Root Rot of Bamboo Palm Caused by *Phytophthora arecae*

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ABSTRACT

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Phytophthora arecae caused a blackened, water-soaked necrosis on roots and shoots (formed beneath the soil) of bamboo palm (*Chamaedorea seifrizii* × *erumpens*). Under controlled conditions, the fungus primarily infected apical portions of roots of this ornamental palm. Root rot severity and recovery of the pathogen from necrotic root tissue were reduced by both fosetyl-Al and metalaxyl fungicides. Dry matter accumulations and root:shoot ratios were not influenced by treatment with either of these fungicides, however. Watering schedules of every 1, 2, 4, or 6 days had no effect on dry matter accumulations, root:shoot ratios, root rot severities, or recovery of the pathogen. Although *P. arecae* may be a soilborne pathogen of only minor concern on bamboo palm, its soilborne role with palms on which it can induce a foliar disease phase may be important epidemiologically.

Additional keywords: bud rot and nut fall of coconut palm

Species of *Phytophthora* have been identified infrequently as pathogens of members of the family Palmae. In Florida, *P. palmivora* Butl. was reported as the cause of a bud rot of Washingtonia palm (*Washingtonia robusta* Wendl.) (2) and, before the appearance of lethal yellowing, caused the most important disease of coconut palm (*Cocos nucifera* L.), bud rot, in nurseries and landscape plantings in the state (17). Bud rot of coconut palm has also been reported in India (9), the Philippines (15), and Puerto Rico (20). In India, *P. meadii* McRae was recently reported as the incitant of koleroga, or fruit rot, of the arecanut palm (*Areca catechu* L.) (16), although an earlier report of this disease identified *P. omnivora* de Bary (*P. arecae* (Coleman) Pethy.) as the causal agent (19). *P. arecae* also has been reported to cause nut fall of coconut (18). A collar and trunk rot of *W. robusta* caused by unidentified species of *Phytophthora* was reported in California (3,8). In these and other reports (6), root diseases caused by the different species of *Phytophthora* were not noted.

In two recent editions of the *Index of Plant Diseases in Florida* (1,21), *Phytophthora* spp. were associated with root diseases of *Chamaedorea seifrizii* Burret and several other palms. Cause and effect relationships between these fungi and root rots were not established, however, and the fungi isolated were not speciated. A brief description of the root rot of bamboo palm (*C. seifrizii* × *erumpens*) caused by *P. arecae* was presented previously (14). The objectives of this study were to identify tissues of this palm that were infected by *P. arecae* and to determine the potential for damage caused by this pathogen and the effects of fungicides and watering schedule on disease development.

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MATERIALS AND METHODS

During the summer and fall of 1986, declining bamboo palms from a nursery in Homestead, FL, were examined for disease and insect damage. Root damage observed on these palms included galleries caused by larvae of the banana moth (*Opogona sacchari* (Bojer)) (7) and blackened and water-soaked tissue associated and not associated with larval damage. Discolored tissue (associated and not associated with galleries) from these roots and root tissue from bamboo palms not damaged by banana moth larvae from three other nurseries in the Homestead area were tested for the presence of *Phytophthora* spp.

Isolation and identification of *Phytophthora* sp. Segments (1–3 cm long) of blackened, water-soaked first- and second-order roots (as defined by the morphometric root analysis system of Fitter [5]) were washed in tap water, surface-disinfested for 30 sec in 95% ethanol, rinsed once in sterile deionized water, blotted dry with sterile paper towels, and placed on a medium (PARPH) selective for the recovery of *Phytophthora* spp. (11). Roots from at least three and as many as 12 plants were examined for each nursery. Root samples were incubated on the medium for at least 2 days at 25 C in the dark before they were examined for growth of fungi. Isolates of a *Phytophthora* sp. recovered from roots were maintained on slants of Difco potato-dextrose agar (PDA) before use in an experiment.

Isolate Pa 3-1 of the *Phytophthora* sp. was grown on V-8 juice agar and broth (10) for observation of hyphae, sporangia, and chlamydospores. Oospore formation in paired crosses with A1 and A2 compatibility types of *P. capsici, P. cinnamomi*, and *P. palmivora* was evaluated on V-8 agar.

Artificial inoculation studies. Unless noted otherwise, the following studies were conducted with isolate Pa 3-1. This isolate has been deposited in the American Type Culture Collection (Rockville, MD) under the accession number 64558.

