no fungus. The beakers were covered with Parafilm (American Can Co., Greenwich, Ct 06830) over aluminum foil and maintained at 21 C in darkness for 7 days. Dormant cherry rootstock shoots from 6-10 mm in diameter were cut into 7 cm segments, disinfested in 0.6% NaOCl for 5 min, rinsed three times in distilled water and dried on paper towels. Five mm of the base of each twig was removed with disinfested pruning shears and a cut 1 cm in length by 2 mm in depth was made in each side at the base to expose the cambium. Single twigs from each cultivar were placed base down into the medium at the edge of the fungal colony, so that the cambium was exposed to the fungus. There were fifteen replicate beakers. The beakers were covered again and maintained as before. After 10 days the twigs were stripped of bark and the length of necrosis in the periderm above the agar level was recorded.

Rootstock material was obtained from Hilltop Nurseries (Route # 2, Hartford, MI) in February of 1983 and 1984. During 1983 dormant shoots of Mahaleb clones 163091, 193688, 193693, 194096, Mazzard, MxM #2, MxM #14, MxM #39, MxM #60, MxM #97, Meteor, and Vladimir were assayed for relative resistance to infection by _Ph_. _cactorum_ (Leb & Cohn) Schroet., _Ph_. _drechsleri_ Tucker, _Ph_. _megasperma_ Drechsler and _P_. _irregulare_. During 1984 dormant shoots of Mahaleb seedlings and clone 163091, Mazzard, MxM #2, MxM #14, MxM #39, MxM #60, MxM #97, Vladimir, and Colt (provided by R. L. Perry, Horticulture) were assayed for relative resistance to infection by _Ph_. _cactorum_, _Ph_. _citricola_ Sawada, _Ph_. _drechsleri_, _Ph_. _megasperma_ and _P_. _irregulare_.

Rootstock trial The experiments were conducted under lath with rootstock cultivars planted in uninfested and infested soil. The basic proceedure was as described by Mircetich and Matheron (10). Inoculum of _Phytophthora_ and _Pythium_ species was prepared by growing each isolate at 21 C for 5 wk on 200 cc vermiculite moistened with 100 ml V-8 juice solution (200 ml V-8 juice, 2 g $CaCO_3$, and 800 ml distilled water) in 500 ml Erlenmeyer flasks. The inoculum was washed with distilled water over cheesecloth in a Buchner funnel to remove unassimilated nutrients, then mixed at the rate of 10 cc per 1000 cc of sterilized soil/sand/peat (1/1/1 by volume). Rootstocks were planted in 4 L plastic pots with six replicates. The controls received the vermiculite mixture but no fungus. The rootstocks were flooded for 48 hr every 2 wk and fertilized biweekly. Dry weights of the roots and shoots were recorded after 15 wk. In an initial experiment dormant 1-yr-old seedlings of Mahaleb and Mazzard and dormant rooted cuttings of MxM #2, MxM #14, MxM #39, MxM #60, MxM #97, and Meteor were planted in uninfested soil and soil infested with Ph. cactorum, Ph. citricola, Ph. megasperma, P. irregulare, and P. sylvaticum. A second experiment used actively growing 1-yr-old seedlings of Mahaleb and Mazzard, and rooted cuttings of MxM #2, MxM #39, MxM #39, MxM #46, and MxM #60 which were planted in uninfested soil and soil infested with Ph. cactorum, Ph. drechsleri, Ph. megasperma, and P. irregulare.

