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Proc. Fla. State Hort. Soc. 91: 240-242. 1978.

PHYTOPHTHORA LEAFSPOT OF BRASSAIA ACTINOPHYLLA¹

GAIL C. WISLER, WILLIAM H. RIDINGS
Florida Department of Agriculture and Consumer Services,
Division of Plant Industry,
Bureau of Plant Pathology,
P. O. Box 1269, Gainesville, FL 32602
AND

ROBERT S. COX
Trop-Ag Consulting Services,
1404 Shirley Court, Lake Worth, FL 33460

Abstract. A *Phytophthora* sp. was isolated consistently from leafspots on *Brassia actinophylla*. Sporangial and papillary characteristics, mycelial growth at 35C, and production of oospores with amphigenous antheridia in paired cultures were used to identify this isolate as *P. parasitica* (*P. nicotiana* var. *parasitica*). All single zoospore cultures were of the A₁ mating type. Isolates of *P. parasitica* known to be pathogens of *Philodendron scandens* subsp. *oxycardium*, *Peperomia obtusifolia*, *Bougainvillea* sp., and *Schefflera arboricola* were used in a series of selected inoculations with *P. parasitica* from *B. actinophylla* to determine specificity of pathogenicity. *P. parasitica* from *B. actinophylla* was pathogenic to *P. scandens* subsp. *oxycardium*, *Petunia* sp., and *Schefflera arboricola*. Isolates from hosts other than *Schefflera arboricola* were not pathogenic to *B. actinophylla*. A comparison of symptoms of the leafspots on *B. actinophylla* caused by *P. parasitica* and *Alternaria* sp. indicated that dark brown, water-soaked lesions produced by both pathogens were similar.

Chemical control of *P. parasitica* on *B. actinophylla* was evaluated using Kocide 101 (83W), Daconil (75W), Dithane M-45 (80W), Ciba-Geigy GA-1-82 (50W), Zineb (75W). Although all fungicides provided adequate control, Ciba-Geigy GA-1-82 and Zineb were the most effective. Zineb also provided excellent control of the leafspot caused by *Alternaria* sp.

Brassia actinophylla Endl. is widely grown in Florida

foliage nurseries as a popular indoor and outdoor ornamental. A *Phytophthora* sp. was consistently isolated from dark brown, water-soaked leafspots on several specimens of *B. actinophylla*.

The purpose of this study was to identify the *Phytophthora* species associated with this leafspot, establish its pathogenicity on *B. actinophylla* and various hosts, determine the efficacy of different fungicides, and compare the leafspot caused by *Phytophthora* sp. with the common leafspot caused by *Alternaria* sp.

Materials and Methods

A *Phytophthora* sp. (Florida Type Culture Collection, FTCC 859) was cultured from leafspots of *B. actinophylla*. Leafspots were surface sterilized in 0.1% sodium hypochlorite for 2 minutes, rinsed with sterile deionized water, and plated onto potato dextrose agar (PDA) (broth from 200g fresh potatoes, 20g dextrose, 15g Difco agar, and deionized water to make 1 liter) and/or the selective medium for pythiaceae fungi (12), CMA-PPV (Difco corn meal agar, pimaricin, pentachloronitrobenzene, vancomycin). The cultures were maintained on hemp agar (HA) (oil extracted from 30g hemp seed, 20g Difco agar/liter water) slants in test tubes at room temperature.

Single zoospored cultures were prepared by inoculating petri plates of lima bean broth with mycelial agar plugs (ca 5 mm diameter) from the initial isolate, FTCC 859. To induce sporangial formation, cultures 15-20 mm in diameter were rinsed 3 times with sterile tap water and placed under continuous fluorescent light at an intensity of 2500 lux for 2 days. To induce zoospore release from sporangia, the cultures were then rinsed with sterile deionized water. After 30 minutes, the zoospore suspension was strained through 16 layers of damp cheesecloth to remove sporangia. A 5 ml sample was agitated for 30 seconds in a vortex mixer prior to averaging 8 sample counts on a standard hemacytometer. One ml samples of a zoospore dilution of 500/ml were uniformly spread onto petri plates of water agar (WA) and incubated at 27C for 3-6 hours, or until single, germinated zoospores could be removed and placed onto HA

¹Contribution No. 457, Bureau of Plant Pathology.

slants. A single zoospore culture selected for use throughout this study was designated as 15C.

