Etiology and Control of Seedling Blight of *Brassaia actinophylla* Caused by *Pythium splendens* in Hawaii

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ABSTRACT

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Phythium splendens was consistently associated with grayish water-soaked lesions on seeding hypocotyls of *Brassaia actinophylla*. Six isolates of *P. splendens* from brassaia and single isolates from schefflera, anthurium, and papaya caused preemergence and postemergence damping-off of brassaia seedlings. Chlorotic leaves and reduced growth rates were symptoms of both root rot and stem canker on inoculated 4-mo-old plants. Metalyxyl at 25 ppm a.i. and etridiazole at 500 ppm a.i. were effective in controlling the disease. Metalaxyl at 500 ppm a.i. and etridiazole at 1,000 ppm a.i. or higher were phytotoxic.

Brassaia actinophylla Endl. (=*Schefflera actinophylla* (Endl.) Harms) is grown commercially in California, Florida, and Hawaii as a potted plant and landscaping tree. In 1978, a serious seedling blight of *B. actinophylla* was observed in a nursery on the island of Oahu. Grayish to brown water-soaked lesions at the stem base were characteristic symptoms of the disease. The fungus isolated from disease tissue was tentatively identified as *P. splendens* Braun based on its large terminal, spherical conidia (sporangia) that did not release zoospores. No sexual structures were observed in single hyphal tip culture.

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Knauss (3) reported *P. splendens* to be the causal agent of a serious seedling decay of *B. actinophylla* in Florida. In New Zealand, Robertson (5) isolated *P. splendens* from cuttings of *Aralia* sp., *Fatsia japonica*, and *Hedera* sp., genera in Araliaceae, the same family as *B. actinophylla*.

This study was undertaken to investigate the etiology of seedling blight of *B. actinophylla* in Hawaii, to determine its cause, and to evaluate fungicidal control measures.

MATERIALS AND METHODS

Diseased tissues from the islands of Oahu and Maui were surface-sterilized with 0.3% sodium hypochlorite for 5 sec and blotted dry before plating on 1.5% water agar. After 24 hr, single hyphal tips were isolated.

Pathogenicity. Pathogenicity of six isolates of *P. splendens*, (924, 925, 928, 929, 932, and 933) obtained from blighted

brassaia seedlings and one isolate (930) obtained from cuttings of *Schefflera arboricola* Hayata was tested. *P. splendens* isolate 117 from *Carica papaya* L. and isolate 461 from *Anthurium scherzerianum* Schott were also included in inoculation studies on preemerged seeds and 3-wk-old seedlings of *B. actinophylla*.

To study the pathogenicity of *P. splendens* on germinated seedlings, 25 seeds of *B. actinophylla* were planted in each of five 10-cm-square pots containing a 1:3 (v/v) mixture of autoclaved peat and vermiculite. Six days after seeding, 10 ml of a conidial suspension were added to each pot as inoculum. The controls were treated with deionized water. The conidial suspension was prepared from 5-day-old cultures, grown on vegetable juice agar (VJA = 100 ml V-8 juice, 2 g $CaCO_3$, 1.8 g agar, 900 ml deionized water) at 24 C under continuous fluorescent illumination at 2,700 lux. The colonies were then scraped with a rubber spatula and the mycelial mass was suspended in deionized water. The suspension was stirred vigorously with the rubber spatula to dislodge the conidia and passed through Kimwipes tissue paper to remove mycelia. The conidial concentrations were adjusted to 2×10^3 conidia per milliliter. The test plants were maintained in a greenhouse with a daily temperature range of 18–36 C. Final assessment of disease severity was based on percentage seed germination 20 days after inoculation.

The ability of *P. splendens* to cause disease on 3-wk-old seedlings was studied by inoculating 30 seedlings in each of five pots with 10 ml of conidial inoculum as described earlier. Final assessment of disease severity was made by recording plant mortality 20 days after inoculation. This test was repeated once and reisolations were made from all diseased plants.

To determine the effect of *P. splendens* on established plants, five pots containing four or five 4-mo-old plants were inoculated as described previously and maintained for 2 mo. Disease assessments were based on top and root mass dry weights. Isolates 925, 928, 461, and 117 were used in this study. Controls were treated with deionized water and reisolations were made from all plants.

