

**Cylindrocladium heptaseptatum sp. nov. on Fronds of
Polystichum adiantiforme from Honduras**

E. K. Sobers, S. A. Alfieri, Jr., and J. F. Knauss

Associate Professor, Department of Plant Pathology, University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton 31794; Assistant Director, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville 32602; and Assistant Plant Pathologist, University of Florida, Agricultural Research Center, Apopka 32703; respectively.

Accepted for publication 11 October 1974.

ABSTRACT

Cylindrocladium heptaseptatum sp. nov. was isolated from fronds of *Polystichum adiantiforme* plants imported into central Florida from Honduras. Its morphology and pathogenicity were compared with that of *C. pteridis* and *C. quinqueseptatum*. The three species produced similar symptoms on leaves and stems of *Annona squamosa*, but

lesions on fronds of *P. adiantiforme* differed in shape and color. The three species are readily distinguished by the length, width, and number of septations of their conidia, by the shape of their phialides, and by the number of conidiophore branches.

Phytopathology 65:331-333

Additional key words: *Calonectria quinqueseptata*, *Cylindrocladium pteridis*, *Cylindrocladium quinqueseptatum*, *Cylindrocladium theae*.

In 1971, inspectors of the Florida Department of Agriculture and Consumer Services quarantined plants of *Polystichum adiantiforme* (Forst.) J. E. Sm. (leatherleaf fern) that exhibited what appeared to be atypical frond lesions caused by *Cylindrocladium pteridis* Wolf. Microscopic examination showed that the fungus was not *C. pteridis*, but somewhat resembled *C. quinqueseptatum* Boedijn & Reitsma (*Calonectria quinqueseptata* Figueiredo & Namekata). Because the plants had arrived recently from Honduras, and *C. quinqueseptatum* had only been reported previously as a pathogen of *Syzygium aroomaticum* Merr. & L. M. Perry in Indonesia (2) and of *Annona squamosa* L. in Brazil (3), all the plants were destroyed.

Further study of the *Cylindrocladium* sp. from Honduras, and comparison with *C. quinqueseptatum*, suggested that it was an undescribed species. Appropriate experiments were initiated to establish pathogenicity of the new fungus, and to compare its morphology with that of *C. pteridis* and *C. quinqueseptatum*.

MATERIALS AND METHODS.—Plants of *P. adiantiforme* used in pathogenicity tests were established in 15.2-cm diameter plastic pots containing a mixture of wood chips and peat moss. Plants of *A. squamosa* were grown from seed in 15.2-cm diameter clay pots containing soil fumigated with methyl bromide. At the time of inoculation, each *A. squamosa* plant had 12 to 16 leaves, and each fern plant had five to nine fronds.

Inocula for pathogenicity tests were prepared by suspending conidia from 10-day-old cultures [grown on PDA (Difco potato-dextrose agar, 39 g/liter in distilled water)] in 10-ml portions of demineralized water. Resulting suspensions were filtered through a single thickness of cheesecloth, adjusted to contain 10,000 conidia per ml, blended for 30 seconds after adding Triton B-1956 (active ingredient, 77% modified phthalic glyceryl alkyl resin) at a rate of 0.05 ml per 20 ml of suspension, and sprayed on fronds or leaves of test plants. A solution containing 0.5 ml of Triton B-1956 per 20 ml of demineralized water was sprayed on control plants. After inoculation, all plants were maintained in a mist chamber for 24 hours at 28 C and 95-100% relative humidity. Results were recorded 14 days after inoculation.

Morphological comparison of the *Cylindrocladium* isolate from Honduras with *C. pteridis* and *C. quinqueseptatum* was made after 7-days of growth on PDA. The culture (ATCC 16550) of *C. quinqueseptatum* was isolated from *Scolopendrium* sp., and *C. pteridis* was a transfer of an isolate described in 1968 (4) from fronds of *P. adiantiforme*.

RESULTS.—*Pathogenicity.*—Symptoms produced by each of the species of *Cylindrocladium* on leaves, petioles, and stems of *A. squamosa* were essentially the same. Leaf lesions were irregularly circular, 3-8 mm in diameter with small tan centers, and wide purple margins. Leaves eventually became uniformly chlorotic and wilted. Petioles were blackened, shrivelled, and bore numerous *Cylindrocladium* fructifications. Stem lesions were raised, circular, dark-purple to black, and up to 2 mm in diameter. Many coalesced to form larger, blackened areas that girdled the stem. All three species were reisolated readily from affected tissues.

