

# Early stages of blue-stain fungus invasion of lodgepole pine sapwood following mountain pine beetle attack

Halvor Solheim

**Abstract:** Invasion of lodgepole pine sapwood by blue-stain fungi was followed for 7 weeks after infestation by the mountain pine beetle, *Dendroctonus ponderosae*. During this period all sapwood was heavily stained blue and blue-stain fungi were always isolated close to the front of visible occlusion. *Ophiostoma clavigerum* and *Ophiostoma montium* were commonly isolated, both of which are known to be carried in the mycangia of the mountain pine beetle. *Ophiostoma montium* was most frequently isolated, but when both fungi were present *O. clavigerum* was always at the leading edge of fungal penetration. On average *O. montium* trailed 7.3 mm behind *O. clavigerum*. Other microorganisms were seldom isolated.

**Key words:** lodgepole pine, *Dendroctonus ponderosae*, fungal succession, blue-stain fungi.

**Résumé :** Suite à l'infestation par le *Dendroctonus ponderosae*, les auteurs ont suivi pendant 7 semaines l'invasion de l'aubier du pin lodgepole par les champignons causant la bleuissement. Au cours de cette période, tout l'aubier devient fortement coloré en bleu et les champignons du bleuissement sont constamment isolés, jusqu'à la limite de l'occlusion visible. On a pu généralement isoler l'*Ophiostoma clavigerum* et l'*Ophiostoma montium*, ces deux champignons étant connus pour transporter les mycangies du *D. ponderosae*. L'*O. montium* a été plus fréquemment isolé, mais lorsque les deux champignons étaient présents, l'*O. clavigerum* se retrouve toujours au front de pénétration par les champignons. Dans l'ensemble, l'*O. montium* a un retard d'environ 7,3 mm derrière l'*O. clavigerum*. On a rarement isolé d'autres microorganismes.

**Mots clés :** pin à encens, *Dendroctonus ponderosae*, succession fongique, champignon du bleuissement. [Traduit par la rédaction]

## Introduction

Bark beetle infestations can enable beetle-associated fungi to be the earliest invaders of bark and wood. The Eurasian spruce bark beetle, *Ips typographus* L., carries spores of numerous fungi in pits on the pronotum and elytra, but in southeastern Norway only four species of fungi were frequently isolated from beetles (Furniss et al. 1990). These fungi were the first to invade the sapwood of Norway spruce trees [*Picea abies* (L.) Karst.] infested by *I. typographus*, and invasion occurred in an obvious succession (Solheim 1992a, 1992b).

Many species of blue-stain fungi have been found associated with infestations of mountain pine beetle, *Dendroctonus ponderosae* Hopk., but only *Ophiostoma clavigerum* (Robins., Jeff. & Davids.) Harrington and *Ophiostoma montium* (Rumb.) von Arx have been isolated from the beetles themselves (Rumbold 1941; Robinson 1962; Reid et al. 1967).

These two species, and associated yeasts, are carried in mycangia of the beetles (Whitney and Farris 1970).

The reaction of lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Engelm.) to attack by the mountain pine beetle and associated blue-stain fungi was thoroughly studied by Reid et al. (1967), and Whitney (1971) studied the development of associated microorganisms in bark in relation to beetle development. Ballard et al. (1982, 1984), using light and electron microscopy, looked at the penetration and growth of hyphae in the sapwood of lodgepole pine following mountain pine beetle attack. However, the species of fungi involved in sapwood colonization and their successional invasion have not been reported.

The purpose of the present study was to determine the early stages of fungal invasion in sapwood of lodgepole pine trees infested by the mountain pine beetle. The study was made at a site near Princeton, British Columbia, up to 7 weeks after the main beetle flight, when the sapwood was totally stained blue.

