Phytophthora Crown Rot of Apple Trees:
Sources of *Phytophthora cactorum* and *P. cambivora* as Primary Inoculum

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ABSTRACT

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A recently developed baiting bioassay that enhanced the detection of *Phytophthora cactorum* was employed to determine the occurrence and distribution of this pathogen on apple nursery stock and in soils from within and around apple orchards. The bioassay also proved to be a sensitive means of detecting *P. cambivora*, a known crown rot pathogen, and *P. citricola*. Over a 2-yr period, 152 of 153 bundles of unbudded clonal rootstocks were found to be infested, 150 with *P. cactorum* and 111 with *P. cambivora*; 25 of 29 samples from unbudded seedling rootstocks were infested with only *P. cactorum*; and 158 of 175 bundles of nursery-grown trees on clonal rootstocks were infested, 158 with *P. cactorum* and only nine with *P. cambivora*. The incidences of *P. cactorum* and *P. cambivora* on 80 individual unbudded rootstocks from four clones were 76 and 73, respectively. All rootstock cultivars, with the possible exception of those grown from seed, were equally infested. All nurseries sampled in the United States, Canada, and Europe had contaminated plants. Naturally occurring inoculum on roots of unbudded rootstocks of Malling-Merton 106 apple caused severe root and crown rot when plants were flooded periodically. *P. cactorum* was detected in 56 of 112 soil samples collected around both symptomatic and healthy-appearing trees in 22 of 36 New York apple orchards. *P. cactorum* also was detected in 17 of 37 nonagricultural soils collected from sites in the vicinity of apple orchards. *P. citricola* frequently was present in these soils also. Isolates of *P. cactorum* recovered from soil were as virulent as those previously isolated from infected apple roots and crowns. Both infested nursery stock and infested orchard soils are potential sources of primary inoculum for Phytophthora crown rot of apple trees.

Additional key words: *Malus pumila*, *Phytophthora megasperma*, *Phytophthora syringae*, soilborne plant pathogens.

Phytophthora crown rot is the most important disease affecting the crown and roots of apple trees (*Malus pumila* Miller) in New York and probably worldwide. It has been reported from most apple-growing regions (5,12,16,28). In isolations from diseased trees during the period 1978–1984, *Phytophthora cactorum* (Lebert & Cohn) Schroeter was the most frequently encountered species (S. N. Jeffers, *unpublished*); other species also have been involved in New York (16) and elsewhere (5,24,29,32).

Little research has been done to identify the effective source of primary inoculum for Phytophthora crown rot. Two possibilities exist: naturally infested orchard soil and young trees infested or infected before being planted in the orchard. *P. cactorum* and other species of *Phytophthora* have been recovered from apple orchard soils since at least 1925 (e.g., references 6, 19, 20, 27, 30, and 33); however, pathogenicity to apple was demonstrated only occasionally in recovered isolates (20,27,33). Recovery of *P. cactorum* from associated nonorchard soils has been investigated infrequently, and the results are not consistent (19,20). The occurrence and distribution of this fungus in soils from apple orchards and surrounding areas in New York are not known.

Contaminated nursery stock is the other potential source of primary inoculum. Heavy losses to nursery stock of apple and other deciduous fruit trees caused by *Phytophthora* spp. were first reported in 1925 from California (31). In 1978 (18), both unbudded apple rootstocks and nursery-grown apple trees coming into North Carolina were found infected with *P. cactorum* (7.8% of samples) and *P. cambivora* (Petri) Buisman (8.7% of samples). Shortly thereafter, 87% of samples of unbudded apple rootstocks surveyed in Georgia were reported to be infested with *P. cactorum* (4). Pathogenicity of the isolates recovered was not demonstrated in either instance. The possibility that apple nursery stock infested with *Phytophthora* spp. has been distributed to nurseries and orchards in New York has not been examined previously.

The goals of this project were to determine the occurrence and distribution of *P. cactorum* in apple orchard soils, in surrounding nonagricultural soils, and on apple nursery stock used by New York nurserymen and orchardists. A preliminary report has been published (13).

MATERIALS AND METHODS

Unbudded rootstocks. Samples from unbudded apple rootstocks and budded nursery-grown trees (see below) were collected and assayed between February and May of 1983 and of 1984. Unbudded rootstocks were sampled from shipments received by local nurseries from the major rootstock suppliers before the plants were planted in New York. A sample consisted of one bundle of vegetatively propagated clonal rootstocks or one or two bundles of seedling rootstocks (grown from open-pollinated apple seed); a bundle contained 25, 50, or 100 plants. Four to six samples of each cultivar from each supplier usually were collected. The cultivars of clonal rootstocks sampled were Malling-Merton (MM.) 106, MM.111, Malling (M.) 7, M.26, M.9, M.27, and a Polish selection, P-22.

The bundles were shaken briefly to remove loosely adhering material, and then the roots were submerged in 4–8 L of distilled water in 15-L plastic pails. After soaking for at least 5 min, the bundles were agitated vigorously to dislodge rhizosphere soil and debris. The pails were covered tightly, transported to the laboratory, and stored at 2–4 C for 4–6 hr, to allow soil and debris to settle. Excess water then was decanted carefully, and approximately 1 L of concentrated rootwash slurry was retained and returned to 2–4 C to continue settling overnight. The following morning the remaining supernatant was discarded, and concentrated rootwashes (300–800 ml) were assayed for *Phytophthora* by three methods (plating to selective agar media and two baiting bioassay procedures).

