

Calonectria Leaf Spot of *Howeia forsterana* in Hawaii

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ABSTRACT

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Grayish brown, nearly black, or reddish brown and zonate leaf spots on Forster sentry palm (*Howeia forsterana*) were shown to be caused by three species of *Calonectria*, namely, *C. theae*, *C. colhounii*, and *C. crotalariae*. Each of these fungi has a *Cylindrocladium* anamorph. Teleomorphic and anamorphic characteristics of isolates of all three species from *Howeia* are given, including macroconidial characteristics of *C. theae*.

Additional keywords: *Metrosideros collinus*

Potted ornamental palms belonging to *Chrysalidocarpus*, *Howeia*, *Chamaedorea*, *Caryota*, and other genera are important export commodities of the Hawaiian foliage industry and also are used extensively for landscaping in Hawaii. *Howeia forsterana* (C. Moore & F. J. Muell.) Becc., a dark green solitary palm, is popular for indoor use throughout the country and is among the top four palms produced in Hawaii. Commonly known as kentia palm, the name Forster sentry palm or sentry palm is more appropriate, so as to avoid confusion with species of *Hedyscepe*, *Gronophyllum*, and other palms also referred to as kentias.

Recently, a severe leaf spot and blight were discovered on Forster sentry palm grown on the island of Hawaii, a major foliage production area of the state. The disease description and pathogen identification is provided herein. A popular account has been published (14).

MATERIALS AND METHODS

Isolations were made from diseased leaves collected at two commercial nurseries. Leaves were washed well with household liquid detergent, immediately rinsed in running tap water, blotted dry, and sectioned. Sections were taken from the margin between diseased and healthy tissue from blights and large spots, or entire spots less than 3 mm in diameter were dissected out.

All tissue sections were dipped momentarily in freshly prepared 0.05–0.25% NaOCl, drained on clean tissue paper, and plated on 1.7% water agar (WA). Isolation plates were incubated at

approximately 25 C under 2,700 lx continuous cool-white fluorescent irradiation. After 2–4 days, single hyphal tips were aseptically transferred to vegetable juice agar (VJA) (6). In other cases, conidial masses were transferred with a glass needle from isolation plates after 5–7 days and spread on a second WA plate. Single germinating conidia were then transferred to VJA plates.

All isolates used for testing started from single conidia or single ascospores. Sectors producing abundant conidia were transferred every 2 wk during this study to fresh VJA plates, and stock cultures were maintained on VJA slants and in sterile distilled water.

Conidiophores, vesicles, and conidia were observed in cultures grown at 24 C on 9 ml of VJA per 60-mm glass petri plate for 5–7 days under continuous cool-white fluorescent irradiation (approximately 2,700 lx). Formation of macroconidia was studied in WA or in WA amended with 0.5% glycerol (1). Perithecia were produced under fluorescent irradiation on VJA at 24 C after 2 wk, on WA on which sections of 4-day-old colonies grown on Mycophil agar (BBL, Becton Dickinson and Co., Cockeysville, MD) had been placed, or on autoclaved sentry palm leaves placed on WA.

Representative isolates ATCC 76634, ATCC 76635, and ATCC 76649 identified as *Calonectria colhounii* Peerally, *C. theae* C. A. Loos, and *C. crotalariae* (C. A. Loos) D. K. Bell & Sobers, respectively, were used in most tests. Other isolates collected from sentry palm that were examined included *C. colhounii* (ATCC 76639 and 1495) and *C. theae* (ATCC 76644 and 1504). *C. theae* isolates ATCC 76641 and 1463 from *Metrosideros collinus* (J. R. Forster) A. Gray were also examined.

Suspensions of conidia for pathogenicity tests were prepared by growing cultures at 24 C for 5–6 days on VJA

with irradiation as described above. Sterile distilled water (5–7 ml) was added to each plate, conidia were gently dislodged with a rubber spatula, and suspensions from several plates were combined and filtered through a layer of tissue paper to remove mycelial fragments. Conidia were quantified with a haemocytometer and the concentration adjusted to 10^4 conidia per milliliter. This inoculum was brushed carefully onto leaves in half-leaf tests. All leaflets on the right of the midrib were inoculated, and all leaflets on the left served as controls. For most tests, young, newly expanded leaves were inoculated.