Determination of mating types of *P. splendens* isolates. The seven test isolates and *P. splendens* testers 461 and 117, previously designated (+) and (−), respectively (N. Visarathanonth, *unpublished*), were paired in all possible combinations on rapeseed-malt agar (RSM) plates prepared by boiling 10 g of washed rapeseed in 100 ml of deionized water for 30 min. The extract was then filtered through Kimwipes tissue paper and diluted 1:4 with deionized water to which 15 g of agar and 2 g of malt extract were added per liter and autoclaved. This was a modification of a medium by Satour and Butler (6). The petri dish cultures were maintained in the dark at 18 C for 2 wk. Production of antheridia, oogonia, and oospores confirmed compatible matings.

Temperature-growth relations. To determine the rate of growth at various temperatures, the seven brassaia isolates and 461 and 117 were grown on VJA for 48 hr at 24 C under continuous fluorescent irradiation of 2,700 lux. Disks 4 mm in diameter were cut from each colony and placed near the edge of 90-mm cornmeal agar (CMA) plates. After inoculations, the plates were incubated at 15, 18, 20, 24, 28, 31, 34, and 37 C in the dark. Radial extension of six colonies was measured after 48 hr of incubation.

Fungicide evaluation. The fungicides evaluated were efosite aluminum, 80% WP, at 1,000 and 2,000 ppm a.i.; metalaxyl, 50% WP, at 5 and 25 ppm a.i.; etridiazole, 30% WP, at 250 and 500 ppm a.i.; and captan, 50% WP, at 250 and 500 ppm a.i.

A preemergence drench test was conducted by planting 35 seeds of *B. actinophylla* per 10-cm-square pot in an autoclaved 1:3 (v/v) peat:vermiculite mixture. Six days after planting, a 10-ml conidial suspension of 2×10^3 conidia per milliliter of isolate 924 was added to each pot. Twenty-four hours later, 40-ml aliquots of each fungicide concentration were added to each of five pots per treatment. The number of surviving plants was recorded 20 days after inoculation as the number of surviving plants per pot.

A postemergence drench test was performed on 3-wk-old brassaia seedlings with expanded cotyledons. Seeds were planted as described previously and 14 days later, the seedling population was thinned to 30/pot. After seven days, plants were inoculated as previously described. Controls and methods to evaluate disease severity were as described earlier. Plants were watered daily, except on the day of fungicide treatment. Both tests were repeated with 10 pots per fungicide treatment.

Phytotoxicity of fungicides to brassaia. This test was designed to determine the maximum nonphytotoxic dosage (single application) of the test fungicides on brassaia seedlings. The concentrations of the various fungicides were 50W and 2E metalaxyl: each at 5, 25, 50, 500, and 1,000 ppm a.i. and etridiazole: 250, 500, 1,000, and 5,000 ppm a.i. Five 10-cm-square pots containing 30 3-wk-old brassaia seedlings each were drenched with 40 ml of each concentration of fungicide. Twenty-four hours later, regular daily watering was instituted. The seedlings were observed for 2 mo, and growth characteristics and appearance were noted as compared with the water control.

RESULTS

Pathogenicity of *Pythium splendens* on brassaia. The seven isolates from brassaia and the two additional *P. splendens* isolates were pathogenic to preemerged seedlings. A few seeds germinated but the seedlings were infected and stunted. No seedlings that emerged survived the 20-day test period. *P. splendens* was readily recovered from discolored decaying seeds.

The nine *P. splendens* isolates were also pathogenic to 3-wk-old brassaia seedlings (Table 1), causing damping-off of seedlings 3 days after inoculation. Water-soaked lesions appeared at the hypocotyl base, followed by wilting. Under humid conditions, infections of seedlings progressed upward and into the cotyledons.

Isolate 924 was the most virulent of the nine isolates, causing 95% mortality after 20 days. The least virulent isolates were 461 and 117, causing 65 and 47% mortality, respectively. *P. splendens* was reisolated from plants in all inoculated pots. The test was repeated with similar results.

All four isolates of *P. splendens* were pathogenic to the 4-mo-old seedlings, causing lesions and girdling at the soil line. Lesions were seen 10 cm above the soil line, associated with infected adventitious roots. *P. splendens* was reisolated from stem and root lesions.

