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# Bark beetles have a decisive impact on fungal communities in Norway spruce stem sections

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## ABSTRACT

To study the importance of insects in the establishment of fungi, stem sections of Norway spruce were placed in mature managed conifer forests in Southeast Sweden. After one or two flying seasons, fungal communities in wood, bark and bark beetle samples were analysed by molecular methods. Excluding insects from stem sections with cages had a significant effect on the fungal community. Small wounds made in the bark to mimic insect activity did not significantly alter the fungal community, indicating that physical holes as such only played a minor role for the insect interaction with the fungal community development. Several white rot species were significantly more abundant in stem sections with insect access and were also detected from bark beetle samples. This suggests that insects do contribute to the development of early fungal succession on dead wood, but that creating small disturbances in the bark only have a minor contributing effect.

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## Introduction

Boreal forests, which are widely distributed across the northern hemisphere, are largely composed of conifers. These forests provide a habitat for a diverse biome of saproxylic organisms that depend on dead or dying wood to complete their life cycle (Siitonen, 2001; de Jong et al., 2004). Fungi, and in particular basidiomycetes, are important decay organisms, as they are able to decompose all the structural components of

wood. Despite their importance for decaying dead wood and recycling nutrients in the ecosystem, it is still unclear how most of the wood-decay basidiomycetes colonize newly dead trees. It is believed that most wood-decay fungi are dispersed primarily by airborne basidiospores. However, intact tree bark is a major structural obstacle to the establishment of mycelia in wood from single fungal spores, for example, the basidiospores of *Heterobasidion annosum* (Lindberg and Johansson, 1991).

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A dying tree can be viewed as an open resource that can, potentially, provide both energy and nutrients (Rayner and Boddy, 1988a). There are several ways that early-succession basidiomycetes can establish on recently dead wood. (i) The species can establish prior to the death of the tree by causing pathogenic bark lesions on roots followed by active root and stem rot. (ii) Establishment can take place prior to tree death via wounds on the living tree leading to stem rot. (iii) Wood decay species can establish, presumably via young bark tissue, and remain inactive as wood endophytes. (iv) The spread of decay fungi can be actively vectored and inoculated by insects that depend on the fungi for survival (Vasiliauskas et al., 1998). (v) Insects may passively carry fungal spores on their bodies (Harrington et al., 1981; Whitney et al., 1982; Pettay and Shaw, 1986; Beaver et al., 1989) and provide entry points through the bark during feeding or breeding activity. (vi) Saproxylic insects may enhance the access of air-disseminated fungal propagules to wood by making holes through the outer bark. (vii) As a null hypothesis, some air-borne spores may be able to penetrate the bark without mechanical disruption of the bark.

In this study we examined the influence of insects on the early fungal colonization of Norway spruce (*Picea abies*) logs. Müller et al. (2002) investigated the role played by insects in the fungal decomposition of spruce logs by comparing exposed logs with logs that were protected by metal netting. They found strong indications that insect attacks, by both cambium-gnawing and deeply boring bark beetles, increased the decomposition rate of spruce logs, but that when the bark beetle *Hylurgops palliatus* was present in high numbers the fungal diversity was reduced.

Few studies have investigated the role played by insects in the colonization success of air-dispersed basidiomycete fungal species or the association between the fruit bodies of particular decay fungi and certain early-succession insect species (Abrahamsson et al., 2008; Weslien et al., 2011). It has been proposed that several bark beetle species, which fly during basidiomycete sporulation periods, may randomly pick up basidiomycete spores and consequently serve as vectors (Pettay and Shaw, 1986). It has also been suggested that rot fungi have an effect on beetle diversity in dead wood (Jonsell et al., 2005). Studies of Norway spruce logs have shown that the brown rot fungus *Fomitopsis pinicola*, which is an early colonizer of conifers in boreal forests, has a significant effect on colonization by other wood-inhabiting organisms (Olsson et al., 2011). Persson et al. (2009, 2011), using T-RFLP technology, reported on the occurrence of some common wood decay basidiomycetes, e.g. *F. pinicola* and *Stereum sanguinolentum*, in association with bark beetles in the genera *Ips*, *Crypturgus* and *Pityogenes*.