## Material and methods

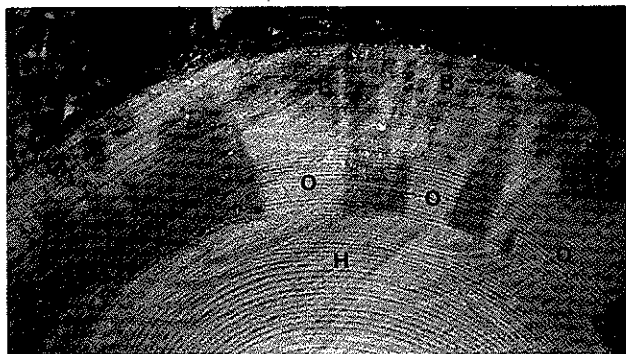
Five, 100- to 150-year-old lodgepole pine trees (diameter at breast height 30–50 cm; sapwood 30–45 mm) were felled at Sunday Creek, near Princeton, B.C. in 1992. The trees were in a natural stand of lodgepole pine in an area that had

Received June 2, 1994.

Halvor Solheim<sup>1</sup> Forestry Canada, Pacific and Yukon Region, 506 West Burnside Road, Victoria, BC V8Z 1M5, Canada.

<sup>1</sup> Present address: Norwegian Forest Research Institute, Høgskoleveien 12, N-1432 Ås, Norway.

**Fig. 1.** Part of a disc of lodgepole pine 5 weeks after mountain pine beetle attack. Some wedges of occlusion (O) had reached the heartwood (H), and blue staining (B) was in progress.



sustained heavy attacks by the mountain pine beetle for the previous 6 years. The main flight of the beetles started during the 3rd week of July 1992, and trees were harvested 2, 5, and 7 weeks later. Bolts, 50–80 cm long, from each tree were brought to the laboratory and stored at 0°C for 1–10 days before fungal isolation took place.

On the day when fungal isolations were made, the bolts were sectioned in 4-cm thick discs, and blocks were taken underneath mountain pine beetle galleries in radii from cambium into heartwood (see Solheim 1992b). Fungi were isolated from the blocks along radii situated 3–5 cm from the beetle entrance holes. Wood samples, 3–5 mm<sup>3</sup>, were taken aseptically at 5-mm intervals until the heartwood was reached. These were placed on malt agar (2% malt, 1.5% agar) in Petri dishes and checked for fungus growth over the next 3 months. Altogether 132 beetle galleries were examined for the presence of fungi when fungal penetration had reached different depths inside the cambium; 11 galleries were examined when fungi had reached 5 mm inside the cambium, 9 galleries were examined when the fungal penetration had reached 10 mm, etc. (see Table 1).

Where possible, sapwood depth, occluded sapwood, and blue-stained sapwood were measured along each radius. Occluded sapwood stands out with a lighter colour (whitish) than functional sapwood (Fig. 1).

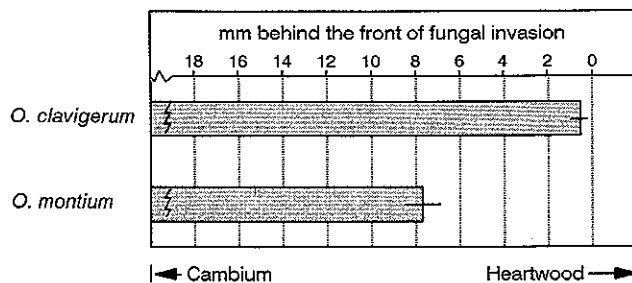
Identification of blue-stain fungi was done by using appropriate literature, which includes the monograph of *Ceratocystis* and *Ophiostoma* (Upadhyay 1981) and the original descriptions of species associated with the mountain pine beetle (Rumbold 1941; Robinson-Jeffrey and Grinchenko 1964; Robinson-Jeffrey and Davidson 1968).

The data were analysed using STATISTICA (StatSoft 1994). Results of *t*-tests or Friedman ANOVA tests are given.

