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Fungi Inhabiting Bark Stripping Wounds Made by Large Game on Stems of *Picea abies* (L.) Karst. in Latvia

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Abstract

In the last decade the populations of large game as moose and red deer have increased in Latvia, and the risk of damage to forest stands has increased simultaneously. The aim of this study was to evaluate the extent of bark stripping wounds, decay incidence and associated fungi in 30-year-old *Picea abies* stems damaged by big game. In total, 90 trees were evaluated and 157 bark stripping wounds of different age (1-10 years) were measured. From each wound margin one wood sample was collected for evaluation of presence of decay and subsequent fungal isolation. Decay was found in 13-50% of investigated wounded *P. abies* trees depending on study site (mean 26.7%). All injuries were open wounds. Area of exposed sapwood was 7 – 6142 cm^2. The most commonly isolated fungi were ascomycetes *Neonectria fuckeliana*, *Sarea difformis* and *Phialocephala* sp., and basidiomycetes *Cylindrobasidium evolvens* and *Amylostereum areolatum*.

Key words: *Picea abies*, bark stripping, decay fungi.

Introduction

Norway spruce (*Picea abies* (L.) Karst.) is one of the most economically important conifer species in Latvia. However, root and stem rot caused by various species of fungi can lead to considerable losses in timber production (Arhipova et al. 2011). The most important fungal species causing stem decay in *P. abies* stands are *Heterobasidion annosum* s.l. and *Stereum sanguinolentum* (Korhonen and Piri 2003, Arhipova et al. 2011). Several studies have shown that stem wounds are an important route for tree infection with decay-causing fungi (Vasiliauskas et al. 1996, 2001, Vasiliauskas 2001).

In the last decades, the populations of large game as moose (*Alces alces* L.) and red deer (*Cervus elaphus* L.) have increased in Latvia (Baumanis 2013). The risk of damage to forest stands has increased simultaneously. One type of damages is bark stripping that can reduce value of final wood harvest (Gill 1992, Vospernik 2006, Andersone-Lilley et al. 2010). The most severe damage usually occurs in trees with DBH (diameter at breast height) 5-20 cm and age 4 to 50 years (Gill 1992, Vasiliauskas et al. 1996, Vospernik 2006, Čermák and Strejček 2007, Månsson and Jarnemo 2013). Bark stripping wounds are usually situated at a height of 1-2 m, while injuries caused by timber harvesting machines are mostly on tree roots and butt (Isomäki and Kallio 1974, Vasiliauskas 2001). The area of bark stripping wounds is very variable, from 2 to 4,815 cm^2 (Vasiliauskas et al. 1996, Čermák and Strejček 2007). Vasaitis et al. (2012) showed that wounds on *P. abies* stems greater then 5 cm width are unlikely to be completely occluded and are more prone to infection by fungi causing stem decay. The most common decay-causing fungus is *Stereum sanguinolentum*, which can cause extensive stem rot resulting not only in reduced timber quality, but increased vulnerability to wind or snow damage (Randveer and Heikkilä 1996, Vasiliauskas et al. 1996, Čermák and Strejček 2007, Vasaitis 2013). Only one study on fungi colonizing bark stripping wounds has been conducted in Latvia (McLaughlin and Šica 1996). The aim of this study was to evaluate the wounding pattern caused by bark stripping on stems of *P. abies* and to identify the associated fungi.

Materials and methods

Field work

The study was conducted in Latvia in three 32-34-year-old *P. abies* monocultures (Figure 1), all growing on mineral podzolic soil: two (Šķēde 1 and 2) in an *Oxalidosa* and

one (Kalsnava) in a *Hylocomiosa* forest type, according to the Latvian classification system (Zālītis and Jansons 2013).

Experimental design was similar to that of related work on *Pinus contorta* (Arhipova et al. 2015). In each study site, 30 living *P. abies* trees with bark stripping injuries were selected by choosing the most adjacent wounded tree to the previous measured one. Each tree was numbered and its diameter at breast height (DBH) measured. The number of individual injuries per stem (wounds separated by sound bark) was recorded for each selected tree. For each of 157 recorded injuries, maximum length and width, as well as heights of the lowest and highest wound margins were measured. To estimate wound area, configuration of small wounds (length < 60 cm) was drawn by a waterproof marker on a transparent paper and in the laboratory area was measured using a Tamaya Digital Planimeter "Planix 10-S". For wounds with length more than 0.6 m, the area was calculated as an ellipse using measured maximum length and width.