Plants were placed in a moisture chamber for 24 hr, then returned to a greenhouse and maintained under 2 sec of mist every 10 min. Pathogenicity tests were monitored for 1–3 mo, and all tests were repeated once.

RESULTS AND DISCUSSION

Sentry palm leaves from the field had abundant dark brown to nearly black circular, elliptical, or irregular lesions (Fig. 1A). Advanced lesions were gray, dry spots with dark borders that coalesced and blighted leaves. Dark elliptical lesions also occurred on petioles (Fig. 1B).

Three species of *Calonectria* (each with a *Cylindrocladium* anamorph) were readily isolated from these leaf spots and blights. The majority of specimens yielded *Cylindrocladium* colonies in 7 days. Following single-spore transfers, pure cultures were identified as *Calonectria colhounii*, *C. theae*, or *C. crotalariae*.

Pathogenicity of all three *Calonectria* species was confirmed on *H. forsterana*. Symptoms were similar for all species (Fig. 1C), although minor differences occurred. All three species produced numerous (50–200+) flecks and spots per pinna within 10 days. *C. colhounii* formed circular to elliptical lesions 1–2 mm long, with some beginning to coalesce. Many 2-mm-diameter spots were zonate, with reddish brown to tan centers surrounded by dark green, water-soaked tissue bordered by a band of light brown tissue. Larger veins limited lesion expansion. *C. crotalariae* formed similar zonate spots but also produced small spots about 0.5 mm wide with broad water-soaked areas (2–3 mm). The *C. theae* isolate produced large numbers of brown flecks surrounded by a narrow

chlorotic zone, or water-soaked tissue with chlorotic zones. By the 14th day, some lesions remained small, while others, especially those produced by *C. colhounii*, coalesced into blights

approximately 80 × 5 mm. These blights were dark brownish black and continued to expand. Large target or zonate spots were common on leaves inoculated with *C. colhounii* or *C. crotalariae* (Fig. 1D).

In general, lesions produced by *C. theae* remained smaller and uniformly reddish brown, in contrast to the larger zonate spots caused by the other species. After 2 mo, many lesions of all three species became tan to light gray with black borders, and blights expanded to over 100 mm in length for *C. colhounii*. Following artificial inoculation and reproduction of typical foliar symptoms, each of the three species was reisolated and identified on the basis of anamorphic and teleomorphic characteristics.

Disease development on *Howeia* was dependent on high humidity. In early tests, inoculated plants were maintained without mist, and no lesions developed. Furthermore, small spots that were formed under mist expanded very slowly when plants were removed from mist. Young leaves were readily diseased, whereas older expanded leaves developed flecks or exhibited no symptoms following inoculation.

C. colhounii (ATCC 76634) produced cylindrical to slightly clavate conidia that were predominantly three-septate, averaging $74.8 \pm 6.6 \mu\text{m} \times 6.2 \pm 0.5 \mu\text{m}$ (Fig. 2A). Vesicles, borne on long stipes, were generally narrowly clavate (Fig. 2B). Perithecia were yellow (yellow to brown macroscopically), subglobose, $316.4 \pm 44.8 \mu\text{m}$ in height and $348.6 \pm 44.8 \mu\text{m}$ in diameter. Asci were $121.8 \pm 14.8 \mu\text{m}$ long, each with four ascospores. Ascospores were three-septate, fusiform, slightly curved, with rounded ends, and averaged $59.3 \pm 5.9 \mu\text{m} \times 7.1 \pm 0.5 \mu\text{m}$. Conidia of ATCC 76639 were considerably shorter, although other reproductive structures were similar in dimensions to those of ATCC 76634.