Identification of the *Phytophthora* isolate was made according to the key of Waterhouse (13). Cultures of *P. parasitica*, FTCC 493 from *Nicotiana tabacum* L. and 494 from *Citrus limon* (L.) Burm. f., were used for comparison in growth studies and compatibility typing. Growth of all isolates was measured on petri plates of lima bean agar (LBA) at 35C. Compatibility types were determined by production of oospores in isolate pairings on HA. Sporangia produced on HA were mounted in lactophenol for measurement. Stem inoculations of *Nicotiana tabacum* cv. Hicks were made with 15C and FTCC 494 (*P. parasitica* var. *nicotiana*).

Plants and seed of *B. actinophylla* obtained from D'Agostino Brothers Nursery in Bradenton, Florida, were planted, fertilized biweekly, and maintained under optimum greenhouse conditions. Inocula for the different experiments consisted of zoospore suspensions standardized at 50,000 zoospores/ml. The inoculum was atomized over the entire surface of the plant till runoff. The inoculated plants were enclosed in plastic bags with a wet paper towel per plant and placed in a greenhouse where temperatures ranged from 20-30C. Bags were removed after 5 days, and plants were placed in a mist chamber. Data were recorded 5 and 14 days following inoculation. Resulting leafspots were excised, surface sterilized, and pieces of tissue plated on CMA-PPV.

For comparison of symptoms, an isolate of *Alternaria* sp. was selected which produced leafspots on *B. actinophylla* (078-812). Inoculum of *Alternaria* was produced on PDA in petri plates. Fully grown cultures were comminuted in a microblender and atomized to runoff onto plants of *B. actinophylla*. At the same time, zoospore inoculum of *P. parasitica* (15C) was atomized onto plants of *B. actinophylla*. Plants were bagged and maintained in a greenhouse at 20-30C.

Isolates of *P. parasitica* known to be pathogenic to *Philodendron oxycardium* (Hort.) (*P. scandens* C. Koch & H. Sello subsp. *oxycardium* (Schott) Bunt.) (FTCC 686), *Peperomia obtusifolia* (L.) A. Dietr. (R-117), *Bougainvillea* sp. (077-2923), *Petunia* sp. (G-14), and *Schefflera arboricola* Hayata (077-2444; Wisler, unpublished data) were employed in a series of selected inoculations with *P. parasitica* (15C) to determine specificity of pathogenicity. Plants of each species were inoculated with zoospore suspensions of 50,000 ml from their respective isolate and from *B. actinophylla*. Plants of *B. actinophylla* were inoculated with each *P. parasitica* isolate listed.

Fungicides were evaluated as foliar protectants of *B. actinophylla* against pathogenicity by *P. parasitica* (15C) and *Alternaria* sp. The fungicides were atomized onto plants twice, one week and one day prior to inoculation with isolate 15C. The fungicides tested were Ciba-Geigy GA-1-82 50W (0.30 g/l), Kocide 101 80W (1.8 g/l), Daconil 75W (1.8 g/l), Dithane M-45 80W (1.8 g/l), and Zineb 75W (1.8 g/l). These fungicide rates were based upon recommended rates used in the foliage industry. In all experiments, three plants were used for each treatment, and each test was repeated at least twice.

Results

Large numbers of ovoid, papillate sporangia were produced on hemp agar and lima bean broth, in the presence or absence of fluorescent light. The average length to width ratio of 30 sporangia was 1.46 to 1.0, with the average length and width being $42.5 \mu \pm 10$ and $29.8 \mu \pm 7$. Papillae were distinct with the average length and width being

$5.2 \mu \pm 0.8 \times 7.5 \mu \pm 1.7$. Mycelial growth of 16.5 mm per 24 hours at 35C on lima bean agar was recorded. Oospores with amphigynous antherida were produced on hemp agar plates when grown in paired culture with FTCC 494 of compatibility type A₂, indicating the isolate 15C to be compatibility type A₁. Stem inoculation of *Nicotiana tabacum* 'Hicks' produced no disease symptoms by *P. parasitica* (15C) whereas isolate 494 (*P. nicotianae* B. deHaan var. *nicotianae*) caused extensive stem necrosis. These data for 15C best fit the description of *P. parasitica* Dastur (*P. nicotianae* B. deHaan var. *parasitica* (Dastur) Waterh.).

