Brown Leaf Necrosis of Mahonia bealei Caused by Cylindrocladium ellipticum species nova

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ABSTRACT

Cylindrocladium ellipticum sp. nov. is designated as the cause of a leaf spot and marginal necrosis of leaves of Mahonia bealei at Monticello, Florida. Pathogenicity to leaves of Rhododendron indicum 'Pride of Dorking' has also been established. Although the fungus superficially resembles C. scoparium, its vesicles are significantly smaller, 9.5-20.4 \times 6.8-8.8 μ (average 15.8 \times 7.6 μ), than those of C. scoparium, 19.0-37.7 \times 8.3-14.5 μ (average

 $29.6 \times 11.9~\mu$), and its conidia are larger, $47.6\text{-}76.2 \times 4.1\text{-}5.4~\mu$ (average $62.7 \times 4.6~\mu$), as compared with $34\text{-}57.1 \times 3.5\text{-}4.8~\mu$ (average $43.6 \times 4.4~\mu$) for *C. scoparium*. Conidiophores of *C. ellipticum* are more numerously branched than those of *C. scoparium*, and its phialides are mostly reniform, whereas those of *C. scoparium* are mostly doliform. A detailed comparison of the two species is made. Phytopathology 60:1212-1215.

In May 1969, a species of *Cylindrocladium* was isolated from leaves of *Mahonia bealei* Carr. collected at Monticello, Florida. The leaves exhibited brown, irregularly circular lesions that were located primarily along the margin and basal portion of the leaves. A preliminary examination of the fungus suggested a similarity to *C. scoparium* Morgan. However, detailed examination revealed that the ellipsoid to oval vesicles of this species were smaller, and its 1-septate conidia larger, than those of *C. scoparium*.

Since species of *Mahonia* are prized in many areas for their ornamental foliage and other uses, and because no previous report of a *Cylindrocladium* pathogenic to species of *Mahonia* was found, work was initiated to establish the pathogenic relationship of the fungus to its suspected host, and to describe and compare its morphology with that of *C. scoparium*. A preliminary report has been made (1).

MATERIALS AND METHODS.—Pathogenicity of the apparently new *Cylindrocladium* was tested on leaves of five *M. bealei* and five *Rhododendron indicum* Sweet 'Pride of Dorking' plants. The latter species was used because of the susceptibility of azaleas to several species of *Cylindrocladium* (2, 3, 4, 5, 6, 7).

Inoculum for pathogenicity tests was prepared by blending 10-day-old cultures of the fungus growing on potato-dextrose agar (PDA, extract of 200 g cooked potatoes, 20 g dextrose, 20 g agar) for 1 min in sterile, distilled water. This mixture was filtered through four thicknesses of cheesecloth and sprayed on the leaves of test plants. A similarly treated mixture consisting of sterile PDA and water was sprayed on leaves of a like number of control plants. All plants were maintained in an environmental growth chamber for 2 days prior to, and 5 days after, inoculation. The chamber was programmed for 12 hr at 21 C with no light, and for 12 hr at 32 C with 5,000 ft-c of light. Humidity was maintained at 88% (±5%).

The following media were used in an attempt to elicit the perfect stage of the pathogen: banana agar (BA), 250 g of peeled and blended bananas, 20 g of agar; carrot juice agar (CJA), 355 ml of commercial carrot juice, 20 g agar; hemp agar (HA), water ex-

tract from 20 g of blended hemp seed, 20 g agar; lima bean agar (LBA), 77.5 g Bacto-lima bean agar (Difco Laboratories); nutrient agar (NA), 23 g Bacto-nutrient agar (Difco Laboratories); potato-dextrose agar (PDA), extract of 200 g cooked potatoes, 20 g dextrose, 20 g agar; V-8 juice agar (VJA), 250 ml V-8 juice, 40 g agar; yeast extract agar (YEA), 10 g yeast, 20 g dextrose, 15 g agar. The constituents of each of these media were made up to 1 liter with distilled water. The commercial preparation of PDA (Baltimore Biological Laboratories, 39 g/liter) was used in comparative morphological studies only.

The description of the fungus is based on a minimum of 500 observations and/or measurements of structures produced on leaves of *M. bealei*. Morphological comparisons of the pathogen with *C. scoparium* are based on structures produced in culture on PDA. The culture of *C. scoparium* was obtained from azalea leaves collected at Ft. Myers, Florida, in 1965.

