Part 1

Leptographium species: tree pathogens, insect associates and agents of blue-stain

INTRODUCTION

The genus *Leptographium* is characterized by dark mononematous conidiophores that give rise to a series of branches, terminating in conidiogenous cells in brush-like heads (Kendrick, 1962). The conidiogenous cells produce single-celled, hyaline or faintly pigmented conidia through enteroblastic ontogeny and holoblastic proliferation. Conidia accumulate in slimy masses at the apices of conidiophores, making them ideal for dispersal by insects (Molnar, 1965; Wingfield, 1993a). In association with the insects, some well known species of *Leptographium* have the ability to cause diseases of trees (Grosmann, 1932; Kendrick, 1962; Barras & Perry, 1971a; Harrington & Cobb, 1988). Numerous other species are typically saprophytic or weakly pathogenic and their ecological role remains to be determined (Harrington, 1988).

Leptographium spp. are known to have teleomorphs in *Ophiostoma*. As in the case of *Ophiostoma, Leptographium* spp. are tolerant to high concentrations of the antibiotic cycloheximide and are characterized by the presence of cellulose, rhamnose and chitin in their cell walls (Rosinski & Campana, 1964; Spencer & Gorin, 1971; Weijman & de Hoog, 1975; Marais & Wingfield, 1999a,b). However, in most cases where the teleomorph is known, the anamorph has not been named and only brief reference has been made to its presence. This often leads to taxonomic confusion, as the teleomorph structures are rarely produced in culture, making identification extremely difficult.

Several authors have reviewed the taxonomy of *Leptographium* and its related teleomorph genera, *Ophiostoma* Sydow & P. Sydow, *Ceratocystis* Halst. *sensu lato* and *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. In addition, several keys to selected species of these genera have been published (Hunt, 1956; Kendrick, 1964a; Upadhyay, 1981; Hutchison & Reid, 1988). However, no comprehensive key, to all species of *Leptographium* or *Ophiostoma* with *Leptographium* states exists, which makes this a most difficult group of fungi to treat.

Most descriptions of *Leptographium* spp. are based on living cultures and herbarium material, which may have deteriorated over time. In some taxa holotype material is altogether lacking (Harrington, 1988). The need for a comprehensive monograph reviewing all the known species of *Leptographium,* and a key to species in this genus is long overdue (Harrington & Cobb, 1988; Harrington, 1988; Wingfield, Capretti & Mackenzie, 1988; Wingfield, 1993a). During the past 20 years, M.J. Wingfield has actively collected and preserved *Leptographium* spp. from a wide variety of sources. These collections form the basis of this study. My aim has been to provide a comprehensive key to all known *Leptographium* spp., or *Ophiostoma* spp. with *Leptographium* states. I have also attempted to support this with detailed descriptions, as well as with photographs and line drawings for all species.

TAXONOMY

Anamorph genera similar to or synonymous with *Leptographium*

Scopularia Preuss

The first anamorph genus associated with the taxonomic history of *Leptographium* is *Scopularia,* based on the single species, *S. venusta* Preuss. The vague description of this genus provided by Preuss (1851), was amended and redescribed by Saccardo (1886) and later again by Lindau (1907). The original illustration by Preuss was, however, the source of considerable confusion and the accuracy of his description was placed in doubt by Saccardo (1886). In addition, the type specimen of *S. venusta* was lost, making comparative studies and verification of characters reported for this genus, impossible (Kendrick, 1964b). In a study of fungi causing blue-stain of timber, Lagerberg, Lundberg and Melin (1927) found that some of their isolates resembled the characters reported for *Scopularia.* However, these could not be verified as a result of the lost type specimen. This led to the establishment of *Leptographium* in 1927, based on the single species, *L. lundbergii* (Lagerberg *et al.*, 1927).

Goidanich (1936) argued against the use of *Leptographium* in place of *Scopularia,*

although the former name was used by most authors at that time. He consequently transferred several species described in *Leptographium* to *Scopularia* (Goidanich, 1936). Shaw and Hubert (1952) reviewed the nomenclature of these related genera and found that *Scopularia* Preuss was a later homonym of *Scopularia* Lindley and was, therefore, invalid. *Leptographium* was thus accepted as the valid name for this genus. Rediscovery of the type material of *Scopularia* led Kendrick (1964b) to conclude that *Scopularia* could have been a synonym of *Leptographium.* The state of the material was, however, poor and it was impossible to make any definite conclusions in this regard (Kendrick, 1964b).

Hantzschia Auersw.

Grosmann (1932) regarded the genus *Scopularia* unsuitable for a new species found on spruce in Europe and concluded that the undescribed species would best reside in *Hantzschia* (Kendrick, 1964b). The genus *Hantzschia* was established in 1862 for a single species, *H. phycomyces* Auersw. (Kendrick, 1964b). However, Grosmann (1932) reduced *Hantzschia* to synonymy with *Leptographium* and retained the latter name because the description for *Hantzschia,* as in the case of *Scopularia,* was unclear and insufficient for taxonomic purposes. *Hantzschia phycomyces* subsequently became *L. phycomyces* (Auersw.) Grosmann. Shaw and Hubert (1952) also declared *Hantzschia* invalid based on the existence of an earlier described algal genus, *Hantzschia* Grunow. Hughes (1953) distinguished *Hantzschia* and *Leptographium* based on their different modes of conidium development, phialidic in the case of *Hantzschia* and annelidic in the case of *Leptographium. Leptographium phycomyces,* was later transferred to a new genus, *Phialocephala* W.B. Kendr. based on the phialidic production of conidia (Kendrick, 1964a).

Phialocephala Kendrick

Phialocephala was established for species producing conidia in phialides with periclinal thickening and prominent collarettes (Kendrick, 1961; 1963a). The type

species was described as *P. dimorphospora* W.B. Kendr., based on its well differentiated conidiophores and unmistakable phialides. The generic description was subsequently amended by Crane (1971) to include species that are once or twice branched at the stipe, while Onofri and Zucconi (1984) included species with conidiogenous cells originating directly from the stipe. Several additional species have been added to the genus (Kendrick, 1961; 1963a,b, 1964a; Crane, 1971; Jong & Davis, 1972; Sivasithamparam, 1975; Onofri & Zucconi, 1984, Siegfried, Seifert & Bilmer, 1992), which now deserves revision.

In contrast to *Leptographium* spp., which occur mainly on coniferous hosts, the habitat of *Phialocephala* is usually decaying wood and bark or processed timber and living trees (Kendrick, 1961). No definite relationship with bark beetles has been established and no connection to any teleomorph genus has been found (Harrington, 1988). This is also in contrast to *Leptographium* spp. that have a definite and unique relationship with insects (Solheim, 1986; Harrington, 1988; Perry, 1991; Malloch and Blackwell, 1993; Harrington, 1993; Krokene & Solheim, 1996) and have teleomorphs in *Ophiostoma* (Grosmann, 1932; Harrington, 1987; Wingfield, 1993a; Van der Westhuizen *et al.*, 1995; Jacobs *et al.*, 1997).

Wingfield, Van Wyk and Wingfield (1987) questioned the placement of anamorphs of *Ophiostoma* in *Phialocephala*. After a study of various species of *Phialocephala*, they concluded that the anamorphs of *Ophiostoma* with *Leptographium*-like conidiophores would best be accommodated in *Leptographium* and not *Phialocephala*. Harrington (1988) supported the exclusion of *Phialocephala* from the anamorphs of *Ophiostoma*. These findings were further supported by Mouton, Wingfield and Van Wyk (1992) who found that closely packed annelations at the apices of conidiogenous cells cannot be seen with the light microscope. These annelations lead to the impression that conidia are produced at the same level, without percurrent proliferation, giving the false interpretation of phialides, when viewed with the light microscope. Based on these findings, they suggested that the only proposed *Phialocephala* anamorph in *Ophiostoma*, i.e. *O. francke-grosmanniae*, should reside in *Leptographium*.

Wingfield *et al.* (1987) found that the genus *Phialocephala* could be divided into two groups based on the mode of conidium development. Species displaying

replacement wall building (Minter, Kirk & Sutton, 1983) remained in *Phialocephala*, whereas those with ring wall building (Minter *et al.*, 1983) were accommodated in *Sporendocladiella* G. Arnaud, Nag Raj & W.B. Kendr. Although *Phialocephala* is now more clearly defined than it was in the past, it remains in need of closer investigation (Wingfield, 1993a).

Verticicladiella S. Hughes

The genus *Verticicladiella* was separated from *Leptographium* based on different modes of conidium development. *Verticicladiella*, together with its type *V. abietina* (Peck) S. Hughes, was established by Hughes (1953) to accommodate species that produce conidia sympodially. Kendrick (1962) provided a re-description for this genus and its type, and transferred several species from *Leptographium* to *Verticicladiella.* Several new species were also described in the genus (Kendrick, 1962).

The separation of *Verticicladiella* and *Leptographium* was not universally accepted. Jooste (1978) commented on the conidiogenesis of *V. abietina* in a study undertaken to compare conidiogenesis of certain species in *Verticicladiella* and *Leptographium*. He noted the delayed secession of conidia observed in species of *Verticicladiella,* as well as annelations characteristic of *Leptographium,* and suggested that further studies would be needed to clarify these discrepancies. Wingfield (1985), after a thorough electron microscope study of many species residing in the two genera, reduced *Verticicladiella* to synonymy with *Leptographium.* This synonymy was based on the fact that species in the two genera were indistinguishable under the light microscope. Scanning electron microscopy revealed that species in both *Leptographium* and *Verticicladiella* displayed annelidic as well as sympodial conidiogenesis. Their findings were confirmed by Van Wyk and Wingfield (1987) and Van Wyk, Wingfield and Marasas (1987) who showed that delayed secession of the conidia, developing percurrently, can lead to a false impression of sympodial development when viewed under the light microscope (Fig. 1). This synonymy was also supported by Harrington (1988), in his review of species in *Leptographium.*

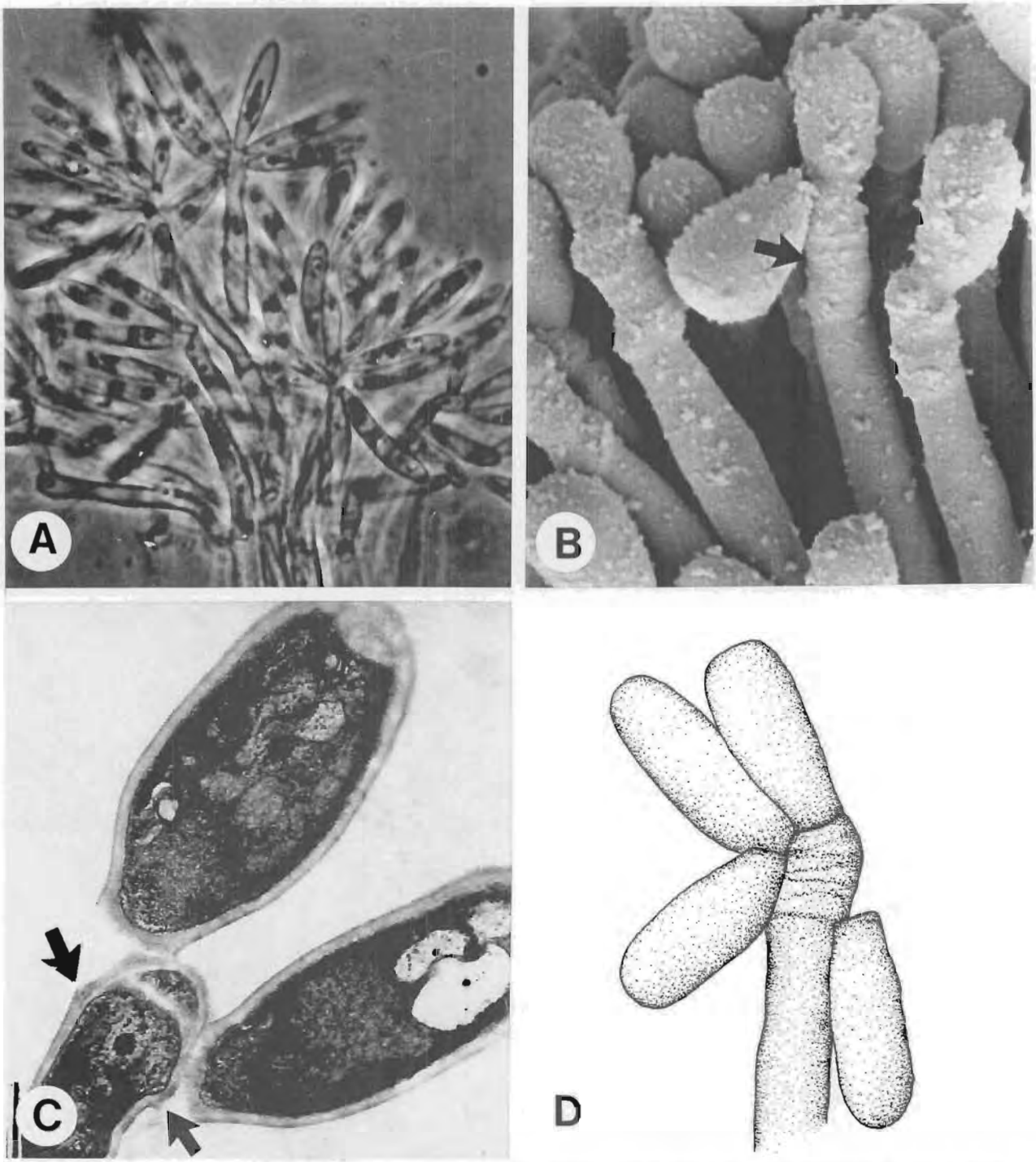

Fig. 1. Conidiogenesis in *Leptographium*. **A.** Light micrograph showing conidiogenous cells and conidia that appear to develop sympodially. **B.** Scanning electron micrograph showing percurrent proliferation of conidiogenous cells. Note the distinct annelations (arrows) and the fact that delayed secession gives a false impression of sympodial conidium development. **C.** Transmission electron micrograph showing annelations (arrows) at the apex of conidiogenous cells. **D.** Schematic representation of conidium development in *Leptographium* spp.

Teleomorphic genera associated with *Leptographium*

Some *Leptographium* spp. have described teleomorphs in *Ophiostoma.* Grosmann (1931, 1932) described the first species of *Leptographium* associated with *Ceratostomella* Saccardo, which was later reduced to synonymy with *Ophiostoma* (Von Arx, 1952). The history of *Ophiostoma* is characterized by several name changes that can be traced back to the early part of the 20th Century. A few years after Grosmann's description of *L. penicillatum,* Goidanich (1936) described the teleomorph genus *Grosmannia* Goid. for all the *Leptographium* species that had been associated with teleomorphs. *Endoconidiophora* had been established for species with *Chalara* - like anamorphs (Samuels, 1993). Von Arx (1952), however, reduced *Grosmannia, Endoconidiophora* Münch and *Ceratostomella* to synonymy with *Ophiostoma,* and transferred all species to that genus. Parker (1957a) described *Europhium* A.K. Parker for one species of *Leptographium, L. trinacriforme,* with a cleistothecial-like teleomorph that lacked the typical long necks of *Ophiostoma* (Parker, 1957a). Robinson-Jeffrey and Davidson (1968) described a further three species in this genus. All of these species were later transferred to *Ophiostoma* (Harrington, 1987).

Ceratocystis is another important genus that has been associated with species of *Leptographium.* There are many similarities between *Ophiostoma* and *Ceratocystis.* Most notable are the long necks of the ascomata and a close association with insects. These similarities have led to considerable debate as to the validity of the genera. This debate has now been resolved and the two genera are widely accepted as being phylogenetically unrelated (Hausner, Reid & Klassen, 1993a, Spatafora & Blackwell, 1994). Thus, *Ceratocystis* can be distinguished from *Ophiostoma* based on its *Chalara* (Corda) Rabenh. anamorphs (Ellis & Halsted, 1890; De Hoog & Scheffer, 1984), intolerance to the antibiotic cycloheximide (Fergus 1956; Harrington, 1981; Marais & Wingfield, 1999b), absence of cellulose, chitin and rhamnose in its cell walls (Smith, Patik & Rosinski, 1967; Spencer & Gorin, 1971; Jewell, 1974; Weijman & de Hoog, 1975; Marais & Wingfield, 1999a) and differences in ascospore development and morphology (Van Wyk & Wingfield, 1990; 1991; Van Wyk, Wingfield & Van Wyk, 1991). In contrast, species of *Ophiostoma* are characterized by anamorphs other than *Chalara* (De Hoog &

Scheffer, 1984). These include *Leptographium, Graphium, Sporothrix* and *Hyalorhinocladiella* (Harrington, 1988; Wingfield, 1993a, Seifert & Okada, 1993; De Hoog, 1993; Mouton, Wingfield & Van Wyk, 1994). *Ophiostoma* spp. are also characterized by a marked resistance to high concentrations of cycloheximide (Fergus, 1956; Hicks, 1973; Harrington, 1988; Marais & Wingfield, 1999b) and the presence of cellulose, chitin and rhamnose in their cell walls (Rosinski & Campana, 1964; Smith, Patik & Rosinski, 1967; Spencer & Gorin, 1971; Jewell, 1974; Weijman & de Hoog, 1975).

The separation of *Ceratocystis* and *Ophiostoma* was debated for many decades. Hunt (1956) considered *Ophiostoma* and *Ceratocystis* to be synonyms and supported the synonymy of *Grosmannia* with *Ophiostoma.* He, however, divided *Ceratocystis* into two groups based on the mode of conidium development of their anamorphs, namely exoconidia (*Leptographium* - like) and endoconidia (*Chalara* - like). This synonymy was supported by Olchowecki and Reid (1974) who placed all species of *Ophiostoma* including those with *Leptographium* anamorphs in *Ceratocystis.* They further divided *Ceratocystis* into four groups based on ascospore shape. Other than being a convenient arrangement of taxa, this situation did not provide an indication of the natural division of species in the genus (Harrington, 1988).

De Hoog (1974) divided *Ceratocystis sensu lato* into *Ophiostoma* and *Ceratocystis sensu stricto.* This separation was based on two distinct anamorph groups (those with exoconidia and those with endoconidia), previously noted by Hunt (1956). Weijman and de Hoog (1975), as well as Samuels and Müller (1978) distinguished between *Ceratocystis* and *Ophiostoma* based on cell wall composition as well as conidium development. In his monograph, Upadhyay (1981) disregarded the separation of *Ceratocystis* and *Ophiostoma* proposed by De Hoog (1974), Weijman and De Hoog, (1975) and Samuels and Müller (1978), and treated all species in these genera as either *Ceratocystis* or *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. Thus, *Leptographium* species were again treated as anamorphs of *Ceratocystis.*

Ceratocystis s.l. was once again split by De Hoog and Scheffer (1984) based on the two different anamorph groups. Species with anamorphs other than *Chalara* were

moved to *Ophiostoma*. Following the same trend a further 11 species with *Leptographium* anamorphs were later transferred to *Ophiostoma* by Harrington (1987). He also suggested that *Leptographium* spp. with a tolerance to cycloheximide implies a strong affinity to *Ophiostoma* (Harrington, 1988).

Ceratocystiopsis was described by Upadhyay and Kendrick (1975) for species with falcate ascospores. Although De Hoog and Scheffer (1984) considered *Ceratocystiopsis* to be a well-defined genus, Wingfield (1988; 1993b) proposed a reconsideration of *Ceratocystiopsis* because this genus is separated from *Ophiostoma* and *Ceratocystis,* based solely on the shape of the ascospores. *Ceratocystiopsis crassivaginata* (H.D. Griffin) H.P. Upadh., was the only species in this genus with a *Leptographium* anamorph and it was consequently transferred to *Ophiostoma* as *O. crassivaginatum* (H.D. Griffin) T.C. Harr. (Harrington, 1987).

Hausner, Reid and Klassen (1993b) compared *Ophiostoma, Ceratocystis* and *Ceratocystiopsis* at the molecular level and concluded that *Ceratocystiopsis* and *Ophiostoma* should be synonomised. Most species previously treated in *Ceratocystiopsis,* were moved to *Ophiostoma*. Currently, and as a result of the above-mentioned studies, all *Leptographium* spp. with known teleomorphs are found in *Ophiostoma*. Studies at the molecular level have provided strong support for the fact that *Ophiostoma* and *Ceratocystis* are distinct and phylogenetically unrelated (Hausner *et al.,* 1993a; Samuels, 1993; Spatafora & Blackwell, 1994; Wingfield *et al.,* 1994; Samuels & Seifert, 1995; Wingfield *et al.,* 1996; Wingfield, Viljoen & Wingfield, 1999).

Ceratocystiopsis is generally treated as a synonym of *Ophiostoma* (Wingfield, 1988; Wingfield, 1993b; Hausner *et al.,* 1993a, b). Two species of *Ceratocystiopsis, C. falcata* and *C. proteae,* were not transferred to *Ophiostoma* by Hausner (1993b). Subsequent studies have treated these species and *C. falcata* now resides in the monotypic genus *Cornuvesica* Viljoen, Wingfield & Jacobs, as *Cornuvesica falcata* Viljoen, Wingfield & Jacobs (Viljoen *et al.,* 1999). *Ceratocystiopsis proteae* resides in *Gondwannamyces* Marais & M.J. Wingf. as *G. proteae* (M.J. Wingf., P.S. van Wyk & Marasas) Marais & M.J. Wingf., together with *G. capensis* (M.J. Wingf. & P.S. van Wyk) Marais & M.J. Wingf. (Marais *et al.,* 1998). It has been suggested that *Ophiostoma* could represent a number of well defined genera, possibly

separated by different ascospore forms, although this has yet to be clearly shown (Wingfield, Viljoen & Wingfield, 1999).

The teleomorph structures of *Ophiostoma* spp. with *Leptographium* states are characterized by small, hyaline ascospores and evanescent asci (Fig. 2). In all cases the ascospores are surrounded by a gelatinous sheath. This is in contrast to certain other *Ophiostoma* spp. that are characterized by ascospores without sheaths (Van Wyk, Wingfield & Van Wyk, 1993). The ascocarps are darkly pigmented with, in most cases, well-developed necks and ostioles. Sticky ascospores accumulate at the apices of the necks, and are well adapted for insect dispersal (Harrington, 1988; Malloch & Blackwell, 1993). Although this similarity in morphology can lead to the impression that *Ophiostoma* and *Ceratocystis* are closely related, this might not be the case at all. These similarities are most probably the result of adaptation to their habitat, which in most cases constitutes the tunnels of insects formed in the inner bark of trees (Lagerberg *et al.*, 1927; Craighead, 1928), and convergent evolution (Wingfield, 1993a).

In a review of *Leptographium* spp., Harrington (1988) listed 20 species of *Ophiostoma* with *Leptographium* anamorphs. Since then several additional species have been described. Many species of *Leptographium* are not associated with a teleomorph or alternatively, the teleomorph has been seen seldom or only once, as in the case of *Ophiostoma wageneri* (Goheen & Cobb) Harrington (Goheen & Cobb, 1978). In such cases, the anamorph might be considered as the holomorph (Wingfield, 1993a). Harrington (1988) suggested that in species of *Ophiostoma* with *Leptographium* anamorphs, a name for the anamorph is unnecessary and that the teleomorph name should preferably be used. This can, however, lead to confusion, as in most cases, the teleomorph is not readily formed in culture. This confusion is compounded where mycologists rely on published names and descriptions for identification. For the purpose of this study, we have chosen to provide names for *Leptographium* states of the small number of *Ophiostoma* spp. where such names have not been provided previously. Although we fully recognized the arguments for not doing so, we believe that this group is exceptional, in that a very small number of species have not been treated in this way. We also believe that this will simplify the task of pathologists who are unlikely to ever see a teleomorph in most of these species.

