# A Root Disease of Pine, Araucaria, and Eucalyptus in Brazil Caused by a New Species of Cylindrocladium

Charles S. Hodges and Luiza Cardoso May

Principal Plant Pathologist, Forest Service, USDA, Southeastern Forest Experiment Station, Forestry Sciences Laboratory, Research Triangle Park, North Carolina 27709; and Pathologist, Seccão de Fitotecnia Parasitológica, Instituto Florestal do Estado de S. Paulo, São Paulo, S. P., Brazil, respectively.

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## ABSTRACT

*Cylindrocladium clavatum* sp. n. has been found associated with a root disease of *Araucaria angustifolia, Eucalyptus saligna,* and several species of *Pinus* in Brazil. The disease has been found thus far in four contiguous states in the south of Brazil, and is pathogenic to all species of *Pinus* planted in Brazil. Unlike most other species of *Cylindrocladium, C. clavatum* affects large trees up to 15 years of age. The disease is characterized by discrete circular infection centers of up to 25 trees. On *Pinus* spp., resin infiltration of the roots and sometimes copious resin exudation is common. *Cylindrocladium clavatum* is characterized by having a clavate vesicle and 1-septate, 42.1- × 4.7-μ conidia.

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In 1969, a species of *Cylindrocladium* was isolated from the roots of dying 10- to 15-year-old *Araucaria angustifolia* (Bert.) Kuntze growing in a plantation of the Horto Florestal da Companhia Melhoramento Norte do Paraná in Jussara, Paraná, Brazil. Subsequently, the same fungus was isolated from roots of several *Pinus* spp. and seedlings of *Eucalyptus saligna* Sm. in the states of Minas Gerais, São Paulo, and Espirito Santo. This fungus was distinctly different morphologically from previously described species of *Cylindrocladium*, and is believed to be a new species. This paper describes the new taxon and discusses its pathogenicity and host range.

*Hosts and symptomatology.*—Although the fungus was first found on *Araucaria angustifolia*, subsequent observations indicate that it was far more common in pine plantations. Shortly after its first discovery, the fungus was isolated from roots of 15-year-old trees in plantations of *Pinus elliottii* Engelm. var. *elliottii* located near Casa Branca and Mogi Mirim in the state of São Paulo. These plantations contained numerous infection centers composed of 2 to 5 dead and dying trees. In the Casa Branca plantation, there were two centers about 15 × 20 m in size with all the trees in the center dead and several trees on the periphery in various stages of decline. Roots of these trees were pitch-soaked, and copious resin exudation had caused large amounts of soil to adhere tightly to the roots. In general, the symptomatology and spread patterns were very similar to those of Fomes annosus root rot (6), but this fungus was not isolated from diseased roots. Similar infection centers also were found in a 15-year-old and an adjacent 7-year-old plantation of *P. elliottii* var. *elliottii* near Santa Rita do Passo Quatro, which is near Casa Branca, but *Cylindrocladium* thus far has not been isolated from roots of these diseased trees.

In 1970, the same *Cylindrocladium* sp. was isolated from dying trees of several *Pinus* spp. in plantations near Itabira in the state of Minas Gerais. Seedlings for these plantations had been obtained in 1968 from a nursery located near the infested plantation at Santa Rita do Passo Quatro and heeled in in a nursery at Itabira before outplanting. In 1971, extensive mortality occurred in plantings of *P. oocarpa* Schiede and *P. insularis* Endl. Twenty-five trees were dead or dying in one infection center in a planting of *P. oocarpa.*

In a large nearby plantation of 3-year-old *P. caribaea* Morelet var. *hondurensis* Barrett & Golfari, numerous infection centers of 2 to 15 trees were found. The seedlings used in establishing this plantation were grown in the same nursery in which the seedlings mentioned above had been stored.

Needles on infected trees of all the *Pinus* spp. first turned bright yellow, drooped, and finally turned brick red. The entire root system was pitch-soaked, but there was very little resin exudation as seen in the older trees. Instead, the root bark was saturated with pitch, giving it a dark appearance. Spread of the disease was in a more or less circular pattern, but it could not be determined whether spread was by root-to-root contact or through the soil. However, the fungus was recovered from soil in infection centers using alfalfa seedlings as a trap crop as reported by Bugbee & Anderson (2).

