Comparative Pathogenicity of Calonectria theae and Cylindrocladium scoparium to Leaves and Roots of Azalea

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ABSTRACT

Calonectria theae was isolated from leaves, root, and stems of several greenhouse cultivars of azalea. Pathogenicity of this fungus was compared with that of *Cylindrocladium scoparium* to roots and leaves of three greenhouse and three hardy cultivars of azalea. No mortality occurred among plants grown for 2 months in soil artificially infested with either species. However, more plants grown in soil infested with *C. scoparium* showed smaller and/or discolored root systems than those grown in soil infested with *C. theae*. The leaves of all cultivars showed various degrees of susceptibility to both species. In general, *C. scoparium* was more virulent to leaves and roots than was *C. theae*. A morphological comparison of *C. theae* and *C. crotalariae* was made.

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Additional key words: Calonectria crotalariae, Candelospora theae, Cercosporella theae, Cylindrocladium crotalariae, Cylindrocladium theae, Rhododendron obtusum.

In 1970, a species of *Cylindrocladium* was found in three widely separated locations in the United States on leaves of *Rhododendron obtusum* (Lindl.) Planch. 'Skylark' and 'Warbler', on roots of Gloria, Kingfisher, and Red Macaw, and on stems of Roadrunner and Skylark. Fertile perithecia were produced abundantly in culture on potato-dextrose agar (PDA) and were identified as those of *Calonectria theae* Loos, causal agent of a serious leaf disease of *Thea sinensis* L. (tea) in Ceylon and India (9). A search of available literature revealed no previous association of the pathogen with azalea or rhododendron, nor any occurrence of the fungus outside of Ceylon or India.

Since preliminary studies (1) showed that *C. theae* was pathogenic to leaves and roots of azalea plants, we compared its pathogenicity with that of *Cylindrocladium scoparium* Morgan, a well-known pathogen of azalea (8, 11, 15). Certain taxonomic aspects of the imperfect state of *Calonectria theae* were considered, and it was compared morphologically with *C. crotalariae* (Loos) Bell & Sob., formerly considered a variety of *C. theae* and an increasingly important pathogen of peanuts in southeastern USA.

MATERIALS AND METHODS.—Three plants each of greenhouse cultivars Kingfisher, Redwing, and Roadrunner, and five plants each of hardy cultivars Duc de Rohan, Formosa, and Mrs. G. B. Gerbering azaleas were established in 6-inch pots containing soil fumigated with methyl bromide at a rate of 454 g/137.2 cm^3. These plants were subjected to comparative leaf and root pathogenicity tests using cultures of *Calonectria theae* and *Cylindrocladium scoparium*. The isolate of *C. scoparium* was obtained from leaves of an unknown cultivar of azalea collected at Fort Myers, Fla., in 1965; and the isolate of *Calonectria theae*, from leaves of the cultivar Roadrunner collected at Fort Myers in 1970. All plants were 6 to 7 months old.

Inocula for leaf pathogenicity tests were prepared by suspending conidia scraped from 10-day-old cultures grown on PDA (Difco, 39 g/liter in distilled water) in 10-ml portions of distilled water. The resulting suspensions were filtered through a single thickness of cheesecloth, adjusted to contain 20,000 conidia/ml, blended for 30 sec after adding Triton B-1956 (active ingredient, 77% modified phthalic glyceryl alkyd resin) at a rate of 0.05 ml/20 ml of suspension, and sprayed on the leaves of test plants. A solution containing 0.05 ml of Triton B-1956/20 ml of distilled water was sprayed on leaves of control plants. All plants were maintained in a mist chamber for 24 hr at 28 C and 95 to 100% relative humidity after applying the inoculum. Results were recorded 7 days after inoculation.

Inocula for root pathogenicity tests were prepared by blending 14-day-old cultures growing on PDA in 30-ml portions of distilled water. The mixtures were then adjusted to contain 70,000-75,000 propagules/ml of suspension. Sixty-five ml of inoculum was mixed into the top 3 cm of soil in each pot. Soil in pots containing control plants was treated in the same manner with a like amount of autoclaved inoculum. Plants were maintained in a greenhouse where temperature varied from 19-36 C during the experiment. Results were recorded 2 months after the tests were initiated.