RESULTS.—Pathogenicity tests.—Lesions on leaves of M. bealei first appeared as faint chlorotic spots that gradually became light brown, irregularly circular, and up to 4 mm in diam, and were occasionally surrounded by a faint area of chlorosis (Fig. 1-A). In some cases the chlorotic area assumed a light orange color. In nature, lesions are found most frequently along the margin and basal portion of the leaves (Fig. 1-B). Necrosis of affected leaves was usually complete 5 to 6 weeks after inoculation (Fig. 1-C).

Leaves of *R. indicum* 'Pride of Dorking' were readily infected by the pathogen. Lesions were greyish green and irregular at first, and later turned brown. Infected leaves abscised readily.

Attempts to induce formation of sexual structures of the pathogen on nine different media were unsuccessful.

The pathogen.—Conidiophores are borne laterally (Fig. 2-A) on a stipe that terminates in a hyaline, ellipsoid to oval, vesicle, 9.5-20.4 \times 6.8-8.8 μ , and average 15.8 \times 7.6 μ (Fig. 2-B). Stipes arise at right angles from the surface of the host, or from procumbent mycelia in culture. They are septate, 4-10 μ wide, mostly hyaline at the base, becoming narrower toward

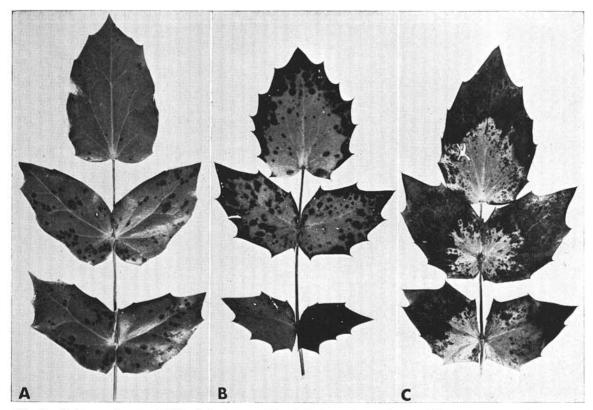


Fig. 1. Lesions on leaves of Mahonia bealei caused by Cylindrocladium ellipticum; A) early infection; B) early stage of marginal leaf necrosis; and C) advanced necrosis of leaf margins.

the apex, and eventually attaining lengths up to 425 μ . Primary and secondary conidiophore branches are mostly nonseptate, and their length is 19.8-30.9 and 15.6-23.5 μ , respectively. Other branches are nonseptate, and 9.5-17.1 μ in length (Fig. 2-C).

One to four phialides, mostly one to two, may develop from the terminal end of any of the branches. They are hyaline, nonseptate, usually reniform, and $6.8-18.6 \times 4.1-5.4 \mu$ (Fig. 2-A, C).

Conidia are formed by budding from the apex of the phialides. They are hyaline, cylindric, straight, granular, rounded at both ends, slightly wider at the base than the apex, $47.6-76.2 \times 4.1-5.4 \mu$, and average $62.7 \times 4.6 \mu$ (Fig. 2-D).

Comparison with C. scoparium.—Cylindrocladium scoparium is the only species known to us that is sufficiently similar to the pathogen morphologically to warrant comparative discussion. Differences between the two, however, are significant and readily apparent. Vesicles of the pathogen are $9.5\text{-}20.4 \times 6.8\text{-}8.8\,\mu$ (average $15.8 \times 7.6\,\mu$), as compared with $19.0\text{-}37.7 \times 8.3\text{-}14.5\,\mu$ (average $29.6 \times 11.9\,\mu$) for C. scoparium (Fig. 2-E). The phialides of C. scoparium are mostly doliform (Fig. 2-F), whereas those of the pathogen are mostly reniform (Fig. 2-A, C). Conidia of the pathogen are $47.6\text{-}76.2 \times 4.1\text{-}5.4\,\mu$, as compared with $34.0\text{-}57.1 \times 3.5\text{-}4.8\,\mu$ for C. scoparium; and its conidiophores (Fig. 2-C) are more numerously branched than those of C. scoparium (Fig. 2-F) or of any of the

previously described species of *Cylindrocladium*. From these comparisons it is also noted that the average conidium size of the pathogen $(62.6\times4.6\,\mu)$ is larger than the largest conidia of *C. sco-parium*, and that the average conidium size of *C. sco-parium* $(43.6\times4.4\,\mu)$ is smaller than the smallest observed conidia of the pathogen. With respect to vesicle size, the average vesicle of the pathogen $(15.8\times7.6\,\mu)$ is smaller in length and width than the smallest vesicle of *C. sco-parium*, and the average vesicle of *C. sco-parium*, and the average vesicle of *C. sco-parium*, $(29.6\times11.9\,\mu)$ is larger in both aspects than the largest vesicle of the pathogen. Based on these differences, the name *Cylindrocladium ellipticum* sp. nov. is proposed for the fungus from leaves of *M. bealei*.