## Results

Two weeks after the main beetle flight, sapwood occlusion extended up to 20 mm (mean 11 mm;  $n = 12$ ), and fungi were isolated up to 15 mm into the sapwood underneath the galleries. No blue staining was visible. Three weeks later sapwood occlusion often had reached the heartwood and blue stain was developing, occasionally reaching the heartwood (Fig. 1). However, still a few 1- to 2-week-old attacks could

**Fig. 2.** The invasion of *Ophiostoma clavigerum* and *Ophiostoma montium* in the sapwood of lodgepole pine infested by mountain pine beetle in relation to the front of fungal penetration. Bars indicate standard errors of means.



be seen with initial sapwood occlusion. Seven weeks after the main flight all sapwood was occluded and stained blue.

Visible occlusion averaged 2.0 mm ( $n = 48$ ,  $t = 3.065$ ,  $p = 0.004$ ) ahead of the front of isolated fungi. This occurred more in the initial stages of fungal penetration, less in the later stages. When blue stain occurred, it developed, on average, 6.2 mm ( $n = 31$ ,  $t = -8.631$ ,  $p < 0.001$ ) behind the observed occlusion.

Two species of fungi, *O. clavigerum* and *O. montium*, were commonly isolated. *Ophiostoma clavigerum* was isolated underneath more than 80% of the 132 inspected galleries. *Ophiostoma montium* was more common and occurred underneath 89% of the 132 galleries examined, or underneath 96% of the 112 inspected galleries where fungi had penetrated more than 10 mm inside the cambium (Table 1). However, *O. clavigerum* was much more common than *O. montium* during the initial stage of fungal succession, when fungal penetration had reached 5 and 10 mm (Table 1).

*Ophiostoma clavigerum* seems also to be more often present at the leading edge of fungal penetration later in the fungal succession. Table 2 shows the situation when fungal penetration was still progressing towards the heartwood and had reached 30, 35, and 40 mm inside the cambium. *Ophiostoma clavigerum* was isolated from the innermost isolation point in 92, 70, and 100% of the samples, while *O. montium* was isolated from the innermost isolation point in 42, 40, and 13% of the samples (Table 2). During these stages of the fungal succession *O. montium* occurred more frequently near the cambium and thus appears to gradually replace *O. clavigerum* (Table 2).

*Ophiostoma clavigerum* and *O. montium* were both present underneath 78 galleries when fungal penetration was progressing towards the heartwood. *Ophiostoma clavigerum* was then at the leading edge of fungal penetration in 67% of the radii, up to 30 mm in front of *O. montium*. The two species occurred together at the leading edge of fungal penetration in 31% of the radii. On two occasions, *O. montium* was in front of *O. clavigerum*. On average, *O. montium* trailed 7.2 mm ( $t = -7.287$ ,  $p < 0.001$ ) behind *O. clavigerum* (Fig. 2). In the Petri dishes, the two species were growing mixed together.

Other microorganisms were isolated from only 25 radii: *Ophiostoma huntii* (Robinson) deHoog & Scheffer in five radii, Basidiomycetes sp. in six radii, bacteria in six, yeasts in three, *Acremonium* sp. in two, light sterile mycelium in two, and Sphaeropsidales sp. in one radius.

**Table 1.** Frequency (%) of radii with *Ophiostoma clavigerum*, *Ophiostoma montium*, and both species together, in the sapwood of lodgepole pine underneath mountain pine beetle galleries when fungal penetration had reached different depths inside the cambium.

Species	Depth of fungus penetration (mm)									Total > 10 mm	
	5	10	15	20	25	30	35	40	45		
<i>O. clavigerum</i>	82	78	29	73	80	89	79	94	100	83	84
<i>O. montium</i>	45	44	100	82	100	93	93	100	100	89	96
Both species	27	22	29	55	80	83	71	94	100	72	80
No. of radii examined	11	9	7	11	15	18	14	32	15	132	112

**Table 2.** Frequency (%) of *Ophiostoma clavigerum* and *Ophiostoma montium* at different depths in sapwood of lodgepole pine infested by the mountain pine beetle.

