Phytophthora Blight of West Indian Holly

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ABSTRACT

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Leaf spot, blight, defoliation, and death of young plants of West Indian holly (*Leea coccinea*) in Hawaii were caused by *Phytophthora meadii*. Both green and red cultivars of *Leea* were diseased and shown to be highly susceptible to several isolates of *P. meadii*. This is a new host record for *P. meadii* and the first report of the fungus in the United States.

Additional keywords: West Indian holly blight

West Indian holly (*Leea coccinea* Planch.) is commonly used for landscaping in the tropics and is also popular as an indoor potted plant in temperate regions of the United States. These plants grow relatively rapidly, have a compact growth habit, and have glossy green foliage. A cultivar with purple-green foliage, known as red *Leea*, also is grown in Hawaii. Except for Calonectria collar rot and leaf spot reported in 1981 (6), *Leea* has been relatively free of disease in Hawaii and elsewhere in the United States (4).

In 1987, a blight of large, potted *Leea* plants was observed at a commercial nursery in Hawaii. This previously undescribed blight occurred sporadically at two nurseries in the ensuing years and caused large losses of emerging seedlings and 2- to 4-mo-old plants. The disease was consistently associated with a *Phytophthora* species new to Hawaii. A blight of *Leea* caused by *P. nicotianae* Breda de Haan with symptoms closely resembling those of the disease in Hawaii has been reported from France (14). This report describes the etiology of the disease in Hawaii and the identification of the causal organism. Preliminary results (13) and a popular account (2) have been published.

MATERIALS AND METHODS

Isolations. Samples of diseased leaves and petioles of large red *Leea* plants and blackened veins, petioles, and stems of small, young green *Leea* plants were collected. Interphase sections between diseased and healthy areas were removed from blighted and spotted tissue and washed in running tap water for 15 min. Tissue sections then were dipped for about 3–5 sec in freshly prepared 0.25% sodium hypochlorite, drained on clean paper towels, and plated immediately on 1.7% water agar. Isolation plates were incubated at approximately 25 C under continuous cool-white fluorescent irradiation (2,700 lx).

Cultures. Single hyphal tips of the presumed *Phytophthora* species were transferred after 1–4 days of growth to vegetable juice agar (VJA) (12) to establish axenic cultures. Nine single zoospore isolates were used in this study and are available from the authors. Three isolates have been submitted to the American Type Culture Collection as voucher specimens (ATCC 90010, 90011, and 90012). Stock cultures were maintained on VJA at 24 C with continuous irradiation as described above, and sporulating sectors were transferred every 2 wk.

Radial growth of all isolates on VJA was determined at 5, 10, 16, 20, 24, 28, 31, and 35 C in darkness. The average growth rate of single colonies on two 60-mm glass petri dishes of each isolate was obtained after 2 and 4 days. All tests were repeated at least once.

Compatibility type was determined by pairing each isolate with known A1 or A2 isolates of *P. capsici* Leonian and *P. palmivora* (E.J. Butler) E.J. Butler. The A1 testers were ATCC 32067 and H463 of each species, respectively, and the A2 testers were ATCC 46319 and H640 of each species, respectively. Gametangia produced from a compatible pair of *Phytophthora* isolates from *Leea*, ATCC 90011 × ATCC 90012, were measured. All inter- and intraspecies crosses were made on rape seed extract–malt agar (12) or on VJA at 24 C in darkness, and plates were examined periodically over a month.

Inoculum. Sporangial measurements were made from 6- to 8-day-old cultures grown on 9 ml of VJA in 60-mm glass petri dishes and maintained as described above. Fifty sporangia per isolate were measured. Sporangia used for pathogenicity tests were produced in 6- to 10-day-old cultures. For inoculum preparation, approximately 7 ml of sterile distilled water was added to each plate and sporangia on the agar surface were dislodged with a rubber spatula. Numerous sporangia just under the agar surface at the edge of the plate were also gently freed into the suspension. The suspensions from three to nine plates were combined, filtered through a layer of Kimwipes tissue paper, and allowed to stand at 20 C for 30 min. Zoospores were then enumerated with a hemacytometer.