Seedlings (6–18 mo old) of the bamboo palm cultivar Florida Hybrid were used in all artificial inoculation studies. Unless noted otherwise, plants were grown in 10-cm-diameter pots. A peat-perlite potting mix (Promix BX, Premier Brand Inc., New Rochelle, NY) was used as a growth medium in all experiments. Plants were fertilized with 20-20-20 fertilizer (Nutri-leaf) approximately every other month and watered about every third day; different fertilizing and watering regimes were used during watering experiments.

Sites of infection. Two experiments were conducted to ascertain which root tissues of bamboo palm were infected by the fungus. In the first experiment, plants were inoculated with either zoospores or 2-wk-old millet seed cultures of the fungus. Zoospores were produced by growing the fungus for 10 days on half-strength (50 ml/L) clarified V-8 broth, washing the resultant hyphal mats three times in deionized water, and incubating the washed mats in cold (about 10 C), deionized water for about 30 min to induce zoospore release.

About an hour before inoculation with

zoospores, potted plants were set in 5 cm of tap water in saucers. Then, 4 ml of a suspension of motile zoospores (containing about 750 zoospores per milliliter) were added to a single location in each pot. Suspensions were slowly poured through a 10-ml widemouthed pipet inserted in pots 3 cm from the edge and to a depth approximating the level of flood water; these inoculation locations were marked for future reference.

For preparation of millet seed inoculum, millet seed was moistened with tap water (30 ml/20 g of seed) and autoclaved for 1 hr on each of two consecutive days before inoculation with mycelial plugs of the fungus from PDA slants. Plants inoculated with millet seed inoculum were transplanted into 15-cm-diameter pots containing potting mix infested with 8.5 g of infested millet seed per liter of mix. Plants were watered thoroughly immediately after transplanting and after 4 days.

Roots exposed to either zoospores or infested millet seed were assayed for infection 8 hr, 1 day, and 5 days after inoculation. Each combination of inoculum and assay time was replicated four times in a randomized complete block design. Four noninoculated plants (controls) were assayed for infection at the beginning of the experiment. For plants inoculated with zoospores, a circular area of roots about 3 cm in diameter from the location of inoculation was assayed. For plants inoculated with millet seed inoculum, all roots on the exterior portions of a root ball were assayed. For plants inoculated with both zoospores and millet seed, only first- and second-order roots (5) were assayed for infection. Entire first-order roots were assayed intact; second-order roots were cut into segments about 2 cm long before being assayed. Higher order roots in these samples were uncommon and not assayed. Infection was determined by plating root segments on PARPH as described above. Roots were observed for fungal growth 2, 3, and 4 days after plating, and the origin of growth on root segments was noted for each segment from which growth was observed.

In the second root infection experiment, only millet seed inoculum was used; rates of millet seed and method for infestation were as described above. Plants were either flooded or not flooded for the duration of the experiment, and roots were assayed for infection 8 hr, 1 day, and 5 days after infestation (Table 1). Each combination of inoculation and assay time was replicated four times in a randomized complete block design. Three nonflooded, noninfested plants were assayed for infection at the beginning of the experiment.

Fungicidal disease control. Two experiments were conducted to determine the efficacy of fosetyl-Al (Aliette 80WP) and metalaxyl (Ridomil 2EC) in controlling root rot of bamboo palm caused by the fungus. In the first experiment, inoculated plants were immersed for 18 hr to a depth of 2 cm in saucers of zoospore suspensions containing about 1,000 zoospores per milliliter, generated as described above. In the second experiment, inoculated plants were transplanted into 15-cm-diameter pots containing potting mix infested with millet seed inoculum as described above. Plants treated with fungicides in each experiment were drenched the day after inoculation; 300 and 600 ml of fungicide were added to each pot during the first and second experiments, respectively. Single applications of fosetyl-Al (1.15 g a.i./L) and metalaxyl (0.015 g a.i./L) were used for inoculated and noninoculated treatments in each experiment, and nontreated, inoculated, and noninoculated controls were used in each experiment (Table 2). Each combination of fungicide and inoculated or noninoculated treatment was replicated six times in a randomized complete block design in each experiment. Both experiments were conducted in an air-conditioned greenhouse.

Two months after inoculation, plants in each experiment were harvested and root necrosis, recovery of the fungus from necrotic tissue, and plant root and shoot dry weights were determined. Eighteen necrotic, first-order root segments, about 1 cm long, were assayed for fungal colonization for each plant as described above. Plant tissue was dried for at least 2 days at 100 C in a drying oven for determination of dry weights.

