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Fungi inhabiting stems of *Picea abies* in a managed stand in Lithuania

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Abstract

Wood inhabiting fungi were investigated in a stand of *Picea abies* (L.) Karst., damaged during selective cuttings and bark peeling. Five sets of stems were sampled: 104 with bark peeling wounds on a stem (above 1.0 m from the ground), 105 with extraction wounds on a butt (below 0.4 m from the ground), 172 with artificial butt wounds inflicted in August, 156 with artificial butt wounds inflicted in January and 40 sound looking stems. Most common fungi infecting wounds were *Stereum sanguinolentum* (Alb. and Schw.: Fr.) Fr. (in 20.3% of wounds), *Ophiostoma* sp. (4.5%), *Cylindrobasidium evolvens* (Fr.) Jül. (3.7%), *Amylostereum areolatum* (Fr.) Boid. (3.5%) and *A. chailletii* (Fr.) Boid. (2.6%). Position of the injury on the stem had a pronounced general impact on the species composition of wound infecting fungi (t=3.73; P<0.01). Most significantly, it affected the infection of *Ophiostoma* sp., that was associated with butt wounds (χ^2 =9.81; P<0.01), and *C. evolvens*, that was more frequent in stem injuries (χ^2=4.12; P<0.05). Season of the injury significantly influenced infection of *Ophiostoma* sp. that was more frequent in August wounds (χ^2=6.75; P<0.01). Basidiomycetes *S. sanguinolentum* (χ^2=23.78; P<0.001) and *C. evolvens* (χ^2=5.84; P<0.025) occurred more often in January wounds. Seventy nine (14.7%) of all wound samples yielded more than one fungus. Most common fungi in sound looking stems were *Nectria fuckeliana* Booth (27.5%), *Heterobasidion annosum* (Fr.) Bref. (22.5%) and *Ascocoryne* sp. (20.0%). Results of the study show that forest and wildlife management can significantly affect occurrence and species composition of wood inhabiting fungi in stands of *P. abies*. © 1998 Elsevier Science B.V.

Keywords: Decay fungi; Wounds; *Picea abies*; Selective cuttings

1. Introduction

Intensive management of forest stands for wood production typically involves clearcutting all the trees and snags, replanting with single species and periodic thinning to maintain vigorous, evenly spaced crop trees. Managed plantations, therefore, are relatively uniform in tree species, size, spacing and contain no snags or fallen trees (Hansen et al., 1991). Following the death of the tree, trunks are removed from a stand, thereby the continuity of natural wood decomposition is destroyed and many wood inhabiting fungi, especially those preferring later stages of decay, are deprived of their substrate. For example, 80% of the total number of basidiomycete species that occur on *Picea abies* (L.) Karst., harbour trunks of later stages of decay (Renvall, 1995). On the other hand, forestry practices in *P. abies* stands may additionally provide

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new infection foci for several wood destroying fungi that infect living trees via wounds (Pechmann and Aufsess, 1971) or adjacent stump surfaces (Hodges, 1969; Stenlid, 1987).

In Lithuania, all year round thinnings, selective and shelterwood cuttings have been in general practice for several decades, especially in the 1960s and 1970s. In 1965, for example, only 15% of the total amount of wood in the republic was harvested by clear cuttings (Matulionis, 1966). *P. abies* is a common understory tree in stands of *Betula*, *Populus*, *Picea* and *Alnus*, and made up a large portion of stands emerging in the region following various non-clear cuttings (Kairiúkštis, 1973). Presently 57.5% of Lithuania's forests consist of *P. abies* stands, often mixed with *Betula verrucosa* Ehrh. and *Populus tremula* L. (Brukas, 1994). Because of the thin bark and superficial root system, spruce suffers severely during harvesting operations. Trees with logging wounds remaining in *P. abies* stands after shelterwood, selective cuttings and thinnings comprised, respectively, 28–46%, 12–23% and 5–16% of total growing stock (Vasiliauskas, 1989; Kovbasa, 1996). Moreover, no protective stump treatment was ever performed in *P. abies* stands in Lithuania.

Another damage problem in todays forests associated with careless management is bark peeling of living stems by red deer (*Cervus elaphus* L.) and moose (*Alces alces* L.). Occurrence of such damage is particularly common in *P. abies* plantations with high uniformity of tree species, size and spacing (Haber, 1961), and in a number of cases was found to increase in stands after thinnings or other non-clear cuttings (Smirnov, 1984; Skogsstyrelsen, 1995; Randveer and Heikkilä, 1996; Padaiga, 1996). High population densities of *C. elaphus* and *A. alces* in Lithuania's forests due to intense wildlife management have also resulted in high incidences of damage. For example, *P. abies* stands containing more than 10% of peeled stems are covering territory of 6446 ha in state forests alone (Padaiga, 1992).