The new *Cylindrocladium* sp. also has been recovered from 1.5-year-old *P. elliottii* var. *elliottii* in a plantation on Fazenda Guará do Pinhal, near Itapetininga, São Paulo State, from 2-year-old *P. elliottii* var. *elliottii* from Fazenda da Companhia Nestlé in Minas Gerais State, and from seedlings of *Eucalyptus saligna* near Santa Maria, São Paulo State, and Aracruz, Espírito Santo State.

*Pathogenicity.*—Inoculation studies were conducted on several tree species in the United States and Brazil. Inoculations with a culture of the new fungus from *A. angustifolia* were made in Brazil on 1-year-old seedlings of *A. angustifolia, P. taeda* L., *P. elliottii* var. *elliottii, P. palustris* Mill., *P. caribaea, P.*

*michoacana* Martinez, *P. montezumae* Lamb., and *P. patula* Schlecht. & Chamb. Small bits of inoculum of the fungus growing on potato-sucrose agar were placed beneath a small flap of bark or over wounds made by pricking the bark several times with a sharp needle. The point of inoculation, on the stem about 4 cm above the soil line, was then covered with moist cotton and wrapped with tape. At least 15 trees of each species were inoculated. The fungus rapidly girdled the stem, and all seedlings inoculated were dead after 2 weeks.

In the USA, 1-year-old seedlings of *P. taeda* L., *Liriodendron tulipifera* L., *Juniperus virginiana* L., and *A. angustifolia* growing in pots were inoculated with an isolate of the fungus from *A. angustifolia* by the placing of wheat grains on which the fungus had been growing for 2 months in a hole adjacent to the taproot. The inoculum was then covered with soil. Other seedlings of these hosts were inoculated with a North Carolina isolate of *Cylindrocladium scoparium* Morgan from *L. tulipifera.* With the exception of *J. virginiana,* all of the 10 seedlings of each species inoculated with the Brazilian isolate were infected after 6 weeks, and about half the seedlings of each species were dead at that time. The cortex of the roots of *A. angustifolia* and *L. tulipifera* was discolored and badly decayed. Roots of *P. taeda* were resin-soaked and rather hard. None of the seedlings of *J. virginiana* was visibly infected. Of the seedlings inoculated with *C. scoparium*, only *L. tulipifera* became infected. Only two of ten seedlings of this species inoculated were dead after 6 weeks. Noninoculated controls in both studies remained healthy. The fungus was recovered from inoculated plants.

*Description of the pathogen.*—No observations of the fungus were made on host material; therefore, the following description is based entirely on cultural characteristics. The fungus grows and sporulates well on malt extract agar, potato-dextrose agar, cornmeal agar, potato-carrot agar, and V-8 juice agar. Colonies on malt extract agar reach a diameter of 9 cm in 6 to 7 days at 24 C. The aerial mycelium is at first white but rapidly turns reddish brown, is 1 to 3 mm deep, and often exhibits numerous reddish exudation drops. The reverse is reddish brown. Small, reddish-brown microsclerotia, typical of many species of *Cylindrocladium,* are formed in the substrate. Conidiophores (Fig. 1-A) arise mainly from the substratum, but often from aerial mycelium as well. They are septate, 4.0 to 6.5 $\mu$ in diameter, and brownish near the base, but become smaller in diameter and hyaline above. They branch dichotomously or trichotomously in 2 to 4 series, with the branches 10 to 25 $\mu$ long. The ultimate branches terminate in a whorl of 2 to 5 bottle-shaped phialides, 8.0 to 12.0 × 3.2 to 3.6 $\mu$. Conidia (Fig. 1-C) are cylindrical, 1-septate, hyaline, 37.6 to 47.9 × 3.4 to 5.6 $\mu$ (average 42.1 × 4.7 $\mu$), and are slightly wider at the distal end. They collect in a sticky, whitish, palisadelike layer at the tips of the phialides. A stipe (Fig. 1-A) arises from one of the branches of most, but not all, conidiophores. These stipes are

