Phytophthora Root Rot and Stem Canker of Peach Trees in Mississippi

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ABSTRACT

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Symptoms of Phytophthora root rot and stem canker were initially detected in Mississippi peach orchards in 1982. Incidence ranged from 1 to 75% of trees in the orchards surveyed. During the following 3 yr, *Phytophthora cinnamomi*, *P. cactorum*, and *P. nicotianae* var. *parasitica* were isolated repeatedly from young, necrotic peach roots. Isolates of all three species, after inoculation to trunks of 1-yr-old container-grown peach trees, caused cankers similar to those observed in the field and also induced root necrosis of peach trees grown in artificially infested soil. This is the first report demonstrating the involvement of *P. nicotianae* var. *parasitica* in root and stem canker of peach trees.

In 1982, numerous commercial peach producers in Mississippi were unable to control apparent borer damage by applying recommended insecticides. Examination of weakened trees with chlorotic and wilted leaves indicated that the clear gum on stem bases was not the result of borer damage but was associated with lesions that extended down into the root system and often up into the scaffold branches. Major portions of the root systems were found to be dead when trees were pulled from their planting sites. Symptoms were not typical of oak root rot, gummosis, peach tree short life, or any other disorder previously reported in Mississippi or surrounding areas. Symptoms were, however, very similar to root rot and stem canker caused by *Phytophthora cinnamomi* Rands (13).

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Several species of *Phytophthora* have been reported as pathogens of peach trees (1,2,8,9,11–13,16,19,20,25). After three *Phytophthora* spp. were recovered from soil and roots in numerous Mississippi peach orchards, a study was initiated to identify the species, determine their role in the decline of peach trees, and conduct an informal survey on the incidence of the disease both among and within commercial peach orchards. A short account of this work was reported previously (4).

MATERIALS AND METHODS

Procedures for isolating *Phytophthora* spp. from infected trees and soils. Isolations were made from roots of trees showing symptoms of root rot in more than 20 commercial peach orchards in Mississippi between 1982 and 1985. From each tree, 10–15 segments 1–2 cm long of decayed roots were dipped in 70% ethanol for 15 sec, rinsed in sterile distilled water for 30 sec, dried on a paper towel, and placed on a selective medium containing cornmeal agar amended with 10 mg of pimaricin, 100 mg of PCNB, 250 mg of ampicillin, 10 mg of rifampicin, and 50 mg of hymexazol per liter (CMA-PPARH) (7) in petri plates. Plates were stored at room temperature for up to 7 days. *Phytophthora* spp. growing on the selective medium were transferred to fresh CMA-PPARH for further studies.

Soil was assayed for species of *Phytophthora* by placing at least one 5-ml soil subsample from each tree site into a well formed by removing a plug about 2 cm in diameter and 1.5 cm deep in apple fruit (cultivar Granny Smith), then replacing the plug. Wet paper towels were placed over the cut apple surfaces in moisture chambers. After 5 days, the apples were examined for decay, and small sections of diseased tissue from advancing margins were placed on CMA-PPARH.

Identification of *Phytophthora* spp. Vegetative growth rates of selected isolates were studied on Difco cornmeal agar (CMA) in polystyrene petri dishes (100 × 15 mm) incubated at 27, 31, and 35 C. Colony morphology was studied on potato-dextrose agar (PDA: extract of 200 g of boiled potatoes, 10 g of glucose, 17 g of agar, and distilled water to make 1