Species of wood inhabiting fungi in damaged stands of *P. abies* have been previously investigated in western Europe (Pechmann and Aufsess, 1971; Pawsey and Stankovicova, 1974; Aufsess, 1978; Ali El Atta and Hayes, 1987) and in Scandinavia (Isomäki and Kallio, 1974; Huse, 1978; Hallaksela, 1984; Vasiliauskas et al., 1996). Currently, there are two corresponding studies in east European region carried out by Igolkina (1990) in Russia's Moscow district and by Kovbasa (1996) in Byelorussia.

The aims of the present work were to determine the species of wood inhabiting fungi that infect stems of *P. abies* on managed forest sites in Lithuania, estimating long term effects of wound position on the trunk and season of the injury in relation to the occurrence of most common species.

2. Materials and methods

The experimental site was located in central Lithuania, 10 km east of Kaunas in Dubrava forest area (54° 55′N, 24° 02′E). The investigated *P. abies* stand was 50 years old and occupied an area of 5.3 ha. The stand was damaged during previous selective cuttings and by big game, and contained 23% of trees with logging and 14% of trees with bark peeling wounds.

The experiment was established in August 1988, when 110 *P. abies* stems with logging and 110 stems with bark peeling wounds were selected and consecutively numbered. Trees were 16–24 cm in diameter at breast height (DBH). All marked logging injuries resulted from extraction and were situated at butt not exceeding 0.4 m height from the ground. All marked peeling injuries were situated above 1.0 m from the ground. No stem among the selected ones was bearing any other visible injury except for the single wound included into the experiment. Furthermore, 180 sound looking stems of *P. abies* were selected and numbered in the same stand. On each of them an artificial injury 300 cm^2 in size (5×20 cm) was inflicted in August at the butt by tearing off the bark with an axe. Another set of 180 sound looking stems was numbered in January 1990 and the similar wounding procedure was then carried out.

Six years later, in August 1994, all the trees numbered in August 1988 were sampled, and in January 1996 all the trees numbered in January 1990 plus 40 more sound looking stems without any visible injury were sampled. During the previous six years, 43 of the initially selected trees had died or had been repeatedly damaged by peeling and were, therefore, excluded from further work.

Sampling of stems was carried out by means of an increment borer as described by Vasiliauskas et al.

(1996). Each wounded tree was sampled by inserting the borer 6–8 cm deep into the stem 1–3 cm away from the wound edge. Similar samples from sound looking stems were taken at 30 cm above the ground. The bore cores were brought to the laboratory in sterilised glass tubes. All woody pieces were then surface sterilised by flaming and placed on Petri dishes containing Hagem agar (HA) medium: 5 g glucose, 0.5 g NH_4NO_3, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 5 g malt extract, 20 g agar, 1000 ml H_2O at pH 5.5. Fungal colonies were subcultured after 10–15 days of growth and species in pure culture were identified according to descriptions by Nobles (1965) and Stalpers (1976).

Overall frequencies of fungal infections were compared between different sets of investigated stems using *t*-statistic. For this purpose statistical analysis of paired comparisons was applied (Eason et al., 1986), designated for comparison of *n*-pairs of observations from two populations influenced by a common factor. For every species of wound invading fungus, χ^2-tests were then calculated (Clarke, 1989) to determine infection rates and to correlate them with the position and the season of the wound.

Identical sampling and isolation procedures in our earlier Swedish study (Vasiliauskas et al., 1996) made it possible to compare the similarity of species invading bark peeling wounds of *P. abies* in Lithuania and Sweden. For this purpose Sørensen's Quotient of Similarity (QS) (Magurran, 1988) was calculated.

3. Results

A total of 537 wounds were sampled during the present work and 84.7% of samples showed fungal growth in the laboratory. Cultures from 77.3% of all samples were identified to species, the remaining 7.4% were unidentified non-basidiomycetes. Among 40 samples taken from sound looking stems 77.5% showed growth that was identified to species in 67.5% of the cases.