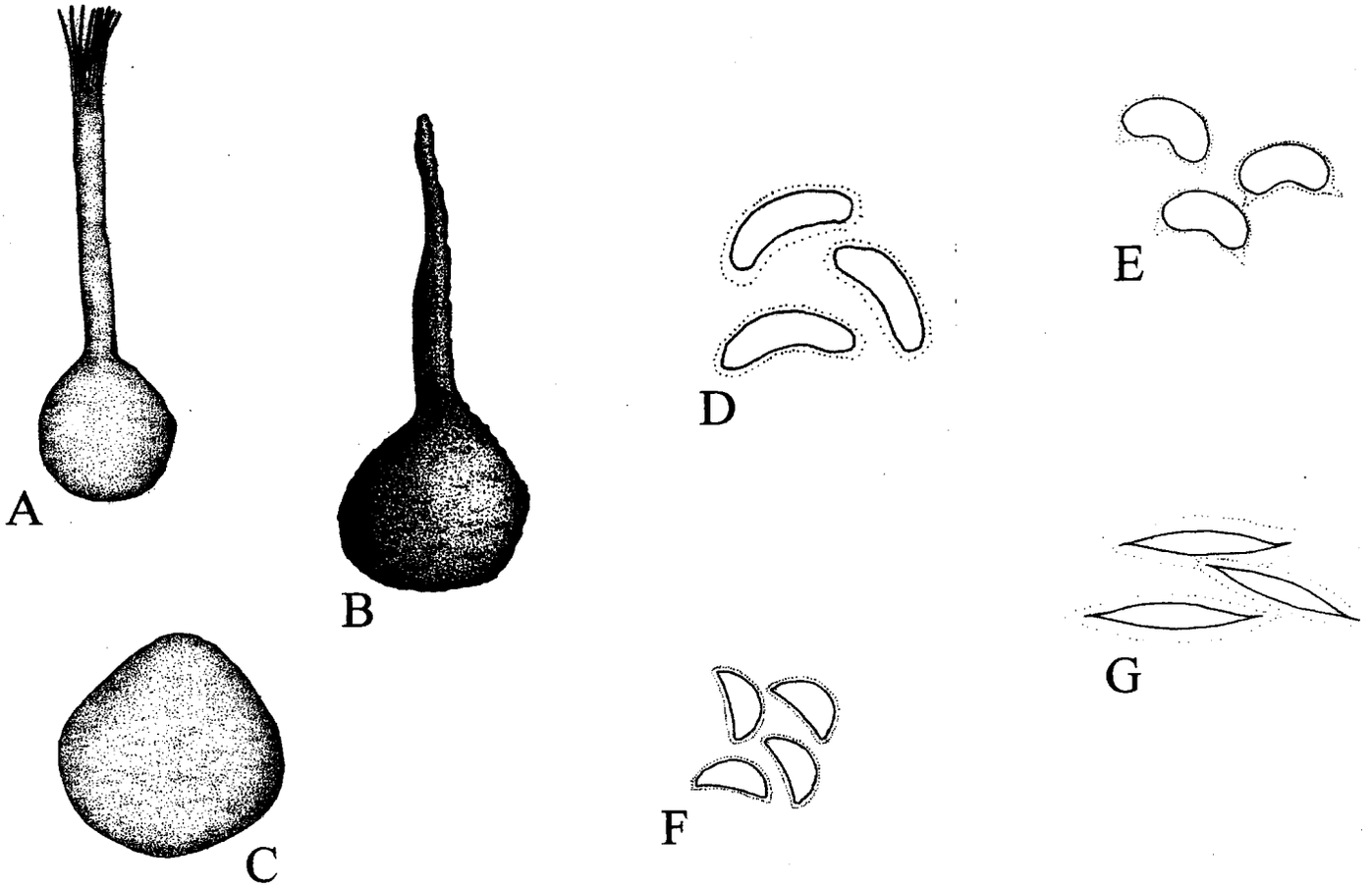

Fig. 2. Teleomorph structures associated with *Leptographium* spp. Perithecia can be with (A,B) or without (C) necks. Ostiolar hyphae can be present (A) or absent (B). Ascospores can be allantoid (D), cucullate (E), orange-section shaped (F) or elongate (G).

DISEASES ASSOCIATED WITH SPECIES OF *LEPTOGRAPHIUM*

Some species in *Leptographium* are associated with serious diseases of trees that cause devastation in forests, resulting in major economic losses (Harrington & Cobb, 1988; Solheim, 1992a,b; Wingfield, Seifert & Webber, 1993). The best known of these are certainly the three varieties of *Leptographium wageneri* that are responsible for black stain root disease (BSRD) of conifers in the North Western United States (Wagener & Mielke, 1961; Cobb, Lawson & Popenuck, 1987; Cobb, 1988; Harrington, 1993). Other species considered to play an important role in disease are *L. procerum*, associated with a root disease of pines, *L. serpens,* associated with pine disease in Italy and South Africa (Wingfield & Marasas, 1980; 1981), *L. terebrantis,* that is known to cause extensive lesions on pines (Wingfield,

1986) and *L. calophylli,* associated with the wilt of the takamaka tree (*Calophyllum inophyllum*) in Mauritius and the Seychelles (Wiehe, 1949; Webber *et al.,* 1999). Most *Leptographium* spp. are, however, best known for their association with blue-stain of sapwood in conifers.

While species of *Leptographium* might have been isolated from diseased trees, their role in causing disease is often unknown (Kulhavy, Chako & Partridge, 1978). The disease complexes in which these fungi are involved, usually include the fungus, the host, which in most cases would be a coniferous tree, and in certain cases insects. Most species of *Leptographium* are, however, non-pathogenic and are probably saprotrophic (Harrington, 1988; Wingfield *et al.,* 1988). Results obtained from wound inoculation studies should also be interpreted with care, as these fungi have extremely complex relationships with insects and the development of lesions need not necessarily imply a primary role in disease (Harrington, 1988; Wingfield *et al.,* 1988). At this stage, only *L. wageneri* and *L. calophylli* are considered to be true primary pathogens. The role of *L. procerum* and *L. serpens* as pathogens is still debated (Wingfield *et al.*, 1988).

Black-stain root disease

Leptographium wageneri is responsible for a disease known as black stain root disease (BSRD). This disease was first recorded in 1939 on *Pinus* spp. in California (Wagener & Mielke, 1961), but was later also described from other conifers (Harrington & Cobb, 1987). Wagener and Mielke (1961) first described the symptoms and factors associated with the disease. Kendrick (1962) provided the name *Verticicladiella wageneri* Kendrick for the causal agent of BSRD. Although several species of *Leptographium* have been isolated from trees showing symptoms of BSRD, Harrington and Cobb (1983) showed conclusively that the disease is caused by the single species, *Leptographium wageneri.* The role of the fungus had probably been overlooked for a considerable time because of the presence of bark beetles in diseased trees and the fact that people attributed tree death to insect infestation (Cobb, 1988). Other *Leptographium* spp. were frequently isolated from trees with BSRD, but these are probably only secondary invaders (Partridge & Bertagnole, 1980).

BSRD is restricted to the western United States (Walters & Walters, 1977; Harrington, 1982; Cobb, 1988). It was found to spread rapidly, and is capable of causing extensive losses in forests (Byler, Cobb & Rowney, 1979; Cobb *et al.*, 1982; Cobb, 1988). Economic impacts are not restricted only to direct losses such as reduced growth and death. Indirect losses also occur through the build-up of populations of secondary fungal pathogens and insects (Smith, 1974). BSRD is also of particular importance since it is capable of killing European conifers, and could be a serious threat to forests of Britain and Europe, if it were to be introduced into that part of the world (Webber & Hansen, 1990).

BSRD occurs on trees of all ages and predisposes the host to further attacks by bark beetles (Helms, Cobb & Whitney, 1971; Morrison & Hunt, 1988). Although BSRD has been grouped with the major root pathogens, it also displays symptoms characteristic of vascular wilt pathogens on hardwoods (Leaphart, 1960; Smith, 1967; Harrington, 1982). These include the fact that it is restricted to the xylem, and the fact that it spreads specifically in the vascular system of trees (Smith, 1967; Goheen & Cobb, 1978; Harrington, 1982; Hessburg & Hansen, 1982; Cobb *et al.*, 1984; Bertagnole, Partridge & LeTourneau, 1987).

The host specificity of strains of *L. wageneri* has been noted by various researchers (Wagener & Mielke, 1961; Smith, 1967; Harrington, 1982; Cobb *et al.*, 1984; Harrington & Cobb, 1984; Cobb, Lawson & Popenuck, 1987). Three varieties of this fungus are currently known and these are referred to as *L. wageneri* var. *wageneri* occurring on pinyon pines (*Pinus monophylla; P. edulis*) (Kendrick, 1962; Harrington, 1993), *L. wageneri* var. *pseudotsuga* occurring on douglas-fir (*Pseudotsuga menziesii*) (Cobb & Platt, 1967; Harrington & Cobb, 1987; Harrington, 1993) and *L. wageneri* var. *ponderosum* occurring on hard pines (*P. ponderosa, P. contorta, P. jeffreyi*) (Harrington & Cobb, 1987; Harrington, 1993). These varieties can be distinguished based on various characters such as morphology (Harrington, 1982), differences in virulence (Otrosina, Cobb & Popenuck, 1987), isozymes (Otrosina, 1986; Otrosina & Cobb, 1987; Zambino & Harrington, 1987; Zambino, Harrington & O'Malley, 1987; Zambino & Harrington, 1989), Random Amplified Polymorphic DNA markers (RAPD's) (Witthuhn *et al.*, 1997) and ribosomal DNA sequences (Jacobs *et al.*, unpublished).

All three varieties of *L. wageneri* are able to infect tree species other than those from which they were isolated, but this characteristic is rare in nature (Cobb, & Platt, 1967; Harrington & Cobb, 1984; Diamandis, Epstein & Cobb, 1987). This can be attributed to several factors, including symptoms that might not be expressed on certain hosts, feeding activities of insects that carry the fungi, and the fact that seedlings used in the pathogenicity tests might not have displayed the resistance expressed in older trees (Harrington & Cobb, 1984). Zambino & Harrington (1989) suggested that the host specialization and designation of three varieties of *L. wageneri* is possibly the result of limited recombination, or the lack thereof, in nature. This conclusion is based on the fact that there is no or very limited sexual recombination in the natural populations of *L. wageneri* (Goheen, 1976; Goheen & Cobb, 1978; Zambino & Harrington, 1989).

Goheen and Cobb (1978) described *Ceratocystis wageneri* as the teleomorph of *L. wageneri.* This state has never been seen again and it is possible that teleomorph structures were not appropriately linked to *L. wageneri.* Zambino and Harrington (1989, 1990) found a low level in gene diversity, suggesting a low level of recombination amongst isolates of the three varieties. Population studies of this species indicate that the three varieties of *L. wageneri* represent homogenous populations with essentially asexual reproduction (Zambino & Harrington, 1990). The presence of a teleomorph in nature, thus, seems unlikely.

Symptoms associated with BSRD include reduced leader and branch growth, chlorosis, reduced needle size, needle retention and resinous lesions on the lower stems (Leaphart, 1960; Hunt & Morrison, 1980; Witcosky, 1981) (Fig. 3). Other symptoms are severe needle chlorosis, needle cast and a pronounced reduction in height growth (Cobb & Platt, 1967; Lawson & Cobb, 1987a). Infected trees appear to form more heartwood than uninfected trees, which reduces water conduction (Lawson & Cobb, 1987b). The pathogen causes severe reduction in photosynthesis and transpiration as a result of water stress and stomatal closure (Helms *et al.*, 1971), which is most probably the result of phytotoxins (Cobb, 1988).

The stain resulting from infection by *L. wageneri* is streaky and occurs in the tracheids (Wagener & Mielke, 1961), extending from the roots upwards in the tree (Cobb, 1988) (Fig. 4). This is the most characteristic symptom of the disease

(Cobb, 1988). The difference between this staining pattern and that of blue-stain, is the result of the hyphae that are located only in the tracheids (Goheen & Cobb, 1978; Harrington, 1982; Hessburg & Hansen, 1982; Cobb *et al.*, 1984; Bertagnole *et al.*, 1987), and not in the parenchyma as is the case of typical blue-stain fungi (Cobb, 1988). This results in the black streaked patterns associated with this disease, in contrast to the wedge-shaped staining patterns associated with blue-staining organisms (Wagener & Mielke, 1961; Cobb, 1988) (Fig. 4).

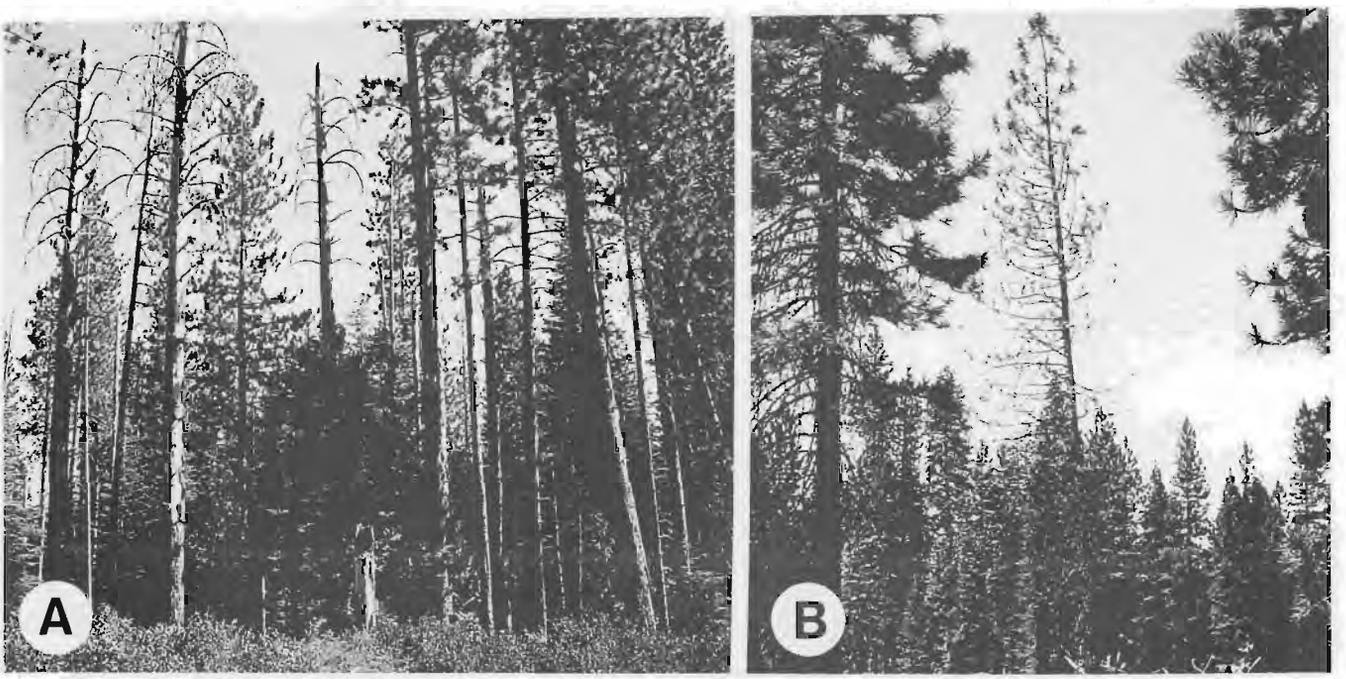

Fig. 3. Symptoms associated with BSRD in *Pinus ponderosa*. **A.** Dying tree in an infection centre. **B.** Dying tree showing distinct crown thinning (Photos taken by Fields W. Cobb).

The invasion of the tracheids by *L. wageneri* leads to a decrease in sap flow, which ultimately results in tree death (Hessburg & Hansen, 1987). Resinosis appears on the outer surface of pine roots, but this is more apparent in douglas-fir than in other conifers. Foliar symptoms can be seen in some cases, but bark beetles usually kill the trees before these symptoms appear (Cobb, 1988). In douglas-fir, the symptoms are generally similar to those found in pine. Growth of trees is reduced for 2-3 years before death, the crown thins and the foliage becomes chlorotic (Hansen *et al*, 1988) (Fig. 3).

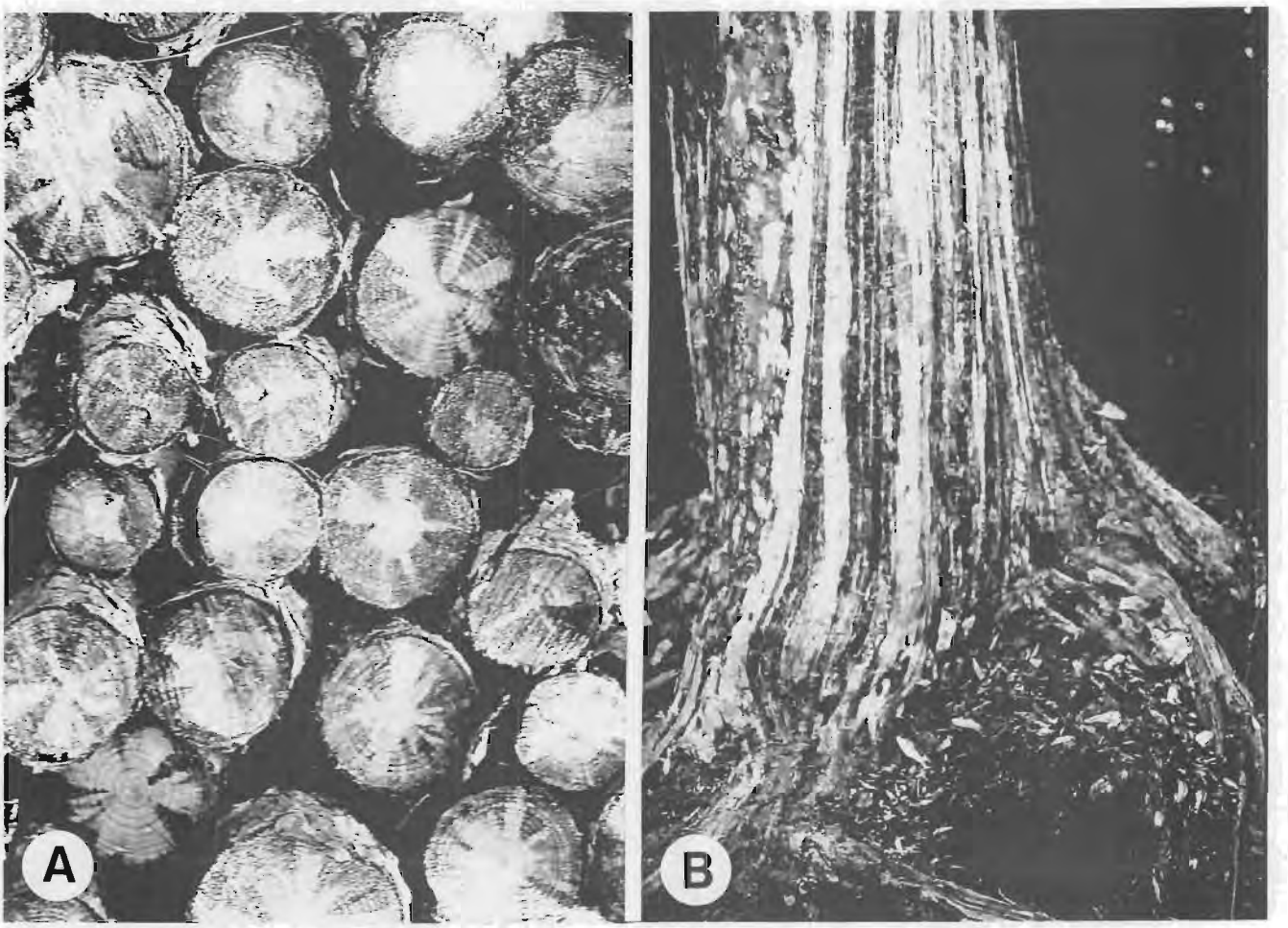

Fig. 4. Patterns of wood colonization associated with *Leptographium* spp. **A.** Pie-shaped lesions associated with most *Leptographium* spp. and other blue stain fungi that colonize both tracheids and ray parenchyma. **B.** Typical staining pattern associated with infection in *L. wageneri*, where the fungus is restricted to tracheids and does not colonize parenchyma (photographs supplied by Fields W. Cobb).

Reports of the mode of infection of *L. wageneri* are conflicting, possibly as a result of different hosts and environmental conditions that are associated with this disease. On the one hand, *L. wageneri* has been found to be able to infect healthy trees in the absence of traumatic wounds (Cobb, 1988). In contrast, the fungus was found to be able to colonize only non-living tracheids and was never found to infect living tissue (Hansen *et al.*, 1988).

Infection by *L. wageneri* occurs through the roots (Cobb & Platt, 1967; Smith,

1967), and because *L. wageneri* is unable to break down or utilize cellulose, spreads through the trees via the pit membranes (Smith, 1969). *Leptographium wageneri* occasionally spreads short distances from tree to tree across root grafts and major contacts. The most common origin of infections is through small rootlets (Wagener & Mielke; 1961; Goheen, 1976; Hansen, 1978; Hessburg & Hansen, 1986a). Although it might increase infection, contact between roots of different trees is not necessary for spread of the disease (Hessburg & Hansen, 1986a). The mechanism of spread between roots is unknown (Hansen *et al.*, 1988), but long distance spread requires insect vectors (Hansen, 1978). *Leptographium wageneri* has also been isolated from soils around diseased roots and might be able to survive saprophytically in this environment (Hicks, 1973).

BSRD can predispose trees to infestation by bark beetles. Thus, diseased trees have been found to be more likely to become bark-beetle infested than healthy trees (Goheen & Cobb, 1980; Goheen *et al.*, 1985; Hansen *et al.*, 1988). Weakened trees then serve as a food base for beetle populations to increase. When these populations become high in number, mass attacks can occur and healthy, as well as diseased trees are affected (Cobb *et al.*, 1974; Cobb, 1988). Diseased trees usually occur in groups or centres (Cobb, 1988). A disease centre appears as a group of dead trees mixed with uninfected trees (Goheen & Hansen, 1978). Disease centres can be established by insect vectors attracted to stressed trees. The disease then spreads further by points of contact between diseased and healthy trees (Morrison & Hunt, 1988). The rate of infection and the expansion of disease centres appears to slow with the aging of the tree (Hansen & Goheen, 1988).

Leptographium wageneri can be found in trees infested by species of *Dendroctonus* (Cobb *et al.*, 1974), *Pissodes fasciatus, Steremnius carinatus* and the root bark beetle, *Hylastes nigrinus* (Cobb *et al.*, 1984; Hansen *et al.*, 1988; Witcosky & Hansen, 1985; Witcosky, Schowalter & Hansen, 1986). Although there was initially no firm evidence for insect transmission (Cobb *et al.*, 1974), insects are now known to serve as vectors for this fungus (Hansen *et al.*, 1988; Harrington, Cobb & Lownsberry, 1985; Witcosky & Hansen, 1985; Witcosky *et al.*, 1986). *Hylastes nigrinus* appears to be the primary vector of the douglas-fir variant of *L. wageneri* (Cobb, 1988).