Pathogenicity tests. Young red and green *Leea* plants approximately 2–6 mo old and 10–30 cm tall were grown from seed in Sunshine Blend No. 1 potting mix and vermiculite (1:2) and fertilized periodically with Osmocote slow-release fertilizer (14-14-14). Plants were sprayed to runoff with a freshly prepared zoospore suspension adjusted to 10^3 zoospores per milliliter, placed immediately in a humidity chamber for 24 hr at approximately 25 C, and returned to a glasshouse for symptom development. Evaluations were made daily for 1 wk, and plants were observed periodically thereafter. Roots of 6-mo-old plants were inoculated by drenching with 144 ml of a freshly prepared zoospore suspension adjusted to 10^4 zoospores per milliliter or 1.4×10^6 zoospores for each pot (380 cm^3). Water was withheld for 24 hr to retain inoculum. Plants were maintained in the greenhouse and evaluated after 2 mo. All pathogenicity and cultural tests were repeated at least once.

RESULTS

Symptoms. Disease symptoms on naturally infected and artificially inoculated plants were similar. Young seedlings (2 to 3 mo old) quickly succumbed to *Phytophthora* infection. Within 24 hr after inoculation, small, irregular, water-soaked flecks, 1 to 3 mm in diameter, were visible on the abaxial leaf surface. These rapidly expanded into spots (Fig. 1) 10 to >40 mm long over the next 12 hr and into severe blight by the second day. Defoliation was common as the

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fungus progressed into petioles and stems. A few plants were dead by the fifth day and nearly all were killed within 2 wk.

Lesion color varied with plant age, nutrition, and growing environment. On young tissue, active and rapidly spreading leaf spots were gray-green to brownish with a scalded appearance on green *Leea* and olive-green to slightly purplish brown on red cultivars. On the abaxial surface of young red *Leea* plants, water-soaked, irregular, oily-appearing green lesions developed, while the uninfected surrounding tissue remained purple or mauve. Under dry conditions, blighted tissue was frequently shriveled and appeared scorched, resembling injury caused by phytotoxicity (e.g., over-fertilization). Spots became darker brown to black, and infected tissue was brittle.

Symptom development on 5- to 6-mo-old plants was slower and plants were rarely killed. Leaf spots were darker gray to brown, sometimes black, and many failed to expand beyond 10 mm in length. Leaf blights and petiole infections occurred but were less common than in younger seedlings. Individual lesions on mature red *Leea* plants were difficult to see because of the blending of dark lesions into the dark purple background. Black lesions, which completely girdled the primary rhachis (midrib) or the secondary rhachis, resulted in collapse of distal parts of the leaf. These wilted leaves were distinctive symptoms of this disease. Many stem infections were restricted at the nodes. Regrowth of healthy new shoots was common following defoliation of older plants, although infection recurred during periods of high moisture. *Leea* roots appeared to be tolerant to the pathogen, since little root rot was evident following root inoculations and growth of inoculated plants was not reduced.

All nine isolates were pathogenic to both red and green *Leea* and all were reisolated from diseased tissue. Isolate ATCC 90011 was the least virulent; it killed fewer plants and had slower blight and stem lesion development than the other isolates.

Pathogen description. All *Phytophthora* isolates from *Leea* barely grew at 10 or 35 C, and none grew at 5 C. All isolates grew well over the range of 20–31 C, with 28 C being the optimum for most isolates.

The fungus produced deciduous, papillate to semipapillate, ovoid, obpyriform, ellipsoid, or asymmetric sporangia, commonly with rounded bases. Collective mean sporangial dimensions for all nine isolates were: length, 46.1 ± 3.5 μm (range, 42.2–55.0 μm); and breadth, 27.3 ± 1.9 μm (range, 25.5– 32.5 μm). The sporangial length-to-breadth ratio was 1.7 for seven isolates, 1.6 for H993-1, and 1.8 for H673-10. Pedicels were of intermediate length and averaged 13.8 ± 1.6 μm for nine isolates.