Wilting during midday and mild chlorosis were also symptoms found on the 4-mo-old inoculated seedlings. Although stems were girdled and accompanied by severe root rot, none of the seedlings died, probably because of the development of adventitious roots. Mean top and root dry weights of seedlings inoculated with *P. splendens* were significantly less than the control (Table 2).

Determination of mating types of *P. splendens* isolates. The seven isolates of *P. splendens* from brassaia were of two

Table 1. Pathogenicity of different isolates of *Pythium splendens* to brassaia seedlings

Isolate	Source	Surviving seedlings (no.) per pot[x]
924	*Brassaia* (seedling)	1.6 a[y]
925	*Brassaia* (seedling)	5.8 b
932	*Brassaia* (seedling)	4.6 ab
928	*Brassaia* (seedling)	4.8 ab
929	*Brassaia* (seedling)	5.8 b
930	*Schefflera* (cutting)	4.2 ab
933	*Brassaia* (seedling)	6.8 bc
117	*Carica papaya*	15.8 d
461	*Anthurium*	10.4 c
Control		30.0 e

[x] Average of five pots, 30 seedlings per pot, inoculated 3 wk after seeding with 2×10^4 conidia per pot, observed 20 days after inoculation.

[y] Means followed by the same letter in the same column do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

mating types. Isolates 925, 932, 929, and 928 were designated mating type (+), whereas isolates 924, 933, and 930 were mating type (−) with the production of oospores when paired with isolates 461 (+) and 117 (−).

Temperature-growth relations. The optimum temperatures of all nine isolates ranged from 24 to 31 C; growth was observed at 15 and 37 C. The maximum growth observed in 48 hr was 72.5 mm obtained by isolate 925 at 28 C.

At 34 C, differences in growth were distinguishable (Table 3). Three groups were formed; isolates 117 and 461 averaged 13.6 mm, isolates 933, 930, 929, and 925 averaged 30.5 mm, and isolates 924, 932, and 928 averaged 54.2 mm. Growth at other temperatures was uniform.

Fungicide evaluation. In the preemergence fungicide application test, etridiazole at 250 and 500 ppm a.i. and metalaxyl at 25 ppm a.i. gave good control of damping-off of brassaia seedlings and were not significantly different from the uninoculated control after 20 days (Table 4). Efosite aluminum at 1,000 ppm a.i. and metalaxyl at 5 ppm a.i. were no better than the inoculated control. Captan at 250 and 500 ppm a.i. was ineffective in control of preemergence damping-off of brassaia.

In the postemergence fungicide drench test, etridiazole at 500 ppm a.i. and metalaxyl at 25 ppm a.i. gave the best control and were not significantly different from the uninoculated control. The other fungicide treatments gave varying degrees of control but were all significantly better than the inoculated control.

Phytotoxicity of fungicides. Metalaxyl in both emulsifiable and wettable powder formulations at 500 and 1,000 ppm a.i., efosite aluminum at 10,000 ppm a.i., and etridiazole at 1,000 and 5,000 ppm a.i. were phytotoxic to 3-wk-old brassaia seedlings. Metalaxyl caused marginal necrosis with chlorotic zones on leaves 3 wk after application. Efosite aluminum at 10,000 ppm caused shortening of the internodes and cupping of leaves 1 mo after application. Five days after treatment, the hypocotyls of several plants drenched with etridiazole at 5,000 ppm collapsed at the soil line. Upon isolation, no pathogen was recovered. No phytotoxic symptoms were observed at concentrations used in the control studies.

DISCUSSION

In this study, the isolates of *P. splendens* obtained from *Brassaia* and *Schefflera* and two additional isolates from papaya and anthurium were pathogenic to brassaia seedlings. Knauss (3) reported that the pathogen caused preemergence and postemergence decay of germinating seedlings of brassaia. Young stems and roots were water-soaked with grayish lesions that developed a brown color and infected seedlings frequently collapsed and rotted. In our study, the disease was not confined to the roots and stem bases, but under moist conditions, the pathogen advanced through the hypocotyl and entered the cotyledons. Older brassaia seedlings were more tolerant to the pathogen.

Isolate 924 was the most virulent among the isolates tested, whereas papaya isolate 117 and anthurium isolate 461 were the least virulent. The latter two cultures were isolated 10 and 5 yr ago, respectively, and may have lost some virulence in culture. The test isolates were all heterothallic. Van der Plaats-Niterink (8) demonstrated *P. splendens* to be both homothallic as described by Braun (1) and heterothallic in nature. In most pairings, few oospores were usually observed. Abundant oogonia and antheridia were formed on the agar surface by compatible mating types. However, a large percentage of the oospores were empty or appeared to have degenerated, probably lacking a nutritional requirement to enhance production.

