Three new *Leptographium* species associated with conifer roots in the United States

MICHAEL J. WINGFIELD[1]
Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa

THOMAS C. HARRINGTON
Department of Plant Pathology, Iowa State University, Ames, IA 50011, U.S.A.

AND

PEDRO W. CROUS
Department of Plant Pathology, University of Stellenbosch, Stellenbosch 7600, South Africa

Received July 5, 1993

WINGFIELD, M.J., HARRINGTON, T.C., and CROUS, P.W. 1994. Three new *Leptographium* species associated with conifer roots in the United States. Can. J. Bot. **72**: 227–238.

Leptographium species are most commonly known as anamorphs of *Ophiostoma* and are usually associated with insects that infest trees. Three new species of *Leptographium* were isolated from conifer roots in various parts of the United States. These three species differ from described species both morphologically and on the basis of their allozyme banding patterns. ***Leptographium albopini*** occurs both in the eastern and western United States on white pine hosts, while ***Leptographium douglasii*** occurs commonly in the western United Sates and has been found only on *Pseudotsuga menziesii*. ***Leptrographium neomexicanus*** occurs in the southwestern United States and has thus far only been collected from *Pinus ponderosa*.

Key words: *Leptographium albopini*, *Leptographium douglasii*, *Leptographium neomexicanus*, *Pinus*, *Pseudotsuga*, systematics, root infesting insects.

WINGFIELD, M.J., HARRINGTON, T.C., et CROUS, P.W. 1994. Three new *Leptographium* species associated with conifer roots in the United States. Can. J. Bot. **72** : 227–238.

Les espèces de *Leptographium* sont plus généralement connues par leurs anamorphes du genre *Ophiostoma* et sont généralement associées à des insectes qui vivent dans les arbres. Les auteurs ont isolé trois nouvelles espèces de *Leptographium* à partir de racines de conifères dans diverses parties des États-Unis. Ces trois espèces diffèrent des espèces déjà décrites, à la fois par leurs caractères morphologiques ainsi que par leurs patrons isozymiques. Le ***Leptographium albopini*** se retrouve aussi bien à l'est qu'à l'ouest des États-Unis sur le pin blanc, alors que le ***Leptographium douglasii*** se retrouve dans l'ouest des États-Unis et n'a été récolté que sur le *Pseudotsuga menziesii*. Le ***Leptographium neomexicanum*** se retrouve dans les sud-ouest des États-Unis et n'a été récolté jusqu'ici que sur le *Pinus ponderosa*.

Mots clés : *Leptographium albopini*, *Leptographium douglasii*, *Leptographium neomexicanus*, *Pinus*, *Pseudotsuga*, systématique, insectes colonisateurs des racines.

[Traduit par la rédaction]

