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Leptographium and *Graphium* species associated with pine-infesting bark beetles in England

MICHAEL J. WINGFIELD

Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa

JOHN N. GIBBS

Forestry Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey, GU10 4LH, England

Isolations for blue-stain fungi were made from *Hylastes ater, H. opacus, Hylurgops palliatus* and *Tomicus piniperda* trapped in *Pinus sylvestris* billets. Five *Leptographium* spp. including one of unknown identity and two apparently undescribed *Graphium* spp. were isolated. One of the *Leptographium* spp. identified as *L. truncatum* is thought to be synonymous with *L. lundbergii*. This is the first record of *Leptographium procerum, L. serpens* and *L. wingfieldii* from the United Kingdom. *Leptographium lundbergii* has previously been found in the country.

Many species of *Ophiostoma* H. & P. Sydow (*Ceratocystis* Ellis & Halst. *sensu lato*) or its anamorphs, *Leptographium* Lagerberg and Melin and *Graphium* Corda cause blue-stain in logs and lumber while others cause disease in standing trees (Boyce, 1961; Upadhyay, 1981; De Hoog & Scheffer, 1984). In some cases definite links have been established with bark beetle vectors but in others, no clear association has been demonstrated. The situation of *Pinus* spp. is of special interest because of the high susceptibility of the wood to blue-staining and because of the number of pine diseases linked with *Leptographium* spp. in a more or less causal role (Wingfield, Capretti & Mackenzie, 1988). Recent developments in the taxonomy of this group of fungi, particularly with respect to the *Leptographium* stage, have made it highly desirable that the various pine-inhabiting bark beetles should be examined to determine which blue-staining fungi they carry (Harrington, 1988; Wingfield *et al.*, 1988).

During Feb. 1986, batches of freshly cut Scots pine (*Pinus sylvestris* L.) billets, approx 30 cm in length and 7 cm in diam. were prepared. These were placed in two compartments in the High Lodge beat of Thetford forest, East Anglia, where 50–60-yr-old Scots pine crops had recently been felled. In order to encourage colonization by root-infesting insects, the billets were sunk into the ground so that half the circumference of each billet was below soil level. After 3 and 4 months, samples of billets were collected for laboratory examination. The bark was carefully removed and adult beetles collected and stored singly in vials at 3 °C. Identification of the beetles was made by T. G. Winter (Entomology Branch, Forestry Commission, Alice Holt Lodge).

A few days after collection, each adult beetle was macerated in sterile water and a dilution of the macerate plated on a medium selective for *Ophiostoma* spp. and their anamorphs (2% Oxoid malt extract agar containing 10 p.p.m. streptomycin and 100 p.p.m. cycloheximide). Young colonies were then subcultured and purified for further examination and identification.

A total of 35 adult insects belonging to three genera and four species were collected. These included *Hylurgops palliatus* Gyll., *Hylastes ater* Payk., *Hylastes opacus* Erichs. and *Tomicus piniperda* L. The first three of these insects are predominantly root-infesting species whereas *T. piniperda* is usually found in above-ground parts of trees. The seven species of *Leptographium* and *Graphium* isolated from these insects are shown in Table 1.

The presence in the United Kingdom of *Leptographium procerum* (Kendrick) Wingfield is of particular interest since this fungus is associated with a root collar disease of eastern white pine (*Pinus strobus* L.) in various parts of the United States (Lackner & Alexander, 1982; Wingfield, 1983), Yugoslavia (Halambek, 1976) and New Zealand (Shaw & Dick, 1980; Wingfield & Marasas, 1983). Neither of the insects carrying *L. procerum* in this study has previously been associated with the fungus. The presence of the fungus in New Zealand (Wingfield & Marasas, 1983), however, suggests that it was introduced into that country either with *Hylurgus ligniperda* Fabr. or *H. ater* which are both of European origin.

Leptographium serpens (Goid.) Siem. (teleomorph *Ophiostoma serpens* (Goid.) Von Arx) is associated with a root disease of pines in Italy (Gambogi & Lorenzini, 1977) and South Africa (Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1981). The vectors of this fungus in Italy have not been identified. In South Africa, *L. serpens* is commonly associated with *H. angustatus* Herbst. believed to have been

Table 1. *Leptographium* and *Graphium* spp. isolated from insects infesting billets of *P. sylvestris*

