HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

The striped ambrosia beetle, *Trypodendron lineatum* (Olivier), and its fungal associates

Heidi Oranen

Pro gradu -thesis

University of Helsinki

Department of Forest Sciences

Forest Ecology, Forest Pathology

April 2013

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta/Osasto – Fakultet/Sektion – Faculty	Laitos/Institution – Department
Faculty of Agriculture and Forestry	Department of Forest Sciences

Heidi Oranen

Työn nimi/Arbetets titel – Title

The striped ambrosia beetle, *Trypodendron lineatum* (Olivier), and its fungal associates

Oppiaine/Läroämne – Subject

Forest ecology

Työn laji/Arbetets art – Level	Aika/Datum – Month and year	Sivumäärä/Sidoantal – Number of pages
Pro gradu -thesis	4/2013	85 p.

Tiivistelmä/Referat – Abstract

Filamentous fungi vectored by *Trypodendron lineatum* were identified. The mycota of flying beetles was distinguished from that of hatching ones. The purpose was also to study to what extent *T. lineatum* is associated with specific fungal partners and opportunistic fungi. The hypotheses stated that: the proportions of beetles carrying epi- and endomycetes (positioned to outer surface and inner parts of insect, respectively) differ (1); proportions of beetles that get attached to fungal propagules vary between collection methods (pheromone traps and stumps) (2); the frequencies of fungal isolates differ between positions (3); the frequencies of isolates are unequal between collection methods (4).

The beetles were collected from two clear felled spruce dominated sites in Southern Finland with pheromone traps in spring and sawing pieces of stumps in summer. The experimental design of this study is unique in studying the effect of trapping time and method on the resulting assemblage of fungi. Identification of fungi rests on morphological characteristics and DNA-based methods. The sequences were compared against BLAST. The hypotheses were tested statistically using odds ratio (logistic regression) for proportions and ratio of averages (Poisson regression) for frequencies. Species richness and diversity for positions, trap types, sites and treatment methods were analyzed using Shannon-Wiener Diversity Index/Shannon Entropy Calculator and EstimateS.

The proportion of beetles carrying epimycetes was greater than that of endomycetes (1). The proportions of vector beetles were not significantly affected by trap type (2). The frequencies of fungal isolates were higher among the epimycota (3). Trap types had no impact on the frequencies of isolated fungi (4). Species richness and diversity analyses supported the outcome of the tested hypotheses. Additionally, they indicated similarity between the mycota of the sites, whereas conclusions concerning the treatment methods could not be made. Seven genera (*Penicillium* sp., *Cadophora* sp., *Cladosporium* sp., *Rhizosphaera* sp., *Sydowia* sp., *Absidia* sp. and *Mucor* sp.) were discovered associated to the striped ambrosia beetle as new. Swarming beetles and those leaving stumps were observed to carry different assemblages of fungi. Origin of the Zygomycota was only traced to the beetles hatching from stumps in midsummer. 61.84 % of the isolates belonged to the Ascomycota, 10.53 % to the Zygomycota and 27.63 % remained unidentified. Best concentrated genera were *Hypocrea*, *Ophiostoma* and *Sydowia*.

Avainsanat/Nyckelord – Keywords *Trypodendron lineatum*, striped ambrosia beetle, epimycota, endomycota, Ascomycota, Zygomycota, *Ophiostoma*

Säilytyspaikka/Förvaringställe – Where deposited

Muita tietoja/Övriga uppgifter – Additional information

Thesis supervisors: docent and university lecturer Risto Kasanen, professor Kari Heliövaara, assistant supervisor Eeva Terhonen

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta/Osasto - Fakultet/Sektion – Faculty	Laitos/Institution – Department
Maatalous- ja metsätieteiden tiedekunta	Metsätieteiden laitos

Tekijä/Författare – Author
Heidi Oranen

Työn nimi/Arbetets titel – Title
Havutikaskuoriainen, *Trypodendron lineatum* (Olivier), ja sen liittolaisena olevat sienet

Oppiaine/Läroämne – Subject
Metsäekologia

Työn laji/Arbetets art – Level	Aika/Datum – Month and year	Sivumäärä/Sidoantal – Number of pages
Pro gradu -tutkielma	4/2013	85 s.

Tiivistelmä/Referat – Abstract

Tarkoituksena oli määrittää havutikaskuoriaisen (*Trypodendron lineatum*) liittolaisena olevia rihmamaisia sienilajeja. Keväällä parveilevien ja keskikesällä kannoista kuoriutuvien kuoriaisten sienilajistoja tutkittiin. Tarkoituksena oli myös selvittää, missä määrin havutikaskuoriaisen sienilajisto koostuu tähän tiukasti sitoutuneista (spesifeistä) lajeista ja löyhemmin sitoutuneista opportunisteista. Hypoteeseissa oletettiin, että: sieniä ulkopinnallaan (epimykeettejä) kantavien hyönteisten osuus eroaa sisällään sieniä (endomykeettejä) kantavien osuudesta (1); sienten vektorina toimivien hyönteisten osuudet eroavat pyyntimenetelmien välillä (2); eristettyjen sienten frekvenssit eroavat sijainnin (epi- ja endomykeetit) suhteen (3); sienten frekvenssit eroavat pyyntimenetelmien välillä (4).

Kuoriaisia kerättiin kahdelta Etelä-Suomessa sijaitsevalta kuusivaltaiselta hakkuualalta feromonipyydyksin ja kannoista. Tutkielman kaikkia menetelmiä ei ole tällaisenaan käytetty aikaisemmin, minkä ansiosta olikin mahdollista selvittää eri ajankohtina toteutettujen pyyntimenetelmien vaikutuksia kuoriaista löydettävään sienilajistoon. Sienten tunnistus perustui niiden ulkonäköön (morfologiaan) ja DNA-sekvensseihin, jotka analysoitiin vertaamalla niitä BLAST -tietokannan sekvensseihin. Osuuksien erilaisuutta testattiin tilastollisesti vetosuhteen (logististen regressio) ja frekvenssien keskiarvojen eroja verrattiin Poisson regression avulla. Lajirunsautta ja monimuotoisuutta analysoitiin sijainnin, pyyntimenetelmien, koealojen ja hyönteisten käsittelymenetelmien välillä. Vertailut toteutettiin Shannon-Wiener Diversity Index/Shannon Entropy Calculator ja EstimateS -sovelluksilla.

Epimykeettejä kantavien hyönteisten osuus oli endomykeettejä kantavien osuutta suurempi (1). Pyydystyypillä ei ollut vaikutusta sieniä kantavien hyönteisten osuuksiin (2). Hyönteisten ulkopinnalta eristettyjen sienten frekvenssi oli suurempi (3). Sienten frekvensseissä ei ollut tilastollisesti merkitsevää eroa pyyntimenetelmien välillä (4). Vertailut lajirunsaudesta ja monimuotoisuudesta tukivat edellä mainittuja tuloksia. Lisäksi havaittiin, että lajiston rakenteessa ei koealojen välillä ollut eroja. Päätelmiä lajiston erilaisuudesta käsittelymenetelmien välillä ei varmuudella voitu tehdä. Kuoriaisen havaittiin kuljettavan mukanaan seitsemää sellaista sienisukua (*Penicillium* sp., *Cadophora* sp., *Cladosporium* sp., *Rhizosphaera* sp., *Sydowia* sp., *Absidia* sp. and *Mucor* sp.), mistä ei ollut aikaisempia havaintoja. Parveilevien ja kannoista kuoriutuvien hyönteisten havaittiin toimivan vektoreina erilaiselle sienilajistolle. Yhtymäsieniä tavattiin ainoastaan kannoista keskikesällä aikuistuvilla kuoriaisilla.

Avainsanat/Nyckelord – Keywords
Trypodendron lineatum, havutikaskuoriainen, epimykeetit, endomykeetit, kotelosienet, Ascomycota, yhtymäsienet, Zygomycota, *Ophiostoma*

Säilytyspaikka/Förvaringställe – Where deposited

Muita tietoja/Övriga uppgifter – Additional information
Ohjaajat: dosentti ja yliopistonlehtori Risto Kasanen, professori Kari Heliövaara ja Eeva Terhonen

Acknowledgements

The process has been challenging but rewarding. It has engaged many persons to whom I am truly grateful. I want to thank docent, university lecturer Risto Kasanen and professor Kari Heliövaara for providing me with the keys for initiating the experiment and helping me many times along the way. I received a lot of valuable information from you and I wish this paper gives you something in its turn.

I also wish to thank professor Fred Asiegbu, Eeva Terhonen and Hui Sun for emphasizing the fact that help was always available. Many times, some major problems were reduced or totally wiped away by Eeva, whose input in this project and importance as my support has been notable. I also wish to thank Riikka Linnakoski for her enthusiasm in recognizing fungi and for sharing her experience. Statistical assistance was kindly provided by university lecturer Hannu Rita. He introduced the logistig regression and Poisson regression models to me and dedicated himself for organizing the complex data with me so that it could be transformed into sentences with common sense.

I appreciate the contribution of the members of the whole forest pathology group for ensuring the functioning of the lab. I'm also grateful to the personnel of The Finnish Forest Research Institute of Vantaa, since they had to take care of the material in their premises. My family and closest friends have also been there for me. They have reminded me of the other forms of life at times, which I do appreciate.

Hopefully, no one whose guidance and help I was enjoying, were here unintentionally forgotten. Thank you all for your patience and support! It has been a great privilege to work with all of you.

Table of Contents

1 INTRODUCTION 1

1.1 Backgrounds of the study 1

1.2 The striped ambrosia beetle, *Trypodendron lineatum*, and related species 1

1.3 Behaviour of insects 3

1.4 Characteristics of the symbiosis between bark beetles and fungi 6

1.5 Fungal associates of bark beetles 7

1.6 Characteristics of *Heterobasidion* species 11

1.6.1 Heterobasidion species complex 11

1.6.2 Impacts of climate on pests and pathogens 12

1.6.3 Spreading strategy of the pathogen 14

1.7 Aims and hypotheses of the study 16

2 MATERIALS AND METHODS 18

2.1 Collection sites 18

2.2 Collecting methods 18

2.2.1 Pheromone traps 18

2.2.2 Pieces of stumps 19

2.3 Treatment of the beetles in laboratory 20

2.3.1 Individually treated beetles 20

2.3.2 Group treatments and second round 21

2.4 Cultivation of fungi 23

2.5 DNA extraction and PCR 24

2.6 Identification of species 27

2.7 Statistical analyses 28

2.8 Species richness and diversity analyses 29

3 RESULTS 31

3.1 The cultivations yielded 122 fungal isolations and 76 DNA extractions 31

3.2 The proportion of beetles carrying epimycetes was distinct from the proportion of beetles carrying endomycetes 31

3.3 The frequencies of fungal isolates were higher among the epimycota 32

3.4 Swarming beetles and those leaving stumps carry different fungi 34
3.5 Epimycota and the mycota of flying beetles were relatively versatile 42
3.6 The associated mycota appeared richer than previously known 44
4 DISCUSSION 46
4.1 Evaluating the methods 46
4.1.1 Treatment of the beetles in laboratory 46
4.1.2 The cultivation of fungi 48
4.1.3 Methods of DNA extraction, PCR and identification of species 49
4.1.4 The statistical methods 50
4.2 Evaluation of the results 51
4.2.1 Fulfillment of the aims and hypotheses of the study 51
4.2.2 Other procedure-related considerations 54
4.2.3 Summarizing the species richness and diversity 57
4.2.4 Results of the DNA extraction and PCR and identification of species . 59
4.2.5 The recovered taxa belonged to the Ascomycota and Zygomycota 60
4.2.5.1 Ascomycota, Pezizomycotina 60
4.2.5.2 Eurotiomycetes 60
4.2.5.3 Leotiomycetes 61
4.2.5.4 Dothideomycetes 62
4.2.5.5 Sordariomycetes 63
4.2.5.6 Ascomycota, Saccharomycetes 64
4.2.5.7 Zygomycota, Mucoromycotina 65
4.3 The study is topical 65
5 CONCLUSIONS 68
APPENDIX

1 INTRODUCTION

1.1 Backgrounds of the study

Different bark beetles species are known to be associated with different assemblages of fungi, among which some tree-killing pathogens may also occur (Bakshi, 1950; Müller et al., 2002; Jankowiak et al., 2009; Linnakoski, 2011). Studies regarding bark beetle-fungi-interactions have greatly focused on economically significant species (Linnakoski, 2011) and only sparse data on associates of non-aggressive beetles is available (Jankowiak et al., 2009). Current knowledge has thus warranted space for further studies and *Trypodendron lineatum* Olivier 1795 was here selected for the purpose of examining the mycobiota of a non-aggressive bark beetle species.

1.2 The striped ambrosia beetle, *Trypodendron lineatum*, and related species

Trypodendron lineatum Olivier 1795 (*Xyloterus lineatus* Ol. (Mathiesen-Käärik, 1953); *Xyloterus lineatum, Tomicus lineatum* (Metsätuho-opas, 2010)) (Coleoptera: Curculionidae: Scolytinae) is a common pest in Finland. The distribution area of the striped ambrosia beetle includes Europe, Siberia, Asia Minor, North Africa and North America (Heliövaara et al., 1998). The scolytid favours shady sites as its habitat and spruce and pine as its host tree species. It may also attack larch (Bakshi, 1950; Löyttyniemi, 1967), fir trees (Park & Reid, 2007), Douglas fir (Chapman, 1966), Western hemlock and Western red cedar (Borden, 1988). The beetle prefers trees that have been felled the previous autumn or winter (Dyer & Chapman, 1965). The 3.0-3.5 mm long striped ambrosia beetle inhabits stumps, dead standing trees, windthrows and unbarked timber. *T. lineatum* is a brownish black bark beetle with light coloured longitudinal stripes on its back (elytra i.e. hard fore wings (Amateur Entomologists'..., 2012)) (Heliövaara et al., 1998).

The swarming period of the striped ambrosia beetle begins as temperature exceeds 15 °C, taking place usually at the end of April or during the first weeks of May (Heliövaara et al., 1998). The generation time of *T. lineatum* is one year (Heliövaara et al., 1998), during which the development of multiple broods is possible (Chapman & Kinghorn, 1958; Annila et al., 1972). After the early initial flights in spring, *T. lineatum* adults typically fly once more in June-July, in order to establish another

brood (Chapman & Kinghorn, 1958). The larvae develop inside the wood (Mathiesen-Käärik, 1953) within approximately a two-month-long period (Shore et al., 1987) but the development is dependent on climatic factors (Jönsson et al., 2007). Broods developing late in the season may die due to encountering winter in vulnerable larval or pupal stage (Christiansen & Bakke, 1988). The beetles abandon their host in the autumn, overwinter in litter or duff close to the base of a trunk or a stump (Borden, 1988; Lindgren, 1990) and may return in late spring (Bakshi, 1950).

Bark beetles usually excavate their galleries and brood chambers near phloem while ambrosia beetles bore deeper into the sapwood (Beaver, 1989; Müller et al., 2002; Six, 2012). The attacks of the striped ambrosia beetle leave small holes (1 mm) on the tree (Heliövaara et al., 1998) that can be detected from white boring dust on the bark (Park & Reid, 2007). The mother cavity of *T. lineatum* goes into the depth of ca. 2 cm and divides into two horizontal cavities that retell annual rings. The vertical larval cavities are ca. 4-5 mm long located in the sapwood above and beneath the mother cavity (Mathiesen-Käärik, 1953; Heliövaara et al., 1998). By engraving deeper galleries the beetle might escape from predators. The feeding habit of ambrosia beetles is termed xylomycetophagy (Beaver, 1989). It means that the beetle inoculates the wood with symbiotic ambrosia fungi (Bakshi, 1950; Beaver, 1989), which serve essentially as nourishment for the larvae and young adults of the striped ambrosia beetle (Mathiesen-Käärik, 1953; Beaver, 1989). The ambrosia fungus of *T. lineatum* is *Ambrosiella ferruginea* L.R. Batra 1968 (Mathiesen-Käärik, 1953).

T. lineatum is a significant pest from economical and ecological perspective. *T. lineatum* favours dying or dead trees preferably found in conifer dominated stands (Borden, 1988) prior to and a while after harvest (Park & Reid, 2007). Stumps of 1- to 2-year-old and logs stored on forest sites or mill yards provide this early successional beetle species with breeding habitat (Lindelöw et al., 1992; Park & Reid, 2007) of suitable moisture content (Orbay et al., 1994). The beetle spreads blue stain fungi, which turn the walls of the galleries and wood into dark coloured (Mathiesen-Käärik, 1953; Heliövaara et al., 1998). Thus, they cause losses by spoiling wood aesthetically, causing import bans and remanufacturing costs (Orbay et al., 1994). From

an ecological point of view, ambrosia beetles are the first invaders of dead wood their cavities functioning as entries for saprotrophic fungi. They are involved in the maintenance of stand dynamics as their attacks can be considered to initiate the decomposition of wood and nutrient recycling (Lindgren, 1990).

Trypodendron species involve *T. signatum* Fabricius 1787 (3.2-3.5 mm long), which prefers deciduous trees as its host. It may attack timber cut over a year ago. The stripes of *T. signatum* are yellow and black the contrast being clear (Heliövaara et al., 1998). The timing of its flight period is the same with *T. lineatum* but *T. signatum* is not as common as *T. lineatum* (Heliövaara et al., 1998; Martikainen, 2000). A third striped beetle, *T. domesticum* Linnaeus 1758 (3.1-3.8 mm), sometimes sympatric with *T. lineatum* (Bakshi, 1950), occurs mainly in southern Finland (Heliövaara et al., 1998). *T. domesticum* favours recently damaged deciduous host trees. Its yellowish brown elytra share one black longitudinal stripe. The swarming of *T. domesticum* begins early in the spring (Heliövaara et al., 1998). Fourth, there is *T. proximum* Nijima 1909 (syn. *T. laeve* Eggers 1939, *T. piceum* Strand 1946) (Heliövaara et al., 1998; Martikainen, 2000), which resembles *T. lineatum* in size (3.0-3.5 mm), host preference and appearance, albeit often lacking clear stripes (Heliövaara et al., 1998). *T. proximum* begins to fly at + 13 °C. It may briefly swarm simultaneously with other *Trypodendron* species before finishing its short flight period. The adults of *T. proximum* may overwinter in tree stems, which could allow them to initiate flying before the snow cover has melted. *T. proximum* is more widespread in Finland and other Nordic countries than has been considered (Martikainen, 2000). These *Trypodendron* species could be distinguished by the colour of their legs (femur), the shape of their antennae and dots or hair on their back, and based on the ecology shortly described above (Heliövaara et al., 1998; Martikainen, 2000).

1.3 Behaviour of insects

Surveys about insect behaviour have yielded different theories. For example, the ideal free distribution (IFD) model introduced by Fretwell & Lucas (1969) suggests that the number of specimens directly corresponds to the resource abundance in a patch. When an IFD has been achieved, changing patch is probably not going to bring any

added value to the individual. The IFD model neglects competition between insects, time lags and costs of searching for the best breeding substrate (Kausrud et al., 2011). Another theory, the resource concentration hypothesis, predicts that herbivores accumulate where their host is more abound (Root, 1973) and takes search costs into account (Park & Reid, 2007). According to the theory, patches that are rich in host plants, will more easily be found and less eagerly left behind by specialized insects (Root, 1973). Kausrud et al. (2011) developed the sequential restricted distribution model (SRD). It suggests that migration and high breeding density in the host tree would restrict the energy reserves of the insect, and that therefore beetles emerging from densely inhabited trees tend to attack those close to the parental host (Kausrud et al., 2011).