Morphological comparison of the imperfect state of *Calonectria theae* with that of *C. crotalariae* was made after 7-days' growth on PDA, and of the perfect state after 21-days' growth on the same medium. Measurements of *C. theae* are based on an average of seven isolates from five cultivars of azalea grown in

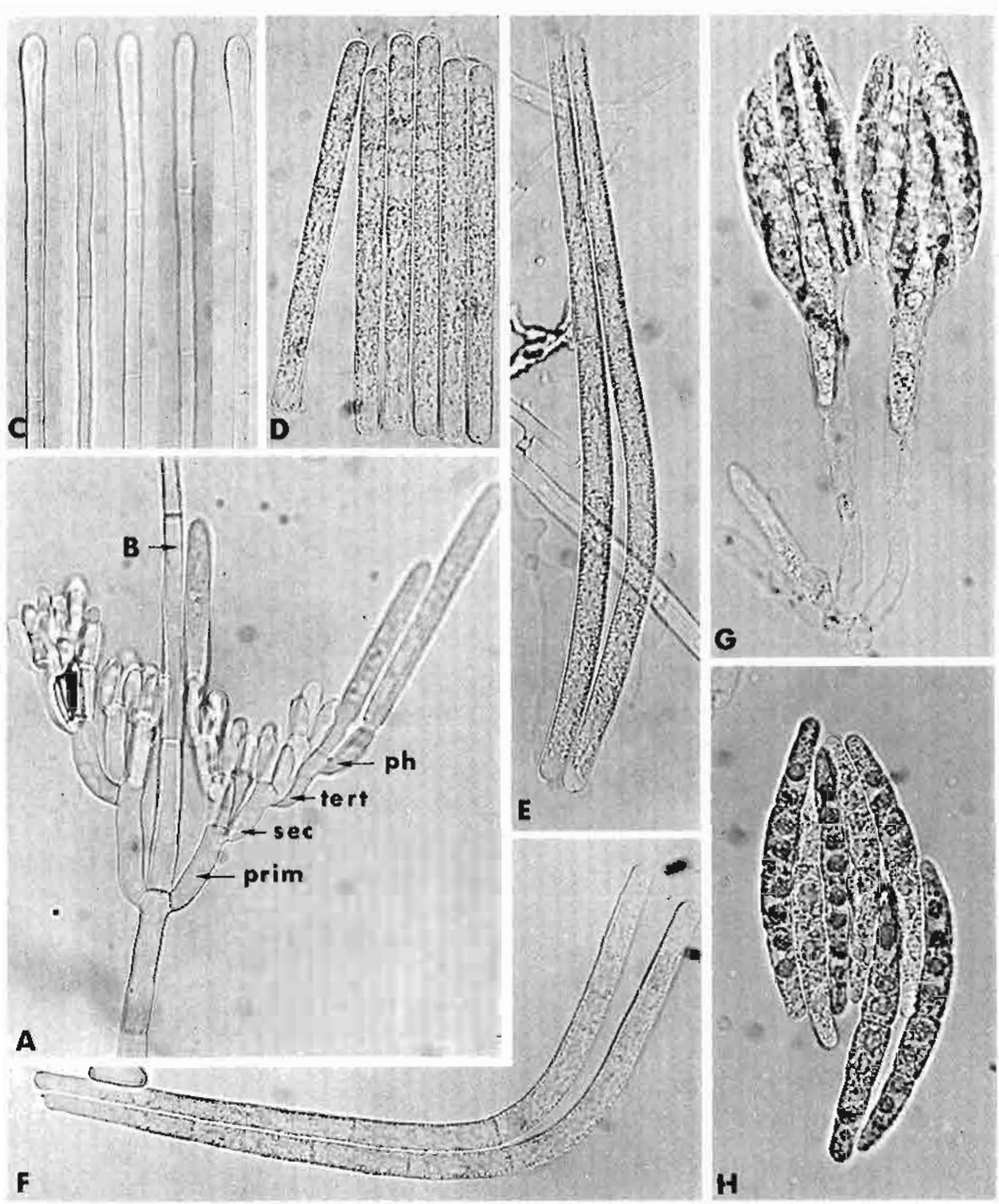

Fig. 1. *Calonectria theae:* A) conidiophore (prim = primary branch; sec = secondary branch; tert = tertiary branch; ph = phialide); B) stipe; C) clavate vesicles; D) normal conidia (range from 88.5 to 94 μ); E, F) macroconidia; G) asci; and H) ascospores.

three different locations in the USA, and those of *C. crotalariae* on 16 isolates from peanuts grown in 13 different locations in Georgia, South Carolina, and Virginia.