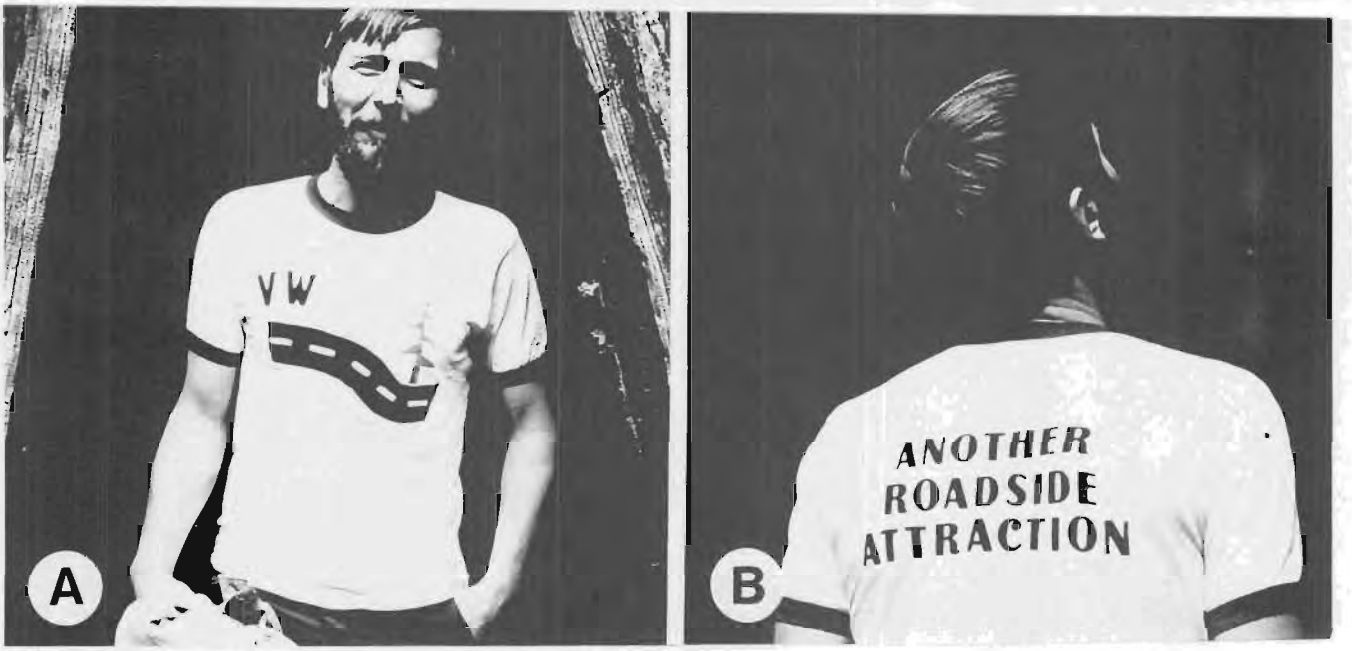

Fig. 5. Black stain root disease centres tend to occur at roadsides. These two pictures are of Dr. Everett Hansen wearing a T-shirt to illustrate this point. **A.** A tree growing at a roadside with VW referring to *Verticicladiella wageneri* (now *Leptographium wageneri*). **B.** The second picture illustrates the roadside nature of the disease.

Cobb (1988) proposed that *L. wageneri* renders sufficient trees susceptible to bark beetle infestation to maintain a high beetle population that is able to attack healthy trees. The adult beetles create wounds through their maturation feeding habits, and introduce the pathogen through these wounds (Harrington *et al.*, 1985; Hansen *et al.*, 1988). How the beetles detect a diseased or stressed tree is still unknown, although the incidence of root disease is directly correlated with the incidence of beetle infestation (Cobb, 1988).

Factors influencing BSRD can, in most cases, be associated with disturbances in the environment (Harrington *et al.*, 1983). BSRD appears to be more severe in places that have been disturbed by human activity, such as near roads or railroad tracks, where logging has occurred or where the thinning of trees is practiced (Fig. 5) (Hansen, 1978; Harrington, 1982; Harrington *et al.*, 1983; Cobb, 1988; Hansen *et al.*, 1988). This feature of the disease is believed to be associated with insect activity.

Leptographium wageneri is a temperature sensitive fungus that grows best at temperatures below 20°C (Wagener & Mielke, 1961; Smith, 1967; Hicks, 1973; Harrington, 1982; Hessburg & Hansen, 1983). Thus, BSRD occurs mostly in soils with bedrock near the surface and on well-drained coarse textured soils that have been disturbed (Morrison & Hunt, 1988). Soil moisture also influences the occurrence of this disease. BSRD is favored by high soil moisture and cooler temperatures (Goheen, 1976; Landis & Helburg, 1976; Goheen, Cobb & McKibbin, 1978; Cobb *et al.,* 1984; Wilks, Gesper & Cobb, 1985; Hessburg & Hansen, 1986a; Cobb, 1988). Fenn, Dunn and Wilborn (1990) found that increased levels of ozone tend to lead to an increase in disease incidence in ponderosa pine. Stressed trees are also especially susceptible to the disease (Hansen, 1978). Virulence of *L. wageneri* appears to increase with the increase of manganese concentrations and soil moisture (Goheen, 1976; Wilks, Gersper & Cobb, 1983).

Disease management strategies may include replacement of old trees with more vigorous trees, less prone to attack by bark beetles and spacing of trees to prevent spread through root contact (Goheen *et al.*, 1978). Some other strategies include planting mixed stands instead of trees in monoculture (Goheen *et al.*, 1978), minimizing stand and site disturbance, and selection of disease-resistant trees (Cobb, 1988; Hansen *et al.,* 1988). Sanitation through the removal of diseased trees or chemical treatment has also been suggested (Witcosky, 1989). In the case of douglas-fir, thinning after insect flight will reduce activity of vectors. If species other than douglas-fir are planted and site disturbances and tree injury are minimized, this will also reduce the incidence of BSRD. *Leptographium wageneri* has a short survival span after infected trees have been felled, indicating that the site where the disease occurs can be regenerated in a short period of time (Hunt & Morrison, 1986). An integrated pest management plan, making use of sanitation, resistant species and desirable cultural practices provides an ideal strategy for reducing the impact of BSRD (Witcosky, 1989).

White pine root decline

White pine root decline (WPRD) was first reported in the Eastern United States and was later found that *Leptographium procerum* is consistently associated with this

disease symptom (Kendrick, 1962; Dochinger, 1967). The role of the fungus in causing this disease has, however, been a matter of considerable debate (Lackner & Alexander, 1982; Harrington & Cobb, 1983; Wingfield, 1983; 1986). White pine root decline refers to a symptom. The consistent association of *L. procerum* with diseased trees need not imply that the fungus causes the disease. The association of the fungus with opportunistic insects that feed in the roots and root collars of stressed trees implies that *L. procerum* is commonly found in these parts of trees displaying symptoms of WPRD (Wingfield, 1983; Wingfield *et al.*, 1988). WPRD results in major economic losses in the Christmas tree industry in the USA (Lackner & Alexander, 1982).

Leptographium procerum is able to infect various species of pine other than *Pinus strobus*, but the symptoms and disease development in these species have been found to differ from those in *P. strobus* (Horner & Alexander, 1983a,b). The fungus has also been isolated from dying red pine (*P. resinosa*) and Scots pine (*P. sylvestris*) (Sinclair & Hudler, 1980). A disease similar to WPRD has been reported from Croatia and New Zealand, and the causal agent was speculated to be *L. procerum* (Orlic *et al.*, 1973; Halambek, 1976; Shaw & Dick, 1980; Halambek, 1981). The presence of WPRD in New Zealand was later confirmed by Mackenzie and Dick (1984). White pine root decline is now known to occur in various parts of the world in various ecosystems, and is not only restricted to forest trees (Livingston & Wingfield, 1982; Morelet, 1986; Alexander, Horner & Lewis, 1988; Morrison & Hunt, 1988; Smith, 1991). The extent of damage associated with WPRD has also not been fully assessed (Towers, 1977; Meyer, Hindal & Quinn, 1983).

Symptoms associated with WPRD include extended periods of bud break, retardation of shoot elongation, crooking of growing shoots, retention of needles, needle wilt, browning of needles and resin soaked black-streaked wood at the bases of stems, as well as basal cankers (Pest Alert, 1977; Towers, 1977; Anderson & Alexander, 1979; Mackenzie & Dick, 1984; Alexander *et al.*, 1988) (Fig. 6). The disease begins with a dark brown discoloration of the cambium at the base of trees. In the case of severe infection, marked resin exudation is observed (Alexander *et al.*, 1988). Colonized roots are resin-soaked and cross-sections of the stems reveal prominent wedges of blue-stained wood. Discoloration of the sapwood is consistent with the patterns and physiology of blue-stain fungi

(Alexander *et al.*, 1988). Electron microscopic examination has shown that *L. procerum* erodes the cell walls, and spreads from cell to cell via pits (Kilbertus, Mangenot & Radtke, 1980). Reduced water potential in symptomatic trees supports the notion that this root disease is associated with xylem dysfunction (Horner, Alexander & Lewis, 1987). Tree death occurs when the xylem is blocked by resin, resulting in desiccation (Alexander *et al.*, 1988).

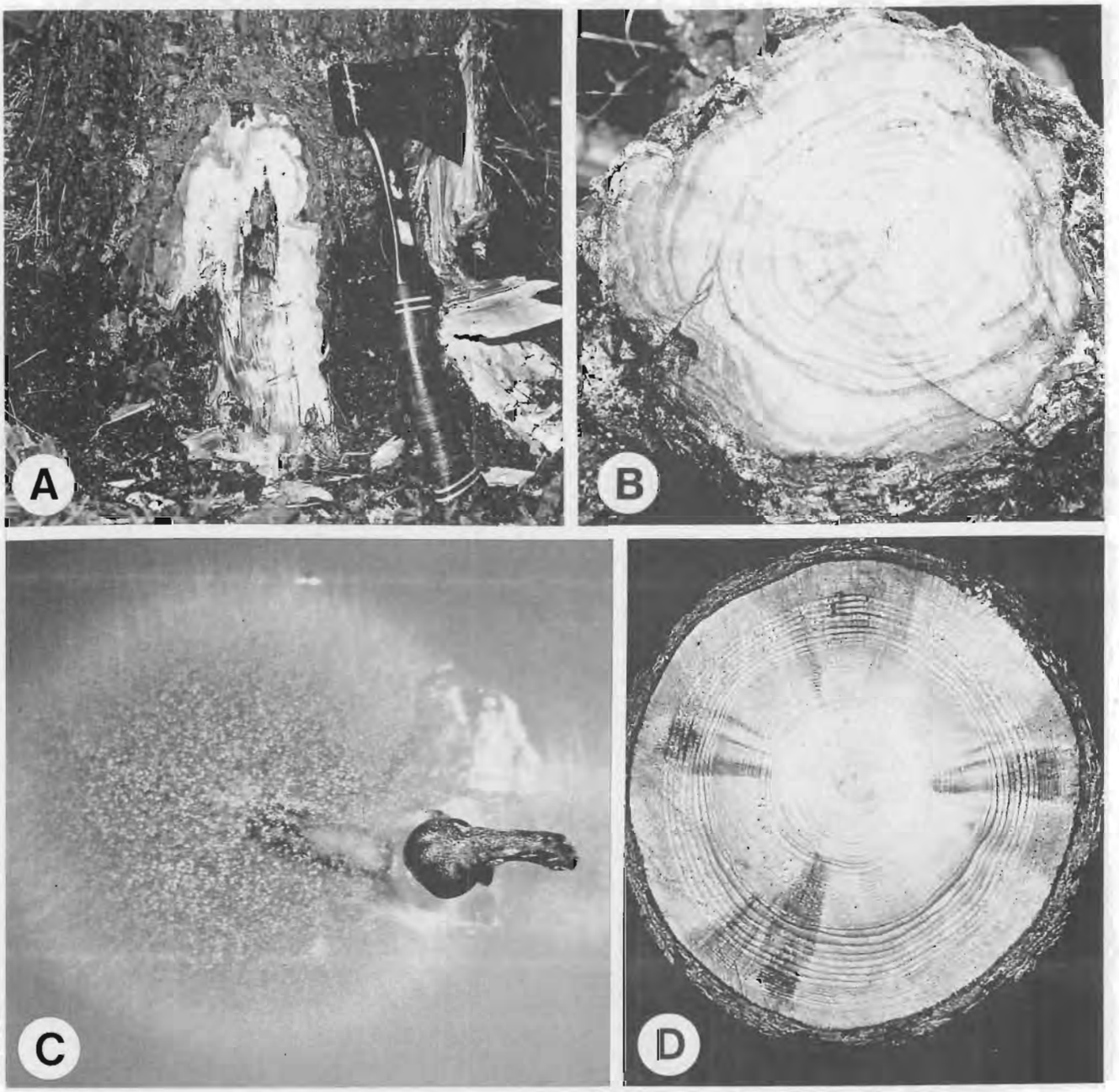

Fig. 6. Symptoms and insects associated with WPRD. **A.** Resinous lesion at the base of a mature *P. strobus* tree. **B.** Base of a *P. sylvestris* tree infested by the pine root collar weevil and colonized by *L. procerum*. **C.** *Leptographium procerum* growing out of body parts of a pine root collar weevil on agar. **D.** Typical staining pattern in pine wood inoculated with *L. procerum*.

Insect activity is evident at the bases of trees infected with *L. procerum* (Alexander *et al.*, 1988). Various reports exist where trees infected with *L. procerum,* were also infested with insects that may act as vectors for the fungus (Alexander *et al.*, 1988). It appears that weevils (Coleoptera: Curculionidae) are the main vectors, with bark beetles less commonly associated with the fungus (Wingfield, 1983; Lewis, 1985; Lewis & Alexander, 1986; Horner *et al.*, 1987; Alexander *et al.*, 1988) (Fig. 6). Volatiles such as ethanol and turpenes are often released from trees infected with *L. procerum.* The release of these volatiles is thought to play an important role in the association of the vectors with the trees (Nevill & Alexander, 1992a). The severity of WPRD is also affected by the breeding and feeding activities of the bark beetles that are secondary invaders (Alexander *et al.*, 1988).

Leptographium procerum is transmitted by insects, and it has also been speculated to spread through the soil. Air-borne dispersal has been ruled out as a means of spread (Alexander *et al.*, 1988). Propagules of *L. procerum* are able to survive in the soil around infected hosts for short periods of time (Lackner & Alexander, 1984; Alexander *et al.*, 1988). It appears that colonized roots are the main source of these propagules in the soil (Alexander *et al.*, 1988). The propagules occurring in the soil were later found to be relatively unimportant in the spread of the pathogen (Lewis, 1985; Lewis & Alexander, 1985; Alexander *et al.*, 1988). *Leptographium procerum* is also not uniformly distributed through the soil and is, therefore, unlikely to be a relevant source of infection. It has, thus, been proposed that insects are the main source of inoculum (Lewis, Alexander & Horner, 1987).

The pathogenicity of *L. procerum* has been a matter of substantial debate, and some studies have indicated that *L. procerum* is only a weak pathogen (Towers, 1977; Livingston & Wingfield, 1982; Wingfield, 1982; Wingfield, 1986; Wingfield *et al.,* 1988; Harrington, 1993). This can be illustrated by the fact that in some cases, only the symptoms of the disease have been reported, without any trace of a vector or *Leptographium* sp. The cause of these symptoms has, therefore, been attributed to other factors such as soil moisture (Prey, 1975) and not the fungus.

Leptographium procerum has been isolated from severely diseased trees (Leaphart, 1960; Dochinger, 1967). However, Houston (1969) found with inoculation studies that *L. procerum* does not kill as many trees as other pathogens.

Sinclair and Hudler (1980) indicated that it is frequently associated with mortality of red pine on poorly draining soils. However, there is no evidence to suggest that *L. procerum* is directly responsible for the mortality. Harrington and Cobb (1983) indicated that *L. procerum* is not virulent and is unable to kill wounded or unwounded douglas-fir. This was confirmed by Wingfield (1983, 1986) who considered *L. procerum* to be a weak pathogen. This is contrast to studies of Lackner (1981) and Lackner and Alexander (1982), who viewed the fungus as the cause of severe losses in Christmas tree plantations. In contrast to the results of Harrington and Cobb (1983) and Wingfield (1983, 1986), pathogenicity tests done on seedlings with isolates of this fungus confirmed its ability to kill seedlings. (Halambek, 1981; Alexander *et al.*, 1988). Nevill and Alexander (1992a) postulated that the lack of foliar symptoms as observed by Wingfield (1986), might be as a result of a long latent period of this fungus. In a separate study, however, *Leptographium procerum* did not produce lesions that were significantly longer than those of the controls in *P. taeda* (Nevill *et al.,* 1995).

Control and management of WPRD includes the planting of trees on sites suitable for the species, the control of weevils and bark beetles, removal of slash in and around the plantation and the control of weeds (Alexander *et al.*, 1988). It is also advisable to allow sites to lie fallow for one year or to consider planting non-susceptible trees (Lewis, 1985). WPRD affects trees more seriously when they are planted on wet sites (Anderson & Alexander, 1979). Poor site drainage has also been reported to promote disease development (Smith, 1991).

Dochinger (1967) speculated that soil moisture and temperature play an important role in the ecology of the fungus that causes WPRD. Excessive soil moisture can increase the severity of WPRD (Alexander *et al.*, 1988). *L. procerum* has also been found to be associated with root damage along roads (Alexander *et al.*, 1988), which is probably due to insect activity as in the case of *L wageneri* (Cobb *et al.,* 1984; Hansen *et al.*, 1988; Witcosky & Hansen, 1985; Witcosky *et al.,* 1986). Lackner (1981) and Lackner and Alexander (1983) found that *P. strobus* trees subjected to air pollution were more susceptible to root disease, presumably caused by *L. procerum* and insect infestation.

The debate surrounding the role of *L. procerum* as a conifer pathogen has perhaps

not fully been resolved. The fungus is substantially less virulent than *L. wageneri* and a general consensus seems that it cannot kill trees independently. It is commonly associated with root and root collar insects (Wingfield, 1983). Symptoms associated with insects such as pine root collar weevil (*Hylobius radicis*) on young trees are similar to those reported for WPRD and this has perhaps led to confusion relating to the role of *L. procerum* as pathogen (Wingfield, 1986). White pine root decline is a distinct disease syndrome on *Pinus strobus,* particularly in Christmas tree plantations and it is probably pertinent to view this disease alone and not confuse it with the occurrence of *L. procerum* on other pine species. The role of *L. procerum* in the development of WPRD and in the ecology of root and root collar insects remains to be fully understood.

Other diseases associated with species of *Leptographium*

Pole blight

The disease known as pole blight occurred exclusively on western white pine (*Pinus monticola* Douglas) in the 1950's (Gill & Andrews, 1949; Gill, Leaphart & Andrews, 1951; Hubert, 1953), where it caused serious damage (French, 1949; Foster, 1957; Leaphart, Copeland & Graham, 1957). Leaphart (1956) isolated a species of *Leptographium* from trees with pole blight symptoms. From the description of the corkscrew-like or wavy appearance of the mycelium, this fungus was thought to be *L. serpens* (Leaphart, 1956). However, inoculation studies on trees with this fungus did not conclusively result in symptoms (Leaphart, 1958).

Hubert (1953) suggested that the *Leptographium* sp. associated with pole blight is not the primary cause of the disease. These findings were supported by Leaphart and Gill (1959) in their study of the effect of several species of *Leptographium* on western white pine. They found that species of *Leptographium* were pathogenic to pine, but that they were not the causal agents of pole blight.

Ophiostoma trinacriforme has also been implicated as a possible cause of pole blight. However, a study by Parker (1957b) showed that this fungus is unable to produce the typical lesions associated with the disease. It is more likely a

secondary invader of lesions created by the causal agent of pole blight.

Leptographium terebrantis - associated disease

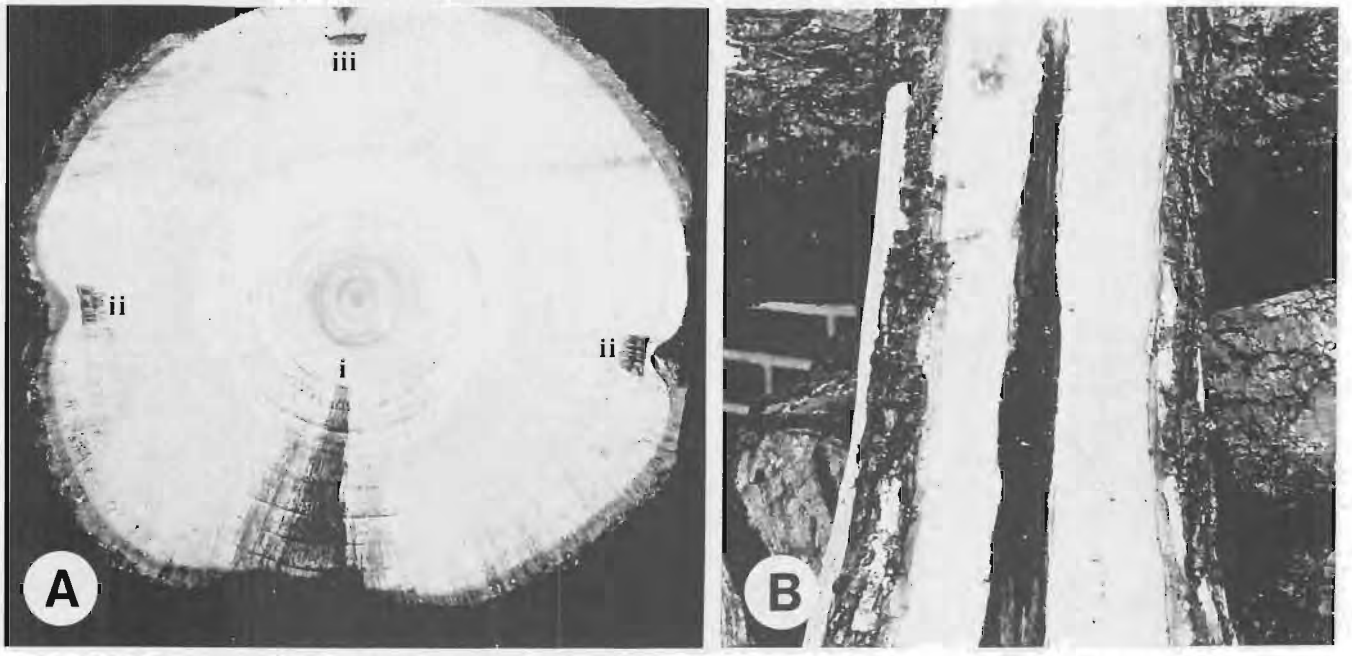

Fig. 7. Lesions in *Pinus strobus* five months after inoculations with *L. terebrantis* and *L. procerum*. **A.** Section through a stem inoculated with *L. terebrantis* (i), *L. procerum* (ii) and control (iii). **B.** Face view of an extensive lesion caused by *L. terebrantis* five months after inoculation.

Leptographium terebrantis is a common blue-stain fungus that is associated with a wide range of bark beetles, particularly *Dendroctonus terebrans* (Bennet & Tattar, 1988). Although the fungus has never been considered as a primary cause of tree disease, it has a high level of pathogenicity. Thus, Harrington & Cobb (1983) were able to kill pine seedlings with this fungus while, in the same study, *L. procerum* was not able to kill the plants. Similarly, Wingfield (1986) showed that *L. terebrantis* could kill inoculated seedlings and cause extensive lesion development in established trees (Fig. 7). This was unlike *L. procerum* that did not kill seedlings and gave rise to very limited lesion development, which was hardly different to the controls. The pathogenicity of this fungus to Japanese and Scots pine was confirmed by Bennet & Tattar (1988), Ross, Fenn & Stephan, (1992) and Nevill *et al.*, (1995). They found that this fungus caused severe resinosis and lesion development. Otrosina *et al.*, (1997) isolated *L. terebrantis* from lesions in trees

attacked by the Southern pine beetle. However, no conclusions were made regarding its pathogenicity to pine.

Leptographium terebrantis has also been found in the roots of *Pinus resinosa* with symptoms of red pine decline (RPD). In association with two other fungi, *O. ips* and *O. nigrocarpum,* it was thought to play a role in red pine death in the Lake States (Smalley *et al.,* 1993). Inoculation studies with other species of *Ophiostoma* and *Leptographium* suggest that *L. terebrantis* is the primary cause of root disease in red pine. This species is also known to be associated with the red turpentine beetle that infests *P. resinosa* (Smalley *et al.,* 1993).

