Prevalence and Pathogenicity of *Phytophthora* spp. from Sour Cherry Trees in Michigan

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ABSTRACT

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In 1986 and 1987, *Phytophthora megasperma*, *P. cactorum*, *P. cryptogea*, *P. syringae*, and *P. cambivora* were isolated from the margins of necrotic root and crown tissue from dying sour cherry trees in orchards planted on heavy, poorly drained soils. *Phytophthora megasperma* was recovered from each of the cherry-growing districts of Michigan and was the most prevalent of the five species. In greenhouse tests involving 66 isolates, all five species caused root rot or canker formation on Mahaleb cherry seedlings that were grown in artificially infested potting medium and periodically flooded. Isolates of *P. cryptogea* and *P. cambivora* were highly virulent. Isolates of *P. megasperma* varied widely in their ability to cause root rot. Isolates of *P. cactorum* caused little root rot, but they caused large cankers on Montmorency scions of grafted trees and on stems of Mahaleb seedlings. Isolates of *P. syringae* also caused cankers on seedlings. These results indicate that infection by *Phytophthora* spp is a significant factor in the death and decline of sour cherry trees in Michigan.

Additional keywords: Phytophthora root and crown rot, *Prunus cerasus*

In numerous commercial sour cherry (*Prunus cerasus* L.) orchards in Michigan, tree mortality has increased in recent years. In orchards planted on sites with light, well-drained soils, three species of *Armillaria* were associated with the death of the trees (5). *Armillaria* was not recovered from dying trees in orchards planted on sites with heavy, poorly drained soils. The symptoms in affected trees in these orchards were similar to those reported for Phytophthora root and crown rot of sweet cherries (3).

In California, *Phytophthora megasperma* Drechsler, *P. cambivora* (Petri) Buisman, *P. drechsleri* Tucker, *P. cryptogea* Pethyb. & Laff., and an unidentified *Phytophthora* sp. were identified as causal agents of Phytophthora root and crown rot of sweet cherries (3,11). In addition, isolates of *P. cinnamomi* Rands and *P. citricola* Sawada from other deciduous fruit and nut crops in California were pathogenic on Mahaleb cherry in greenhouse tests and were considered a potential hazard to cherry (11). Recently, *P. megasperma*, *P. cryptogea*, *P. cambivora*, and an unidentified *Phytophthora* sp. were identified as the causal agents of root and crown rot of sour cherry in New York (10). None of these pathogens has been reported from tree fruit orchards in Michigan.

The objectives of this study were to identify the species of *Phytophthora* associated with declining trees in Michigan sour cherry orchards located on heavy soils and to evaluate their relative pathogenicity to cherry.

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MATERIALS AND METHODS

Isolation of *Phytophthora* spp. from diseased trees. The roots and crowns of Montmorency sour cherry trees with symptoms of infection from *Phytophthora* were removed from 18 commercial orchards in western Michigan during the summer of 1986 and the spring of 1987. All orchards were less than 9 yr old. The samples were transported to the laboratory in plastic bags and were held in a cold room for 1–2 days before isolation. The trees sampled were grafted on *Prunus mahaleb* L. (Mahaleb) seedling rootstocks, except two trees were grafted on *P. avium* L. (Mazzard) seedling rootstocks.

Bark samples 2–10 mm wide and 1–2 cm long were cut from the margin of necrotic root, crown, and stem tissue of each tree. After trimming to obtain clean surfaces, samples were rinsed in sterile distilled water for 1–2 min, blotted on paper toweling, cut into 1- to 2-mm-thick segments, and placed in petri plates on a selective medium for the isolation of *Phytophthora* (2). The medium contained (per liter) 20 ml of clarified V-8 juice adjusted to pH 6.5 before autoclaving, 17 g of agar, 5 mg of benomyl, 5 mg of hymexazol, 5 mg of pimaricin, and 25 mg of rifampicin. Plates were incubated in the dark at 20 C and were examined daily for colonies of *Phytophthora*. All *Phytophthora* colonies were transferred to Difco cornmeal agar (CMA) or to clarified V-8 juice agar (V8A).