Bark beetle populations respond quickly to ecological changes such as decreased host defence due to for example forestry and changes in climate (Kausrud et al., 2011), which allow some beetle species for the development of a second generation (Jönsson et al., 2007). Since reaching an adequate breeding tree could be critical for a beetle (Wermelinger, 2004), ambrosia beetles can occupy their woody habitat at high densities (e.g. the average for *T. lineatum* is 150-215 attacks m^{-2}) (Borden, 1988). However, heavy attacks have been observed to alter logs unattractive (Chapman & Kinghorn, 1958) for further settlement, whereas no reduction of log attractiveness has resulted after light attacks by *Trypodendron* (Chapman, 1966). Consequently, it has been considered that only at high population densities it would be beneficial for a female to seek for a new habitat (Hoover et al., 2000). As it intends to do so, the xylomycetophagous scolytid, *T. lineatum*, can search for a suitable breeding patch at least 1.5 km away (Salom & McLean, 1991). Hibernation may have reduced the stored lipids of the beetle by 25 %, which during search for a new habitat may become decreased by another quarter (Nijholt, 1967). This kind of a decline in energy reserves may have negative implications on reproduction. It has been assessed that beetles make a trade-off between their somatic and reproductive condition as they propose to mate (Elkin & Reid, 2005). The best reproductive success is most likely gained at low or intermediate breeding densities (Økland & Bjørnstad, 2006), and, depending on the species, enhanced fitness achieved when attacks are focused on living trees (Kausrud et al., 2011).

In short, host colonization includes phases of dispersal, selection, concentration and establishment (Wood, 1982). During these phases of its life, *T. lineatum* relies on a combination of factors. As the temperature allows the beetles to fly after hibernation (Chapman & Kinghorn, 1958), they respond positively to light (Chapman & Kinghorn, 1958; Borden, 1988) and semiochemicals (Salom & McLean, 1990). Beetles respond to olfactory stimuli, which refers to their ability of sensing message-bearing odors (Wood, 1982; Ranger et al., 2011). Bark beetles are attracted to host volatiles, which are produced following stagnation of normal metabolism in a host (Graham, 1968), and furthermore, released after wounding and felling of trees (Lindelöw et al., 1992). Ethanol, for example, serves as the primary attractant (Borden, 1988) especially for the female striped ambrosia beetles (Bakke, 1983). It is liberated from stressed, dying and decaying hosts and is known to lure wood borers, including ambrosia beetles (Graham, 1968; Borden, 1988). Another host-derived attractant, α-pinene (Borden, 1988), is at a certain release rate known to increase catches of *T. lineatum* (Klimetzek et al., 1981; Bakke, 1983). Lineatin, instead, is the aggregation pheromone of *T. lineatum* acting as the secondary stimulus (Borden, 1988).

All of the three components mentioned above (ethanol, α-pinene and lineatin) seem to act synergistically and influence the behaviour of *T. lineatum* in its European populations (Klimetzek et al., 1981; Bakke, 1983). Ethanol has been suggested to attract the beetles at short and lineatin at longer distances. Since females are the pioneering sex for *T. lineatum*, they are attracted to both ethanol and lineatin during their flight and landing on a host (Salom & McLean, 1990). Male beetles are primarily attracted to lineatin (Salom & McLean, 1990) synthesized from host chemicals and liberated from the female hindgut (Borden, 1988). This kind of a system, where one semiochemical (e.g. ethanol) speeds up the finding of a host and another (lineatin) has slightly different influence on the two sexes, enhances their survival and breeding (Salom & McLean, 1990).

Sympatric species may be attracted to the very chemicals but reproductive isolation could be gained through enantiospecificity. For example, in British Columbia, the

response of *T. lineatum* to different combinations of (+)-lineatin and (-)-lineatin was solely a result of presence or absence of (+)-lineatin (Hoover et al., 2000). α-pinene, instead, may have the potential of keeping conifer-attacking *Trypodendron* species separate from those attacking hardwoods (Klimetzek et al., 1981). In addition to the points discussed above, the behaviour of *T. lineatum* is affected by many other factors. Abundance of host material, air temperature, air-mass movements, altitude and melting of snow (Chapman & Kinghorn, 1958), are examples of these.

1.4 Characteristics of the symbiosis between bark beetles and fungi

Entrance holes of bark beetles not only allow for access to the wood for fungi (Lindgren, 1990) but the beetles act as vectors for fungi (Bakshi, 1950; Viiri & Lieutier, 2004; Jankowiak et al., 2009; Linnakoski, 2011). Fungal propagules may be dispersed on several insect body parts such as pronotum (first upper section of the central bodypart (Amateur Entomologists'..., 2012)), mandibles (pair of mouthparts (Amateur Entomologists'..., 2012)), elytra, legs (Bueno et al., 2010) but also in digestive tract (Whitney, 1982; Furniss et al., 1990). Ambrosia beetles have specialized organs called mycangia that seal the fungal cells, and that locate in the prothoracic cuticle or elsewhere in the insect body (Francke-Grossmann, 1967; Borden, 1988; Beaver, 1989; Kajimura & Hijii, 1992; Fraedrich et al., 2008; Yuceer et al., 2011). In *T. lineatum*, the tubelike mycangia are in the integument of the prothorax their openings close to the precoxal cavities. Most generally it is only the sex that begins the tunneling in the host that possesses mycangia (Francke-Grossmann, 1967).

A fungal symbiont that provides the developing larvae and mature beetles with food will be introduced to the wood attacked by ambrosia beetles (Mathiesen-Käärik, 1953; Francke-Grossmann, 1967; Yuceer et al., 2011). Feeding on the ambrosia fungi is essential for the maturation of the gonads of ambrosia beetles. Therefore the development of a subsequent offspring is dependent on the establishment of the fungus (Beaver, 1989). Vectoring aggressive fungi may be advantageous to bark beetles in terms of preventing the host from expressing its resistance against the attack and from recovering from the invasion, which could be detrimental to the developing offspring. The associated organisms may modify the wood more suitable substrate for

oviposition (Whitney, 1982). The fungi may also be involved in the production of beetle pheromones (Beaver, 1989). The fungal partner of the symbiosis in its turn is assessed in the dispersal and establishment in wood (Whitney, 1982). As an indication of coevolution are, for example, the sticky spores produced by the blue stain fungi (Mathiesen-Käärik, 1953). They attach easily on the insect body (Mathiesen-Käärik, 1953) and get reliably transported to a new environment (Beaver, 1989). It is contributed to continue its existence (Whitney, 1982), since the beetle may maintain the dominance of ambrosia fungi in the galleries at the expence of other fungal species (Beaver, 1989). The fungal spores may be protected from desiccation and deleterious UV-light (McKee, 1969) as they are sealed within mycangia during the transportation.

The description above lets one believe the relationship is beneficial for both of the members (i.e. the beetle and the fungus). However, the relationship can sometimes be other than mutualistic (Whitney, 1982), since certain ophiostomatoid fungi may be antagonistic to the beetle vector (Beaver, 1989; Hofstetter et al., 2006). On the other hand, being strictly depedent on each other will have costs. If the beetle dies during swarming, for example, the fungus will be lost as well. If the fungus fails to establish in the wood, the beetle cannot copulate (Beaver, 1989).

1.5 Fungal associates of bark beetles

It has been considered that aggressive beetle species are more often associated with more virulent fungi, whereas nonaggressive beetle species are more commonly associated with less pathogenic fungi (Krokene & Solheim, 1996; Krokene & Solheim, 1998a). Pathogenic fungi may be of high importance for bark beetles in killing initially healthy trees (Krokene & Solheim, 1998a). Nonaggressive bark beetle species, instead, may not need pathogenic sapstain fungi to be able to colonize a tree since they prefer weakened trees (Jankowiak et al., 2009). The pioneer fungi settling on a living host must have the ability of intruding into the vital tissues of the plant (Six & Wingfield, 2011). An exception to the above-mentioned trends is brought along the fact that nonaggressive bark beetles may vector virulent or moderately virulent fungi (Krokene & Solheim, 1996; Krokene & Solheim, 1998a; Jankowiak et al., 2009).

Also, in certain conditions a bark beetle may colonize a tree without pathogenic fungi (Persson et al., 2009). The composition of the mycota associated with bark beetles is linked to the prevailing circumstances. During low insect population levels the mycobiota of a given pest seem to include less virulent fungi (Viiri & Lieutier, 2004) than during epidemic levels. The fungal communities inhabiting different woody niches change along succession and vary under different conditions (Lygis et al., 2004; Viiri & Lieutier, 2004), which can be reflected as variation in the composition of the fungal flora associated with beetles (Müller et al., 2002; Persson et al., 2009).

Many bark beetle species transport ophiostomatoid fungi from genera such as *Ophiostoma*, *Graphium* and *Leptographium* (Mathiesen-Käärik, 1953; Jankowiak et al., 2009; Linnakoski, 2011), which can be either pathogenic capable of killing healthy trees or saprotrophic (non-pathogenic) (Krokene & Solheim, 1998b; Bueno et al., 2010). Ophiostomatoid fungi may cause blue-stain in wood (Krokene & Solheim, 1998b) while others cause no staining (Krokene & Solheim, 1998a). Discolouration of wood is related to higher virulence of the fungus (Krokene & Solheim, 1998a). Staining of wood is caused by brown pigment particles deposited in the hyphae (Whitney, 1982) and seems to be connected with the achieved density and stage of hyphal development within the wood tissue (Whitney, 1982; Krokene & Solheim, 1998a). The terms ophiostomatoid and bluestain or sapstain fungi are often used as synonyms, even if all ophiostomatoid species do not cause bluestain.

In Finland, Müller et al. (2002) isolated *Antrodia serialis* (Fr.) Donk 1966, *Trichoderma* sp. and *Phlebiopsis gigantea* (Fr.) Jülich 1978 from spruce logs that were attacked by *T. lineatum*. Interestingly, *Heterobasidion annosum* (Fr.) Bref. 1888 and *Sporothrix* sp. were also found associated to *T. lineatum* colonization, yet less frequently (Müller et al., 2002). *Ceratocystis piceae* (Münch) B.K. Bakshi 1950, *Leptographium lundbergii* Lagerb. & Melin 1927 and *Heterobasidion* sp. have also been observed in the galleries of *T. lineatum* (Bakshi, 1950; Bakshi, 1952). More recently, Linnakoski et al. (2010) found four ophiostomatoid species from *T. lineatum*. Of these, *Ophiostoma canum* (Münch) Syd. & P. Syd. 1919 has been described beforehand, but *O. canum*-like, *O. pallidulum* Linnak., Z.W. de Beer & M.J. Wingf.

2010 and *O. rachisporum* Linnak., Z.W. de Beer & M.J. Wingf. 2010 were new to science. None of these species could be detected from galleries of *T. lineatum*, while *O. minus* was (Linnakoski et al., 2010).

The spruce bark beetle *Ips typographus* Linnaeus 1758 may succeed in passing the constitutive protection mechanisms of a tree (Christiansen & Bakke, 1988; Persson et al., 2009). High diversity of ophiostomatoid fungi has often been present with *I. typographus* (Viiri & Lieutier, 2004; Persson et al., 2009). Although with sometimes low frequency, the pest has been reported to be associated with *Ceratocystis polonica* (Siemaszko) C. Moreau 1952 (Jankowiak et al., 2009) and *Grosmannia europhioides* (E.F. Wright & Cain) Zipfel, Z.W. de Beer & M.J. Wingf. 2006, which are among the most virulent bluestain fungi (Persson et al., 2009). The study by Viiri & Lieutier (2004) has revealed the pest associated with *O. bicolor* R.W. Davidson & D.E. Wells 1955, *O. penicillatum* (Grosmann) Siemaszko 1939, *O. piceaperdum* (Rumbold) Arx 1952, (synonym for *O. europhioides* (E.F. Wright & Cain) H. Solheim 1986 (Jacobs et al., 2000)), *O. ainoae* H. Solheim 1986, *Ceratocystiopsis minuta* (Siemaszko) H.P. Upadhyay & W.B. Kendr. 1975 and to lesser extent with *O. piceae* (Münch) Syd. & P. Syd. 1919 and *O. cucullatum* H. Solheim 1986. *Pesotum* species have also been discovered related to *I. typographus* in France (Viiri & Lieutier, 2004). The name *Pesotum* refers to anamorphs of *Ophiostoma* (Harrington et al., 2001; Geldenhuis et al., 2004). Persson et al. (2009) detected a wide range of *I. typographus*-associated ascomycetes, within which *Penicillium* sp. and *Trichoderma* sp. were very concentrated (Persson et al., 2009). Dissemination of yeasts (Furniss et al., 1990; Persson et al., 2009), basidiomycetes such as *P. gigantea*, *Fomitopsis pinicola* (Sw.) P. Karst. 1881, *Trichaptum abietinum* (Dicks.) Ryvarden 1972, *Bjerkandera adusta* (Willd.) P. Karst. 1879 and *Stereum sanguinolentum* (Alb. & Schwein.) Fr. 1838, and *Mucor* sp. as a member of the less common group of zygomycetes by *I. typographus* has been observed (Persson et al., 2009).

Bueno et al. (2010) used different methods to study the fungal communities associated with *Ips sexdentatus* Börner 1776 attacking Maritime pine, *Pinus pinaster* Aiton 1789, in Spain. From pines naturally colonized by *I. sexdentatus*, they found four (in

total six with the imperfect states included) species of ophiostomatoid fungi (the pathogenic *Ophiostoma ips* (Rumbold) Nannf. 1934, *O. brunneo-ciliatum* Math., *Ophiostoma* sp., *Ceratocystiopsis minuta* (and the imperfect states *Graphium* and *Sporothrix*)) and one species of Deuteromycetes. Divergent spectra of fungi were recovered by the different treatments. *Trichoderma* sp., *Penicillium* sp., *Aspergillus niger* Tiegh. 1867, *Verticillium* sp., *Gliocladium viride* Matr. 1893 and *Sporothrix* sp. were recovered when a beetle had walked on the medium or the water that had been used for washing a beetle had been placed on the medium. They also distinguished the fungi on different insect body parts and found the differences statistically significant. The species discovered on different body parts were *Penicillium* sp. (elytra), *Mucor* sp., *Scopulariopsis brevicaulis* (Sacc.) Bainier 1907 (only from female mandibles), *O. ips* (only from male pronotum and elytra) and eight taxa of *Sporothrix*. Based on the method of introducing beetles into the pine wood, they found five different ophiostomatoid species (*Sporothrix* spp.) and three saprotrophic fungal species (*Trichoderma* sp., *Penicillium* sp., and *Mucor* sp.) (Bueno et al., 2010).

Leptographium lundbergii Lagerb. & Melin 1927, *Mariannaea elegans* (Corda) Samson 1974 and *Mortierella* sp. have been isolated from dead pine (Lygis et al., 2004) without confirmation of relationship with insects. The possibility of such an event should not be excluded because Bakshi (1950) reported the former species associated to *T. lineatum*, and Persson et al. (2009) traced *Mortierella* spp. and *M. elegans* associated with *I. typographus*, which despite of preferring spruce may attack pine as well (Heliövaara et al., 1998).

The studies cited above should at least to some extent demonstrate which fungal phyla are the typical associates of bark and ambrosia beetles attacking spruce and pine. Based on those, one could postulate that ascomycetes including ophiostomatoid fungi and yeasts (both ascomycetes and basidiomycetes) benefit from their winged partners more often than mycelial basidiomycetes and zygomycetes or chytridiomycetes and glomeromycetes do.

Although only rarely has *Heterobasidion* been detected from an insect (Kirschner, 1998), insect vectors of the pathogen have been confirmed. *Heterobasidion* has been detected on large pine weevil, *Hylobius abietis* Linnaeus (Kadlec et al., 1992; Viiri, 2004) and from wood attacked by *T. lineatum* (Bakshi, 1950; Bakshi, 1952; Müller et al., 2002). In more detail, conidiophores of *Heterobasidion* have been detected from the galleries of the striped ambrosia beetle, *T. lineatum* (Bakshi, 1950; Bakshi, 1952).

1.6 Characteristics of *Heterobasidion* species

1.6.1 *Heterobasidion* species complex

Heterobasidion species cause significant economical losses to forestry. In EU region, the losses caused by *Heterobasidion* species reach about 790 million Euros every year (Woodward et al., 1998). The annual losses in Finland have been estimated to range from 35 to 50 million Euros (Woodward et al., 1998; Finland´s National Forest Programme 2015, 2008) while in Sweden the losses have been reported to vary between 50 and 100 million Euros (Oliva et al., 2010).

The genus *Heterobasidion* belongs to Bondarzewiaceae (family), Russulales (order), Incertae sedis (uncertain taxonomic position), Agaricomycetes (class), Agaricomycotina (subphylum) and Basidiomycota (phylum) (Dictionary of…, 2012). The conidial state of *Heterobasidion* has been termed *Oedocephalum lineatum* Bakshi 1952 and *Spiniger meineckellus* (A. J. Olson) Stalpers 1974 (Bakshi, 1952; Niemelä & Korhonen, 1998; Dictionary of…, 2012).

In biology, the term species refers to a group of individuals capable of reproducing with each other. Taxonomical classification of fungi largely rests upon the morphological features rather than breeding relationships. If a taxonomic species consists of more than one biological species, the species (biological species within a taxonomic classification) are termed intersterility groups (ISGs) (Mitchelson & Korhonen, 1998). *Heterobasidion sensu lato* (s.l.) has been used to refer to the *Heterobasidion* species complex (Niemelä & Korhonen, 1998). The complex of *Heterobasidion annosum* s.l. has been divided into three Eurasian and two North American

intersterility groups including pathogens and saprobes with a wide host range (Korhonen & Stenlid, 1998).

The Eurasian species complex includes P-, S- and F-types of fungi (intersterility groups, ISGs P, S and F). These subtypes have been described as distinct species (Korhonen, 1978; Capretti et al., 1990). The P-type, *H. annosum sensu stricto* (s.s.) (Fr.) Bref., is present all over the Europe despite the most dry and northern sites (Korhonen & Lipponen, 2001). *H. annosum* s.s. infects different species of pine, spruce, fir (Schönhar, 1995; Korhonen & Stenlid, 1998) and juniper (Niemelä & Korhonen, 1998) but larches, Douglas firs (Filip & Morrison, 1998) and birches as well (Lygis et al., 2004). The S-type, *H. parviporum* Niemelä & Korhonen 1998 (Korhonen & Lipponen, 2001), infects mainly spruce in its natural distribution area (Niemelä & Korhonen, 1998). It is common in Central and Northern Europe, Southern Siberia, China and Japan. The southernmost appearance of *H. parviporum* in Asia may be restricted to eastern Himalayas, where it seems to remind the F-type, *H. abietinum* Niemelä & Korhonen 1998. In Europe, *H. abietinum* may cause disease in firs originated from Southern or Central Europe but may infect spruces as well. The distribution area of *H. abietinum* is restricted to Poland in the north, Bulgaria in the east, Italy and Greece in the south and France in the west (Korhonen & Lipponen, 2001).

The North American ISGs have been given new names: ISG P is *H. irregulare* Garbel. & Otrosina 2010 and ISG S is *H. occidentale* Otrosina & Garbel. 2010 (Otrosina & Garbelotto, 2010). Five economically negligible *Heterobasidion* species are known. These are *H. araucariae* P.K. Buchanan 1988, *H. insulare* (Murr.) Ryv. 1972, *H. perplexum* (Ryv.) Stalpers 1996, *H. pahangense* Corner 1989 and *H. rutilantiforme* (Murrill) Stalpers 1996 (Niemelä & Korhonen, 1998).

1.6.2 Impacts of climate on pests and pathogens

Along the longer periods of high air temperatures the development of multiple broods of bark beetles may become possible (Christiansen & Bakke, 1988; Jönsson et al., 2007). During the summers 2010 and 2011 in Finland, for example, the popu-

lation size of *Ips typographus* has reached epidemic levels at some southern and southeastern parts of the country (Kirjanpainajahyönteisten määrä…, 2012). The possibility of bivoltinism within *T. lineatum* populations should not be excluded. Simultaneously with the growing population size grows the risk of beetles taking over living trees.

Witzell et al. (2011) studied the effect of temperature regime on the potential spreading of *H. annosum* in Scandinavia. They assumed that temperature is the key factor affecting the distribution of the pathogen, provided that susceptible host trees exist in the area. Based on their results obtained from inoculation experiments in southern locations, they concluded that warming climate might not push *H. annosum* towards the north. Climatic factors hardly are the sole agents influencing the distribution of pathogens (Witzell et al., 2011). In agreement with the previous was the study by Kasanen et al. (2011), in which no correlation between annual mean temperature and infection rate of stumps was discovered. However, it was stated that the impact of temperature sum should not be totally ignored (Kasanen et al., 2011).