Lesions produced by the three species on fronds of *P. adiantiforme* differed sufficiently to provide a means of distinguishing between the species. Those caused by the Honduras isolate were circular to irregularly circular, reddish brown, up to 10 mm in diameter, with or without a reddish margin, and occasionally with a slight chlorotic area surrounding the lesion (Fig. 1-A, B). Eventually the centers of the larger lesions became gray, particularly those occurring at the tips of the leaflets. Lesions caused by *C. quinqueseptatum* were irregular, light-brown to tan, and blended into extensive chlorotic areas involving large portions of the leaflets. Lesions caused by *C. pteridis* were irregular and reddish-brown, with distinct, but limited, areas of chlorosis surrounding the lesions. The three species were readily isolated from affected tissues.

The pathogen.—Conidiophores arise laterally from a stipe that terminates in hyaline, clavate vesicle, 17.1-41.3 × 4.1-7.1 μm (Fig. 2-A). The stipes arise at right angles from the surface of the host, or from procumbent mycelia in culture. They are hyaline, septate, and 137-272 × 3.5-6.5 μm. Primary branches are mostly nonseptate, hyaline, and 18.9-31.3 × 5.3-8.3 μm. Secondary branches, when present, are hyaline, nonseptate, and 16.5-24.2 × 4.7-8.3 μm. Tertiary branches have not been observed. Two-to-

