MYCOTAXON

Vol. XXVI, pp. 151-164 July-September 1986

CALONECTRIA SPATHULATA SP. NOV.

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Calonectria spathulata, with its anamorph Cylindrocladium spathulatum, is described as a new species from leaf spots on Eucalyptus viminalis from Brazil.

Key Words: Cylindrocladium spathulatum, Eucalyptus viminalis, homothallism.

INTRODUCTION

Species nomenclature of the Calonectria/Cylindrocladium connection, is based on conidiophore morphology, branching, phialide morphology, conidial morphology and septation, characteristics of a vesicle (if produced) at the apex of the conidiophore and characteristics of the teliomorph, if produced, (ascospore morphology and septation, and anatomy of the perithecial wall).

Recently, species of Cylindrocladium are being differentiated on the basis of aminopeptidase profiles (Stevens et al. 1985), and on protein and isoesterase profiles (A. C. Alfenas, Universidade Federal De Vicosa, 36570-Vicosa-MG-Brazil: personal communication).

In September 1983, with the approval of the U. S. Department of Agriculture, Animal and Plant Health Inspection Service, leaves of Eucalyptus viminalis Labill., exhibiting discrete leaf spots and variable necrotic lesions,

were received for diagnosis from Brazil by the Florida Department of Agriculture and Consumer Services. Isolations from symptomatic leaf tissues yielded Colletotrichum gloeosporioides Penz., a Dothiorella sp., and a Cylindrocladium sp. which closely resembled C. ellipticum Alf., Seymour, and Sob. or C. scoparium Morgan. However, there were subtle but distinct differences between them. Comparative morphological observations of the teleomorphic and anamorphic states were made to determine the identity of the Cylindrocladium isolate, and a technique on the use of isozymes to differentiate between our Brazilian isolate and C. scoparium was performed by Alfenas. As a result, a new species is described herein.

METHODS AND MATERIALS

Initial isolations were made on freshly prepared acidified potato dextrose agar (APDA). The potato dextrose agar (PDA) was prepared from the broth of 200 g freshly peeled, diced, and boiled Irish potatoes supplemented with 20 g dextrose, 1 g KH_2PO_4 and 18 g Difco bacto-agar, and made up to 1 liter with deionized water. Fifteen drops of 50% lactic acid were added to 250 ml of cooled (50 C), autoclaved (121 C for 15 min) PDA to inhibit bacterial growth.

Morphological comparisons were made on subcultures grown on peanut stem water agar (PSWA) which was used to enhance conidial production and promote formation of perithecia. It was prepared in the following manner: cooled, autoclaved water agar was poured over dried, propylene oxide-fumigated (Hansen and Snyder, 1947) stem pieces of peanut (Arachis hypogaea L.). With careful handling, the stem pieces floated and became partially submerged upon solidification of the agar. Single-conidial and single ascospore cultures of the Brazilian isolate were obtained using the method described by Hansen and Smith (1932). Cultures of the Cylindrocladium spp. were grown at 25 $\pm$ 2 C under continuous fluorescent light (General Electric F40 LW-RS-WMII at approximately 1000 lux) for 4 weeks or longer on PSWA.

The following live transfers from stored Florida cultures were used in this study: C. ellipticum isolated in 1969 (Log Number 069-952 = Florida Type Culture Collection FTCC 439 = American Type Culture Collection ATCC 38227) from leaves of Mahonia bealei Carr., C. scoparium isolated in 1984 (084-1091) from leaf spots on Callistemon viminalis (Soland. ex Gaertn.) Cheel, C. floridanum Sobers and Seymour

isolated in 1980 (080-500 = ATCC 42971) from leaf spots on Rumohra adiantiformis (G. Forst.) Ching, C. floridanum isolated in 1982 (082-2546) from Trifolium pratense L. roots, C. floridanum isolated in 1982 (082-1984) from Malus sylvestris Mill. roots, and C. floridanum first isolated in 1965 (FTCC 394) from Prunus persica (L.) Batsch roots. The type culture of C. floridanum (ATCC 18882) from peach roots was also included in this investigation. These cultures are stored at 12 C in the Department of Agriculture and Consumer Services, Division of Plant Industry, 1911 S. W. 34th Street, Gainesville, Florida 32608, U.S.A.