Influence of watering regime. Two experiments were conducted to determine the influence on root rot severity of watering schedules bamboo palm may

Table 1. Location on first-order roots of bamboo palm infected by *Phytophthora arecae*[x]

Treatment of plants	Time after infestation	Proportion infected: Root segments[y]	Proportion infected: Distance behind root tip[z] 0–5 mm	5–10 mm	>10 mm
Not flooded	0	0/101	0	0	0
	8 hr	0/231	0	0	0
	1 day	13/480	8/13	4/13	1/13
	5 days	24/302	20/24	2/24	2/24
Flooded	0	...	...	...	...
	8 hr	0/221	0	0	0
	1 day	0/414	0	0	0
	5 days	15/377	12/15	2/15	1/15

[x] Results of experiment 2. Palms were planted in a peat-perlite potting mix containing millet seed infested with *P. arecae*. Roots in contact with inoculum were assayed for infection with PARPH (11).
[y] Total number infected/total number assayed for infection.
[z] Total number of infected root segments for that distance/total number infected for a given combination of treatment and assay time.

Table 2. Influence of fungicides on root rot development, recovery of *Phytophthora arecae*, and plant dry matter accumulation in bamboo palm[u]

Treatment[v]	Experiment	Necrosis (%)[w]	Recovery (%)[x]	Dry matter accumulations (g): Root	Shoot	Root:shoot ratio
Noninoculated control	1	0 c[y]	0 b	2.47 a	5.42 a	0.46 a
	2	1.0 c	0 b	5.93 a	13.01 a	0.47 a
Noninoculated fosetyl-Al	1	0 c	...[z]	2.64 a	6.76 a	0.41 a
	2	1.8 bc	0 b	4.20 ab	10.98 ab	0.39 a
Noninoculated metalaxyl	1	0 c	...	2.93 a	6.30 a	0.42 a
	2	1.5 bc	0 b	5.46 a	12.79 a	0.42 a
Inoculated control	1	10.5 a	12.7 a	2.59 a	5.85 a	0.46 a
	2	3.7 a	13.8 a	3.71 ab	9.72 ab	0.39 a
Inoculated fosetyl-Al	1	2.2 b	0.5 b	2.70 a	5.76 a	0.47 a
	2	2.0 ab	0 b	2.73 b	7.07 b	0.40 a
Inoculated metalaxyl	1	1.8 b	1.5 b	3.05 a	7.21 a	0.43 a
	2	2.0 ab	0.9 b	5.37 a	11.55 ab	0.46 a

[u] Mean values for six replications in each experiment.
[v] Plants were inoculated or not inoculated with *P. arecae*. Controls were not treated with fungicides. Plants treated with fungicides were drenched with solutions containing 1.15 g a.i./L of fosetyl-Al or 0.015 g a.i./L of metalaxyl.
[w] Percent of entire root system that was necrotic.
[x] Percent recovery of *P. arecae* from 18 necrotic, 1-cm long first-order roots from each plant on PARPH (11).
[y] For a given experiment, means within a column followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[z] Recoveries not performed.

encounter in nursery practice. In each experiment, plants were transplanted to 20-cm-diameter pots containing potting mix. The day after transplanting, plants were inoculated by pouring 20 ml of an inoculum slurry into each of five holes (1 cm in diameter and 10 cm deep), punched on a uniform basis, on the periphery of the root ball of each plant. Inoculum was prepared by growing the fungus in 100 ml of half-strength V-8 broth in 250-ml Erlenmeyer flasks for 1 wk. For each experiment, the resultant mycelial mats from each of five flasks were washed in deionized water and comminuted in 200 ml of deionized water for 1.5 min at high speed in a Waring Blendor. Fourfold dilutions of the blended mats were then pooled and used as inoculum.

Four watering schedules were chosen to represent extreme and moderate levels of irrigation; plants were watered every day, every other day, every fourth day, or every sixth day. At a depth of 12 cm in the pots in which these experiments were conducted, minimum gravimetric water contents resulting from these watering schedules were 607, 543, 477, and 458%, respectively, and water (matric) potentials were 0, 0, −0.03, and −0.13 bars, respectively. Water potentials were determined with tensiometers. Watering treatments in both experiments were replicated six times in a randomized complete block design and were continued until the experiments were terminated.