C. theae (ATCC 76635) produced cylindrical to slightly clavate conidia, which were one- to three-septate and averaged $90.8 \pm 9.0 \mu\text{m} \times 6.2 \pm 0.5 \mu\text{m}$ (Fig. 2C) and formed narrowly clavate vesicles (Fig. 2D) and red globose perithecia $375 \pm 35 \mu\text{m}$ in height and $315 \pm 37.5 \mu\text{m}$ in diameter. Asci were $113 \pm 15.5 \mu\text{m}$ in length, each with eight mostly three-septate ascospores, which were fusiform, slightly curved, with rounded ends, and averaged $52.8 \pm 4.8 \mu\text{m} \times 5.5 \pm 0.5 \mu\text{m}$. Conidia of *C. theae* (ATCC 76641) from *Metrosideros* were $90.0 \pm 4.8 \mu\text{m} \times 6.0 \pm 0.5 \mu\text{m}$. Ascospores were $51.2 \pm 5.2 \mu\text{m} \times 5.2 \pm 0.2 \mu\text{m}$. ATCC 76644, 1463, and 1504 produced conidia of similar dimensions.

All *C. theae* isolates also formed macroconidia, which averaged $199 \pm 18 \mu\text{m} \times 6.5 \pm 0.5 \mu\text{m}$ in ATCC 76635 (Fig. 2E). These macroconidia were three- to 14-septate and sharply curved or bent at right angles, formed on dichotomously branching conidiophores in loose clusters of up to 16 spores, and were produced readily in WA by most isolates in 7 days under fluorescent irradiation. Macroconidia were commonly formed in the agar and infrequently observed on