In the plating procedure, 1-ml aliquots of concentrated rootwash slurries were pipetted onto each of five to 10 plates of

pimaricin, ampicillin, and rifampicin (PAR) and PAR plus hymexazol (PARH) selective media (17), and the plates were incubated in the dark at 19 C. After 4–5 days, the surface of each plate was washed free of debris under a gentle stream of tap water, and macroscopic colonies of *Phytophthora* spp. were counted. Hyphal tips from representative colonies were transferred to fresh PAR or PARH for later identification.

All of the remaining rootwash slurry concentrate was assayed, first by a direct baiting procedure and then by an extended baiting procedure, as described previously by Jeffers and Aldwinckle (14). In the direct baiting procedure, the slurry was divided into three or four 100- to 200-ml aliquots, each in a disinfested 475-ml glass jar. Approximately 200–300 ml of distilled water was added to each jar to resuspend the rootwash debris, and apple cotyledon baits were floated on the surface. The jars then were placed in a growth chamber (20 C and 16-hr photoperiod). After 7 days, cotyledons were removed and examined, and the water was decanted. In the subsequent extended baiting procedure, rootwash debris first was allowed to thoroughly air-dry (6–8 days) at room temperature (25–28 C); the debris in each jar then was remoistened with just enough distilled water to wet but not saturate the entire amount as it was mixed with a spatula. The actual volumes of water added to the jars varied, depending on the volume of the air-dried debris. Next, the jars, containing the remoistened rootwash debris, were covered and placed back in the growth chamber for 3 days. Lastly, the debris samples were flooded and baited as described above for the direct baiting procedure.

P. cactorum or other species of *Phytophthora* were determined to be present in a rootwash sample if morphologically characteristic sporangia developed on apple cotyledon baits (14). If *P. cactorum* was not detected in a sample, the sample was baited by the extended procedure again. *Phytophthora* spp. were isolated from cotyledons on PAR or PARH selective medium. From each nursery source, representative isolates recovered from each rootstock cultivar were saved for identification.

Baiting rootwash slurries with apple cotyledons was consistently more successful at recovering *Phytophthora* spp. than was plating small aliquots of slurry concentrate onto selective media. Consequently, the plating procedure was discontinued while assaying unbudded apple rootstocks in 1983.

From each of the rootstock clones Budagovsky 9, Budagovsky 57-490, P-22, and MM.106 (all from the same rootstock supplier), rootwashes were collected from 20 individual unbudded rootstocks. Each rootstock was agitated in 300 ml of distilled water in a 500-ml flask, and the resulting debris was concentrated as above. Rootwash slurries (5–10 ml from each rootstock) were poured into disinfested 120-ml glass jars and resuspended in 50 ml of distilled water. Each slurry was baited directly and then by the extended procedure, with two cotyledons per jar.

Nursery-grown trees. Rootwashes were collected from individual bundles of nursery-grown trees prior to planting in commercial apple orchards. Samples were collected, in a manner similar to that used for unbudded rootstocks, from three local and three out-of-state nurseries in 1983 and from two local and 11 out-of-state nurseries in 1984. A bundle of nursery-grown trees contained five to 25 plants. The rootstock cultivars sampled were MM.106, MM.111, M.26, M.9, and M.2; nursery-grown trees on seedling rootstocks also were sampled. Rootwashes were assayed as described above, and again representative isolates were saved.

Pathogenicity of inoculum associated with unbudded rootstocks. Inoculum naturally occurring on roots of unbudded apple rootstocks was tested for pathogenicity by the following procedure. Susceptible MM.106 rootstocks were planted in sterile vermiculite (one part coarse-textured to one part fine-textured, v/v) in 15-L plastic pails drilled with a single drain hole. The plants were grown outside or in a greenhouse. Care was taken to avoid splash or runoff contamination from one container to another or from other sources. The only inoculum present was that which occurred naturally on the roots of the test plants. When all the rootstocks had actively growing shoots, half of the plants were flooded (with 5–10 cm of standing water) for 48 hr once every 2 wk. After four flooding cycles, the plants were rated for symptoms, and

root washings from each plant were baited by both the direct and the extended procedures. In addition, isolations were made from symptomatic plants onto PAR and PARH selective media. The experiment was conducted three times with rootstocks from two different suppliers.

Identification of isolates. Isolates were identified by growing cultures on cornmeal agar (CMA) and on amended and clarified V-8 juice agar (8), with the amount of clarified V-8 broth reduced to 100 ml/L. The cultures were incubated at 19 or 22 C in the dark. Sporangia were produced in the light at 19 C on V-8 juice agar plugs flooded with 1.5% nonsterile soil extract (14). Isolates not forming oospores in single-strain cultures were paired on separate plates of the amended clarified V-8 juice agar with the A1 mating type of *P. cryptogea* Pethybridge & Lafferty (isolate P1088) and the A2 mating type of *P. drechsleri* Tucker (isolate P1087), both from the *Phytophthora* collection of the University of California at Riverside. Oospores were observed in crosses after 2 wk and were characterized at 5 wk.

Nursery soils. Soil samples were collected from three local nurseries in fall 1983. Samples were taken from within tree-rows, from between rows, and from adjacent areas not previously planted in nursery stock. From each sample site, 15–20 subsamples were collected to a depth of 20 cm with an Oakfield soil tube (Forestry Suppliers, Inc., Jackson, MS), having a 2-cm inner diameter, to make a composite soil sample. The samples were screened and processed as described below and then baited by both the direct and the extended procedures.

