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Pathogenicity of five species of *Ophiostoma* fungi to Douglas-fir

Fem blåvedsoppers patogenitet på Douglas

Erik Christiansen and Halvor Solheim

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Abstract

CHRISTIANSEN, E. and SOLHEIM, H. 1993. Pathogenicity of five species of *Ophiostoma* fungi to Douglas-fir. (Fem blåvedsoppers patogenitet på Douglas). Medd. Skogforsk. *47*(1):1-12.

The blue-stain fungus *Ophiostoma polonicum*, which is associated with the bark beetle *Ips typographus* in Scandinavia, can kill various species of spruce when artificially inoculated under the bark. Douglas-fir, normally not a host for this beetle, may also be conquered by *O. polonicum*. Here, we investigate whether *Ophiostoma* species other than *O. polonicum* can kill Douglas-fir after artificial inoculation. Young Douglas-fir trees growing in South Norway were mass-inoculated with five species of fungi, *O. bicolor, O. europhioides, O. minus, O. penicillatum*, and *O. polonicum*. Whereas *O. polonicum* killed three out of four trees, none of the other fungi proved lethal at the same load of infection.

Key words: Pathogenicity, *Ophiostoma*, blue-stain fungus, Douglas-fir, *Pseudotsuga menziesii*, artificial inoculation.

Utdrag

CHRISTIANSEN, E. and SOLHEIM, H. 1993. Pathogenicity of five species of *Ophiostoma* fungi to Douglas-fir. (Fem blåvedsoppers patogenitet på Douglas). Medd. Skogforsk. *47*(1):1-12.

Blåvedsoppen *Ophiostoma polonicum*, som er assosiert med granbarkbillen i Skandinavia, kan drepe ulike arter av gran når den inokuleres kunstig under barken. Douglas, som normalt ikke utnyttes av granbarkbillen, kan også bli erobret av denne soppen. Her undersøker vi om andre *Ophiostoma*-arter kan drepe Douglas etter kunstig inokulering. Unge Douglas-trær som vokste i Ås, Akershus, ble masse-inokulert med fem sopparter, *O. bicolor, O. europhioides, O. minus, O. penicillatum* og *O. polonicum*. Mens *O. polonicum* drepte tre av fire trær, var det ingen av de andre soppene som var dødelige ved den samme inokuleringsdosen.

Nøkkelord: Patogenitet, *Ophiostoma*, blåvedsopp, Douglas, *Pseudotsuga menziesii*, kunstig inokulering.

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Preface

Field work was carried out by Gunnar Fjone and Torfinn Sæther. G. Fjone also did laboratory work and computing. Lawrence R. Kirkendall read the manuscript critically, and also improved the language. The Department of Forestry of the Agricultural University of Norway provided experimental trees. All this assistance is gratefully acknowledged.

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Contents

Introduction

Fungi of the genus *Ophiostoma* H. Syd. & Syd. are commonly associated with bark beetles. Their sticky spores are carried externally on the beetles or on their phoretic mites, or pass through the digestive tract (Levieux et al. 1989; Moser et al. 1989; Furniss et al. 1990).

The pathogenicity of these fungi varies considerably. Of the species associated with the spruce bark beetle *Ips typographus* L. in Scandinavia, *Ophiostoma polonicum* Siem. stands out as the most pathogenic. When artificially mass-inoculated under the bark this fungus invades both xylem and phloem of Norway spruce (*Picea abies* (L.) Karst. and may, unlike other *Ophiostoma* species, kill the trees (Horntvedt et al. 1983; Christiansen 1985; Solheim 1988).

In spruce attacked by *I. typographus*, *O. polonicum* is consistently found at the leading edge of fungal penetration into the sapwood (Solheim 1992a). Its competitive superiority could be due to its ability to grow under oxygen-deficient conditions, i.e., in wet sapwood (Solheim 1991).

When artificially inoculated under the bark, *O. polonicum* may also kill other species of *Picea*, as well as Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. In Douglas-fir, the fungus can enter the sapwood and there spread tangentially, without producing a distinctive resinous reaction in phloem or xylem (Christiansen and Solheim 1990).

We wanted to investigate whether *O. polonicum* is superior to other *Ophiostoma* species in invading the sapwood of the non-host Douglas-fir, or if this superiority is limited to its conquest of spruce. Additional points of interest were the capability of the different fungal species to colonize phloem, and the apparent absence of an induced defence reaction in Douglas-fir.