Perithecia produced on PSWA by the Brazilian isolate were compared to those of Calonectria kyotensis Terashita produced on the same medium by four isolates of C. floridanum mentioned above which had been previously obtained from leaves of leatherleaf fern and roots of red clover, apple, and peach.

A strip of peanut stem epidermis and cortex from PSWA bearing mature perithecia (exuding ascospores) was removed for microtome sectioning. The material was mounted vertically in a drop of 40% mucilage on a cold brass chuck in a -20 C freezing microtome. Sections (14 µm thick) were stained in lactophenol cotton blue. Cells of the perithecial wall were then measured under oil immersion at the equatorial level of longitudinally sectioned ascocarps. Eight ascocarps per isolate were selected at random, and no less than 100 perithecial wall cells per isolate were measured.

TAXONOMY AND DISCUSSION

The Cylindrocladium from E. viminalis has 1- to 3-septate conidia and a single central stipe (an extension of the main axis of the conidiophore stalk) terminating in a spathulate vesicle (Fig. 1), differing from C. ellipticum and C. scoparium which have ellipsoid to oval vesicles and only 1-septate conidia (Alfieri et al., 1970). The isolate under study has doliform phialides. Phialides of C. ellipticum are mostly reniform (Alfieri et al., 1970). Alfenas ran two gels with our isolate from Brazil. He showed that its protein and isoesterase patterns differ from those of C. scoparium (personal communication). The Cylindrocladium under study produced perithecia containing asci and ascospores on PSWA within 3-4 weeks (Fig. 2). Ascospores with up to five septa were extruded from induced perithecia of our Brazilian isolate as compared to the one-septate asco-