In contrast to the relatively unstudied insect–basidiomycete connection, there are many studies dealing with the interaction between pathogenic ophiostomatoid ascomycete species and insects such as *Ips typographus*, a destructive pest known to facilitate the establishment of a variety of *Ophiostoma* spp. and non-pathogenic fungal species in Norway spruce (Solheim, 1991, 1993; Persson et al., 2009).

Most knowledge about decay fungi has been obtained from observations of sporocarps on different dead wood substrata (Niemela et al., 1995; Renvall, 1995; de Jong et al., 2004; Johansson et al., 2007), which do not reveal the entire

species richness in dead wood. Some fungal species do not form fruit bodies but are present as mycelia that can only be detected by culturing mycelia from wood pieces on selective media (Rayner and Boddy, 1988b) or by molecular methods (Allmer et al., 2006). For molecular identification of fungal species, the ribosomal internal transcribed spacer (ITS) region is the first choice because it has a high interspecific and a low intraspecific variation, and is enclosed by highly conserved regions of ribosomal encoding genes (Begerow et al., 2010).

The aim of the study was to investigate the influence of insect colonisation on the diversity of the fungal flora in newly dead *P. abies* (i.e., 1 and 2 yr after cutting), particularly the basidiomycete decay fungi. We tested the hypothesis that fungal diversity is driven by insects and surveyed the mycobiota by 454 pyrosequencing of the fungal ITS region followed by community analysis using multivariate statistics. We studied the effect of: (1) excluding insects by enclosing fresh logs in metal net cages to test whether any fungal species in early succession were exclusively favoured by insects; (2) mimicking insect boring by mechanically creating holes through the bark to assess the facilitation effect of bark penetration; and (3) inducing insect colonization with pheromone treatment to enhance any insect contribution to colonisation.

## Materials and methods

The experiment was set up in Apr. 2008 in two mature Norway spruce stands, approximately 20 km apart (Skarphärad: 60° 09'N, 18° 04'E; Släsby: 60° 05'N, 17° 49'E), in a managed forest landscape in the county of Uppland in Southeast Sweden. A total of 30 Norway spruce stem sections, 1 m long and 15–30 cm in diameter, were cut from three living trees in each stand and randomly assigned to the different treatments. All sections were free from any visual damage. Experimental treatments included caging (cages 40 × 50 × 120 cm in size with steel net with mesh width of 0.43 mm and a stainless steel bottom) to prevent insect colonization; 40 mechanically drilled 1.5 mm-diameter holes through the bark to simulate bark beetle entry holes; and baiting with *Pityogenes chalcographus* pheromone dispensers to ensure bark beetle colonization. The pheromones were released from plastic pouches (Chalcosan®, PheroNova AG) with at least 8 weeks of lifetime. Thus, each stem section was subjected to one of the five following treatments: (i) no holes and no cage (hereafter denoted NN); (ii) holes and no cage (HN); (iii) no holes and cage (NC); (iv) holes and cage (HC); and (v) pheromone baiting and no cage (Pheromone). In all, each treatment included six stem sections in each of the two locations. Stem sections belonging to the two caged treatments (NC and HC) were placed pairwise in the same cage, but at least 20 cm apart within each cage (a total of six cages per stand). The two uncaged treatments (NN and HN) were also placed pairwise on 10 cm high plastic cylindrical constructions with a convex upper part to prevent contact with the ground and positioned 1 m away from the closest cage. All cages and pairs of uncaged stem sections were spaced evenly apart in a circular formation with 30 m diameter, except for the stem sections baited with *P. chalcographus* pheromones, which were placed 40 m away from the outer edge of the closest cage.

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