Leptographium serpens - associated diseases

Leptographium serpens has been associated with a root disease of *Pinus pinea* in Italy (Lorenzini & Gambogi, 1976). A similar disease was later found in on *Pinus radiata* and *P. pinaster* in South Africa (Wingfield & Knox-Davies, 1980a). The causal agent of the root disease in South Africa was described as *Leptographium alacris* M.J. Wingf. & Marasas (Wingfield & Marasas, 1980), but this species was later synonomised with *L. serpens* (Wingfield & Marasas, 1981). There have been some reports of this fungus from the USA, although these are of doubtful authenticity (Harrington, 1988).

Wingfield *et al.* (1988) concluded that the pathogenicity of *L. serpens* has not been conclusively established and that the combined feeding activity of the insects and the subsequent colonization by the fungus may result in tree death. *Leptographium serpens* colonizes both the ray parenchyma as well as the tracheids resulting in a wedge shape discoloration of infected wood (Wingfield *et al.*, 1988). Two root feeding insects, *Hylurgus ligniperda* and *Hylastes angustatus,* are associated with this fungus and can act as possible vectors. The disease, thought to be associated with *L. serpens* is also characterized by distinct infection centers in plantations (Wingfield *et al.,* 1988).

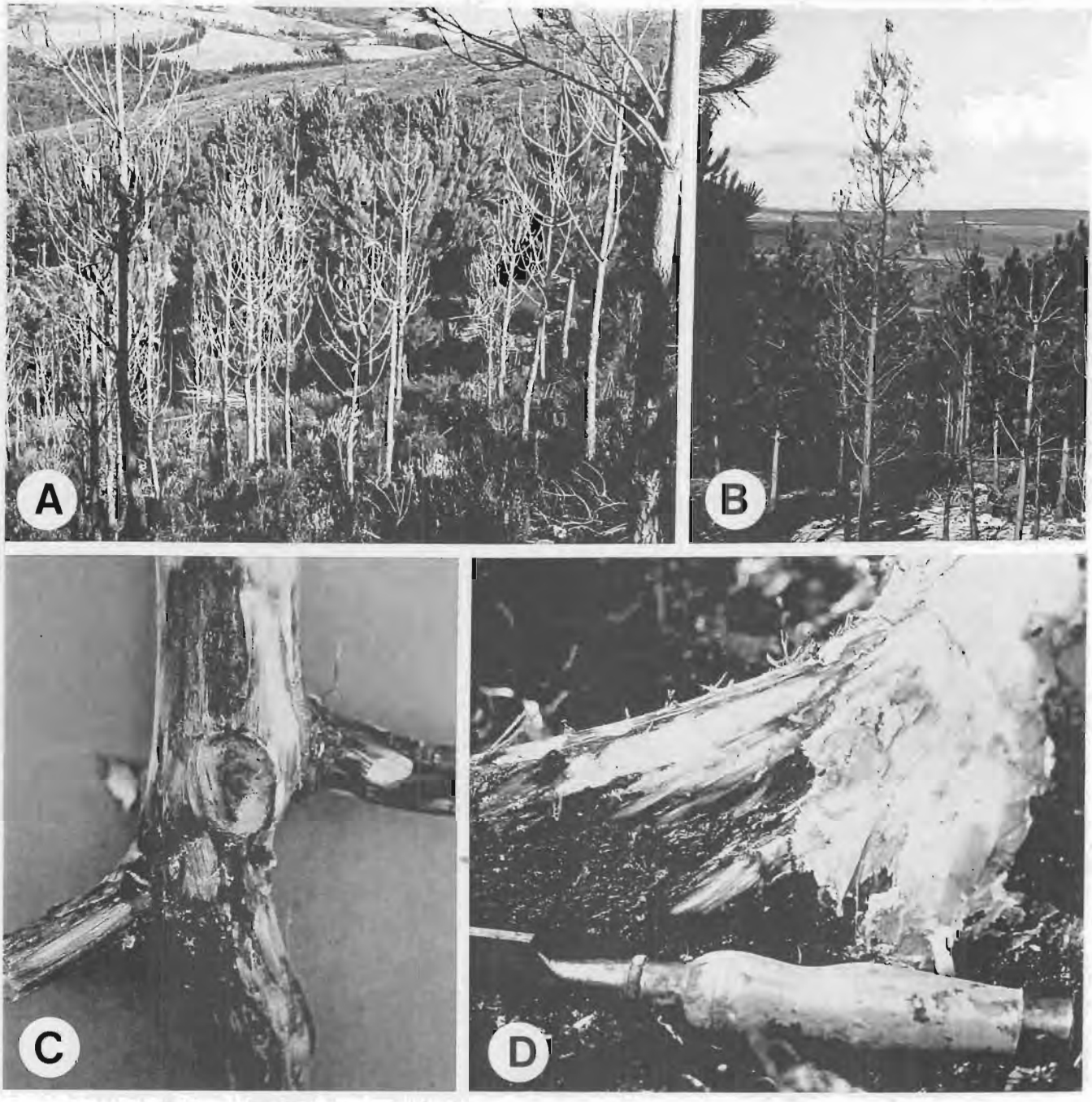

Fig. 8. Symptoms associated with *P. pinaster* trees infected with *L. serpens* in South Africa. **A.** Dead trees in a discrete patch-like infection centre. **B.** Dying tree at the edge of an infection centre. **C.** Diseased root system of young tree. **D.** Stained root on trees.

Symptoms of the disease associated with *L. serpens* includes scant, yellowish foliage in the upper crown of trees, reduced needle length, sudden marked

decrease in height growth and dark stained areas on roots (Wingfield & Marasas, 1983 Wingfield *et al.*, 1988). The trees retain their dead needles after death, in contrast to other diseases where trees will loose needles before they die (Wingfield & Knox-Davies, 1980a, b) (Fig. 8).

Takamaka disease

Takamaka disease occurs on Takamaka (*Calophyllum inophyllum*) trees, which are indigenous to the Seychelles and Mauritius (Wiehe, 1949; Wainhouse *et al.*, 1998; Webber *et al.*, 1999). The fungus associated with this severe wilting disease was initially identified as a species of *Haplographium* (Wiehe, 1949), and Gams (1971) transferred it to *Verticillium*. In recent study of this fungus, it was shown that it is unlike other *Verticillium* spp. and was subsequently transferred to *Leptographium* as *L. calophylli* (Webber *et al.*, 1999).

Fig. 9. Symptoms Takamaka disease on *Calophyllum inophyllum* in the Seychelles. **A.** Dying trees on beach front. **B.** Thinning crown of a dying tree. (Photographs supplied by Dr. D. Wainhouse).

Takamaka disease is characterized by wilting of the crowns of trees. The leaves loose their shine, curl inwards and dry out (Fig. 9). The leaves dry suddenly and remain attached to the trees for up to two weeks (Wiehe, 1949). No apparent lesions on the trunks, branches or roots are associated with this disease. However, brownish streaks are visible in the tracheids of trees (Wiehe, 1949).

Fungal infection occurs through wounds on the branches and twigs. These wounds can be as a result of mechanical wounding by strong winds or bark beetle activity. Bark beetle tunnels are frequently associated with this disease and their feeding and breeding habit can cause wounds (Wiehe, 1949). The bark beetle, *Cryphalus trypanus,* has been identified as the principal vector of *L. calophylli* (Wainhouse *et al.*, 1998)

Blue-stain

Blue-stain of conifer wood refers to the discoloration of sapwood that results from the presence of fungal hyphae (Münch, 1907; Lagerberg, 1927; Seifert, 1993) and can be recognized by its wedge-shaped appearance in the logs (Gibbs, 1993). The discoloration can range from bluish to grey (Seifert, 1993). However, the color of the mycelium does not necessarily influence the color of the stain (Lagerberg *et al.,* 1927). Two different categories of blue-stain are recognized, namely log-blueing and surface-blueing, and different fungi are associated with these symptoms (Lagerberg *et al.,* 1927). Blue-stain fungi generally do not kill trees, although Nelson (1934) found with experiments using dye that the stained areas in the wood interfered with transpiration.

Many species of *Leptographium* are associated with blue stain in conifer lumber (Lagerberg *et al.,* 1927; Solheim, 1992a,b, 1995a,b,c; Solheim, Långström & Hellqvist, 1993). This was first recognized, when Lagerberg *et al.* (1927) studied the causal agents of blue-stain in pine and spruce. This study led to the description of *Leptographium* (Lagerberg *et al.,* 1927) and its type species, *Leptographium lundbergii*, as discussed earlier. Various examples of *Leptographium* spp. causing blue-stain are known, for example *L. penicillatum* and *L. piceaperdum* associated with *Ips typographus* L. on Norway spruce (Solheim, 1992b; Wingfield *et al.,* 1993).

Leptographium wingfieldii and *L. terebrantis* have, apart from their blue-stain properties, also been shown to be pathogenic to their hosts (Wingfield, 1986; Solheim & Långström, 1991; Gibbs & Inman, 1991; Solheim *et al.,* 1993).

Insect activity is also associated with blue-stain and the frequency of the blue-stain is determined by the frequency of the beetle attack (Highley & Tattar, 1985). Insects lower the resistance of trees and allow fungi to colonize trees (Francke-Grosmann, 1965; Livingston *et al.,* 1983; Kulhavy, Partridge & Stark, 1984; Wingfield *et al.,* 1988; Lieutier, Cheniclet & Garcia, 1989; Solheim, 1993a; Krokene & Solheim, 1996). Hobson, Parmeter and Wood (1991) found that blue-stain fungi were generally absent from the xylem of dying pine trees. These fungi were found to colonize trees later when the xylem had been debilitated.

INSECT ASSOCIATIONS

Insects are commonly associated with *Leptographium* spp. (Münch, 1907; Lagerberg *et al.,* 1927; Kendrick, 1962; Harrington, 1988; Wingfield & Gibbs, 1991; Wingfield, Harrington & Crous, 1994) (Table 1) (Fig. 10). Currently, there are two hypotheses to explain the relationship between *Leptographium* spp. and insects. One is that these fungi are mostly transported, with little primary benefit to the insects (Leach, Orr & Christensen, 1934; Bramble & Holst, 1935, 1940; Mathre, 1964; Hinds, 1972; Goheen & Cobb, 1978; Witcosky & Hansen, 1985; Lewis & Alexander, 1986). The fungus on the other hand might serve as a source of food for the insects or play some role in the development of the brood (Nelson, 1934; Leach *et al.,* 1934). A second hypothesis is that the association of the insects and the fungi might be co-incidental. The fungi would then be considered as "weeds" in the habitat of the beetles (Harrington, 1993).

The conidia of *Leptographium* spp. are sticky and adhere easily to the body surfaces of insects (Harrington, 1993; Malloch & Blackwell, 1993) (Fig. 10). However, several species of *Ophiostoma* and *Leptographium* are carried in the mycangia of their associated insects (Francke-Grosmann, 1965; Whitney & Farris, 1970; Barras & Perry, 1971b; Ross & Solheim, 1995; Six & Paine 1996; Solheim, 1995a). Mycangial fungi have been shown to be important to the beetles and the

removal of these structures can lead to a reduction of the progeny and development of the pine beetle brood (Barras, 1973). Some evidence is also available to suggests that the fungi provide nutrition for the beetles (Batra, 1963; Francke-Grosmann, 1967; Hinds, 1972; Brand *et al.* 1976; Six & Paine, 1996).

Phoretic mites associated with bark beetles might serve as a vectors of blue stain fungi. It has for example been found that the mites associated with *Ips typographus,* carry one or more spores of different fungi (Moser, 1985; Moser, Perry & Solheim, 1989) and these represent an example of secondary phoresy (Blackwell *et al.,* 1986). However, the role of the fungi in the life cycle of the insects is still uncertain and much debated (Robinson, 1962; Lieutier *et al.,* 1988; Redfern, 1989; Paine, Raffa & Harrington, 1990; Hobson, Parmeter & Wood, 1991; Lévieux *et al.,* 1994; Raffa, 1995; Six & Paine, 1995; Wingfield, Harrington & Solheim, 1995; Otrosina *et al.,* 1997).

Insects associated with species in *Leptographium* mostly occur on conifers, especially bark beetles (Coleoptera: Scolytidae) (Grosmann, 1931; Harrington, 1988; Paine *et al.*, 1990). These insects can be primary bark beetles that attack and kill healthy trees, or secondary bark beetles that rarely kill their hosts (Berryman, 1972; Paine *et al.,* 1990). Most insects associated with *Leptographium* spp. are quite specific to the fungi they carry. Although one species of insect may carry two or more *Leptographium* spp., these relationships give a very clear insight into the taxonomy of the fungi (Grosmann, 1931; Leach *et al.,* 1934; Mathiesen, 1951; Griffin, 1968; Olchowecki & Reid, 1974; Horntvedt *et al.,* 1983; Harrington, 1988; Wingfield *et al.,* 1988; Furniss, Solheim & Christiansen, 1990; Gibbs & Inman, 1991). In other cases, the insects associated with the fungi can be diverse and the relationship appears to be casual (Olchowecki & Reid, 1974; Harrington, 1988) (Table 1). It is, however, important to distinguish between the pathogenic cycle where the insect introduces a pathogenic fungus into a tree, and a saprophytic cycle, where the dying trees provide food and brood material for the insects and sites for sporulation of the fungi (Brand *et al.,* 1976; Wingfield *et al.,* 1988).

Several studies indicate that root disease and blue stain fungi predispose the trees to further attack by bark beetles (Francke-Grosmann, 1965; Livingston *et al.,* 1983;

Kulhavy *et al.*, 1984; Lieutier, *et al.*, 1989; Solheim, 1993a; Krokene & Solheim, 1996). Fungi infecting the roots, such as *L. terebrantis* and *L. procerum,* might also predispose trees to further beetle-attack by diminishing the tree defenses as a result of the lesions caused by these fungi (Otrosina *et al.*, 1997). Cobb *et al.* (1974) showed a high degree of association between root disease and species of *Dendroctonus* that infest trees. Krokene (1996) and Krokene & Solheim, (1996) indicted that aggressive beetles vector pathogenic fungi, whereas non-aggressive beetles tend to carry less pathogenic fungi.

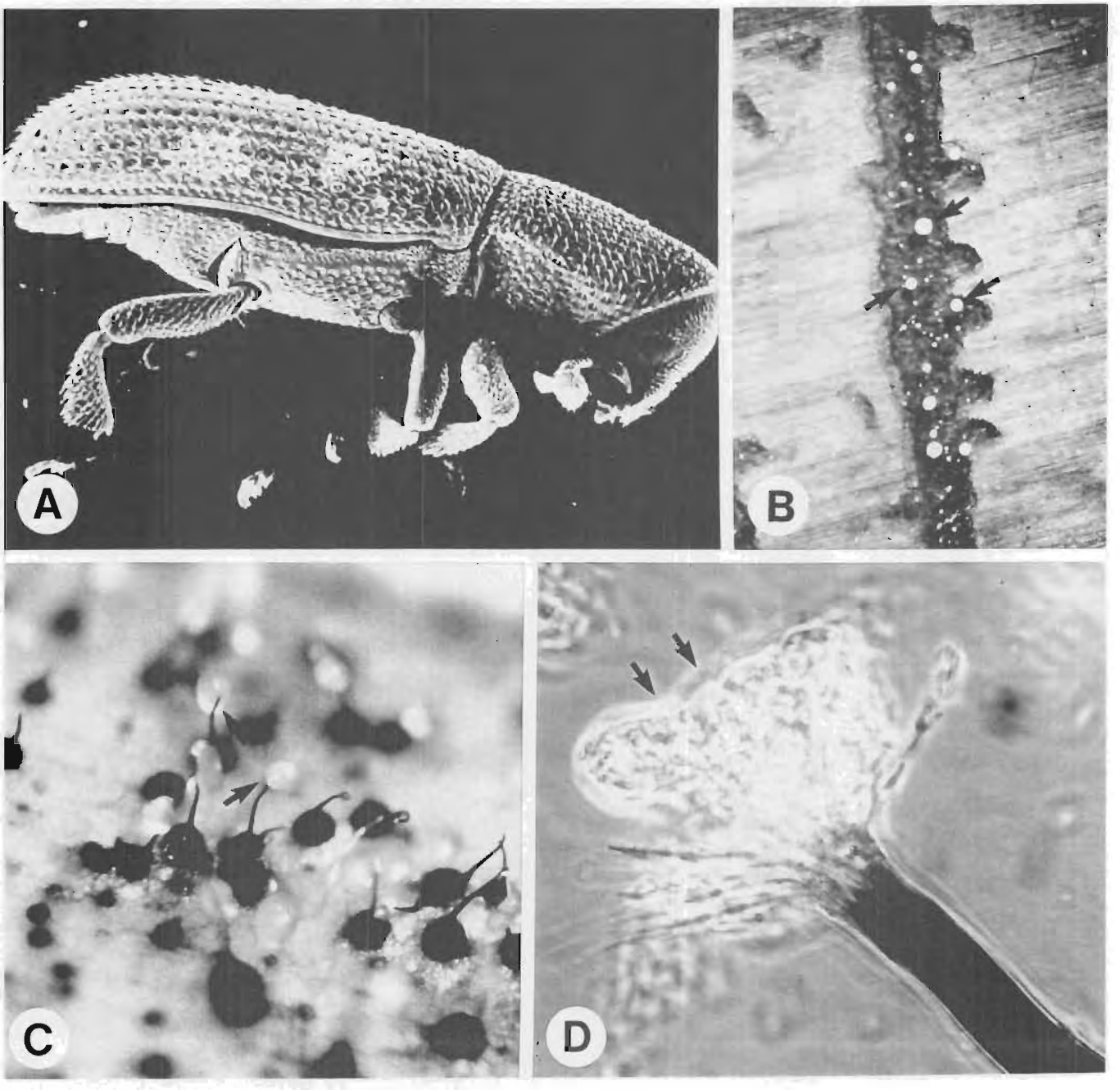

Fig. 10. Most *Leptographium* spp. are vectored by bark beetles such as the root-feeding beetle *Hylastes angustatus* (A). Fungal structures are adapted to insect dispersal with conidiophores (B) and perithecia (C, D) produced in galleries with spores in slimy masses (arrows) at the apices.

Table 1. Insects associated with *Leptographium* spp. and *Ophiostoma* spp. with *Leptographium* anamorphs.

Fungus	Insect	Reference
Leptographium abietinum	*Dendroctonus rufipennis*	Davidson, 1955; Kendrick, 1962; Harrington, 1988; Perry, 1991; Reynolds, 1992; Solheim, 1995a,b; Werner, 1995
	Dendroctonus pseudotsugae	Harrington, 1988; Perry, 1991; Lewinsohn *et al.*, 1994; Ross & Solheim, 1995; Solheim & Krokene, 1998
	Hylastes longicollis	Harrington, 1982, 1988
	Hylurgops porosus	Wagner, 1977
	Hylurgops planirostris	Harrington, 1988
Leptographium abicolens	*Korscheltellus gracilus*	Jacobs, Wingfield & Bergdahl, 1999
Ophiostoma abiocarpum	*Ips* spp.	Davidson, 1966
	Polygraphus rufipennis	Harrington, 1988
	Dryocoetus confusus	"
Ophiostoma aenigmaticum	*Ips typographus* f. *japonicus*	Jacobs *et al.*, 1998
Leptographium albopini	*Hylastes* spp.	Wingfield *et al.*, 1994
Leptographium alethinum	*Hylobius abietis*	Jacobs *et al.*, (1999)
Ophiostoma americanum	*Dendroctonus simplex*	Jacobs, Wingfield & Bergdahl, 1997
Ophiostoma aureum	*Dendroctonus sp.*	Robinson-Jeffrey & Davidson, 1968; Perry, 1991
	Hylurgops porosus	Harrington, 1988
Ophiostoma brevicolle	*Trypodendron retusus*	Davidson, 1958; Harrington, 1988
Leptographium calophylli	*Cryphalus trypanus*	Webber *et al.*, 1999
Ophiostoma crassivaginatum	*Trypodendron retusus*	Harrington, 1988
	Epuraea spp.	Hinds, 1972
	Colopterus truncatus	"
	Glischrochilus moratus	"

Table 1. cont.

Ophiostoma crassivaginatum (cont.)	*Glischrochilus vittatus*	"
	Rhizophagus brunneus	"
	Nudobius coricalis	"
Leptographium douglasii	*Hylastes nigrinus*	Wingfield *et al.*, 1994
Ophiostoma dryocoetidis	*Dryocoetus confusus*	Kendrick & Molnar, 1965; Molnar, 1965; Harrington, 1988
Leptographium euphyes	*Tomicus piniperda*	Jacobs *et al.*, 1999
Ophiostoma francke-grosmanniae	*Hylecoetus dermestoides*	Davidson, 1971
Leptographium guttulatum	*Dryocoetus autographus*	Jacobs *et al.*, 1999
	Hylastes ater	Wingfield & Gibbs, 1991
	Hylastes opacus	"
	Hylurgops palliatus	Mathiesen, 1950; Harrington, 1988; Wingfield & Gibbs, 1991; Jacobs *et al.*, 1999
	Hylurgops glabratus	Jacobs *et al.*, 1999
	Ips typographus	Mathiesen, 1950
	Tetropium sp.	"
	Tomicus piniperda	Jacobs *et al.*, 1999
Ophiostoma huntii	*Dendroctonus ponderosae*	Robinson-Jeffrey & Grinchenko, 1964; Harrington, 1988; Perry, 1991; Solheim, 1995c
	Hylastes ater	Jacobs *et al.*, 1998
	Hylastes macer	Harrington, 1988
	Ips pini	Davidson & Robinson-Jeffrey, 1965; Harrington, 1988
	Tomicus piniperda	Gibbs & Inman, 1991
Ophiostoma laricis	*Ips cembrae*	Van der Westhuizen *et al.*, 1995; Yamaoka *et al.*, 1998
Leptographium lundbergii	*Bursaphelenchus xylophilus*	Kaneko & Harrington, 1990
	Blastophagus minor	Mathiesen-Käärik, 1953
	Blastophagus piniperda	"

Table 1. cont.

Leptographium lundbergii (cont).	*Dendroctonus ponderosae*	Rumbold, 1931
	Hylastes angustatus	Wingfield & Marasas, 1983; Harrington, 1988; Wingfield *et al.*, 1988
	Hylastes ater	Harrington, 1988
	Hylastes opacus	Wingfield & Gibbs, 1991
	Hylurgus ligniperda	Harrington, 1988
	Hylurgops palliatus	Wingfield & Gibbs, 1991
	Ips acuminatus	Mathiesen-Kääńk, 1953; Harrington, 1988
	Myelophilus minor	Harrington, 1988
	Myelophilus piniperda	"
	Orthotomicus proximus	Mathiesen-Käärik, 1953; Harrington, 1988
	Pissodes pini	Mathiesen-Käärik, 1953
	Pityogenes quadridens	"
	Tomicus piniperda	Gibbs & Inman, 1991
	Trypodendron lineatum	Harrington, 1988; Bakshi, 1950
Ophiostoma penicillatum	*Dendroctonus rufipennis*	Perry, 1991
	Hylastes ater	Mathiesen, 1950; Mathiesen-Käärik, 1953; Harrington, 1988
	Hylastes cunicularis	Mathiesen-Käärik, 1953; Harrington, 1988
	Hylurgus ligniperda	"
	Hylurgops porosus	Wagner, 1977
	Hylurgops palliatus	Mathiesen, 1950; Mathiesen-Käärik, 1953; Harrington, 1988
	Dryocoetus confusus	Davidson, 1958
	Ips typographus f. *japonicus*	Yamaoka *et al.*, 1997
	Ips typographus	Goidanich, 1936; Kendrick, 1962, Mathiesen, 1950; Grosmann, 1931; Rennerfelt, 1950; Mathiesen-Kääńk, 1953; Solheim, 1986, 1992a; Harrington, 1988; Furniss *et al.*, 1990; Solheim, 1993b; Krokene, 1996; Krokene & Solheim, 1996; Viiri, 1997
	Ips duplicatus	Valkama, 1995; Krokene, 1996; Krokene & Solheim, 1996
	Pityogenes chalcographus	Goidanich, 1936; Mathiesen, 1950; Grosmann, 1931; Mathiesen-Käärik, 1953
	Pityogenes quadridens	Harrington, 1988
	Polygraphus poligraphus	Krokene, 1996; Krokene & Solheim, 1996

Table 1. cont.