Species	Distance inside cambium (mm)							
	5	10	15	20	25	30	35	40
Fungal front 30 mm								
<i>O. clavigerum</i>	83	75	75	92	92	92		
<i>O. montium</i>	92	83	75	67	58	42		
Fungal front 35 mm								
<i>O. clavigerum</i>	40	55	60	60	60	70	70	
<i>O. montium</i>	90	90	90	80	70	60	40	
Fungal front 40 mm								
<i>O. clavigerum</i>	75	79	88	88	100	100	100	100
<i>O. montium</i>	100	100	100	96	91	70	43	13

NOTE: Results from 12, 10, and 24 samples (radii) taken when fungal penetration had reached 30, 35, and 40 mm inside the cambium, respectively.

## Discussion

The study was done in only one area, and only a few trees were included, so the results must be interpreted carefully. However, the two most prevalent blue-stain fungi found, *O. clavigerum* and *O. montium*, are the same fungi associated with mountain pine beetle attack on lodgepole pine elsewhere (Robinson 1962; Reid et al. 1967; Whitney 1971). Also the development of occlusion and blue stain observed in this study was similar to that observed by Reid et al. (1967). Thus the results of this study may be representative for larger areas.

The two earliest invaders of sapwood, *O. clavigerum* and *O. montium*, occur in mycangia of the mountain pine beetle (Whitney and Farris 1970), which seems to enable them to be the primary invaders of both phloem (Whitney 1971) and sapwood. Even though *O. clavigerum* and *O. montium* are generally simultaneously introduced by the beetles (Whitney 1971), *O. clavigerum* is the primary invader of fresh sapwood and apparently has the same successional role as *Ophiostoma polonicum* Siem. has in Norway spruce attacked by the bark beetle *I. typographus*. *Ophiostoma polonicum* is always at the leading edge of fungal penetration, while the other species introduced by the beetle trail behind (Solheim 1992a, 1992b). In contrast, Caird (1935) and Bramble and Holst (1940) reported a more complex invasion pattern after infestations of shortleaf pine (*Pinus echinata* Mill.) by southern

pine beetle, *Dendroctonus frontalis* Zimm. Many species were involved, and none of them prevailed against the others to be the primary invader.

It comes as no surprise that *O. clavigerum* invaded the sapwood in advance of *O. montium*. Reid et al. (1967) noted that *O. clavigerum* was the more aggressive of the two fungi but did not provide evidence. Owen et al. (1987) found that *O. clavigerum* killed more ponderosa pine (*Pinus ponderosa* Laws.) seedlings than did *O. montium*,<sup>2</sup> and monitored by a heat pulse velocity instrument, the former species was more aggressive to lodgepole pine trees than the latter (Yamaoka et al. 1990). However, Basham (1970), Mathre (1964b), and Strobel and Sugawara (1986) found that *O. montium* killed loblolly (*Pinus taeda* L.), ponderosa, and lodgepole pines, respectively. The methods used in these studies were questionable, as large areas of bark were removed. It seems that *O. clavigerum* plays the same role that *O. polonicum* apparently plays in the tree-killing process after bark beetle attack. The latter fungus penetrates the sapwood and kills Norway spruce trees, while the other species introduced simultaneously by *I. typographus* (Furniss et al. 1990) are not as aggressive (Horntvedt et al. 1983; Solheim 1988).

In this study, *O. montium* was more prevalent than *O. clavigerum*. In earlier studies the frequency of this fungus has varied (Robinson 1962; Whitney and Farris 1970). However, Robinson (1962) always isolated *O. montium* from beetles, and Whitney (1971) isolated it from all the galleries that he inspected. The variation in frequency may have many explanations. For example, the materials and methods used may influence the results. Another factor is related to the population dynamics of the bark beetles and how the species composition of fungus associates may change under varying conditions (see Solheim 1992a, 1993). Rumbold (1941) previously observed differences in the fungal flora associated with mountain pine beetles collected in epidemic and endemic areas.