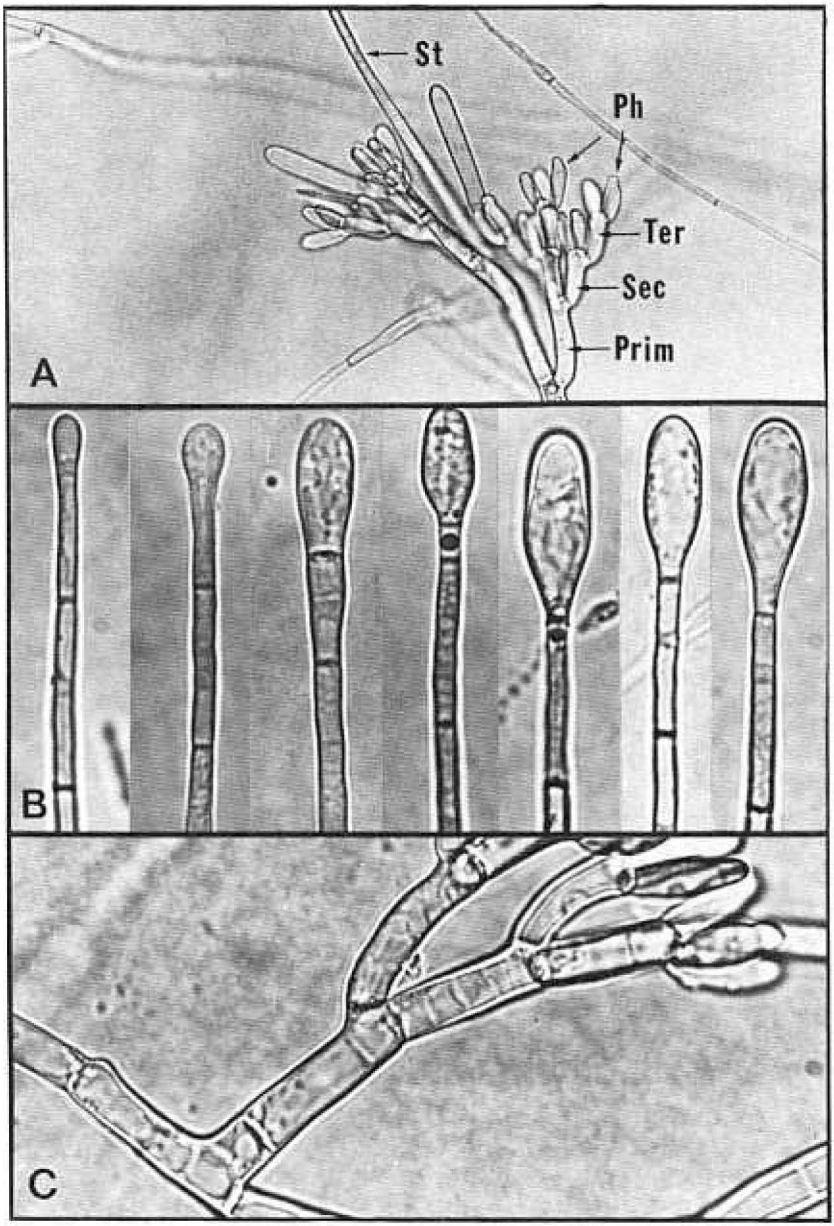

Fig. 1. Cylindrocladium spathulatum: A) Portion of conidiophore showing branching and phialides and a single central stipe, 462 X. (Prim = primary, Sec = secondary, Ter = tertiary, Ph = phialide, St = stipe). B) Stipes and spathulate vesicles, 1155 X. C) Conidophore arising from procumbent mycelial cell, 1155 X.