Cylindrocladium ellipticum Alf., Seymour, & Sob.

Conidiophori stipe a latere surgentes; rami primarii plerumque non septati, 19-34 μ ; rami secundarii plerumque non septati, 15-24 μ ; alii rami non septati, 9-17 μ ; phialidae reniformes, non septatae, hyalinae, 6.8-18.6 \times 4.1-5.4 μ . Stipes septatus, hyalinus, apice tenuior, latitudinia 4-10 μ proceritatis usque ad 425 μ , in vesiculum ab elliptico ad ovatum, 9.5-20.4 \times 6.8-8.8 μ fininens. Conidia hyalina, granularia, cylindrata, recta, pede quam apice latiora, utroque termino globosa, in quibus unum septatum, 46-77 \times 4.1-5.4 μ .

Segregatum a foliis Mahonia bealei Carr. in Monti-

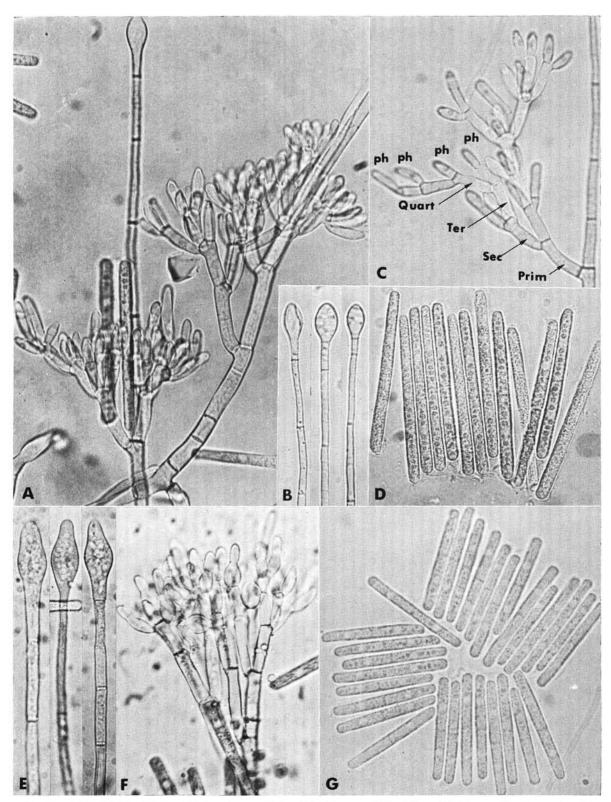


Fig. 2. A-D) Cylindrocladium ellipticum from a 5-day-old culture on PDA; A) conidiophores with stipe and vesicle; B) stipes with vesicles; C) portion of conidiophore showing branching and phialides; D) conidia; E-G) Cylindrocladium scoparium from a 5-day-old culture on PDA; E) stipes and vesicles; F) conidiophores; and G) conidia.

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Cylindrocladium ellipticum Alf., Seymour, & Sob. sp. nov.

Conidiophores arise laterally on a stipe, primary branches mostly nonseptate, $19\text{-}34\,\mu$; secondary branches mostly nonseptate, $15\text{-}24\,\mu$; other branches nonseptate, $9\text{-}17\,\mu$; phialides reniform, nonseptate, hyaline, $6.8\text{-}18.6\times4.1\text{-}5.4\,\mu$. Stipes septate, hyaline and narrower toward the apex, $4\text{-}10\,\mu$ wide, up to $425\,\mu$ in length, terminating in an ellipsoid to oval vesicle $9.5\text{-}20.4\times6.8\text{-}8.8\,\mu$. Conidia hyaline, granular, cylindric, straight, wider at the base than the apex, rounded at both ends, one-septate, and $46\text{-}77\times4.1\text{-}5.4\,\mu$.

Isolated from the leaves of Mahonia bealei Carr. in Monticello, Florida. Deposited in the herbaria of the New York Botanical Garden, The National Fungus Collection, and The University of Florida.

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