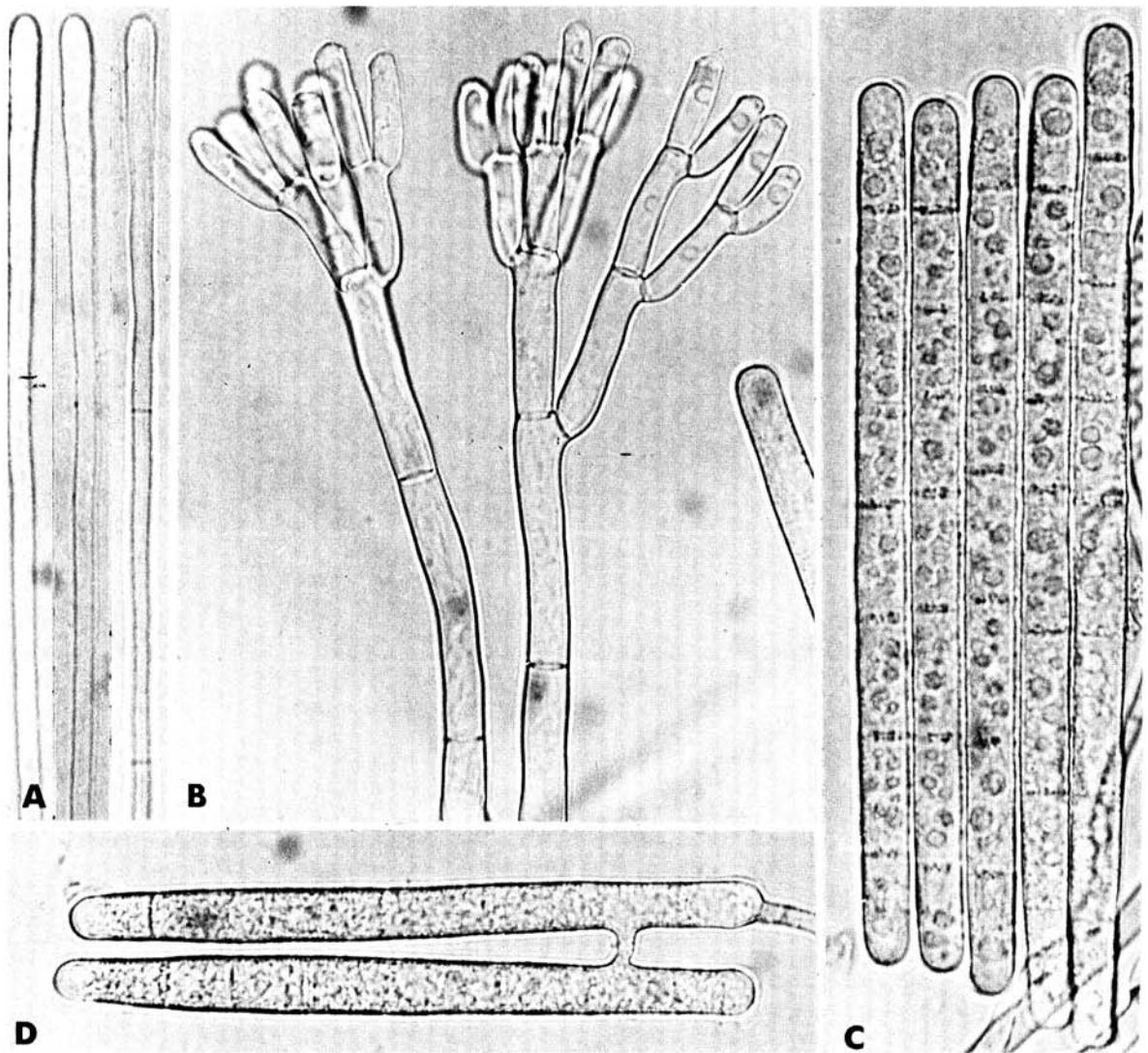


Fig. 1-(A, B). Lesions on leaflets of *Polystichum adiantiforme* caused by *Cyindrocladium heptaseptatum*; A) 5-days, and B) 14-days after inoculation.

TABLE I. Comparative morphology of *Cyindrocladium heptaseptatum*, *C. pteridis*, and *C. quinqueseptatum* after 7 days of growth on potato-dextrose agar. All measurements are in micro-meters.

Structure	<i>C. heptaseptatum</i>	<i>C. pteridis</i>	<i>C. quinqueseptatum</i>
Vesicles	Clavate 17.1-41.3 × 4.1-7.1	Clavate 24.2-47.2 × 5.3-9.4	Clavate 20.1-38.4 × 4.1-7.1
Stipes	137-272 × 3.5-6.5	167-287 × 4.7-8.3	126-299 × 3.5-6.5
Branching			
Primary	18.9-31.3 × 5.3-8.3	23.6-35.4 × 5.3-7.7	19.5-30.1 × 4.7-7.7
Secondary	16.5-24.2 × 4.7-8.3	17.7-28.9 × 4.7-7.7	17.1-24.2 × 4.1-6.5
Tertiary	None	16.5-24.8 × 4.1-6.5	14.2-23.1 × 4.1-5.9
Phialides	Cylindric 12.9-21.8 × 4.7-7.7	Reniform 10.6-23.0 × 4.7-7.1	Reniform 9.4-18.9 × 4.7-7.1
Conidia	7-Septate 80.2-131.9 × 6.5-9.4 108.9 × 7.9 (Avg)	1-Septate 61.2-119.7 × 4.1-7.1 88.3 × 5.7 (Avg)	5-Septate 62.6-95.2 × 4.7-7.8 80.1 × 6.4 (Avg)

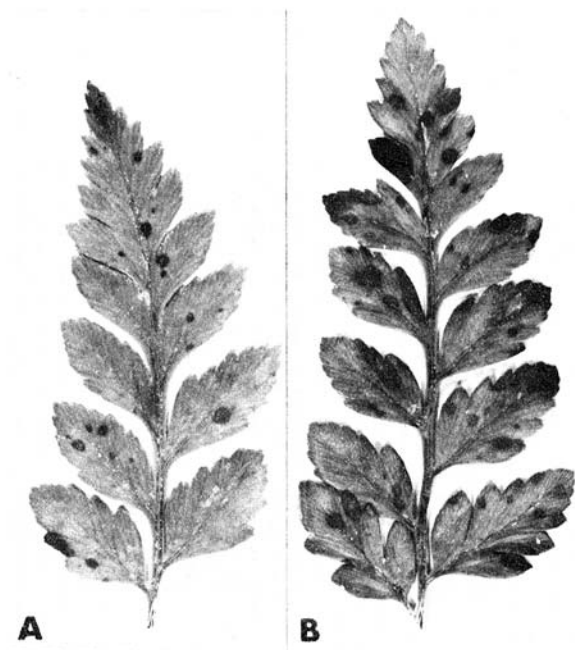


Fig. 2-(A to D). *Cylandrocladium heptaseptatum*: A) stipes and vesicles; B) conidiophores; C) conidia left to right, 111.5 μ m, 110.2 μ m, 115.6 μ m, 119.7 μ m, and 129.2 μ m; and D) anastomosing conidia.

three hyaline, nonseptate, essentially cylindric phialides, 12.9-21.8 \times 4.7-7.7 μ m, develop at the distal end of primary and/or secondary branches (Fig. 2-B).