Identification of *Phytophthora* spp. Preliminary identification of the isolates was made directly on the selective medium based on colony morphology. Most isolates of *P. cactorum* (Lebert & Cohn) Schroet. and many isolates of *P. megasperma* produced oospores and many isolates of *P. cactorum*, *P. syringae* (Kleb.) Kleb., and *P. cryptogea* produced sporangia on the selective medium. The final identity of all isolates was confirmed on the basis of the morphology of colonies on CMA and V8A; of sporangia on disks taken from 5- to 7-day-old cultures on V8A and flooded with 1.5% nonsterile soil extract at 17 C for 12–48 hr (3); and of oospores, particularly for heterothallic species, on V8A amended with β-sitosterol, tryptophan, and thiamine (βV8A) (1). We also compared the morphology of our isolates with known isolates from sweet and sour cherry supplied by S. N. Jeffers, S. M. Mircetich, and W. F. Wilcox, and to the descriptions of Waterhouse (9), Ribeiro (6), and Tucker (8).

Pathogenicity tests. Wound inoculations were made into the bark to determine the pathogenicity of four of the species. The isolates used are M117, M354, C-21, M120, C-23, M28, M246, M270, M318, and M364. One-year-old Montmorency sour cherry trees on Mahaleb rootstocks were grown in 11.4-L plastic pots containing Pro-Mix potting medium (Premier Brands, Inc., New Rochelle, NY) and were maintained in the greenhouse. Four-mm-diameter disks from 7- to 10-day-old colonies on V8A were placed into holes cut with a cork borer in the scion or rootstock to the depth of the cambium region. Following

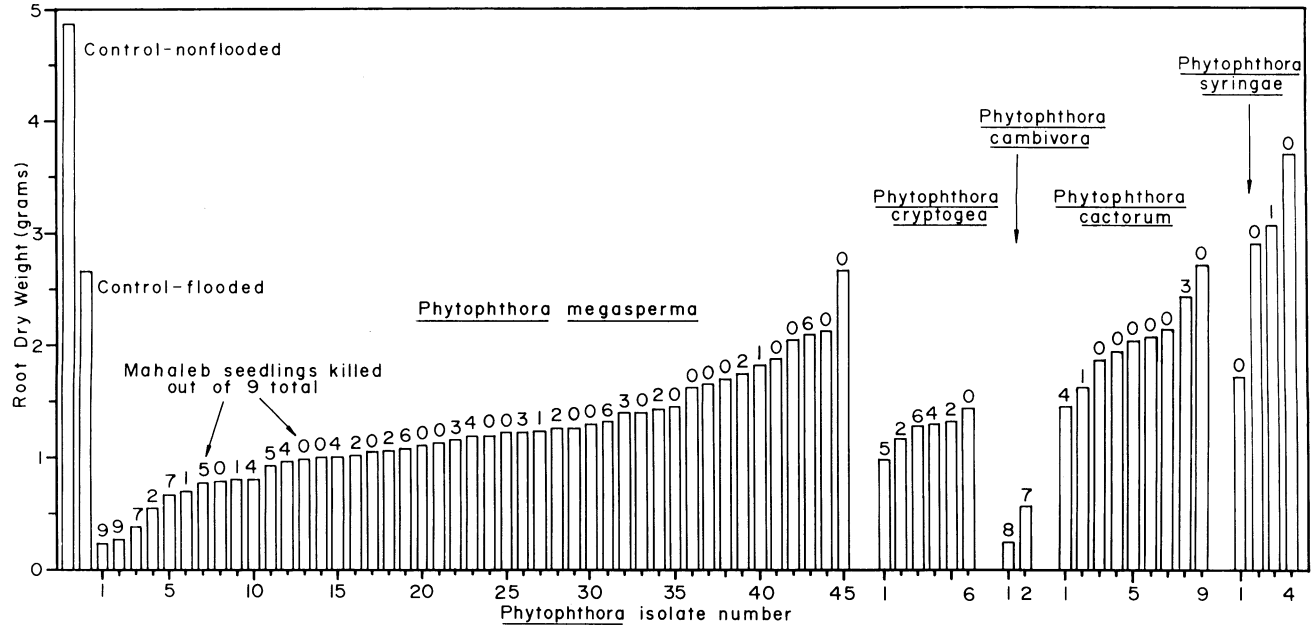

Fig. 1. Pathogenicity of isolates of five species of *Phytophthora* recovered from sour cherry trees in Michigan to 3-mo-old Mahaleb seedlings grown in artificially infested potting medium for 3 mo in the greenhouse and flooded at 2-wk intervals. Isolates were arranged within each species by root dry weights from lowest to highest and renumbered to simplify presentation. Original isolate numbers are available from A. L. Jones.