Pathogenicity to leaves.—Lesions were found in varying size and number on leaves of all cultivars 3-4 days after inoculation with either species. By the 7th day, plants of the least susceptible cultivar (Duc de Rohan) exhibited only a few irregularly circular, purplish black lesions 1-2 mm in diam with no leaf abscission. Susceptible cultivars had larger lesions of similar shape and color, frequently with dark brown centers, each surrounded by a purplish black margin, and with some leaf abscission apparent. Very susceptible cultivars were moderately to severely defoliated, and exhibited lesions that occasionally covered over half the leaf surface.

Leaves of cultivars Kingfisher, Redwing, Roadrunner, and Formosa were very susceptible to *Cylindrocladium scoparium*, whereas Duc de Rohan and Mrs. G. B. Gerbering were susceptible. Leaves of cultivars Kingfisher, Redwing, Roadrunner, Formosa, and Mrs. G. B. Gerbering were susceptible to *Calonectria theae*, and leaves of Duc de Rohan were only slightly susceptible. It was impossible to distinguish visibly between the lesions produced by the two species, even though the isolate of *Cylindrocladium scoparium* was apparently more virulent than that of *Calonectria theae*.

Pathogenicity to roots.—None of the plants grown for 2 months in soil artificially infested with either species died. However, significant root discoloration was observed among plants of cultivars Kingfisher and Roadrunner grown in soil infested with *C. theae*. The fungus was reisolated from Redwing, Duc de Rohan, and Formosa plants that had apparently healthy roots, but not from roots of the cultivar Mrs. G. B. Gerbering. Roots of Kingfisher and Roadrunner plants grown in soil infested with *Cylindrocladium scoparium* were discolored and significantly smaller than those of the control plants. Plants of cultivars Redwing, Formosa, and Mrs. G. B. Gerbering exhibited slightly to moderately discolored roots with no significant reduction in size. The fungus was reisolated from all plants grown in soil infested with *C. scoparium* except those of the cultivar Duc de Rohan, which had apparently healthy roots. The isolate of *C. scoparium* was also more virulent on roots.

The pathogen.—Conidiophores (Fig. 1-A) of *Calonectria theae* are borne laterally on a stipe (Fig. 1-B) that terminates in a hyaline, clavate vesicle 4.1-8.2 × 13.6-28.7 μ (Fig. 1-C). Stipes arise at right angles from the surface of the host or from procumbent mycelia in culture. They are septate, 5.4-9.5 μ wide at the base, hyaline, narrowing to 2.4-4.1 μ at the apex, and up to 550 μ in length.

Primary conidiophore branches are mostly nonseptate and 17.7-31.3 μ, secondary branches are nonseptate and 13.6-24.5 μ, and tertiary branches are nonseptate and 6.8-17.7 μ. Phialides are hyaline, nonseptate, mostly reniform, and 7.7-16.5 × 3.5-5.3 μ (Fig. 1-A).

Conidia are hyaline, cylindric, granular, rounded at both ends, slightly wider at the base than the apex, mostly three-septate, 57.1-95.2 × 4.7-7.1 μ, and average 79.3 × 5.8 μ (Fig. 1-D). When this species is grown on glycerol water agar, however, conidia may be straight to acutely curved (Fig. 1-E, F), 3-14 septate, and up to 3 times the length attained on conventional media or on host tissue. Although the reason for this phenomenon is not known, it was observed that conidia remained attached to the phialides longer than is normally expected. It was suggested that because of prolonged attachment to the phialides, the conidia grew longer with a greater number of septations, and became bent because of the increased weight of the much larger conidium (12).

