

Phytophthora Root and Crown Rot of Apple Trees in Arizona

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ABSTRACT

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Five *Phytophthora* spp. were isolated from declining apple trees in commercial orchards in Arizona. *Phytophthora cactorum*, *P. cambivora*, *P. drechsleri*, *P. parasitica*, and an unidentified *Phytophthora* sp. (isolate A46) were recovered from 43, seven, three, one, and two trees, respectively, of 56 trees from 36 different orchard sites. Additionally, *P. cactorum* and *P. cambivora* were recovered from nursery-grown apple rootstocks and trees. All five *Phytophthora* spp. were pathogenic to apple seedlings grown in artificially infested potting mix in greenhouse tests. *P. cactorum* and *P. cambivora* were highly virulent, causing rapid plant decline and death. *Phytophthora drechsleri*, *P. parasitica*, and *Phytophthora* sp. (isolate A46) were less virulent, causing root necrosis and occasional plant death. These results implicate *Phytophthora* spp. in the decline and death of apple trees in Arizona. The association of *P. cactorum* and *P. cambivora* with roots of nursery-grown apple plants may partially explain the occurrence and spread of *Phytophthora* root and crown rot in this state.

Phytophthora root and crown rot is a destructive and widespread disease in most areas of the world where apples are grown. Species of *Phytophthora* recovered from affected trees or apple orchard soils include *P. cactorum* (Lebert & Cohn) Schroet. (3,4,8,10,13,15,19,20), *P. cambivora* (Petri) Buisman (4,6,10,14,17), *P. citricola* Sawada (4,13,15), *P. cinnamomi* Rands (10), *P. cryptogea* Pethyb. & Laff. (4,6), *P. drechsleri* Tucker (10), *P. megasperma* Drechsler (4,8,10,16), and *P. syringae* (Kleb.) Kleb. (4). *Phytophthora cactorum*, *P. cambivora*, and *Phytophthora* spp. also have been recovered from nursery-grown apple plants (5,9).

During the past 3 years, dead and declining apple trees have been observed in several orchards in southeastern Arizona. Symptoms associated with declining trees included the presence of small, pale-green leaves, sparse foliage, and the absence of vigorous growth of terminal shoots. In early autumn, leaves of declining trees became reddish in color, while neighboring healthy trees retained dark-green foliage. Examination of the trunk area, from 1 to 20 cm below the soil surface, usually revealed the presence of a crown canker. This necrotic tissue had completely girdled trees showing severe symptoms of decline. Various degrees of root rot also were observed. Several *Phytophthora* spp. were recovered from the decayed root

and crown tissue, as well as from soil around declining apple trees. Two *Phytophthora* spp. also were recovered from five of 12 tested nursery-grown apple trees from Maryland.

The present investigations were initiated to identify the *Phytophthora* spp. associated with declining apple trees and to determine their role in the incidence and severity of root and crown rot of apple trees in southeastern Arizona. A short account of this work was reported previously (11).

MATERIALS AND METHODS

Isolation of *Phytophthora* spp. from infected trees and soils. Apple orchards sampled were planted between 1979 and 1985 and included trees on M.7, MM.106, MM.111, and apple seedling rootstocks. From three to 10 declining trees were sampled in 36 different orchard sites in southeastern Arizona. Isolations were made from decayed root and crown tissues, and from soils surrounding diseased apple trees by using previously described methods (12,13). Segments of decayed roots 1–2 cm long were rinsed in tap water, dipped in 70% ethanol, dried on a paper towel, and pressed into a selective medium containing cornmeal agar (CMA) amended with 5 mg of pimarin, 300 mg of vancomycin hydrochloride, and 25 mg of pentachloronitrobenzene per liter (PVP) (12). Small pieces (2 × 5 mm) of bark collected from the margins of active cankers on the trunk were processed in a similar manner. Twenty root and/or bark pieces were plated for each tree. The plates were incubated in darkness at 21 C and examined daily for 5–7 days for growth of *Phytophthora* spp.

Isolation of *Phytophthora* spp. from soil was achieved by collecting soil from three locations within the drip line of each apple tree, then thoroughly mixing the three subsamples. Approximately 500 cm³ of this combined soil sample was placed in a container. Two ripe but green-colored, unblemished Bartlett or Anjou pear fruits were placed on the surface of the soil sample. Enough water was added to establish a 1- to 2-cm layer of free water at the soil surface (12). After incubation at 21 ± 1 C for 48 hr, the fruits were removed from the soil, washed, and incubated for an additional 24–72 hr at 25 ± 2 C. Firm brown spots developed on pear fruits invaded by *Phytophthora* spp. Small pieces of tissue from the advancing margin of the brown spot were placed on PVP, incubated at 21 C in darkness, and observed for growth of *Phytophthora*.

