Leptographium eucalyptophilum, a new species from *Eucalyptus* in the Congo

K. Jacobs*, M.J. Wingfeld and J. Roux

Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, Republic of South Africa

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.

*To whom correspondence should be addressed. (E-mail: kjacobs@fabi.up.ac.za)

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; Jooste 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: Scolytinae) 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 (Turnbull 1991). Currently approximately 8–9 million hectares of exotic *Eucalyptus* plantations exist in tropical and sub-tropical countries of the world (Turnbull 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

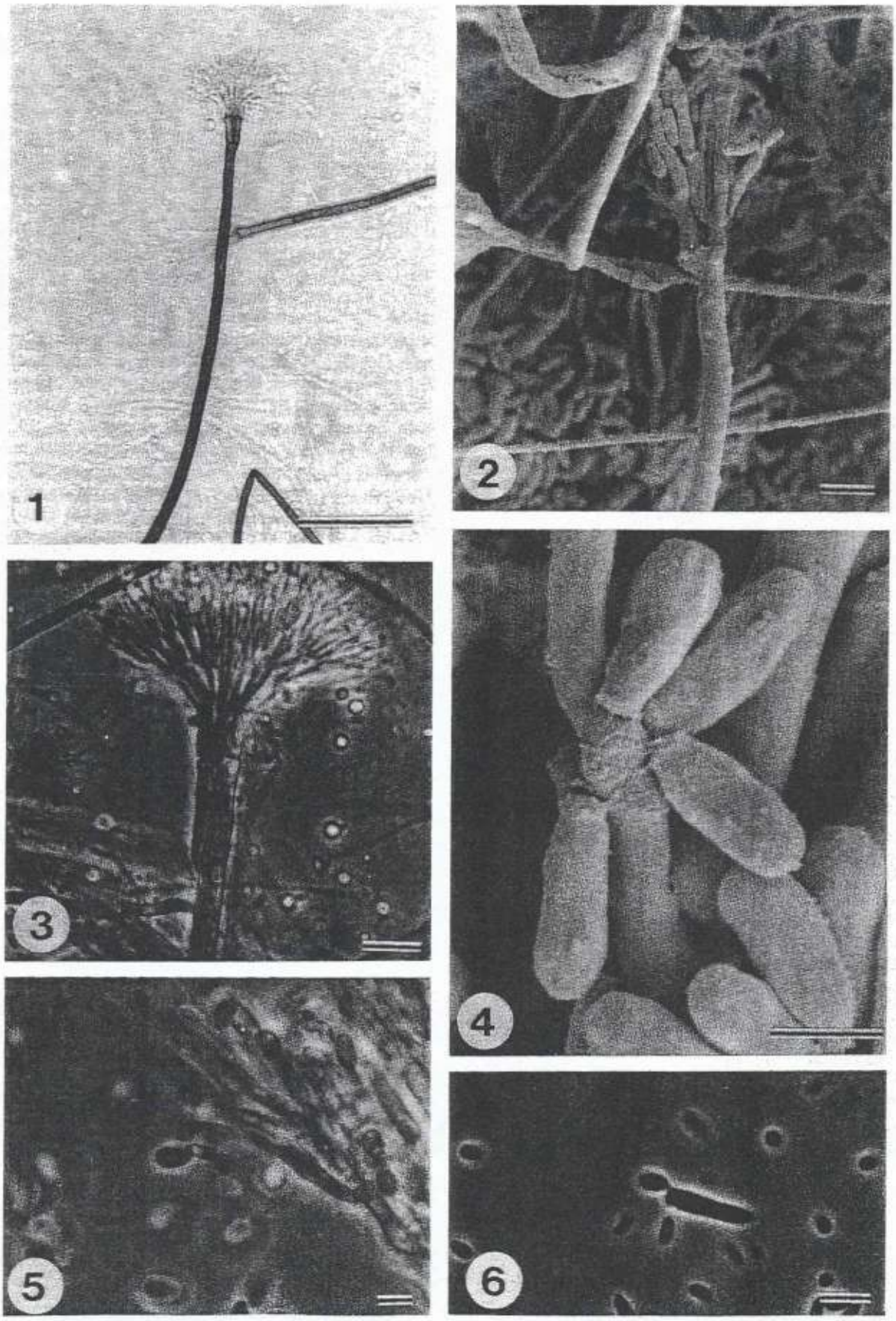
A survey of the diseases of *Eucalyptus* trees in the Point Noire area of the Republic of Congo resulted in the consistent isolation of an unknown *Leptographium* sp. Isolates were found sporulating in the xylem of diseased *E. urophylla* × *E. pellita* hybrid trees from the Kissoko plantation. Spore masses were transferred from the apices of conidiophores to 2% malt extract (MEA) plates (20 g Biolab malt extract, 20 g Biolab agar and 1000 ml distilled water) amended with 0.5 g/l cycloheximide. Resulting colonies were transferred to clean 2% 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 Jeol JSM 840 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, 2.5 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 on to 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.