Ophiostoma penicillatum (cont).	*Tetropium* sp	Mathiesen, 1950; Mathiesen-Kääńk, 1953
	Trypodendron lineatum	Harrington, 1988
Leptographium peucophilum	*Korscheltellus gracilus*	Jacobs *et al.*, 1999
Ophiostoma piceaperdum	*Dendroctonus ponderosae*	Perry, 1991
	Dendroctonus pseudotsugae	Solheim & Krokene, 1998
	Dendroctonus rufipennis	Harrington, 1988; Perry, 1991
	Dendroctonus valens	Perry, 1991
	Dryocoetus sp.	Davidson & Robinson-Jeffrey, 1965; Harrington, 1988
	Hylurgops palliatus	Harrington, 1988; Krokene & Solheim, 1996
	Ips typographus f. *japonicus*	Yamaoka *et al.*, 1997
	Ips typographus	Harrington, 1988; Solheim, 1986, 1992a, 1993b; Harding, 1995; Viiri, 1997
	Ips duplicatus	Krokene, 1996; Krokene & Solheim, 1996
	Pityogenes chalcographus	Harrington, 1988
	Polygraphus poligraphus	Krokene, 1996; Krokene & Solheim, 1996
Leptographium pineti	*Ips* spp.	Jacobs *et al.*, 1999
Leptographium procerum	*Dendroctonus frontalis*	Otrosina *et al.*, 1997
	Dendroctonus valens	Wingfield, 1983; Harrington, 1988
	Dendroctonus terebrans	Harrington, 1988; Perry, 1991
	Hylastes sp.	Lewis & Alexander, 1986; Alexander *et al.*, 1988
	Hylastes ater	Mackenzie & Dick, 1984
	Hylastes opacus	Wingfield & Gibbs, 1991
	Hylobius abietis	Lévieux *et al.*, 1994
	Hylobius pales	Lackner & Alexander, 1982; Wingfield, 1983; Lewis & Alexander, 1986; Alexander *et al.*, 1988; Nevill & Alexander, 1992a, b
	Hylobius radicis	Wingfield, 1982; Wingfield, 1983; Alexander *et al.*, 1988
	Hylobius rhizophagus	"
	Hylurgus ligniperda	Mackenzie & Dick, 1984
	Hylurgops palliatus	Wingfield & Gibbs, 1991
	Hylurgops porosus	Wagner, 1977

Table 1. cont.

Leptographium procerum (cont.)	*Ips typographus*	Harrington, 1988
	Orthotomicus spp.	Lewis & Alexander, 1986; Alexander *et al.*, 1988
	Pachylobius picivorus	Wingfield, 1983; Alexander *et al.*, 1988
	Pissodes spp.	Lewis & Alexander, 1986
	Pissodes approximatus	Lackner & Alexander, 1982; Alexander *et al.*, 1988
	Pissodes nemorensis	Nevill & Alexander, 1992a, b
	Pissodes pini	Kendrick, 1962; Livingston & Wingfield, 1982
	Pityokteines sp.	Lackner & Alexander, 1984; Alexander *et al.*, 1988
	Pityogenes sp.	Lackner & Alexander, 1984; Lewis & Alexander, 1986; Harrington, 1988; Alexander *et al.*, 1988
	Pityophthorus sp.	Lackner & Alexander, 1984; Alexander *et al.*, 1988
	Tomicus piniperda	Gibbs & Inman, 1991
	Xyleborus sp.	Lewis & Alexander, 1986; Alexander *et al.*, 1988
Leptographium pyrinum	*Dendroctonus adjunctus*	Davidson, 1978; Harrington, 1988; Perry, 1991; Six & Paine, 1996
Ophiostoma robustum	*Dendroctonus sp.*	Robinson-Jeffrey & Davidson, 1968; Perry, 1991
Ophiostoma serpens	*Hylastes angustatus*	Harrington, 1988; Wingfield *et al.*, 1988
	Hylastes ater	Wingfield & Gibbs, 1991
	Hylastes linearis	Harrington, 1988
	Hylobius pales	Nevill & Alexander, 1992
	Hylurgus ligniperda	Harrington, 1988; Wingfield *et al.*, 1988; Wingfield & Knox-Davies, 1980a
	Myelophilus piniperda	Siemaszko, 1939; Harrington, 1988
	Orthotomicus erosus	Wingfield & Knox-Davies, 1980a
	Pissodes nemorensis	Nevill & Alexander, 1992
Leptographium sibiricum	*Monochamus urrusovi*	Jacobs *et al.*, 1999
Leptographium terebrantis	*Dendroctonus frontalis*	Otrosina *et al.*, 1997
	Dendroctonus pseudotsugae	Lewinsohn *et al.*, 1994

Table 1. cont.

Leptographium terebrantis (cont.)	*Dendroctonus terebrans*	Barras & Perry, 1971a; Wingfield, 1983; Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988; Harrington, 1988; Perry, 1991
	Dendroctonus valens	Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988; Perry, 1991
	Hylurgops porosus	Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988
	Hylobius radicis	Wingfield, 1983
	Hylobius rhizophagus	"
	Ips pini	Bennet & Tattar, 1988
Leptographium wageneri	*Dendroctonus brevicomis*	Wagener & Mielke, 1961; Goheen, 1976; Goheen & Cobb, 1980
	Dendroctonus ponderosae	Goheen, 1976; Goheen & Cobb, 1980; Hunt & Morrison, 1986; Morrison & Hunt, 1988
	Dendroctonus valens	Goheen, 1976; Harrington & Cobb, 1983; Harrington, 1988; Perry, 1991
	Hylastes macer	Goheen, 1976; Goheen & Cobb, 1978; Harrington, 1982; Harrington & Cobb, 1983; Harrington, 1988
	Hylastes nigrinus	Witcosky, 1981, 1989; Harrington, 1982; Harrington & Cobb, 1983; Witcosky *et al.*, 1986; Harrington, 1988; Jacobi, 1992
	Hylurgops porosus	Wagner, 1977; Harrington, 1982
	Ips latidens	Morrison & Hunt, 1988
	Ips mexicanus	"
	Pissodes fasciatus	Witcosky, 1981, 1989; Witcosky *et al.*, 1986; Jacobi, 1992
	Steremnius carinatus	"
Leptographium wingfieldii	*Hyalstes opacus*	Wingfield & Gibbs, 1991
	Hylurgops palliatus	"
	Tomicus piniperda	Morelet, 1988; Lieutier *et al.*, 1989a,b; Wingfield & Gibbs, 1991; Gibbs & Inman, 1991; Solheim & Långström, 1991; Masuya *et al.*, 1998
Leptographium yunnanensis	*Tomicus piniperda*	Zhou *et al.*, 1999

HOSTS AND GEOGRAPHIC DISTRIBUTION OF *LEPTOGRAPHIUM* SPP.

Species of *Leptographium* are known from various parts of the world and occur on a wide variety of hosts. In the northern hemisphere, *Leptographium* spp. have been recorded from the U.S.A (Davidson, 1942; Davidson, 1958; Robinson-Jeffrey & Davidson, 1968; Wingfield *et al*, 1994), Canada (Hunt, 1956; Parker, 1957a; Wright & Cain, 1961; Kendrick, 1962; Robinson-Jeffrey & Grinchenko, 1964; Olchowecki & Reid, 1974), Europe [Croatia (Halambek, 1981), Germany (Grosmann, 1932), Italy (Goidanich, 1936) and Norway (Solheim, 1986, 1992a)] and Asian countries such as Japan (Van der Westhuizen *et al.*, 1995; Yamaoka *et al.*, 1997), Vietnam (Jacobs *et al.,* 1999), Indonesia (Jacobs *et al.,* 1999) and Taiwan (Wingfield, Crous & Tzean, 1994). In the southern hemisphere, *Leptographium* spp. have been reported from New Zealand (Shaw & Dick, 1980; Wingfield & Marasas, 1983; Mackenzie & Dick, 1984; Hutchison & Reid, 1988; Farrell *et al.,* 1997), South Africa (Wingfield & Knox-Davies, 1980a, b; Wingfield & Marasas, 1980; 1983), Central Africa (Jacobs, Wingfield & Roux, 1999), and Australia (Jacobs *et al.*, 1998) (Fig. 11).

In most cases, *Leptographium* spp. occur on conifers (Kendrick, 1962; Harrington, 1988; Wingfield *et al.,* 1994) (Table 2). Only a small number of species occur on deciduous trees, or other substrates (Davidson, 1958, 1971, 1976; Jooste, 1978; Kendrick, 1962, Jacobs *et al.,* 1998). Some *Leptographium* spp. are highly specific, and are often closely linked to insects that infest trees. Host, insect associations and area of occurrence, can thus be helpful in species identification. In some cases, the host can be used to distinguish between different species, for example the *L. wageneri* varieties (Kendrick, 1962; Harrington & Cobb, 1986, 1987). Thus, Hunt (1956) used this host specificity as a character in his key to species in several genera, including *Ophiostoma* and *Ceratocystis.*

Most *Leptographium* spp. are known from the Northern Hemisphere where conifers are native (Kendrick, 1962; Harrington, 1988). Virtually all species that have been recorded from the Southern Hemisphere have been introduced into that region with pine infesting bark beetles. Thus, a number of species of *Leptographium* (*L. lundbergii, L. procerum* and *L. huntii*) have been introduced into New Zealand and Australia from Europe with *Hylastes ater* and *Hylurgus ligniperda.* In South Africa,

L. serpens, *L. procerum* and *L. lundbergii* have been introduced into exotic pine plantations together with *H. angustatus* and *Hylurgus ligniperda* (Table 1, 2).

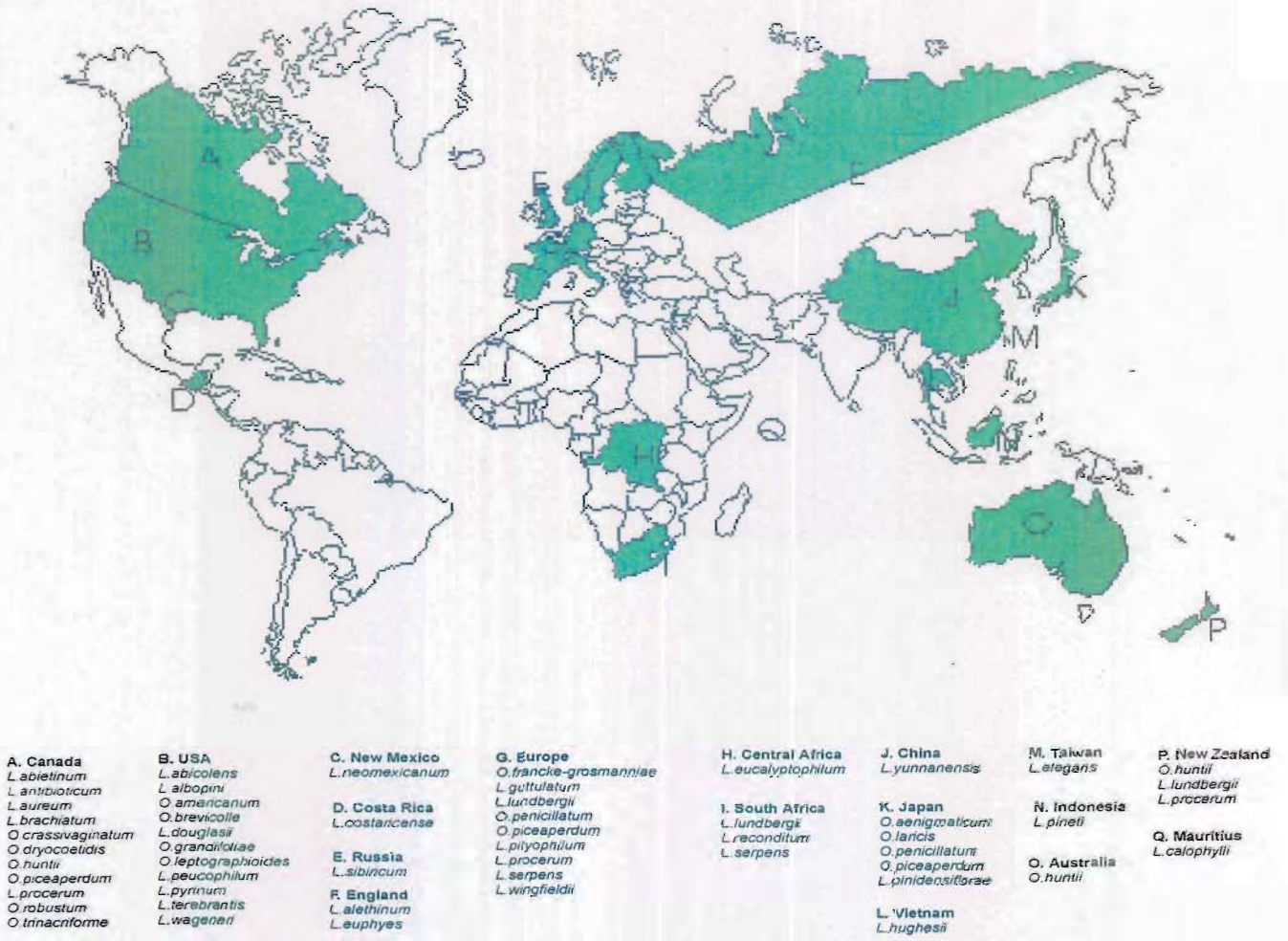

Fig. 11. World map showing the distribution of known *Leptographium* spp.

Table 2. Hosts associated with *Leptographium* spp. and *Ophiostoma* with *Leptographium* anamorphs.

Fungus	Host	Reference
Leptographium abietinum	*Melia* sp.	Kendrick, 1962
	Picea mariana	"
	Picea engelmannii	Davidson, 1955; Solheim 1995b
	Picea glauca	Solheim, 1995b
	Pseudotsuga menziesii	Mielke, 1979; Lewinsohn *et al.*, 1994; Solheim & Krokene, 1998
	Pinus contorta	Mielke, 1979
	Pinus monticola	Kulhavy *et al.*, 1978; Mielke, 1979
	Pinus sylvestris	Mielke, 1979
	Pinus ponderosa	"
	Pinus aristata	"
	Pinus mugo	"
Leptographium abicolens	*Abies balsamea*	Jacobs *et al.*, 1999
Ophiostoma aenigmaticum	*Picea jezoensis*	Jacobs *et al.*, 1998
Leptographium albopini	*Pinus edulis*	Wingfield *et al.*, 1994
	Pinus strobus	"
Leptographium alethinum	*Picea* spp.	Jacobs *et al.*, 1999
Ophiostoma americanum	*Larix decidua*	Jacobs *et al.*, 1998
Leptographium antibioticum	*Pinus contorta*	Mielke, 1979
	Pinus monticola	Kulhavy *et al.*, 1978; Mielke, 1979
	Abies lasiocarpa	Mielke, 1979
	Abies balsamea	Harrington, 1988
	Pinus albicaulis	Mielke, 1979
Ophiostoma aureum	*Pinus contorta var. latifolia*	Robinson-Jeffrey & Davidson, 1968
	Pinus ponderosa	Harrington, 1988

Table 2. cont.

Leptographium brachiatum	*Pinus edulis*	"
	Pseudotsuga menziesii	Kendrick, 1962
	Picea mariana	"
	Pinus pinaster	Morelet, 1986
	P. strobus	"
	P. sylvestris	"
Ophiostoma brevicolle	*Populus tremuloides*	Davidson, 1958
Leptographium calophylli	*Calophyllum inophyllum* var. *tacamaha*	Webber *et al.*, 1999
Leptographium costaricense	Rhizosphere of *Talauma sambuensis*	Weber, Spaaij & Wingfield, 1996
Ophiostoma crassivaginatum	*Picea mariana*	Griffin, 1968; Olchowecki & Reid, 1974
	Picea glauca	Olchowecki & Reid, 1974
	Pinus resinosa	"
	Pinus strobus	"
	Pinus sylvestris	"
	Populus grandidentata	Griffin, 1968
	Populus tremuloides	Griffin, 1968; Hinds, 1972
	Fraxinus nigra	Olchowecki & Reid, 1974
Leptographium douglasii	*Pseudotsuga menziesii*	Wingfield *et al.*, 1994
Ophiostoma dryocoetidis	*Abies lasiocarpa*	Kendrick & Molnar, 1965; Molnar, 1965
Leptographium elegans	*Chamaecyparis formosensis*	Wingfield *et al* ., 1994
Leptographium eucalyptophilum	*Eucalyptus urophylla* X *E. pellita* hybrid	Jacobs *et al.*, 1999
Leptographium euphyes	*Pinus* spp.	Jacobs *et al.*, 1999
Ophiostoma francke-grosmanniae	*Quercus* spp.	Davidson, 1971

Table 2. cont.

Ophiostoma grandifoliae	*Fagus grandifolia*	Davidson, 1976
Leptographium guttulatum	*Pinus sylvestris*	Wingfield & Gibbs, 1991; Jacobs *et al.*, 1999
	Picea abies	Jacobs *et al.*, 1999
Leptographium hughesii	*Parashorea plicata*	Kendrick, 1962; Jacobs *et al.*, 1999
Ophiostoma huntii	*Pinus contorta* var. *latifolia*	Robinson-Jeffrey & Grinchenko, 1964; Solheim, 1995c
	Pinus strobus	Davidson & Robinson-Jeffrey, 1965
	Pinus ponderosa	"
	Pinus monticola	"
	Pinus banksiana	Olchowecki & Reid, 1974
	Picea mariana	"
Ophiostoma laricis	*Larix* sp.	Van der Westhuizen *et al.*, 1995
	Larix kaempferi	Yamaoka *et al.*, 1998
Ophiostoma leptographioides	*Quercus* spp.	Davidson, 1942
Leptographium lundbergii	*Pinus* spp.	Lagerberg *et al.*, 1927
	Pinus densiflora	Kaneko & Harrington, 1990
	Pinus ponderosae	Rumbold, 1931
	Pinus taeda	Wingfield & Marasas, 1983; Wingfield *et al.*, 1988
	Pinus pinaster	Morelet, 1986
	Pinus radiata	Wingfield & Marasas, 1983
	Pinus strobus	Wingfield & Marasas, 1983; Morelet, 1986
	Pinus sylvestris	Morelet, 1986; Wingfield & Gibbs, 1991
	Pinus thunbergii	Kaneko & Harrington, 1990
	Larix leptolepis	Bakshi, 1950
	Picea spp.	Lagerberg *et al.*, 1927
	Picea abies	Bakshi, 1950; Hallaksela, 1977
Leptographium neomexicanum	*Pinus ponderosa*	Wingfield *et al.*, 1994

Table 2. cont.

Ophiostoma penicillatum	*Abies lasiocarpa*	Davidson, 1958
	Picea sp.	Mathiesen, 1951; Mathiesen-Käärik, 1960; Aoshima, 1965
	Picea abies	Grosmann, 1931; Goidanich, 1936; Siemaszko, 1939; Kendrick, 1962; Solheim, 1986; 1992a, 1993
	Picea jezoensis	Yamaoka *et al.*, 1997
	Pinus sp.	Mathiesen, 1951; Mathiesen-Käärik, 1960; Aoshima, 1965
	Pinus contorta	Mielke, 1979
	Pinus monticola	Kulhavy *et al.*, 1978; Mielke, 1979
	Pinus strobus	Morelet, 1986
	Pinus sylvestris	Mielke, 1979; Morelet, 1986
	Pinus pinaster	Morelet, 1986
	Pinus ponderosa	Mielke, 1979
Leptographium peucophilum	*Picea rubra*	Jacobs *et al.*, 1999
Ophiostoma piceaperdum	*Picea abies*	Solheim, 1986, 1992a; 1993
	Picea glauca	Rumbold, 1936
	Picea mariana	Wright & Cain, 1961
	Picea jezoensis	Yamaoka *et al.*, 1997
	Pinus glauca	Wright & Cain, 1961
	Pinus nigra	Hutchison & Reid, 1988
	Pinus radiata	"
	Pinus resinosa	Wright & Cain, 1961; Griffin, 1968
	Pinus strobus	"
	Pinus sylvestris	"
	Pinus taeda	Hutchison & Reid, 1988
	Pinus banksiana	Olchowecki & Reid, 1974
	Pseudotsuga menziesii	Davidson & Robinson-Jeffrey, 1965
Leptographium pineti	*Pinus* spp	Jacobs *et al.*, 1999
Leptographium pityophilum	*Pinus nigra*	Jacobs *et al.*, 1999

Table 2. cont.

Leptographium procerum	*Abies grandis*	Lane & Goheen, 1979
	Picea abies	Hallaksela, 1977; Alexander *et al.*, 1988
	Picea fraseri	Alexander *et al.*, 1988
	Pinus banksiana	Kendrick, 1962; Wingfield, 1982, 1983; Alexander *et al.*, 1988
	Pinus contorta	Mielke, 1979; Alexander *et al.*, 1988
	Pinus clausa	Barnard *et al.*, 1985; Alexander *et al.*, 1988
	Pinus echinata	Horner & Alexander, 1983a; Alexander *et al.*, 1988
	Pinus elliotii	"
	Pinus monticola	Alexander *et al.*, 1988
	Pinus nigra	Lackner & Alexander, 1982; Wingfield, 1982; Alexander *et al.*, 1988
	Pinus pinaster	Morelet, 1986
	Pinus ponderosa	Mielke, 1979; Wingfield, 1982; Alexander *et al.*, 1988
	Pinus radiata	Mackenzie & Dick, 1984
	Pinus resinosa	Kendrick, 1962; Towers, 1977; Sinclair & Hudler, 1980; Halambek, 1981; Wingfield, 1982; Harrington, 1988; Alexander *et al.*, 1988
	Pinus strobus	Kendrick, 1962; Houston, 1969; Towers, 1977; Shaw & Dick, 1980; Sinclair & Hudler, 1980; Livingston & Wingfield, 1982; Wingfield, 1982; Lackner & Alexander, 1982; Horner & Alexander, 1983a, b; Lackner & Alexander, 1984; Mackenzie & Dick, 1984; Alexander *et al.*, 1983, 1988; Smith, 1991
	Pinus sylvestris	Wingfield & Gibbs, 1991; Wingfield, 1982; Lackner & Alexander, 1984; Horner & Alexander, 1983b; Harrington, 1988; Alexander *et al.*, 1988
	Pinus taeda	Horner & Alexander, 1983a; Alexander *et al.*, 1988
	Pinus virginia	"
	Pseudotsuga menziesii	Mielke, 1979; Morrison & Hunt, 1988; Alexander *et al.*, 1988
Leptographium pyrinum	*Pinus ponderosa*	Davidson, 1978
Leptographium reconditum	*Triticum* rhizosphere	Jooste, 1978

Table 2. cont.