Other fungi were seldom isolated in this study, but *O. huntii* was occasionally found entering the sapwood. This fungus, together with a few other blue-stain fungi, has been isolated from mountain pine beetle attacked trees but mostly from older galleries (Robinson 1962; Mathre 1964a; Robinson-Jeffrey and Grinchenko 1964; Robinson-Jeffrey and David-

<sup>2</sup> Upadhyay (1981) treated *O. montium* as a synonym of *Ophiostoma ips* (Rumb.) Nannf. Owen et al. (1987) named the fungi isolated from mountain pine beetle *O. ips* but referred to it as one of two blue-stain fungi transmitted by the mountain pine beetle.

son 1968; Whitney 1971). These blue-stain fungi are not found in the mycangia of mountain pine beetles (Whitney and Farris 1970) and may thus be introduced by secondary bark beetles, other insects, or mites.

Yeasts are common associates of bark beetles. This is also true for the mountain pine beetle (Robinson 1962; Whitney and Farris 1970), and Whitney (1971) found that yeasts always entered the phloem together with blue-stain fungi. However, yeasts are almost certainly not early invaders of lodgepole pine sapwood attacked by mountain beetles. The same holds true for Norway spruce attacked by *I. typographus* (Solheim 1992a, 1992b).

Occlusion of sapwood seems to be close to the leading edge of fungus penetration as shown by Caird (1935) and Mathre (1964b). The method used in the present study was not designed to determine the relation between occlusion and fungal penetration, but fungi were isolated on average only 2 mm behind the visible occlusion.

Blue stain always seems to trail behind the leading edge of fungus penetration. This lag in staining may be influenced by many factors such as temperature, oxygen influx, gas exchange, and the species of fungi involved. Mathre (1964b) inoculated Ponderosa pine with the blue-stain fungi *O. ips* and *Ophiostoma minus* (Hedge.) H. et P. Syd. and found that in the blue-stained area 90–100% of the rays were infected by hyphae of the inoculated fungi, but the abundance of hyphae decreased sharply toward the water-conducting sapwood. The amount of brownish hyphae seems thus to be important for the observation of visible blue stain in sapwood (see also Whitney 1982). When fungi invade the sapwood in an obvious succession with one species of blue-stain fungus at the leading edge of fungal penetration, as observed here and earlier (Solheim 1992a, 1992b), the blue-stained area will not be far behind the frontier of fungus penetration. The situation may be different when a more complex invasion pattern occurs, as was observed after attack by the southern pine beetle where a whole group of fungi act as primary invaders and some of the species are not blue-staining fungi (Caird 1935; Bramble and Holst 1940).

## Acknowledgements

This study was carried out at the Pacific Forestry Centre (P.F.C.), Forestry Canada, Pacific and Yukon Region, Victoria, B.C. and was funded by a scholarship from The Agricultural Council of Norway. Support was given by the P.F.C. and the Norwegian Forest Research Institute. Robert Betts and Doug Linton, P.F.C., provided infested lodgepole pine bolts. Les Safranyik and Jack Sutherland, P.F.C., critically read the manuscript and gave valuable suggestions. The above-mentioned persons and institutions are gratefully acknowledged.

