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Do bark beetles facilitate the establishment of rot fungi in Norway spruce?

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ABSTRACT

Bark beetles, mycelia and wood were sampled from the vicinity of insect galleries in the bark of *Picea abies* high stumps of four different age classes in southeastern Sweden. Molecular methods were used for fungal species identification. From 203 samples a total of 21 fungal taxa were found, including 12 ascomycetes and 9 basidiomycetes. Of the filamentous fungal species, 50 % were found both in bark and bark beetles, and 37 % were found in bark, wood and bark beetles. Yeasts dominated in stumps that were 1-yr old and in control samples without insect activity. In 2- and 3-yr-old stumps, filamentous ascomycetes were present but also common wood decay basidiomycetes such as *Stereum sanguinolentum*, *Phlebiopsis gigantea*, *Trichaptum abietinum* and *Fomitopsis pinicola* were found as mycelia associated with insect galleries and on bark beetles. The results indicate that insect facilitation of the establishment of wood decay fungi cannot be neglected.

Introduction

About 30 % of the 25 000–30 000 Fennoscandian multicellular forest species depend on dead or dying wood for some part of their life cycle, and it is thus a key component for biodiversity in these forests (de Jong *et al.* 2004). The most species-rich groups of saproxylic organisms in Fennoscandia are fungi and insects, represented by more than 2 500 and 3 000 taxa, respectively (Petersen 2003; de Jong *et al.* 2004). Due to the introduction of intensive forestry practices at the beginning of the 20th century, the amount of dead wood in forests has decreased (Fridman & Walheim 2000). In accordance with forest certification schemes such as the Programme for the Endorsement of Forest Certification (PEFC) and the Forest Stewardship Council (FSC), one way of increasing the amount of dead or dying wood in boreal forest is to leave high stumps after clear felling (Jonsell *et al.* 2004).

Dead wood is a suitable habitat for both insect and fungal species and the interactions range from fungal pathogens of insects to insect grazing of fungi, and from ephemeral connections to truly mutualistic relationships (Carlile & Watkinson 1994). The symbiotic relationship between ambrosia fungi and ambrosia beetles is well known (Muller *et al.* 2002). Ambrosia beetles make up around 3 400 of the 7 500 species in the weevil subfamily *Scolytinae* (Mueller *et al.* 2005), and can significantly increase the fungal decomposition of bolts of spruce trees by boring into the wood (Muller *et al.* 2002). Ambrosia beetles feed exclusively, or near exclusively, on fungi (Mueller *et al.* 2005), including basidiomycetes, ascomycetes, yeasts and mitosporic fungi (Carlile & Watkinson 1994). The

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female beetle carries the ambrosia fungi in glandular invaginations on the surface of their body called mycangia (Carlile & Watkinson 1994; Paine *et al.* 1997). The wood is inoculated when the female beetle bores tunnels into the sapwood and exudes yeast-like fungal cells from the mycangia before laying eggs. This ensures that there is a ready supply of food available when the larvae hatch (Carlile & Watkinson 1994).

Some of the primary saproxylic bark beetles, including members of *Pityogenes* sp. and *Crypturgus* sp. that colonize living conifer trees, also carry fungi in unspecialized structures on their body surface. Primary scolytid species can sometimes kill a considerable number of living trees if there is an explosion in the population (Paine *et al.* 1997). Attacks by some species, for example, *Dendroctonus ponderosae* and *Ips typographus*, are thought to be aided by aggressive blue-stain fungi vectored by the beetles (Paine *et al.* 1997; Krokene & Solheim 1998; Rice & Langor 2009).

Important factors that affect the fungal community involved in wood decomposition are the physical and chemical properties of the host tree, the micro climate of the forest site and how the tree died (Sippola & Renvall 1999). Wood is made up of lignin, cellulose and hemicelluloses, which are attractive to different fungal species depending on their ability to decompose the different wood components (Petersen 2003). Brown rot fungi, such as *Fomitopsis pinicola*, preferentially decompose the wood cellulose and hemicellulose, whereas white rot fungi, such as *Stereum sanguinolentum*, are able to utilize lignin as well as cellulose and hemicellulose (Rayner & Boddy 1988).