From each of 157 wounds a single wood sample (10-16 cm long bore core) was taken using an increment borer. Depending on wound height and accessibility, the sample was taken either 1 cm above or below the injury. Wood samples were assessed for the presence/absence of decay or discolouration, individually placed in sterile plastic tubes, and transported to the laboratory for further fungal isolation.

Standard statistics (means, standard deviation) of measurements of wounds and discolourations were calculated (Fowler et al. 1998).

Isolation and identification of fungi

In the laboratory, the bore core was split into two pieces, each piece flame-sterilized and individually placed in 9-cm diameter plastic Petri dishes on malt agar media (15 g malt extract, 12 g agar, 1l H_2O) (300 Petri dishes in total). Petri dishes were incubated at room temperature and inspected twice a week for fungal growth; all emerging mycelia were subcultured on individual Petri dishes and grown as pure cultures. After 3-4 weeks of growth, all pure cultures were examined under a microscope and grouped into mycelial morphotypes according to microscopic features of mycelium.

One to two representatives of each distinct mycelial morphotype were subjected to molecular identification following procedures from Arhipova et al. (2011). In brief, DNA extraction and PCR amplification were made according to established protocols (Kåren et al. 1997). The ready PCR products were purified using FastAP Thermosensitive Alcaline Phosphatase and *Escherichia coli* exonuclease I (Thermo Scientific) and sent to Macrogen Europe (Amsterdam, the Netherlands) for further Sanger sequencing. Sequencing was done in one direction using universal primer Its4 for every specimen. All sequences were manually edited using the Bioedit software (version 7.0.9.0). BLAST searches were performed using the GenBank sequence database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For delimiting fungal taxon (presumed species), the Internal Transcribed Spacer (ITS) sequence homology was set at 98-100 %. For delimiting at genus level, the ITS sequence homology was set at 94-97 %. All obtained ITS sequences were deposited in GenBank (accession numbers KR072493-KR072507).

Results

Mean parameters of surveyed trees and bark stripping wounds are presented in Table 1. Stem DBH of examined trees varied from 9 to 29.5 cm. The maximum number of wounds per stem was four. All 157 injuries represented open wounds. Area of exposed sapwood was in the range from 7 to 6.142 cm^2 of sapwood (589 ± 1.173 cm^2 on average). Wound age varied from 1 to 10 years, and on individual stems wound age varied between 1 and 6 years. The lowest wound height was (0 - 0.4 m) at root collar and the highest one was 199 cm. However, typically the lowest wound margin was recorded at stem height 80 – 1.4 m (102 wounds or 65 %). Decay was found in 13-50 % of the selected *P. abies* trees depending on study site (26.7 % on average).

Table 1. Average parameters (mean ± *SD*) of analyzed *Picea abies* trees (n=90) and bark stripping wounds (*n* = 157)

Parameters / site	Kalsnava	Šķēde1	Šķēde 2	All
Stem diameter at breast height (cm)	17 ± 4	17 ± 5	19 ± 5	18 ± 5
Wounds per stem (no.)	2.4 ± 0.9	1.4 ± 0.6	1.5 ± 0.6	1.7 ± 0.8
Wood discolouration (% of stems)	50.0	13.3	20.0	26.7
Wound width (cm)	9 ± 5	10 ± 5	11 ± 5	10 ± 5
Wound length (cm)	28± 20	38 ± 21	37 ± 19	33 ± 20
Height of lower wound margin (cm)	97 ± 32	100 ± 30	62 ± 23	88 ± 33
Height of upper wound margin (cm)	125 ± 27	139 ± 23	98 ± 24	121 ± 30
Exposed sapwood per wound (cm^2)	591 ± 1206	541 ± 1095	631± 1211	589 ± 1173
Time since damage (years)	5 ± 2	5 ± 1	6 ± 2	5 ± 2

Of 157 wood samples, 92 (59 %) resulted in fungal growth and yielded 160 fungal isolates representing 25 fungal taxa (Table 2). The most common fungi isolated from bark stripping wounds were ascomycetes *Neonectria fuckeliana* (24.8 % of all wounds), *Sarea difformis* (13.4 % of all wounds) and *Phialocephala* sp. (13.4 % of all wounds). Eight species of basidiomycetes were occasionally isolated. The most common species of basidiomycetes were *Cylindrobasidium evolvens* (10.8 % of all wounds) and *Amylostereum areolatum* (3.8 % of all wounds).