Ophiostoma robustum	*Pinus ponderosa*	Robinson-Jeffrey & Davidson, 1968
Ophiostoma serpens	*Pinus monticola*	Gill *et al.*, 1951
	Pinus nigra	Morelet, 1988
	Pinus taeda	Gill *et al.*, 1951
	Pinus sylvestris	Goidanich, 1936; Kendrick, 1962; Morelet, 1988; Wingfield & Gibbs, 1991
	Pinus pinaster	Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1980; Wingfield *et al.*, 1988
	Pinus pinea	Wingfield *et al.*, 1988
	Pinus radiata	Wingfield & Knox-Davies, 1980; Wingfield & Marasas, 1980; Wingfield *et al.*, 1988
	Pseudotsuga menziesii	Mielke, 1979
Leptographium sibiricum	*Abies sibirica*	Jacobs *et al.*, 1999
Leptographium terebrantis	*Pinus sylvestris*	Highley & Tattar, 1985; Highley & Tattar, 1987; Bennet & Tattar, 1988
	Pinus thunbergiana	"
	Pinus taeda	Barras & Perry, 1971a
	Pinus banksiana	Wingfield, 1983
	Pinus ponderosa	Harrington, 1988
	Pinus resinosa	Wingfield, 1983; Bennet & Tattar, 1988; Harrington, 1988
	Pinus edulis	Harrington, 1988
	Pinus strobus	Wingfield, 1983; Harrington, 1988
	Pseudotsuga menziesii	Harrington, 1988
Ophiostoma trinacriforme	*Pinus monticola*	Parker, 1957a
Leptographium wageneri	*Abies grandis*	Mielke, 1979
	Larix occidentalis	"
	Picea glauca	Morrison & Hunt, 1988
	Picea engelmannii	"
	Pinus aristata	Mielke, 1979

Table 2. (cont.)

Leptographium wageneri (cont.)	*Pinus attenuata*	Smith & Graham, 1975
	Pinus contorta	Cobb & Platt, 1967; Smith & Graham, 1975; Goheen & Hansen, 1978; Mielke, 1979; Hunt & Morrison, 1986
	Pinus edulis	Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Smith & Graham, 1975; Landis & Helburg, 1976; Walters & Walters, 1977
	Pinus jeffreyi	Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Smith & Graham, 1975
	Pinus lambertiana	Smith & Graham, 1975
	Pinus monophylla	"
	Pinus monticola	Kulhavy *et al.*, 1978; Smith & Graham, 1975; Mielke, 1979
	Pinus ponderosa	Wagener & Mielke, 1961; Kendrick, 1962; Cobb & Platt, 1967; Goheen, 1976; Goheen & Cobb, 1978; Goheen & Hansen, 1978; Mielke, 1979
	Pinus strobus	Smith & Graham, 1975
	Pinus sylvestris	Mielke, 1979
	Pseudotsuga menziesii	Miller & Veirs, 1968; Mielke, 1979; Smith & Graham, 1975; Hansen, 1978
	Tsuga heterophylla	Morrison & Hunt, 1988
	Tsuga mertensiana	Leaphart, 1960; Byler *et al.*, 1983; Goheen & Hansen, 1978
Leptographium wingfieldii	*Pinus sylvestris*	Morelet, 1988; Wingfield & Gibbs, 1991; Solheim & Långström, 1993
	Pinus brutia	Morelet, 1988
	Pinus strobus	"
	Pinus densiflora	Masuya *et al.*, 1998
Leptographium yunnanensis	*Pinus yunnanensis*	Zhou *et al.*, 1999
	Pinus gaoshanensis	"
	Pinus shimaonensis	"

LABORATORY METHODS FOR *LEPTOGRAPHIUM*

Leptographium spp. can be isolated from four main sources. These include lesions associated with disease symptoms, soil around roots of diseased trees, insects such as bark beetles and from within beetle galleries, inluding blue-stained wood underneath beetle galleries. *Leptographium* spp. sporulate profusely on wood, and cultures can be obtained through direct transfer of the gloeioid conidial masses. Their presence in soil and on insects is not obvious and specialized media and techniques have been developed for their isolation. The ability of *Leptographium* spp. to tolerate high concentrations of cycloheximide provides a valuable aid in isolation (Fergus, 1956, Harrington, 1981; Marais, 1996). Most other fungi can not grow on cycloheximide, and this antibiotic is, therefore, routinely included in media for the isolation of *Leptographium* or *Ophiostoma* spp.

Some *Leptographium* spp. are conspicuous due to their large, dark, macronematous conidiophores, whereas others have small, more lightly pigmented conidiophores, which are not readily observed. Conidiophore morphology can vary, depending on the type of medium used. Malt extract agar (1-2%) normally results in good sporulation (Harrington, 1992; Wingfield & Marasas, 1980; Wingfield *et al.,* 1994; Jacobs *et al.,* 1998). Some species, such as *L. wageneri,* sporulate best when the fungus is cultured on a rich medium (MEA) before being transferred to water agar (Harrington, 1992). Some species only sporulate well in the presence of host tissue. This can be achieved by using pine twig medium (PTM) or by placing sterilized de-barked pine twigs on the surface of the growth medium. This is particularly helpful for isolates of, for example *O. huntii* and *O. piceaperdum.* In cases where teleomorphs are associated with *Leptographium* spp., using PTM or pine twigs on the medium sometimes induces the formation of perithecia. Some authors have also reported using potato dextrose agar (PDA) to grow *Leptographium* spp., but we have found that this medium leads to the formation of abundant aerial mycelium. This makes the identification and study of *Leptographium* difficult.

Culture media for *Leptographium*

Malt extract agar (MEA)

Malt extract	20 g
agar	15 g
distilled water	1000 ml

MEA (1-2%) is generally sufficient to support the growth and sporulation of most *Leptographium* spp.

Potato dextrose agar (PDA) (Singleton, Mihail & Rush, 1992).

Peeled potatoes	200 g
agar	15 g
dextrose	20 g
distilled water	1000 ml

Add the peeled potatoes to 500 ml of the water and autoclave. Strain the autoclaved potatoes through cheesecloth. Add the rest of the water to a final volume of 1 l. Add the agar and dextrose and autoclave again. It is important to note that the cultural characters differ when grown on MEA and PDA. PDA induces the formation of abundant aerial mycelium which can mask the production of conidiophores. Commercially available PDA also gives results different to those associated with laboratory prepared PDA.

Cycloheximide-streptomycin-malt-agar (CSMA) (Harrington, 1992).

Malt extract	10 g
agar	15 g
cycloheximide	200 mg
streptomycin	100 mg
distilled water	1000 ml

This medium should be used when isolations are made from natural substrates, soil or insects. Both cycloheximide and the streptomycin should be added after autoclaving (Harrington, 1992). For the isolation of *L. wageneri,* using 800 ppm (0.8 g/l) cycloheximide and 200 ppm (0.2 g/l) streptomycin sulfate in PDA (pH 4.0) has been suggested (Hicks, 1973; Hicks, Cobbs & Gersper, 1980). For the production

of perithecia in culture, Hutchison and Reid, (1988), suggested the addition of thiamine (100 µg/ml), pyridoxine (100 µg/ml) and biotin (50 µg/ml) to the medium.

Pine twig medium (PTM) (Harrington, 1992).

debarked pine twigs	
agar	15 g
distilled water	1000 ml

Debarked pine twigs are cut to 1-2 cm pieces and split longitudinally. The twigs are autoclaved for 30 minutes (or alternatively twice for 15 minutes with a 24 h interval). The autoclaved twigs are aseptically placed, facing upwards, in Petri dishes. Autoclaved water agar is poured over the twigs until they are just covered. Cycloheximide and streptomycin (see CSMA) can be added to the medium to minimize contamination during prolonged incubation (Harrington, 1992). This medium promotes sporulation and in some cases induces the formation of perithecia.

***Leptographium procerum* selective medium (LPSM)** (Swai & Hindal, 1981).

Glucose	2.0 g
Fe^{++}	0.2 mg
Zn^{++}	0.2 mg
Mn^{++}	0.1 mg
chlortetracycline hydrochloride	50 mg
cycloheximide	50 mg
streptomycin sulfate	50 mg
agar	20 g
distilled water	1000 ml

This selective medium has been used to isolate *L. procerum* from symptomatic trees as well as from soil.

Media used to produce *nit*-mutants (Zambino & Harrington, 1990).

Basal medium (BM)

Glucose	20 g
KH_2PO_4	1.0 g

$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$CaCl_2$	0.1 g
trace element solution	0.2 ml
vitamin solution	10 ml

Trace element solution

Citric acid	5.0 g
$ZnSO_4$	5.0 g
$Fe(NH_4)_2(SO)_4.6H_20$	1.0 g
$CuSO_4.5H_2O$	0.25 g
$MnSo_4.H_2O$	50 mg
H_3BO_4	50 mg
$NaMoO_4.2H_2O$	50 mg
distilled water	95 ml

Vitamin solution

Thiamin HCl	0.1 mg
pyridoxine HCL	0.075 mg
biotin	0.005 mg per 1.0 % ethanol

Complete medium (CM)

Basal medium with 1.0 g asparagine added

Nitrate minimal medium with Triton X-100 (MMT)

Basal medium with 1.0 g $NaNO_3$ and 2 ml Triton X-100 added.

Nit-mutants are obtained by growing wild type isolates on CM that contains 1.5 % $KClO_4$. Fast growing areas are hyphal tipped and incubated on malt-yeast extract medium containing chlorate. A complementation test is done by placing two mutant strains adjacent to each other on minimal medium (MMT). After a few weeks of growth, the plates can be examined for a dense band of aerial mycelium, indicating complementation (Puhalla, 1985; Zambino & Harrington, 1990).

Isolations from natural substrates

Most species of *Leptographium* occur on conifers. These species can be found associated with lesions on stems or roots, sporulating in the galleries of bark beetles or in the soil surrounding roots. Isolations from samples should be made as soon as possible after collection, because more aggressive secondary fungi tend to colonize the specimens. Samples can, however, also be stored at 4 °C for up to two weeks (Harrington, 1992).

Methods for isolation of *Leptographium* spp. have been described by several authors. Samples are taken from the canker face or blue-stained area after the bark has been removed. Small pieces of wood can be placed in moisture chambers (wet filter paper in a Petri dish) and incubated for 10 days to induce conidiophore production (Anderson & Alexander, 1979; Solheim, 1986). Conidial masses form at the apices of conidiophores and can then be transferred to agar (MEA or WA) using a sterile needle (Seifert, *et al.,* 1993).

Slivers of wood or small pieces of diseased tissue or cambium adjacent to beetle galleries can be placed directly on CSMA. The cycloheximide and streptomycin inhibit most other fungi as well as bacteria and allow *Leptographium* spp. to grow (Wingfield, 1983; Solheim & Långström, 1991; Harrington, 1992). Conidiophores develop on the host tissue, or arise from the mycelium, that has grown onto the medium. Drops of conidia can then be lifted from conidiophores and transferred onto MEA or WA. An alternative means to purify cultures is to cut hyphal tips and to transfer these to new plates (Harrington, 1992; Seifert, *et al.,* 1993). Isolates of *Leptographium* spp. can be incubated between 20 and 25 °C. Harrington (1992) noted that most species, other than *L. wageneri,* grow well at these temperatures. *Leptographium wageneri* grows best at 15 °C and temperatures above 30 °C can be lethal to isolates of this species.

When isolations are made from ascospores at the apices of perithecia, it is a good practice to make a permanent slide of the perithecium from which the isolation has been made. In this way, morphology of the teleomorph can be correlated with anamorph features. This is especially useful in isolates where the teleomorph is not readily produced in culture and might never be seen after the isolation is made (Seifert, *et al.,* 1993).

Isolations from soil

Leptographium spp. occurring in soil are generally found in close proximity to the roots of infected trees. After collection of the soil sample, a dilution series is made and plated on CSMA (Swai & Hindal, 1981; Wingfield, 1983). For isolations of certain *Leptographium* spp. such as *L. wageneri,* Hicks *et al.* (1980) proposed a medium containing 800 µg/ml cycloheximide and 200 µg/ml streptomycin sulfate. Swai and Hindal (1981) used a selective medium (LPSM) with great success to isolate *L. procerum* from the soil.

Isolations from insects

Several methods have been described for trapping of insects that carry *Leptographium* spp. and *Ophiostoma* spp. (Wingfield, 1983; Bèdard *et al.,* 1990; Krokene, 1996) and these will not be discussed in any detail here. Harrington (1992) recommended the use of "Stickem-special" sticky traps because these do not appear to be toxic to *Leptographium* spp. Other methods include pitfall traps, trap logs or freshly cut wood bolts, buried in the soil (Harrington, 1992).

After the insects have been collected, there are several techniques that can be used to isolate *Leptographium* spp. from them. Insects can be crushed and placed directly on CSMA (Gibbs, & Inman, 1991; Wingfield & Gibbs, 1991; Harrington, 1992). To minimize contamination from other sources, the insects are washed in 1% sodium hypochlorite solution containing Tween 80 for 5 min before they are placed on CSMA (Wingfield, 1983). Alternatively, the insects can be ground in a small amount of sterile distilled water. From this slurry of water and insect parts, a dilution series can then be made and plated onto CSMA. This technique is useful when quantifying the number of propagules that are transmitted by beetles (Harrington, 1992).

Insects that carry these fungi, can be place on natural media, such as logs. The fungi are then allowed to colonise the logs. Isolations can be made from these media (Furniss *et al.,* 1990; Krokene & Solheim, 1996).

Genetic studies

Mating compatibility

Leptographium spp. have *Ophiostoma* teleomorphs and typically have a heterothallic mating system. In some species where *Ophiostoma* states are known, it is possible to determine the mating compatibility between different strains of the same species or between different species. In order to do mating studies, it is necessary to work with single ascospore cultures. To make single ascospore cultures a single drop of ascospores is removed from the apex of a perithecium. The ascospores are suspended in 5 ml of sterile water and shaken vigorously. In some cases it might be necessary to use a vortex mixer to disperse spores. The spore suspension can then be transferred to plates (MEA or WA) and dispersed thoroughly using an inoculating needle or a glass rod with the basal end bent at 90° to the main axis ("hockey" stick) and incubated for 12-24 hours. After incubation, germinating ascospores can be viewed under a dissection microscope, and can be aseptically transferred, using a sterile needle, to fresh plates. After about 24 h, single ascospore cultures are usually visible. From these small colonies, hyphal tips can be aseptically transferred onto fresh plates (Fig. 12).

To test mating compatibility, single ascospore isolates can be paired in different combinations as well as with themselves. Small blocks of medium are cut from the single ascospore isolates, and placed alongside each other on fresh plates, and incubated. PTM is recommended for these studies, as most *Ophiostoma* spp. do not produce teleomorphs readily in culture. Pine twigs (or other relevant host tissue) placed alongside the inoculum can also induce the formation of perithecia. Where perithecia form in single ascospore cultures, that have not been paired with other isolates, this is usually an indication of homothallism (Jacobs *et al.,* 1998; Seifert *et al.,* 1993). Physically wounding the medium can also stimulate the formation of perithecia.

An alternative technique to test for mating compatibility, is to incubate one mating type of an *Ophiostoma* sp. until it covers the plate. A spore suspension is made from the opposite mating type culture and this is then spread onto the recipient

culture, which then results in the formation of perithecia on the plates (Seifert *et al.*, 1993). Using this technique and reciprocal paring, it is possible to determine whether isolates are female fertile (Leslie & Klein, 1996; Britz, 1997).

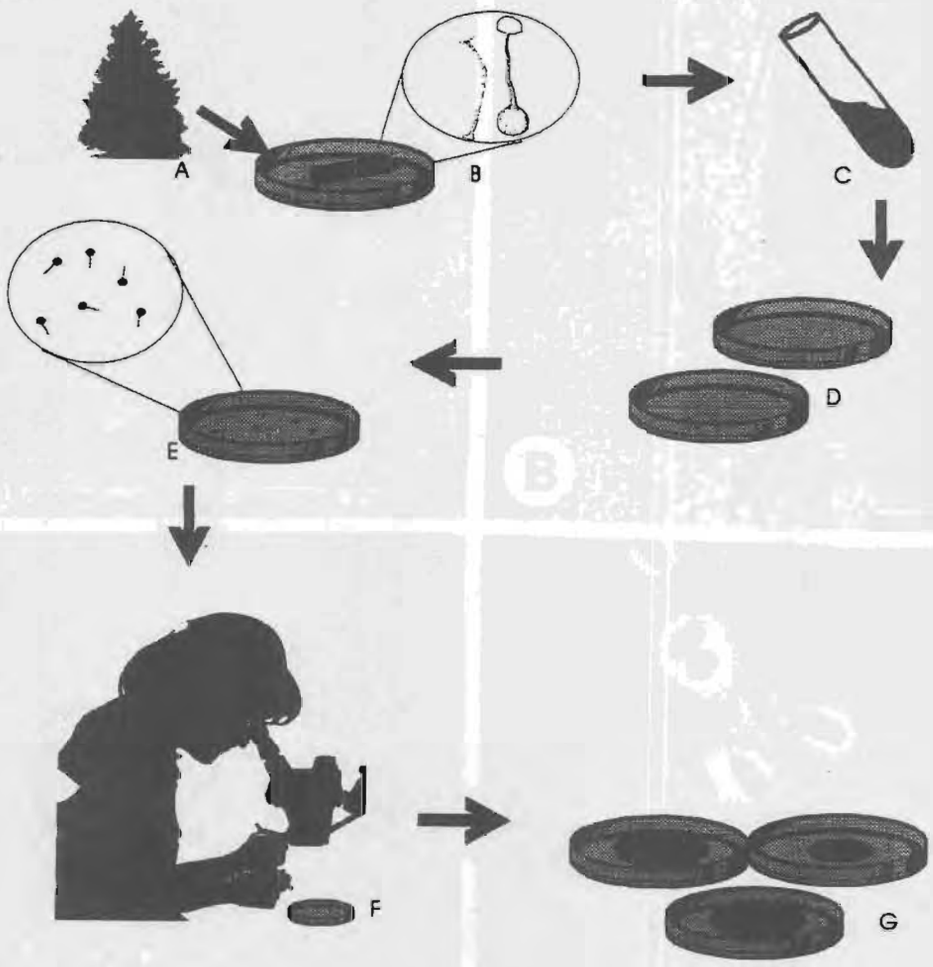

Fig. 12. Preparation of single ascospore/conidial cultures. **(A)** Plant material (pieces of bark including beetle galleries or wood pieces) are placed into moisture chambers **(B)** until the onset of sporulation. The gloeioid masses of spores at the apices of the conidiophores or perithecia are then carefully lifted from these structures with a sterile needle and suspended in sterile water **(C)**. The water is then spread over the surface of 2% MEA plates amended with 0.5 g/l cycloheximide and incubated **(D)**. The germinating spores can be lifted from plates with a needle after approximately 12 -24 hours **(E)** and transferred to clean MEA plates **(F)**.

Vegetative compatibility

In studies of vegetative compatibility, the choice of medium is important and it is necessary to test many media in order to find one in which VCG's and be visualised (Seifert *et al.,* 1993). To adequately test media, wild-type single ascospore isolates of a species can be paired against themselves and other isolates to observe interaction zones (Seifert *et al.,* 1993). Vegetative compatibility tests have not been extensively used in studies of *Leptographium.*

Zambino and Harrington (1990) used *nit*-mutants to study vegetative compatibility in *Leptographium wageneri.* This method exploits the use of nitrate non-utilizing (*nit)* mutants to indicate compatibility between isolates. Paring of complementary *nit*-mutants on minimal medium results in the development of abundant aerial mycelia. Cultures are examined for a dense band of aerial mycelia between the plugs, indicating complementation (Seifert *et al.,* 1993). This method has proved to be especially useful in *Fusarium,* as well as several other genera (Puhalla, 1985; Corell, Klittich & Leslie, 1987; 1989; Klittich & Leslie, 1988; Leslie, 1993; Hawthorne & Rees-George, 1996).

Storage of cultures

Efficient maintenance and long term storage of cultures of *Leptographium* spp. are extremely important. Cultures can be stored in the number of different ways. Generally, best results are achieved by duplicates stored using a variety of techniques, although this might not always be economically feasible. Fungi with complex conidiophores, such as *Leptographium* spp., tend to lose the capacity to produce these structures during the process of extended subculturing. To reduce this degradation, conidia, rather than mycelial plugs should be transferred to fresh plates (Seifert, *et al.,* 1993).

Most *Leptographium* spp. survive well on 2 % MEA slants, maintained at 4 °C. In our laboratory, we store all our isolates in triplicate in small McCartney bottles on MEA slants. One of each set is sealed with cigarette paper to prevent mite infestation (Snyder & Hansen, 1946). In a second bottle, an agar slant, covered

with mycelial growth, is overlaid with sterile mineral oil. A third isolate is stored in water. In the case of storage in water, the cultures are grown on MEA or PDA. Small blocks are then cut from the agar and transferred to sterile water. These are then maintained at 4 °C. Although water storage appears to be efficient, a common problem with this technique is contamination.

Lyophilisation provides an excellent method to store *Leptographium* spp. and we maintain a subset of isolates in this form. The method for storage that we recommend is described by Joubert and Britz (1987). A conidial suspension is prepared by adding 2 ml sterile, antibiotic-and endospore-free skim milk/lactose (12 %/ 5% m/v) solution to the culture. This solution is then added to sterile 6.0 mm assay disks in small ampoules. The tubes with the solution are freezed at - 20 °C and dried under vacuum. The ampoules are then sealed under vacuum and stored at -20 °C. Cultures have been shown to remain viable for up to 35 years using this method (Joubert & Britz, 1987).

SPECIES AND THEIR IDENTIFICATION

Leptographium spp. are notoriously difficult to identify. This is primarily because these fungi are morphologically similar and a comprehensive treatment of the group has not been available since the monograph of Kendrick (1962). In addition, numerous species can grow together in nature and mixed cultures are a common problem. The use of single spore cultures is, therefore, an absolute necessity (Wingfield *et al.*, 1988) (Fig. 12). This ensures that isolates are pure. In our key to *Leptographium* spp., emphasis has been placed on conidial morphology, primary branch patterns, presence and absence of rhizoids and conidiophore lengths. We have found that these characters are relatively stable and enable accurate identification of species. Correct interpretation of these characters (Figs. 13-16) is, however, crucial.

Hughes (1953) recognized the importance of conidial morphology and conidium development as taxonomic characters for Hyphomycetes including members of the *Leptographium* complex. Based on different modes of conidium development, he placed various genera of Hyphomycetes in groups. In *Leptographium* spp., conidia

are all produced through sympodial development of the conidiogenous cells but with delayed secession. Distinct scars representing the outer conidial walls, give a false appearance of percurrent proliferation. (Wingfield, 1985; Van Wyk *et al.*, 1988). Conidium development does not appear to provide a useful taxonomic characteristic in *Leptographium.*

Conidial shape

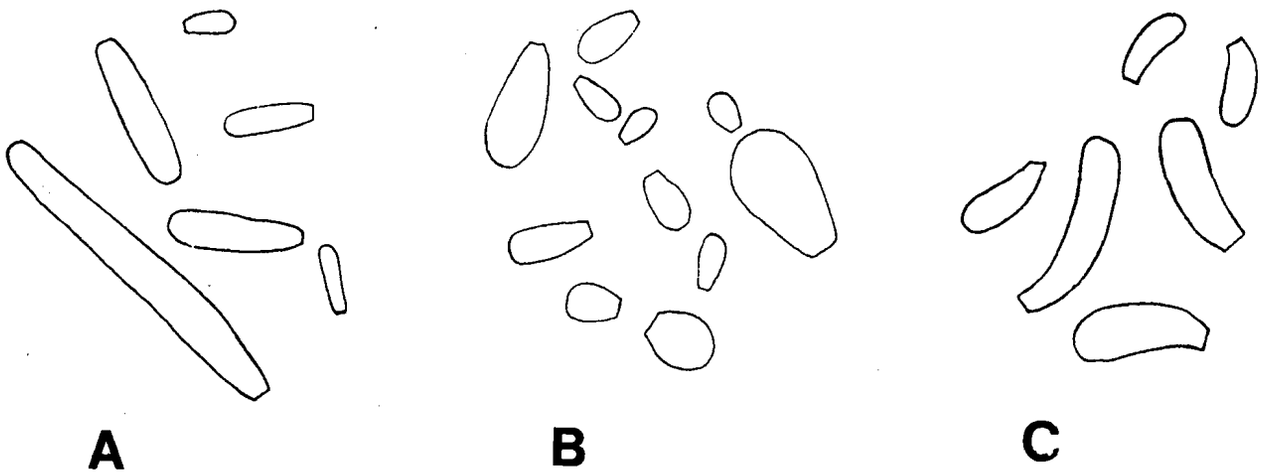

Fig. 13. Three categories of conidial shape found in *Leptographium* spp. **Type A** represents all species with long oblong to obovoid conidia. **Type B** represents species with obovoid conidia. **Type C** represents those with distinctly curved conidia.