## References

- Ballard, R.G., Walsh, M.A., and Cole, W.E. 1982. Blue-stain fungi in xylem of lodgepole pine: a light-microscope study on extent of hyphal distribution. *Can. J. Bot.* **60**: 2334–2341.
- Ballard, R.G., Walsh, M.A., and Cole, W.E. 1984. The penetration and growth of blue-stain fungi in the sapwood of lodgepole pine attacked by mountain pine beetle. *Can. J. Bot.* **62**: 1724–1729.
- Basham, H.G. 1970. Wilt of loblolly pine inoculated with blue-stain fungi of the genus *Ceratocystis*. *Phytopathology*, **60**: 750–754.
- Bramble, W.C., and Holst, E.C. 1940. Fungi associated with *Dendroctonus frontalis* in killing shortleaf pines and their effect on conduction. *Phytopathology*, **30**: 881–899.
- Caird, R.W. 1935. Physiology of pines infested with bark beetles. *Bot. Gaz.* **96**: 709–733.
- Furniss, M.M., Solheim, H., and Christiansen, E. 1990. Transmission of blue-stain fungi by *Ips typographus* (Coleoptera: Scolytidae) in Norway spruce. *Ann. Entomol. Soc. Am.* **83**: 712–716.
- Hornthvedt, R., Christiansen, E., Solheim, H., and 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.
- Mathre, D.E. 1964a. Survey of *Ceratocystis* spp. associated with bark beetles in California. *Contrib. Boyce Thompson Inst.* **22**: 353–362.
- Mathre, D.E. 1964b. Pathogenicity of *Ceratocystis ips* and *Ceratocystis minor* to *Pinus ponderosa*. *Contrib. Boyce Thompson Inst.* **22**: 363–388.
- Owen, D.R., Lindahl, K.Q., Jr., Wood, D.L., and Parmeter, J.R., Jr. 1987. Pathogenicity of fungi isolated from *Dendroctonus valens*, *D. brevicornis*, and *D. ponderosae* to ponderosa pine seedlings. *Phytopathology*, **77**: 631–636.
- Reid, R.W., Whitney, H.S., and Watson, J.A. 1967. Reaction of lodgepole pine to attack by *Dendroctonus ponderosae* Hopkins and blue stain fungi. *Can. J. Bot.* **45**: 1115–1126.
- Robinson, R.C. 1962. Blue stain fungi in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) infested by the mountain pine beetle (*Dendroctonus monticolae* Hopk.). *Can. J. Bot.* **40**: 609–614.
- Robinson-Jeffrey, R.C., and Davidson, R.W. 1968. Three new *Europhium* species with *Verticicliadiella* imperfect state on blue-stained pine. *Can. J. Bot.* **46**: 1523–1527.
- Robinson-Jeffrey, R.C., and Grinchenko, H.H. 1964. A new fungus in the genus *Ceratocystis* occurring on blue-stained lodgepole pine attacked by bark beetles. *Can. J. Bot.* **42**: 527–532.
- Rumbold, C.T. 1941. A blue stain fungus, *Ceratostomella montium* n.sp., and some yeasts associated with two species of *Dendroctonus*. *J. Agric. Res.* **62**: 589–601.
- 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. 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. Pathol.* **22**: 136–148.
- Solheim, H. 1993. Fungi associated with the spruce bark beetle *Ips typographus* in an endemic area in Norway. *Scand. J. For. Res.* **8**: 118–122.

- StatSoft. 1994. STATISTICA. Tulsa, Okla.
- Strobel, G.A., and Sugawara, F. 1986. The pathogenicity of *Ceratocystis montia* to lodgepole pine. *Can. J. Bot.* **64**: 113–116.
- Upadhyay, H.P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press, Athens, Ga.
- Whitney, H.S. 1971. Association of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) with blue stain fungi and yeasts during brood development in lodgepole pine. *Can. Entomol.* **103**: 1495–1503.
- Whitney, H.S. 1982. Relationship between bark beetles and symbiotic organisms. In *Bark beetles in North American conifers*. Edited by J.B. Mitton and K.B. Sturgeon. University of Texas Press, Austin, Tex. pp. 183–211.
- Whitney, H.S., and Farris, S.H. 1970. Maxillary mycangium in the mountain pine beetle. *Science (Washington, D.C.)*, **167**: 54–55.
- Yamaoka, Y., Swanson, R.H., and Hiratsuka, Y. 1990. Inoculation of lodgepole pine with four blue-stain fungi associated with mountain pine beetle, monitored by a heat pulse velocity (HPV) instrument. *Can. J. For. Res.* **20**: 31–36.