Table 2. Fungi isolated from bark stripping wounds of *Picea abies* in Latvia

Fungal taxa	GenBank accession no.*	Frequency of occurrence, %			
		in wounds (*N*=157)	in trees with discolouration (*N*=24)	in trees without discolouration (*N*=67)	in trees, (*N*=90)
Basidiomycetes					
Amylostereum areolatum (Chaillet ex Fr.) Boidin	KR072496	3.8	25.0	-	6.7
Climacocystis borealis (Fr.) Kotl. & Pouzar	KR072506	0.6	4.2	-	1.1
Cylindrobasidium evolvens (Fr.) Jülich	KR072493	10.8	25.0	13.4	16.7
Gymnopilus penetrans (Fr.) Murrill	KR072502	2.5	12.5	1.5	4.4
Heterobasidion parviporum Niemelä & Korhonen	-	0.6	4.2	-	1.1
Peniophorella praetermissa (Karst.) Larss	KR072503	0.6	4.2	-	1.1
Pholiota spumosa (Fr.) Singer	KR072505	0.6	4.2	-	1.1
Stereum sanguinolentum (Alb. & Schwein.) Fr	KR072495	0.6	4.2	-	1.1
All Basidiomycetes	-	4.7	11.8	6.8	7.8
Ascomycetes and anamorphic fungi					
Ascocoryne cylichnium (Tul.) Korf	-	13.4	29.2	11.9	16.7
Aspergillus sp.	-	0.6	-	1.5	1.1
Aureobasidium pullulans (de Bary) G. Arnaud	-	1.3	4.2	-	1.1
Eutypa sp. K7	KR072494	5.1	20.8	4.5	8.9
Neobulgaria sp. K35	KR072504	0.6	4.2	-	1.1
Hypocrea pachybasioides Yoshim	-	1.3	4.2	1.5	2.2
Hormonema dematioides Lagerb. & Melin	-	0.6	-	1.5	1.1
Lachnum sp. K48	KR072507	0.6	-	1.5	1.1
Neonectria fuckeliana (C. Booth) Castl. & Rossman	-	24.8	45.8	17.9	25.6
Ophiostoma piceae (Münch) Syd. & P. Syd	-	0.6	4.2	-	1.1
Grosmannia piceaperda (Rumbold) Goid	KR072497	0.6	4.2	-	1.1
Penicillium sp.	-	1.9	-	4.5	3.3
Pezicula eucrita (Karst.) Karst.	KR072499	1.3	-	3.0	2.2
Phaeomoniella effusa Damm & Crous	KR072500	1.3	4.2	1.5	2.2
Phialocephala sp. K19	KR072498	13.4	33.3	13.4	18.9
Sarea difformis (Fr.) Fr	KR072501	13.4	25.0	19.4	21.1
All Ascomycetes and anamorphic fungi	-	55.6	82.4	56.2	61.1
Zygomycetes					
Umbelopsis isabellina (Oudem.) W. Gams	-	0.6	4.2	-	1.1
All Zygomycetes	-	8.8	29.4	8.2	12.2