Changing climate might affect the distribution of different forest pathogens in different manner. This is expected to take place especially in the marginal areas of their current distribution area (Witzell et al., 2011). In Finland, *H. annosum* s.s is more common in the south-eastern parts of the country (Fig. 1.). Because the pathogen may spread towards west, the area where stump treatment costs can be compensated by public funds, has been widened (Piri, 2011; Juurikäävän torjunta…, 2012).

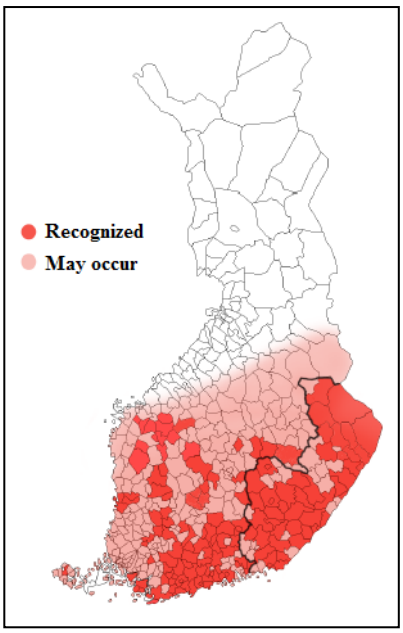

Figure 1. Current distribution area of *H. annosum* s.s. in Finland. The pathogen has been recognized in the dark red areas and probably occurs in the light red areas (Modified from Piri, 2011).

1.6.3 Spreading strategy of the pathogen

Fresh stump surfaces (Rishbeth, 1948) and wounds are the most important routes for primary infection of root rot (Korhonen & Stenlid, 1998). Spores of *Heterobasidion* are produced in basidiocarps that most often hide in stumps, logs and on the roots of wind thrown trees. Humid conditions appear fundamental for germination of basidiospores (Rishbeth, 1951; Korhonen & Stenlid, 1998). Once a basidiospore germinates into homokaryotic mycelium, it will be heterokaryotized with another homokaryon allowing fruiting structures to emerge. More virulent heterokaryotic mycelium is capable of overcoming the resistance mechanisms of the target host. Secondary growth may allow *Heterobasidion* genets to linger and stay infectious for at least half a century, provided that there is a suitable nutrient base available (Korhonen & Stenlid, 1998).

From an infected stump or stem, secondary spreading of the mycelium into adjacent trees may take place via root contacts (Rishbeth, 1948). Varying observations have been reported about the rate at which the fungus can spread. The spreading is faster in tissues lacking active defence mechanisms (Schönhar, 1978) and immediately after establishment (Korhonen & Stenlid, 1998). In roots of living spruce, the average

growth rate has been 9-12 cm per year (Stenlid & Johansson, 1987; Bendz-Hellgren et al., 1999). Rishbeth (1948) has reported growth rates in root system of pines lying between 30 cm and 80 cm per year. In stump roots the growth has been 25 cm $year^{-1}$ and even one meter per year (Rishbeth, 1948; Bendz-Hellgren et al., 1999). The advance of decay in stem may reach 70 cm $year^{-1}$ (Jørgensen et al., 1939). However, environmental factors may modify the spreading patterns (Garbelotto et al., 1999). For example, cold weather may reduce mycelial growth and development of decay in wood (Cowling & Kelman, 1964). As summarized by Piri (2003) frequency of root contacts, competition and antagonism also influence secondary spreading. Resistance and physiological status, on the other hand, are such host-related factors that have an impact on secondary spreading of the pathogen. Size of inoculum and virulence are the corresponding pathogen-related factors. The development of the disease is also affected by age of the both counterparts (Piri, 2003). On unsuitable soils, lack of advantageous mycorrhiza could predispose planted trees to disease (Piri, 2011).

Spore deposition has diurnal and seasonal variation. In southern Finland, *Heterobasidion* spores are present in highest amounts from April to November and are absent from December to mid-April. The amount of deposited spores in the vicinity of infection centre in southern Finland can vary between 30 000 and in most cases 200 000 spores dm^{-2} h^{-1} (Kallio, 1970). Despite that the basidiospores tolerate 80 °C below zero (Kasanen, 2009), they cannot be liberated from frozen fruiting bodies (Rishbeth, 1951). At another time, the spores can spread into distances of 50-500 km but most settle within a few meters from the basidiocarp (Kallio, 1970). Consequently, basidiospores constitute the major part of the inoculum (Korhonen & Stenlid, 1998). *Heterobasidion* mycelium may not substantially spread freely in the soil (Rishbeth, 1948; Hodges, 1969). Instead, both basidiospores and conidia landing on soil with moisture content of 30 % have retained the ability to germinate for at least 12 months (Schönhar, 1980).

1.7 Aims and hypotheses of the study

According to the literature, ascomycetes including ophiostomatoid fungi and yeasts (both ascomycetes and basidiomycetes) are more commonly vectored by bark beetles than are filamentous basidiomycetes and zygomycetes. Reports have revealed sparse data on the remaining two fungal phyla chytridiomycetes and glomeromycetes. The previous studies suggest also that basidiomycetes might be more closely associated with the striped ambrosia beetle, *T. lineatum*, than ambrosia and bark beetles in general (see 1.5).

Several reports suggest (Bakshi 1950, 1952; Müller et al., 2002) that *T. lineatum* commonly carries spores of *Heterobasidion* spp. and therefore the pathogen was expected to be found. It was also assumed that *Phlebiopsis gigantea* could be transported by the striped ambrosia beetle. Recovery of the ambrosia fungus *Ambrosiella ferruginea* (former *Monilia ferruginea*) (Mathiesen-Käärik, 1953) was expected, because the species should be a vital part of the fungal garden *T. lineatum* cultivates (Klimetzek et al., 1981). However, the previous studies provided no information, whether the fungal partners are more often associated to swarming or hatching insects.

In the present study, the mycota of flying beetles was distinguished from that of hatching ones. The experimental design of this study with the different cultural methods is unique in studying the effect of trapping method and time (see 2.2) of insects on the resulting assemblage of fungi. The study aimed to identify filamentous fungal species that could be isolated from *T. lineatum*. Yeasts, bacteria, nematodes or other organisms associated with this ambrosia beetle species were not on focus in the study.

The first hypothesis of this experiment was set to reveal to what extent *T. lineatum* is associated with specific fungal partners and opportunistic fungi. Consequently, it stated that the proportions of beetles carrying epi- and endomycetes (positioned to outer surface and inner parts of insect, respectively) differ (1). It was also hypothesized that the proportions of beetles that get attached to fungal propagules vary be-

tween collection methods (pheromone traps and stumps) (2). The frequencies of fungal isolates were assumed to be different between positions (3). The frequencies of fungal isolates were considered to be unequal between collection methods (4). Statistical tools were used to study proportions of beetles carrying certain types of fungi as well as to study frequencies of fungal isolates. Species richness and diversity were analyzed between positions, trap types, sites and treatment methods.

2 MATERIALS AND METHODS

2.1 Collection sites

The insects were collected from two sites located in the county of Uusimaa in Myrskylä. The sites were called Paavola (P) (60°40´N, 25°47´E) and Kreivilä (K) (60°39´N, 25°56´E) and had been clear felled the previous autumn in 2009. The sites were fertile (at least of *Vaccinium myrtillus* (MT) forest site type) but somewhat stony. Site P located at a rocky slope heading south. The estimated thermal autumn in the reforestation areas had begun at the earliest on 24th September 2009 (Ilmatieteen laitos, 2012b). The stumps had not been treated with control agents.

2.2 Collecting methods

2.2.1 Pheromone traps

Two pheromone baited funnel traps were set on two sites, one per each, on April 23rd 2010. According to the climatic data for 2010, the estimated thermal spring begun around April 26th in the collection area (Ilmatieteen laitos, 2012a). The traps were removed after two weeks. The traps consisted of six plastic funnels that were upside down and partly within each other. On the top of the funnels there was a roof and the pheromone specific for *Trypodendron lineatum* (Trypowit®, Bio/Technik/Chemie Witasek, PflanzenSchutz GmbH, Feldkirchen) was put hanging below the roof. A container was attached below the funnels and at the bottom of the container there was a net that would let rainwater to exit. The length of the trap was approximately one meter. The traps were fastened up with a wire to small stems that had remained at the sites after the clear cut. The traps were attached so that the lowest end of each trap was somewhat higher than a meter above the ground (Fig. 2.). The catches were named after the name of the sites: P (Paavola) and K (Kreivilä). The insects were left in the containers sealed with film. The beetles were temporarily stored at +5 °C in Viikki, University of Helsinki. Afterwards a portion of the beetles was pre-sterilized with 70 % ethanol and sterile MQ-water and frozen but a portion was frozen without any treatment.

Figure 2. Hanging up the pheromone trap in Paavola (Kasanen, 2010).

2.2.2 Pieces of stumps

Pieces of stumps of spruce were sawn on June 14th 2010 from the same sites as where the pheromone traps were used. The summer was warm and rainfall less than average (Ilmatieteen laitos, 2012b, c). The stumps were first checked for attacks of *T. lineatum.* The gnawings could be detected by the typical boring dust on the dark bark of the stump (Park & Reid, 2007). The sawn pieces were labeled after the name of the site with a running number (P1-P6, K1-K6). The pieces of twelve stumps (six per site) were transferred to the premises of the Finnish Forest Research Institute of Vantaa. Pieces of stumps were wrapped up in plastic bags, which were sealed tightly to prevent any insect from escaping. All beetles hatching from the wood were collected with aid of adhesive tape and put on eppendorf-tubes. The tubes were named respectively to the wood sample. The hatched insects were picked on July 6th, 7th and 9th and were first stored at +4 °C in Vantaa and were later removed to Viikki and stored at +5 °C. A part of the beetles were pre-sterilized with 70 % ethanol and rinsed with MQ-water before freezing.

2.3 Treatment of the beetles in laboratory

2.3.1 Individually treated beetles

Beetles of the pheromone traps

The frozen beetles were let to thaw at room temperature and were handled individually. Ten insects lured by the pheromone traps from each site were picked for further treatment (during the first round). Each insect was rolled on a separate petri dish of which five were filled with malt agar (1.5 %) and five with hagem agar (see 2.4). Then, the insects were surface sterilized with 70 % ethanol and 10 % sodium hypochlorite, 10-15 seconds each, after which the insects were rinsed well with sterile MQ-water. The same insects were used in order to be able to separate the epimycota from the endomycota. The beetles were crushed with a pair of sterilized tweezers and put on a dish different from the one that was used before sterilization. The beetles were either unsterilized and rolled on both types of plates, or sterilized and placed on both types of plates. All plates were given a running number. A capital letter 'N' was given to indicate when the insect was not sterilized. The N-plates did not have an insect on them. Another capital letter reflected the collection site. Indication of the collecting method was included in the plate label and date of cultivation was marked. All plates were sealed with parafilm and incubated at room temperature. The sterilized insects as well as fungal isolates were held in the dark. Plates were inspected regularly and all emerging hyphae were isolated (Fig. 3. (AxioCamICc3)) on to a hagem dish. Each isolate were given the same label as its mother-plate but also an additional running number, so that the last number would tell the order of the isolates. This section of the experiment resulted in a total of 40 petri dishes of which 20 contained a sterilized beetle. The counts involve both sites.

Figure 3. Hyphae are growing from *T. lineatum*. This picture was taken during a preliminary study but the principle of isolating fungi in the present study was exactly the same.

Beetles from pieces of stumps

The beetles were treated similarly to the scolytids from pheromone traps. The number of hatched insects varied between the pieces of stumps on both sites (stumps 5 yielded no beetles). In principle, ten beetles per site, two from each stump, were rolled on a dish individually (N-plates). The insects were then sterilized and crushed on the other type of dish. The labeling of the plates was similar as described earlier. Additional letters separated the insects from the same stump. When both sites were employed, this part yielded 40 petri dishes of which half contained a sterilized beetle.

2.3.2 Group treatments and second round

Beetles were handled with different methods in order to allow divergent fungi to emerge (Persson et al., 2009; Linnakoski et al., 2010). Group treatments involved 20 beetles collected by each method (40 in total) from site P. Same number of beetles was treated from site K. Beetles were treated as groups of five of which half were disinfected similarly as was described earlier. Groups were crushed with a sterile ceramic rod on a sterile plate that was changed for a clean one after each group. The pulverized beetles were placed on malt agar and/or hagem plates. Now, as an excep-

tion to the previous treatments, also the N-plates did contain beetles. This part of the study resulted in 16 dishes containing 80 crushed beetles altogether. (Fig. 4.)

Another round of cultivation (II) was carried out concerning only the beetles from pheromone traps. Twenty insects from Paavola and twenty from Kreivilä were treated as groups of five, yielding eight sets of beetles. Half of the scolytids from each site were sterilized and both media were used. Then, two insects from each site were rolled individually on both types of plates. The beetles were then sterilized and put crushed on a same type of plate. In total, 44 beetles were used here yielding eight plates with groups of insects and eight plates of individually treated insects of which four had a beetle on it.

Altogether, 164 beetles were used and placed on 112 plates (Table 1.).

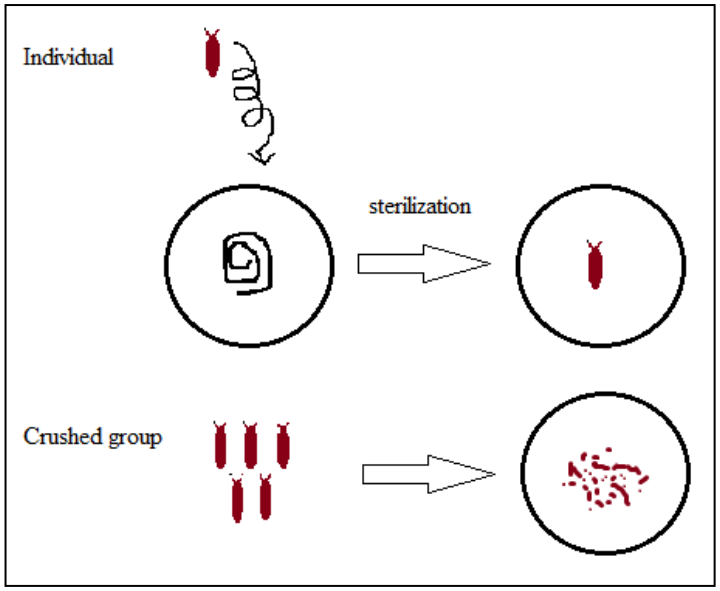

Figure 4. Simplified demonstration of the treatments. Beetles were treated individually and in groups but also unsterilized and sterilized.

Table 1. Division between treated beetles on a site and the sums from both sites.

Pheromone		Beetles	%(w)	%(a)	Plates
Individual	N	(12)			12
	Ster.	12	23,0	14,6	12
Group	N	20	38,5	24,4	4
	Ster.	20	38,5	24,4	4
Sum		52	100,0	63,4	32
Sum from both sites		104			64

Stump		Beetles	%(w)	%(a)	Plates
Individual	N	(10)			10
	Ster.	10	33,3	12,2	10
Group	N	10	33,3	12,2	2
	Ster.	10	33,3	12,2	2
Sum		30	99,9	36,6	24
Sum from both sites		60			48
Total		**164**			**112**

N=not sterilized; %(w)=percentage of treated insects within the collection method; %(a)=percentage from all beetles. Individual N-plates have no insects on them and thus the number of treated beetles is in brackets.

2.4 Cultivation of fungi

Fungi were grown on malt and hagem agar. The malt agar medium consisted of agar (15 g), (Bacto™ Agar, Difco Laboratories; Becton, Dickinson and Company, MD USA); malt extract (20 g), (Bacto™ Malt extract, Difco Laboratories; Becton, Dickinson and Company, MD USA) and 1 l of MQ-water. The hagem medium included $MgSO_4$ (0.5 g), (Riedel-de Haën®, Germany); NH_4Cl (0.5 g), (Merck KGaA, Germany); KH_2PO_4 (0.5 g), (Merck KGaA, Germany); D-glucose (5 g), (BDH Ltd., Poole, England); malt extract (5 g), agar (15 g), and 1 l of MQ-water. pH of the hagem medium was adjusted between 5.2 and 5.4 with 10 % HCl and KOH. The acidity was set using UltraBasic pH-Meter Ordior (Denver Instrument) calibrated the same day. Reagents less than a gram, were weighed on an analysis scale (Mettler Toledo, AB204-S/FACT, Switzerland). Other reagents were measured with toploader balances (Precisa 1000C-3000D, PAG OERLIKON AG, Switzerland; and Sartorius BP310S).

Most of the plates were regularly checked for about six months. The cultivations of the crushed groups from both rounds were initiated two or two and a half months lat-

er and were thus observed a shorter period of time. All different hyphae were isolated onto a new hagem plate. Once the cultures were pure, a piece of young hyphae was transferred on a plate having a sterile cellophane on it. The membrane allowed peeling off the hyphae without any remnants of the medium, which would have disturbed the extraction of DNA. The cultures were divided into three categories based on their quality. The isolates that most obviously were pure were designated to class 1. The plates that were slightly contaminated but could be purified were placed on class 2. The third class included more severely contaminated plates but efforts were made in order to make them pure. Some of the pure cultures within each class were grouped based on the morphology, colour of the hyphae and on possible sporulation on the plate in order to direct certain pure cultures to the DNA extraction. Some isolates were studied under a microscope (Olympus CX31RBSF, Philippines). Class 1 included 38 morphologically dissimilar groups consisting of 43 isolates. Class 2 included 44 groups and was composed of 55 fungal isolates. Class 3 had 24 groups/fungi.

Ten fungal isolates were grown on malt agar containing streptomycin for a part of the cultivation period. The antibiotic was used for inhibiting the growth of unwanted organisms. Streptomycin powder (0.1 g) was weighed on an analysis scale (Mettler Toledo, AB204-S/FACT, Switzerland) and mixed with 1000 µl of sterile MQ-water. In principle, the solution was diluted to 10 ml of sterile liquid malt agar, which was aseptically filtered through a sterile syringe into 500 ml of sterile malt agar. The medium was delivered on petri dishes. The ten isolates were (sample numbers) 6, 11, 12, 14, 16, 17, 18, 20, 21 and 23 (see Table 6.).

2.5 DNA extraction and PCR

It is a common practice in mycological research, that the internal transcribed spacer (ITS) regions of the nuclear small ribosomal RNA subunit are used for the species level designation (e.g. Linnakoski, 2011). ITS regions occur at high copy numbers (Bruns & Shefferson, 2004; Nilsson et al., 2008) and they possess conserved primer sites, which makes them a suitable target for DNA sequencing (Bruns & Shefferson, 2004; Nilsson et al. 2008; Nilsson et al. 2009; Nilsson et al., 2010). Particularly, the

nuclear small and large subunits (nSSU and nLSU) are good primer anchors for ITS1 and ITS2, respectively (Nilsson et al., 2010). Here, instead of ITS2, ITS4 was used with ITS1 allowing longer gene fragments for being synthesized (Fig. 5-6.). The nuclear ribosomal ITS region is roughly 650 base pairs (bp) long. The ITS region includes three subloci: ITS1 (ca. 180 bp), ITS2 (ca. 170 bp) and the 5.8S gene (ca. 160 bp) (Nilsson et al., 2010).

ITS1 and ITS2 regions show variability (Nilsson et al., 2008), whereas the intercalary 5.8S gene is highly conserved (Bruns & Shefferson, 2004; Nilsson et al., 2008; Nilsson et al., 2009). Therefore, it has been suggested that ITS1/ITS2 sequences should be analyzed in isolation with the highly conserved genes left out (Nilsson et al., 2008; Nilsson et al., 2009; Nilsson et al., 2010).