Material and methods

Twenty trees were selected in a stand of *P. menziesii*, of the "gray" inland form (formerly termed *var. caesia*), located in an arboretum at Ås, 30 km south of Oslo (latitude ca. 60° N). The stand was planted in 1958 and thinned during the winter 1988-89. The tree chosen for the experiment were among the smaller in the stand, with a DBH of 11.9 ± 2.1 cm and a height of 11.6 ± 2.1 m (mean ± SD).

The trees were randomly assigned to five treatments, four trees per treatment. The treatments were inoculation with five species of *Ophiostoma: O. bicolor* Davids. & Wells, *O. europhioides* (Wright & Cain) H. Solheim, *O. penicillatum* (Grosm.) Siem., *O. polonicum*, and *O. minus* (Hedgc.) H. & P. Syd.. The first four species are all associated with *I. typographus* in Norway (Solheim 1992b), and have not been found in connection with Douglas-fir. *O. minus* is associated with different species of *Dendroctonus, Ips*, and *Tomicus* beetles (Käärik 1980; Solheim and Långström 1991) and occurs in Douglas-fir attacked by *Dendroctonus pseudotsugae* Hopkins (Rumbold 1936; Upadhyay 1981). Our strain of *O. minus*

originated from Scots pine (*Pinus sylvestris* L.) attacked by *T. piniperda* (L.) (SOLHEIM & LÅNGSTRÖM 1991).

On 25-27 June 1990 the trees were artificially inoculated with the fungi. On the stem section between 120 and 180 cm above ground, inoculation sites were marked out at a density of 8 per dm^2. Even spacing of the sites was achieved by using a template. Thus a tree with a DBH of 10 cm had about 150 inoculation points. At each site, a bark plug was removed with a 5 mm cork borer, malt agar holding fresh mycelium placed in the hole, and the plug returned. The particular inoculation density was used because this dose of *O. polonicum* had killed three out of four Douglas-firs in an earlier study (CHRISTIANSEN and SOLHEIM 1990).

The trees were felled on 27 September 1991, i.e., after 15 months. In the laboratory, two thin cross-sectional discs were cut from the inoculated stem sections (20 cm from the upper and lower margin, respectively). Stained and non-stained areas of the sapwood were measured with a planimeter, and percent blue-stained sapwood used as a measure of fungal proliferation. Diameter increment was measured along four radii on one disc, and growth recorded for the periods 1980-1984 and 1985-1989.

Within the inoculated stem sections, the outer bark was removed to expose necrotic zones on the phloem surface. The length of 40 or more zones per tree were measured.

Results

The stem increment was generally similar in the experimental trees, however, two *O. bicolor* and two *O. minus* trees showed a better growth than the others during the period 1985-1989.

Three trees began loosing their foliage during June-July 1991 and were completely denuded upon felling. They had all been inoculated with *O. polonicum*. The fourth tree treated with this fungus retained a green crown, as did all 16 trees inoculated with the other four fungi.

On the stem discs from the three dead trees all sapwood proved blue-stained (Fig. 1A). The discs from the fourth *O. polonicum* tree showed that the fungus had penetrated straight into the inner sapwood, which was completely stained (Fig. 1B). However, disregarding local necroses around the points of inoculation, the cambium had survived, and had laid down a fairly wide annual ring both during the summer of inoculation, and in the following year. This ring of fresh wood, amounting to 21% of the total sapwood cross-sectional area, had conducted sufficient water for the crown to survive.

The discs from trees inoculated with the four other fungi exhibited large sectors of desiccated sapwood but no staining. The degree of desiccation was about the same for these four treatments, and on an average less than 50% of the sapwood was recorded as fresh. The proportion of fresh to dry sapwood was not influenced by stem increment in the years prior to inoculation.

Resin exudation on the bark surface was slight, and the necrotic zones in bark and outer sapwood near the points of infection generally appeared to hold very little resin (not quantified). In some cases, small resin-filled pockets had developed in the phloem and outer xylem. Such pockets also occurred in trees killed by *O. polonicum*.