Identification of *Phytophthora* spp. Colony morphology and cardinal temperatures for vegetative growth were determined on CMA. Sporangia were produced when 6-mm-diameter V-8 agar (containing 200 ml of commercial V-8 juice; 2 g of CaCO₃; 17 g of agar; and 800 ml of distilled water) disks with mycelia from the edge of an actively growing colony were placed in petri plates, flooded with a nonsterile soil extract, and incubated at 21–24 C for 8–48 hr. Soil extract was prepared by mixing 15 g of sandy orchard soil in 1 L of distilled water with a magnetic stirrer for 8 hr at 24 ± 1 C. After an additional 16-hr incubation period, soil extract was decanted and separated from large soil particles remaining in the bottom of the flask. Further clarification of the soil extract was achieved by centrifugation at 5,000 g for 20 min or by filtration through Whatman No. 2 filter paper.

Production of sexual structures and determination of compatibility types of the apple isolates were performed on clarified V-8 juice agar. Clarified V-8 juice agar was prepared by mixing 17 g of agar with 50 ml of clarified V-8 juice (centrifuging the supernatant from V-8 juice for 10 min at 1,000 g), 20 mg of β-sitosterol dissolved in 5 ml of warm 95% ethanol, 111 mg of CaCl₂, and 950 ml of distilled water. The medium was adjusted to pH 5.5 with 0.1 N KOH before autoclaving. Isolates from apple trees were identified on the basis of descriptions by Tucker (18) and Waterhouse (21–26).

Pathogenicity and relative virulence tests. Pathogenicity and virulence of apple isolates of *P. cactorum*, *P. cambivora*, *P. drechsleri*, *P. parasitica* Dastur, and one unidentified *Phytophthora* sp. (isolate A46) to apple seedlings were determined under greenhouse conditions with soil temperatures of 15–24 C. Apple seedlings were grown in sterile potting mix (45% peat:45% vermiculite:10% sand) in 10-cm-diameter × 10-cm-deep plastic pots. Representative species of *Phytophthora* were grown on V-8 agar for 7 days at 24 C. Ten 6-mm-diameter agar disks were removed from the edges of actively growing cultures of each isolate and placed randomly on the potting mix surface around the 2- to 6-wk-old apple seedlings growing in the pots. The agar disks contained only actively growing mycelium and no sporangia or oospores. Uninoculated control plants received agar disks without mycelium. The pots containing seedlings and agar disks were placed for 48 hr in water-filled plastic pans 20 cm wide × 25 cm long × 13 cm deep, so that 1 cm of water covered the potting mix surface in each pot. During the 48-hr period of flooding, the agar disks were not touching the stems of trees. Pots were removed from the pans of water after 48 hr and allowed to drain freely. To enhance disease development, the 48-hr flood treatment was repeated every 2 wk during the test. Between flooding treatments, the seedlings were watered as needed and the potting mix was allowed to drain freely. After 3 mo, the experiments were terminated and disease severity was assessed by recording fresh weight of roots and percent of root system that was decayed.

The pathogenicity and virulence tests were performed five times, with the number of single plant replicates per treatment ranging from five to 15. Disease was confirmed as resulting from infection by the appropriate *Phytophthora* sp. by reisolating the pathogen from test apple seedlings. Plants were fertilized weekly with water-soluble Miracle-Gro fertilizer.

Excised stem inoculations. Representative isolates of the same species of

Phytophthora were grown on V-8 agar for 7 days at 24 C. Stem pieces of dormant or actively growing rooted cuttings of MM.106 apple rootstock were cut into 10-cm-long pieces. The stem pieces were wounded by removing a 6-mm-diameter plug of phloem, then inoculated by placing a 6-mm-diameter mycelial disk into the wound. Excised stems were then placed in a moist chamber (100% relative humidity) and incubated for 7 days in darkness at 21 C. Disease severity was determined by measuring the lengths of cankers that developed at the inoculation sites. This experiment was repeated twice, and, because each experiment yielded comparable results, data from only one experiment are presented in the results section.