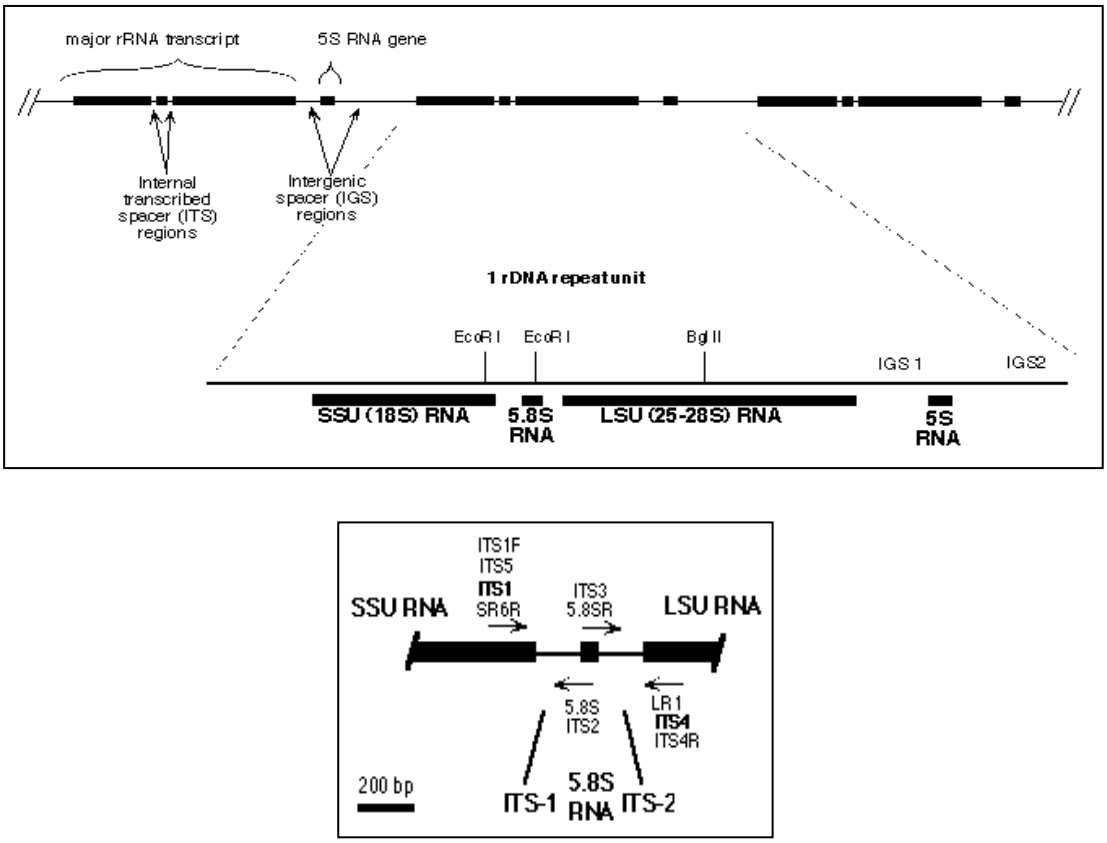

Figure 5-6. The upper picture describes a repeat unit of rDNA while the picture below demonstrates the ITS region where commonly used primers are shown bold (ITS1 and ITS4) (Conserved primer…2012).

A standard cetyl–trimethyl ammonium bromide (CTAB) method (Chang et al., 1993) was modified for the extraction of fungal DNA from 76 isolates. Small amount of

fresh hyphae was peeled from a pure culture and put in a sterilized 1.5 ml eppendorf tube. The tissue was homogenized with sterilized sand and 100 µl of 2 % CTAB (Sigma-Aldrich, USA) using a sterile micropestle. A volume of 500 µl of CTAB was added and the sample was heated to 65 °C. One volume of chloroform:IAA (isoamyl alcohol) (24:1) was added. The sample was shaken for 7 minutes and centrifuged (Heraeus Pico17, Thermo Fisher Scientific, Germany) at 13000 rpm for 15 minutes. The supernatant was transferred to a new eppendorf tube followed by one volume of chloroform:IAA. After the sample was manually mixed for another 7 minutes, it was centrifuged at similar speed for the same period of time. The subsequent supernatant was pipetted into an eppendorf tube and the DNA was precipitated by two volumes of cold isopropanol. The sample was held on ice for 30 minutes or stored in freezer. Next, the sample was centrifuged at 13000 rpm for 20 minutes and the supernatant was carefully removed. The pellet was washed by adding 200 µl of 70 % cold ethanol and centrifuging the sample at half speed for 5 minutes. The supernatant was poured away and the pellet was left to dry after which the DNA was resuspended in 40 µl of TE-buffer (10:1) containing 1 M TRIS-HCl (1 ml), 0.5 M EDTA (pH 8) (0.2 ml) and MQ-water (100 ml). The sample was either stored in freezer or continued to the next step.

Concentration and quality of the samples were analyzed with a spectrophotometer (NanoDrop ND-1000 Spectrophotometer, Thermo Fisher Scientific, USA). A drop, 1 µl, of each sample was used for the evaluation. The concentration of DNA was measured in order to be able to calculate the reaction volume for the following step of PCR (polymerase chain reaction). PCR is a method for amplifying a certain region within the DNA molecule. The targeted fragment can be distinguished with primers, which in this study were ITS1 and ITS4. The sequences for the primers of the ITS region were: (ITS1) 5'-TCCGTAGGTGAACCTGCGG-3' and (ITS4) 5'-TCCTCCGCTTATTGATATGC-3' (Conserved primer…, 2012). In principle, the PCR proceeds in steps of strand denaturation, primer annealing, DNA synthesis and extension. PCR is fast but sensitive to disturbances and failures may occur. PCR mixture per one sample included autoclaved MQ-water (30.5 µl), buffer (5 µl), dNTP's (1 µl), ITS1 (1 µl), ITS4 (1 µl), $MgCl_2$ (1 µl) and polymerase (0.5 µl) (Dynazyme, Finnzymes). A sufficient quantity of each sample was transferred to a

new tube and diluted with sterilized MQ-water in order to obtain a final reaction volume of 50 µl with a concentration of 10 ng μl^{-1} of the template. Negative controls were used to detect possible contaminations. In those cases, MQ-water was added to the PCR mixture instead of the template.

The PCR conditions for ITS1 and ITS4 were: an initial denaturation step at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C for 45 s and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min and holding at 4 °C for one hour. In some cases (samples 43-48), another protocol was followed: an initial denaturation step at 95 °C for 2 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s, extension at 72 °C for 1 min; and a final extension at 72 °C for 8 min and holding at 4 °C for 55 min. The final reaction volumes were smaller, 25 µl, with the same concentration of the template. The PCR was run using BIOER XP-E Thermal cycler. Each PCR product and ladder (Thermo Scientific GeneRuler™, 100 bp) was confirmed by running the samples on 1.5 % agarose gel in TAE buffer. A quantity of 5 µl of each PCR product (DNA) was pipetted mixed with 1 µl of loading buffer into the gel. The samples were run in an electric field of 120 V with a current of 400 mA for ca. 30 minutes. Verification of DNA was based on ethidium bromide stain and ultraviolet illumination (Molecular Imager®, GelDoc™ XR+ Imaging system, Bio Rad). Good quality samples were sent for sequencing to the Haartman Institute, Helsinki (Finland).

2.6 Identification of species

Identities of the 76 sequences were defined using BLAST (Basic Local Alignment Search Tool) provided by the NCBI (The National Center for Biotechnology Information). BLAST compares the sequences against those in GenBank. For each sequence ten best alternatives obtained from GenBank were taken into account. When the alternatives were given at species level but the percentages for query coverage or sequence similarity were slightly under 90 %, the suggested definitions of this study were expressed at class level. If the percentages were between 90 % and 96 % and seemed relevant, the suggestion was expressed at order level. If the percentages reached 97-100 %, the suggestion was given at genus level. In case the al-

ternatives by GenBank were given at other than species level, the specificity of the suggestions was reconsidered and given at level, which combined and best described the great majority of the given alternatives. If the values were under 80 %, the specimen was considered unidentified. The principles for the delimitations were applied from a study by Persson et al. (2009) with some modifications. Taxonomy of the fungi was inspected and determined using Dictionary of the Fungi (www.indexfungorum.org/). Comparative data was obtained from NCBI Taxonomy Browser (www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi).

2.7 Statistical analyses

Odds ratio (OR) was used as the statistical tool for describing differences in proportions of vector beetles between collection methods (pheromone traps and stumps) and positions (epi- and endomycetes) in general. Proportions of vector beetles were also compared between the same variables (collection methods, positions) within a certain group of fungi (Ascomycetes, Zygomycetes and unidentified fungi). The comparisons were carried out with logistic regression. Similarly, the differences in mean frequencies of isolated fungi were compared with Poisson regression between trap types and positions. The data was analyzed with Statistix 9.0.

The separation of position of isolation could only be done when the groups of crushed beetles were removed, since the position could not be determined from the groups. Observations from the second round were also excluded because no beetles of stump origin had remained. Isolates from the second round involved thus only pheromone baited beetles and using that data in the analyses could have twisted the outcome.

Odds ratio, as a standard output of logistic regression models, is a suitable tool for describing and comparing proportions and likelihoods (Rita, 2004; Rita & Komonen, 2008). Odds ratio is the ratio of two odds and in order to calculate the OR, the proportions on focus must be transformed into odds. Odds reflect the probability of an incident occurring to the probability of the incident is absent. Simultaneously, OR is both statistical and ecological means for characterizing the magnitude and direction

of difference between two proportions (Rita & Komonen, 2008). Rita & Komonen (2008) have suggested that odds ratios less than one in the output of the analysis should be reported as their inverse values with an exponent indicating the direction of the change. The effect of a factor may seem meaningless because of the initially small value of OR. The inverse presentation thus should help see the true effect of the factor (Rita & Komonen, 2008). Three values of OR in Table 3. have been transformed into inverse. Ratios of averages are reported for the frequencies of fungal isolates (Lindsey, 1995).

The sign of each coefficient in Table 4 and 5. indicate the direction of the shift because the variables were coded for the statistical analyses. Pheromone trap was 0 and stump was 1, endomycetes were 0 and epimycetes were 1. For example, when the coefficient is positive and the corresponding OR is over one, the proportion will increase as the focus turns from 0 to 1. If the coefficient is negative and the initial OR smaller than one, the proportion will decrease as the focus turns in same order (from 0 to 1). Note that the ORs less than one have been transformed into inverse values (Rita & Komonen, 2008).

2.8 Species richness and diversity analyses

Data on species richness and Shannon-Wiener and Simpson diversity indices were obtained for the purpose of assessing species richness and diversity within the fungal communities between different positions (epi- and endomycetes), collection methods (pheromone trap and stump), sites (Paavola and Kreivilä) and treatments of beetles (as individuals and in groups). Indices were calculated using Shannon-Wiener Diversity Index/Shannon Entropy Calculator (http://www.changbioscience.com/genetics/shannon.html). Diversity was also assessed via similarity indices (Classic Jaccard, Classic Sorensen, Morisita-Horn and Bray-Curtis) calculated by EstimateS version 7.5.2 (Colwell, 2009), a software application measuring numerous indices for the evaluation of biodiversity. However, as it has been observed that frequent occurrence of rare specimens in the data may restrict the applicability of Jaccard's and Sorensen similarity indices in the diversity assessment (Chao et al., 2005), Morisita-Horn

(MH) and Bray-Curtis (BC) indices were here considered as the more suitable estimates.

3 RESULTS

3.1 The cultivations yielded 122 fungal isolations and 76 DNA extractions

The original 112 plates that had the total of 164 beetles on them yielded 122 fungal isolates (Table 2.). DNA was extracted from 76 isolates of which three escaped the identification despite the use of two PCR protocols. The numbers of DNA extractions in quality classes of pure cultures 1, 2 and 3 were 35, 36 and five, respectively. On average, each beetle was associated with 0.74 (122/164) fungi. Of all original plates, 34 yielded no isolations. Thus, the fungal isolations concern only 78 plates instead of the original 112. Furthermore, these 78 plates contained 107 beetles. In that case, the ratio would be somewhat higher, 1.14 (122/107) fungi per beetle.

Table 2. Division of isolates and DNA extractions in total and between collection methods and sites.

			Site P		Site K	
Source	Total f	Total D	Total f	Total D	Total f	Total D
Pheromone	48	37	14	13	34	24
Stump	51	22	24	12	27	10
Pheromone g	18	14	6	5	12	9
Stump g	5	3	4	3	1	0
	122	76	48	33	74	43

Total f=total count of fungal isolates. Total D=total number of DNA extractions. Small case g indicates crushed groups of beetles.

3.2 The proportion of beetles carrying epimycetes was distinct from the proportion of beetles carrying endomycetes

The results of the logistic regression including odds ratios that were used for describing differences in the proportions of beetles vectoring fungi are displayed in Table 3. In general, fungi were more often isolated from the outer surface of the beetles. The difference in the proportions between positions was statistically significant (OR 4.72; p-value 0.0030). The proportions of vector beetles were not significantly affected by trap type (OR 1.14; p-value 0.8012), but a bigger proportion of beetles exposing their mycota were collected from stumps as compared to beetles from pheromone traps. However, the situation was opposite as the focus was solely on beetles with ascomycetes. The trap type was statistically significant (OR 6.67^{-1}; p-value 0.0523)

and position was not (OR 2.33^{-1}; p-value 0.4274) (notation described in Rita & Komonen (2008). See also 2.7). The pheromone trapped beetles were more often carrying ascomycetes than were the stump originated beetles. (See also Table 5., where it is shown that the origin of zygomycetes can only be traced to the beetles collected from stumps.) Differences in the proportions of beetles carrying zygomycetes were statistically insignificant between traps (OR 6579.36; p-value 0.5964) and positions (3056.70; p-value 0.6326). These two ORs are unreliable and result from zero frequencies. No significant differences could be caught between traps (OR 1.02^{-1}; p-value 0.9813) and positions (1.66; p-value 0.6048) for the beetles carrying isolates that remained unidentified.

Table 3. Coefficients and odds ratios (with corresponding p-values) of logistic regression for proportions of beetles associated with certain fungi.

	Beetles with fungi		Beetles with A		Beetles with Z		Beetles with U	
Variable	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Trap	0,12685	0,8012	-1,90056	0,0523	8,79169	0,5964	-0,01931	0,9813
Position	1,55190	0,0030	-0,83635	0,4274	8,02509	0,6326	0,50573	0,6048
	Beetles wf		Beetles with A		Beetles with Z		Beetles with U	
	OR		OR		OR		OR	
Trap	1,14		$6,67^{-1}$		6579,36		$1,02^{-1}$	
Position	4,72		$2,33^{-1}$		3056,70		1,66	

Beetles wf=Beetles with fungi. A=Ascomycetes, Z=Zygomycetes, U=unidentified. OR=Odds ratio. ORs with negative exponents have been transformed into inverse (Rita & Komonen, 2008).

3.3 The frequencies of fungal isolates were higher among the epimycota

The results of Poisson regression and ratios of averages (Lindsey, 1995) for the frequencies of fungi are shown in Table 4. The total frequencies of isolated fungi were higher among the epimycota than endomycota (ratio of averages 3.45; p-value 0.0003). The stumps appeared less frequent source of inoculum, but the difference between trap types was statistically insignificant (ratio of averages 0.81; p-value 0.4757) (Fig. 7.). The changes in the frequencies between the two combinations of trap types and positions were alike (data not shown). The situation remained the same for the counts of ascomycetes. Thus, the position was significant (ratio of averages 3.29; p-value 0.0056), but trap type was not (ratio of averages 0.58; p-value 0.1486). Ascomycetes were isolated in higher numbers from the outer surface of the

beetles (and from beetles from pheromone traps). There was indication of interaction because the shifts between the combinations of trap types and positions were not alike: the change in the frequencies of isolated ascomycetes between positions from beetles from pheromone traps was greater than the corresponding change among the beetles from stumps. The effect of trap type and position on the frequencies of zygomycetes was insignificant (ratios of averages for both variables 8111.92; p-value 0.6866). Changes were parallel from pheromone traps to stumps and from the inside to the outside. Similarly were the differences in frequencies of unidentified fungi statistically insignificant. Ratio of averages for trap and position were 0.63 (p-value 0.4096) and 2.25 (p-value 0.1770), respectively.

Table 4. Coefficients and ratios of averages (with corresponding p-values) of Poisson regression for frequencies of isolated fungi.

Variable	All fungi (DNA ext.)		Ascomycetes		Zygomycetes		Unidentified	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Trap	-0,20479	0,4757	-0,54655	0,1486	9,00109	0,6866	-0,47000	0,4096
Position	1,23969	0,0003	1,18952	0,0056	9,00109	0,6866	0,81093	0,1770
	Ratio of averages							
Trap	0,81482		0,57894		8111,92110		0,62500	
Position	3,45454		3,28550		8111,92110		2,24999	

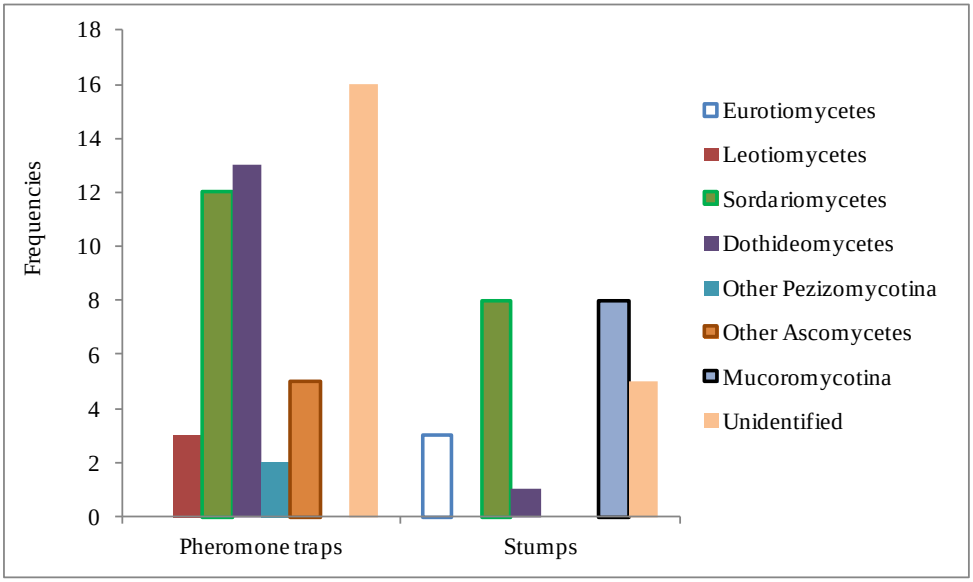

Figure 7. Total frequencies of fungal isolates were higher among the beetles from pheromone traps, but the difference was statistically insignificant.

3.4 Swarming beetles and those leaving stumps carry different fungi

The three additional hypotheses were not confirmed since *Heterobasidion* spp., *Phlebiopsis gigantea* and *Ambrosiella ferruginea* were not isolated. However, the results revealed that the composition of fungi of those beetles that fly and attack timber or stumps was clearly different from that of those beetles that leave the stumps. For example, members of the Zygomycota (and Mucoromycotina) were solely isolated from beetles from stumps (Table 5.).

From the 76 fungi, 61.84 % belonged to the Ascomycota and 10.53 % belonged to the Zygomycota. Many isolates (27.63 %) remained unidentified (see Appendix, Fig. 14-15.). Under the phylum Ascomycota, two subphyla could be distinguished. From the 76 fungi, Pezizomycotina covered over a half (55.26 %) while Saccharomycotina were clearly less common (2.63 %). The most ordinary taxa were Sordariomycetes (Fig. 8.) and Dothideomycetes (Fig. 9.). Within these, the most abundant genera were *Hypocrea* and *Ophiostoma* belonging to the former class, and *Sydowia* sp. as a representative of the latter class. The phylum Zygomycota had one subphylum, Mucoromycotina, which in general was among the most abundant groups of fungi. (See 4.2.5 for descriptions.) Frequencies of certain taxa for combinations of collection methods and positions are shown in Fig. 10. Suggested identities for the 76 isolates are in Table 6.