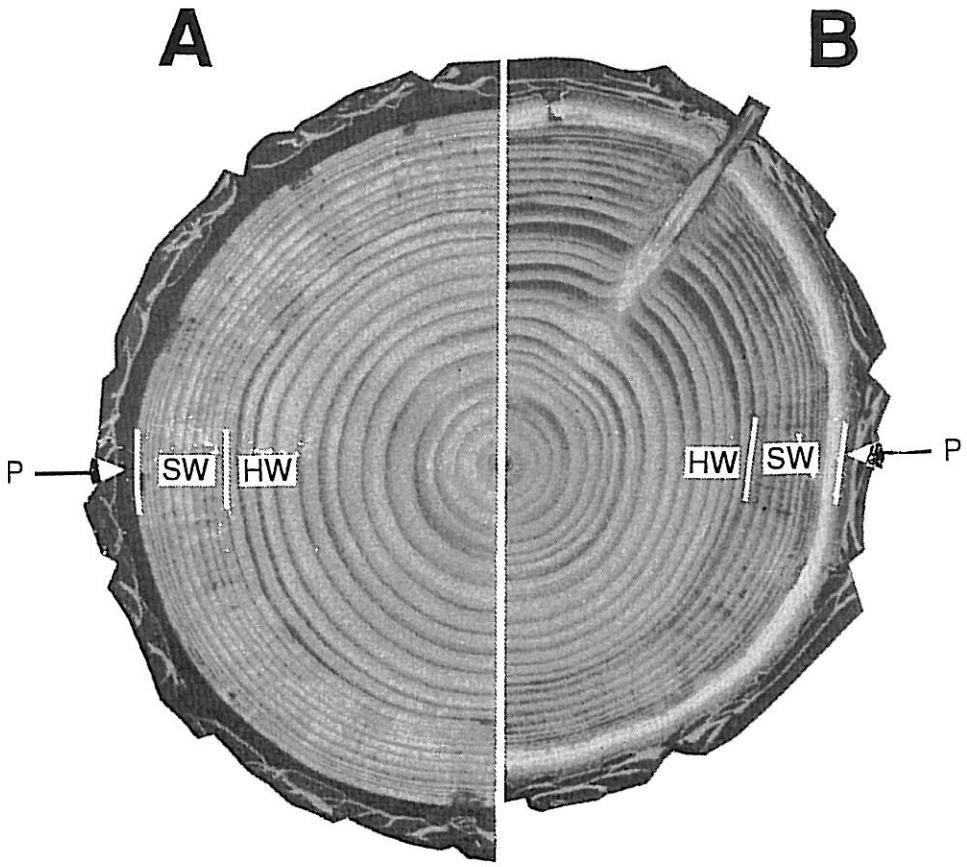

Figure 1. Cross-sectional discs from stem sections inoculated with *Ophiostoma polonicum*. A. Disk from a killed tree; the phloem (P) is dead (dark), and all sapwood (SW) is invaded by the fungus. B. Disk from the surviving tree; phloem and two outer annual rings (post-inoculation) are fresh, whereas the inner sapwood is permeated by the fungus. HW = heartwood.

Phloem necroses varied greatly in size (length) among individual trees infected with *O. polonicum*, *O. europhioides*, or *O. penicillatum*, but less so among those with *O. minus* or *O. bicolor* (Table 1). Generally speaking, average necrosis length varied as much among trees of a specific treatment as between treatments. On an average, the former three fungi had spread more in the bark than the two latter. Within a tree, necrosis length variation was slight. Necrosis length was not related to stem increment.

Table 1. Length of necrotic zones (mean ± SD) in the phloem of Douglas-fir inoculated with five species of *Ophiostoma* fungi. In three trees that were killed by *O. polonicum* (marked D), the table refers to darker zones that were distinguisable from other dead phloem. N = number of zones measured per tree.

Species	Tree no.	N	Necrosis length (mm)
O. polonicum	2 D	50	14.4 ± 3.9
"	3	50	23.9 ± 3.6
"	11 D	48	24.6 ± 6.3
"	12 D	50	37.6 ± 9.5
O. europhioides	4	49	33.0 ± 5.9
"	9	49	23.0 ± 3.2
"	13	41	18.5 ± 4.0
"	16	49	27.7 ± 4.8
O. penicillatum	1	46	12.5 ± 1.7
"	5	53	22.4 ± 4.9
"	10	50	17.6 ± 2.5
"	14	52	36.4 ± 8.0
O. minus	7	49	27.7 ± 6.4
"	8	47	16.7 ± 3.2
"	17	52	18.2 ± 6.3
"	20	48	13.8 ± 1.5
O. bicolor	6	50	15.2 ± 5.7
"	15	48	11.6 ± 1.8
"	18	52	17.0 ± 2.6
"	19	51	11.5 ± 1.5

Discussion

The study confirms that *O. polonicum* may grow straight into the inner sapwood of Douglas-fir, where it spreads out in the tangential direction, as described by Christiansen and Solheim (1990). This is contrary to the behaviour of the fungus in spruce, where infections form wedge-shaped zones on a stem cross-section, the tip of the wedge pointing towards the pith.