Species of *Leptographium* can be divided into three distinct groups based on conidial shape (Fig. 13). The first of these (type A), includes all the species with oblong to obovoid conidia. This group is characterized by oblong conidia where the base of the conidium approximates the same size as the apex of the spore. In some cases, obovoid and oblong conidia are observed in the same isolate. Obovoid conidia have bases that are narrower than their apices. The second group (type B) includes those species with only obovoid conidia. No oblong conidia are observed in isolates of these species. Conidia in these species can, in most cases, also be placed in the category of *Leptographium* spp. with small conidia. The last group (type C) is characterized by species with distinctly curved conidia. Conidia in

Conidial size

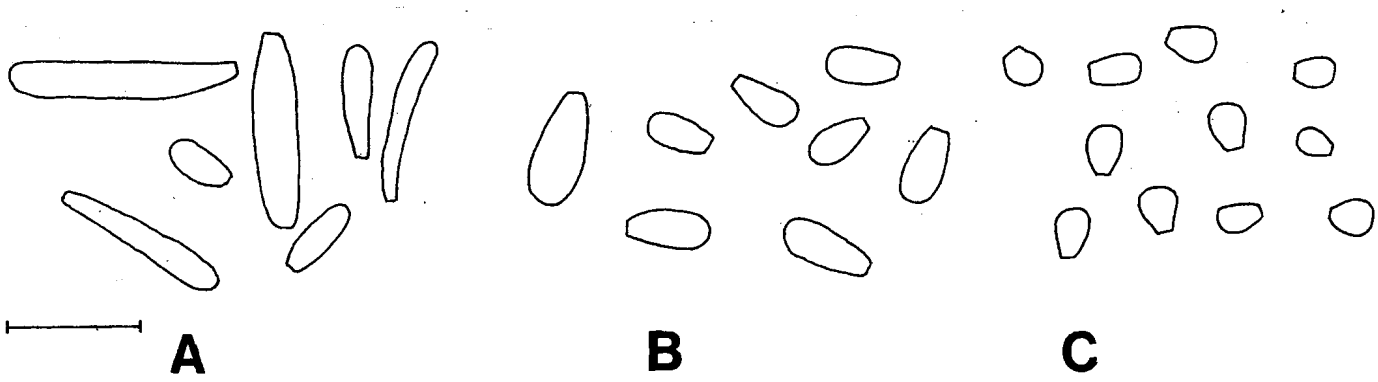

Fig. 14. Three different categories of conidial length. **A.** Long conidia are between 6 and 20 µm. **B.** Medium-sized conidia are between 5 and 12 µm. **C.** Small conidia are between 3 and 6 µm.

Conidia of *Leptographium* spp. can be divided into three groups based on conidium size. Although three distinct size groups can be distinguished, namely short, medium and long, the ranges within species can also overlap. Therefore, the sizes of the groups are given as ranges and 15-30 conidia need to be measured in order to determine the appropriate category of conidial length for an isolate (Fig. 14).

Primary branch patterns

Primary branch patterns provide a useful character for identifying *Leptographium* spp. Three distinct patterns of primary branching are found. Type A includes all species with only two primary branches. Type B includes species with two or more primary branches. Type C includes all species with more than two branches, where one of these branches is a large central branch at least twice as thick as the other primary branches. In this group, a single isolate can also display occasional

conidiophores with only two branches resembling type A or B. The majority of conidiophores should, however, be of type C (Fig. 15).

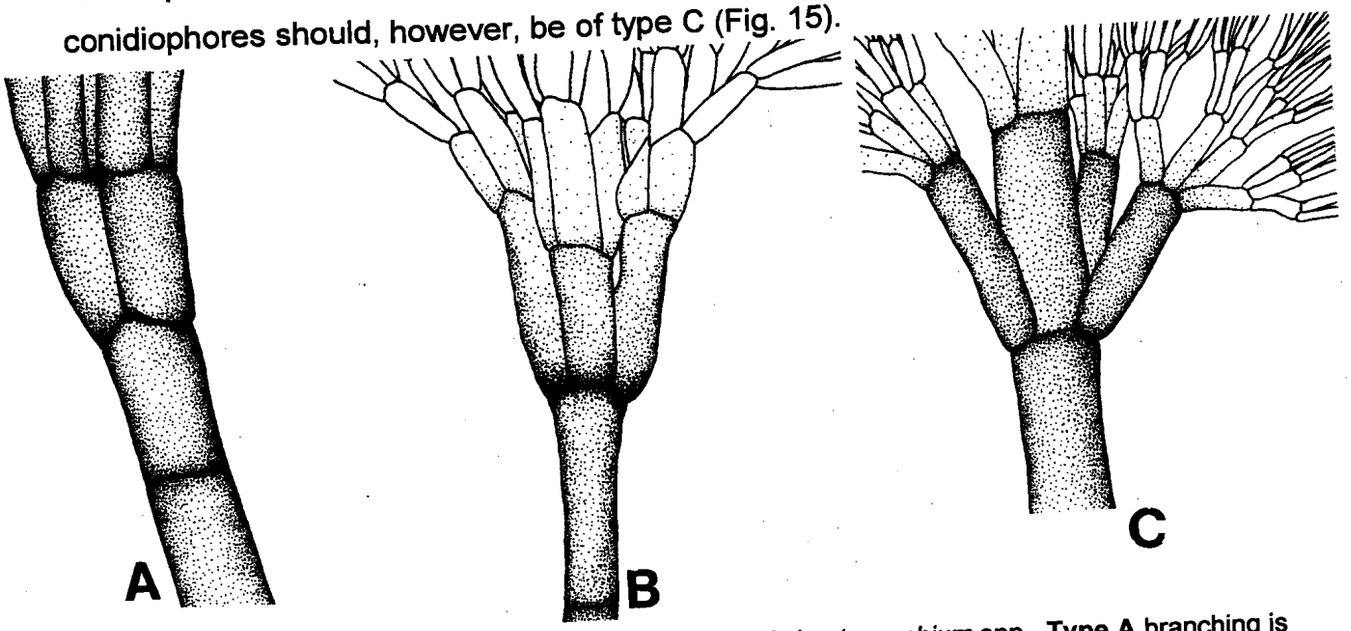

Fig. 15. Primary branch patterns can be used to distinguish *Leptographium* spp. **Type A** branching is found in species with only two branches. **Type B** branching is characterized by two or more branches. **Type C** branching is characterized by more than two branches with a single large branch in the middle.

Rhizoids

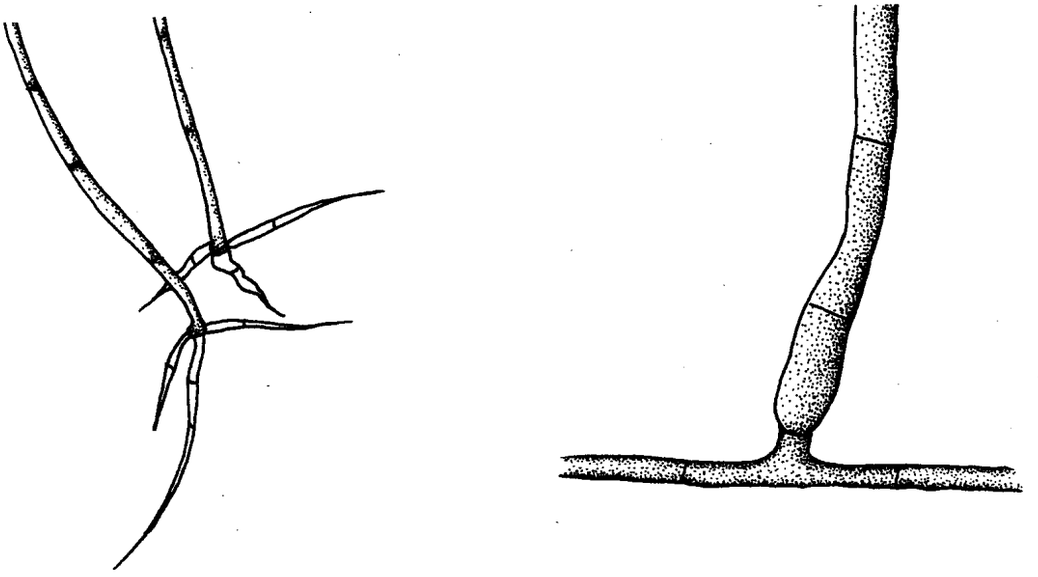

Fig. 16. Rhizoids in *Leptographium* spp. can either be present or absent.

The presence or absence of rhizoids at the base of conidiophores is a useful character in identifying *Leptographium* spp. Here, rhizoids are defined as mycelium-like outgrowths at the bases of conidiophores. Where rhizoids have been indicated as absent, the cell at the base of conidiophores grows continuos with the mycelium that gives rise to the conidiophore (Fig. 16

Cycloheximide tolerance

Species of *Ophiostoma* and *Leptographium* are able to tolerate high concentrations of cycloheximide in culture (Harrington, 1981; Marais, 1996). This antibiotic is, therefore, frequently included in selective media, when these fungi are isolated (Swai & Hindal, 1981). Tolerance to high levels of cycloheximide is a consistent character for most species of *Leptographium* although there are a small number of species that are sensitive to low concentrations of the antibiotic (e.g. *L. antibioticum, L. brachiatum* and *L. costaricense*) (Harrington, 1981, 1988; Weber *et al.,* 1996). This might suggest that these species are not appropriately placed in *Leptographium* and are not members of the Ophiostomatales. In the case of *L. costaricense,* this suggestion is strengthened by the fact that this species occurs in soil, in contrast to most other species of *Leptographium* that predominantly occur on woody substrates associated with insect activity. In this study, cycloheximide tolerance was tested at a concentration of 0.05 g/l. The tolerance of is expressed as a percentage of the control.

Cycloheximide tolerance provides a useful taxonomic characteristic for *Leptographium* spp. It also appears to be correlated with the presence of cellulose in the cell walls of most of the fungi (Horner, Alexander & Julian, 1986; Marais, 1996). *Leptographium* spp. are also characterized by the presence of rhamnose,

mannose, galactose and glucose in their cell walls. This is similar to the cell walls of *Ophiostoma* spp., and confirms the close association of these genera (Marais & Wingfield, 1999a).

Molecular characteristics

Zambino and Harrington (1992) distinguished between different species in *Leptographium* using isozyme analysis. Although this technique was shown to be valuable in distinguishing between species, variable success has been obtained in other genera of fungi. The data of Zambino and Harrington (1992) supported the synonymy of *L. serpens* and *L. alacris* as proposed by Wingfield and Marasas (1981) as well as the suggestion that *L. abietinum* and *L. engelmannii* Davidson are synonyms (Harrington, 1988; Jacobs *et al.* 1999). Furthermore, a low level of relatedness was observed among species representing the four ascospore morphology groups as defined by Olchowecki and Reid (1974). Isozyme analysis also proved useful in distinguishing between *L. douglasii, L. albopini* and *L. neomexicanum,* which are morphologically very similar (Wingfield *et al.,* 1994). This technique could also differentiate between the three varieties of *L. wageneri* (Zambino & Harrington, 1992). Similarly, Witthuhn *et al.* (1997) could distinguish between the varieties of *L. wageneri* using RAPD's.

Strydom, Wingfield & Wingfield (1997) used ribosomal DNA sequences to support the synonymy of *L. truncatum* and *L. lundbergii.* Isolates of these species had been shown to be morphologically similar and indistinguishable from each other. This similarity was confirmed through the phylogenetic analysis of sequence data for isolates of these species. Ribosomal DNA sequences have also proved to be useful in distinguishing *L. guttulatum* from *L. penicillatum.* Isolates of *L. guttulatum*

were thought to be what Mathiesen (1950) had described as a variety of *L. penicillatum,* known as *L. penicillatum* f.sp. *palliati.* DNA analysis, however, showed that *L. guttulatum* is a distinct taxon, and not related to *L. penicillatum* (Jacobs *et al.,* 1999).

Recent studies have compared a large number of *Leptographium* spp. based on sequences of the ITS2 and 28S genes of the ribosomal DNA operon (Jacobs, Wingfield & Wingfield, unpublished). Large sub-unit sequence showed that all species considered are members of the Ophiostomatales and are most likely anamorphs of *Ophiostoma.* ITS sequence data confirmed that 43 species considered, represents distinct taxa. Species previously synonomised (e.g. *L. abietinum* and *L. engelmannii*) were confirmed to be the same. No clear natural groupings emerged, although pathogenic species appeared to be most closely related to each other. There was no apparent correlation between groups defined based on sequence data and those emerging from phylogenetic analysis of morphological features.

MATERIALS AND METHODS

All available herbarium type specimens, in addition to living isolates of described *Leptographium* spp. were examined in this study. Cultures of *Leptographium* spp., included in this study have been collected over a period of approximately 20 years by M.J. Wingfield. Most of these specimens were isolated during field studies in many parts of the world and others were obtained from a variety of culture collections and colleagues. Working with herbarium specimens included the typical limitations of incomplete collections and poor specimens. *Leptographium* spp. in general do not keep well as herbarium specimens due to the fact that

conidiogenous apparatuses tend to break off, or fall apart, leaving only stipes and parts of the conidiophores intact. In a small number of instances, herbarium material could not be traced or appears not to exist, and these species have not been included in this study.

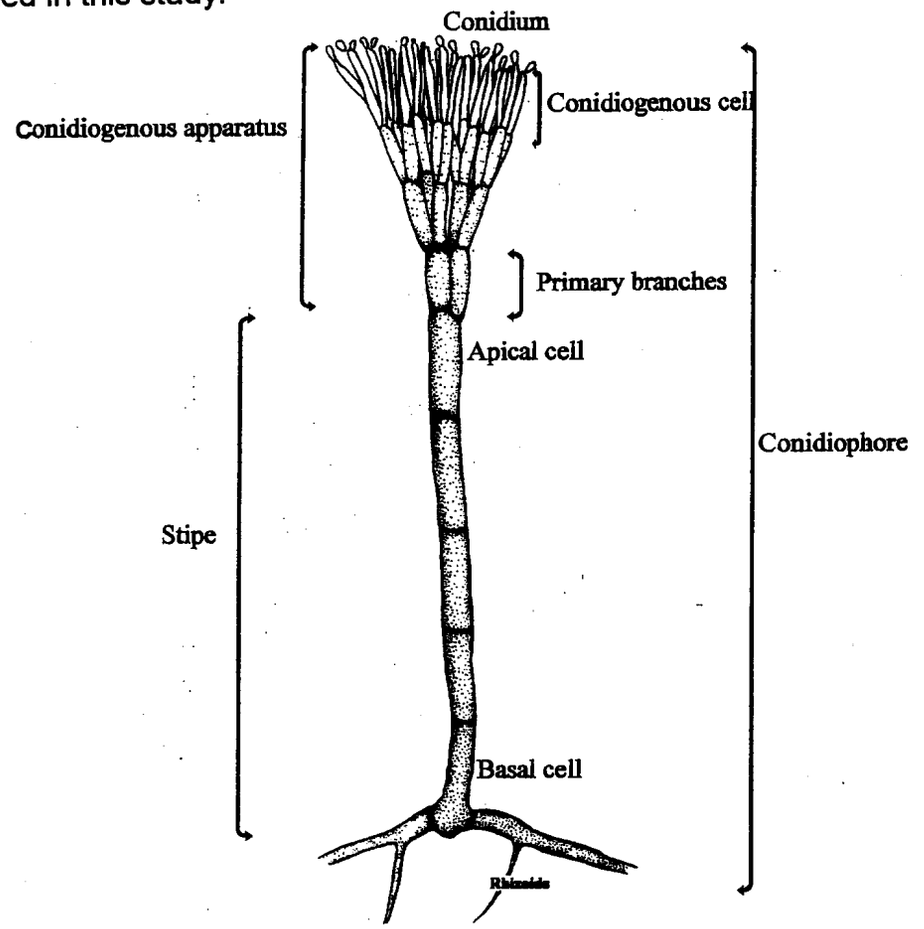

Fig. 17. Typical conidiophore of a *Leptographium* spp.

Descriptions of species were done from fungal cultures grown on 2% MEA. For microscopy, relevant structures were mounted in lactophenol, as well as in distilled water on glass slides. Herbarium specimens were examined by placing a drop of 1% KOH on the dried tissue. After five minutes, small pieces of fungal tissue were removed and mounted in lactophenol on glass slides. Fifty measurements of each relevant morphological structure were made. For some species, teleomorph structures were not produced in culture, and herbarium material included only a

small number of perithecia. In these cases, we referred back to previous studies to provide complete descriptions. Colors were determined using the colour charts of Rayner (1970). Structures that were measured and that are considered useful characteristics of *Leptographium* spp., are shown in Fig. 17.

Typical isolates of all the *Leptographium* spp. under consideration were examined using 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, dehydrated in a graded acetone series and critical-point dried. Specimens were mounted and coated with gold palladium alloy and examined using a JSM 840 Scanning Electron Microscope.

Isolates chosen to determine growth characteristics were those that sporulate best and are representative for the species. The optimal growth temperatures for these isolates were determined by inoculating eight MEA plates with 6.0 mm diam. agar disks taken from the actively growing margins of fresh isolates. Plates were incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Colony diameters were measured after 4, 7 and 9 days (unless indicated otherwise) and growth was computed as an average of eight readings. Cycloheximide tolerance of these isolates was determined on MEA plates (8 per isolate) amended with 0.5g/l cycloheximide. The plates were incubated at 25°C and colony diameters were measured after 8 days.

In this study we include 46 taxa including the three varieties of *L. wageneri.* Our dichotomous key to all species includes not only morphological characteristics, but also details of hosts or substrates. This might be considered unusual but many *Leptographium* spp. are highly host or substrate specific and we argue strongly that this information is crucial to species identification. We also provide a separate dichotomous key to those species with known *Ophiostoma* states and a synoptic key to all species. We believe that the three sets of keys and detailed descriptions will make it possible for researchers to identify species of *Leptographium.*

KEY TO SPECIES BASED ON HOST AND MORPHOLOGY

1. Host/substrate non-coniferous _____ 2
   1'. Host coniferous _____ 10

2. Conidia oblong (type A) or obovoid (Type B) _____ 3
   2'. Conidia oblong, occasionally curved (Type C); colony with abundant aerial mycelia _____ *L. hughesii*

3. Conidia oblong (type A) _____ 4
   3'. Conidia obovoid (type B) _____ 5

4. Arrangement of primary branches (Type A) _____ *O. brevicolle*
   4'. Arrangement of primary branches (Type B) _____ 8

5. Arrangement of primary branches (Type B) _____ 6
   5'. Arrangement of primary branches (Type C) _____ *L. reconditum*

6. Conidiophore length: (50-)100-250(-300) µm _____ 7
   6'. Conidiophore length: (150-)250-1000(-1500) µm _____ *L. costaricense*

7. Conidiogenous cell appearing phialidic _____ *O. francke-grosmanniae*
   7'. Conidiogenous cells proliferating percurrently _____ *O. leptographioides*

8. Conidiophore length: (-50)100-300(-500) µm _____ 9
   8'. Conidiophore length: 50 - 100 µm _____ *L. calophylli*

9. Conidial size 2.5 - 5 µm; rhizoids present _____ *O. grandifoliae*
   9'. Conidial size 6 - 9 µm, rhizoids absent _____ *L. eucalyptophilum*

10. Conidia oblong to allantoid, occasionally curved (Type C) _____ 11
    10'. Conidia oblong or obovoid, occasionally ellipsoid (type A or B) _____ 12

11. Conidial size: (3-)4-6(-7) µm _____ *L. abietinum*

11'. Conidial size: (4-)6-10(-12) µm _____ *O. penicillatum*

12. Conidia obovoid to ellipsoid (type A) _____ 13

12'. Conidia obovoid (type B) _____ 28

13. Conidial size: (3-)4-8(-12) µm _____ 14

13'. Conidial size: (6-)10-20(-22) µm _____ 27

14. Conidial size: (3-)4-6(-8) µm _____ 15

14' Conidial size: (4-)6-8(-12) µm _____ 17

15. Arrangement of primary branches (Type B) _____ 16

15'. Arrangement of primary branches (Type C) _____ *L. neomexicanum*

16. Conidiophore length: (50-)100-250(-400) µm _____ 22

16'. Conidiophore length: (150-)250-1000(-1500) µm _____ *L. albopini*

17. Arrangement of primary branches (Type A) _____ *L. brachiatum*

17'. Arrangement of primary branches (Type B) _____ 18

18. Conidiophore length: (50-)100-250(-400) µm _____ 19

18'. Conidiophore length: (150-)250-1000(-1500) µm _____ 20

19. Hyphae smooth _____ *L. terebrantis*

19'. Hyphae roughened with granular appearance _____ *L. yunannensis*

20. Conidiogenous apparatus consisting of distinct series of branches, no teleomorph present _____ 21

20'. Conidiogenous apparatus consisting of a long indistinct series of branches, teleomorph in the genus *Ophiostoma* _____ *O. aureum*

21. Conidia prominently guttulate _____ *L. guttulatum*

21'. Conidia not guttulate _____ *L. wingfieldii*

22. Rhizoids present _____ 23
22'. Rhizoids absent _____ 24

23. No association with insects _____ *L. antibioticum*
23'. Associated with insects _____ 26

24. Hyphae smooth, no teleomorph present _____ 25
24'. Hyphae roughened by granular material _____ *O. crassivaginatum*

25. Prominent *Sporothrix* synanamorph present _____ *L. elegans*
25'. Prominent *Sporothrix* synanamorph absent _____ *L. sibiricum*

26. Optimal growth temperature below 20°C, colonies slow growing, associated with the conifer swift moth _____ *L. abicolens*
26'. Optimal growth temperature 25°C, associated with bark beetle activity _____ *L. euphyes*

27. Arrangement of primary branches (Type A)
Conidiophore length: (150-)250-650(-800) µm _____ *O. americanum*
27. Arrangement of primary branches (Type B)
Conidiophore length: (25-)50-250(-300) µm _____ *O. dryocoetidis*

28. Conidia 2 - 6 µm long _____ 29
28'. Conidia frequently more than 6 µm and longer _____ 38

29. Arrangement of primary branches (Type B) _____ 30
29' Arrangement of primary branches (Type C) _____ 34

30. Rhizoids present _____ 31
30'. Rhizoids absent _____ 32

31. Colonies fast growing and characterized by concentric rings in culture _____ *L. procerum*
31' Colonies slow growing, concentric rings in culture not present _____ *L. peucophilum*

32. Primary branches lower on stipes _____ *L. lundbergii*
32'. Primary branches on the apex of the stipes _____ 33

33. Conidiophore length: 50 - 100 µm,
Ophiostoma teleomorph present _____ *O. robustum*
33'. Conidiophore length: 100 - 200 µm,
Ophiostoma teleomorph absent _____ *L. pineti*

34. Isolates with distinctly serpentine hyphae _____ *L. serpens*
34'. Isolates without serpentine hyphae _____ 35

35. Only found on *Pinus* spp. _____ 36
35'. Only found on *Pseudotsuga menziesii* _____ *O. wageneri* var. *pseudotsugae*

36. Only found on *Pinus ponderosa* _____ *L. wageneri* var. *ponderosum*
36'. Only found on soft pines i.e. *Pinus*
monophylla, P. monticola and *P. sylvestris* _____ 37

37. Conidiophore length: 600 - 1000 µm _____ *L. wageneri* var. *wageneri*
37'. Conidiophore length: 100 - 600 µm _____ *L. pityophilum*

38. Conidiophores up to 400 µm long _____ 39
38'. Conidiophores frequently much longer than 400 µm _____ 42

39. *Ophiostoma* teleomorph known _____ 40
39'. No teleomorph present _____ *L. pyrinum*

40. Ascospores hat-shaped _____ 41
40'. Ascospores not hat-shaped but reniform _____ *O. laricis*

41. Perithecia with distinct neck, up to 800 µm long _____ 44
41'. Perithecia with no or very short neck _____ *O. trinacriforme*

42. Rhizoids present _____ *L. douglasii*

42'. Rhizoids absent _____ 43

43. *Ophiostoma* teleomorph present _____ *O. huntii*
43'. Teleomorph absent _____ *L. charles*

44. Hat-shaped ascospores with elongated brims, occurs on *Larix* sp. _____ *O. aenigmaticum*
44'. Hat-shaped ascospores without elongated brims, occurs on species of *Pinus* and *Picea* _____ *O. piceaperdum*

Many *Leptographium* spp. are known to have *Ophiostoma* teleomorphs. In most cases these structures are not regularly produced in culture. When the teleomorphs are present, these can aid in the identification of *Leptographium* spp. However, the absence of a teleomorph does not necessarily imply that a teleomorph does not exist.