Orchard soils. Between 1978 and 1984, 112 soil samples from 36 apple orchard sites were collected; most of the samples were collected in 1982 and 1983. Ninety-seven collections came from western New York (Onondaga, Ontario, Wayne, Monroe, Orleans, and Niagara counties), and 15 collections came from the Hudson Valley area of eastern New York (Orange, Ulster, and Columbia counties). Composite soil samples were collected next to the trunk, in the area of the root crown, by taking four to eight cores with an Oakfield soil tube to a depth of 20 cm. Alternatively, if soils were too rocky, a hand trowel was used to take several scoops around the trunk to a similar depth. The soil tube or trowel was disinfested in 70% ethanol between samples.

Samples were collected around 50 healthy-appearing and 62 symptomatic trees. One to three trees of each type were sampled per orchard; both types of trees were not sampled in all orchards. If no symptomatic trees were present in an orchard, a composite row sample was collected by taking one core from each tree or from alternate trees, depending on the row length.

All soil samples were placed in polyethylene bags, transported in a cooled ice chest, and stored at 2–4 C in the dark. Some samples were stored only 24 hr; most samples were stored 1–2 yr; and a few samples were stored for 5–6 yr. Each soil sample was passed first through a 6-mm-mesh screen and then through a 3-mm-mesh screen and was thoroughly mixed prior to assaying. All samples were assayed for *P. cactorum* by the extended baiting procedure only.

Nonagricultural soils. From all locations, composite soil samples were collected with an Oakfield soil tube to a depth of 20 cm. At each site, 15–20 soil cores were taken in a random pattern, with each core at least 10 m away from any other. In fall 1983, a total of 37 soil samples were collected, with the aid of county soil survey maps, from sites at least 1 km away from any apple orchard. Eight to 10 samples were collected from each of the principal apple-growing counties of western New York—Wayne, Monroe, Orleans, and Niagara; 11 more soil samples were collected from four locations immediately adjacent to apple orchards containing symptomatic trees. All nonagricultural soil samples were stored and screened as described previously and then were assayed with the extended baiting procedure. Representative isolates were recovered and identified as described above.

Based on county soil survey maps and descriptions, all 37 soil samples collected in the vicinity of apple orchards were from soil series and textures similar to those used for apple cultivation. Soil textures were classified as silt loams (18 sites), gravelly and fine sandy loams (13 sites), silty clay loams (five sites), and loamy fine

sand (one site). Natural drainage classes ranged from poorly drained to somewhat excessively drained; most soils were either moderately well drained (16 sites) or somewhat poorly drained (15 sites). Soil series at all sites except two were characterized by seasonally high or perched water tables, most of which were within 15–48 cm of the soil surface. Plant cover was either hardwood forest or mixed shrubby and herbaceous native flora. In only two sites was there evidence of recent agricultural use (e.g., decomposing cornstalks).

Data analysis. Data on the detection of *P. cactorum* and *P. cambivora* from nursery stock and in soil were summarized as the number of samples from which the fungus was recovered out of the total number of samples assayed. Comparisons then were made in contingency tables by calculating a chi-square statistic (χ^2) and determining the probability of a greater chi-square value occurring (*P*), i.e., the significance level. Chi-square values with one degree of freedom were corrected for continuity.

Virulence of isolates of *P. cactorum* recovered from soil. Five isolates recovered from each soil source (symptomatic trees, healthy-appearing trees, and nonagricultural sites) were compared quantitatively in an excised twig assay (15) with five isolates previously recovered from infected apple roots and crowns. Each isolate was grown on CMA amended with pimaricin (20 mg/L) and agar (5 g/L) in Pyrex storage jars for 12 days in the dark at 22 C. One jar of CMA was left sterile and served as the control. Previous season's woody shoots from unbudded MM.106 apple trees were collected and inoculated on 2 and 11 February 1984 for trials 1 and 2, respectively. Ten twigs, each 65 mm in length and with the basal end pared, were pushed vertically into the agar at the advancing edge of each colony. An additional 10 twigs were pushed into sterile agar in the control jar. Net necrosis lengths (lesion lengths above the agar surface) were measured after 21 and 17 days in the dark at 22 C for trials 1 and 2, respectively. Sources of isolates were compared in separate one-way analyses of variance of the individual trials; each source had 50 observations (five isolates × 10 twigs per isolate). The values for net necrosis length of the uninoculated twigs were not included in the analyses, so that the variability among sources could be assessed more accurately.

RESULTS

***Phytophthora* spp. on nursery stock.** Two distinctive types of sporangia of *Phytophthora* were distinguished on apple cotyledons. Characteristically papillate sporangia that frequently occurred in close sympodia, typical of *P. cactorum*, were one type, and larger, nonpapillate, internally proliferating sporangia on simple or irregularly branched sporangiophores, characteristic of Waterhouse's Groups V and VI (35), were the other type. Isolation and identification (35) of *Phytophthora*-like fungi resulted in only two species, *P. cactorum* and *P. cambivora*. Of 92 isolates of *P. cambivora* identified from both unbudded rootstocks and nursery-grown trees, 89 were of mating type A1 and three were of mating type A2. *P. cambivora* was detected only by direct baiting, whereas *P. cactorum* was detected predominantly by extended baiting.

Unbudded rootstocks. Sampled rootstocks were relatively free of visible symptoms; no evidence of typical crown or root rot was observed. However, succulent roots usually were discolored (orange-brown) and damaged due to handling and oxidation during digging, wrapping, and transporting. These roots are superfluous for new growth; some nurserymen routinely remove them prior to planting.

Samples came from eight sources in the Pacific Northwest, one source in New York, and two sources in the Netherlands (Table 1). The majority came from Oregon and Washington. Sampled bundles from all rootstock nurseries were infested with both *P. cactorum* and *P. cambivora*.