No staining was seen after inoculations with the four other fungi although large areas of the sapwood were occluded. The experiment did not include a control with sterile agar, and we therefore cannot assertain whether the considerable sapwood embolism found in these trees resulted from mechanical injury caused by the cork borer, or was an effect of a biochemical influence from the fungi (cf. Sperry and Tyree 1988). Usually, control inoculations on conifers result only in small necrotic zones in the phloem and sapwood (Molnar 1965; Reid et al. 1967; Wong and Berryman 1977; Horntvedt et al. 1983; Stephen and Paine 1985; Solheim 1988; Lieutier et al. 1989). In a single-inoculation experiment, control inoculations with sterile agar produced small resinous zones protruding only 0.5 mm or less into the sapwood (Solheim, unpublished).

The role of blue-stain fungi in the tree-killing process after attack on Douglas-fir by *D. ponderosae* is uncertain. No comprehensive study has been undertaken although blue-stain is observed (Kimmey and Furniss 1943; Wright and Harvey 1967). Sporadic isolation of fungi has been done and the only species mentioned in connection with Douglas-fir beetles are *O. pseudotsugae* (Rumb.) von Arx and *Leptographium abietinum* (Peck) Wingf. (Rumbold 1936; Harrington 1988). *O. pseudotsugae* is later synonymized with *O. minus* (Upadhyay 1981).

The ability of *O. polonicum* to penetrate fresh sapwood may be caused by its tolerance for low oxygen conditions (Solheim 1991). However, *O. minus*, which is also known to tolerate low oxygen pressures (Scheffer 1986), was not able to conquer any of the four Douglas-fir trees in our experiment. The *O. minus* strain used in this study proved capable of killing Scots pines when inoculated in a dose similar to that used in the present experiment (Solheim and Långström 1991; Solheim et al. 1993). This strain may, however, be quite different from those associated with the Douglas-fir beetle in North America: the possibility remains that specific strains are adapted to different host trees or, alternatively, *O. minus* from Douglas-fir may be another species.

Growth performance of the different fungi in the phloem had little bearing on their pathogenicity in the wood: average necrosis length in the three *O. polonicum* trees that died varied from 14.4 to 37.6 mm, while that of the surviving tree was intermediate (23.9 mm). For trees inoculated with the four other fungi there was no correlation between necrosis length and relative sapwood desiccation.

Variation in phloem necrosis length among individual trees inoculated with the same fungus could be caused by differences in substrate quality, e.g., contents of nutrients or secondary metabolites. The magnitude of this variation indicates that genetical traits may be involved.

We do not want to extrapolate our results to Douglas-fir trees growing under natural conditions. Our trees were growing at a high latitude, and also had suffered a serious set-back from an infection by the needle fungus *Phaeocryptopus gaeumannii* (Rhode) Petr. However, despite their relatively poor vigour, *O. polonicum* was the only fungus that was able to kill the trees.

Our experiment emphasizes the hazards connected with accidental introduction of foreign insects and their microbial associates: should *O. polonicum* be introduced into North America and there be picked up by *D. ponderosae*, it might become a great threat to native Douglas-fir forests. Likewise, an introduction of the Douglas-fir beetle into Europe poses a serious threat, should it become associated with a fungus like *O. polonicum*.

Summary

Ophiostoma polonicum, a blue-stain fungus associated with the spruce bark beetle *Ips typographus* in Scandinavia, can kill various species of spruce when inoculated under the bark. Douglas-fir, which is normally not infested by *I. typographus*, also succumbs to artificial infection with this fungus.

In the present study we investigated whether *O. polonicum* is superior to other *Ophiostoma* species in invading the sapwood of the non-host Douglas-fir, or if this superiority is limited to its conquest of spruce. In addition to *O. polonicum* we used three other *Ophiostoma* species that are transmitted by *I. typographus* and one species associated with the Douglas-fir beetle, *Dendroctonus pseudotsugae*.