Figures 1–6 *Leptographium eucalyptophilum* (PREM 56312). **1.** Conidiophore with a dark olivaceous stipe and complex conidiogenous apparatus (Bar = 100 µm). **2.** Scanning electron micrograph of the conidiogenous apparatus (Bar = 10 µm). **3.** Light micrograph of the conidiogenous apparatus (Bar = 10 µm). **4.** Conidiogenous cells showing false sympodial conidiogenesis (Bar = 5 µm). **5.** Light micrograph of conidiogenous cells (Bar = 10 µm). **6.** Conidia with occasional rhamoconidia (Bar = 10 µm).

Results

The *Leptographium* isolates from *Eucalyptus* were characterized by an optimal growth temperature of 30°C. Conidiophores were found to be long and slender and not as dark as those of other *Leptographium* spp. These isolates were further characterized by their long, oblong conidia. In some instances rhamoconidia were observed. Such structures have not been seen in other *Leptographium* spp. and they appear to be unique to the isolates from *Eucalyptus*.

Pathogenicity tests on a *Eucalyptus* hybrid indicated that the *Leptographium* sp. is not pathogenic to *Eucalyptus*. No external lesions were produced, but the fungus did prevent wounds from healing as quickly as those associated with the control inoculations. In the xylem, a blue discolouration was found in association with the *Leptographium* inoculations. This species is most probably a saprotroph and may be able to cause blue stain on dead wood. Comparison with other known species of *Leptographium*, revealed that this species has not been described previously and it is, therefore, described as follows:

Leptographium eucalyptophilum K. Jacobs, M.J. Wingf. and J. Roux sp. nov.

Teleomorph state: Not known.

Coloniae optime in temperature 30°C crescentes, atroviride olivaceae. Hyphae immersae vel emersae in medio solido, cum myceliis aeriis abundantibus. Conidiophora singula vel ad terna, e mycelio recta exorientia, erecta, macronematosa, mononematosa, (180–)323(–500) µm longa, cum 2 vel 3 seriebus ramorum cylindricorum; 2–3 ramis primariis; sine structuris rhizoidiformibus. Conidia aseptata, oblonga vel obovoidea, (6.0–)8.0(–9.0) × (3.0–)3.0(–5.0) µm.