All of the clonal rootstock cultivars we examined were infested equally with *Phytophthora* spp.; in all, 99.3% (152/153) of the bundles were infested (Table 2). A significantly greater proportion (*P* < 0.005) of clonal rootstock bundles was infested with *P. cactorum* (150/153 = 0.98) than with *P. cambivora* (111/153 =

TABLE 1. Sources of unbudded apple rootstocks and nursery-grown apple trees assayed for *Phytophthora* spp. in 1983 and 1984

	Rootstocks		Trees	
Location	Number of nurseries	Samples with *Phytophthora*	Number of nurseries	Samples with *Phytophthora*
California	—[a]	—	1	3/3
Georgia	—	—	2	2/2
Michigan	—	—	1	11/11
Missouri	—	—	1	8/8
New York	1	5/5	3	118/139
Oregon	2	83/83	—	—
Pennsylvania	—	—	2	5/5
Washington	5	77/81	4	9/9
British Columbia	1	5/5	1	3/4
Netherlands	2	7/8	—	—
Total	11	177/182	15	159/181
Proportion		0.97		0.88

$\chi^2 = 10.24$, $P < 0.005$[b]

[a] No samples from this location were assayed.

[b] χ^2 = chi-square statistic, corrected for continuity and with one degree of freedom, for comparing total numbers of rootstock and tree samples infested with *Phytophthora* spp.; *P* = probability of a greater chi-square value occurring.

TABLE 2. Detection of *Phytophthora cactorum* and *P. cambivora* associated with bundles of unbudded apple rootstocks in 1983 and 1984[a]

	Number of samples infested[c]					
	P. cactorum		*P. cambivora*		*P. cactorum* or *P. cambivora*	
Rootstock[b]	1983	1984	1983	1984	1983	1984
Clonal rootstocks						
MM.106	19/21	19/19	17/21	14/19	21/21	19/19
MM.111	21/22	20/20	15/22	11/20	21/22	20/20
M.7	17/17	11/11	5/17	10/11	17/17	11/11
M.26	5/5	15/15	5/5	15/15	5/5	15/15
M.9	5/5	10/10	5/5	8/10	5/5	10/10
M.27	—[d]	5/5	—	3/5	—	5/5
P-22	1/1	2/2	1/1	2/2	1/1	2/2
Total	68/71	82/82	48/71	63/82	70/71	82/82
Proportion	0.96	1.00	0.68	0.77	0.99	1.00
Seedling rootstock	11/14	14/15	0/14	0/15	11/14	14/15
Proportion	0.79	0.93	0.00	0.00	0.79	0.93

Comparison[e]	χ^2	*P*
Clonal rootstocks infested with *P. cactorum* in 1983 vs. 1984	1.65	0.21
Clonal rootstocks infested with *P. cambivora* in 1983 vs. 1984	1.19	0.29
Clonal rootstocks infested with *P. cactorum* vs. *P. cambivora* in 1983 and 1984	37.62	<0.005
Seedling rootstocks infested with *P. cactorum* in 1983 vs. 1984	0.42	0.52

[a] Concentrated root washings from rootstock bundles were assayed sequentially by direct and then by extended baiting procedures using apple cotyledons.

[b] The first seven cultivars are vegetatively propagated clonal rootstocks. MM. = Malling-Merton; M. = Malling. Seedling rootstocks were grown from open-pollinated seed.

[c] A sample contained 25, 50, or 100 clonal rootstocks (one bundle) or 100 or 200 seedling rootstocks (one or two bundles). Data are reported as (number of samples infested)/(number of samples assayed).

[d] No samples were assayed.

[e] Comparisons between total numbers of clonal or seedling rootstock samples infested. χ^2 = chi-square statistic, corrected for continuity, with one degree of freedom; *P* = probability of a greater chi-square value occurring.

0.73) in 1983 and 1984 combined (Table 2). However, there was no significant difference between the proportion infested with *P. cactorum* ($P= 0.21$) or with *P. cambivora* ($P= 0.29$) in 1983 and the proportion infested in 1984 (Table 2).

Because unbudded seedling rootstocks had much less debris associated with their roots, two bundles were often washed in the same pail to produce one sample. In 1983 and 1984 combined, 86.2% (25/29) of the samples were infested with only *P. cactorum*; no difference ($P = 0.52$) was observed between the proportion of samples infested in 1983 and the proportion infested in 1984 (Table 2). In addition to apple, 11 samples from unbudded seedling rootstocks of pear (*Pyrus communis* L.) were assayed. These came from the same rootstock suppliers as did the apple seedling rootstocks. *P. cactorum* was detected in eight of these samples.

Phytophthora spp. frequently were detected on individual unbudded clonal rootstocks also (Table 3); 98% of all plants were contaminated with one or both species. There was no significant difference between the total proportion infested with *P. cactorum* and that infested with *P. cambivora* ($P = 0.54$, Table 3).

In 1984, a shipment of unbudded M.27 rootstocks had a high incidence of stem canker occurring well above the root zone. Associated with each canker was a 5- to 10-mm-long vertical crack in the bark, apparently the result of low-temperature damage. *P. syringae* (Klebahn) Klebahn consistently was isolated from these cankers on PAR selective medium. In 1983, this species also was recovered from one bundle of unbudded MM.106 rootstocks by plating rootwash slurry concentrate directly onto PARH medium.

Nursery-grown trees. Nursery-grown trees, ready for planting in apple orchards, also appeared healthy and free of any crown rot symptoms. Sampled trees came from 14 nurseries across the United States and from one nursery in Canada; the majority were from three nurseries in New York (Table 1). Trees from all nurseries, regardless of geographical location, were infested. Although the proportion of nursery tree samples infested with *Phytophthora* spp. was relatively great (0.88), it was significantly less ($P < 0.005$) than the proportion of unbudded rootstock samples infested (0.97) (Table 1).