DICHOTOMOUS KEY TO SPECIES WITH *OPHIOSTOMA* TELEOMORPHS

1. Species characterized by cucullate sheaths around the ascospores _____ 2
1'. Species characterized by curved sheaths around the ascospores _____ 11

2. Conidia of *Leptographium* state less than 5 µm long _____ 4
2'. Conidia of *Leptographium* state more than 5 µm long _____ 8

3. Perithecial necks less than 500 µm long _____ 4
3'. Perithecial necks more than 500 µm long _____ 6

4. Perithecial necks 150-500 µm in length _____ 5
4'. No obvious perithecial neck _____ *O. robustum*

5. Occurs on conifers _____ *O. brevicolle*
5'. Occurs on non-coniferous host _____ *O. francke-grosmanniae*

6. Occurs on conifers _____ 7
   6'. Occurs on non-coniferous hosts _____ *O. grandifoliae*

7. Conidia of the *Leptographium* state needle-shaped _____ *O. americanum*
   7'. Conidia of the *Leptographium* state obovoid _____ *O. serpens*

8. Perithecial necks 150-500 µm long _____ 9
   8'. Perithecial necks 500-1000 µm long _____ 10

9. Ostiolar hyphae present _____ *O. dryocoetidis*
   9'. Ostiolar hyphae absent _____ *O. penicillatum*

10. Habitat mainly on *Pinus* spp. _____ *O. wageneri*
    10'. Habitat mainly on *Larix* spp, infested with *Ips* spp. _____ *O. laricis*

11. Perithecial neck less than 500 µm long _____ 12
    11'. Perithecial necks more than 500 µm long _____ 16

12. Perithecial necks distinct and 150-500 µm long _____ 13
    12'. Perithecial neck absent _____ 15

13. Conidia of the *Leptographium* state up to 5 µm long _____ *O. aenigmaticum*
    13'. Conidia of the *Leptographium* state more than 5 µm long _____ 13

14. Habitat mostly conifers _____ *O. crassivaginatum*
    14'. Habitat non-coniferous _____ *O. leptographioides*

15. Conidiogenous apparatus with indistinct branches,
    conidial masses appearing bright yellow in culture _____ *O. aureum*
    15'. Branches of conidiogenous apparatus distinct _____ *O. trinacriforme*

16. Perithecia readily formed in culture,
    homothallic, colony with serpentine hyphae _____ *O. piceaperdum*
    16'. Perithecia not readily formed in culture, _____

heterothallic, colony with straight hyphae _____ *O. huntii*

SYNOPTIC KEY TO *LEPTOGRAPHIUM* SPECIES

Synoptic keys are not as widely used as dichotomous keys. These keys can, however, be valuable in the identification of *Leptographium* spp. Use of synoptic keys in conjunction with dichotomous keys and species descriptions, should enable the user to correctly identify species, even in the absence of the teleomorph. These keys are especially useful, where some data for important characteristics are lacking. The value of synoptic keys versus dichotomous keys was discussed in detail by Korf (1972) and the relevant arguments will not be repeated here.

The synoptic key used in this monograph has been based on those proposed and used by P.W. Leenhout (Jacobs, 1966), Korf (1972), Korf & Zhuang (1985) and Wolfaardt, Wingfield and Kendrick (1992). The key can be entered at any point. When a character has been identified, the numbers listed under the character should be noted. The user should then proceed to the next character that corresponds to the unknown species. The numbers under the second character state that do not occur in the first set of the numbers should be omitted. The user should then proceed to the next character and repeat the procedure. This should be repeated until only one or two numbers remain. The numbers correspond to species listed at the end of the key (Jacobs, 1966; Korf, 1972). The unknown species should then be compared with the description of those species

Teleomorph characters
a. Teleomorph absent: 1, 2, 4, 5, 7, 9, 11, 12, 14, 16 - 18, 21, 22, 26, 27, 29, 31 - 36, 39, 40, 43 - 46
b. Teleomorph present: 3, 6, 8, 10, 13, 15, 19, 20, 23, 24, 25, 28, 30, 37, 38, 41, 42

Perithecial characters:
Base diameter
a. 50 -100 µm: 13, 42
b. 100 -300 µm: 3, 6, 10, 15, 19, 20, 23 - 25, 28, 30, 37, 41, 42
c. 300 - 500 µm: 6, 8, 23, 24, 30, 37, 38, 41, 42

Perithecial neck
a. Absent or very short (less than 10 µm): 8, 37, 41
b. Present: 3, 6, 10, 13, 15, 19, 20, 23 - 25, 28, 30, 38, 42

Perithecial neck length
a. 50 - 100 µm: 13
b. 100 - 300 µm: 3, 10, 15, 19, 23, 25, 30
c. 300 - 500 µm: 3, 15, 23, 24, 28, 30, 38, 42
d. 500 - 700 µm: 6, 15, 20, 23, 24, 30, 38, 42
e. 700 - 900 µm: 6, 20, 23, 24, 30, 42
f. more than 900 µm: 6, 20, 24
g. no neck: 8, 37, 41

Ascospore shape
a. cucullate appearance: 3, 8, 19, 23, 30, 41
b. curved appearance: 6, 10, 13, 15, 20, 24, 25, 28, 37, 38, 42

Ascospore length
a. 2 - 4 µm: 6, 8, 19, 20, 23, 30, 37, 38, 41
b. 4 - 6 µm: 6, 3, 8, 10, 15, 20, 30, 37, 38, 41
c. 6 - 8 µm: 15, 24, 25, 28
d. more than 8 µm: 13, 24

Ascospore width
a. 1 -2 µm: 6, 10, 19, 20, 23, 38, 41
b. 2 - 3 µm: 3, 6, 8, 15, 24, 28, 30, 37, 41
c. 3 - 4 µm: 3, 8, 24, 25
d. 4 - 5 µm: 13

Anamorph characters
Hyphae
a. constricted at the septa: 3, 4, 8, 13 - 15, 18, 23, 26, 34, 38, 40, 41, 43, 45,
b. not constricted at the septa: 1 - 3, 5 - 7, 9 - 13, 16 - 46

Conidiophore length
a. less than 100 µm: 2, 9, 11, 13, 14, 15, 19, 20, 25, 27, 31, 37, 46
b. 100 - 200 µm: 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 35, 36, 37, 39, 40, 41, 45, 46
c. 200 - 400 µm: 1, 2, 3, 4, 6, 7, 8, 10, 12, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 38, 39, 40, 41, 45, 46
d. 400 - 600 µm: 2, 4 - 6, 8, 12, 14, 16, 17, 21 - 23, 26 - 29, 33, 34, 36, 38, 40 - 42, 45
e. 600 - 800 µm: 4 - 6, 8, 12, 21 - 23, 26, 27, 33, 34, 36, 38, 41 - 45
f. 800 - 1000 µm: 4, 5, 8, 22, 27, 38, 42 - 44
g. 1000 - 1500 µm: 5, 8, 22, 38, 43, 44

Stipe length
a. less than 100 µm: 1 - 3, 7 - 11, 13 - 16, 19, 20, 22, 24 - 28, 30 - 32, 35, 37, 39 - 41, 46
b. 100 - 200 µm: 1 - 4, 6 - 10, 12, 14, 15 - 36, 39 - 41, 45, 46
c. 200 - 400 µm: 1, 2, 4, 6 - 8, 10, 12, 14, 16, 17, 18, 20 - 23, 26 - 29, 33 - 36, 38, 40, 41
d. 400 - 600 µm: 2, 4 - 6, 8, 12, 14, 17, 21 - 23, 26, 27, 29, 33, 34, 36, 38, 40 - 45
e. 600 - 800 µm: 4, 5, 6, 8, 21 - 23, 34, 36, 38, 42 - 45
f. 800 - 1000 µm: 4, 5, 22, 38, 42 - 44

g. 1000 - 1500 µm: 5, 22, 38

Stipe smooth
1, 2, 4 - 12, 14 - 46

Stipe constricted at the septa
3, 13, 46

Conidiogenous apparatus length
a. 10 - 30 µm: 2, 6, 7, 9, 10, 12 - 16, 19, 20, 22, 23, 25, 27, 31, 34, 39
b. 30 - 50 µm: 1 - 20, 22, 23, 25 - 29, 31 - 34, 36 - 41, 46
c. 50 - 80 µm: 1 - 8, 11, 13 - 18, 21, 22, 24, 26 - 34, 36 - 46
d. 80 - 100 µm: 1 - 4, 8, 16, 18, 21, 22, 24, 26, 28 - 30, 33 - 36, 40 - 46
e. more than 100 µm: 4, 8, 21, 24, 26, 29, 30, 35, 40, 43, 44 - 46

Rhizoids
a. present: 1, 3, 7, 9, 14, 18, 19, 20, 22, 25, 27, 29, 31, 34 - 36, 38
b. absent: 2, 4 - 6, 8, 10 - 13, 15 - 17, 21, 23, 24, 26, 28, 30, 32, 33, 37, 39 - 46

Primary branch type
a. Type A: 6, 9, 10
b. Type B: 1 - 8, 11 - 26, 28 - 32, 34, 35, 37, 39 - 41, 45, 46
c. Type C: 27, 33, 36, 38, 42 - 44

Number of primary branches
a. 2 branches: 1 - 46
b. 2 to 3 branches: 1 - 8, 11 - 46
c. 3 to 4 branches: 4, 5, 7, 12, 21, 27, 28, 31, 33, 35, 36, 38, 42 - 45
d. 4 to 5 branches: 4, 7, 28, 33, 36, 38, 42 - 44
e. more than 5 branches: 4, 38

Primary branch length
a. less than 10 µm: 1, 2, 4, 6, 7, 9 - 11, 13 - 15, 19, 20, 22, 23, 25, 29, 31, 36, 37, 39, 42, 46
b. 10 - 15 µm: 1 - 4, 6, 7, 9 - 20, 22 - 44, 46
c. 15 - 20 µm: 1 - 4, 6, 8 - 18, 21 - 46
d. 20 - 25 µm: 1 - 4, 8, 9, 13, 14, 16 - 18, 21 - 24, 26 - 33, 34 - 36 38 - 45
e., 25 - 30 µm: 1, 3, 5, 8, 17, 18, 21 - 24, 26, 28, 30, 34, 35, 37, 40 - 44
f. 30 - 35 µm: 5, 8, 18, 21, 22, 26, 30, 34, 35, 37, 40 - 44

Secondary branch length
a. less than 10 µm: 1, 2, 4, 6, 7, 9 - 20, 22, 23, 25 - 29, 32 - 34, 36 - 40, 42 - 46
b. 10 - 15 µm: 1 - 7, 9 -11, 13 - 20, 22 - 30, 32 - 46
c. 15 - 20 µm: 2 - 6, 13, 14, 18, 21 - 24, 26 - 30, 33, 35 - 46
d. 20 - 25 µm: 3, 4, 5, 21, 24, 26, 28, 30, 35, 40 - 45
e. 25 - 30 µm: 5, 21, 24, 26, 35, 40, 43, 44
f. structure beyond primary branches long: 8

Tertiary branch length
a. less than 10 µm: 1 - 3, 6, 7, 11 - 18, 20, 22 - 24, 26 - 30, 32 -, 34, 36, 38 - 46
b. 10 - 15 µm: 1 - 7, 9, 11, 14 - 16, 18, 21 - 24, 26 - 30, 32 - 36, 38 - 46

c. 15 - 20 µm: 3 - 5, 11, 21, 23, 24, 26 - 28, 30, 35, 40, 41, 43 - 46
d. more than 20 µm: 3, 21, 24, 26, 30, 35, 40, 45, 46
e. to complex to measure: 8
f. not present: 10, 19, 25, 37

Quaternary branch length
a. less than 10 µm: 1, 2, 4 - 6, 14, 15, 18, 21 - 24, 28 - 30, 33, 34, 38, 40, 41, 45
b. 10 - 15 µm: 1, 2, 4 - 6, 15, 18, 21, 23, 24, 28 - 30, 33 - 35, 38, 41, 45, 46
c. 15 - 20 µm: 4, 5, 6, 21, 24, 30, 35, 45, 46
d. more than 20 µm: 21, 35
e. too complex: 8
f. not present: 3, 7, 9, 10 - 13, 16, 17, 19, 20, 25 - 27, 32, 36, 37, 39, 42 - 44

Conidiogenous cell length
a. less than 10 µm: 1, 6, 7, 8, 11 - 14, 17, 19, 22, 25, 27, 29, 32, 36, 37, 39, 42
b. 10 - 15 µm: 1 - 45
c. 15 - 20 µm: 1 - 9, 11, 12, 14 - 16, 18, 20 - 24, 26 - 31 33 - 42, 44 - 46
d. more than 20 µm: 1 - 6, 8, 11, 14, 16, 21, 23, 24, 26 - 28, 30, 35 - 37, 40, 41, 45, 46

Conidium shape
a. oblong to obovoid: 1, 4, 6 - 11, 13, 15, 16 - 19, 21, 22, 25, 26, 31, 34 - 39, 41 - 46
b. obovoid: 3, 5, 12, 14, 20, 23, 24, 27, 29, 30, 32, 33, 40
c. distinctly curved: 2, 22, 28

Conidial length
a. 3 - 5 µm: 1 - 7, 9 - 14, 16, 18 - 34, 36, 38 - 46
b. 5 - 7 µm: 1 - 6, 8, 10, 11, 13, 14, 17, 18, 21, 23, 24, 25, 28 - 31, 33, 35, 39 - 46
c. 7 - 10 µm: 3, 5, 6, 8, 13, 15, 17, 21, 23 - 25, 28, 30, 31, 35, 37, 40, 43, 44, 46
d. 10 - 12 µm: 6, 8, 15, 25, 31, 35, 37, 46
e. more than 12 µm: 6, 15, 37

Associated hosts/substrate
a. *Pinus* spp: 2, 4, 7, 8, 13, 18, 21, 23, 26 - 28, 30 - 35, 37, 38, 40 - 42, 44 - 46
b. *Picea* spp.: 2, 3, 9, 13, 21, 23, 26, 28, 29, 30, 34
c. *Larix* spp.: 6, 24, 26, 44
d. *Pseudotsuga* spp.: 2, 9, 14, 30, 34, 38, 40, 43
e. *Abies* spp.: 1, 5, 7, 15, 28, 34, 39, 44
f. other conifers: 7, 16
g. non-conifers: 10, 11, 17, 19, 20, 22, 25, 36

Association with insects
a. Associated with insects: 1 - 6, 8, 10, 13 - 15, 18, 19, 21, 23, 24, 26, 28, 29 - 32, 34, 35, 37 - 40, 42 - 46
b. Not associated with insects: 7, 9, 11, 12, 16, 17, 20, 22, 25, 27, 33, 36, 41

Optimum growth temperature
a. 15 °C: 1, 29
b. 20 °C: 3, 5, 6, 8, 14, 31, 33, 36, 42 - 44
c. 25 °C: 2, 4, 9, 12, 15, 16, 18 - 24, 26, 27, 30, 32, 34, 35, 37 - 41, 45, 46
d. 30 °C: 7, 10, 11, 17, 25, 28

Ratio of the conidium length: width

a. 1.5:2:35, 37
b. 2:1: 3, 12, 17 - 19, 29, 33, 34, 38 - 40, 45, 46
c. 2.5:1: 1, 4, 7 - 11, 13, 14, 20, 22 - 24, 27, 30 - 32, 36, 41 - 44
d. 3:1: 5, 21, 25, 28
e. 4:1: 6
f. 5:1: 15
g.4:3: 26

1. *L. abicolens*
2. *L. abietinum*
3. *O. aenigmaticum*
4. *L. albopini*
5. *L. alethinum*
6. *O. americanum*
7. *L. antibioticum*
8. *O. aureum*
9. *L. brachiatum*
10. *O. brevicolle*
11. *L. calophylli*
12. *L. costaricense*
13. *O. crassivaginatum*
14. *L. douglasii*
15. *O. dryocoetidis*
16. *L. elegans*
17. *L. eucalyptophilum*
18. *L. euphyes*
19. *O. francke-grosmanniae*
20. *O. grandifoliae*
21. *L. guttulatum*
22. *L. hughesii*
23. *O. huntii*
24. *O. laricis*
25. *O. leptographioides*
26. *L. lundbergii*
27. *L. neomexicanum*
28. *O. penicillatum*
29. *L. peucophilum*
30. *O. piceaperdum*
31. *L. pinidensiflorae*
32. *L. pineti*
33. *L. pityophilum*
34. *L. procerum*
35. *L. pyrinum*
36. *L. reconditum*
37. *O. robustum*
38. *O. serpens*
39. *L. sibiricum*
40. *L. terebrantis*
41. *O. trinacriforme*
42. *O. wageneri* var. *ponderosum*
43. *L. wageneri* var. *pseudotsuga*
44. *L. wageneri* var. *wageneri*
45. *L. wingfieldii*
46. *L. yunnanensis*

GENERIC DESCRIPTION FOR *LEPTOGRAPHIUM*

Leptographium Lagerb. & Melin Svenska Skogsvårdsföreningens Tidskrift. **25**, 249 1927.

= *Scopularia* Preuss. 1851.

= *Hantzschia* Auersw. 1862.

= *Verticicladiella* S. Hughes. *Canadian Journal of Botany* **31**, 653. 1953.

Teleomorph: *Ophiostoma* Sydow & P. Sydow. *Annales Mycologici* **17,** 43. 1919.

= *Rostrella* Zimmerm. *Meded's Lands Plantentuin* **37,** 24. 1900.

= *Endoconidiophora* Münch *Naturw. Zeitschrift Forst und Landw.* **6,** 34. 1908.

= *Linostoma* Von Höhnel. *Annales Mycologia* **16,** 91. 1918.

= *Grosmanniae* Goidanich. *Boll. Staz. Pat. Veg. Roma.* **16,** 26. 1936.

= *Ceratocystiopsis* H.P. Upadhyay & W.B. Kendr. *Mycologia* **67,** 800. 1975.

Etymology: Lep-to-grá-phi-um: a thin, small brush. From the greek adjective, λεπτος: thin and the greek noun γραφισν: a small brush. The generic name refers to the conidiophores of the genus that resemble small brushes.

Known distribution: U.S.A., Canada, Europe, Japan, East Asia, South Africa, Central Africa, New Zealand, Australia and Mauritius.

Conidiophores occurring singly or in groups of up to eight, arising directly from the mycelium or on aerial mycelium, erect, macronematous, mononematous, 30 - 1350 µm in length, rhizoid-like structures present or absent. *Stipes* smooth or occasionally constricted at septa, cylindrical, simple, 0-18 septate, apical and basal cells occasionally swollen. *Conidiogenous apparatus* 15 - 200 µm long, excluding the conidial mass, with 2 to 4 series of cylindrical branches, 2-6 primary branches,

cylindrical or barrel shaped, 0-2 septate. *Conidiogenous cells* discrete, 2-3 per branch, cylindrical, tapering slightly at the apex. Conidium development occurring through replacement wall building with holoblastic ontogeny and percurrent proliferation and delayed secession giving the false impression of sympodial proliferation (Minter *et al.*, 1982; 1983; Van Wyk *et al.*, 1988). *Conidia* hyaline, aseptate, obovoid to broadly ellipsoid with truncated ends and rounded apices occasionally prominently curved, 3 - 22 µm. Conidia accumulating in slimy droplets at the apex of conidiogenous apparatus. *Sporothrix* synanamorph only present in *Leptographium elegans.*

Colonies with optimal growth temperatures between 15°C and 30°C on 2% MEA. Able to withstand high concentrations of cycloheximide with no more than 80% reduction in growth on 0.5 g/l cycloheximide. Colony colour ranging from cartridge buff (19'f) to olivaceous (21''m). *Colony margins* smooth, laciniate, sinuate or effuse. *Hyphae* submerged on solid medium with very sparse aerial mycelium to abundant aerial mycelium in some species, olivaceous (21''m) to hyaline, smooth or roughened by granular material, straight, in certain cases serpentine, occasionally constricted at the septa.

Perithecial bases black, globose and smooth walled, unornamented or sparsely ornamented, 143 - 420 µm in diam., necks present or absent, necks dark brown to black, cylindrical with a slight apical taper, smooth, 117 - 1700 µm long, *ostiolar hyphae* present or absent. *Asci* prototunicate, hyaline, evanescent. *Ascospores* reniform, allantoid, cucullate or pillow -shaped, aseptate, hyaline, invested in a sheath, 3 - 11 µm.

Hosts/substrate: *Abies* spp., *Calophyllum* sp., *Chamaecyparis* sp., *Eucalyptus* spp., *Fagus* spp., *Larix* spp., *Melia* spp., *Parashorea* sp., *Picea* spp., *Pinus* spp., *Populus* spp., *Pseudotsuga* spp., *Quercus* spp., *Talauma* sp., *Triticum* rhizosphere, *Tsuga* spp.

Associated animals: **Nematodes**: *Bursaphelenchus* spp. **Insects**: **Coleoptera: Scolytidae**: *Dendroctonus* spp., *Dryocoetus* spp., *Ips* spp., *Hylastes* spp., *Hylurgops*

spp., *Myelophilus* spp., *Orthotomicus* spp., *Pachylobius* spp., *Pityogenes* spp., *Pityokteines* spp., *Pityophthorus* spp., *Polygraphus* spp., *Tomicus* spp., *Trypodendron* spp., *Xyleborus* spp. **Coleoptera: Lymexylidae**: *Hylecoetus* spp. **Coleoptera: Curculionidae**: *Hylobius* spp., *Pissodes* spp., *Steremnius* spp. **Coleoptera: Cerambycidae**: *Tetropium* spp., *Monochamus* spp. **Hymenoptera: Agaonidae:** *Blastophagus* spp. **Lepidoptera: Hepialidae**: *Korscheltellus* spp.

Type: *Leptographium lundbergii* (PREM 50548). See detailed description on page 220