Leptographium eucalyptophilum, a new species from Eucalyptus in the Congo

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Received 21 June 1999; revised 20 September 1999

Leptographium spp. are known mostly from the Northern hemisphere where they have been described mainly from coniferous hosts. Few Leptographium spp. have been described from the southern hemisphere and the tropics. During a recent survey of fungal diseases on Eucalyptus in the Republic of Congo, West Africa, an unidentified Leptographium sp. was isolated from stems of Eucalyptus hybrids. Comparison with known Leptographium spp. led us to conclude that this is a previously undescribed species. It is, therefore, described in this paper as Leptographium eucalyptophilum sp. nov.

Keywords: Eucalyptus, Leptographium, West Africa, Congo, fungal description.

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Introduction

Leptographium spp. are characterised by dark mononematous conidiophores with complex series of branches. These branches terminate in conidiogenous cells that produce conidia through percurrent proliferation (Kendrick 1962; Wingfield 1993). However, delayed secession of the conidia can create the impression of sympodial conidium development (Van Wyk et al. 1988). Approximately half of all Leptographium spp. are known to be associated with an Ophiostoma teleomorph (Harrington 1988; Wingfield 1993). As in the case of Ophiostoma, Leptographium spp. are also known to be able to tolerate high concentrations of cycloheximide in culture media (Harrington 1981).

Most Leptographium spp. occur on conifers (Lagerberg et al. 1927; Kendrick 1962, Harrington 1988), although a few exceptions have been described (Davidson 1942, 1958, 1971, 1976; Jocote 1978; Weber et al. 1996). Leptographium spp. are essentially saprotrophic (Harrington 1988; Wingfield et al. 1988) and are known to be causative agents of blue-stain on conifers (Lagerberg et al. 1927; Morrison & Hunt 1988; Solheim 1995). In only a few instances, Leptographium spp. are known as primary pathogens, capable of causing considerable losses (Cobb 1988; Harrington 1993).

Leptographium spp. are well-adapted for insect dispersal (Nelson 1934; Harrington 1988, 1993). The most common insect associates of these fungi are bark beetles (Coleoptera; Scolytidae) in the genera Hylastes and Hylurgops (Harrington 1988; Perry 1991). These insects generally feed on roots of conifers, but the nature of the association is currently not clear.

Plantation forestry, based on exotic Eucalyptus spp., forms an important part of the export market of many countries (Tumbln 1991). Currently approximately 8-9 million hectares of exotic Eucalyptus plantations exist in tropical and subtropical countries of the world (Tumbln 1991; Wingfield & Wingfield 1998). Although Eucalyptus is an unusual niche for Leptographium spp., recent surveys of diseased trees in the Republic of Congo, have resulted in the isolation of a Leptographium sp. of unknown identity. The aim of this study was to identify this Leptographium sp., and to consider its pathogenicity to Eucalyptus.

Materials and Methods

A survey of the diseases of Eucalyptus trees in the Point Noire area of the Republic of Congo resulted in the consistent isolation of an unknown Leptographium sp. Isolates were found sporulating in the xylem of diseased E. urophylla × E. polieta hybrid trees from the Kisoko plantation. Spore masses were transferred from the apices of conidiophores to 2% malt extract (MEA) plates (20 g Bioblab malt extract, 20 g Bioblab agar and 1000 ml distilled water) amended with 0.5 g/ℓ cycloheximide. Resulting colonies were transferred to clean 9% MEA plates and incubated at 25°C until the onset of sporulation. Fungal structures were imbedded in lactophenol and mounted on glass slides for microscopic examination. Fifty measurements were taken of each relevant morphological structure and ranges and averages computed. Colours were determined with the aid of a colour chart (Rayner 1970).

The optimal growth temperatures of representative isolates (PREM 56312, PREM 56313) were determined by inoculating eight MEA plates for each temperature (5-35°C at 5°C intervals) with a 6.0 mm diameter agar disk taken from the actively growing margin of a two week old isolate. Colony diameters were measured four and eight days after commencing the experiment. The colony diameter was computed as an average of eight readings.

For scanning electron microscopy (SEM), small blocks of agar cut from sporulating colonies, were fixed in 3% glutaraldehyde and 0.5% osmium tetroxide in a 0.1 M phosphate buffer. The material was dehydrated in a graded acetone series and dried using a critical-point drier. Specimens were mounted and coated with gold palladium alloy and examined using a lsof JSM 630 scanning electron microscope.

Cycloheximide tolerance of isolates (PREM 56312, PREM 56313) was determined by placing them on 2% MEA with different concentrations of cycloheximide (0, 0.05, 0.1, 0.5, 1.25 and 5 g/ℓ). Petri dishes were incubated in the dark at 25°C for eight days. Five replicate plates were prepared for each concentration and the growth rate (mm/day) was determined based on the average of ten diameter readings.