Conidia are hyaline, granular, cylindric, straight, wider at the apex than at the base, rounded at both ends, mostly seven-septate, and 80.2-131.9 \times 6.5-9.4 μ m (Fig. 2-C). Anastomosing conidia (Fig. 2-D) are frequently encountered in this species, and are usually smaller than the average size conidium. The fungus produces a significant number of long, sharply bent conidia on 2% glycerol water-agar, and to a lesser extent on PDA. These conidia are similar to those described for *Cylandrocladium theae* (Petch) Subram. (1, 5), which have as many as 11 septations and are up to 247 μ m long.

Morphology compared.—The three species of *Cylandrocladium* differ in the length, width, and number of conidial septations; in the shape of the phialides; and in the number of conidiophore branches (Table 1). Conidia of the Honduras fungus are mostly seven-septate as compared to five septations for *C. quinqueseptatum*, and one for *C. pteridis*. Conidia of the Honduras fungus

average approximately 25% wider and longer than *C. quinqueseptatum*, and over 30% wider than *C. pteridis*. Phialides of *C. quinqueseptatum* and *C. pteridis* are reniform, as compared to the nearly cylindric and blunt phialides of the fungus from Honduras. Conidiophores of *C. quinqueseptatum* and *C. pteridis* exhibit primary, secondary, and tertiary branching, whereas the Honduras *Cylandrocladium* produces only occasional secondary, and no tertiary branches. Other characteristics of the three species of *Cylandrocladium* are listed in Table 1.

The *Cylandrocladium* isolated from fronds of *P. adiantiforme* is considered to be morphologically distinct and is easily distinguished from existing species. It is therefore proposed as a new species and described as follows:

Cylandrocladium heptaseptatum
Sob., Alf., & Knauss sp. nov.

Conidiophora stipitata a latere oriunda, dichotome ramosa; rami plerumque eseptati, primariis 18.9-31.3 \times 5.3-8.3 μ m, secundariis 16.5-24.2 \times 4.7-8.3 μ m; phialides teretiuscule, hyalinae, eseptatae, 12.9-21.8 \times 4.7-7.7 μ m; stipites septati, hyalini, 137-272 \times 3.5-6.5 μ m; vesiculis clavatis 17.1-14.3 \times 4.1-7.1 μ m; conidia hyalina, granulata, cylindrata, apicibus latioris quam basibus; utrumque rotundata, plerumque septeseptata, 80.2-131.9 \times 6.5-9.4 μ m.

Sejunctum frondis *Polystichum adiantiforme* (Forst.) J. E. Sm. In herbariis *New York Botanical Gardens* (NY), et *The University of Florida* (FLAS). Holotypus: *Polystichum adiantiforme* NY Isotypus: FLAS.

LITERATURE CITED

- ALFIERI, S. A., JR., R. G. LINDERMAN, R. H. MORRISON, and E. K. SOBERS. 1972. Comparative pathogenicity of *Calonectria theae* and *Cylandrocladium scoparium* to leaves and roots of azalea. *Phytopathology* 62:647-650.
- BOEDIJN, K. B., and J. REITSMA. 1950. Notes on the genus *Cylandrocladium*. *Reinwardtia* 1:51-60.
- FIGUEIREDO, M. B., and T. NAMEKATA. 1970. Constatacao de *Calonectria quinqueseptata* n. sp. forma perfeita de *Cylandrocladium quinqueseptatum* Boedijn & Reitsma, sobre *Annona squamosa* L. e *Eucalyptus* sp. *Arq. Inst. Biol., São Paulo* 34:91-96.
- SOBERS, E. K. 1968. Morphology and host range of *Cylandrocladium pteridis*. *Phytopathology* 58:1265-1270.
- SOBERS, E. K. 1971. A macro-conidial form of *Cylandrocladium theae* occurring on glycerol-water agar. *Bull. Ga. Acad. Sci.* 29:98 (Abstr.).