Most basidiomycetes have small (4–24 μm), often asymmetric, spores that are actively discharged when mature. The most common method of spore dispersal is thought to be passively by wind (Rayner & Boddy 1988; Edman *et al.* 2004). However, insects may facilitate the spread by carrying spores on their body and transporting them to a suitable site. Insects may also help fungal spores to penetrate the bark of trees. With parental tracking, Guidot *et al.* (2003) have shown that the sexual cycle of the fire-dependent fungus *Daldinia loculata* can only be completed with the aid of pyrophilous insects. The insects acted as vectors for the male and female fungal gametes within a localized burned forest site by grazing on the conidia and flying between nearby trees, enabling the male gametes to fertilize the female gametes (Guidot *et al.* 2003). The beetle *Dendroctonus pseudotsugae* has also been shown to act as a vector in the transfer of the brown rot fungus *F. pinicola* to trees and may thereby significantly advance the initiation of wood decay (Harrington *et al.* 1981).

Our aim was to study the fungal flora and its possible association with bark- and wood-boring insects in mechanically created high stumps of Norway spruce (*Picea abies*). The main questions were whether bark beetles have the potential to facilitate the establishment of wood decay fungi and whether the fungal flora differs between 1-, 2- and 3-yr-old high stumps.

Materials and methods

Collecting mycelia, wood and insect material

Samples were collected from mechanically created high stumps (approx. 3 m in height, and 20–40 cm in diameter at 1.3 m above ground) of Norway spruce of four different ages. The high stumps were created in the springtime of consecutive years at four clear cut sites in southeast Sweden: the stumps of newly cut trees (i.e. year zero) were located at Kungstomt (N60°7′56.73" E17°48′59.80"); 1-yr-old stumps (i.e. the trees were cut 1 yr before samples were collected) were located at Skyttorp (N60°5′27.00" E17°44′32.11"); 2-yr-old stumps were located at Gimo (N60°11′31.65" E18°7′20.53"); and 3-yr-old stumps were located at Österbybruk (N60°9′48.65" E18°2′38.30). In total, 66 mycelia samples, 87 wood samples, 18 wood control samples (collected where no insect activity was noted) and 32 insect samples were collected from 16 high stumps, divided equally between the four clear cut sites plus two control trees.

Bark at breast height (approx. 1.3 m above the ground) with signs of bark beetle activity was lifted off using a knife, and samples of mycelia were scraped off the interface of the collected bark sample and placed in a microcentrifuge tube. Bark samples (approx. 15 × 15 cm) were collected where mycelia and bark beetle galleries were visible. The bark was carefully examined and any bark beetles in the bark were collected for identification and further examination. The totally dominating species of bark beetles were *Pityogenes chalcographus* and *Crypturgus* sp., whereas e.g. *I. typographus* was absent. The beetles were examined molecularly to verify whether they were carrying fungal propagules. Wood samples were collected in association with the mycelial area in the bark and the bark beetle marks by drilling three holes 2–4 cm into the high stump. Care was taken to remove all remnants of bark before sampling the wood. The drill chips from the three holes were pooled and collected in plastic bags attached to the stump below the drill holes before being moved to 2-ml screw cap tubes. Each sample was collected in a separate plastic bag. Wood control samples were collected from high stumps in a newly felled area (year zero) that were without any trace of bark beetle activity or mycelial outgrowth. To estimate the natural fungal flora that was not directly associated with bark beetle activity in 0-, 1-, 2- and 3-yr-old high stumps, samples were taken from areas of the stump that had no sign of bark beetle activity or mycelial outgrowth. The wood control samples were collected by drilling three holes into each high stump as described above.

To sterilize the instruments before each sample was collected the instruments were sprayed with alcohol and then left to dry. All the samples were stored in a cooling bag in the field for transportation back to the laboratory where they were stored at −20 °C before further analysis.

Molecular identification of fungal species

DNA was extracted according to the CTAB protocol (Gardes & Bruns 1993) with some modifications. Each wood sample was prepared by filling approximately half a 2-ml screw cap tube with drill chips. A screw and a nut were added and the wood was homogenized by shaking for 30 sec in a bead beater (Fast prep FP 120, Savant Instrument Inc., NY, USA). Next, 1.2 ml of CTAB-buffer (3 % cetyltrimethylammonium bromide, 2 mM EDTA, 150 mM Tris–HCl, 2.6 M NaCl, pH 8) was added to each tube and the sample was heated at 65 °C for 1 hr. After centrifugation the supernatant was sequentially extracted with an equal volume of chloroform, centrifuged for 7 min at