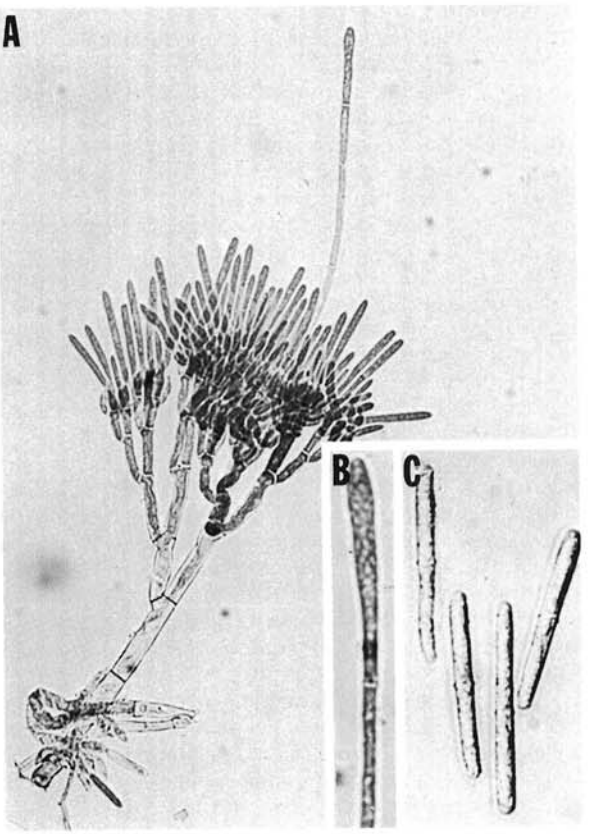


**Fig. 1.** *Cylindrocladium clavatum:* **A)** Conidiophore with stipe and clavate vesicle (× 540). **B)** Clavate vesicle (× 850). **C)** Conidia (× 1,000).

hyaline, septate, 2.5 to 3.5 $\mu$ in diameter, and range from 115 to 220 $\mu$ long, measured from the attachment to the conidiophore. They terminate in a clavate, 0- to 1-septate vesicle (Fig. 1-B) which measures 35 to 60 × 4.0 to 4.8 $\mu$. The vesicle usually stains much darker in cotton blue than does the stipe. In many cases, the stipe has the appearance of originating directly from the substrate with conidiophores arising at several points along its length.

No significant differences in morphology were found among isolates from different hosts or localities, although some isolates varied slightly in growth rate or conidial production.

*Taxonomy of the pathogen.*—Although not mentioned in the original description of the genus, the stipe, a hypha of determinate growth originating from one of the branches of the conidiophore or sometimes from the substratum, and extending laterally or vertically beyond the level of the phialides and spores, is the most distinguishing morphological character of the genus *Cylindrocladium*. The apex of the stipe may be enlarged into a structure of various shapes termed a "vesicle". Until recently, the presence or shape of the vesicle was not considered in delineation of species in the genus. However, Sobers

& Seymour (8), in describing *C. floridanum*, separated that fungus from *C. scoparium* on the basis of difference in size and shape of the vesicle, and presented evidence of the stability of this character in these and other *Cylindrocladium* spp. They considered the presence and shape of the vesicle the most important characters by which species of *Cylindrocladium* can be identified.

The currently known species of *Cylindrocladium* can be placed into five groups based on presence and shape of the vesicle (E. K. Sobers, *personal communication*). These are clavate, elliptical, globose, hastate, and avesiculate. The Brazilian fungus belongs to the group with clavate vesicles.

Five species of *Cylindrocladium* with clavate vesicles have been described previously. Three of these can be separated easily from the Brazilian species because they have 3-septate (*C. macrosporium* Sherb. var. *hederae* Arn. and the *Cylindrocladium* state of *Calonectria theae* Loos) or 5-septate (*C. quinqueseptatum* Boedijn & Reitsma) conidia. Although both have 1-septate conidia, *C. parvum* Anderson has significantly smaller (15 to 21 X 2 to 3 $\mu$) and *C. pteridis* Wolf has significantly larger (63 to 85 X 6.5 to 7.5 $\mu$) conidia than the Brazilian fungus.

Three species and one variety of *Cylindrocladium* previously have been reported from Brazil. *Cylindrocladium scoparium* is a common agent of root rot and damping-off on seedlings of *Eucalyptus* spp. in many areas of Brazil, and has been collected by the writers on numerous occasions. This species also has been isolated occasionally from the roots of *Pinus densiflora* and *P. elliottii* (5). It differs from the new fungus in having an elliptical vesicle and larger spores. *Cylindrocladium scoparium* var. *brasiliensis* Batista & Ciferri was described from *Eucalyptus* spp. and distinguished from *C. scoparium* by smaller spores (1). No mention is made of the vesicle shape in the original description. The type of *C. scoparium* var. *brasiliensis*, obtained from the herbarium of the Instituto de Pesquisas Agrônomicas in Recife, Brazil, did not have any conidiophores or conidia of the fungus. This was also true of two additional specimens obtained from the herbarium of the Instituto de Mycologia, Universidade Federal de Pernambuco, Recife. Three cultures labeled *C. scoparium* var. *brasiliensis*, obtained from the same institute (No. 05, 95, and 1643), all had ellipsoidal vesicles and spores that fell within the range of *C. scoparium*. A culture of *C. scoparium* var. *brasiliensis* (CBS 230.51) obtained from the Centraalbureau voor Schimmelcultures was sterile. Viegas (9) described *C. candelabrum* from leaves of *Luma* sp. collected near Rio Claro, São Paulo, in 1946. The type of this species was obtained from the herbarium of Viegas at Campinas, São Paulo State, and found to be identical with *C. scoparium*. *Cylindrocladium quinqueseptatum* and its perfect stage, *Calonectria quinqueseptata*, has also been reported from Brazil (3).