Discussion and conclusions

A large proportion (26.7 %) of young spruce (32-34 years) examined in this study had decay and discolouration. Several basidiomycetes commonly isolated from decayed trees usually use open stem wounds as infection courts. *Amylostereum areolatum*, *Stereum sanguinolentum* and *Heterobasidion parviporum* can cause stem decay, which can significantly decrease wood quality. For example, in a ten-year period after injury, average vertical spread of decay due to *A. areolatum* was observed to be 2.8 m, with decay affecting 30-40% of total stem cross area (Vasiliauskas 1999). This fungus is one of few species of basidiomycetes that uses insects for transmission, and usually is introduced to fresh bark wounds by siricid woodwasps during their oviposition (Vasaitis 2013). *Stereum sanguinolentum* is the most important decay-causing fungus colonizing bark stripping wounds of different origin (Roll-Hansen and Roll-Hansen 1980, McLaughlin and Šica 1996, Vasiliauskas et al. 1996, Čermák and Strejček 2007). Rate of vertical spread of decay by *S. sanguinolentum* was observed to be between 13.3 and 19.5 cm per year (Čermák and Strejček 2007). Seven years after wounding, the decay column in spruce stems can reach 1-4 m height and can affect 3 - 84 % of the stem cross-section (Vasiliauskas and Stenlid 1998a). Vasiliauskas et al. (1996) found a positive correlation between wound age and frequency of wounds infected by *S. sanguinolentum*. In Norway, 16% of stem wounds were found to be infected 5-7 years after wounding and 39 % of trees with 15-20-years-old wounding scars were infected by *S. sanguinolentum* (Solheim 2006). The low incidence of this fungus in the current study could be associated with rather young wound age (the mean is five years), and the infection incidence might be expected to increase with time. The method used for detection of decay was not very precise, and approximately 30 % of decayed trees might remain unnoticed, especially in cases of lateral rot (Stenlid and Wästerlund 1986). *Heterobasidion parviporum*, which usually infects trees through root contacts, is able to infect open wounds, especially on roots or close to tree base (Redfern and Stenlid 1998). In the current study *H. parviporum* was isolated from a wound with the lowest margin at 18 cm from root collar. However, stem and root wounds are not as important infection courts for this pathogen as freshly cut stumps (Redfern and Stenlid 1998 and references therein, Rönnberg 2000). Nevertheless, wound infection can play a significant role in unmanaged forest, where stumps are absent (Garbelotto and Gonthier 2013). *Cylindrobasidium evolvens*, the most abundant wound colonizing basidiomycete in the current study, is usually associated with large recent wounds and without considerable stem decay (McLaughlin and Šica 1996, Vasiliauskas et al. 1996). *Climacocystis borealis* typically infects wounds on roots and the lower part of the trunk, causing root and butt rot of spruce in old grown forests (Hallaksela 1984, Solheim 2006), but we isolated it from a wound at height 70 cm. In Scandinavia this fungus is an indicator species of natural forest (Nitare 2000).

The most common fungus isolated from bark stripping wounds was ascomycete *Neonectria fuckeliana.* This species is a common wound invader of several tree species (Vasiliauskas et al. 1996, Vasiliauskas and Stenlid 1998b), but it is not associated with decay and can be isolated also from sound-looking wood (Roll-Hansen and Roll-Hansen 1979, Huse 1981). However, this fungus is a weak pathogen and can cause bark necrosis of spruce (Philips and Burdekin 1982). *Neonectria fuckeliana* has been reported as a significant pathogen causing Nectria flute canker in *Pinus radiata* plantations in New Zealand (Dick and Crane 2009). An experiment established in Latvia in 2011 showed that *N. fuckeliana* can cause cankers on spruce bark three years after artificial inoculation (Brūna, unpublished data).

As spruce trees are vulnerable to bark stripping from the age of 4 years up to 50 years (Gill 1992 and references therein, Vasiliauskas et al. 1996, Gill et al. 2000, Čermák and Strejček 2007), subsequent damage after bark stripping will increase with time. Fresh bark stripping wounds were observed on some of the sampled trees in current study, which means that the number of trees containing stem decay can be expected to increase up to final harvest. The extent of stem decay and frequency of infection is strongly correlated with stand age (Vasiliauskas et al. 1996, Čermák and Strejček 2007, Gaitnieks et al. 2008). In a study conducted in the Czech Republic (Čermák and Strejček 2007), 44% of spruce trees were damaged by red deer and stem decay was observed in 68 % of damaged trees. Bark stripping damage in young conifer stands (including bark stripping and browsing damages) is positively correlated with deer and moose population density (Gill 1992 and references therein, Kiffner et al. 2008, Baumanis 2013).

In comparison with the exotic tree species *Pinus contorta* Douglas ex Loudon, which also is susceptible to bark stripping by deer and moose (Arhipova et al. 2015), our data showed that damage on *P. abies* trees of the same size and age can be much more severe. The maximum area of exposed wood in the current study was considerably higher than in studies made by other authors (Vasiliauskas et al. 1996, Čermák and Strejček 2007), which might be because spruce pure cultures are more severely damaged than mixed stands (Gill 1992 and references therein, Baumanis 2013). The results of this study repeatedly emphasize risks posed to forest stands in areas, where the populations of the big game are disproportionally large.

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