Species	Insect	No. of insects	Accession nos[a]
L. procerum	*Hylastes opacus*	4	IMI 341 358
	Hylurgops palliatus	1	CBS 342.90
			CMW 825
L. truncatum	*H. opacus*	2	IMI 341 360
	H. palliatus	4	CBS 344.90
			CMW 835
L. serpens	*Hylastes ater*	1	IMI 341 363
			CBS 347.90
			CMW 853
L. wingfieldii	*Tomicus piniperda*	1	IMI 341 361
	H. opacus	1	CBS 345.90
	H. palliatus		CMW 840
Leptographium sp. 'GG'	*H. ater*	2	IMI 341 359
	H. opacus	3	CBS 343.90
	H. palliatus	6	CMW 831
Graphium sp. A.	*H. opacus*	3	IMT 341 362
	H. palliatus	1	CBS 346.90
	T. piniperda	2	CMW 849
Graphium sp. B.	*H. palliatus*	1	IMI 341 364
			CBS 348.90
			CMW 857

[a] Accession numbers relate to the culture collections of the International Mycological Institute (IMI); the Centraalbureau voor Schimmelcultures (CBS) and the culture collection of the senior author (CMW).

accidentally introduced with its fungal associates from Europe. Its presence on *H. ater* here goes some way to supporting this view.

Leptographium truncatum (Wingfield & Marasas) Wingfield was first described from South Africa and New Zealand (Wingfield & Marasas, 1983; Wingfield, 1985). It was associated with the roots of dying trees infested with *H. angustatus* in South Africa (Wingfield & Marasas, 1983) as well as *H. ater* and *H. ligniperda* in New Zealand (Wingfield *et al.*, 1988). When inoculated into pine roots, the fungus causes distinct lesions (Wingfield & Marasas, 1983). The precise role of this fungus in pine root disease has, however, not been established (Wingfield *et al.*, 1988).

Leptographium wingfieldii Morelet was recently described from France (Morelet, 1988) as part of a study of bark beetles and blue-stain fungi in dying Scots pine stands. It was found on *T. piniperda* adults with a low but uniform frequency (Lieutier *et al.*, 1989). A similar situation was found in studies of windthrown pine in southern England conducted after the severe storm of October 1987 (Gibbs & Inman, 1991). No definite pathological role has been ascribed to the fungus.

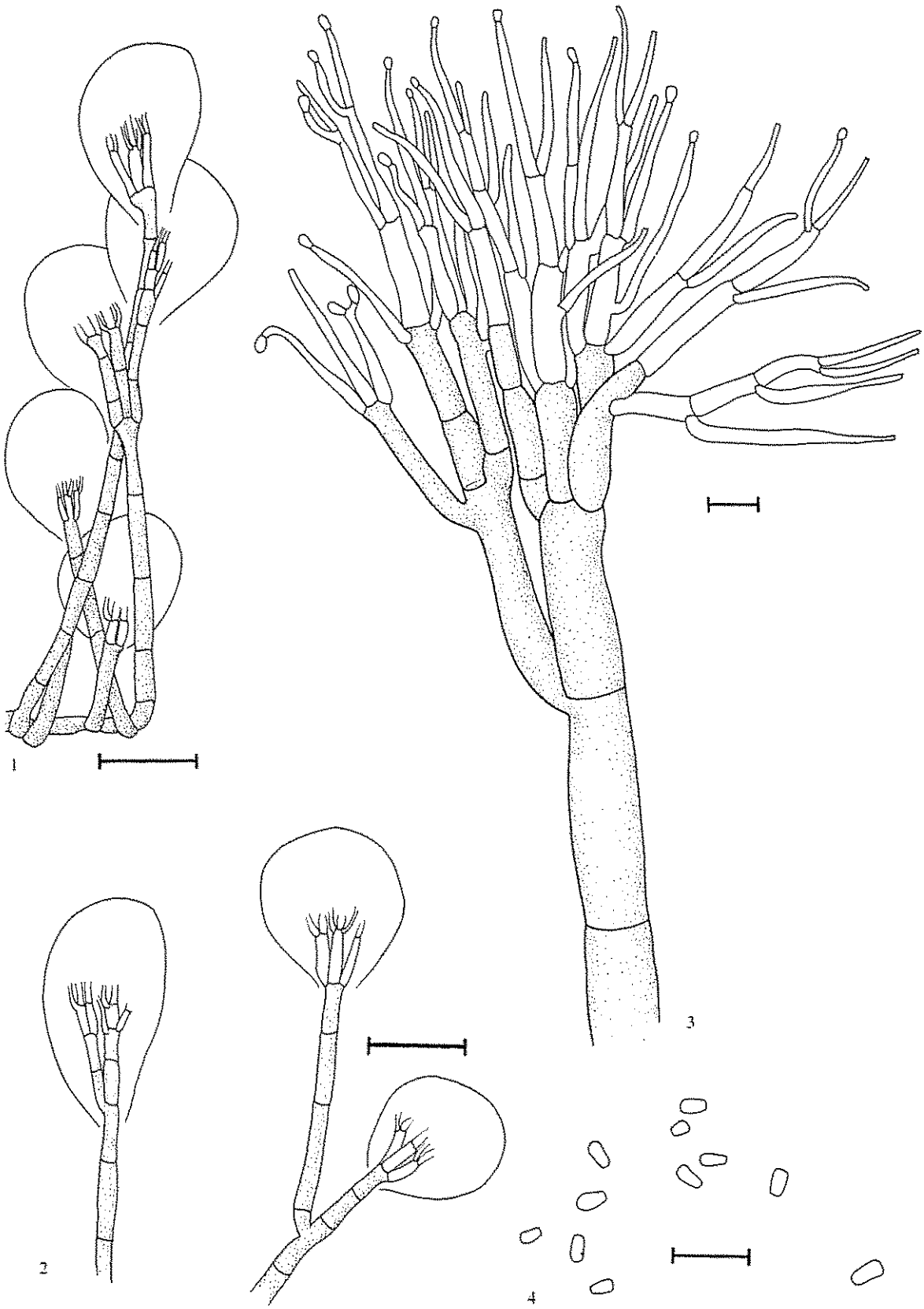
The unidentified *Leptographium* sp., code-named 'GG', has vacuolate spores and has affinities with the fungus identified as *L. penicillatum* f.sp. *palliati* by Mathiesen (1950). The association of this fungus with *H. palliatus* also supports this tentative identification.