Young Douglas-fir trees growing in South Norway were administered a standardized mass-inoculation with *O. bicolor*, *O. europhioides*, *O. minus*, *O. penicillatum*, and *O. polonicum*, to test their susceptibility to the infection.

O. polonicum killed three out of four trees, whereas none of the other fungi proved lethal at the given dose. The study also confirms that *O. polonicum* may grow straight into the inner sapwood of Douglas-fir, where it spreads tangentially. This is contrary to the behaviour of this fungus in spruce, where the infection appears as wedge-shaped zones on a stem cross-section, the tip of the wedge pointing towards the pith.

The growth performance of the different fungi in the phloem was not related to their pathogenicity in the wood.

Resin exudation on the bark surface was slight, and bark necroses resulting from infection appeared to hold little resin.

The experiment emphasizes the hazards connected with accidental introduction of foreign insects and their fungal associates: should *O. polonicum* be introduced into North America, and there be picked up by *D. ponderosae*, it might create a serious problem in native Douglas-fir forests. Likewise, an introduction of the Douglas-fir beetle into Europe poses a serious threat, should it become associated with a fungus like *O. polonicum*.

Fem blåvedsoppers patogenitet på Douglas

Blåvedsoppen *Ophiostoma polonicum*, som normalt er assosiert med granbarkbillen *Ips typographus* i Skandinavia, kan drepe ulike granarter når den inokuleres under barken. Douglas, som normalt ikke angripes av *I. typographus*, kan også drepes med kunstig inolulering med denne soppen.

I dette arbeidet undersøker vi om *O. polonicum* er andre *Ophiostoma*-arter overlegen også når det gjelder å drepe Douglas, som ikke er et naturlig vertstre, eller om denne overlegenheten er begrenset til det å drepe gran. Vi sammenliknet *O. polonicum* med tre andre *Ophiostoma*-arter som overføres av *I. typographus* og en art som er assosiert med Douglas-billen *Dendroctonus pseudotsugae*.

Unge Douglas-trær i arboretet ved Norges Landbrukshøgskole i Ås, Akershus, ble masse-inokulert med en standard dose av soppene *O. bicolor, O. europhioides, O. minus, O. penicillatum* og *O. polonicum* for å teste deres mottakelighet for infeksjonen.

O. polonicum drepte tre av fire trær, og i disse var all yteved blå (Fig. 1A). Ingen av de andre soppene var dødelige ved den gitte dosen. Undersøkelsen bekrefter også at *O. polonicum* kan vokse rett inn i den indre delen av yteveden hos Douglas, hvor den sprer seg i tangensial retning (Fig. 2B). Det gjør den ikke i gran; der spres infeksjonen i soner som i et stammetverrsnitt framtrer som kiler med spissen inn mot margen.

Soppenes spredningsevne i barken var ikke relatert til dens evne til å spre seg i yteveden. For alle soppenes vedkommende varierte barknekrosenes lengde betydelig fra tre til tre (Tabell 1). Douglas-trærne skilte ut lite kvae på barkoverflaten, og barknekrosene syntes også å inneholde lite kvae.

Forsøket understreker hva slags problemer som kan oppstå når fremmede insekter og deres assosierte sopper innføres til et land: Skulle f.eks. *O. polonicum* bli innført til Nord-Amerika, og der komme i kontakt med Douglas-billen *Dendroctonus ponderosae*, kunne det skape store problemer i Douglas-skogene der. Likeledes er en eventuell innføring av Douglas-billen til Europa en trussel: Skulle dette insektet bli assosiert med en sopp som *O. polonicum*, ville det skape store problemer for dyrkingen av Douglas, som av mange ses på som et viktig framtidstre i Europa.

Literature

CHRISTIANSEN, E., 1985. *Ceratocystis polonica* inoculated in Norway spruce: Blue-staining in relation to inoculum density, resinosis and tree growth. Eur. J. For. Path. *15*: 160-167.

CHRISTIANSEN, E. & SOLHEIM, H., 1990. The bark beetle-associated blue-stain fungus *Ophiostoma polonicum* can kill various spruces and Douglas fir. Eur. J. For. Path. *20*: 436-446.

FURNISS, M. M.; SOLHEIM, H. & CHRISTIANSEN, E., 1990. Transmission of blue-stain fungi by *Ips typographus* (Coleoptera: Scolytidae) in Norway spruce. Ann. Entomol. Soc. Amer. *83*: 712-716.