Table 1. Species of *Phytophthora* recovered from symptomatic Montmorency sour cherry trees from orchards in the western Michigan fruit belt during 1986 and 1987

County	Orchard code	Trees with *Phytophthora*/ trees sampled[z]	Species recovered
Antrim	A	1/3	*P. megasperma*
Grand Traverse	B	2/4	*P. megasperma*
	C	3/4	*P. megasperma*
Leelanau	D	1/3	*P. megasperma*
	E	2/3	*P. megasperma*
	F	1/3	*P. megasperma*
	G	4/4	*P. megasperma, P. cactorum*
	H	2/3	*P. megasperma*
	I	3/3	*P. megasperma, P. cryptogea, P. syringae*
Benzie	J	2/3	*P. megasperma*
Manistee	K	2/2	*P. megasperma*
Mason	L	2/2	*P. cactorum, P. syringae*
	M	4/4	*P. megasperma*
Kent	N	4/4	*P. megasperma, P. cryptogea, P. cambivora*
	O	2/4	*P. megasperma, P. cryptogea, P. cambivora*
Van Buren	P	3/4	*P. megasperma*
	Q	2/2	*P. cryptogea*
Berrien	R	1/3	*P. megasperma*

[z]Number of sour cherry trees that yielded *Phytophthora* over the total number of symptomatic trees from which isolations were attempted.

inoculation, the holes were wrapped with Parafilm (American Can Co., Greenwich, CT). Measurements of cankers on the Montmorency scion were made 14 days after inoculation by removing the bark above and below the site of inoculation and measuring the length of discoloration in the wood. Measurements of cankers on the Mahaleb rootstock were made 3 mo after inoculation.

In addition to the wound inoculation tests, pathogenicity of isolates listed in Figure 1 was determined in artificially infested potting medium as described by Mircetich and Matheron (3). Each isolate was grown for 6 wk at 21 C in 1-L flasks containing 500 ml of sterile vermiculite thoroughly moistened with V-8 juice broth. The inoculum was rinsed with sterile water and 100 cm was placed in a hole in the center of each 3-L metal pot containing 3-mo-old Mahaleb seedlings (three seedlings per pot) growing in Pro-Mix potting medium. Three pots were inoculated with each isolate. Pots were flooded every 2 wk during the first 2 mo of the experiment by immersing them in water for 48 hr. During the experiment, seedlings were removed from the pots as they died, and isolations were made on the selective medium to verify the species of *Phytophthora* present. Seedlings alive 3 mo after inoculation were removed from the pots and the roots were washed to remove the potting medium. After drying for 2 wk at room temperature, the roots were weighed.

RESULTS

Isolations from cherry. *Phytophthora* spp. were recovered from 41 of 58 symptomatic sour cherry trees from 18 orchards in nine Michigan counties (Table 1). Based on the general morphology of cultures, morphology of sporangia and oospores, and taxonomic descriptions, the isolates were identified as *P. megasperma*, *P. cactorum*, *P. cryptogea*, *P. syringae*, and *P. cambivora*. Two or three species of *Phytophthora* were often isolated from the same orchard.

Phytophthora megasperma was the species recovered most often (34 of 58 trees and 16 of 18 orchards). *Phytophthora cryptogea* was recovered from four trees and four orchards, *P. cactorum* from four trees and two orchards, *P. syringae* from three trees and two orchards, and *P. cambivora* from two orchards. The symptoms were as described for sweet cherry by Mircetich and Matheron (3), except that cankers on the crown rarely extended above the graft union, which in most orchards was located belowground. Most trees had root rot together with crown rot, whereas trees in orchard L had crown rot and apparently healthy roots.

Losses ranged from a few to several hundred trees per orchard.