Phytophthora spp. initially were not detected on the two five-tree bundles received from two Georgia nurseries. To determine if these were actually pathogen-free, each tree was planted in a 15-L pail containing autoclaved vermiculite and grown in a greenhouse for 3 mo. Care was taken to avoid splash or runoff contamination from one container to another or from other sources. During this time, the trees were flooded for a 48-hr period once every 2 wk (for a total of six flooding periods). The plants were allowed to go dormant for 15 wk at 2–4 C and then were placed in a growth chamber (16–20 C and 16-hr photoperiod) to resume growth. Periodic flooding was continued after buds broke, and apple

seedlings were floated in flooded pots to bait zoospores of *Phytophthora* spp. (14). *P. cactorum* was detected around eight flooded plants and *P. cambivora* around one; one tree remained free of detectable *Phytophthora* spp.

Rootstock cultivars of nursery-grown trees assayed for *Phytophthora* spp. (Table 4) were similar but not identical to the cultivars of unbudded rootstocks assayed (Table 2). All cultivars of clonal rootstocks had a high incidence of *Phytophthora* spp.; 90.3% (158/175) of all sampled bundles from both years were infested (Table 4). This proportion (0.90) was significantly less ($P < 0.005$) than a similar proportion (0.99) for infested unbudded clonal rootstocks (Table 2), based on $\chi^2 = 11.24$, with one degree of freedom. The incidences of *P. cactorum* in 1983 and 1984 were equally high on clonal rootstocks of nursery-grown trees (Table 4), and the incidences of *P. cambivora* were equally low (Table 4) and much less than that on unbudded clonal rootstocks (Table 2). *P. cambivora* was detected from only nine of 175 bundles for both years combined; this incidence did not contribute to the total number of bundles infested with either species. Only six samples were collected from nursery-grown trees on seedling rootstocks (Table 4). *P. cactorum* and *P. cambivora* were detected in just one of these samples.

Pathogenicity of inoculum associated with unbudded rootstocks. In all three trials, all 33 naturally infested unbudded MM.106 rootstocks that were not flooded grew vigorously and were free of crown rot symptoms (Fig. 1). However, *P. cactorum*, *P. cambivora*, or both species were detected from 28 of these plants. Of the 33 naturally infested rootstocks that were flooded periodically, 32 had severe root and crown rot and were either dead or dying (Fig. 1). Either *P. cactorum*, *P. cambivora*, or both species were detected from all of these plants.

Nursery soils. *P. cactorum* but not *P. cambivora* was detected in nursery soils at all three locations. This species was detected in four

TABLE 3. Detection of *Phytophthora cactorum* and *P. cambivora* associated with 20 individual unbudded apple rootstocks from each of four cultivars[a]

Rootstock cultivar	Number of plants infested		
	P. cactorum	*P. cambivora*	*P. cactorum* or *P. cambivora*
Malling-Merton 106	20	19	20
P-22	20	20	20
Budagovsky 9	18	19	20
Budagovsky 57-490	18	15	18
Total	76/80	73/80	78/80
Proportion	0.95	0.91	0.98

$\chi^2 = 0.39$, $P = 0.54$[b]

[a]Concentrated root washings from individual plants were assayed sequentially by direct and then by extended baiting procedures using apple cotyledons.

[b]χ^2 = chi-square statistic, corrected for continuity and with one degree of freedom, for comparing the total number of plants infested with *P. cactorum* and the total number infested with *P. cambivora*; P = probability of a greater chi-square value occurring.

TABLE 4. Detection of *Phytophthora cactorum* and *P. cambivora* associated with bundles of nursery-grown apple trees in 1983 and 1984[a]

	Number of bundles infested[c]					
	P. cactorum		*P. cambivora*		*P. cactorum* or *P. cambivora*	
Rootstock[b]	1983	1984	1983	1984	1983	1984
Clonal rootstocks						
MM.106	24/24	29/30	1/24	2/30	24/24	29/30
MM.111	23/24	14/14	0/24	0/14	23/24	14/14
M.7	12/15	8/12	0/15	0/12	12/15	8/12
M.26	20/22	10/12	5/22	1/12	20/22	10/12
M.9	6/7	7/10	0/7	0/10	6/7	7/10
M.2	—[d]	5/5	—	0/5	—	5/5
Total	85/92	73/83	6/92	3/83	85/92	73/83
Proportion	0.92	0.88	0.07	0.04	0.92	0.88
Seedling rootstock	—	1/6	—	1/6	—	1/6
Proportion		0.17		0.17		0.17

Comparison[e]	χ^2	P
P. cactorum in 1983 vs. 1984	0.51	0.48
P. cambivora in 1983 vs. 1984	0.30	0.61
P. cactorum vs. *P. cambivora* in 1983 and 1984	250.86	<0.005

[a]Concentrated root washings from individual bundles were assayed sequentially by direct and then by extended baiting procedures using apple cotyledons.

[b]The first six cultivars are vegetatively propagated clonal rootstocks. MM. = Malling-Merton; M. = Malling. Seedling rootstocks were grown from open-pollinated seed. Rootstocks were budded to numerous scion cultivars.

[c]A bundle contained five to 25 trees. Data are presented as (number of bundles infested)/(number of bundles assayed).

[d]No bundles were assayed.

