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

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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 States and has been found only on Pseudotsuga menziesii. Leptographium 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.


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’ouest qu’à l’est des États-Unis sur le pin blanc, alors que le Leptographium douglasii se retrouve dans l’ouest des États-Unis et n’a été décrit que sur le Pseudotsuga menziesii. Le Leptographium neomexicanus se retrouve dans les sud-ouest des États-Unis et n’a été décrit que sur le Pinus ponderosa.

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

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 accumulation 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, 1993a, 1993b). A number of species have been associated with root diseases of conifers (Hunt and Morrison 1979; Mielke 1981; Harrington and Cobb 1983, 1987; Harrington 1993b), and Leptographium wagneri (Kendr.) Wingf. is the causal agent of the serious malady commonly known as black stain root disease in the western United States (Harrington 1993b).

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.

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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

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Conc. of cycloheximide (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>L. albopini</td>
<td>32.0</td>
</tr>
<tr>
<td>L. douglasii</td>
<td>16.6</td>
</tr>
<tr>
<td>L. neomexicanus</td>
<td>65.4</td>
</tr>
</tbody>
</table>

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°C 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.

Coloniae primum hyalinae ad margines atro-olivaceous et in MEA dum senescentes. Hyphae immersae in medio eti mycelium sparsum et aerium quoque adest. Atro-olivaceae parietibus distincte asperis, 2–15 μm diametro. Conidiophora plerumque solitaria sed aliquando bina trinaque aggregata, macronematos, mononematos, rizoides plerumque praesentibus bene evolutisque ad basim. Stipes erectus, olivaceus paulum pallescens ad apicem, 205–621 (x = 395) μm longus, 9–16 (x = 13) μm latus ad basem paulumque tumidus ad apicem. Apparatus conidiogenus 50–84 (x = 59 μm) longus massa conidica ex vasa constans ex tubus usque quinque seriebus ramorum. Tres usque sex rami primae saepe ramus distinctus centralis paulo maiore quam ceteris, pallidiores colores, 11–30.5 (x = 14) μm longae, 4–10 (x = 6) μm lati. Cellae conidiogenae discretae, hyalinae, attenuatae a basi ad apicem, 12–20.5 (x = 14) μm longae, 1.5–3.5 (x = 2) μm latae. Auctus conidii constructio parietum ad restituendum ontogenie.
holoblastica percurrentique ontogenie, secessione retardata
dante pseudo-aspectum sympodialis proliferationis. Conidia
hyalina, oblonga vel obpyriformia paulumque cuorvata versus
locum afficiens, 4.5–6.5 (x = 5) μm longa, 2–4.5 (x = 3) μm
lata. Conidia accumulant circa apparatus conidiogenum in
massa mucilaginosa quae cremicorescit dum senescit.

HOLOTYPE: U.S.A., New Mexico, Mescalero, Pini pond-
Colonies on MEA grew optimally at 25°C, reaching 65.5 mm
in diameter in 4 days. Growth rate declined considerably at
20°C, and no growth occurred below 15°C or above 25°C.

The fungus was able to withstand high concentrations of cyclo-
heximide with growth at 25°C reduced by approximately 50%
on MEA containing 2.5 g/L of the antibiotic (Table 1). Coloni-
ies at first hyaline at the margins, becoming dark olivaceous,
23°k (Rayner 1970) on MEA. Hyphae immersed in the
medium, although a sparse aerial mycelium is also present.
dark olivaceous with distinctly roughened walls, 2–15 μm in
diameter. Conidiophores (Figs. 1, 3, 4) usually single but
sometimes in groups of two or three, mononematous,
mononematous with rhizoids usually present and well de-
veloped at the base (Figs. 1, 5). Stipe erect, olivaceous becoming

Figs. 10–13. Conidiophore, conidiogenous cells, and conidia of Leptographium neomexicanus as seen using scanning electron microscopy. Fig. 10. Mononematous, macronematous conidiophore. Scale bar = 9 μm. Fig. 11. Conidiogenous cells with conidia appearing to have
developed sympodially. Scale bar = 5 μm. Fig. 12. Conidiogenous cells showing distinct annellations. Scale bar = 5 μm. Fig. 13. Oblong
to obpyriform conidia. Scale bar = 2.5 μm.
slightly lighter at the apex. 205–621 (x̄ = 395) μm long and 9–16 (x̄ = 13) μm wide at the base and slightly swollen at the apex. Conidiogenous apparatus (Figs. 3, 6–8, 12) 50–84 (x̄ = 59 μm) long (excluding conidial mass) consisting of between three and five series of branches. Three to six primary branches are present, often with a distinct central branch slightly larger than the others, paler in color than the stipe, 11–30.5 (x̄ = 14) μm long, 4–10 (x̄ = 6) μm wide. Conidiogenous cells (Fig. 12) discrete, hyaline, tapering from base to apex, 12–20.5 (x̄ = 14) μm long, 1.5–3.5 (x̄ = 2) μm wide. Conidium development replacement wall building with holo-blastie ontogeny and percurrent ontogeny with delayed secession, giving a false appearance of sympodial proliferation. Conidia (Figs. 2, 9, 13) hyaline, oblong to obpyriform and slightly curved towards the point of attachment, 4.5–6.5 (x̄ = 5) μm long, 2–4.5 (x̄ = 3) μm wide. Conidia accumulating around the conidiogenous apparatus in a clear mucilaginous mass becoming pale yellow when dry.