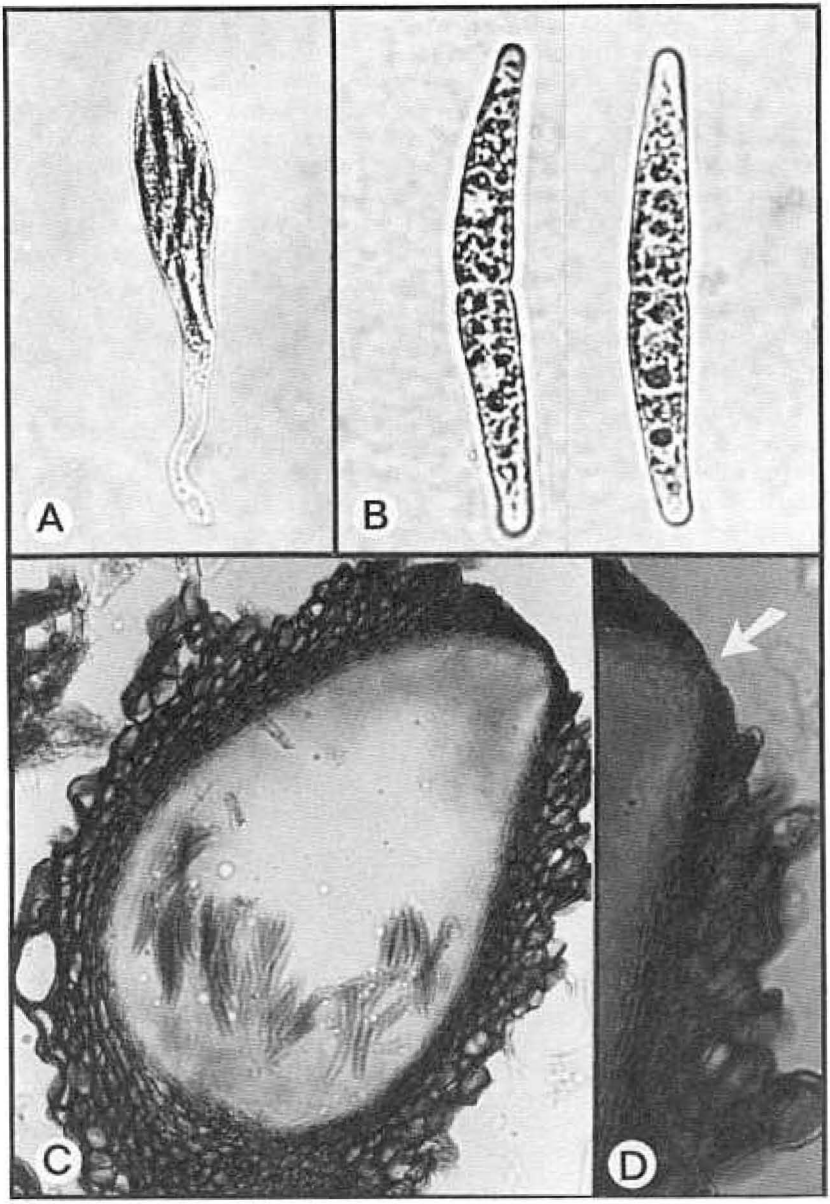

Fig. 2. Calonectria spathulata: A) Ascus containing 8 ascospores, 462 X. B) 1- and 3-septate ascospores showing constriction at the central septum, 1155 X. C) Longitudinal section of an ascocarp showing multiple inner layers of narrow cells. Outer cells oppressed, 231 X. D) close-up of Fig. 2C. Outer cells tapering toward the outside near the ostiole area, 462 X. Ostiole location is indicated by arrow.

spores from induced perithecia of Calonectria kyotensis on PSWA, under the same conditions. Ascospores of C. kyotensis are rarely more than one-septate (Terashita, 1968) and according to Gerlach (1968) they are always one-septate when still in the ascus. Twenty-seven single ascospore cultures from the Brazilian isolate were found to be identical to the culture that produced them. Each culture produced perithecia on PSWA. Forty-one hyphal tips taken from germ tubes of single cells of 24 additional germinating ascospores all developed into self-fertile cultures, clearly establishing the homothallic nature of this fungus. Descriptions were based on the original culture and on six monoascosporic isolates.

Sobers (1973) crossed isolates of C. scoparium from various hosts and locations and obtained orange or yellow perithecia of a Calonectria or a mixture of both colors in approximately equal numbers. Measurements of perithecia, asci, and ascospores were given. Although approximately 85% of the ascospores (which were mostly one-septate) germinated, they soon died and no viable cultures were obtained. Ribeiro (1978) crossed cultures of C. scoparium and obtained perithecia containing asci and ascospores. A new species was then proposed (Calonectria scoparia Ribeiro & Matsuoka sp. nov.), and the heterothallic nature of the fungus was demonstrated. Ascospores (which were mostly one-septate) of this species were pathogenic on detached leaves of Eucalyptus cloeziana F. J. Muell. and seedlings of E. grandis W. Hill ex Maiden under laboratory conditions.

However, Rossman (1983) listed C. scoparium (Fig. 3A & 3B) as the anamorph of Calonectria kyotensis. She further maintains that C. floridanum (Fig. 3C & 3D) is a synonym of C. scoparium and we disagree. We are in agreement with Sobers and Seymour (1967), and Morrison and French (1969), in that C. floridanum and C. scoparium are different and distinct species. Cylindrocladium floridanum is known to have in addition to the central stipe, lateral stipes from secondary conidiophores terminating in sphaeropedunculate (Snell and Dick, 1971) vesicles. We are aware of the variability in vesicle morphology (Hunter and Barnett, 1978), however, vesicle characteristics of all C. floridanum mentioned under Methods and Materials (including the type culture) have been consistently stable on all media used in our studies (APDA, PDA, PSWA), and such characteristics were not observed in C. scoparium. Lateral vesiculate stipes from secondary conidiophores were found in all isolates of C. floridanum included in our studies, but in none of 25 iso-