The frequency of fungal species isolation was subdivided into five sets, reflecting fungi in stem (bark peeling) wounds, fungi in butt (extraction) wounds, fungi in artificial butt wounds inflicted in August, fungi in artificial butt wounds inflicted in January and fungi in intact stems (Table 1). All injuries in the first two sets varied in size and were more than 6 years of age, and all injuries in the following two sets were of similar initial size (300 cm^2) and of similar age (6 years).

Statistically significant differences (t=2.40; P<0.05) were noted between fungal infection to wounded and to sound looking trees. Three species were identified in sound stems, while wounded stems were infected by at least 19 species (Table 1).

Wounding did not enhance the incidence of the four fungi listed first in Table 1 (t=1.70; P>0.05), indicating that all of them can invade the stems of *P. abies* independently of wounding. This is well illustrated by infection rates of *Ascocoryne* sp. and *H. annosum*, which were more frequent in externally sound looking stems (respectively, 20.0% and 22.5%) as compared with injured ones (respectively, 4.3% and 18.6%). In the case of *N. fuckeliana*, the difference in infection frequency between wounded and intact trees was 1.2% (Table 1) and statistically non-significant (χ^2=0.03; P>0.05). Therefore, these three species along with *P. chrysoloma* that is known to invade living *P. abies* via dead branches (Butin, 1995) were excluded from further analyses of wound infection dynamics regarding position and season of the injury.

Position of the injury on the stem had a pronounced impact on the species composition and infection frequencies of wound infecting fungi (t=3.73; P<0.01). According to the χ^2-test it affected significantly the infection of *Ophiostoma* sp., which was mostly associated with low butt wounds (χ^2=9.81; P<0.01) and *C. evolvens*, which on the contrary, was more likely to infect higher located stem injuries (χ^2=4.12; P<0.05) (Table 1).

Season of the injury in general had no statistically significant effect on species composition and their occurrence in wounded *P. abies* (t=1.91; P>0.05), although infection of the three species was influenced significantly. *Ophiostoma* sp. was more likely to invade trees damaged in August (χ^2=6.75; P<0.01). On the contrary, the basidiomycetes *C. evolvens* (χ^2=5.84; P<0.025) and, *S. sanguinolentum* in particular (χ^2=23.78; P<0.001) were more frequently isolated from January wounds. For example, occurrence in non-vegetation period (January) wounds of *S. sanguinolentum* was higher by 20.7% than occurrence in vegetation period (August) wounds (Table 1). Seventy five of all wound samples (14.0%) gave

Table 1
Isolation frequency (%) of fungi from *Picea abies* stems

Fungus	With wounds					Intact
	Stem	Butt	Aug.[a]	Jan.	All	
Occurring in intact trees:						
Ascocoryne sp.	–	1.0	2.3	11.5	4.3	20.0
Nectria fuckeliana Booth	22.1	15.2	43.6	25.6	28.7	27.5
Heterobasidion annosum (Fr.) Bref.	4.8	7.6	32.6	19.9	18.6	22.5
Phellinus chrysoloma (Fr.) Donk	–	–	0.6	–	0.2	–
Associated with wounds and insects:						
Ophiostoma sp.	1.0	11.5	5.8	0.6	4.5	–
Amylostereum areolatum (Fr.) Boid.	2.9	5.7	2.9	3.2	3.5	–
Amylostereum chailletii (Fr.) Boid.	1.0	2.9	4.1	1.9	2.6	–
Associated with wounds only:						
Stereum sanguinolentum (Alb. and Schw.: Fr.) Fr.	26.0	21.9	8.1	28.8	20.3	–
Cylindrobasidium evolvens (Fr.) Jül.	3.8	–	2.3	7.7	3.7	–
Sistotrema brinkmannii (Bres.) Erikss.	1.0	4.8	1.2	–	1.5	–
Peniophora incarnata (Fr.) Karst.	2.9	1.0	–	–	0.7	–
Peniophora pithya (Pers.) Erikss.	–	–	–	1.3	0.4	–
Resinicium bicolor (Alb. and Schw.: Fr.) Parm.	–	1.9	–	–	0.4	–
Coniophora arida (Fr.) Karst.	–	1.0	–	–	0.2	–
Fomitopsis pinicola (Fr.) Karst.	–	–	0.6	–	0.2	–
Postia stiptica (Pers.: Fr.) Jül.	1.0	–	–	–	0.2	–
Hyphoderma sp.	1.0	–	–	–	0.2	–
Basidiomycete sp. no. 1	–	–	–	0.6	0.2	–
Basidiomycete sp. no. 2	–	–	0.6	–	0.2	–
No. of trees sampled	104	105	172	156	537	40

[a] Artificial butt wounds inflicted in August.
[b] Artificial butt wounds inflicted in January.

growth to two, and four samples (0.7%) gave growth to three different fungal species. There were 24 different combinations of fungi and nine of them were noted more than once. Most common was *N. fuckeliana* + *H. annosum* combination, that occurred in 3.7% of all wounds. However, according to χ^2-test, infection rates of different fungal species were not associated with each other in any case.