The temperature-growth relationships were similar for the test isolates, except at 34 C. The optimum temperture for growth ranged from 24 to 31 C, indicating that the pathogen is most active at warmer temperatures. Hancock (2) reported that *P. splendens* caused the highest levels of cotton damping-off at 27 C in contrast with *P. ultimum* and *P. debaryanum*, which were favored by 21 C. At 34 C, the isolates separated into three groups with different growth rates, which may serve as a marker in genetic studies.

Maximum temperatures for most of the brassaia isolates were close to 37 C. Braun (1) and Middleton (4) reported 37.5 and 34–37 C, respectively, as the maximum temperatures for growth of *P. splendens*. Middleton (4) observed that the upper temperature limits are uniform in conspecific isolates.

Knauss (3) obtained 88 and 78% survival of *P. splendens* inoculated brassaia seedlings that were treated with metalaxyl at 6 ppm a.i. in two tests. He was not able to demonstrate significant root rot control of brassaia seedlings inoculated with *P. splendens* with etridiazole at 270 ppm a.i. In our studies, metalaxyl at 5 ppm a.i. was ineffective. However, metalaxyl at 25 ppm a.i. and etridiazole at 500 ppm a.i. applied as a preemergence or postemergence drench were the most effective in controlling brassaia seedling damping-off. Efosite aluminum and captan were ineffective at

Table 2. Vegetative growth of brassaia seedlings inoculated with *Pythium splendens*

Isolates	Tops[x]	Roots[x]
Control	10.1 a[y]	3.9 a
461	5.9 b	1.3 b
117	5.3 b	1.2 b
925	5.0 b	1.1 b
928	3.5 b	0.7 b

[x] Mean dry weight (g) of tops and roots per pot of 4-mo-old seedlings, four or five per pot, were inoculated with 2×10^4 conidia per pot and observed 2 mo later.

[y] Means followed by the same letter do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

Table 3. Characteristics of test isolates of *Pythium splendens*

Isolate no.	Mating reaction	Growth at 34 C[a]
924	(−)	59.7 ± 2.3
925	(+)	30.8 ± 5.3
932	(+)	51.7 ± 3.8
928	(+)	51.2 ± 3.4
929	(+)	29.0 ± 5.2
930	(−)	34.5 ± 4.6
933	(−)	27.8 ± 3.4
117	(−)	12.7 ± 2.3
461	(+)	14.5 ± 2.3

[a] Average radial extension (mm) and standard deviation of six colonies per isolate in 48 hr on VJA.

Table 4. Fungicidal control of brassaia damping-off caused by *Pythium splendens*

Treatment	Concentration[w] (ppm a.i.)	No. of surviving plants: Preemergence[x] application	No. of surviving plants: Postemergence[y] application
Control	···	34.1 a[z]	30.0 a
Etridiazole	500	32.8 a	29.0 ab
Metalaxyl	25	31.3 a	28.9 ab
Etridiazole	250	31.2 a	27.4 b
Efosite aluminum	2,000	5.6 b	15.8 c
Metalaxyl	5	2.6 c	6.1 f
Captan	500	2.3 c	11.6 e
Efosite aluminum	1,000	0.7 c	13.8 d
Captan	250	0.1 c	11.1 e
Inoculated control	···	0.2 c	1.3 g

[w] Fungicides were applied 24 hr after inoculations, 40 ml/pot.

[x] Average of 10 pots, 35 seeds per pot, 6 days after seeding; conidia were added at 2×10^4 per pot and observed for 20 days after inoculation.

[y] Average of 10 pots, 30 seedlings per pot, inoculated 3 wk after seeding at 2×10^4 conidia per pot and observed for 20 days after inoculation.

[z] Means followed by same letter in a column do not differ significantly ($P = 0.01$) according to Duncan's multiple range test.

the concentrations applied. The possibility of metalaxyl resistance (7) in oomycetes alerts applicators to institute a procedure to forestall this development. Alternate application of metalaxyl and etridiazole is one possible way of delaying development of fungicide resistance.

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