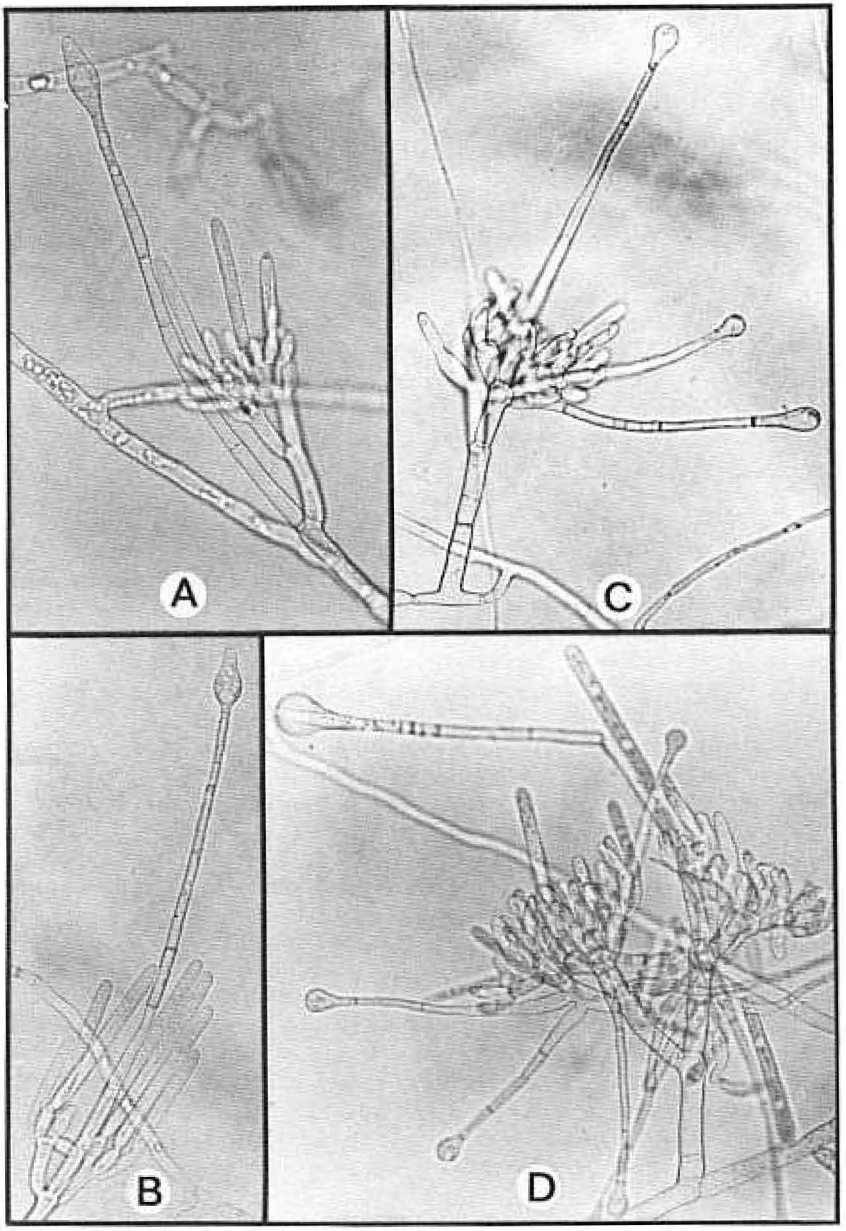

Fig. 3. Cylindrocladium scoparium isolated from Callistemon viminalis leaf spots: A) & B) conidiophores showing a single central stipe terminating in an ellipsoid to oval vesicle, 462 X, and Cylindrocladium floridanum isolated from Rumohra adiantiformis leaf spots: C) & D) conidiophores showing lateral stipes terminating in sphaeropedunculate vesicles, 462 X.

lates of C. scoparium collected in Florida and North Carolina.