Similarity of the fungal species invading bark peeling wounds on *P. abies* in Lithuania and in Sweden appeared to be high (QS=0.67) and generally there was no significant difference between the frequencies of their infection (t=1.58; P>0.05).

4. Discussion

Sampling of tree stems with an increment borer does not always reveal the presence of existing stem rot. The rot can remain undetected if the decay has not advanced to the vertical level at which the core sample is taken, or if decay is in a lateral position in a cross-section of the stem (Stenlid and Wästerlund, 1986). At early stages, wound decay in living *P. abies* is laterally oriented close to the wound site and only after several years of extension, both vertical and horizontal, it becomes a decay column that closely resembles heart-rot. During all development stages fungal activity is most intense near the wound edges (Vasiliauskas, 1989). Consequently, sampling at this part of a tree allows to detect the most common fungi associated with stem damages.

High similarity between occurrence of fungal species invading wounded *P. abies* in Lithuania and in Sweden indicated that there is a number of wood inhabiting fungi constantly associated with open injuries. These species, therefore, gain advantage over other fungi for distribution and spread in stands damaged by both careless forestry practices and wildlife management. Our findings are supported by data from related studies in other parts of Europe, where *S. sanguinolentum* was the most common decayer in wounds of *P. abies*, followed by *C. evolvens*, *H. annosum*, *Amylostereum* spp. and several other basidiomycetes of less importance (Pechmann and Aufsess, 1971; Isomäki and Kallio, 1974; Pawsey and Stankovicova, 1974; Schönhar, 1975; Aufsess, 1978; Roll-Hansen and Roll-Hansen, 1980; Hallaksela, 1984; Ali El Atta and Hayes, 1987; Igolkina, 1990). In natural forests all these fungi are mere decomposers of snags and/or fallen tree trunks (Breitenbach and Kränzlin, 1986; Renvall, 1995).

Previous investigations in Lithuania had revealed that fruiting bodies of decay fungi *Postia caesia* (Schrad.: Fr.) Karst. and *P. stiptica* may frequently appear on injured *P. abies*, comprising 44.4% among all fruiting bodies of basidiomycetes growing on wounds (Vasiliauskas, 1989). Both these fungi were also common on wounds of *P. abies* also in former Czechoslovakia and consequently were regarded to be important spruce pathogens in the region (Prihoda, 1957; Hašek, 1965; Soukup, 1985; Cerny, 1989). *P. stiptica* was noted on wounded conifers also in Great Britain (Pawsey, 1971) and it was found to be present in 62% of wounded *P. abies* in Byelorussia (Kovbasa, 1996), indicating that it is a common wound invader in the neighbouring region. Contrary to expectations we failed to isolate *Postia* spp. except for one case in stem wound, during the present study (Table 1).

Several previous studies had shown decreasing fungal infection rates to trunk injuries situated high above the ground. According to Parker and Johnson (1960), infection frequency to wounds at the soil level was 100%, when butt wounds were infected upto 70% and stem wounds upto 38%. Hašek (1965) found decay in 26–28% of extraction injuries at the root collar as compared with 4–11% decay infected peeling wounds on the stem. Isomäki and Kallio (1974) reported, that root collar injuries offer more favourable starting points for the onset and spread of decay than root and trunk injuries. Some species of fungi are preferring low and others high situated injuries. *S. sanguinolentum* and *C. evolvens*, for example, were more often associated with higher stem wounds (Kallio, 1976; Roll-Hansen and Roll-Hansen, 1980) as it was also noted during the present study (Table 1). *H. annosum*, on the contrary, was more frequently associated with root and root collar injuries (Vasiliauskas and Pimpè, 1978). This indicates that environmental conditions affecting fungi may differ at various levels from the ground in forest stands. Our study also showed that such differences do indeed exist.