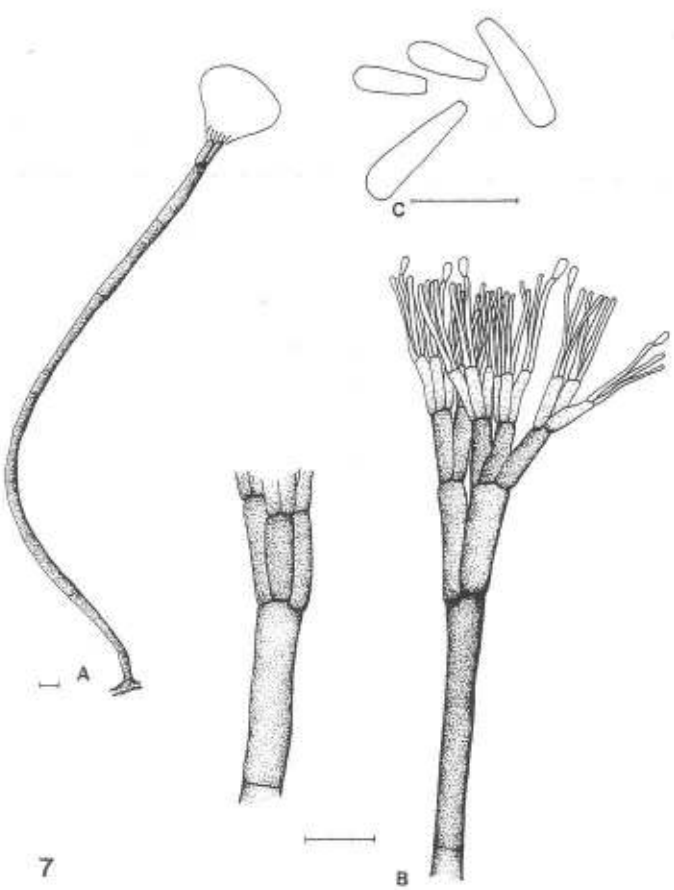


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Figure 7 *Leptographium eucalyptophilum* (PREM 56312). **A.** Habit sketch of the conidiophore. **B.** Conidiogenous apparatus (Bar = 10 µm). **C.** Conidia (Bar = 10 µm).

Colonies with optimal growth at 30°C on 2% MEA, reaching 27 mm in diameter in 6 days. No growth below 10°C or above 35°C. Able to withstand high concentrations of cycloheximide with a 15% reduction in growth on 0.1 g/l cycloheximide 8 after days at 30°C in the dark. Colonies dark green olivaceous (23''') with a smooth margin. *Hyphae* submerged and on top of solid medium with abundant aerial mycelia, light olivaceous to hyaline, smooth, not constricted at the septa, (2.0–)3.0(–5.0) µm diameter. *Conidiophores* occurring singly or in groups of up to three, arising directly from the mycelium, erect, macronematous, mononematous, (180–)323(–500) µm in length, rhizoid-like structures absent (Figure 1, 7a). Stipe, light olivaceous, smooth, cylindrical, simple, 4–9 septate, (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. *Conidiogenous apparatus*, excluding the conidial mass, (30–)52(–80) 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, (5.0–)7.0(–10) µm long, 2.0–3.0 µm wide (Figure 2, 7b). Conidiogenous 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. Conidium development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession 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 conidiogenous apparatus, aseptate, oblong to obovoid, (6.0–)8.0(–9.0) × 3.0–5.0 µm (Figures 5, 6 and 7c).

Holotype: PREM 56312, isolated from the xylem of diseased *Eucalyptus urophylla* × *E. pellita* hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Democratic Republic of Congo, June 1998. **Paratypes**: PREM 56313, PREM 56314, PREM 56315, PREM 56316, PREM 56317, PREM 56318, PREM 56319, PREM 56320, isolated from the xylem of diseased *Eucalyptus urophylla* × *E. pellita* hybrid, collected: J. Roux, Kissoko plantation, Point Noire area, Democratic Republic of Congo, June 1998.

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

Leptographium eucalyptophilum is characterized by its long, oblong conidia. Other species with similar long conidia, are *L. americanum* Jacobs, Wingfield & Bergdahl and the *Leptographium* anamorphs of *O. penicillatum* (Grosmann) Siemaszko and *O. dryocoetidis* (Kendrick & Molnar) Harrington (Grosmann 1932; Kendrick & Molnar 1965; Jacobs *et al.* 1997). The conidia of *L. eucalyptophilum* can, however, easily be distinguished from *O. penicillatum* and *O. dryocoetidis* that are twice as broad as those in *L. eucalyptophilum* (Grosmann 1932; Kendrick & Molnar 1965). In addition, *O. penicillatum* and *O. dryocoetidis* are known to produce perithecia, which were never seen in the case of *L. eucalyptophilum*. Both *O. penicillatum* and *O. dryocoetidis* occur on conifers in the northern hemisphere and are associated with severe staining of host tissue (Solheim 1986; Molnar 1965).

Leptographium americanum, has long and almost needle shaped conidia that are most similar to those of *L. eucalyptophilum* (Jacobs *et al.* 1997). The conidia are, however, much

longer than those found in *L. eucalyptophilum*. *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. americanum*. *Leptographium eucalyptophilum* and *L. americanum* can also be distinguished based on their host preferences and insect associations. *Leptographium americanum* 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 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 Unite de Recherche sur la Prodictivite des Plantations Industrielles (UR2PI) for financial assistance, Madame Rosalie Safou for assistance with the disease survey and isolations and Dr. Hugh Glen for providing the Latin diagnosis.

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