Because *P. syringae* is rare in orchards of the eastern United States, and may be confused with *P. cactorum* or *P. citricola*, two homothallic species with paragynous antheridia, maximum temperatures for growth were established on CMA (7,14). Isolates M119, M120, M213, M446, and M447 from Michigan and isolates NY218, NY219, and AP81 of *P. syringae* from the culture collection of S. N. Jeffers failed to grow at 28 C, and grew extremely slowly (2–3 mm per day for isolates M119, M446, and M447) over 4 days at 25 C. For most isolates, mycelial plugs on media held at 28 C for 10 days failed to grow when transferred to 20 C. After 4 days, colonies of isolates of *P. cactorum* (R-15, M130, and M354) increased in diameter by 8.8 and 8.1 mm per day, at 25 and 28 C, respectively, and an isolate of *P. citricola* (ATCC 42885) increased by 12.5 and 12.9 mm per day at 25 and 28 C, respectively. Similar results were obtained when the cultures were grown on V8A, but on lima bean agar most isolates of *P. syringae* grew well at 25 C.

Single cultures of *P. cactorum* and *P. citricola* produced oospores on βV8A within 10 days at 20 C. All isolates of *P. syringae*, except AP81 and M120, produced oospores within 1 mo at 15 and 20 C. Isolate M120 produced oospores within 3 mo at 15 C. The oospores were 21–32 μm in diameter, smooth, yellowish, and indistinguishable from oospores of *P. cactorum*. Sporangia of *P. syringae* were 23 to 38 × 31 to 57 μm, sparse in number compared with *P. cactorum*, and more irregular in size and shape. Isolates M446 and M447 produced compact colonies with radial zonation similar to NY218 and typical for *P. syringae*, whereas isolates M119, M120, and M213 grew faster and produced more aerial mycelium, and were intermediate between *P. syringae* and *P. cactorum* in colony type on V8A at 20 C (Fig. 2). Small catenate swellings occurred in several cultures of *P. syringae* on CMA, as described by Sewell and Wilson (7).

No oospores were produced by single cultures of *P. cambivora* and *P. cryptogea* on βV8A within 2 mo in the dark at 20 C. Oogonia and oospores with bullate protuberances, as pictured by Mircetich and Matheron (3), were formed within 20 days at 20 C when cherry isolates of *P. cambivora* (C-45 and M-72) were paired on βV8A with known A^1 compatibility types of *P. cryptogea* (isolate O-1 from Poland) and *P. cambivora* (isolate NY216 from S. N. Jeffers). Oospores were formed within 20 days at 20 C when cherry isolates of *P. cryptogea* A^1 (M172, M417, and M440) were paired on βV8A with known A^2 compatibility type of *P. cryptogea* (isolate 9-1-5 from S. M. Mircetich).

Pathogenicity test. In the wound inoculation test, isolates of *P. cactorum* were significantly more aggressive than isolates of *P. cryptogea*, *P. syringae*, and *P. megasperma*, both on the Montmorency scion and the Mahaleb rootstock of grafted trees (Table 2). Trees inoculated with *P. cactorum* often had gumming, in addition to extensive necrosis, in the outer bark. *Phytophthora cactorum* was reisolated from the cankers at the end of the experiment.

The virulence of a large number of isolates of *P. megasperma*, *P. cactorum*, *P. cryptogea*, *P. syringae*, and *P. cambivora* to Mahaleb seedlings was compared in artificially infested potting medium (Fig. 1). In noninfested, flooded potting medium, Mahaleb seedlings were stunted and had significantly ($P = 0.05$) lower dry root weights than nonflooded seedlings. None of the nonflooded seedlings in infested potting medium died, but three seedlings in potting medium infested with one isolate of *P. cactorum* developed cankers on stem tissue above the soil line.