Introduction

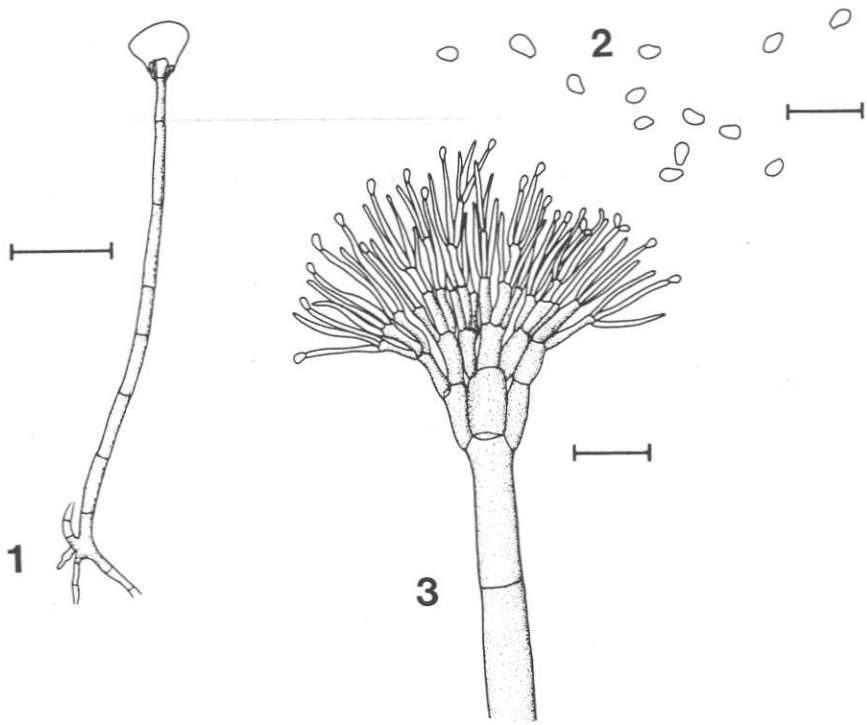
Species of *Leptographium* Lagerberg & Melin are dematiaceous Hyphomycetes characterized by dark, robust mononematous and macronematous conidiophores that terminate in a penicillately branched conidiogenous apparatus. Conidia are hyaline, aseptate, and produced in a slimy matrix through holoblastic extension of conidiogenous cells (Hughes 1953; Kendrick 1961, 1962; Wingfield 1993). Conidiation results in the accumination of slimy conidial masses at the apices of conidiophores to facilitate insect dispersal (Wingfield 1993). Many species of *Leptographium* are anamorphs of *Ophiostoma* Sydow & Sydow (Upadhyay 1981; Wingfield 1985; Harrington 1987, 1988) and therefore are also characterized by being tolerant to high concentrations of cycloheximide in culture (Harrington 1981) and having rhamnose in their cells (Jewell 1974; Weijman and De Hoog 1975; Horner et al. 1986). These fungi are adapted to insect dispersal and commonly associated with scolytid bark beetles (Coleoptera: Scolytidae), many of which infest conifers (De Hoog and Scheffer 1984; Harrington and Cobb 1983; Harrington 1988, 1993*a*, 1993*b*). A number of species have been associated with root diseases of conifers (Hunt and Morrison 1979; Mielke 1981; Harrington and Cobb 1983, 1987; Harrington 1993*b*), and *Leptographium wageneri* (Kendr.) Wingf. is the causal agent of the serious malady commonly known as black stain root disease in the western United States (Harrington 1993*b*).

Although generic circumscription of *Leptographium* is now reasonably well defined, species concepts are still clouded (Harrington 1988). Delimitation of species within the *Leptographium* complex is difficult, as there are only a few taxonomic criteria that can be used, and these may change with prolonged storage and subculturing (Zambino and Harrington 1992). Furthermore, many described species are poorly known. Indeed, even the type species *Leptographium lundbergii* Lag. & Melin lacks type material and requires further clarification (Wingfield and Gibbs 1991).

Harrington (1988) expressed the opinion that based on his collections over an extended time period, many species of *Leptographium* have yet to be described. Indeed, three undescribed species have commonly been collected in the United States in recent years. These three species have been subject to both morphological scrutiny as well as detailed comparisons based on allozyme analyses (Zambino and Harrington 1992) supporting the contention that they are new. The aim of this paper is to provide descriptions and names for these three *Leptographium* spp. associated with conifer roots.

[1]Author to whom all correspondence should be addressed.

Printed in Canada / Imprimé au Canada



FIGS. 1–3. Conidiophores and conidia of *Leptographium neomexicanus*. Fig. 1. Mononematous, macronematous conidiophore with rhizoids at the base. Scale bar = 75 μm. Fig. 2. Oblong to obpyriform conidia. Scale bar = 15 μm. Fig. 3. Conidiogenous apparatus showing series of metulae terminating in conidiogenous cells. Scale bar = 15 μm.