RESULTS

Excised twig assay. Necrosis developed on twigs of all rootstock cultivars inoculated with Phytophthora species and

P. irregulare. No necrosis developed on uninoculated rootstock cultivars. The length of necrosis produced differed significantly between cultivars (Table 1-2). The results varied between the two years, but a correlation coefficient of 0.396 gave a significant (P=0.05) correlation between the two assays. Necrosis length was generally greater during 1984 than 1983. During 1983 the longest necrotic area was produced on Mahaleb 193688 by Ph. cactorum, Ph. drechsleri, and Ph. megasperma, and on Mahaleb 194096 by P. irregulare. A significantly longer necrotic area was produced by the Phytophthora species on MxM #2 and MxM #60 than on the other MxM cultivars. The shortest necrotic area was produced on Vladimir by Ph. drechsleri and Ph. megasperma. During 1984, the longest necrotic area was produced on MxM #2 by Ph. cactorum and Ph. citricola, and on Mahaleb 163091 by Ph. drechsleri, Ph. megasperma, and P. irregulare. The shortest necrotic area was produced on Colt by Ph. cactorum and Ph. citricola, on Mazzard by Ph. drechsleri and P. irregulare, and on Vladimir by Ph. megasperma.

Rootstock trial Results from the initial experiment were not significant due to poor establishment of the rootstocks. In the second experiment shoot dry weights of Mahaleb were reduced in soil infested with Ph. cactorum, Ph. drechsleri, Ph. megasperma, and P. irregulare, and root dry weights in soil infested with Ph. cactorum, and Ph. megasperma (Table 3). Shoot dry weights of Mazzard were not significantly

Table 1. Relative susceptibility of dormant excised shoot tissue of cherry rootstock cultivars to infection by *Phytophthora* species and *Pythium irregulare* during 1983

	Necrosis length on shoot (mm)[y]			
Cultivar	*Ph. cactorum*	*Ph. drechsleri*	*Ph. megasperma*	*P. irregulare*
Mahaleb				
163091	11.2 c[z]	11.1 e	12.5 bc	8.2 bc
193688	23.8 a	24.6 a	19.2 a	11.6 b
193693	11.0 c	7.9 e	7.3 d	10.6 b
194096	16.0 b	18.1 bc	17.2 ab	18.8 a
Mazzard	18.5 b	9.6 e	7.1 d	4.6 c
MxM #2	16.5 b	18.9 b	15.6 ab	4.1 c
MxM #14	1.1 d	10.3 e	4.5 de	4.0 c
MxM #39	9.2 c	8.1 e	6.2 d	3.6 c
MxM #60	19.6 ab	13.8 cd	16.9 ab	3.5 c
MxM #97	6.4 c		7.9 cd	
Meteor	1.3 d	6.8 e	3.2 e	2.9 c
Vladimir	7.5 c	1.6 f	0.1 e	7.8 bc

[y]Mean length of necrosis above agar level from 15 replicate shoot pieces.
[z]Values followed by the same letter are not significantly different using Duncan's multiple range test (P=0.05).

Table 2. Relative susceptibility of dormant excised shoot tissue of cherry rootstock cultivars to infection by *Phytophthora* species and *Pythium irregulare* during 1984

	Necrosis length on shoot (mm)[Y]				
Cultivar	*Ph. cactorum*	*Ph. citricola*	*Ph. drechsleri*	*Ph. megasperma*	*Py. irregulare*
Mahaleb	26.9 ab[Z]	25.3 ab	16.5 a	16.4 a	12.1 bc
163091	22.3 bc	25.9 ab	15.5 a	16.1 a	17.7 a
Mazzard	17.3 cd	18.3 c	3.5 d	9.5 b	5.2 d
MxM #2	29.0 a	30.1 a	12.1 abc	11.8 ab	17.3 ab
MxM #14	14.8 d	24.9 ab	7.7 cd	9.5 b	11.9 bc
MxM #39	17.3 cd	21.9 bc	9.2 bc	11.7 ab	11.3 c
MxM #60	24.4 ab	28.7 a	13.5 ab	11.7 ab	14.3 abc
MxM #97	17.7 cd	22.1 bc	11.3 abc	12.9 ab	13.3 abc
Vladimir	17.7 cd	27.9 a		9.0 d	9.7 cd
Colt	9.3 e	17.0 c	11.8 abc	11.4 ab	13.4 abc

[Y]Mean length of necrosis above agar level from 15 replicate shoot pieces
[Z]Values followed by the same letter are not significantly different Duncan's multiple range test (P=0.05).