In 1967, C. floridanum was designated as a new species occurring on peach roots in Florida (Sobers and Seymour, 1967), and in 1969, its perfect state was described as Calonectria floridana Sob. (Sobers, 1969). In January 1968, Calonectria kyotensis Terashita was described with a Cylindrocladium conidial state from leaves of Robinia pseudoacacia L. and leaves of Acacia dealbata Link (Terashita, 1968), and in April 1968, Calonectria uniseptata Gerlach was described as the perfect state of C. scoparium (Gerlach, 1968). Sobers (1972) later conducted morphological comparisons of the perfect and imperfect states of these three fungi. In addition, he performed comparative pathogenicity tests with these fungi and a known isolate of C. scoparium. Cylindrocladium scoparium differed from the three isolates under study in that it was slightly virulent to three cultivars of peach roots and highly virulent to roots of three species of Lupinus, whereas the three isolates under study were highly virulent to roots of peach and slightly virulent to roots of lupines. The three Calonectria isolates revealed only minor differences, which suggested that they were taxonomically identical (Sobers, 1972). Vesicles of the imperfect state of C. uniseptata were sphaeropedunculate and typical of C. floridanum rather than ellipsoidal as is typical for C. scoparium. Sobers (1972) therefore concluded that the imperfect states of the three species of Calonectria are C. floridanum and that Gerlach (1968) incorrectly identified the imperfect state of C. uniseptata as C. scoparium. Sobers (1972) also concluded that C. floridana, C. kyotensis and C. uniseptata are synonymous and we concur. Because C. kyotensis predates C. floridana and C. uniseptata, C. kyotensis is correctly assigned as the perfect state of C. floridanum.

All isolates of C. floridanum (including the type culture) mentioned under Methods and Materials, produced C. kyotensis perithecia on PSWA under conditions of our study. The anatomy and morphology of all perithecia produced by these isolates were consistently identical. Perithecia produced by the Brazilian isolate (Calonectria spathulata) on PSWA, under the same conditions were anatomically and morphologically different as follows:

Calonectria spathulata: the inner layers of longitudinally sectioned perithecial walls are composed of narrow cells. The number of inner layers varies from 3 to 6.

Outer cells are oppressed (less inflated) (Table 1), tapering toward the outside near the ostiole area (Fig. 2C & 2D).

Calonectria kyotensis: 1-4 inner cell layers in the perithecial wall with fewer and larger cells. Outer cells are more inflated. (Table 1). These characteristics are consistent among isolates which were derived from different hosts (Fig. 4).

Based on characteristics of the anamorphic and teleomorphic states of these fungi, on the difference in protein and isoesterase patterns, and on the basis of the differences noted as outlined in Table 1, this isolate from Brazil is considered anatomically, morphologically, and physiologically distinct from previously described species in this genus, and we propose the following new species:

Calonectria spathulata El-Gholl, Kimbrough, Barnard, Alfieri, et Schoulties, sp. nov.

Perithecia lutea vel rufa, globosa vel subglobosa (318-) 422 (-536) µm alta x (273-) 367 (-457) µm lata, muro exteriore aspere verrucoso. Perithecia superficialia singulatim portata vel in globulis et ostiolo papillato. Asci hyalini, (87-) 128 (-162) x (10-) 18 (-24) µm. Ascosporae hyalini, granulares, recti vel falcati, plurimi 1-septati, in medio septo constricti, (14.0-) 40.0 (-70.0) x (3.2-) 5.3 (-7.4) µm, et (30.0-) 48.0 (-64.0) x (4.0-) 5.3 (-7.4) µm, et (36.0-) 48.0 (-76.0) x (3.0-) 5.3 (-6.9) µm, singulis 1-, 2-, et 3-septatis ascosporis. Ascosporae 4 et 5 septorum raro visae.