L) and CMA in plastic petri dishes incubated at 20 C. Sporangia were induced by first placing the fungal isolates on clarified V-8 juice agar (CV8A). CV8A was prepared by adding 3 g of $CaCO_3$ to 255 ml of V-8 juice, which was then heated to boiling for 5 min and centrifuged for 10 min at 850 *g*. Seventeen grams of agar was then combined with 200 ml of CV8A and 800 ml of distilled water. Five-millimeter disks of mycelium grown on CV8A were placed into nonsterile 1.5% soil extract, sterile tap water, or 0.1% KNO_3 and incubated for 2–3 days under alternating light (fluorescent CW40) and darkness at room temperature on a laboratory bench. Oogonia, antheridia, and oospores were produced by certain isolates on CV8A. Isolates of heterothallic species were paired with the following A^1 and A^2 compatibility type isolates: *P. nicotianae* (*P. n.*) B. de Haan var. *nicotianae* (Florida Type Culture Collection [FTCC] 493, A^1); *P. n.* var. *parasitica* (Dast.) Waterh. (FTCC 692, A^2), and *P. palmivora* (Butler) Butler (FTCC 491, A^2). Isolates of *P. palmivora* (FTCC 491, 739, 742, and 744), *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leon. (FTCC 693, 694, and 745), *P. n.* var. *nicotianae* (FTCC 493), and *P. n.* var. *parasitica* (FTCC 692) were used in comparing colony morphologies. Isolates from peach roots were identified on the basis of descriptions given by Frezzi (3), Ho (5), Leonian (10), Newhook et al (17), Tucker (21), Waterhouse (22,23), and Waterhouse et al (24).

Pathogenicity tests. Pathogenicity of two isolates each of *P. cinnamomi* (Le and Mo, collected in Leake and Monroe counties, respectively) and *P. n.* var. *parasitica* (H1 and H2, collected from Hinds County) to peach were tested in 1983. The experiment was repeated in 1984 using isolates *P. cinnamomi* (Le) and *P. n.* var. *parasitica* (H1). In 1985, the pathogenicity of the isolates *P. cinnamomi* (Le), *P. n.* var. *parasitica* (H1) and (H2), and *P. cactorum* (A1, collected in Alcorn County) were tested.

Peach trees (2–3 cm caliper on Lovell rootstocks) were planted in plastic containers (25 × 25 cm) containing a steam-sterilized medium composed of two parts clay-loam soil, one part peat moss, and one part sand. The pH of the medium was 6.0. The trees were placed on benches in a lathhouse and fertilized and watered as needed. Sixteen weeks after the trees were planted, the pathogenicities of the selected isolates were determined by two methods. In the first method, the soil around five trees was infested with each isolate, and in the other method, referred to as the wound-plug technique, the trunks of five trees were inoculated with each of the same isolates.

Soil inoculum was prepared by growing the isolates for 6 wk at 21 ± 1 C in 1-L flasks containing 500 cm^3 of sterile vermiculite thoroughly moistened with V-8 juice broth as described by Mircetich and Matheron (14,15). Control flasks contained the same medium but were not infested. For each isolate, the top 5 cm of soil was removed from around five trees, thoroughly combined with the contents of the respective flask, and replaced. Pots were flooded by immersing individual containers in water for 48 hr every other week for 2 mo (14,15). Three months after inoculation, the trees were uprooted and isolations were made from roots as described previously. Percentage of roots decayed was estimated on the basis of fibrous root abundance and root color. The wound-plug technique was used to determine the ability of the isolates to colonize and kill tissue above and below the point of inoculation. Inoculum was prepared by growing the isolates on V-8 juice agar (V8A) (14) 7 days at 21 ± 1 C. Plugs 0.5 cm deep were cut with a sterile no. 3 cork borer (6.5 mm in diameter) from tree trunks about 8 cm above the soil level. Similarly, plugs of inoculum and of uninfested agar were removed and inserted in the trunk wounds. The bark was replaced, and the wound was wrapped successively with absorbent cotton, cheesecloth, and plastic film. The wrapping was held in place with plastic ties at the bottom and rubber bands at the top to facilitate insertion of a water bottle nozzle to moisten the absorbent wrap as needed. The wrapping was removed from the trees 8 wk later. Three months after inoculation, the bark was removed both above and below the wounds, and lesion size was measured. Isolations were made at least 2 cm from the point of inoculation.