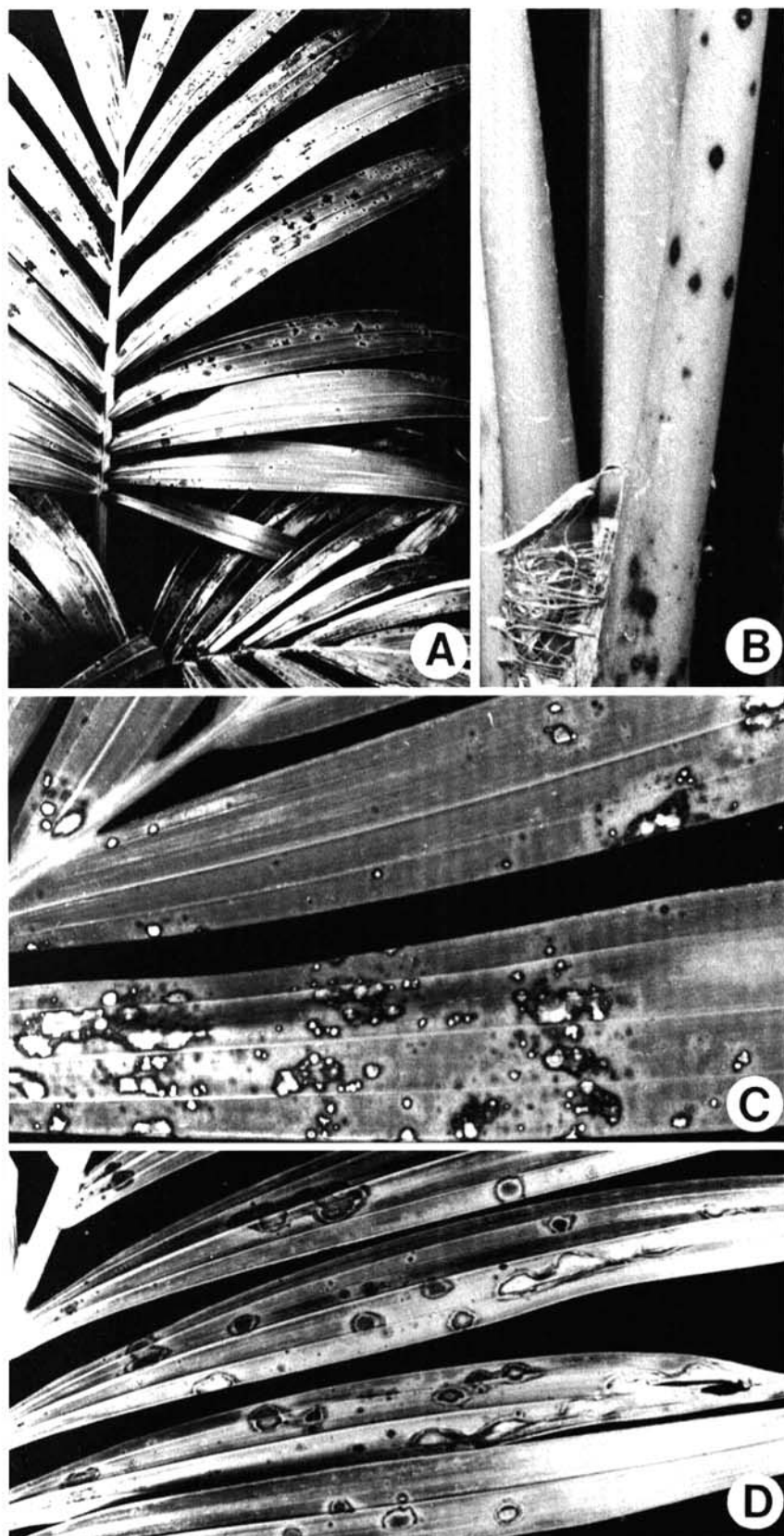


Fig. 1. Calonectria leaf spots on *Howeia forsterana*. (A) Leaf spots and blighting caused by *C. theae* collected from the field. (B) Petiole lesions caused by *C. theae*. (C) Close-up of leaf spots caused by *C. theae*. (D) Leaf spots and blighting 2 mo after inoculation with *C. crotalariae*.

the agar surface. In repeated transfers, most single-spored isolates continued to produce many macroconidia, but isolate 1463, which produced a few initially, formed less than 100 per 90-mm plate. Macroconidia are a diagnostic component of the anamorph of *C. theae*.

C. crotalariae (ATCC 76649) produced three-septate, cylindrical to slightly clavate conidia averaging $64.5 \pm 3.8 \mu\text{m} \times 7.0 \pm 0.2 \mu\text{m}$; formed sphaeropedunculate vesicles (Fig. 2F), a shape distinguished from globose by Schoulties et al (9); and produced red to reddish brown perithecia $450 \pm 50 \mu\text{m}$ in height and $377.5 \pm 55 \mu\text{m}$ in width. Asci contained eight ascospores each. Ascospores were fusiform, slightly curved, with rounded ends, averaged $44.2 \pm 5.8 \mu\text{m}$ by $5.8 \pm 0.5 \mu\text{m}$, and were mostly one- to three-septate, although four-septate ascospores were occasionally observed.

Few pathogenicity studies on foliar diseases of ornamental palms have been published. *Cylindrocladium pteridis* F. A. Wolf (syn. *C. macrosporum* Sherb.) has been established as a pathogen of *Cocos nucifera* L. (11), *Washingtonia robusta* H. Wendl. (10,12), *Chamaedorea elegans* Mart. (12), *H. belmoreana* (F. Muell.) Becc. (12), and *H. forsteriana* (12) and has also been isolated from *Arecastrum romanzoffianum* (Cham.) Becc. (13). *Cylindrocladium* species, primarily *C. scoparium*, were reported to occur naturally on *W. robusta*, *H. forsteriana*, and *Ptychosperma elegans* in Australia (3). Although both *C. pteridis* and *C. scoparium* Morg. are known to occur in Hawaii, neither has been associated with any palm disease. This is the first report of *Calonectria colhounii*, *C. theae*, and *C. crotalariae* as pathogens on palms.

C. theae has been isolated from diseased ohia (*M. collinus*) (6), *M. excelsus* Soland. ex Gaertn., *Strelitzia*, and leather leaf fern (*Rumohra adiantiformis* (G. Forst.) Ching) in Hawaii (unpublished). Isolates from diseased *M. collinus* were equally pathogenic to *Howea* as ATCC 76635 from *Howea*. ATCC 76635 was pathogenic to ohia seedlings and caused defoliation. Young stems were frequently killed, but apparently healthy regrowth emerged 1-3 mo later from older, uninfected stem tissue. The inoculum potential in the ohia forest canopy needs investigation, since these forests surround several palm nurseries.

C. crotalariae is common in Hawaii and has been known to cause serious collar rot diseases of papaya (5), alfalfa (7), leea (4), koa (2), and many potted ornamental plants. It has also been reported as a foliar pathogen of leea (4).