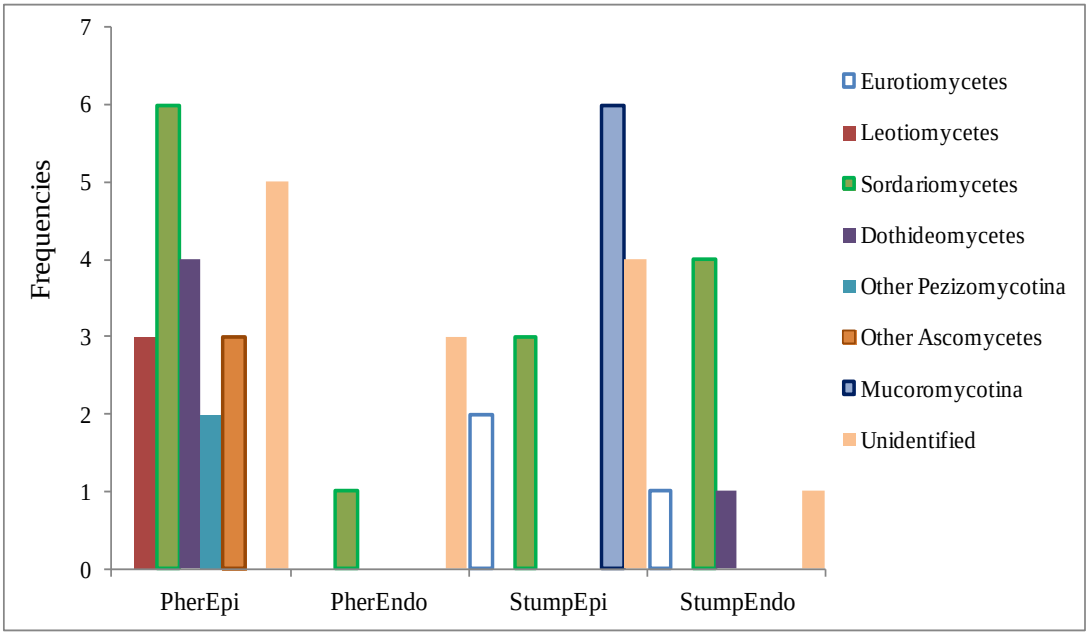

Figure 10. Isolated fungi between combinations of trap types and positions. Sordariomycetes related to stumps and pheromone traps accommodated different genera (*Hypocrea* sp. and *Ophiostoma* sp., respectively.) (Pher=Pheromone trap.)

A total of 55 fungal isolates were identified from the 76 isolates (Table 5.). For instance, class 3, Sordariomycetes, divided into two orders of which both revealed one genus. Order 31, Hypocreales, was composed of 7 isolates, of which 6 could be designated to genus level and one remained at less specific taxonomic level. All of the 7 isolates originated from beetles from stumps from round I. From the 6 samples that were designated to Other Sordariomycetes, 3 originated from beetles caught by pheromone traps at round I, 1 was recovered during the second round (pheromone traps only), and 2 were isolated from crushed groups of which one represented one collection method (P=pheromone trap) and the other represented the other collection method (S=stump). Class 4, instead, accommodated 14 Dothideomycetes. These isolates divided such that 5 were recovered during the first round, 6 during the second round and 3 originated from crushed groups.

Table 5. Frequencies of fungal taxa. The Total-column on the left shows the number of specimens per each taxon. These totals are composed of other totals on the same row showing the division between stages of the work. Simultaneously, the underlined totals on the left sum upwards constituting an appropriate taxon close above. Inside the stage related columns are subcolumns titled with small P (pheromone) and/or S (stump), where the small case counts refer to the origin of the isolates and sum upwards to the left within the same column and within a taxonomic unit on focus. The columns distinguish individually (ind.) handled beetles from rounds I and II from groups.

		Total	Round I ind.			Round II ind.		Crushed groups		
			Total	P	S	Total	P	Total	P	S
Phylum	**Ascomycota**	47	30			8		9		
Subphylum	Pezizomycotina	42	27			8		7		
Class 1	Eurotiomycetes	3	3							
Order 11	Eurotiales	2								
Genus 111	*Penicillium*	2			2					
	Other Eurotiomycete	1			1					
Class 2	Leotiomycetes	3	3							
Order 21	Helotiales	2								
Genus 211	*Cadophora*	2		2						
	Other Leotiomycete	1		1						
Class 3	Sordariomycetes	20	14			2		4		
Order 31	Hypocreales	7	7							
Genus 311	*Hypocrea*	6			6					
	Other Hypocreales	1			1					
Order 32	Ophiostomatales	7	4			1		2		
Genus 321	*Ophiostoma*	5		3					2	
	Other Ophiostomatales	2		1			1			
	Other Sordariomycetes	6	3	3		1	1	2	1	1
Class 4	Dothideomycetes	14	5			6		3		
Order 41	Capnodiales	4	3					1		
Genus 411	*Cladosporium*	4		3					1	
Order 42	Pleosporales	2	1			1				
Genus 421	*Rhizosphaera*	2		1			1			
Order 43	Dothideales	6				5		1		
Genus 431	*Sydowia*	6					5		1	
	Other Dothideomycetes	2	1		1			1	1	
	Other Pezizomycotina	2	2	2						
Subphylum	Saccharomycotina	2						2		
Class 1	Saccharomycetes	2							2	
	Other Ascomycota	3	3	3						
Phylum	**Zygomycota**	8	6					2		
Subphylum	Mucoromycotina	8	6					2		
Order 11	Mucorales	2	1					1		
Genus 111	*Absidia*	1								1
Genus 112	*Mucor*	1			1					
	Other Mucoromycotina	6	5		5			1		1

Table 6. Suggested definitions for isolated fungi. Six isolates are shown on two rows in case the first alternative was not indicative enough in order to support the suggestion given by this study.

Sample number	GenBank accession number for best matches	Qc, %	Mi, %	Description of best matches	Suggestion of the present study	Ster.	Site	Inoculum source
1					Unidentified	N	K	S4
2	AY649782.1	90	100	*Leptographium guttulatum*	Sordariomycetes sp.	N	P	Pg
3	AM943896.1	98	89	*Ophiostoma piceae*	Sordariomycetes sp.	N	K	P3
	AB200423.1	98	89	*Ophiostoma breviusculum*				
4	FR837917.1	99	98	*Zalerion arboricola*	Pezizomycotina sp.	N	P	P4
5	HQ661098.1	100	99	*Cadophora melinii*	*Cadophora* sp.	N	K	P3
6	AM999755.1	92	96	Uncultured fungus	Unidentified	N	K	P5
7	HQ829165.1	97	99	*Mucor sp.*	Mucoromycotina sp.	N	K	S6
8	HQ829165.1	97	99	*Mucor* sp.	Mucoromycotina sp.	N	P	S2
9	HM051062.1	100	99	*Hypocrea viridescens*	*Hypocrea* sp.	Y	K	S6
10	FR846480.1	87	100	*Epicoccum nigrum*	Dothideomycetes sp.	Y	P	S6
11	AY944875.1	98	100	*Absidia glauca*	*Absidia* sp.	Y	P	Sg
12	AY944889.1	18	89	*Absidia cylindrospora*	Unidentified	N	P	S2
13	JF449891.1	100	89	Uncultured *Trichoderma*	Sordariomycetes sp.	Y	P	Sg
14	FJ430783.1	100	99	*Hypocrea koningii*	*Hypocrea* sp.	N	P	S2
15					Unidentified	N	K	S3
16	DQ677652.1	100	99	*Hypocrea rufa*	*Hypocrea* sp.	Y	P	S6
17	AF125942.1	100	86	*Penicillium* sp.	Eurotiomycetes sp.	Y	P	S6
18	GU566252.1	100	99	*Penicillium spinulosum*	*Penicillium* sp.	N	P	S2
19	HQ829165.1	97	99	*Mucor* sp.	Mucoromycotina sp.	N	P	Sg
20	JN032542.1	100	99	Uncultured fungus	Ascomycota sp.	N	K	P2
21	HM776418.1	99	99	*Cladosporium cladosporioides*	*Cladosporium* sp.	N	K	P9
22	AY183366.1	99	100	*Rhizosphaera kalkhoffii*	*Rhizosphaera* sp.	N	K	P1
23	JN851037.1	100	100	*Cladosporium uredinicola*	Unidentified	N	K	P1

Table 6. continued.

Sample number	GenBank accession number for best matches	Qc, %	Mi, %	Description of best matches	Suggestion of the present study	Ster.	Site	Inoculum source
25	FJ437230.1	96	99	*Ophiostoma pluriannulatum*	Ophiostomatales sp.	Y	K(II)	P2
27	GQ412728.1	99	99	*Sydowia polyspora*	*Sydowia* sp.	N	K(II)	P2
28	AB128012.1	89	84	*Sporothrix schenckii* var. *luriei*	Sordariomycetes sp.	Y	P(II)	P3
29	EU725713.1	99	100	Fungal sp.	*Cladosporium* sp.	N	P(II)	Pg10
	EU272532.1	100	99	*Cladosporium cladosporioides*				
30	FR822985.1	100	99	*Sydowia polyspora*	*Sydowia* sp.	N	K(II)	P2
31	AY805557.1	95	100	*Dipodascus* sp.	Saccharomycetes sp.	N	K	Pg
32	FJ437230.1	96	99	*Ophiostoma pluriannulatum*	Ophiostomatales sp.	N	P	P6
33	FJ437230.1	85	99	*Ophiostoma pluriannulatum*	Sordariomycetes sp.	N	P	P7
34	GU966516.1	97	100	*Mucor circinelloides*	*Mucor* sp.	N	K	S3
35	HQ829165.1	99	99	*Mucor* sp.	Mucoromycotina sp.	N	K	S6
36	HQ829165.1	97	99	*Mucor* sp.	Mucoromycotina sp.	N	K	S6
37	HM051062.1	99	99	*Hypocrea viridescens*	*Hypocrea* sp.	Y	K	S6
38	JN790248.1	99	99	*Hypocrea rufa*	*Hypocrea* sp.	N	K	S3
39	DQ838531.1	99	99	*Hypocrea rufa*	*Hypocrea* sp.	N	P	S2
40	HQ829165.1	97	99	*Mucor* sp.	Mucoromycotina sp.	N	P	S2
41	FR822985.1	100	99	*Sydowia polyspora*	*Sydowia* sp.	N	K(II)	P2
42	FR717223.1	100	99	*Sydowia polyspora*	*Sydowia* sp.	N	K(II)	P2
43	AM943896.1	100	97	*Ophiostoma piceae*	*Ophiostoma* sp.	N	P	P3
44	AM944646.1	100	97	*Ophiostoma piceae*	*Ophiostoma* sp.	N	P	P3
45	AB200423.1	99	97	*Ophiostoma breviusculum*	*Ophiostoma* sp.	Y	P(II)	Pg12
46	AB200422.1	99	97	*Ophiostoma breviusculum*	*Ophiostoma* sp.	N	K	P8
47					Unidentified	Y	K(II)	P1
48	AB200421.1	100	97	*Ophiostoma breviusculum*	*Ophiostoma* sp.	Y	P	Pg

Table 6. continued.

Sample number	GenBank accession number for best matches	Qc, %	Mi, %	Description of best matches	Suggestion of the present study	Ster.	Site	Inoculum source
49	GQ412722.1	100	99	*Sydowia polyspora*	*Sydowia* sp.	N	P(II)	Pg10
50	HQ661097.1	100	99	*Cadophora melinii*	*Cadophora* sp.	N	K	P3
51	AY805557.1	53	100	*Dipodascus* sp.	Unidentified	N	K	Pg
52	AY805557.1	95	100	*Dipodascus* sp.	Saccharomycetes sp.	N	K	Pg
53	FR837917.1	100	99	*Zalerion arboricola*	Ascomycota sp.	N	P	P4
54	FR837917.1	99	99	*Zalerion arboricola*	Ascomycota sp.	N	P	P4
55	AF169307.1	79	81	*Zalerion arboricola*	Unidentified	N	P	P4
56	FJ824626.1	64	82	*Mariannaea elegans*	Unidentified	Y	P	S2
57	FJ872069.1	100	99	*Nectria mariannaeae*	Hypocreales sp.	Y	P	S2
58	AB190400.1	89	98	*Phialocephala lagerbergii*	Leotiomycetes sp.	N	K	P3
59					Unidentified	Y	P	P3
60	GU934556.1	99	99	*Penicillium thomii*	*Penicillium* sp.	N	P	S2
61	GQ153122.1	100	99	Dothideomycetes sp.	*Sydowia* sp.	N	K(II)	P2
	GQ412722.1	99	99	*Sydowia polyspora*				
62	AB128012.1	89	84	*Sporothrix schenckii* var. *luriei*	Sordariomycetes sp.	Y	P	P9
63	EU516725.1	100	98	Uncultured ascomycete	Unidentified	Y	K	Pg
64	EU516812.1	67	88	Uncultured Sarcosomataceae	Unidentified	Y	K	Pg
65	HM123708.1	100	97	Fungal sp.	Unidentified	Y	K	Pg
66	DQ979774.1	86	90	Fungal endophyte	Dothideomycetes sp.	Y	K	Pg
	FJ904465.1	85	90	*Herpotrichia juniperi*				
67	EU516725.1	100	97	Uncultured ascomycete	Unidentified	Y	K	Pg
68	DQ979774.1	74	95	Fungal endophyte	Unidentified	Y	K	Pg
69	AM901875.1	95	96	Uncultured ascomycete	Pezizomycotina sp.	N	K	P
	DQ525481.1	82	87	*Pleopsidium chlorophanum*				

Table 6. continued.

Sample number	GenBank accession number for best matches	Qc, %	Mi, %	Description of best matches	Suggestion of the present study	Ster.	Site	Inoculum source
70	AM999755.1	92	88	Uncultured fungus	Unidentified	N	K	P5
71	HQ696029.1	100	100	*Cladosporium* sp.	*Cladosporium* sp.	N	K	P9
72					Unidentified	N	K	S1
73	EU725713.1	100	99	Fungal sp.	*Cladosporium* sp.	N	K	P9
	JN986781.1	100	99	*Cladosporium cladosporioides*				
74	JN032542.1	97	100	Uncultured fungus	Unidentified	N	K	P2
75					Unidentified	Y	P	P3
76					Unidentified	Y	P	P3
77	AY183366.1	100	100	*Rhizosphaera kalkhoffii*	*Rhizosphaera* sp.	N	K(II)	P2
78	JN032542.1	99	99	Uncultured fungus	Unidentified	N	K(II)	P1

Qc=query coverage, Mi=maximum identity. Ster.=sterilization: Y=yes, N=no. Inoculum source: beetle from P=pheromone trap, S=stump. Lower case g indicates groups of crushed beetles. Round II is indicated simultaneously with the site.

Figure 8. Isolate 46 (K pheromone 8a N) was designated to the *Ophiostoma* sp. (as a member of the Sordariomycetes).

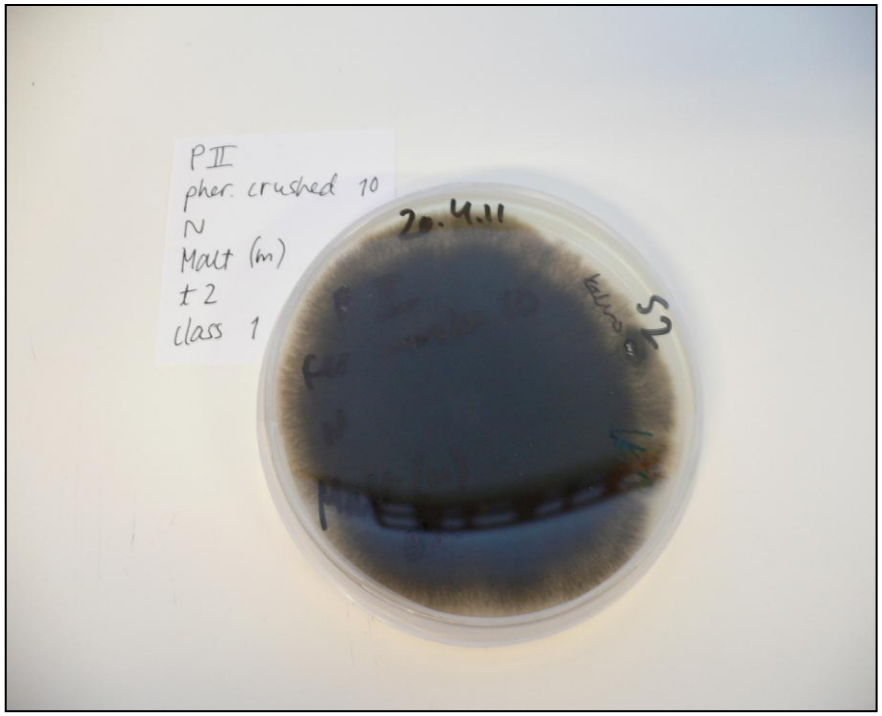

Figure 9. Isolate 49 (PII pheromone crushed group 10 N) was designated to *Sydowia* sp. (as a member of the Dothideomycetes).

3.5 Epimycota and the mycota of flying beetles were relatively versatile

The species richness varied between positions, collection methods, sites and treatment methods (Fig. 11.). The numbers of exclusive specimens for epi- and endomycota, pheromone traps and stumps, sites P and K, and beetles treated individually and as groups were 11 and 3, 10 and 7, 4 and 5, 11 and 2, respectively. The numbers of shared specimens were 3, 3, 11 and 7, respectively. Species richness was thus higher among the epimycota, beetles from pheromone traps, site K and among the individually treated beetles. Species richness indicators are listed in Table 7.

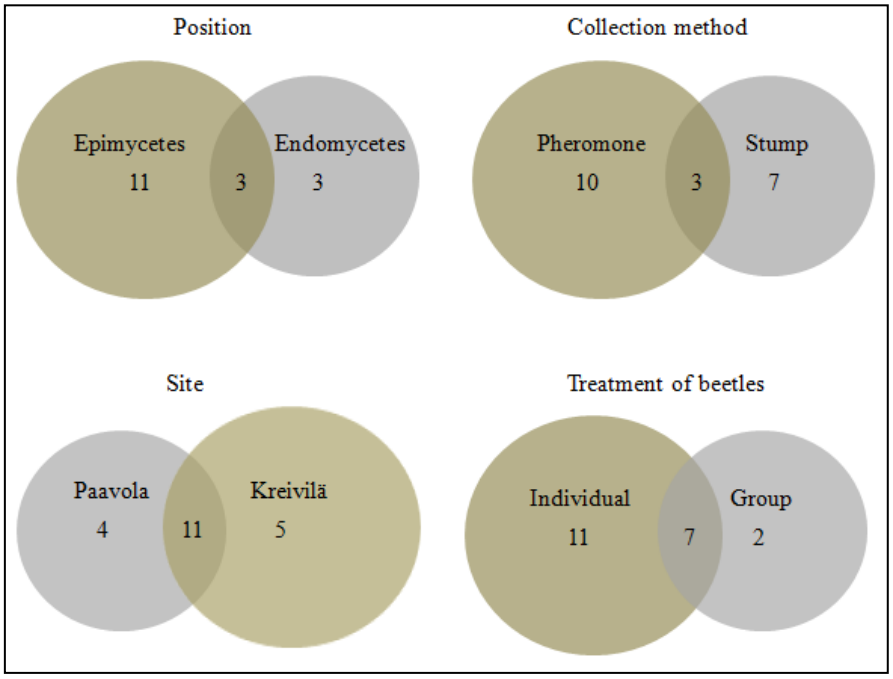

Figure 11. Venn diagrams show the numbers of exclusive and shared specimens for positions, sites and collection and treatment methods.

Table 7. Species richness and shared species between positions, trap types, sites and treatment methods.