Perithecia of the *Calonectria* state are scattered-to-gregarious, and are seated on a small black stroma that breaks free from the host with the perithecium. They are oval-to-occasionally globose, orange to orange-red, 248-481 μ high by 227-383 μ wide, average 374 × 313 μ, and have a papillate ostiolum consisting of small columnar cells. The

TABLE 1. A comparison of *Calonectria theae* and *C. crotalariae*

	C. theae	*C. crotalariae*
Perithecia	Orange to red orange 248-481 × 227-382 μ 374 × 313 μ	Red orange to red 313-475 × 286-405 μ 382 × 348 μ
Asci	99.2-145.5 × 16.3-25.8 μ 122.6 × 21.3 μ	95.2-138.7 × 13.6-19.0 μ 114.3 × 16.4 μ
Ascospores	42.2-77.5 × 5.9-8.3 μ 54.7 × 7.4 μ, mostly 3-septate	28.6-58.9 × 6.3-7.8 μ 40.1 × 7.1 μ, mostly 3-septate
Vesicles	13.6-28.7 × 4.1-8.2 μ, clavate	6.5-11.2 × 5.9-11.2 μ, globose
Conidia	57.1-95.2 × 4.7-7.1 μ 79.3 × 5.8 μ, mostly 3-septate	58.5-107.4 × 4.7-7.1 μ 78.9 × 5.9 μ, mostly 3-septate

perithecial wall is composed of pseudoparenchymatous cells 16.3-36.7 X 13.6-24.5 μ. Asci contain eight ascospores that become aggregated in the upper half of the ascus at maturity. Asci are hyaline, clavate, thin-walled, long-stalked, somewhat pointed and undifferentiated at the apex, 99.2-145.5 X 16.3-25.8 μ, and average 122.6 X 21.3 μ (Fig. 1-G).

Ascospores are hyaline, fusoid to falcate, granular, mostly three-septate when extruded from the ascus, slightly to moderately constricted at the septa, 42.2-77.5 X 5.9-8.3 μ, and average 54.7 X 7.4 μ (Fig. 1-H).

Comparison of Calonectria theae and C. crotalariae.—The most significant difference in the imperfect state of the two species is the shape of the vesicle. Those produced by *C. theae* are clavate; those of *C. crotalariae* are globose. No appreciable differences were found in the size or shape of conidia, conidiophore branches, and phialides of the two species.

Perithecia of *C. theae* are mostly orange, whereas those of *C. crotalariae* are more nearly red. Perithecia of *C. crotalariae* are consistently wider than those of *C. theae*, and consequently are more nearly globose as compared with oval for the latter species. Although the average ascus of *C. theae* is only slightly larger than that of *C. crotalariae*, their ascospores are longer and are more rounded at the ends (Table 1).

Taxonomy of C. theae.—The imperfect state of *C. theae* was initially described as *Cercosporella theae* Petch in 1917 (10). In 1927, Gadd found perithecia of the perfect state in culture, but offered no description other than to indicate that they were red or reddish yellow spheres about the size of a pin head (4). Later, he reported empty perithecia on tea leaves (5), and in 1937, he found *Calonectria theae* and perithecia of a *Calonectria* on diseased plants of *Tephrosia radicans* (6). Subba Rao (13, 14) reported that sterile perithecia were found on the undersurface of fallen tea leaves; and that, when placed on culture media, they developed into colonies of *Cercosporella theae*. In 1949, Loos finally described the perfect state as *Calonectria theae* along with a form of the fungus from stems of *Crotalaria anagyroides* and *Tephrosia vogelii* which he named *Calonectria theae* var. *crotalariae*. The name of the imperfect state was changed to *Candelospora theae* (Petch) Wakefield ex Gadd (7). A study of the genus *Cylindrocladium*, published by Boedijn & Reitsma (3) in 1950, reduced the genus *Candelospora* to synonymy with *Cylindrocladium*. However, *Calonectria theae* and its variety were not included in this work. In 1967, Bell & Sobers (2) found *C. theae* var. *crotalariae* to be pathogenic to peanuts in Georgia, accorded the pathogen-specific rank as *C. crotalariae*, and designated its imperfect state as *Cylindrocladium crotalariae*. At this time it seems proper to identify the imperfect state of *Calonectria theae* as *Cylindrocladium theae*.

Cylindrocladium theae (Petch) Alf. & Sob., comb. nov. *Candelospora theae* (Petch) Wakefield ex Gadd, Monographs on tea production in Ceylon, p. 59-60. 1949.

Cercosporella theae Petch, Ann. Roy. Bot. Gdns., Peradeniya 6:246. 1917.

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