Plants in each experiment were harvested 3 mo after inoculation, and root necrosis, recovery of the fungus, and root and shoot dry weights were determined as described above for disease control experiments.

RESULTS

The fungus was recovered from a low percentage (never more than 15%) of roots from 10 of 21 plants sampled. Isolate Pa 3-1 and seven other isolates recovered from blackened, water-soaked root tissue of bamboo palm during these assays were identified as *P. arecae* on the basis of speciation according to Newhook et al (12). This pathogen formed oospores in paired crosses with A2 compatibility types of *P. cinnamomi* and *P. palmivora*, but oospores were not formed with A1 compatibility types of these fungi or with either compatibility type of *P. capsici*. Although Pa 3-1 showed all the characteristics of *P. arecae*, it differed from the description of *P. palmivora* only in producing almost spherical to obturbinate, occasional intercalary sporangia with distorted shapes and occasional lateral attachments, forming sympodia only in water, having thicker oospore walls, and lacking abundant chlamydospores. Critical taxonomic studies with *P. arecae* and *P. palmivora* may not confirm the differentiation of these two species based on the characteristics used in the tentative keys presently available (12).

In artificial inoculation studies, the fungus caused blackened, water-soaked tissue of first- and second-order roots. In addition, a similar necrosis was observed on adventitious shoots (which originate from the base of this plant below the soil line) on some of the plants that were watered daily. *P. arecae* was recovered from symptomatic tissues of these shoots and roots but not from noninoculated control plants.

Sites of infection. In each experiment, infection of first-order roots by *P. arecae* occurred predominantly near the root tip in the zone of elongation (Table 1). The type of inoculum used and whether or not the plants were flooded had no effect on the predominant location of infection in these experiments. Infections that occurred more than 5 mm behind the root tip were relatively infrequent and were often associated with injuries on the root surface.

Fungicidal disease control. Both fosetyl-Al and metalaxyl fungicides provided significant control of root rot symptoms and reduced recovery of *P. arecae* from symptomatic tissue (Table 2). Phytotoxicity was not observed after treatment of the noninoculated control plants with either of these fungicides. There were generally no significant differences detected among these treatments when plant dry matter accumulations were measured, however. In addition, root:shoot ratios were not altered by root rot or by the fungicide treatments.

Influence of watering regime. In general, no consistent differences were detected among the different watering treatments for root rot severity, recovery of *P. arecae* from symptomatic tissue, or plant dry matter parameters. There was a significantly higher recovery of the pathogen from second- than from first-order root segments in both experiments (t tests; $P<0.05$).

DISCUSSION

We believe this is the first detailed report of root disease caused by a species of *Phytophthora* on a member of the Palmae. During our work, *P. arecae* behaved as a typical root-pathogenic species of *Phytophthora* on bamboo palm; nonwounded apical portions of roots and wounded portions of first- and second-order roots were infected. Other species of *Phytophthora* infect roots in the same locations (22).

Although the severity of symptoms caused by this pathogen can be reduced by applying fosetyl-Al or metalaxyl fungicides, none of the treatments affected plant dry matter accumulations or root:shoot ratios. Also, although high soil moisture has been shown to influence the development of other diseases caused by species of *Phytophthora* (4), frequent irrigation in the present work had no consistent effect on the development of root rot of bamboo palm caused by *P. arecae*. Less frequent irrigation (drier potting mix) may have resulted in different root rot severities in drier vs. wetter treatments. However, root rot was not a severe disease under the conditions tested in these experiments.

In nurseries in which bamboo palms are grown, *P. arecae* is probably an opportunistic pathogen capable of causing only minimal root disease in the absence of damage from other sources (such as that caused by the banana moth). Although this fungus would appear to be of minimal concern on bamboo palm, its role as a pathogen of roots of other palms may be more important. For example, *P. arecae* also colonizes roots of coconut palm in the Fort Lauderdale and Homestead areas (*unpublished*). In light of recent isozyme analyses suggesting that *P. arecae* and *P. palmivora* may be the same species (P. V. Oudemans, *personal communication*), it is possible that infected roots of this palm may act as reservoirs of inoculum that could cause bud rot and nut fall. Although it is not known whether other species of *Phytophthora* that cause foliar diseases on other palms also infect roots of their respective hosts, other *Phytophthora* spp. that incite foliar disease on nonpalmaceous hosts are known to also colonize roots of their hosts (13,23).

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