**Leptographium douglasi** Wingfield, Harrington et Crous sp. nov.

Coloniae primum hyalinae atro-olivacescentes vei nigrescentes dum senescunt in MEA. Margines coloniarum effusae impares. Hyphae serpentineae, atro-olivaceae late ramosae crassis parietibus externis tectis atro exsudato, paene omneo immersae in illo medio, typice aggregatae in catervas inter se conglutinatatas exsudatis parietum. Conidiophora solitaria sed saepe in catervis disparate dispositis in superficie agari, macro-nematosa, mononematosa rhizoides plurumque praesentibus beneque evolutis at basim. Stipes erectus, atro-olivaceus pae-llum pallescens ad apicem, 148–650 (x̄ = 402.5) μm longus 11.5–28.5 (x̄ = 16.5) μm latus ad basim paulum tenuis ad api-cem. Apparatus conidiogenus 46.5–119 (x̄ = 74.5) μm longus massa conidica exclusa, constans ex duabus usque quattuor seriebus ramorum. Usque sex rami principales saepe distincto ramo centrali paulo maiores quam ceteris, pallidiores colore quam stipes, 11–27 (x̄ = 19) μm longi, 5.5–10 (x̄ = 7) μm lati. Cellae conidiogenae discreteae, attenuatae a basi ad apicem, 10.5–24 (x̄ = 16) μm longae, 2–4.5 (x̄ = 3) μm latae mediam ad partem. Auctus conidii constructio parietum ad restituendi holoblastie ontogenie percurrentique ontogenie cum secéssione tardata danti pseudo-aspectum proliferationis sympodiorum. Conidia hyalinae, late ellipsoidea late curvata ad extremum alterum, distincte curvata ad locum afflictionis, 4.5–7 (x̄ = 6) μm longa, 2–4 (x̄ = 3) μm lata ad medium par-
Figs. 23—26. Conidiophore, conidiogenous cells, and conidia of Leptographium douglasii. Fig. 23. Mononematous, macronematous conidiophore subtending branching metulae and conidiogenous cells. Scale bar = 9 μm. Figs. 24 and 25. Conidiogenous cells showing distinct annellations, and conidia appearing to have been produced sympodially owing to delayed secession. Scale bars = 5 μm. Fig. 26. Broadly ellipsoidal conidia. Scale bar = 5 μm.

tem. Conidia accumulant circa apparatum conidiogenum in massa mucilaginosa.


Colonies on MEA grew optimally at 20°C, reaching 22.5 mm in diameter in 4 days. Growth rate dropped consider-
ably at 15 and 25°C, and no growth occurred below 15°C or above 25°C. The fungus was able to withstand high concentrations of cycloheximide with growth at 25°C reduced by approximately 50% on MEA containing 2.5 g/L of the antibiotic (Table 1). Colonies hyaline, becoming dark olivaceous to black, 23⁰k—27⁰m (Rayner 1970) on MEA. Colony

Figs. 17—22. Conidiophores, conidiogenous apparati, conidiogenous cells, and conidia of Leptographium douglasii. Fig. 17. Mononematous, macronematous conidiophores. Scale bar = 70 μm. Figs. 18—20. Conidiogenous apparati showing central primary metulae surrounded by slightly smaller metulae. Scale bars = 10 μm. Fig. 21. Conidiogenous cells giving rise to conidia. Scale bar = 6 μm. Fig. 22. Conidia. Scale bar = 6 μm.
ance of sympodial proliferation. Conidia (Figs. 15, 22, 28) hyaline, broadly ellipsoidal, broadly rounded at one end with distinct taper, curving towards the point of attachment, 4.5–7 (μ = 6) μm long, 2–4 (μ = 3) μm wide at the center. Conidia accumulating around the conidiogenous apparatus in a mucilaginous mass.


Leptographium albopini Wingfield, Harrington et Crous sp. nov.