[e]Comparisons between total numbers of bundles of trees on clonal rootstocks infested. χ^2 = chi-square statistic, corrected for continuity, with one degree of freedom; P = probability of a greater chi-square value occurring.

of four samples from within the tree-row of first-season trees, in four of five samples from within the tree-row of second-season trees, in one of three samples from between rows of first-season trees, and in four of five samples from areas never before planted in nursery trees. Three of these last five sites were hardwood forest and two were cropped to corn. *P. citricola* Sawada was recovered from one sample collected in a forested area. A chi-square analysis with three degrees of freedom showed no significant difference ($P = 0.10$) in the detection of *P. cactorum* among sample sites.

Orchard soils. Detection of *P. cactorum* in orchard soil samples did not appear to be affected adversely by the duration of storage at 2–4 C. In soil samples in which the fungus was detected, recovery of *P. cactorum* by the extended baiting bioassay was no different after 5–6 yr of storage than after several days or after 1–2 yr of storage. The proportion of samples with detectable *P. cactorum* after 5–6 yr (0.47) was equivalent to the total proportion of all samples infested (see below).

P. cactorum was detected in 56 soil samples (50%) in 22 orchards; 63% (39/62) of the samples collected around symptomatic trees and 34% (17/50) of the samples collected around healthy-appearing trees had *P. cactorum* (Fig. 2). These values were significantly different ($x^2 = 8.13$, with one degree of freedom;

$P < 0.005$), and 95% confidence intervals were distinct (Fig. 2). In samples collected around healthy-appearing trees compared to those collected around symptomatic trees, *P. cactorum* frequently colonized fewer cotyledon baits and was detected in fewer replicate subsamples.

In 16 orchards where both healthy-appearing and symptomatic tree sites were sampled, the detection of *P. cactorum* in soil samples often was not associated with symptom development. *P. cactorum* was detected around both healthy-appearing and symptomatic trees in seven orchards, around neither type of tree in five orchards, and around only symptomatic trees in four orchards. In no orchard was *P. cactorum* detected around healthy-appearing trees and not around symptomatic trees, nor was *P. cactorum* ever recovered from soils in orchards without symptomatic trees. *P. cambivora* was recovered from soil samples from two orchards. One sample was collected in a newly replanted orchard in eastern New York, and the other was collected in an established, mature orchard in western New York.

Nonagricultural soils. *P. cactorum* was detected in 17 of 37 (46%) nonagricultural soil samples collected in the vicinity of apple orchards (Fig. 2). Detection was judged independent of either plant cover ($x^2 = 1.60$, with one degree of freedom; $P = 0.22$) or drainage class ($x^2 = 1.17$, with three degrees of freedom; $P = 0.76$). Based on the proportion of soil samples in which *P. cactorum* was detected, there was no significant difference between nonagricultural soils and either soils around healthy-appearing trees ($x^2 = 0.79$, with one degree of freedom; $P = 0.40$) or soils around symptomatic trees ($x^2 = 2.03$, with one degree of freedom; $P = 0.17$). The overlapping 95% confidence intervals in Figure 2 substantiate this. *P. citricola* and *P. megasperma* Drechsler were detected in 14 and two of the 37 nonagricultural soil samples, respectively. The presence of either species was not associated with the presence of *P. cactorum*.

P. cactorum was detected in 10 of 11 soil samples collected from sites immediately adjacent to apple orchards. The collection sites were noticeably downslope from the adjacent orchard in all but three sites. Therefore, runoff water from the orchard would be expected to flow into these areas.

Virulence of isolates of *P. cactorum* recovered from soil. All isolates of *P. cactorum* assayed were virulent on excised MM.106 apple twigs. An analysis of variance showed no significant treatment effect in either trial (Table 5). Consequently, isolates recovered from the three different soil sources—symptomatic trees, healthy-appearing trees, and nonagricultural sites—were as virulent as isolates recovered from infected apple trees. Variability

Fig. 1. Pathogenicity of naturally occurring inocula of *Phytophthora cactorum* and *P. cambivora* on unbudded rootstocks of Malling-Merton 106 apple after no flooding (left) and after four flooding cycles (right), during which plants were flooded for 48 hr once every 2 wk: **A**, above-ground symptoms on a representative plant from each treatment; **B**, symptoms on root systems of the same plants.
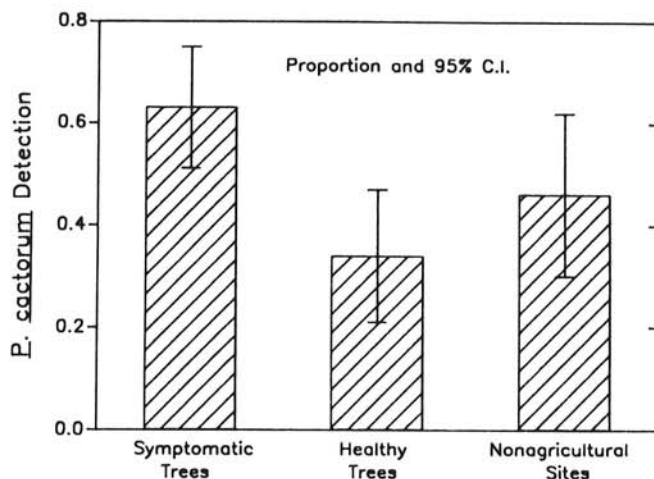

Fig. 2. The proportion of soil samples from three sources in which *Phytophthora cactorum* was detected by apple cotyledon baits and an extended baiting bioassay procedure. Samples were collected from around apple trees either with (symptomatic) or without (healthy) typical symptoms of Phytophthora crown rot or from nonagricultural sites in the vicinity of apple orchards. The total proportions and corresponding 95% confidence intervals (bars) are based on 62, 50, and 37 samples for each soil source, respectively.

in net necrosis lengths within sources was considerable, but the relative ranking of isolate sources remained constant between trials. Isolates from infected apple trees ranked lowest, and those from soil around symptomatic trees ranked highest (Table 5). The twigs in control jars containing only agar developed no necrosis.