TABLE 1. Comparison of the extent of growth of *L. albopini*, *L. douglasii*, and *L. neomexicanus* after 4 days at 25°C on malt extract agar emended with various concentrations of cycloheximide

	Concn. of cycloheximide (g/L)						
Fungus	0	0.005	0.1	0.5	1.0	2.5	5.0
L. albopini	32.0	28.6	27.8	24.7	21.5	15.4	11.4
L. douglasii	16.6	18.5	15.8	13.5	11.6	8.8	6.6
L. neomexicanus	65.4	62.9	60.6	50.7	47.2	24.9	16.0

NOTE: Measurements (mm) represent averages of colony diameter derived from six readings from three plates.

Materials and methods

Descriptions were based on cultures of the holotypes after growth at 25°C on 2% malt extract agar (MEA) (20 g Merck agar plus 20 g Merck malt extract per 1000 mL water) for approximately 3 weeks.

Growth rates were compared on MEA in Petri dishes after 4 days in the dark at temperatures ranging from 10 to 35°C in 5° intervals. Colony diameters were measured twice perpendicular to each other and growth rates were taken as an average of two readings on each of three Petri dishes.

Growth rates on various concentrations (0, 0.05, 0.1, 0.5, 1.0, 2.5, and 5.0 g/L) of cycloheximide added to 2% MEA were compared. Comparisons were made in Petri dishes at 25°C in the dark. Growth on cycloheximide emended MEA was determined on the basis of colony diameter by taking the average of two measurements from each of three Petri dishes after 4 days of growth.

The three fungi were also examined using scanning electron microscopy to ascertain the mode of conidium development. Sporulating colonies on agar disks were fixed in 2.5% glutaraldehyde and 1.5% osmium tetroxide in a 0.1 M phosphate buffer, dehydrated in a graded acetone series, and critical point dried. Specimens were coated with gold–palladium and examined using a JSM 6400 scanning electron microscope.

New species

Leptographium neomexicanus Wingfield, Harrington et Crous sp.nov. Figs. 1–13

Coloniae primum hyalinae ad margines atro-olivacescentes in MEA dum senescent. Hyphae immersae in medio etsi mycelium sparsum aerium quoque adest, atro-olivaceae parietibus distincte asperis, 2–15 μm diametro. Conidiophora plerumque solitaria sed aliquando bina trinaque aggregata, macronemaosa, mononematosa, rhizoideis plerumque praesentibus bene evolutisque ad basim. Stipes erectus, olivaceus paulum pallescens ad apicem, 205–621 ($\bar{x}$ = 395) μm longus 9–16 ($\bar{x}$ = 13) μm latus ad basem paulumque tumidus ad apicem. Apparatus conidiogenus 50–84 ($\bar{x}$ = 59 μm) longus massa conidica exclusa constans ex tribus usque quinque seriebus ramorum. Tres usque sex rami primae saepe ramus distinctus centralis paulo maiore quam ceteris, pallidiores colore, 11–30.5 ($\bar{x}$ = 14) μm longae, 4–10 ($\bar{x}$ = 6) μm lati. Cellae conidiogenae discretae, hyalinae, attenuatae a basi ad apicem, 12–20.5 ($\bar{x}$ = 14) μm longae, 1.5–3.5 ($\bar{x}$ = 2) μm latae. Auctus conidii constructio parietum ad restituendum ontogenie

FIGS. 4–9. Conidiophores, conidiogenous apparati, conidiogenous cells, and conidia of *Leptographium neomexicanus*. Fig. 4. Mononematous, macronematous condiophores. Scale bar = 70 μm. Fig. 5. Rhizoids at the base of conidiophores. Scale bar = 10 μm. Figs. 6 and 7. Conidiogenous apparati showing arrangement of metulae. Scale bars = 10 μm. Fig. 8. Conidiogenous cells showing apparent sympodial proliferation. Scale bar = 10 μm. Fig. 9. Oblong to obpyriform conidia. Scale bar = 10 μm.