C. colhounii has been recently isolated from diseased *Leucospermum* sp. and is known to cause shoot blight of *M. collinus* (unpublished) in Hawaii. It was

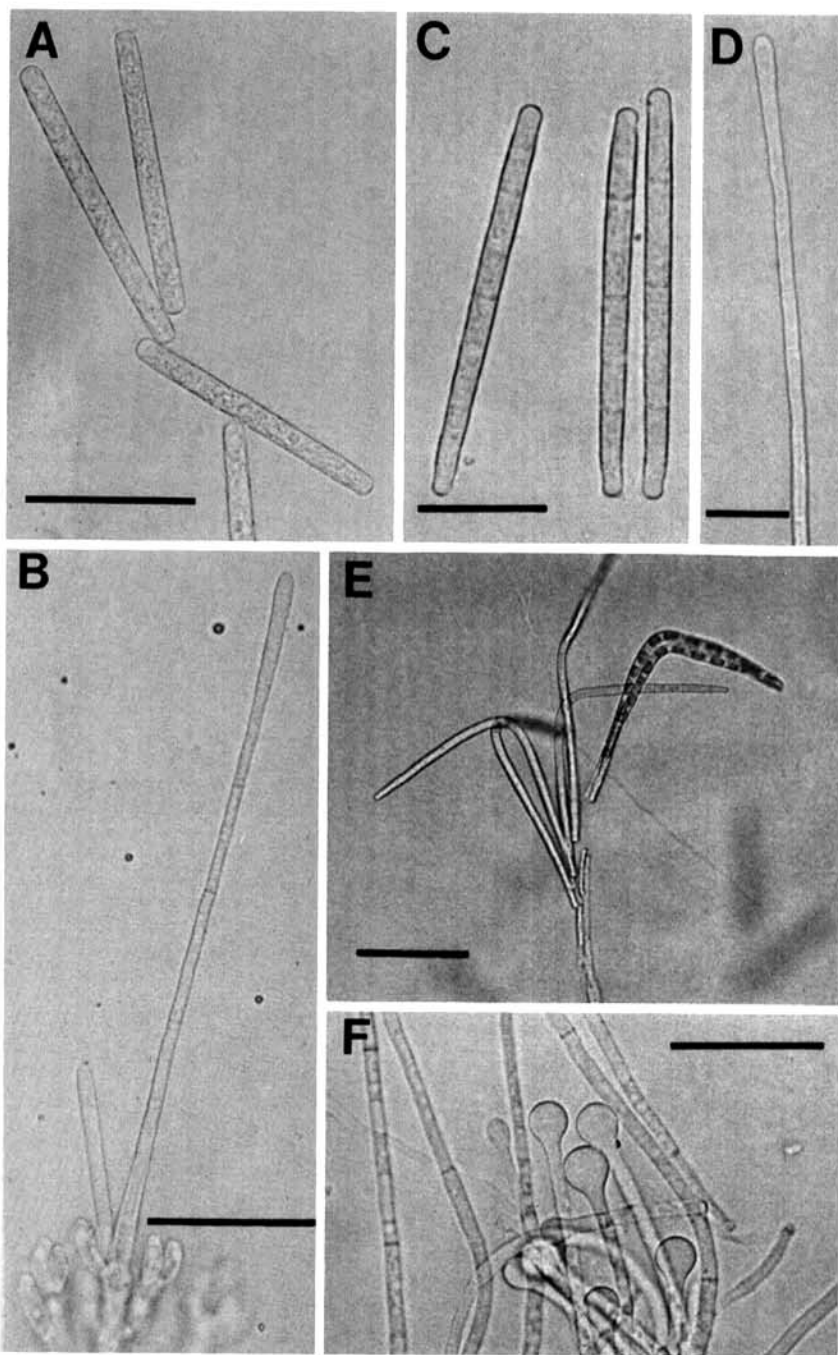


Fig. 2. Anamorph characteristics of *Calonectria* spp. (A) Conidia of *C. colhounii*. Bar = 40 μm . (B) Conidiophore, stipe, and vesicle of *C. colhounii*. Bar = 40 μm . (C) Conidia of *C. theae*. Bar = 30 μm . (D) Vesicle of *C. theae*. Bar = 20 μm . (E) Macroconidia of *C. theae*. Bar = 70 μm . (F) Sphaeropedunculate vesicles of *C. crotalariae*. Bar = 70 μm .

originally described on tea (*Thea sinensis* L.) from Mauritius (8).

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