DISCUSSION

The results of this study would not have been as meaningful without the recent development of an improved baiting bioassay that has greatly enhanced the detection of *P. cactorum* in naturally infested soils (14). With this technique, *P. cactorum* was recovered from a diverse array of soil and rootwash samples. In addition, apple cotyledon baits proved successful at detecting both *P. cambivora* and *P. citricola*, as previously suggested (14). As expected, though, *P. megasperma*, another important crown rot pathogen (16), was detected very infrequently (in only two nonagricultural soil samples); this species does not colonize apple cotyledon baits (14).

Infested apple nursery stock is one source of primary inoculum for Phytophthora crown rot of apple trees. Of the bundles of unbudded rootstocks (clonal and seedling) coming into New York apple nurseries in 1983 and 1984, 97% were contaminated with either *P. cactorum*, *P. cambivora*, or both species. After two growing seasons in the nursery, 88% of the bundles of young apple trees destined for orchards throughout the United States and Canada were infested, primarily with *P. cactorum*. We have demonstrated that this inoculum is pathogenic. It appears that Julis et al (18) were correct in suspecting their estimates of the incidences of *P. cactorum* (7.8%) and *P. cambivora* (8.7%) on apple nursery stock were "conservative." The 87% of samples of apple rootstocks infested with *P. cactorum* reported by Brown and Hendrix (4) also may have been conservative, and they did not detect *P. cambivora*. Our greater detection of both *P. cactorum* and *P. cambivora* is most likely due to the sensitivity and effectiveness of apple cotyledon baits and the extended baiting bioassay.

The rootstock cultivars assayed vary in resistance to Phytophthora crown rot under New York orchard conditions (S. N. Jeffers, *unpublished*). However, all clonal rootstock cultivars, regardless of their field performance, appear to be equally infested. The incidence of *Phytophthora* spp. on unbudded rootstocks and nursery-grown trees was not restricted to any one nursery or geographical location. Even unbudded rootstocks imported from Europe were infested with *P. cactorum*.

P. cactorum, the species isolated most frequently from symptomatic trees in New York apple orchards between 1978 and 1984 (S. N. Jeffers, *unpublished*) and most commonly associated with crown rot worldwide (28), probably becomes associated with roots of apple plants in rootstock propagation beds, remains associated with the roots through two seasons' growth in apple tree nurseries, and then is carried to orchards with the young apple trees. Infested nursery-grown trees lacked obvious symptoms, as reported previously (18), and *P. cactorum* was detected in rootwash slurries almost exclusively by extended baiting. This suggests that this species was present not in an active form but primarily as inactive, quiescent oospores (14).

At this stage, the association between *P. cactorum* and apple roots may not be parasitic or pathogenic. Instead, the organism merely may survive or persist in the rhizosphere of apple roots. We believe that environmental and physiological stresses play an important role in initiating, and may be prerequisite for, the development of Phytophthora crown rot in New York apple orchards. Such stresses may include temperature extremes, excess or shortage of water, cold injury, and the onset of flowering. The importance of flooding to disease incidence was demonstrated in this study and was suspected previously (16). Cother and Griffin (9) hypothesized a similar situation for the existence of *P. drechsleri* in the rhizosphere of herbaceous hosts.

P. cambivora, on the other hand, was associated only with the roots of unbudded clonal rootstocks and infrequently remained associated with the roots of nursery-grown trees ready for planting. This species apparently did not survive well on apple roots during two seasons in the nursery. One hypothesis is that *P. cambivora* does not tolerate cold winter temperatures. *P. cinnamomi*, a closely related species (10,35), was inactivated by temperatures below 0 C (2). Both species are heterothallic (35) and, therefore, normally do not produce durable oospores for survival (see below and reference 38), as does *P. cactorum* (14), a homothallic species (35). Another hypothesis is that *P. cambivora*, lacking oospores, is less capable of surviving or persisting nonparasitically in the rhizosphere of apple, because of the biological pressures of competition and antagonism. That we did not detect *P. cambivora* in any nursery soil sample, regardless of whether the planted trees had been through a winter, may support this hypothesis. However, it has survived sufficiently to cause crown rot in Australia (6), California (24), and Japan (32). For whatever reason, the absence of *P. cambivora* on roots of nursery-grown trees is perhaps why this organism has been recovered from only one diseased apple tree in New York orchards (S. N. Jeffers, *unpublished*).

The recovered isolates of *P. cambivora* were almost exclusively of mating type A1; only three of 92 isolates were A2. A similar situation was reported in Japan (32). Such an imbalance between A1 and A2 mycelia greatly reduces the potential for oospore formation and, therefore, long-term survival in nursery and orchard soils. Failure to detect *P. cambivora* after air-drying rootwash debris supports this hypothesis. However, the recovery of *P. cambivora* from two soil samples by extended baiting is evidence that some propagules of this fungus were durable enough to withstand air-drying. One sample was collected from a mature orchard, which indicates that *P. cambivora* may establish, although infrequently, in New York orchards. Both recovered isolates were of mating type A1. This is believed to be the first report of *P. cambivora* from soils in New York.

The recovery of *P. syringae* both from one rootwash slurry and from stem cankers on unbudded apple rootstocks shows that this species occurs in rootstock nurseries and also may be moved to apple orchards with nursery-grown trees. It has not been recovered from New York orchards to date but is an important crown rot pathogen in Europe (29).