All three experiments yielded similar results for the pathogenicity of the *P. cinnamomi* and *P. n.* var. *parasitica* isolates. Data from the final experiment are presented because a *P. cactorum* isolate was included and results of the pathogenicity tests were more definitive.

RESULTS

Symptoms and incidence of disease and isolation of *Phytophthora* spp. Isolates of *P. cactorum*, *P. n.* var. *parasitica*, and *P. cinnamomi* were identified from two, three, and four orchards, respectively. Symptoms of Phytophthora root rot and stem canker observed in Mississippi were very similar to those described by Mircetich and Keil (13). However, cankers on trees infected with *P. cactorum* (Leb. & Cohn) Schroet. generally extended less than 0.5 m up from the soil line, whereas cankers on trees infected with *P. cinnamomi* and *P. n.* var. *parasitica* often extended up into the scaffold branches. Initial wilt symptoms typically appeared in late spring or early fall. Gum exudation often occurred and was heaviest 1 or 2 days after a rain of 1 cm or more. A large percentage of diseased trees did not form leaves in spring the year after symptoms were first observed; however, some trees lived until early to midsummer of the next year and then died. A few symptomatic trees survived and continued to bear fruit.

Incidence of diseased trees within orchards visited during the informal surveys varied from 1 to 75%. Although diseased trees ranged from 2 to 9 yr old, 3- to 4-yr-old trees had the greatest incidence of disease. With only one exception, the surveyed peach orchards had been planted where peaches had not been grown previously. Disease incidence was as high in orchards that had been planted in newly cleared ground as in those planted where grasses had grown for more than 20 yr. However, *P. cinnamomi* was usually isolated from peach roots in areas that had been cleared of a natural stand of *Pinus* spp. and *Quercus* spp. before planting or that were near natural stands of pine and oak.

High disease incidence occurred on both poorly drained and well-drained soils. Most Mississippi orchards are planted over rolling terrain, and almost as many trees on hilltops as in low areas died from the disease.

Pathogenicity tests. *P. cinnamomi*, *P. nicotianae* var. *parasitica*, and *P. cactorum* readily colonized trunk tissues above and below wound inoculation sites and produced symptoms very similar to those observed on infected trees in orchards. The average lesion lengths on trunks inoculated with the *P. n.* var. *parasitica* and *P. cinnamomi* isolates ranged from 21.8 to 27.0 cm (Table 1). Inoculation with the *P. cactorum* isolate resulted in an average length of 11.6 cm. Necrosis extended less than 2 cm from wounds treated with sterile V8A plugs. The *Phytophthora* spp. were isolated from necrotic tissue 2 to 5 cm above and below the inoculated wounds of the respective treatments whereas no *Phytophthora* spp. were isolated from the necrotic areas surrounding the wounds treated with sterile V8A plugs.

Average necrosis ratings of roots in soils infested with *P. cactorum* and *P. n.* var. *parasitica* isolates ranged from 54 to 64%, whereas infestation of soils with *P.*

Table 1. Lesion lengths on seedling peach rootstocks (Lovell) induced by isolates of *Phytophthora cinnamomi*, *P. nicotianae* (*P. n.*) var. *parasitica*, and *P. cactorum* inoculated into trunk wounds

Treatment	Lesion length[z] (cm)
P. cinnamomi (Le)	26.0 a
P. cactorum (A1)	11.6 b
P. n. var. *parasitica* (H1)	27.0 a
P. n. var. *parasitica* (H2)	21.8 a
Control (sterile agar plug)	3.8 c

[z] Based on five replicates per treatment. Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Root rot on seedling peach rootstocks (Lovell) grown in a lathhouse in periodically flooded soil artificially infested with isolates of *Phytophthora cinnamomi*, *P. nicotianae* (*P. n.*) var. *parasitica*, and *P. cactorum*

Treatment	Root rot[z] (%)
P. cinnamomi (Le)	88 a
P. cactorum (A1)	54 b
P. n. var. *parasitica* (H1)	60 b
P. n. var. *parasitica* (H2)	64 b
Control (sterile agar plug)	30 c

[z] Average visual assessment of five replicates per treatment. Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

cinnamomi resulted in an average rating of 88% (Table 2). Ratings of the treatments inoculated with sterile vermiculite media averaged 30%.