The Brazilian *Cylindrocladium* is considered to be morphologically distinct from previously described species in this genus and is herein described as a new species as follows:

*Cylindrocladium clavatum* Hodges & May

*Coloniae in agaro braciato 6 vel 7 diebus calore 24 C accrescunt diametro usque ad 9 cm, primo albae, denique badiae, altitudine 1-3 mm; facie retroversa et badia. Conidiophori e substratu vel mycelio aeriali exorientes, ad pedem fusci, supra hyalini, septati, in 2-4 series ramosi, ramis longitudine 10-25 $\mu$; phialidia in 2-5 spiras verticillata, 8.0-12.1 X 3.2-3.6 $\mu$; conidia cylindrata, uniseptata, hyalina, 3.4-5.6 X 37.6-47.9 (plerumque 42.1 X 4.7); stipes hyalinus, septatus, e ramo conidiophori exoriens, 115-220 X 2.5-3.5 $\mu$; vesiculum clavatum hyalinum, uniseptatum, 35-60 X 4.0-4.8 $\mu$.*

*Holotypus: Cultura isolati exsiccata,* FSL-511 *e radicibus Pini caribaea,* Horto Conceicão, *ad* Itabira, Minas Gerais, Brazil. (BPI).

Cultures (FSL-511), deposited in the American Type Culture Collection, Centraalbureau voor Schimmelcultures, and Commonwealth Mycological Institute.

DISCUSSION.—Reforestation of cleared tropical hardwood sites with fast-growing species of pine has greatly accelerated in the past 5 years in Brazil. *Pinus elliottii* var. *elliottii* and *P. taeda* from the southern United States, and *P. patula* from Mexico, are the most commonly planted species in the southern part of the country, whereas north of the city of São Paulo, *P. caribaea*, especially the variety *hondurensis* from Central America, is favored.

While currently known to be distributed in only a relatively few locations in four states in southern Brazil, the root disease caused by *C. clavatum* must be considered a potential threat to plantations of exotic pines planted in Brazil for a number of reasons. Firstly, the fungus appears to be highly virulent to all the species of pine most commonly planted in Brazil as well as to some of the less commonly planted ones. Secondly, the fungus spreads very rapidly in a plantation once an infection center is established. Centers containing 15 to 25 dead and dying trees were noted in 3- and 4-year-old plantations. Thirdly, *C. clavatum* is unusual among species of *Cylindrocladium* which affect forest tree species in that it is damaging in plantations rather than nurseries. Previously, only Ross (7) has reported a species of *Cylindrocladium* causing death of large trees. Fourthly, since planting pine in many areas of Brazil is still in the experimental stage, it is common practice to obtain seedlings for many of these new plantings from established nurseries. One incidence of the moving of infected seedlings or infested soil to a distant location has been elaborated earlier in this paper. If this practice is continued, it could result in a wider distribution of the fungus.

*Eucalyptus* spp. are also widely planted in Brazil. Although *C. clavatum* has been found on *E. saligna* seedlings from two locations, this fungus thus far has not been found on large trees in plantations. The ubiquitous *C. scoparium* seems to be the most important pathogen on *Eucalyptus* in Brazilian nurseries.

*Araucaria angustifolia* is the only native Brazilian tree species on which *C. clavatum* has been found.

Thus far, the only known locality is where the original collection of the fungus was made in the state of Paraná.

There is currently no control recommendation for this disease in the plantation. Attempts are being made to eradicate discrete infection centers with methyl bromide, but results are not yet available. Control in the nursery is also difficult, but based on results obtained with *C. scoparium* in the southern United States, good control may be obtained with methyl bromide at twice the rate normally used in the nursery for other diseases (4).

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