HARRINGTON, T. C., 1988. *Leptographium* species, their distribution, hosts and insect vectors. Pp. 1-39 *in*: T. C. HARRINGTON & F. W. COBB JR. (Eds.) *Leptographium* root diseases on conifers. APS press, St. Paul, Minnesota.

HORNTVEDT, R.; CHRISTIANSEN, E.; SOLHEIM, H. & WANG, S., 1983. Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. Medd. Nor. inst. skogforsk. *38(4)*: 1-20.

KÄÄRIK, A., 1980. Fungi causing sap stain in wood. Sveriges lantbruksuniversitet, Inst. f. virkeslära. Rapp. *114*: 1-112. Uppsala.

KIMMEY J. W. & FURNISS R. L., 1943. Deterioration of fire-killed Douglas-fir. U.S. Dept. Agr. Tech. Bull. 851, 61 pp.

LEVIEUX J.; LIEUTIER, F.; MOSER, J. C. & PERRY, T. J., 1989. Transportation of phytopathogenic fungi by the bark beetle *Ips sexdentatus* Boerner and associated mites. J. Appl. Ent. *108*: 1-11.

LIEUTIER, F.; YART, A.; GARCIA, J.; HAM, M. C.; MORELET, M. & LEVIEUX, J., 1989. Champignons pytopathogène associés à deux coléoptères du pin sylvestre (*Pinus sylvestris* L.) et étude préliminaire de leur aggressivité envers l'hote. Ann. Sci. For. *46*: 201-216.

MOLNAR, A. C., 1965. Pathogenic fungi associated with a bark beetle on alpine fir. Can. J. Bot. *43*: 563-570.

MOSER, J.C.; PERRY, T.J. & SOLHEIM, H., 1989. Ascospores hyperphoretic on mites associated with *Ips typographus*. Mycol. Res. *93*: 513-517.

REID, R. W.; WHITNEY, H. S. & WATSON, J. A., 1967. Reactions of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue-stain fungi. Can. J. Bot. *45*: 1115-1126.

RUMBOLD, C. T., 1936. Three blue-staining fungi, including two new species, associated with bark-beetles. J. Agric. Res. *52*: 419-437.

SCHEFFER, T. C., 1986. O_2 requirements for growth and survival of wood-decaying and sapwood-staining fungi. Can. J. Bot. *64*: 1957-1963.

SOLHEIM, H., 1988. Pathogenicity of some *Ips typographus*-associated blue-stain fungi to Norway spruce. Medd. Nor. inst. skogforsk. *40(14)*: 1-11.

SOLHEIM, H., 1991. Oxygen deficiency and spruce resin inhibition of growth of fungi associated with *Ips typographus*. Mycol. Res. *95*: 1387-1392.

SOLHEIM, H., 1992a. The early stages of fungal invasion in Norway spruce infested by the bark beetle *Ips typographus*. Can. J. Bot. *70*: 1-5.

SOLHEIM, H., 1992b. Fungal succession in sapwood of Norway spruce infested by the bark beetle *Ips typographus*. Eur. J. For. Path. *22*: 136-148.

SOLHEIM, H. & LÅNGSTRÖM, B., 1991. Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity. Ann. Sci. For. *48*: 149-156.

SOLHEIM, H.; LÅNGSTRÖM, B. & HELLQVIST, C., 1993. Pathogenicity of the blue-stain fungi *Leptographium wingfieldii* and *Ophiostoma minus* to Scots pine: effect of tree pruning and inoculum density. Can. J. For. Res. *23*: 1438-1443.

SPERRY, J. S. & TYREE, M. T., 1988. Mechanism of water stress-induced xylem embolism. Plant Physiol. *88*: 581-587.

STEPHEN, F. M. & PAINE, T. D., 1985. Seasonal patterns of host tree resistance to fungal associates of the southern pine beetle. Z. angew. Entomol. *99*: 113-122.

UPADHYAY, H. P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. Univ. Georgia Press, Athens. 176 pp.

WONG, B. L. & BERRYMAN, A. A., 1977. Host resistance to the fir engraver beetle. 3. Lesion development and containment of infection by resistant *Abies grandis* inoculated with *Trichosporium symbioticum*. Can. J. Bot. *55*: 2358-2365.

WRIGHT, K. H. & HARVEY, G. M., 1967. The deterioration of beetle-killed Douglas-fir in Western Oregon and Washington. U. S. Dep. Agric. For. Serv. Res. Pap. PNW-50. 20 pp.