	Species richness	SS	Abundance
Epi	14	3	38
Endo	6		11
Pheromone	13	3	51
Stump	10		25
Paavola	15	11	33
Kreivilä	16		43
Individual	18	7	59
Group	9		17

SS=Shared species.

The Shannon-Wiener (SW) diversity indices indicated greater species diversity within the epimycota (2.41) compared to that within the endomycota (1.59) (Table 8.). Species diversity was higher within the fungal community that was associated with beetles collected by pheromone traps (2.23) than those of stump origin (1.98). Diversities were higher in site Paavola (2.48) than in Kreivilä (2.30) and within the individually treated beetles (2.55) than those treated as groups (1.96). The SW index may vary between 0 and 4 but is typically restricted between 1.5 and 3.5. The upper limit indicates higher diversity. These indices were supported by the Simpson diversity indices that in contrast show lower values for higher diversity, and higher values for lower diversity. The Simpson index may range between 0 and 1.

Table 8. Diversity and similarity indices for the assessment of species diversity for/between positions, trap types, sites and treatment methods.

	SW	SI	MH	BC	JC	SC
Epimycota	2,41	0,11	0,64	0,33	0,18	0,30
Endomycota	1,59	0,24				
Pheromone	2,23	0,15	0,42	0,18	0,15	0,26
Stump	1,98	0,17				
Site P	2,48	0,10	0,70	0,53	0,55	0,71
Site K	2,30	0,16				
Individual	2,55	0,11	0,81	0,37	0,35	0,52
Group	1,96	0,18				

SW=Shannon-Wiener diversity index, SI=Simpson diversity index; MH=Morisita-Horn similarity index, BC=Bray-Curtis similarity index, JC=Classical Jaccard's similarity index, SC=Classical Sorensen's similarity index.

In addition to Shannon-Wiener and Simpson diversity indices, species diversity was evaluated by certain similarity indices. Similarity indices of the pairwise comparisons conducted between positions, trap types, sites and treatment methods of the beetles are shown in Table 8. Due to the limitations (Chao et al., 2005; see also 2.8), most attention should here be paid to Morisita-Horn (MH) and Bray-Curtis (BC) indices. MH and BC indices for the fungal communities between the two positions indicate subtle dissimilarity (MH and BC 0.64 and 0.33, respectively) and yet lower similarity between trap types (0.42 and 0.18, respectively). Thus, the epi- and endomycota seemed to be composed of rather dissimilar fungal communities, and the fungal communities between trap types were more versatile. MH and BC indices for

the mycota between sites indicate uniformity (0.70 and 0.53, respectively). The indices for the treatment methods show conflicting results (0.81 and 0.37, respectively). The beetles collected at the different sites were thus associated with similar assemblages of fungi. Conclusions of the diversity of the communities recovered by the different treatment methods can not be readily drawn due to skewed data. These similarity indices may range from zero to one, the latter limit indicating higher similarity. However, Jaccard and Sorensen similarity indices seem to be in line with the MH and BC indices.

3.6 The associated mycota appeared richer than previously known

The recovered mycota was composed of a richer assemblage of fungi than previously characterized (Table 9.). Seven genera (*Penicillium* sp., *Cadophora* sp., *Cladosporium* sp., *Rhizosphaera* sp., *Sydowia* sp., *Absidia* sp. and *Mucor* sp.) were newly discovered associated to the striped ambrosia beetle. A richer spectrum of fungi could be considered participating relationships if one took the less specific taxa (than genera) to the inspection. Saccharomycetes sp., at least, would then be involved but yet sparse. This might be an artefact since yeasts, of which many belong to the Saccharomycetes, were not on focus in the present study.

Except for *Ophiostoma* spp. and *Trichoderma* spp., of which the latter genus was represented by the teleomorphic state (*Hypocrea* sp.) in the present study, many of the recovered taxa have not been reported to be associated to the striped ambrosia beetle earlier. It must be noted that both *Ophiostoma* spp. (member of the Ophiostomatales) and *Trichoderma*/*Hypocrea* spp. (members of the Hypocreales) belong to the Sordariomycetes, Pezizomycotina and Ascomycota. These less specific taxa (accommodating *Trichoderma* sp. and *Ophiostoma* spp.) have therefore been earlier recovered by Müller et al. (2002) and Linnakoski et al (2010) in connection to the beetle and are indicated with superscripts in Table 9.

Table 9. Confirmed associates of *T. lineatum*.

Specimen	Phylum	Isolation source		Reference
Heterobasidion annosum	B	Galleries	Spruce, Japanese larch	Bakshi, 1950
		Logs	Spruce	Müller et al., 2002
Ceratocystis piceae	A	Galleries	Spruce, Japanese larch	Bakshi, 1950
Leptographium lundbergii	A	Galleries	Spruce, Japanese larch	Bakshi, 1950
Antrodia serialis	B	Logs	Spruce	Müller et al., 2002
Phlebiopsis gigantea	B	Logs	Spruce	Müller et al., 2002
Sporothrix sp.	A	Logs	Spruce	Müller et al., 2002
Trichoderma sp.	A	Logs	Spruce	Müller et al., 2002
Hypocrea sp. (*)	A	Stump	Spruce	Present study
Ophiostoma canum	A	Beetle	Spruce, pine	Linnakoski et al., 2010
O. canum -like	A	Beetle	Spruce, pine	Linnakoski et al., 2010
O. pallidulum	A	Beetle	Spruce, pine	Linnakoski et al., 2010
O. rachisporum	A	Beetle	Spruce, pine	Linnakoski et al., 2010
O. minus European lineage	A	Galleries	Spruce, pine	Linnakoski et al., 2010
Ophiostoma sp. *	A	Swarming		Present study
Pezizomycotina sp. $^{S/T/H/O}$	A	Swarming, stump	Spruce	Present study
Saccharomycetes sp	A	Swarming		Present study
Eurotiomycetes sp.	A	Stump	Spruce	Present study
Penicillium sp.	A	Stump	Spruce	Present study
Leotiomycetes sp.	A	Swarming		Present study
Cadophora sp.	A	Swarming		Present study
Dothideomycetes sp.	A	Swarming, stump	Spruce	Present study
Cladosporium sp.	A	Swarming		Present study
Rhizosphaera sp.	A	Swarming		Present study
Sydowia sp.	A	Swarming		Present study
Sordariomycetes sp. $^{T/H/O}$	A	Swarming, stump	Spruce	Present study
Hypocreales sp. $^{T/H}$	A	Stump	Spruce	Present study
Ophiostomatales sp. ^{O}	A	Swarming		Present study
Mucoromycotina sp.	Z	Stump	Spruce	Present study
Absidia sp.	Z	Stump	Spruce	Present study
Mucor sp.	Z	Stump	Spruce	Present study

(*) Anamorphs of *Hypocrea* spp. (*Trichoderma* spp.) have been recovered by Müller et al. (2002). * Many *Ophiostoma* spp. have been recovered by Linnakoski et al. (2010). In relation to the findings of the cited authors, the superscripts indicate, which of the listed taxa are accommodated by the particular specimen. The superscripts S, T, H and O are for Sordariomycetes, *Trichoderma*, *Hypocrea* and *Ophiostoma*, respectively. A=Ascomycota, B=Basidiomycota, Z=Zygomycota.

4 DISCUSSION

4.1 Evaluating the methods

4.1.1 Treatment of the beetles in laboratory

Identities of each treated insect were not confirmed under a microscope since at that time the specificity of the pheromone was considered reliable. This may have lead to mistakes because three additional *Trypodendron* species exist in Finland. The colour of young bark beetles is typically lighter or yellowish (Heliövaara & Mannerkoski, 1987), which would help in the identification of beetle species, but so are *T. signatum* adults as well. Consequently, if the pheromone was not truly specific for *T. lineatum*, it is possible that other *Trypodendron* species were treated incorrectly as *T. lineatum*. Nevertheless, the other *Trypodendron* species are not as common as *T. lineatum* and some possess different host preference indicating of differences also in their affection for dissimilar sets of volatiles (Klimetzek et al., 1981). Despite the confusing factors, most treated beetles obviously were individuals of *T. lineatum*. However, the impact of isolating fungi from incorrect beetle species on the results must be considered and it must be admitted that the spectrum of fungi associated with *T. lineatum* cannot be fully characterized. However, in forest or log storage, the effect of this mix-up would be less remarkable. If a fungus is associated with some other insect species (than *T. lineatum*), the attack on a tree would in any case enable the spreading of the fungus and influence its future development. The target tree of the invasion may provide suitable conditions for the fungus, but if not, the establishment of the fungus could be hindered.

Storing of the insects in freezer might have narrowed the species range and decreased the number of different fungi but at least basidiospores of *Heterobasidion* sp. should have survived freezing. The beetles from stumps 2 and 3 from site K had been pre-sterilized with 70 % ethanol and rinsed with MQ-water before freezing. This might not have significantly affected the results concerning the insects from those two stumps that were later sterilized with even stronger sterilizing agents. On the other hand, the insects that were not sterilized afterwards with the stronger agent, had been pre-sterilized and were from those stumps, might have lost some fungal species they otherwise would have carried on their surface. Nevertheless, according to the obser-

vations on all isolations, the pre-sterilization did not appear to have influenced the number of isolated fungi.

Individual beetles and groups of beetles were treated aseptically. In order to prevent undesired contaminations from occurring, any of the materials used for treating the beetles from a site were not used for treating the beetles from the other site. To consider the 34 plates on which no growth was observed (blank plates), clear difference between the sterilization (done or not) could not be made because 19 plates had sterilized insects and 15 plates had been in contact with unsterilized insects. Most of the blanks were derived from the sterilized beetles collected by pheromone traps at both locations. This suggests effective sterilization method but also that the possibly hidden fungi did not prefer establishing in the provided circumstances or that the flying beetles just did not get attached to high numbers of spores during their swarming in the spring. The latter postulation may well hold true, since it is typical that beetles swarm in the spring with empty guts (Francke-Grossmann, 1967; Beaver, 1989). The proper sterilization of the beetles should have been effective in reducing the emergence of occasionally coexisting species. Those fungi could, anyway, have been associated with and detected from the unsterilized beetles. Whether the destructive effect of sterilization reached fungal specimens transported via mycangia is not known for sure. Perhaps, at least to some extent, the organ was broken exposing what was sealed within it to the sterilizing agents during the different treatments.

The fungal flora on bark beetles is typically studied by isolating fungal cells from the exoskeleton (Persson et al., 2009; Bueno et al., 2010). The isolation method has been observed to have an impact on the information gained (Persson et al., 2009; Linnakoski et al., 2010). Bueno et al. (2010) detected a low frequency of ophiostomatoid fungi by letting the beetles walk on a medium and by placing the water used for washing the beetles on a medium. Considering the method of rolling unsterilized beetles on a medium in the present study, it is possible that the contact to the medium was insufficient for inoculation and cultivation if some fungal propagules were hidden within the organs of *T. lineatum*. Therefore, other techniques were here used in order to permit different groups of fungi to expose themselves. For

example, squashing the beetle individuals with sterile tweezers compared with crushing them with a sterile ceramic refiner could result in dissimilar pulverization of the beetles yielding different spectrum of fungi. One could assume that the finer the particles of the insects are, the easier and faster the propagules that were sealed within the organs might grow into hyphae. Some species may thus have gained competitive edge by the different treatments but some might have escaped isolation by being exposed to UV-light, higher level of oxygen or unfavourable humidity.

4.1.2 The cultivation of fungi

The use of two different growth media might have affected the results by giving opportunities for fungi with different preferences. The hagem plates should have been of suitable pH for *Heterobasidion* sp. but if the medium and other growth conditions did not appeal to one's requirements in nutritional or gaseous terms, pH, lightness, humidity or some other factor, perhaps those species could not be found. Insufficient oxygen level, for example, is known to lead to reduction of fitness (Korhonen & Stenlid, 1998). Despite that high relative humidity (above 92 %) is essential for *H. annosum* establishment (Rishbeth, 1951), too high humidity inside the petri plates could have worsened the vitality of certain fungi (Schönhar, 1980). Some species may have been dependent on certain coemerging species and on the subsequent conditions for which the previous was responsible (fungal succession). Being unable to meet the first one's demands, loss of the second successional fungus would follow. Competition within the mycota most probably has influenced the spectrum of the arising specimens and antagonism may have deprived the growth of certain species.

The incubation period during which the plates were regularly inspected was relatively long. The plates were observed for about six months with the exception of the crushed groups from both rounds that were initiated two or two and a half months later than the other treatments. The long period of observation might have given the opportunity for the most slowly growing specimens such as the mycangial fungi (Beaver, 1989) to emerge, especially in case antagonists or stronger competitors were absent. Despite the many efforts made, recovery of the slow growing species may have been unsuccessful due to faster growing species covering them with their

hyphal mat. Their establishment could also have required certain special conditions, which would have been created in their natural environment (involving other organisms such as bacteria, of which many were destroyed during sterilization and probably freezing).

Antibiotics were used to prevent bacterial growth. In principle, the antibiotic was diluted into 10 ml of malt agar and was filtered into 500 ml of malt agar, but in practice only half of the streptomycin entered the 500 ml of agar. Even if the amount of the antibiotic was smaller than was meant to, it seemed to have a positive impact on purification of the cultures. Those ten fungi grown on a plate containing streptomycin were directed into each of the three (quality) classes. All the five fungi from class 3 and one from class 2 from which DNA was extracted, had grown on an antibiotic plate. More interestingly, four of the ten could be designated into class 1 for DNA extraction. The use of the antibiotic is thus validated because the cultures were successfully purified and directed to DNA extraction. Linnakoski (2011) has used streptomycin with species belonging to *Ceratocystis*.

4.1.3 Methods of DNA extraction, PCR and identification of species

The method of DNA extraction (Chang et al., 1993) in the very study has been previously applied in mycological studies perhaps with some modifications (Terhonen et al., 2011). The morphology-based groupings within class 1 were neglected during the DNA extractions, and several reports suggest this reasonable. Linnakoski (2011) has reported that some morphologically similar species were phylogenetically distinct. Also, according to Paulus et al. (2007), the morphological similarities are not always supported by the ITS sequence data. On the contrary, Linnakoski (2011) found also that some morpohologically different species had similar ITS sequences. The ITS region may occasionally fail to distinguish between closely related phylogenetic species (Bruns & Shefferson, 2004) such as *Ophiostoma piceae* and *O. canum* (Harrington et al., 2001). Consequently, it was considered that a greater increase in the current knowledge of the associated fungi could be gained by ignoring the subgroupings.

The application of another PCR protocol succeeded in the amplification of some samples that failed during the running of the first protocol. Nevertheless, it did not work for all fungi. Identification of species rests upon the sequence similarities found by BLAST. Persson et al. (2009) considered a similarity of 94-97 % sufficient for the identification of a sample to genus level. In order to determine the specimen to species level, they considered the ITS sequence homology accurate enough with the sequence similarity of 98-100 % (Persson et al., 2009). In the present study, the principle of determination of a specimen to a taxon was somewhat similar to that reported by Persson et al. (2009) but more critical since the taxon was never determined to the species level.

The present experiment has possibly failed to catch all members of the fungal flora associated with *T. lineatum*. Failures arise from the used materials and methods, unfulfilled cultural requirements of the mycota and interactions within the divergent assemblages of fungi. It must be mentioned that the identification of species, when based on the values of query coverage and maximum identity, is artificial. In order to obtain more trustworthy designation of species, morphological features should be characterized in more detail and additional genes such as β-tubulin and EF1-α gene regions (Linnakoski et al., 2010; Linnakoski, 2011) should be sequenced. Based on the points above, there were many factors suggesting it reasonable to rather designate the specimens to less specific taxa than to species level even when the negative consequence of this may have lead to an increased number of unidentified fungi.

4.1.4 The statistical methods

Due to the data being composed of multiple hierarchies, all of it could not be included in the analyses. The statistical analyses focused on the proportions of beetles carrying fungal propagules. Separation of epi- and endomycetes required the fungi from round II and crushed groups to be excluded from the data used for the analyses (see 2.7). The results concerning the position of the fungi were obtained from 49 fungi revived from 27 beetles. The DNA extractions divided unevenly between fungi that could be considered endomycetes (11) and epimycetes (38). However, the data and

the outcome represent the phenomenon, in which the fungi were more frequent on the outer surface of the beetles.

Odds ratio (OR), in which the proportions are transformed into odds, was utilized in the statistical analyses. The method is respectful of the phenomenon, and it has been proposed that OR should be reported more commonly in the research papers of ecological studies (Rita, 2004; Rita & Komonen, 2008).

4.2 Evaluation of the results

4.2.1 Fulfillment of the aims and hypotheses of the study

On the level of the entire data the results for the proportions of beetles and frequencies of isolated fungi can be considered reliable. Analyzing only parts of the whole data (Ascomycetes, Zygomycetes and unidentified isolates) may possess weaker reliability due to insufficient data. The proportion of scolytids that were the origin of epimycetes was greater than that of endomycetes. The beetles were thus more often and independent of the trap type vectoring epimycetes than endomycetes. Therefore it can be concluded that *T. lineatum* was to a larger extent associated with opportunistic fungi, since the epimycota may be more easily influenced by the environment. This was supported by the conclusion that obligate endomycetes were not detected (explained later). Moreover, the pheromone traps lured more effectively beetles carrying ascomycetes (on both positions) in the spring, whereas in midsummer, the stumps released less such beetles that were vectoring ascomycetes.

Even though the proportions of beetles carrying ascomycetes were significantly dissimilar between the different trap types, the frequencies of ascomycetes were not with statistical significance influenced by the trap type. The statistical insignificance (in relation to trap type and ascomycetes) is explained by the fact that the beetles from stumps were also vectoring ascomycetes. The proportions and frequencies change at unequal rate leveling off the differences in the frequencies between trap types. As the proportions of beetles carrying ascomycetes at different positions did not differ with statistical significance, but that the frequencies of ascomycetes did, is

because more than one ascomycete has been isolated from each beetle. Ascomycetes, a large group of fungi accommodating several different taxa, were more frequently isolated as members of the epimycota.

In general, the proportions of vector beetles caught by the different trap types at different times did not significantly differ in their efficiency of attracting and capturing beetles. Stumps caught a slightly greater proportion of beetles than pheromone traps. However, the frequencies of isolated fungi were smaller among the beetle population of the stumps. The conclusion, by which the stumps could be considered to hold a species-poor mycota, was not considered significant by the statistical analyses.

The purpose of rolling unsterilized beetles on a dish was to obtain the epimycota occurring together with *T. lineatum*. The sterilized beetles, instead, should have provided the endomycota from inside of the beetle. The fungi recovered from the groups of crushed beetles could not differentiate between endo- and epimycota but serve for additional data on the fungal diversity vectored by the beetle. This has been taken into account during the statistical analyses. It could be assumed that the epimycota might include opportunistic species, while those occurring only among endomycetes are associated to the beetle in a more strict sense. If some fungi could not be found within any of the unsterilized beetles but were found among the sterilized ones, they most obviously preferred dispersing as obligate endomycetes. On the contrary, if a species could be detected from both positions, assuming that the sterilization was carried out successfully, the species would appear more loosely dependent on the position of transportation indicating facultative lifestyle for at least part of the time span. Being vectored at both positions could be maximizing the success of dispersal but if the cases were few, coincidence might make the same result.

Obligate endomycetes were not confirmed, whereas many of the findings were detected from both positions. However, all of the Ophiostomatales (including *Ophiostoma* spp.) and Leotiomycetes (including the genus *Cadophora*) and most Dothideomycetes (*Cladosporium* sp. and *Rhizosphaera* sp.) were found among the epimycetes from the beetles from pheromone traps. All of the members of the

Zygomycota and Mucoromycotina originated from the beetles from stumps. As members of the Mucoromycotina, *Mucor* sp. was derived among the epimycetes while the preferred position concerning *Absidia* could not be determined. The ascomycetes *Penicillium* sp. and *Hypocrea* sp., originated solely from beetles from stumps. *Hypocrea* sp. was confirmedly recovered from both positions. The position of *Penicillium* sp., although it was verified as an epimycete, remained uncertain since Eurotiomycetes accommodating *Penicillium* sp. were recovered from both positions. Bueno et al. (2010) reported that, for example, *Penicillium* sp. was easily dispersed on elytra and that ophiostomatoid fungi were most often carried on pronotum. The results of the positions of the present study seem to be in line with those reported by Bueno et al. (2010).