The two *Graphium* spp. appear to be undescribed. The genus *Graphium* is used here in the broad sense and encompasses the genera *Graphium*, *Pesotum* Crane & Schoknecht and *Phialographium* Upadhyay & Kendrick (Crane & Schoknecht, 1973). Generic concepts in the *Graphium* complex require revision (Wingfield, 1985) and many species remain undescribed.

At the time of its description, *L. truncatum* was considered to be a species of *Verticicladiella* (Wingfield & Marasas, 1983) and it was compared with existing species in this genus. Isolation of the fungus in this study has prompted us to re-examine it in detail, especially in terms of its occurrence in Europe. The fungus bears a strong resemblance to *L. lundbergii* Lagerberg & Melin, the type species of *Leptographium* that was first collected and described from Sweden (Lagerberg *et al.*, 1927).

We have been unable to find any reference to or source of type material of *L. lundbergii* and comparison of *L. truncatum* with type material has thus been impossible. However, a culture of *L. lundbergii* collected by Melin, an author of the original description of the species, has been obtained from the Centraalbureau voor Schimmelcultures (CBS 352.29). This fungus appears identical to isolates of *L. truncatum* from South Africa and New Zealand. We therefore believe that *L. truncatum* should be reduced to synonymy with *L. lundbergii*. Making such a synonymy and designating new type material must receive careful consideration due to the status of *L. lundbergii* as the type of the genus. We therefore abstain from formal proposal here and will base a final judgement on detailed studies that are currently under way. However, in the interim, illustrations of *L. lundbergii* based on CBS 352.29 are provided (Figs 1–4) to assist in identifications. Dried cultures and slide preparations of this isolate have been deposited in the National Fungus Collection, Pretoria (PREM 50548).

The probable synonomy of *L. lundbergii* and *L. truncatum* would provide answers to various questions concerning the



Figs 1–4. Conidiophores and conidia of *L. lundbergii* drawn from the collection by Melin (CBS 352.29).
Fig. 1. Groups of conidiophores on hyphae. **Fig. 2.** Variation in arrangement of primary metulae on conidiophores. **Fig. 3.** Detailed illustration of branching metulae with terminal conidiogenous cells on a typical conidiophore. **Fig. 4.** Truncate based conidia. Scale bars, Figs 1, 2 = 20 μm, 3, 4 = 10 μm.

biogeography of both fungi. *L. truncatum* was described from South Africa and New Zealand where it was associated with introduced insects of European origin (Wingfield *et al.*, 1988). Occurrence of the same fungus in Europe is, therefore, not surprising although a record from Australia (Webb, 1946) is probably not correct (Kile, pers. comm.). *Leptographium lundbergii* has also previously been reported from Britain associated with *Hylastes ater* (Dowding, 1973) and *Trypodendron lineatum* (Bakshi, 1950). Harrington (1988) has recently confirmed the occurrence of *L. truncatum* in Canada. Assuming that *L. lundbergii* and *L. truncatum* are conspecific, it seems likely that the fungus occurs throughout the boreal region associated with conifer-infesting insects. A record from the United States (Rumbold, 1931) is therefore also likely to be accurate.

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