Beitzen-Heineke and Dimitri (1981) found that due to resin flow, wounds inflicted during vegetation period were better protected against fungi and infection frequency was lower by 40% in summer than in winter injuries. Therefore, wounding season appeared to be an important factor influencing fungal infection to *P. abies*. The present study showed a profound effect of the season on the infection frequency of active decayer *S. sanguinolentum*. Wounds made in August were much better protected against the fungus (Table 1). This is in agreement with reports from other studies. In Canada, susceptibility of wound surfaces of *Abies balsamea* (L.) Mill. to *S. sanguinolentum* fell off rapidly and irreversibly after a few days of exposure to high summer temperatures (Etheridge, 1969). In Finland, most suitable temperature range for *S. sanguinolentum* infections appeared to be between -8.3 and $+5.0^{\circ}$C, and the fungus was a very common *P. abies* stump coloniser almost year round except during June–July, when other fungi were active as competitors (Kallio and Hallaksela, 1979). Competition of other fungi is the critical factor for the successful establishment of *S. sanguinolentum* and the dominance of the fungus in wounds is due to the relative inability of competitors to tolerate certain substrate properties, particularly at low temperatures (Etheridge, 1969).

Two other species more frequently found in winter than in summer wounds were *C. evolvens* and *P. pithya* (Table 1). Both these fungi can successfully colonise *P. abies* stumps at temperatures below the freezing point (Kallio and Hallaksela, 1979). Accordingly, the present work indicates relatively high susceptibility of non-vegetation period injuries to decay fungi. However, practical advice to carry out non-clear cuttings during summer is not justified, since the number of trees damaged and the wound dimensions are bigger when harvesting is carried out in summer as compared to the winter harvest (Kallio, 1984).

Fungi from the genera *Ophiostoma* and *Amylostereum* can be introduced to living conifers by bark beetles (Solheim, 1993) and wood wasps (Talbot, 1977), respectively. None of these fungi inhabited sound looking trees in our study area, so damaged stems were likely to be more attractive for the insects. As shown in the Table 1, all three insect transferred fungi more often occurred in *P. abies* bearing low extraction wounds than in trees with stem wounds. Infections of *Ophiostoma* sp. and *A. chailletii* were more pronounced in stems with vegetation period injuries (Table 1), what may be related to intense resin flow from the wounded trees during vegetation. Trees exuding resin are particularly attractive both to bark beetles (Vasechko, 1978) and to wood wasps (Talbot, 1977).

Sound looking stems in the studied stand were frequently found to contain *N. fuckeliana*, *H. annosum* and *Ascocoryne* sp. (Table 1). During other investigations *N. fuckeliana* occurred very commonly in wounded *P. abies* and in some cases was isolated from 60–79% of the injuries, leading to the conclusion that it is a typical wound invader (Bazzigher, 1973; Schönhar, 1975; Roll-Hansen and Roll-Hansen, 1980). However, in some cases the fungus was also found in 8–40% of sound looking *P. abies* stems and was repeatedly isolated from dead branches (Roll-Hansen and Roll-Hansen, 1979; Huse, 1981). These findings along with the results of our work indicate that *N. fuckeliana* is not at all dependent on wounds for the infection and branch stubs, for example, may be a relatively more important path for the fungus. Recent study provided evidence that airborne ascospores are the main source for infections of *N. fuckeliana* (Vasiliauskas and Stenlid, 1997).

Although *H. annosum* is not a common wound invader, it is still a very typical species for managed forest stands, gaining entrance to living trees via stumps that are absent in natural forests (Hodges, 1969). Study of Stenlid (1987) in a stand of *P. abies* had revealed that 58% of the *H. annosum* decayed trees were infected from old stumps, 16% from thinning stumps, and 26% from adjacent trees. High occurrence of *H. annosum* in the studied stand raises general concern about potential volume losses caused by the fungus in Lithuania's forests and suggests further research on the distribution of the pathogen in the region. Fungi from the genus *Ascocoryne* are reported to be typical endophytes inhabiting sound *P. abies* stems (Roll-Hansen and Roll-Hansen, 1979; Huse, 1981).

During the present study *N. fuckeliana* was the most common fungus inhabiting the wounds together with other fungal species. When two or three species were detected in one tree, they were always growing together from the same wood sample; so it may be assumed that they must be living in close contact within the stem. However, earlier studies on mutual influence between fungal species within *P. abies* stems also did not reveal any more pronounced relationships (Roll-Hansen and Roll-Hansen, 1980; Hallaksela, 1993).

Results of our work showed that forest and wildlife management can influence species composition of wood inhabiting fungi in *P. abies* stands. In forthcoming studies, populations of the most common of them will be investigated in more detail.

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