Figs. 27–39

Colonies primum hyalinae pallido-olivaceae contene colore in MEA. Hyphae immersae in illo medio parietibus distincte asperis crassisque, erectae satis late separatae inter se, 3–12 (μ = 6.5) μm diametro. Nullum mycelium aerium formatum est. Conidiophora solitaria, macronematosa, mononematosa rhizoideis plerumque dense intertextis in nodo. Stipes erectus, pallido-olivaceus paulum pallescens ad apicem, 70–960 (μ = 323) μm longus 8–14 (μ = 10.5) μm latus ad basim paulumque tumidus ad apicem. Apparatus conidiogenus 40–92 (μ = 67) μm longus conidica massa excusa constans ex tribus usque sex plerumque tribus vel quattuor seriesbus ramorum. Primi rami dui usque quattuor sex plerumque dui inter se adjacentes, nullus ramus distinctus centralis, 12–30 (μ = 24) μm longi 5–11 (μ = 7.5) μm lati. Cellae conidiogenae discreteae, hyalinae. attenuatae a basi ad apicem. 9–52 (μ = 20.5) μm longae. 1.5–3 (μ = 2) μm latae. Auctus conidii constructum parietum ad restitutum on too aggregatio holoblastica proliferatationeque percurrenti. secundione reticulatae sano pseudo-aspectum auctus sympholalis. Conidia hyalinae, oblonga apice obtusa, attenuatae distincte versus locum affinicos 3.5–8 (μ = 4.5) μm longa 1.5–4 (μ = 2) μm lata. Conidium accumulant circa apparatum conidiogenum in massa mucilaginosae crenilaclorescence in senescence.


Colonies on MEA grew optimally at 25°C, reaching 33 mm diameter in 4 days. Growth rate was almost the same at 20°C and decreased slightly to 25 mm at 15°C. Minimal growth occurred at 10°C, and no growth was observed above 30°C. The fungus was able to withstand high concentrations of cycloheximide with growth at 25°C reduced by approximately 50% on MEA containing 2.5 g/L of the antibiotic (Table 1). Colonies first hyaline becoming light olivaceous, 23°/°°/°° (Rayner 1970) (as for L. neomexicanus but lighter in color) on MEA. Hyphae immersed in the medium with distinctly roughened margins effuse and uneven. Hyphae serpentine, dark olivaceous, extensively branched with thick outer walls. Covered by a dark exudate; almost entirely immersed in the medium, typically aggregated in groups, cemented together by the wall exudates. Conidiophores (Figs. 14, 16, 17, 23) single but often also in loosely arranged groups on the agar surface, macronematous, mononematous, with rhizoids usually present and well developed at the base. Stipe erect, dark olivaceous, becoming slightly lighter at the apex, 148–650 (μ = 402.5) μm long and 11.5–28.5 (μ = 16.5) μm wide at the base and slightly swollen at the apex. Conidiogenous apparatus (Figs. 16, 18–21, 24) 46.5–119 (μ = 74.5) μm long (excluding conidial mass), consisting of between two and four series of branches. Up to six primary branches present, often with a distinct central branch slightly larger than the others, paler in color than the stipe, 11–27 (μ = 19) μm long, 5.5–10 (μ = 7) μm wide. Conidiogenous cells (Figs. 21, 25) discrete, hyaline, tapering from base to apex, 10.5–24 (μ = 16) μm long, 2–4.5 (μ = 3) μm wide at the middle. Conidium development replacement wall building with holoblastic ontogeny and percurrent ontogeny with delayed secession giving a false appearance of sympodial proliferation. Conidia (Figs. 15, 22, 28) hyaline, broadly ellipsoidal, broadly rounded at one end with distinct taper, curving towards the point of attachment, 4.5–7 (μ = 6) μm long, 2–4 (μ = 3) μm wide at the center. Conidia accumulating around the conidiogenous apparatus in a mucilaginous mass.

and thick walls, straight and reasonably broadly separated from each other. 3–12 (μm = 6.5) μm in diameter. No aerial mycelium formed. Conidiophores (Figs. 27, 29, 30, 36) single, macronematous, mononematous with rhizoids usually densely interwoven in a knot (Figs. 30, 31). Stipe erect, light olivaceous becoming slightly lighter at the apex. 70–960 (μm = 323) μm long and 8–14 (μm = 10.5) μm wide at the base and slightly swollen at the apex. Conidiogenous apparatus (Figs. 29, 32–34) 40–92 (μm = 67) μm long (excluding conidial mass) consisting of between three and six but mostly three or four series of branches. There are usually two to four primary branches, but mostly only two adjacent to each other

without a distinct central branch. 12–30 (μm = 24) μm long and 5–11 (μm = 7.5) μm wide. Conidiogenous cells (Figs. 37, 38) discrete, hyaline, tapering from base to apex. 9–52 (μm = 20.5) μm long, 1.5–3 (μm = 2) μm wide. Conidium development replacement wall building with holoblastic ontogeny and percurrent proliferation with delayed secession giving a false appearance of sympodial development. Conidia (Figs. 28, 35, 38, 39) hyaline, oblong with an obtuse apex and distinctly tapered to the point of attachment. 3.5–8 (μm = 4.5) μm long and 1.5–4 (μm = 2) μm wide. Conidia accumulating around the conidiogenous apparatus in a clear mucilaginous mass becoming cream colored when dry.