The compatibility type for eight isolates was A1 and that for isolate ATCC 90011 was A2. Oospore production was low for all crosses with *Phytophthora* from *Leea* when compared with oospore numbers formed by compatible pairs of other species such as *P. capsici*. Fewer than 200 oospores were formed per dish in a cross between ATCC 90011 and ATCC 90010, whereas several thousand were formed between ATCC 46319 × ATCC 32067, two *P. capsici* isolates. No evidence of homothallism was observed in isolates from *Leea*. Oogonia were 27.2 ± 2.2 μm (23.3–32.5 μm) in diameter and 25.5 ± 1.8 μm (20.8–28.3 μm) in length, and antheridia were 16.5 ± 2.8 μm (13.3–23.3 μm) in diameter and 14.0 ± 1.8 μm (10.5–17.2 μm) in length. Oospore diameter averaged 23.5 ± 1.8 μm (20.5–26.8 μm). Chlamydospores were not seen in cultures of any *Leea* isolate on VJA in darkness.

The fungus causing blights on *Leea* was identified as *P. meadii* McRae (7).

DISCUSSION

In 1918, McRae (7) described *P. meadii* sp. nov. on rubber in India. This species was grouped with *P. palmivora* by Tucker (11) in 1931. In 1963, Waterhouse presented *P. meadii* as distinct from *P. palmivora* (15), and Dantanarayana et al (3) also reestablished *P. meadii* as a distinct species based in part on pedicel length, sporangial morphology, and chlamydospore production. *Phytophthora* isolates from *Leea* in Hawaii have characteristics of *P. meadii* as described originally (7) and in recent taxonomic treatments (3,5,10).

Average pedicel lengths ranged from 11.3 to 16.5 μm for isolates from *Leea* and are similar to the lengths reported by Dantanarayana (3). Sporangial size and asymmetry also conform to *P. meadii*. Although McRae mentioned a few self-fertile cultures (7), oospores were not observed in solo or unpaired cultures of *Leea* isolates in this study. The rare or infrequent occurrence of "homothallic" or self-fertile cultures is not useful as a diagnostic feature for this species (3,11). Furthermore, oospores have been reported or observed in pure cultures of certain single-zoospore isolates of other known heterothallic species such as *P. palmivora* (9; J. Y. Uchida, *unpublished*), *P. capsici* (9,12), and *P. nicotianae* (1,9).

Chlamydospores were not observed in any isolate during the course of the present study. Chlamydospore formation is a characteristic of some isolates only, and their rarity in pure culture has been noted by Dantanarayana et al (3) and others (8). This is the first report of *P. meadii* as a pathogen of *L. coccinea* and the first record of this pathogen in the United States.

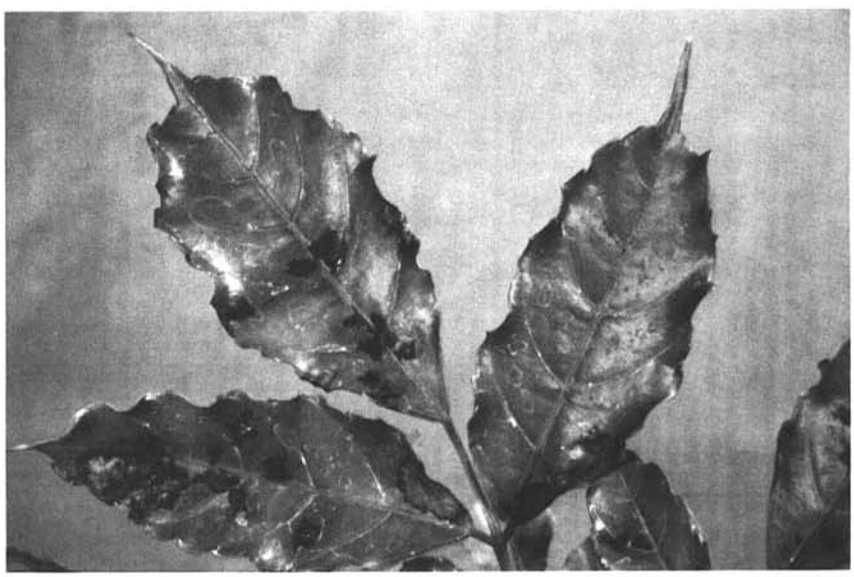

Fig. 1. Mature leaflets of *Leea coccinea* with dark spots caused by *Phytophthora meadii*.

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