To determine the possible role of the Leptographium sp. in disease development on Eucalyptus spp., an isolate (PREM 56312) was inoculated onto 20 clones of Eucalyptus grandis × E. camaldulensis hybrid saplings. The experiment was conducted in a glasshouse with an average daily temperature of 25°C with ambient day/night light periods. The test isolate was cultured on MEA agar for 14 days. The bark of approximately one-year-old trees was removed with a 4 mm diameter cork borer. An agar plug of equal size, overgrown with the test fungus, was inserted into the wounds. All wounds were sealed with parafilm to prevent desiccation of the wound and inoculum. Ten trees were inoculated in a similar fashion, using sterile agar plugs to serve as controls. Lesion development was assessed after 6 weeks by investigating both the outer bark and xylem.
The graphium, revealed that this species has not been described cylindricorum; 2-3 ram is primariis; sine structuris rhizoidiformis, (180-)323(-500) μm longa, cum 2 vel 3 seriebus ramorum e mycelia recta exarientia, erecta, macronematosa, mononematovaceae. Hyphae immerse vel emerse in media solido, cum Coloniae optime in temperature 30°C crescentes, atroviride oli~

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tions. In the xylem, a blue discoloration was found in associa-
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eucalyptus. Conidia aseptata, oblonga vel obovoidea, (6.0-)8.0(-9.0) x
in the xylem of diseased Eucalyptus urophylla hybrid, collected: J. Roux, Kis-
soko plantation, Point Noire area, Democratic Republic of

30°C in the

Holotype: PREM 56312, isolated from the xylem of diseased Eucalyptus urophylla x E. pellita hybrid, collected: J. Roux, Kis-

Eucalyptus. Conidiophores occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (180-)223(-500) μm in length, rhizoid-like structures absent (Figure 1, 7a). Stipe, light olivaceous, smooth, cylindrical, simple, 4–5 septa, (140–272–440) μm long from first basal septum to below primary branches, 4.0–5.0 μm wide below primary branches, apical cell of stipe not swollen; (5.0–6.5–10) μm wide at base, basal cell not swollen. Conidigenous apparatus, excluding the conidioma mass, (30–)52–80) μm long, with 2 to 3 series of cylindrical branches; 2–3 primary branches, light olivaceous to hyaline, smooth, cylindrical, aseptate, (12–17–26) μm long and (3.0–4.0–6.0) μm wide. Secondary branches hyaline, aseptate, (7.0–10–13) μm long, (1.0–2.0–4.0) μm wide; tertiary branches hyaline, aseptate, (3.0–7.0–10) μm long, 2.0–3.0 μm wide (Figure 2, 7b). Conidigenous cells discrete, 2–3 per branch, cylindrical, tapering slightly at the apex, (7.0–10–13) μm long and (1.0–1.5–2.0) μm wide. Conidiolm development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed succession giving a false impression of sympodial proliferation (Minter, Kirk & Sutton, 1982, 1983; Van Wyk, Wingfield & Marasas 1988) (Figures 3 and 4). Conidia accumulating in slimy droplets at the apex of conidigenous apparatus, aseptate, oblong to obovoid, (6.0–8.0–9.0) x 3.0–5.0 μm (Figures 5, 6 and 7c).

Dried cultures of the holotype and paratypes have been deposited in PREM. Subcultures of the type strain have been deposited in CBS and BUCL.

Discussion
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cycloheximide, reaching 27
days at

3.0–5.0 μm.

Colonies with optimal growth at 30°C on 2% MEA, reaching 27

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longer than those found in L. eucalyptphilum. Leptographium eucalyptophilum is characterized by two to three primary branches on the stipe, in contrast to two branches that are consistently found in isolates of L. americana. Leptographium eucalyptophilum and L. americana can also be distinguished based on their host preferences and insect associations. Leptographium americana is known only on larch in North America associated with the bark beetle Dendroctonus simplex (Jacobs et al. 1997). Leptographium eucalyptophilum, is found on Eucalyptus and has no association with any insect has been observed.

Leptographium eucalyptophilum has an optimal growth temperature of 30°C. This is unlike most other species in Leptographium with optimal growth temperatures of between 20°C and 25°C. This phenomenon has also been observed in Leptographium calophylli that occurs in the Seychelles (Webber et al. in press) and appears to be characteristic of Leptographium spp. from tropical areas.

Pathogenicity trials showed that L. eucalyptophilum most likely does not play a primary role in disease development on Eucalyptus trees. This fungus was found to occur on lesions caused by Ceratocystis fimbriata Ell. & Halst. (Roux et al. 1999), a fungus that has recently been shown to be pathogenic to Eucalyptus spp. and was isolated in abundance from dying trees in the Republic of Congo (Roux et al. 1999). Ceratocystis fimbriata is characterised by the production of fruity aromas and insects, carrying this fungus, might accidentally also serve as vectors of L. eucalyptophilum.

Acknowledgments
We thank the National Research Foundation (NRF), and members of the Tree Pathology Co-operative Programme for financial support. We also thank the Université de Recherche sur la Productivité et les Biotechnologies pour l'Alimentation et l'Environnement, Madame Rosalie Safou for assistance with the disease survey and isolations and Dr. Hugh Glen for providing the Latin diagnosis.

References