RESULTS

***Phytophthora* spp. associated with apple orchard trees.** Five species of *Phytophthora* were isolated from decayed roots and crown cankers of apple trees and from the surrounding soil (Table 1). Occasionally, more than one *Phytophthora* sp. was recovered from the same tree. Isolates recovered from apple trees were *P. cactorum* (ATCC 62934), *P. cambivora* (ATCC 62935), *P. drechsleri* (ATCC 62936), *P. parasitica* (ATCC 62937), and one unidentified *Phytophthora* sp. (isolate A46). The cardinal temperatures, morphology of sporangia, and general morphology of isolates of the identified *Phytophthora* spp. were similar to or within the limits reported for these species (18,21–26). The isolate of *P. cambivora* formed no sexual reproductive structures in single culture on clarified V-8 juice agar, but formed bullate oogonia when paired with A2 mating types of *P. cryptogea* and *P. drechsleri*. The isolate of *P. drechsleri* also formed oospores when paired with A2 mating types of *P. cryptogea* and *P. drechsleri*. The isolate of *P. parasitica* formed oospores when paired with the A2 mating types of *P. cinnamomi* and *P. drechsleri* as well as the A1 mating types of *P. cryptogea* and *P. palmivora* (Butler) Butler.

The unidentified *Phytophthora* sp.

(isolate A46) had nonpapillate, ovate sporangia that proliferated internally after discharge of zoospores. Minimum, optimum, and maximum temperatures for growth on CMA were <6, 27–30, and 36 C, respectively. Numerous gold-brown oogonia (mean diameter 33 μm) and oospores were produced in a single culture on clarified V-8 juice agar. Further collection, examination, and evaluation of isolates resembling this pathogen are necessary to determine its true taxonomic status.

Phytophthora cactorum was recovered from 43 of 56 trees from which *Phytophthora* spp. were isolated, while *P. cambivora*, *P. drechsleri*, *P. parasitica*, and *Phytophthora* sp. (isolate A46) were recovered from seven, three, one, and two trees, respectively. *Phytophthora cactorum* and *P. cambivora* were recovered from crown cankers, decayed roots, and soil; *P. drechsleri* was recovered from decayed roots and soil; and *P. parasitica* and *Phytophthora* sp. (isolate A46) were recovered from soil (Table 1).

***Phytophthora* spp. associated with nursery-grown stock.** *Phytophthora cactorum* and *P. cambivora* were recovered from decayed roots and adhering soil of apple trees purchased from a nursery in Maryland as replants for an existing orchard. *Phytophthora cactorum* was recovered from five of 12 trees sampled. *Phytophthora cambivora* also was recovered from one of the five plants from which *P. cactorum* was isolated. Trees from which *Phytophthora* was recovered were propagated on M.7 and apple seedling rootstocks.

Clonal rootstock liners (MM.106) were obtained from another nursery in Oregon and planted in sterile potting mix. Within a month, 20 of 100 rootstock liners leafed out, then suddenly collapsed. Rapidly developing crown cankers were evident on the declining apple plants. *Phytophthora cactorum* and *P. cambivora* were readily recovered from decayed root and crown tissue.

Pathogenicity and relative virulence tests. In these experiments, we examined the pathogenicity and compared the relative virulence of isolates of *P. cactorum*, *P. cambivora*, *P. drechsleri*, *P. parasitica*, and *Phytophthora* sp. (isolate A46). Different isolates of these pathogens were used for some experiments, but the relative virulence of each *Phytophthora* sp. was similar in each experiment. The results of one of these experiments are summarized in Table 2. All five fungi were pathogenic to 3-wk-old apple seedlings, but significant differences in virulence were evident among the *Phytophthora* spp. *Phytophthora cactorum* and *P. cambivora* were highly virulent and caused severe destruction of roots and crown after 3 mo, resulting in tree death in 75 and 100% of test apple seedlings, respectively. *Phytophthora*

Table 1. Isolation of *Phytophthora* species from diseased apple trees in Arizona

<i>Phytophthora</i> sp.	Frequency of isolation ²		
	Crown cankers	Decayed roots	Soil
<i>P. cactorum</i>	14	6	36
<i>P. cambivora</i>	1	2	6
<i>P. drechsleri</i>	0	2	3
<i>P. parasitica</i>	0	0	1
<i>Phytophthora</i> sp. (isolate A46)	0	0	2

²Samples of crown, roots, and soil (72, 53, and 149, respectively) from 153 declining orchard trees were tested for the presence of *Phytophthora* species. Methods of isolation are fully described in the text.

drechsleri, *P. parasitica*, and *Phytophthora* sp. (isolate A46) were significantly less virulent than *P. cactorum* and *P. cambivora*, causing moderate levels of root decay and plant death in 9, 6, and 0% of test seedlings, respectively.

Excised stem inoculations. Canker development on excised stems inoculated with *P. cactorum* or *P. cambivora* was significantly influenced by the physiological status of the apple plant (Table 3). Inoculation of dormant stems of MM.106 rootstock resulted in development of small cankers, whereas inoculation of actively growing stem pieces of the same plant material resulted in development of much larger cankers. The physiological status of apple stem tissue did not significantly affect canker development on stem pieces inoculated with *Phytophthora* sp. (isolate A46), *P. drechsleri*, or *P. parasitica* (Table 3).