Following inoculation of *B. actinophylla* plants with zoospore suspensions, distinct, translucent cells developed on the leaves within 2-3 days. These areas expanded to large, irregular, brown, water-soaked lesions, often accompanied by some leaf abscission (Fig. 1). Subculturing of the resulting leafspots on CMA-PPV yielded isolates identical to the original. Early symptoms of *B. actinophylla* inoculated with *Alternaria* sp. showed distinct areas of blackened cells. As the disease progressed, these leafspots were virtually indistinguishable from those caused by *P. parasitica*.



Fig. 1. *Phytophthora parasitica* leafspot of *Brassia actinophylla* showing irregular, brown, water-soaked lesion..

Results from selected inoculation tests revealed that *P. parasitica* from *B. actinophylla* was pathogenic to *P. oxycardium*, *Petunia* sp., and *Schefflera arboricola*. Isolates of hosts other than *S. arboricola* were not pathogenic to *B. actinophylla* (Table 1).

Results from fungicide tests showed Zineb and Ciba-Geigy GA-1-82 were most effective, preventing infection completely. Daconil, Kocide 101, and Dithane M-45 were nearly equal in disease control efficacy (Table 2). Zineb showed extremely effective control of both the leafspot caused by *P. parasitica* and by *Alternaria* sp.

Table 1. Selected host inoculation tests with different isolates of *Phytophthora parasitica*.

Isolates of <i>P. parasitica</i> (source)	Inoculated Hosts ^a					
	<i>Brassia actinophylla</i>	<i>Philodendron oxycardium</i>	<i>Bougainvillea sp.</i>	<i>Petunia sp.</i>	<i>Peperomia obtusifolia</i>	<i>Schefflera arboricola</i>
<i>Brassia actinophylla</i>	+	+	—	+	—	+
<i>Philodendron oxycardium</i>	—	+	X	X	X	X
<i>Bougainvillea</i> sp.	—	X	+	X	X	X
<i>Petunia</i> sp.	—	X	X	+	X	X
<i>Peperomia obtusifolia</i>	—	X	X	X	+	X
<i>Schefflera arboricola</i>	+	X	X	X	X	+

^a + = infection; — = no infection; X = not tested.

Table 2. Effectiveness of fungicides as foliage protectants of *Brassia actinophylla* against infection by *Phytophthora parasitica*.

Treatment and Concentration	Infection (%) ^z	Control (%) ^y
Ciba-Geigy (0.30 g/l)	0	100
Kocide 101 (1.8 g/l)	3	97
Daconil (1.8 g/l)	3	93
Dithane M-45 (1.8 g/l)	6	98
Zineb (1.8 g/l)	0	100
Inoculated control	88	0
Noninoculated control	0	100

^z = percent infection = ratio of number of leaves showing lesions versus total number of leaves x 100.

^y = percent control = % infection of inoculated control minus % infection with fungicide divided by % infection of inoculated control x 100.

Discussion

This study appears to be the first report of *P. parasitica* causing a leafspot disease on *B. actinophylla*. This isolate of *P. parasitica* was found to be pathogenic to *P. oxycardium*, *Petunia* sp., and *S. arboricola*. However, isolates of *P. parasitica* from other hosts except *S. arboricola* showed no pathogenicity to *B. actinophylla*, indicating that *B. actinophylla* is differentially susceptible to isolates of *P. parasitica*. The cross-infectivity by the isolate from *S. arboricola* was not surprising since this host appears to be closely related to *B. actinophylla*.

P. parasitica has been found to be a highly variable and notorious pathogen on roots, stems, and leaves of *Bougainvillea* sp. (1), *Peperomia* sp. (9), *P. oxycardium* (7), *Petunia* (4), tomato (6), grape (10), *Citrus* sp. (8), and other hosts (2, 3, 11).

Fungicidal control of *P. parasitica* on *B. actinophylla* was achieved adequately with all fungicides tested. Excellent control was obtained with Ciba-Geigy GA-1-82 and Zineb, indicating their potential use in providing greater control.

The data obtained on control with Daconil and Dithane M-45 were very similar to those reported by Ridings and McRitchie for *P. parasitica* on *P. oxycardium* (7). The percent control with Zineb is very encouraging since it provided dual protection against both *P. parasitica* and *Alternaria* sp. Zineb is commonly used for control of *Alternaria* sp. leafspot on *B. actinophylla* (5).

Distinction between the leafspot on *B. actinophylla* caused by *P. parasitica* and *Alternaria* sp. cannot be made easily by symptoms alone. Although the very early stages of infection indicated slight differences, the leafspots were virtually indistinguishable as infection progressed, making a laboratory diagnosis essential.

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