In flooded potting medium (Fig. 3), isolates of *P. megasperma* caused a decay of the lateral roots followed by decay of the main roots (Fig. 3E). Dry root weights for seedlings inoculated with all but a few isolates were reduced significantly from the dry root weights for seedlings in the flooded control (Fig. 1). Twenty-six of 45 isolates killed one or more of the nine seedlings within the 3-

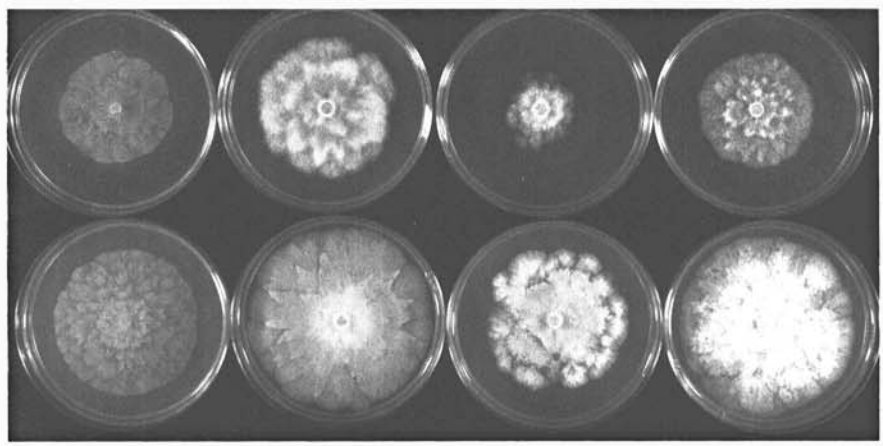

Fig. 2. Colony morphology of isolates of *Phytophthora syringae* grown for 10 days at 20 C on lima bean agar (top) and V-8 juice agar (bottom). Isolates from left are NY218, M119, M120, and M213.

Table 2. Size of cankers formed on 1-yr-old Montmorency cherry trees on Mahaleb rootstock following wound inoculation with four species of *Phytophthora* isolated from sour cherry orchards

		Montmorency scion		Mahaleb rootstock	
Species	Isolate[v]	Length of lesion[w] (mm)	*Phytophthora* reisolated[x] (no.)	Lesion size[y] (cm^2)	Cankers with *Phytophthora*/ total no. of cankers[z]
P. cactorum	M117	···	···	25.4 a	4/6
	M354	80 a	3	15.7 b	2/6
P. cryptogea	C-21	25 b	3	6.5 c	6/6
P. syringae	M120	20 bc	3	5.0 c	3/6
P. megasperma	C-23	18 bc	3	···	···
	M28	···	···	3.7 c	5/6
	M246	13 bc	3	3.4 c	6/6
	M270	···	···	3.1 c	4/6
	M318	10 c	3	···	···
	M364	12 bc	3	1.7 c	3/3
Control		no necrosis	0	no necrosis	0/0

[v] Isolates C-21 and C-23 were obtained from soil collected around necrotic roots of cherry trees using the procedures of Harris and Bielenin (2). The remaining isolates were obtained directly from cherry tissue.

[w] Data were taken 2 wk after inoculation. Each value is the average of three replications. Values with the same letter do not differ from each other ($P = 0.05$) according to Duncan's multiple range test.

[x] Number of cankers out of a total of three plants per treatment.

[y] Data were taken 3 mo after inoculation. Each value is the average of six replications. Values with the same letter do not differ from each other ($P = 0.05$) according to Duncan's multiple range test. Control not included in analysis.

[z] Number of cankers out of the total number of cankers on six plants per treatment from which *Phytophthora* was reisolated.