The occurrence of *Phytophthora* species on nursery stock is not a new problem. In addition to previous reports of *P. cactorum* and *P. cambivora* on apple nursery stock (4,18,31), *P. cactorum* and other species of *Phytophthora* have been reported on nursery stock of other deciduous fruit crops worldwide, including stone fruits (11,26,31,37), raspberries (36), and grapes (34). Infested unbudded rootstocks and nursery-grown trees undoubtedly are playing an

TABLE 5. Virulence of isolates of *Phytophthora cactorum* from different sources on excised twigs of Malling-Merton 106 apple at 22 C[a]

Source of isolates[b]	Net necrosis length (mm)[c]	
	Trial 1	Trial 2
Apple tree	20.1 ± 15.2[d]	13.3 ± 11.4
Soil		
Symptomatic trees	27.4 ± 17.1	17.9 ± 16.0
Healthy-appearing trees	23.1 ± 16.9	13.6 ± 12.3
Nonagricultural	24.0 ± 13.5	16.2 ± 15.9
Control	No necrosis	No necrosis
F statistic[e]	1.49	1.22

[a] The results from five distinct isolates for each source were combined.

[b] Isolates were recovered from infected apple tree roots and crowns, soil around apple trees exhibiting typical Phytophthora crown rot symptoms, soil around healthy-appearing apple trees, and nonagricultural soil in the vicinity of apple orchards. Twigs in control jars were not inoculated.

[c] Ten 65-mm twigs were placed vertically in a 12-day-old agar culture of a test isolate. Net necrosis length was the length of the resulting lesion after subtracting the agar depth, at 21 days in trial 1 and at 17 days in trial 2.

[d] Data are the means of 50 observations (five isolates per source with 10 twigs used per isolate) plus or minus the pooled standard deviation.

[e] *F* statistics, computed from a one-way analysis of variance of each trial, were not significant. An *F* statistic with 3 and 196 degrees of freedom equals 2.65 for $P = 0.05$.

important role in the dispersal of Phytophthora crown rot pathogens and the increasing incidence of this disease. An effort is needed to eradicate *Phytophthora* spp. from nursery plantings.

Naturally infested soil is another source of primary inoculum for Phytophthora crown rot of apple trees. *P. cactorum* was widely distributed in both orchard and nonagricultural soils in the apple-growing regions of New York. The fungus was recovered from soil around healthy-appearing and symptomatic trees, although the frequency of recovery was higher around the latter. These data are in agreement with some earlier reports (1,30,33), but other reports indicated occurrence of *P. cactorum* was associated more closely with symptom expression (3,22). Julis et al (19), on the other hand, found optimum detection of *Phytophthora* spp., including *P. cactorum*, around trees without symptoms. We found *P. cactorum* was detected in more replicate soil subsamples and colonized more cotyledon baits in assays of samples from around symptomatic trees than of samples from around healthy-appearing trees, which suggests a higher inoculum density around symptomatic trees.

That *P. cactorum* was not detected around numerous symptomatic trees does not necessarily mean the fungus was absent. The population density of *P. cactorum* may have been below the detection threshold for the apple cotyledon baiting bioassay; such limits have not been determined. In addition, our sampling scheme for orchard trees may not have taken advantage of zones of highest populations. Based on previous reports (1,22), our soil samples were collected around the trunk, close to the root crown, where relatively few roots occur. If *P. cactorum* survives quiescently in the rhizosphere as we have hypothesized, soil samples collected from under the drip line, where root density is higher, may have yielded a higher proportion of *P. cactorum*. Alternatively, other species of *Phytophthora* not detected by extended baiting with apple cotyledons (14) could be involved. Most notable is *P. megasperma*, which has been isolated frequently from symptomatic trees in New York (16). In two orchards where *P. cactorum* was not recovered from soil samples, *P. megasperma* was isolated previously from symptomatic trees (S. N. Jeffers, *unpublished*).

The question arises whether *P. cactorum* is an indigenous organism in orchard soils or whether the fungus was introduced, presumably on infested nursery stock. There is previous evidence for the occurrence of *P. cactorum* in forest soils (7,23), but it is unclear whether these sites were distant from possible sources of infestation. *P. citricola* (syn. *P. cactorum* var. *applanata* Chester), a species quite similar to *P. cactorum* (30,35), does appear to be indigenous to western New York soils. It was never detected on nursery stock but often occurred in nonagricultural soils. This suggests that *P. cactorum* also could be indigenous. To our knowledge, this is the first report of *P. citricola* in New York soils.

It is possible that *P. cactorum* was introduced and has spread from contaminated nursery stock. This species has a broad host range (25) and easily could become established in the absence of apple. Widespread dispersal of *P. cactorum* is likely. The fungus has been reported to occur in both orchard runoff and irrigation waters (3,12,21), and dissemination by movement of infested soil also is possible. We detected *P. cactorum* in all nonagricultural soil samples collected downslope from infested orchards and from all sites in and around apple nurseries where irrigation and cultivation were used extensively. Isolates of *P. cactorum* recovered from all three soil sources—healthy-appearing trees, symptomatic trees, and nonagricultural sites—were as virulent to MM.106 apple twigs as were isolates recovered from infected trees. McIntosh (20), investigating a similar situation, speculated that *P. cactorum* was not indigenous to apple orchards and other fields in the Okanagan and Similkameen valleys of British Columbia but instead was introduced and disseminated by infested irrigation water.

The origin of *P. cactorum* in the soils of western New York's apple region remains unclear. However, we have demonstrated that both infested apple nursery stock and naturally infested soils are sources of primary inoculum for Phytophthora crown rot of apple trees. The relative importance of each inoculum source in the incidence of this disease needs to be determined.

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