DISCUSSION

Results of these investigations show that *P. cactorum* and *P. cambivora* are associated with declining apple trees affected with root and crown rot, whereas *P. drechsleri*, *P. parasitica*, and *Phytophthora* sp. (isolate A46) are associated with apple trees affected with root rot. Pathogenicity studies reveal that all tested isolates can cause root decay on apple seedlings, while *P. cactorum* and *P. cambivora* can cause crown rot as well.

Jeffers and Aldwinckle (6) have recently reported a seasonal variation in degree of colonization of apple rootstocks by *Phytophthora* spp. The increase in canker development on actively growing stem tissue, when compared with dormant stem tissue, agrees with their findings concerning inoculation of MM.106 apple rootstock with *P. cactorum* or *P. cambivora*. Excised-stem assays have been used to study the susceptibility of apple trees to *P. cactorum* and to compare relative virulence of isolates within a species (1,2,7). Our data, as well as earlier findings (6,14), suggest that the physiological status of apple rootstock tissue can have a profound influence on the apparent susceptibility of this plant material to colonization by *P. cactorum* or *P. cambivora*.

Phytophthora cactorum and *P. cambivora* were highly virulent and caused rapid decline and death of apple seedlings. These pathogens also are the most frequently isolated *Phytophthora* spp. in commercial apple orchards in Arizona. The presence of these highly virulent pathogens within orchards suggests that *P. cactorum* and *P. cambivora* are potentially the most devastating *Phytophthora* spp. that can attack apple trees in Arizona.

The relative distribution of *Phytophthora* spp. in diseased apple orchards differs considerably between geographical

Table 2. Growth and disease development in apple seedlings grown in potting mix infested with five *Phytophthora* species and flood-irrigated biweekly for 48 hr

Potting mix infestation	Plant growth and disease severity ^y	
	Fresh wt of roots (g)	Root rot ^z (%)
Control (uninfested)	1.04 a	15 a
<i>P. cactorum</i>	0.11 e	95 b
<i>P. cambivora</i>	0.12 e	87 b
<i>P. drechsleri</i>	0.50 d	34 a
<i>P. parasitica</i>	0.77 b	33 a
<i>Phytophthora</i> sp. (isolate A46)	0.61 c	24 a

^y Average of 14 replicate plants per treatment. Numbers in each column with the same letter do not differ according to Duncan's multiple range test ($P=0.01$).

^z Percent of root system rotted as estimated by visual observation 3 mo after inoculation. *Phytophthora* spp. were reisolated from decayed roots of all plants in infested potting mix, but not from those grown in uninfested potting mix.

Table 3. Canker development on excised stems of MM.106 clonal rootstock inoculated with five *Phytophthora* species

Excised stem inoculation	Length of canker ^y (mm)		
	Inoculation of dormant stems	Inoculation of actively growing stems	Increased in canker length ^z (mm)
Uninoculated control	0	0	0 a
<i>P. cactorum</i>	0	23	22 b
<i>P. cambivora</i>	3	64	60 b
<i>P. drechsleri</i>	2	2	3 a
<i>P. parasitica</i>	8	13	6 a
<i>Phytophthora</i> sp. (isolate A46)	1	4	4 a

^y Average of 5 replicates per treatment.

^z Increase in canker length when comparing dormant to actively growing excised stems 7 days after inoculation. Average of five replicate pairs of stems per treatment. Numbers with the same letter do not differ according to Duncan's multiple range test ($P=0.05$).

regions of the United States. In New York, Jeffers et al (8) recovered *P. megasperma* more frequently than *P. cactorum* (from eight and three trees, respectively). By comparison, Julis et al (10) recovered *P. cactorum* and *P. cambivora* from 15 and 4% of sampled trees, respectively, in North Carolina, while *P. megasperma* was recovered from only one sample. In Arizona, of all samples from which *Phytophthora* spp. were recovered, 75% of the samples contained *P. cactorum*, while 13% contained *P. cambivora*. Variation in recovery of *Phytophthora* spp. may be due to the isolation procedures used, the climate variation between various regions of apple production, and the means of immigration or introduction of *Phytophthora* spp. into apple orchards.

The recovery of *Phytophthora* spp. from nursery-grown apple trees shipped to Arizona and elsewhere (5,9) emphasizes the importance of obtaining nursery stock free of these pathogens. Before apple orchards were established in southeastern Arizona, the land was either native grassland or planted to crops not known to be hosts to *P. cactorum* or *P. cambivora*. The association of *P.*

cactorum and *P. cambivora* with nursery-grown apple plants may partially explain the establishment of *Phytophthora* root and crown rot in Arizona.

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