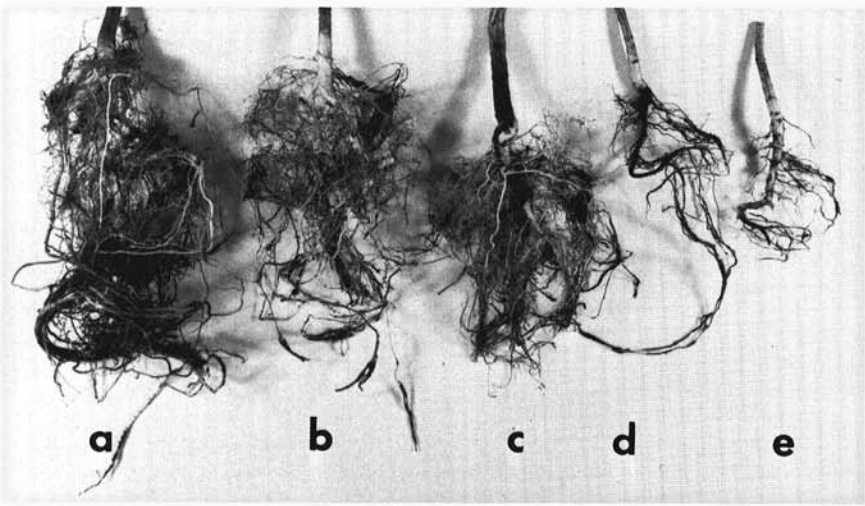

Fig. 3. Roots of representative 6-mo-old Mahaleb seedlings after growth for 3 mo in noninfested potting medium **(A)** without flooding, **(B)** flooded for 48 hr every 2 wk in the first 2 mo, or flooded and infested with **(C)** *Phytophthora cactorum* (note canker on stem of seedling), **(D)** *P. cryptogea*, or **(E)** *P. megasperma*.

mo test period. The isolates of *P. megasperma* evaluated in this experiment were typical of the low-temperature and large oogonial type reported for cherry isolates of *P. megasperma* in California (3,13).

Phytophthora cryptogea caused extensive root rot on lateral and main roots (Fig. 3D). All but one isolate killed two to six of the nine Mahaleb seedlings. Seedlings inoculated with most isolates of *P. cryptogea* died as quickly as seedlings inoculated with the most virulent isolates of *P. megasperma*. *Phytophthora cactorum* was a consistent crown invader but caused very little root necrosis or seedling death (Fig. 3C). The numbers of seedlings out of nine that were cankered (and the mean length of cankers in millimeters) were 7 (59.4), 5 (39.0), 5 (40.0), 3 (45.6), 5 (39.4), 2 (37.5), 7 (40.3), 7 (51.8), and 7 (15.0) for isolates 1–9, respectively. Root dry weights for seedlings inoculated with all isolates except one were not significantly ($P = 0.05$) different from weights for seedlings in the flooded control. *Phytophthora syringae* caused little root rot or seedling mortality (Fig. 1). *Phytophthora cambivora* was virulent, causing extensive decay of the root system, invasion of the stem, and high mortality of the seedlings within 2 mo (Fig. 1).

DISCUSSION

Of the species of *Phytophthora* recovered from declining sour cherry trees, *P. megasperma* was the most prevalent and widely distributed in moribund sour cherry trees in Michigan. This research confirms previous reports that *P. megasperma* and *P. cambivora* are serious pathogens of cherry (3,10,11). In addition, our results confirm the report by Wilcox and Mircetich (11) that *P. cryptogea* is highly virulent on Mahaleb cherry, and we provide additional data for implicating *P. cactorum* and *P. syringae* in Phytophthora crown and root rot of cherry. Both species were previously recovered from diseased sweet cherry trees in single orchards in California (11), and *P. cactorum* was isolated from cherry orchard soils but not directly from cherry in New York (10). This study is the first to establish the role of *Phytophthora* as the cause of death of a tree-fruit crop in Michigan. In addition, we have recovered *P. megasperma*, *P. cactorum*, and *P. cambivora* from apple trees (*Malus sylvestris* Mill.) with root and crown rot. There is a possibility that the species of *Phytophthora* associated with sour cherry are also a threat to commercial apple production in Michigan.

Most sour cherry trees grown in Michigan are grafted on Mahaleb rootstock. The high incidence of dead and dying trees among trees in Michigan orchards planted on Mahaleb, the observation in California of fewer dead trees in orchards planted on Mazzard than on Mahaleb (3), and the demonstration in pathogenicity studies that Mazzard is more resistant than Mahaleb to several species of *Phytophthora* (3,4,11), suggest that the planting of cherry trees on Mahaleb rootstock on sites with poor soil drainage should be avoided.

The isolation of several species of *Phytophthora* that differ in pathogenic behavior may complicate efforts to develop control methods for root and crown rot based on host resistance or fungicides. Where rootstocks or fungicides that control a limited number of the species of *Phytophthora* are used to control Phytophthora root and crown rot, outbreaks of those species not controlled by the rootstock or fungicide could result. Because Phytophthora root and crown rot is associated with poorly-drained soils and can be increased experimentally by prolonged and periodic soil saturation (12), the most logical control strategy is to eliminate wet soil conditions through cultural practices such as site selection, use of drainage systems, and raised planting beds, along with the use of nursery stock on Mazzard rootstock.

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