Habitat: Secreta ex maculis folii in Eucalypto viminali; perithecia in partibus stirpis Arachis hypogaea.

Locus typi: Santa Catarina, Brazilia.

Holotypus: Teleomorphosis FLAS F54257, anamorphosis FTCC 1001, September 1983.

Cylindrocladium spathulatum El-Gholl, Kimbrough, Barnard, Alfieri, et Schoulties anam. nov.

Conidiophora lateraliter portata uno medio stipite qui vesicula hyalina et spathulata terminatur (11.0-) 20.0 (-38.0) x (3.5-) 7.0 (-12.0) µm. Stipites recte orientes ex myceliis procumbentibus in cultura. Stipites septati, (4.5-) 6.0 (-6.9) µm ad basim lati, angustiores (2.5-) 2.9 (-3.2)

Table 1. Measurements of inner and outer cells of longitudinally sectioned ascocarp periderms.

SPECIES	HOSTS	INNER CELLS[1]		OUTER CELLS	
		LENGTH(µm)	WIDTH(µm)	LENGTH(µm)	WIDTH(µm)
Calonectria spathulata	Eucalyptus viminalis	(5.9-)12.9(-26.7)	(1.0-)2.6(-4.0)	(5.0-)13.8(-32.7)	(4.0-)10.5(-26.7)
Calonectria kyotensis	Malus sylvestris	(6.9-)16.9(-27.7)	(2.5-)4.3(-6.4)	(6.9-)22.5(-41.6)	(5.0-)14.1(-30.7)
	Rumohra adiantiformis	(5.0-)15.6(-28.7)	(3.0-)5.0(-6.9)	(8.9-)18.9(-29.7)	(5.0-)13.9(-27.7)
	Trifolium pratense	(9.9-)18.7(-33.7)	(3.0-)5.0(-6.9)	(8.9-)23.8(-51.5)	(5.0-)14.9(-29.7)

[1]3-6 inner cell layers in C. spathulata
1-4 inner cell layers in C. kyotensis

septate, (4.5-) 6.0 (-6.9) µm wide at the base, becoming narrower (2.5-) 2.9 (-3.2) µm at the base of the vesicle, (172-) 202 (-231) µm long including the vesicle. Primary conidiophore branches mostly nonseptate, (14-) 28 (-52) µm. Secondary conidiophore branches mostly nonseptate, (13.0-) 15.5 (-20.0)µm. Tertiary conidiophore branches nonseptate and seldom observed. Two to four phialides may develop from the terminal end of any of the branches. Phialides hyaline, nonseptate, doliform, (9.0-) 13.0 (-17.0) x (4.0-) 4.8 (-6.4) µm. Conidia formed from the apex of the phialides. Conidia hyaline, cylindrical, straight, granular, rounded at both ends, (39.0-) 58.0 (-78.0) x (4.0-) 5.1 (-6.0) µm, (54.0-) 66.0 (-84.0) x (4.5-) 5.5 (-5.9) µm and (55.0-) 68.0 (-86.0) x (5.0-) 5.6 (-6.0) µm for the 1-, 2-, and 3-septate conidia, respectively.

Habitat: Isolated from leaf spots on Eucalyptus viminalis.

Type locality: Santa Catarina, Brazil.

ACKNOWLEDGMENT

The authors thank Dr. Walter E. Forehand, Chairman and Professor, Department of Classics, The Florida State University, Tallahassee, FL, for rendering the Latin diagnosis, Dr. Jack L. Gibson, Post Doctoral Associate in Botany, University of Florida, Gainesville, FL, for sectioning perithecia, Dr. N. C. Schenck, Professor of Plant Pathology, University of Florida, Gainesville, FL, for his review, Drs. John J. McRitchie, John W. Miller, and Timothy S. Schubert, Bureau of Plant Pathology, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL for their reviews, and Janet C. Temple, Cynthia A. Edwards and Judy M. Mattes for typing the manuscript.

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