It is noteworthy that basidiomycetes in general were not observed. The additional aims of recovering *Heterobasidion* species and *Phlebiopsis gigantea* associated with *T. lineatum* were realistic in the light of previous studies. Müller et al. (2002), for example, found both species connected to the beetle and to the same host tree. Other indications of *Heterobasidion* being vectored by an insect have been provided (Bakshi, 1950; Bakshi, 1952; Kadlec et al., 1992; Viiri, 2004). Another unexpected finding was that the detection of the ambrosia fungus, *Ambrosiella ferruginea*, of *T. lineatum* failed. According to an earlier notice by Mathiesen-Käärik (1953), the ambrosia fungi readily escape detection but the recovery should be possible. In order to successfully isolate the ambrosia fungi, more attention should be paid to their preferences. The growth form of the mycangial fungi may vary under different conditions and is often yeast-like (Beaver, 1989). Isolation and upkeeping of the yeast-like fungi should therefore not be avoided, which was done during this experiment. This is not the only study escaping the detection of the fungus, since Linnakoski (2011) did not report it as an associate of the striped ambrosia beetle.

Kallio (1970) observed high seasonal and diurnal variation in the amount of spores on open areas. In southern Finland the deposition of *H. annosum* spores were absent from December to mid April (Kallio, 1970). Humidity and rainfall around the time of fellings and sampling might have affected the frequency and spectrum of fungi. Red-

fern et al. (2001) demonstrated that heavy rainfall decreased infections. The clear cuts at the collection sites had been carried out in the autumn 2009 but the data on rainfall is lacking. The collection of insects for the present experiment was carried out after mid April and midsummer. According to Kallio (1970) and recent climatic data (Ilmatieteen laitos, 2012b, c), *H. annosum* infections could have occurred. The weather was, anyway, relatively cool during the time of collecting the beetles with the pheromone traps and, thus, it is possible that sporulation of some fungi, including those expected, was not at sufficiently high levels during the time the beetles were swarming and caught by the traps. This could inevitably be reflected in the composition of mycota and in the counts. The rainfall in summer 2010 when the pieces of stumps were sawn was less than average, reaching 70-90 % of the rate of the period of comparison (Ilmatieteenlaitos, 2012c). According to Rishbeth´s (1951) observations, exceptionally dry weather curtails the spore discharge of several fungi although with smaller influence on *Heterobasidion* than on other competing fungi.

Some of the numerous reasons why these fungi were not found within the limits of this work have previously been discussed. To speculate further the relationships between certain discovered genera, the following was considered important since many indications of specimens with inhibitory characteristics towards other fungi were here observed. *Heterobasidion* antagonists *Penicillium* and *Trichoderma* (Rishbeth, 1948), whose teleomorph is *Hypocrea*, could have had a competitive edge in the beginning of the trial in laboratory. The presence of those specimens could have reduced the fitness of *Heterobasidion*, basidiomycetes in general, and some other species. According to the GenBank data, *Hypocrea* has been used as a biocontrol agent against *Fusarium* head blight and species of *Zalerion* are known to produce antifungals. From these genera, *Penicillium* and *Hypocrea*, were found in this experiment, but *Zalerion* could only be designated to the subphylum level.

4.2.2 Other procedure-related considerations

The growth rate of hyphae in dead wood material varies. In case of *Heterobasidion* infesting stems of *Picea abies* (Linnaeus) Karsten 1881, growth rates of 0.22 to 0.4 meters per year have been reported (Stenlid & Redfern, 1998). The impact of sawing

the stumps with the same chain saw should be considered. The healthy pieces of stumps were stored at room temperature for about a month after which they were temporarily taken into a cool storage. During this one month, the conditions for fungal growth could have been quite ideal, since the plastic wrapping must have kept the wood moist and warm. If the blade was contaminated and inoculated the next stump and the fungi established, the propagules may have got attached to the beetles. However, the growth speed of some of these fungi in wood material is unknown. Therefore it is difficult to say if the hyphae ever reached the sites where the beetles were in the stumps and if the beetles were contaminated by this means. Nevertheless, sporulation outside of the stumps could have contaminated the beetles as they have left the tunnels. Consequently, the frequency of some fungal species that originated from the beetles from stumps could be overestimated in this study. Also, the beetles in the containers of the pheromone traps have been in contact with each other possibly spreading fungal cells within the trapped community. The endomycota might have been better protected from this kind of transmission of inoculum though.

The route and timing of possible infection and the point of the beetle invading the wood could influence the further dispersal of a disease causing agent. Here, if the cut surfaces of the healthy stumps had been infected by fungal spores by natural means (in contrast to the reasoning above) and the beetles colonized the stumps at the very basal areas of the stumps, it is very likely that the fungus and the beetle did not have enough time for creating an intimate contact before the beetle left its chamber. Theoretically, it could be possible that a bark beetle would introduce compatible mating types as it enters an already infected stump. This would affect the fitness of the fungi since at least among basidiomycetes the homokaryotic phase is short whereas heterokayotic mycelium is longer-lived and possess greater aggressiveness. However, it is obvious that certain basidiomycetes and other species as well have escaped detection due to the previously described phenomenon, in which the potential vector and the fungus did not make a contact. The possibility of bringing different mating types together by bark beetles could be examined in the future studies.

The observed diversity during the first round seemed lower than was initially assumed and as a consequence, another round of cultivation (II) was carried out. Finally, from the insects from pheromone baits, 23 % were sterilized and handled individually, 38.5 % were treated as groups with and 38.5 % without sterilization. (Table 1.) From the stump-derived beetles 33.3 % fell in each treatment. Bigger shares within the pheromone trapped groups of beetles result from the second round. Otherwise, equal amounts of beetles were treated from both sites. According to the results, trap type did not influence the frequencies of fungal isolates (Table 4). Concerning the site P, insects from only three pieces of stumps (2, 3, and 6) were used for cultivation. Insects from stumps 2 and 6 were more abundant and have therefore been used more frequently. The condition of these stumps will have affected the exposed mycota with a slight overemphasis on them but the number of stumps was small anyway. Beetles from the remaining two stumps (1 and 4) had unfortunately been utilized beforehand.

The stumps seemed healthy during sawing. Based on the observations carried out almost two years post-collection, the condition of stumps K2 and P2 gives a reason to give another thought on the fungi recovered from those because the condition of the stumps could directly be reflected in the composition of the mycota. Stump K2 was in a relatively good condition but had a little resupinate fruiting body (possibly *Heterobasidion*). Fungi had not been isolated from beetles from stump K2, which probably is one of the reasons for *Heterobasidion* escaping the recovery. Slight heartwood decay was detected in stump P2. The sterilized and unsterilized beetles from the stump had given rise to isolates 8, 12, 14, 18, 39, 40, 56, 57 and 60, (Table 6.). These isolates were designated to the Mucoromycotina sp., Hypocreales sp. (and *Hypocrea* sp.) and *Penicillium* sp. These taxa were never found from beetles collected by pheromone traps. In case of including the higher taxa of these specimens (e.g. Sordariomycetes, the class accommodating *Hypocrea* sp.) to the inspection, one could notice that Sordariomycetes were also discovered from beetles from pheromone traps. However, these Sordariomycetes had been designated to the genus *Ophiostoma*. This fact demonstrates that the niche that the beetles accommodate will be reflected in the composition of its associated organisms, which is also influenced by the methods and materials used for examining the very topic (Müller et al., 2002;

Lygis et al., 2004; Viiri & Lieutier, 2004; Persson et al., 2009; Bueno et al., 2010; Linnakoski et al., 2010). Additionally, stump P5 was severely decayed and K5 had little heartwood decay but beetles from those stumps were never used in the treatments because none of them had hatched in time. It was a misfortune that the beetles of these stumps that according to the recheck were bearing more aggressive decay fungi were not liberated with their mycota in time.

4.2.3 Summarizing the species richness and diversity

The epimycota was rather versatile. According to the species richness evaluation, the number of different taxa was higher among the epimycota than among the endomycota. Only a few taxa were shared by the communities of the two positions. The Shannon-Wiener (SW) and Simpson indices reflected greater species diversity among the epimycota. Also, the Morisita-Horn (MH) and Bray-Curtis (BC) indices proved that the similarity between epi- and endomycota was not the highest observed. The richness and diversity analyses support the outcome for the frequencies of isolated fungi.

The species richness was higher among the mycota of the pheromone baited beetles than the stump originated beetles. Relatively sparse specimens were common between trap types. The SW and Simpson indices showed that greater diversity of fungi was associated to the swarming beetles lured by the pheromone traps. Thus, the composition of the recovered mycota differed between trap types, which was supported by the lowest similarity indices observed in the study. These results are in agreement with those obtained for the frequencies, which showed that the stumps were hosting less abundant fungal community. However, the fact that many isolates of stump origin had been directed to class 3, the class of the worst quality, must have influenced the outcome. Only a few of these cultures proceeded to DNA extraction.

The number of different species was higher (only by one specimen) at site K. However, the species diversity was higher at the other site (P). Since species richness is simply a measure of different taxa observed, whereas in the diversity assessment the relative abundances of each taxa are taken into account, these may not be conflicting

results. The sites had the highest number of shared species, and the MH and BC indices showed relatively high similarity between the sites. This seems relevant as the sites located close to each other, were similar in site type and the collections were carried out during the same days. Poisson regression was not used for studying the differences in the frequencies of isolated fungi between collection areas due to their closeness. According to the relatively high similarity indices, by doing so, would not have given added value to the results.

The species richness was higher among the beetles treated individually than among the groups of insects (see Appendix, Fig. 16.). Apparently, the treatments recovered quite many shared specimens. Species diversity was higher among the individually treated beetles. This result can hardly be generalized, since the isolates directed to DNA extraction divided unevenly between individual and group treatments. Most of the isolates were from the individually treated beetles, which must have skewed the results. Also the MH and BC similarity indices seem to disagree on this issue, which can be due to the twisted data. Poisson regression was not used at this point, and propably did not lead to losing information. More even data would have given more reliable results concerning the treatment methods, which undoubtedly do influence the outcome.

The total number of fungal isolates was 122. If the subgroupings within the three quality classes of pure cultures are taken into account, the counts of certain taxa will increase. The frequency of *Hypocrea* sp. would (instead of 6) be 14, and the count of *Ophiostoma* sp. would (instead of 5) be 7 (see Table 5.). The additional *Hypocrea* group members originated from sterilized and unsterilized beetles solely from stumps similarly as the official isolates (for which DNA extraction was carried out). The additional members for the *Ophiostoma* group were derived from sterilized beetles from pheromone traps as the original isolates were from unsterilized ones. Thus, the origin of these species would not change even if the group members were included but the position might involve both. The sterilized groups of insects gave also rise to *Ophiostoma* sp. confirming the possibility of the genus being transported at both po-

sitions. (See fifth paragraph under 4.2.1 for details.) The frequencies of other taxa would remain as they were presented in Table 5.

On the average, each beetle was associated with less than one fungus (ratio of fungi per beetle was 0.74). Some of the plates and beetles yielded no fungal isolations, and when these were ignored, the ratio was higher (1.14). The ratios are slightly lower than those of some other studies. Ratios of 1.4 fungi per beetle and 2.7 fungal taxa per beetle have been reported (Jankowiak et al., 2009; Persson et al., 2009). Bueno et al. (2010) observed ratios of 0.8 and 1.0 fungi per male and female beetles, respectively. They let the insects walk on a medium and placed the water that was used for washing the beetles on a medium (Bueno et al., 2010). In the present study, the method of rolling unsterilized beetles on a medium could be comparable to the method Bueno et al. (2010) used because in both cases the beetles have been allowed to make a direct contact with the plate. As a consequence, especially the ratios reported by Bueno et al. (2010) are parallel to those observed in the present study.

4.2.4 Results of the DNA extraction and PCR and identification of species

Multiple copies of some fungi may have been isolated and directed to DNA extraction, since they have been considered different species or individuals (strains). After the repeated isolates were excluded, the number of DNA extractions was 76. In one case, two fungal isolates that originated from the same source were designated to different taxa. The other of these specimens had been isolated from the first fungal isolate, and thus, both classes were included in the calculations. This clearly indicates it wise to consider all isolates as different species or strains, and suggests that the first isolate was propably faster in initiating growth than the second one.

The number of DNA extractions was lower than the number of fungal cultures due to some fungi sharing similar morphological features and ending up grouped together. Contaminations reduced also the number of isolates, to which DNA extraction could be carried out. Despite of using two different PCR protocols, the DNA of three fungal specimens could not be amplified, so they remained unidentified.

NCBI Taxonomy Browser gave slightly different taxonomic location for the genera *Absidia*, *Mucor*, *Rhizosphaera* and *Sydowia*. This kind of uncertainties in the classification of fungi makes it reasonable not to present the findings at very specific taxa unless additional analyses were carried out. The fungi recovered in the present study are described according to the GenBank data and additional sources in the following section (4.2.5). Many of the suggested specimens of the very study were related to soil. Because *T. lineatum* may invade stumps beneath moss layer and hibernate in litter, contacts with soil become inevitable and the suggested taxa appear relevant.

4.2.5 The recovered taxa belonged to the Ascomycota and Zygomycota

4.2.5.1 Ascomycota, Pezizomycotina

Most of the Ascomycetes perform both sexual and asexual forms of life. Sexual ascospores are haploid and produced in ascus (pl. asci). The sexual phase is called teleomorph. During the anamorphic phase the asexual conidiospores emerge from hyphal structures termed conidiophores. Traditionally the taxonomy has been based on fruiting bodies (Carlile et al., 2001). Here, four classes were found under the phylum Ascomycota and subphylum Pezizomycotina. These were Eurotiomycetes, Leotiomycetes, Dothideomycetes and Sordariomycetes.

4.2.5.2 Eurotiomycetes

Only a few members of the Eurotiomycetes were found in the study. The class consists of two subclasses, Chaetothyriomycetidae and Eurotiomycetidae. The former taxon includes some lichenized fungi and some black yeast fungi pathogenic to human and animals. Nevertheless, majority of human pathogenic Pezizomycotina belong to the latter group, Eurotiomycetidae, and more specifically, to Eurotiales. The members of the Eurotiomycetidae produce both toxins and useful metabolites. They serve as sources of agents that are utilized in the production of food and enzymes. *Aspergillus nidulans* (Eidam) G. Winter 1884, a member of the Eurotiomycetidae, has been used as a model organism in genetics. Some of the members tolerate drought (xerophiles) and high temperatures (psychrophiles) (Geiser et al., 2006).

The order Eurotiales includes the family Trichocomaceae, the family of *Penicillium* sp. recovered in the very study (sample numbers 17, 18 and 60 in Table 6). The GenBank often suggested *Penicillium* spp. connected to rhizosphere. The family is characterized by its saprotrophic preference in the acquisition of food. They are well adapted and capable of rapidly colonizing their habitats. Members of the Trichocomaceae are very common in soils and on rotten plant material. Some other members of the Eurotiales enter ectomycorrhizal hypogeous associations (Cannon & Kirk, 2007).

4.2.5.3 Leotiomycetes

Leotiomycetes include the orders Cyttariales, Erysiphales, Helotiales, Rhytismatales and two families of uncertain position, Myxotrichaceae and Pseudeurotiaceae (Wang et al., 2006b). Here, under the order Helotiales was recovered *Cadophora* sp.. Members of the Helotiales have been observed as plant pathogens, plant parasites and endophytes (Shoemaker et al., 2002; Grünig & Sieber, 2005; Cannon & Kirk, 2007). They have relatively recently began to form lichen symbiosis (Platt, 2000) and form mycorrhizal relationships (Okuda et al., 2011). They are distributed in a variety of ecosystems as the saprotrophic members can be found both from terrestrial and aquatic environments (Wang et al., 2006a).

The recovered *Cadophora* sp. lacks the family taxon. Thus, in order to name some other examples of the class Leotiomycetes and the order Helotiales, they are here demonstrated through other families belonging to the Helotiales. The family Hemiphacidiaceae including the genera *Didymascella* and *Rhadocline*; Leotiaceae accommodating the genus *Leotia*; and Helotiaceae, the family of *Calycina, Godronia, Hymenoscyphus* and *Tympanis* are here mentioned. Common for these examples is that they are saprotrophic organisms with minor known economic importance except for the poorly known Helotiaceae that host also pathogens of woody plants. The Helotiaceae include some mychorriza forming and fungicoulous fungi (that grow on other fungi or are mycoparasites (Hawksworth et al., 1995)). Helotiaceae is typical in tropical and temperate regions. *Leotia* is a soil inhabiting fungus favouring decaying plant material. It is widespread but more concentrated in

temperate zones. *Didymascella* and *Rhadocline* can be detected as parasites on conifers in the north temperate region (Cannon & Kirk, 2007).

4.2.5.4 Dothideomycetes

Dothideomycetes most obviously constitute the major class within the phylum Ascomycota hosting more than 19 000 species. Dothideomycetes are distributed to a variety of niches in aquatic and terrestrial environments where they are best recognized as agents causing crop diseases. Many members of the Dothideomycetes are endophytes and epiphytes of plants, and saprotrophs. Dothideomycetes are, yet to lesser extent, known as hyperparasites and related to lichens (Schoch & Hyde, 2011). According to the GenBank, *Herpotrichia juniperi* (Sacc.) Petr. 1925, which in this study remained at the class level (Dothideomycetes sp., sample number 66), has been related to leaf litter of *Pinus arizonica* Engelmann ex Rothrock 1878 var. *arizonica*, *Picea mariana* (Miller) Britton, Sterns, et Poggenburg 1888 and soil.

In the present study, three orders were designated beneath the class Dothideomycetes (see Appendix, Fig. 12.). These orders were Capnodiales, Dothideales and Pleosporales. The families under these orders for the recovered genera *Cladosporium*, *Sydowia* and *Rhizosphaera* were Davidiellaceae, Dothioraceae and Venturiaceae, respectively. Davidiellaceae has been suggested as a new family to accommodate the anamorphic *Cladosporium* and *Davidiella* (Schoch et al., 2006). In the present study, four isolates (sample numbers 21, 29, 71 and 73.) were designated to the genus *Cladosporium*. According to the GenBank data, *C. uredinicola* Speg. 1912 has been reported as a hyperparasite on *Cronartium fusiforme* Hedgc. & N.R. Hunt ex Cummins 1956. *Cladosporium* species have also been observed as endophytes of *Rhizophora apiculata* Blume growing in mangrove forest in India (Kumaresan & Suryanarayanan, 2002), and of western white pine, *Pinus monticola* Douglas ex D. Don (Ganley & Newcombe, 2006). The ubiquitous Dothioraceae include biotrophic and necrotrophic fungi. Some members are important plant pathogens and they are typically found from woody plants. *Hormonema* species (synonym for the teleomorphic *Sydowia*, which in this study was recovered six times (sample numbers 27, 30, 41, 42, 49 and 61), are common spoilage fungi but also recognized

as human pathogens. The family Venturiaceae is widespread but more common in temperate zones. They are weak parasites and endophytes of aerial plant parts such as wood, bark, leaves and fruits. Some members are more aggressive pathogens causing symptoms in the tissues they invade (Cannon & Kirk, 2007). *Rhizosphaera* sp. was here found twice (sample numbers 22 and 77). The GenBank data suggests *R. macrospora* Gourb. & M. Morelet 1979 and *R. kalkhoffii* Bubák 1914 as conifer pathogens and associated with fir bark beetle, *Cryphalus piceae* Ratzeburg 1837. *R. kalkhoffii* has also been found as an endophyte of *P. abies*, *P. pungens* Engelmann 1879 and *Abies beshanzuensis* Wu 1976.