Table 3. Growth of cherry rootstock cultivars planted in soil infested with *Phytophthora* species and *Pythium irregulare* under lath

	Shoot dry weight (g)[y]					Root dry weight (g)				
Cultivar	Control	*Ph. cactorum*	*Ph. drechsleri*	*Ph. megasperma*	*P. irregulare*	Control	*Ph. cactorum*	*Ph. drechsleri*	*Ph. megasperma*	*P. irregulare*
Mahaleb	17.2 a[z]	9.8 b	14.0 bc	14.0 bc	12.3 bc	8.9 a	4.0 b	7.4 a	5.0 b	8.0 a
Mazzard	13.8 b	13.5 b	17.0 a	13.1 b	14.3 ab	9.9 a	3.7 b	11.7 a	4.5 b	12.3 a
MxM #2	5.1 a	4.6 a	4.6 a	6.4 a	4.7 a	3.8 a	3.0 a	3.9 a	4.7 a	3.6 a
MxM #39	4.3 a	3.3 a	4.9 a	5.3 a	4.7 a	4.5 a	2.7 a	4.5 a	4.3 a	3.4 a
MxM #46	7.7 a	4.8 a	6.7 a	5.3 a	7.2 a	6.3 a	4.3 a	4.7 a	5.1 a	6.2 a
MxM #60	6.4 a	3.7 b	3.1 b	3.6 b	7.9 a	5.6 a	3.0 b	2.6 b	3.5 ab	5.5 a

[y]Actively growing rootstocks were transplanted in soil infested with 10 cc of inoculum per 1000 cc of soil. The experiment was conducted under lath. There were six replicates.
[z]Values across rows followed by the same letter are not significantly different using Duncan's multiple range test (*P*=0.05).

reduced by soil infested by any isolate, although root dry weights were reduced with *Ph. cactorum*, and *Ph. megasperma*. Shoot and root dry weights of MxM #60 were reduced in soil infested with *Ph. cactorum*, *Ph. drechsleri*, and *Ph. megasperma*. Shoot and root dry weights of MxM #2, MxM #39, and MxM #46 were not significantly reduced in infested soil.

Correlation coefficients were calculated to compare results from the rootstock trial with those from the 1983 and 1984 twig assays. Results from the rootstock trial were expressed as percent loss in dry weight as compared to the control. Correlation coefficients were 0.463 for 1983, and 0.521 for 1984; both figures show significant correlation (P=0.05).

DISCUSSION

Cherry rootstock cultivars in the twig assay differed in relative resistance to *Phytophthora* species and *P. irregulare*. As the resistance of the host tissue to colonization is only one of several components of resistance, rootstock trials are required for complete evaluation. Mahaleb clones varied in resistance to infection in the twig assay, but overall Mazzard was more resistant than Mahaleb. Dry weights of Mahaleb seedlings were significantly reduced in the rootstock trial, while those of Mazzard were less effected. Mahaleb has been reported to be more susceptible than Mazzard to *Phytophthora* infection (10,11,17). The vertically oriented root system of Mahaleb is believed to predispose it to 'wet feet' problems on heavy soils (7). Dry weights of MxM #2, MxM #39, and MxM 46 were not reduced by growth in infested soil, while dry weights of MxM #60 were significantly reduced.

The variation in results for the twig assays between 1983 and 1984 is believed to be due to variations in environmental conditions. An extremely cold period occured during February of 1984, and extreme environmental conditions , including low temeratures, predispose plants to infection (15).

P. irregulare produced necrosis on rootstock cultivars in the twig assay although to a lesser extent than _Phytophthora_ species. In the rootstock trial _P. irregulare_ caused a significant reduction in shoot dry weight with Mahaleb but not the other cultivars. _P. irregulare_ has been shown to be most pathogenic at temperatures below 21 C (5). The rootstock trial was conducted outside under shade produced by lath during the summer with no regulation of temperatures.

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