Discussion

Leptographium albopini, L. douglasii and L. neomexicanus (referred to as species I, F, and E in Zambino and Harrington 1992) are well defined and based on numerous collections over an extended period of time. All three species are associated with roots of conifers, which we consider to be an important distinguishing characteristic. Furthermore, these species can also be distinguished from other similar Leptographium spp. based on their allozyme banding patterns (Zambino and Harrington 1992).

Leptographium albopini appears to be found on the white pines Pinus strobus and Pinus edulis, and unlike L. douglasii and L. neomexicanus, has been collected both in the eastern and western United States. Superficially, L. albopini resembles Leptographium procumerum (Kendr.) Wingf., which also occurs commonly on Pinus strobus in the eastern United States. It is, however, easily distinguished from L. procumerum primarily by the fact that conidiophores are produced singly and never in groups. Although conidia of L. albopini are more or less the same shape as those of L. procumerum, they tend to be larger in the former species. Mycelium of L. albopini is also typically surrounded by a thick verrucose sheath, which is absent in L. procumerum. Leptographium albopini has also been found to be morphologically similar to Leptographium serpens (Goid.) von Arx (Zambino and Harrington 1992). However, it was found to have narrower conidiogenous cells (Harrington 1988) and manduclating, curved hyphae (Zambino and Harrington 1992), which are different from the serpentine hyphae of L. serpens.

These results were also supported by distinct zymograms of isolates of L. albopini.

Leptographium douglasii, which is apparently restricted to Douglas-fir, occurs commonly in the western United States. It is commonly associated with Hylastes nigrinus (Mann.) (Byler et al. 1983; Harrington and Cobb 1983), a root-feeding bark beetle known to occasionally vector Leptographium wageneri var. pseudosuga Harrington & Cobb (Harrington et al. 1985). Leptographium douglasii was previously confused with Leptographium wageneri var. pseudosuga as both species form serpentine hyphae. Indeed, a culture of this fungus was incorrectly used as L. wageneri in comparisons with L. serpens and L. procumerum in a previous study (Wingfield and Marasas 1980). Although L. douglasii occurs alongside L. wageneri var. pseudosuga in nature, it can easily be distinguished from the latter species. Leptographium douglasii has a more robust conidiogenous apparatus with more primary metulae than that of L. wageneri var. pseudosuga. Conidia are also narrower and longer in L. douglasii and have a distinct curve towards the point of attachment, unlike L. wageneri var. pseudosuga. Also, L. douglasii grows well at 25°C, whereas L. wageneri grows little or not at all at this temperature.

Leptographium neomexicanus was found only in the southwestern United States on Pinus ponderosa Laws. Unlike other Leptographium spp., it produces an abundance of grey aerial mycelium in culture. It is superficially similar to L. douglasii but is easily distinguished from the latter species in having distinctly shorter conidiophores with shorter primary metulae. Although conidia are slightly curved in this species, they are much less so than those of L. douglasii. Leptographium neomexicanus is also considerably faster growing in culture and has a higher optimum temperature for growth than L. douglasii. Finally, hyphae of L. neomexicanus are less serpentine than hyphae of L. douglasii.

Both L. neomexicanus and L. douglasii were shown to be weakly pathogenic to P. ponderosa and Douglas-fir seedlings but much less pathogenic than L. wageneri (Harrington and Cobb 1983). Neither L. neomexicanus nor L. douglasii gave rise to notable lesion development in inoculations, nor were they restricted to the tracheids. They can, therefore, also easily be distinguished from L. wageneri based on their lower virulence and mode of host colonization. The zymograms of L. douglasii and L. neomexicanus were similar to each other and L. wageneri but were distinct from all other Leptographium species compared by Zambino and Harrington (1992), showing them to represent distinct taxa within the L. serpens cluster.

These three Leptographium species can be reasonably distinguished from other described Leptographium species on conifers, but there are many other morphologically similar species that await description. The Leptographium species with small, more or less ellipsoidal conidia are particularly problematic. As with the species described above, allozyme and molecular analyses will help to clearly delineate new taxonomic units for morphological description.