DISCUSSION

Several species of *Phytophthora* have been reported pathogenic to peach seedlings (1,8,11,13,19,20,25). Few accounts of Phytophthora root rot and stem canker of bearing peach trees have been published. In 1965, Powell et al (18) isolated *P. cinnamomi* and *P. parasitica* from soils in Georgia peach orchards and suggested their association with peach tree death under conditions of excessive moisture; however, the pathogenicity of the isolates to peach roots was not determined. Mircetich and Keil (13) isolated *P. cinnamomi* from peach orchards in Maryland and Pennsylvania in the late 1960s and demonstrated its pathogenicity to peach. Mircetich (12) later reported the association of eight species of *Phytophthora* with peach tree death in California. More recently, several species of *Phytophthora* have been associated with the death of peach trees in Pennsylvania and West Virginia (2,9). The lack of additional published reports may be because the disease has not been a major problem, or perhaps it has been present and misdiagnosed as damage by waterlogged soil or the peach tree borer. Phytophthora root rot and stem canker may be causing greater damage than is presently recognized, and more emphasis should be placed on the potentially destructive nature of this disease.

Symptoms caused by *P. cactorum* on peach trees in Mississippi orchards were different from those caused by *P. cinnamomi* and *P. n.* var. *parasitica*. Lesions caused by *P. cactorum* normally extended less than 0.5 m above the soil line, whereas those caused by *P. cinnamomi* and *P. n.* var. *parasitica* often extended into the scaffold branches. This relationship was consistent with our lathhouse study, where inoculation of *P. cactorum* to peach trunks caused significantly shorter lesions than did inoculation with *P. cinnamomi* or *P. n.* var. *parasitica*.

Mircetich and Keil (13) isolated *P. cinnamomi* consistently from trees in orchards planted on newly cleared land where the predominant natural stand consisted of *Quercus* spp. and *Pinus* spp. This was also true in our study and suggests that *P. cinnamomi* is a natural inhabitant of soils in Mississippi peach orchards. It is possible that other *Phytophthora* spp. not isolated in this study are present in Mississippi peach orchards. Perhaps different selective media and isolation techniques should be employed in the future.

Some of the species of *Phytophthora* isolated in Mississippi may have been introduced on peach rootstocks obtained from nurseries in other states. This is of particular concern with *P. cactorum* because it has been isolated from commercially available apple rootstocks (6), from soil around peach seedlings stored in packing sheds (18), and from peach seedlings collected from several commercial nurseries (R. C. Lambe, *personal communication*). It is therefore important that growers examine peach tree roots and trunks closely before planting and return or destroy trees that have necrotic roots or root lesions that suggest the presence of a root rotting fungus.

Large portions of numerous peach root systems were destroyed by *Phytophthora* in well-drained areas of several orchards. This was unexpected because root rots caused by *Phytophthora* are usually associated with heavy or poorly drained soils. In greenhouse studies, Mircetich and Keil (13) demonstrated little difference between root weights of peach trees grown in field soil naturally infested with *P. cinnamomi* and saturated periodically and those of trees grown in soil that was not saturated.

Of the three species of *Phytophthora* isolated in our study, *P. cinnamomi* and *P. cactorum* have been previously reported and demonstrated to be pathogens of peach (2,8,11–13). The pathogenicity of *P. n.* var. *parasitica* to peach was demonstrated for the first time in this study with both the wound-plug and soil inoculation techniques.

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