4.2.5.5 Sordariomycetes

Sordariomycetes include pathogens and endophytes of plants, arthropods and mammals, but also saprotrophs and fungicoulous fungi. Most plant pathogens belong to the orders Hypocreales, Ophiostomatales, (which were recovered in the present study), Microascales, Diaporthales, Phyllachorales and Xylariales. The endophytic Sordariomycetes may provide the infected host with increased drought resistance, reduced feeding by insects and protection against pathogens (Zhang et al., 2006).

Hypocreales includes a family of Hypocreaceae that is the family of the genera *Hypocrea* and *Mariannaea,* for example. *Hypocrea* sp. was suggested six times (sample numbers 9, 14, 16, 37, 38 and 39) by the present study. According to the GenBank, *H. rufa* (Pers.) Fr. 1849 has previously been used as a biocontrol tool against *Fusarium* head blight. There were weak indications of *Mariannaea elegans* (sample number 56), which has been isolated from several sources. It has been observed associated with *Ips typographus* (after hibernation under bark), alder (*Alnus glutinosa* (L.) Gaertn.), spruce stump and in the root collar xylem of a declining ash tree (*Fraxinus excelsior* L.). The family is globally distributed performing a diversity of life styles. Saprotrophic family members thrive on senescing wood and other plant material while some inhabit soils and a minority is coprofilous fungi (Cannon & Kirk, 2007), which live on dung (Hawksworth et al., 1995). Some members of the Hypocreales may be associated to insects (Rossman et al., 1999). Their proteins could serve for fungicides and antibiotics (Cannon & Kirk, 2007). Mycoparasitism

performed by some species of the Hypocreaceae (e.g. *Trichoderma* sp.) has been utilized in biocontrol of many agents from different taxa (e.g. Mucorales, Eurotiales, Cantharellales) causing crop disease (Guédez et al., 2009). *Hypocrea* sp. recovered in the present study is the teleomorph of *Trichoderma* sp..

Among the other observed orders here (Ophiostomatales) was a family, Ophiostomataceae, bearing the genus *Ophiostoma*. Many members of the Ophiostomataceae are vectored specifically by certain insects. (Some characteristics of ophiostomatoid fungi are discussed in 1.5.) They are aggressive pathogens of conifers and hardwoods. One of the most devastating outbreaks has been the Dutch elm disease epidemics in the UK caused by *O. ulmi* (Buisman) Nannf. 1934 or *O. novo-ulmi* Brasier 1991 (Hawksworth et al., 1995; Cannon & Kirk, 2007). Members of the Ophiostomataceae may also infect human (Cannon & Kirk, 2007).

In the present study, *Zalerion arboricola* Buczacki 1972 was suggested four times (sample numbers 4, 53, 54 and 55) but was designated to less specific taxa. However, *Zalerion* is a member of the Sordariomycetes. According to the description obtained from the GenBank, it is a foliar endophyte of wind-fallen spruce and known for its production of antifungals.

4.2.5.6 Ascomycota, Saccharomycetes

Saccharomycetes are yeasts (Hawksworth et al., 1995; Suh et al., 2006). These saprotrophs are often associated with plants, animals and insects (Müller et al., 2002; Geiser et al., 2006; Suh et al., 2006; Persson et al., 2009) but some are pathogenic to human and plants (Suh et al., 2006). Yeasts have been used in industrial and biotechnical processes such as baking and synthesis of recombinant proteins. *Saccharomyces cerevisiae* Meyen ex E.C. Hansen 1883 is among the most important model organisms in the field of research. Typical of yeasts is their reproduction by budding and lack of formation of fruiting body structures during the sexual state (Suh et al., 2006). Some indications of *Dipodascus* spp. (sample numbers 31, 51 and 52) were obtained in the present study. However, these specimens were designated to the Saccharomycetes (sample numbers 31 and 52) and one remained unidentified. Ac-

cording to the GenBank data, *Dipodascus* sp. has been discovered from mycorrhizal root tip of *P. sylvestris* L. and from spruce wood disk.

4.2.5.7 Zygomycota, Mucoromycotina

The phylum Zygomycota includes the order Mucorales. As members of the Mucorales and Mucoraceae were here recovered the genera *Mucor* (see Appendix, Fig. 13.) and *Absidia*. Most members of the very abundant and widespread Mucorales are saprotrophs. They tolerate high temperatures and the distribution area thus includes the tropics and temperate regions (Cannon & Kirk, 2007). They are common in soil and on decaying mushrooms, toadstools and plants but cause also rot of fruits. Seldom can the members of the Mucorales break down chitin or cellulose but they utilize the readily assimilable sugars. Hyphal spreading of the Mucorales is usually fast and production and germination of their spores occurs easily. *Absidia* is capable of spreading fast through its aerial hyphae (stolons) and rhizoids. Some species (e.g. *Mucor hiemalis* Wehmer 1903) have been observed on decayed wood material but probably were not the agents causing the decay. The Mucorales also include mycoparasites that attack other fungi. Some other orders of the Zygomycota are of more limited but yet recognized importance (Carlile et al., 2001). For example, strains of *Rhizopus* and *Amylomyces* are used in fermentation processes (Cannon & Kirk, 2007). Some other Zygomycetes form mycorrhizas, and some are predators, parasites or entomopathogens that kill insects (Carlile et al., 2001).

4.3 The study is topical

In the future studies, it remains to be elucidated whether *T. lineatum* introduces compatible mating types of a fungus as it begins its tunneling in the stump. (The issue has been considered in 4.2.2). Use of protective chemicals and oils in log storages could in certain cases offer tools for protecting timber from bark beetles, but the profitability of such means should be studied (Ranger et al., 2011). It has been postulated that another beetle species flying earlier in the season, *Pseudohylesinus nebulosus* LeConte 1859, could indicate emergence of *T. lineatum* (Chapman & Kinghorn, 1958). These data could be useful if preventative measures were to be employed at log storages but the requirement for futher studies is clear as new circumstances are

created by environmental changes. Also, it would be important to study if pest and pathogen populations could easily be controlled via their symbiotic organisms. Due to the fact that *T. lineatum* is so tightly bonded to its ambrosia fungus, *A. ferruginea*, one could conclude that attacking against the fungus would lead to reduction in the beetle populations. However, it should first be studied in laboratory (if possible), and yet, its applicability in practice could raise ethic dilemmas and thus remain reduced.

Warming climate and longer summers may allow reproduction of *T. lineatum* twice a year in Finland. As the striped ambrosia beetle prefers already damaged wood material for its breeding, it may cause significant damage to timber. Moreover, in more frequent numbers the beetle would cause more serious losses in forestry by lowering the quality of the most valuable butt logs. Therefore it would be beneficial to monitor the development of the population size of this and other insect pests so that necessary measures can be taken before and if the predictions of climate change come true. The shift to a higher population level would increase the possibility of beetles spreading more frequent pathogens of trees. Simultaneously, however, could increase the chance of the beetles transporting such beneficial fungi that possess antagonism or mycoparatizism against the forest pathogens. The new environmental conditions would also raise some new stress factors influencing the standing trees and their susceptibility. Therfore it might be challenging to predict what kind of consequences climate change may have in the complex plexus of relationships in a given environment.

Among the most important measures of avoiding losses in forestry in Finland is obeying the law of the prevention of damages caused by insects and fungi in forests (Laki metsän…2001), which at the time of writing is under revision. The improved law aimed at minimizing forest damages should take effect in the beginning of 2014 (Metsälainsäädäntöön tulevia…, 2012). The new Forest law would provide forest owners with more alternatives considering forest management practices. It might encourage forest owners to gap fellings and to uneven-aged forestry management (Hallituksen esitys…, 2013). Final cuttings of smaller surface areas (gap fellings) might, in total, create longer edges of the forests (in relation to bigger surface areas).

The trees standing close to the longer edges of woods might get exposed to several stress factors such as changes in temperature and microclimate, increased light and leaching of nutrients, which might predispose the trees to secondary agents of damage such as increased populations of insect pests. In an uneven-aged forest, logging injuries on the other hand could weaken the health of some trees making them more susceptible to subsequent damage. Since the long-term data dealing with the aforementioned changes is currently poor, further research is required (Finland´s National Forest Programme 2015, 2008). Thus, studies similar to this are fairly topical at the moment and linked to a larger entity.

5 CONCLUSIONS

Characteristics of a given pest must be known for enabling sound forest management, which needs to be updated and complemented at times. An effort has here been made for establishing a better understanding of the mycota associated to *T. lineatum*. This study was successful in providing new data on the fungal assemblage *T. lineatum* might coexist with (See Table 9.). Seven genera were newly discovered associated to the striped ambrosia beetle: *Penicillium* sp., *Cadophora* sp., *Cladosporium* sp., *Rhizosphaera* sp., *Sydowia* sp., *Absidia* sp. and *Mucor* sp.. Based on the results of this and previous studies, it appears that ascomycetes are more commonly vectored by bark beetles than are members of the other phyla (see 1.5 for details). The outcome of the present study is quite nicely in agreement with the cited reports.

It is noteworthy that the teleomorphic state of *Trichoderma* i.e. *Hypocrea* sp. and *Penicillium* sp. were among the recovered specimens. They may have shaped the spectrum of the recovered mycota by their ability of suppressing certain competing species such as the root rot pathogen *Heterobasidion* and other basidiomycetes. Even though the particular pathogen was not detected on *T. lineatum*, some other studies have found *Heterobasidion* associated with the beetle and the possibility should not be excluded. Neither was *T. lineatum* discovered to carry the most virulent bluestain fungi. In the light of the results of the study, the striped ambrosia beetle does not act as a vector for aggressive forest pathogens and may thus retain its image as a nonaggressive pest.

SOURCES

Annila, E., Bakke, A., Bejer-Petersen, B. & Lekander, B. 1972. Flight period and brood emergence in *Trypodendron lineatum* (Oliv.) (Col., Scolytidae) in the Nordic Countries. Communicationes Instituti Forestalis Fenniae 76.4. 28 p.

Amateur Entomologists' Society, 2012. [Electronic source]. Available at: http://www.amentsoc.org/insects/glossary/. [Cited May 10th 2012].

Bakke, A., 1983. Dosage response of the ambrosia beetle *Trypodendron lineatum* (Oliver) (Coleoptera, Scolytidae) to semiochemicals. Zeitschrift für Angewandte Entomologie 95: 158-161.

Bakshi, B. K. 1950. Fungi associated with ambrosia beetles in Great Britain. Transactions of the British Mycological Society 33: 111-120.

Bakshi, B. K. 1952. *Oedocephalum lineatum* is a conidial stage of *Fomes annosus*. Transactions of the British Mycological Society 35: 195.

Beaver, R. A. 1989. Insect–Fungus Relationships in the Bark and Ambrosia Beetles. In: Wilding, N., Collins, N. M., Hammond, P. M. & Webber, J. F. (eds.). Insect–Fungus Interactions. Academic Press, London, UK. pp. 121-143. ISBN 0-12-751800-2.

Bendz-Hellgren, M., Brandtberg, P-O., Johansson, M., Swedjemark, G. & Stenlid, J. 1999. Growth rate of *Heterobasidion annosum* in *Picea abies* established on forest land and arable land. Scandinavian Journal of Forest Research 14: 402-407.

Borden, J. H. 1988. The striped ambrosia beetle. In: Berryman, A. A. (ed.). Dynamics of Forest Insect Populations – Patterns, Causes, Implications. Plenum Press, New York. pp. 579-596.

Bruns, T. D. & Shefferson, R. P. 2004. Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. Canadian Journal of Botany 82: 1122-1132. Doi: 10.1139/B04-021.

Bueno, A., Diez, J. J. & Fernández, M. M. 2010. Ophiostomatoid fungi transported by *Ips sexdentatus* (Cloeoptera; Scolytidae) in *Pinus pinaster* in NW Spain. Silva Fennica 44: 387-397. Available at: http://www.metla.fi/silvafennica/full/sf44/sf443387.pdf.

Cannon, P. F. & Kirk, P. M. 2007. Fungal Families of the World. CAB International, Wallingford, Oxfordshire, UK. 456 p. ISBN 9780851998275.

Capretti, P., Korhonen, K., Mugnai, L. & Romagnoli, C. 1990. An intersterility group of *Heterobasidion annosum* specialized to *Abies alba*. European journal of forest pathology 20: 231-240.

Carlile, M. J., Watkinson, S. C. & Gooday, G. W. 2001. The Fungi. 2nd Edition. Academic Press, London. 588 p.

Chang, S., Puryear, J. & Cairney, J. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reporter 11: 113-116.

Chao, A., Chazdon, R. L., Colwell, R. K. & Shen, T-J. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8: 148-159. Doi: 10.1111/j.1461-0248.2004.00707.x.

Chapman, J. A. 1966. The effect of attack by the ambrosia beetle *Trypodendron lineatum* (Olivier) on log attractiveness. The Canadian Entomologist 98: 50-59.

Chapman, J. A. & Kinghorn, J. M. 1958. Studies of Flight and Attack Activity of the Ambrosia Beetle, *Trypodendron lineatum* (Oliv.), and other Scolytids. The Canadian Entomologist 90: 362-372.

Christiansen, E. & Bakke, A. 1988. The Spruce Bark Beetle of Eurasia. In: Berryman, A. A. (ed.). Dynamics of Forest Insect Populations – Patterns, Causes, Implications. Plenum Press, New York. pp. 479-503.

Colwell, R. K. 2009. EstimateS: Statistical Estimation of Species Richness and Shared Species from samples. Version 7.5.2 User's Guide and application. [Electronic source]. Available at: http://viceroy.eeb.uconn.edu/estimates.

Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA, 2012. [Electronic source]. Available at: http://www.biology.duke.edu/fungi/mycolab/primers.htm. [Cited Mar 6th 2012].

Cowling, E. B. & Kelman, A. 1964. Influence of temperature on growth of *Fomes annosus* isolates. Phytopathology 54: 249-372.

Dictionary of the Fungi, 2012. [Electronic database]. Available at: http://www.indexfungorum.org/. [Cited Jan 24th 2012].

Dyer, E. D. A. & Chapman, J. A. 1965. Fligth and Attack of the Ambrosia Beetle, Trypodendron lineatum (Oliv.) in Relation to Felling Date of Logs. The Canadian Entomologist 97: 42-57.

Elkin, C. M. & Reid, M. L. 2005. Low energy reserves and energy allocation decisions affect reproduction by Mountain Pine Beetles, *Dendroctonus ponderosae.* Functional Ecology 19: 102–109.

Filip, G. M. & Morrison, D. J. 1998. North America. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds). *Heterobasidion annosum*: biology, ecology, impact and control. CAB International. Wallingford. pp. 405-427.

Finland´s National Forest Programme 2015, 2008. More Welfare from Diverse Forests – Government Resolution. No. 3b. Publications of the Finnish Ministry of Agriculture and Forestry. Helsinki, Finland. [PDF-document]. Available at: http://www.mmm.fi/attachments/metsat/kmo/5yGFtgJQ5/Finlands_National_Forest_Programme_2015_final.pdf. [Cited Sep 5th 2012].

Fraedrich, S. W., Harrington, T. C., Rabaglia, R. J., Ulyshen, M. D., Mayfield, A. E. III, Hanula, J. L., Eickwort, J. M., and Miller, D. R. 2008. A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Disease 92: 215-224.

Francke-Grosmann, H. 1967. Ectosymbiosis in wood-inhabiting insects. In: Henry, S. M. (ed.). Symbiosis, Vol. II. Academic Press. New York. pp. 141-205.

Fretwell, S. D. & Lucas, H. L. 1969. On territorial behavior and other factors influencing habitat distribution in birds. 1. Theoretical development. Acta Biotheoretica 19: 16-36.

Furniss, M. M., Solheim, H. & Christiansen, E. 1990. Transmission of Blue-Stain Fungi by *Ips typographus* (Coleoptera: Scolytidae) in Norway Spruce. Annals of the Entomological Society of America 83: 712-716.

Ganley, R. J. & Newcombe, G. 2006. Fungal endophytes in seeds and needles of *Pinus monticola*. Mycological Research 110: 318–327. Doi: 10.1016/j.mycres.2005.10.005.

Garbelotto, M., Cobb, F. W., Bruns, T. D., Otrosina, W. J., Popenuck, T. & Slaugter, G. 1999. Genetic Structure of *Heterobasidion annosum* in White Fir Mortality Centers in California. Phytopathology 89: 546-554.

Geiser, D. M., Gueidan, C., Miadlikowska, J., Lutzoni, F., Kauff, F., Hofstetter, V., Fraker, E., Schoch, C. L., Tibell, L., Untereiner, W. A. & Aptroot, A. 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. Mycologia 98: 1053–1064. Doi: 10.3852/mycologia.98.6.1053.

Geldenhuis, M. M., Roux, J., Montenegro, F., De Beer, Z. W., Wingfield, M. J. and Wingfield, B. D. 2004. Identification and pathogenicity of *Graphium* and *Pesotum* species from machete wounds on *Schizolobium parahybum* in Ecuador. Fungal diversity 15: 137-151.

Graham, K. 1968. Anaerobic induction of primary chemical attractancy for ambrosia beetles. Canadian Journal of Zoology 46: 905-908.

Grünig, C. R. & Sieber, T. N. 2005. Molecular and phenotypic description of the widespread root symbiont *Acephala applanata* gen. et sp. nov., formerly known as dark-septate endophyte Type 1. Mycologia 97: 628–640.

Guédez, C., Cañizález, L., Castillo C. & Olivar, R. 2009. Antagonist effect of *Trichoderma harzianum* over some strawberry (*Fragaria* spp.) post harvesting fungi pathogens. Revista de la Sociedad Venezolana de Microbiologia 29: 34-38. (Abstract.)

Hallituksen esitys eduskunnalle laeiksi metsälain ja rikoslain 48 a luvun 3 §:n muuttamisesta. [Electronic source]. Available in Finnish at: http://www.mmm.fi/attachments/mmm/lausuntopyynnot/6EH4q2JkE/Hallituksen_esitys_metsalain_ja_rikoslain_muuttamisesta_luonnos.pdf. [Feb 8th 2013]. [Cited Mar 18th 2013].

Harrington, T. C., McNew, D., Steimel, J., Hofstra, D. & Farrel, R. 2001. Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 93: 111-136.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. & Pegler, D. N. 1995. Ainsworth and Bisby's Dictionary of the Fungi. 8th edition. CAB International. Wallingford, UK. ISBN 0851988857.

Heliövaara, K. & Mannerkoski, I. 1987. Metsähyönteiset. Lajintuntemus. University of Helsinki. Department of Agricultural and Forest Zoology. Reports 12. Yliopistopaino, Helsinki, Finland. 90 p.

Heliövaara, K., Peltonen, M., Mannerkoski, I. & Siitonen, J. 1998. Suomen kaarnakuoriaiset. (Coleoptera: scolytidae). University of Helsinki. Department of Applied Zoology. Reports 25. Yliopistopaino, Helsinki, Finland. 91 p.

Hodges, C. S. 1969. Modes of infection and spread of *Fomes annosus*. Annual Review of Phytopathology 7: 247-266.

Hofstetter, R. W., Cronin, J., Klepzig, K. D., Moser, J. C. & Ayres, M. P. 2006. Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. Oecologia 147: 679–691. Doi: 10.1007/s00442-005-0312-0.

Hoover, S. E. R., Lindgren, B. S., Keeling, C. I. & Slessor, K. N. 2000. Enantiomer preference of *Trypodendron lineatum* and effect of pheromone dose and trap length response to lineatin-baited traps in interior British Columbia. Journal of Chemical Ecology 26: 667-677.

Ilmatieteen laitos, 2012a. Kevättilastot. [Electronic source]. Available at: http://ilmatieteenlaitos.fi/kevattilastot. [Cited Feb 29th 2012].

Ilmatieteen laitos, 2012b. Syksytilastot. [Electronic source]. Available at: http://ilmatieteenlaitos.fi/syksytilastot. [Cited Feb 29th 2012].

Ilmatieteenlaitos, 2012c. Vuosi 2010. [Electronic source]. Available at: http://ilmatieteenlaitos.fi/vuosi-2010. [Cited Feb 29th 2012].

Jacobs, K., Wingfield, M. J. & Crous, P. W. 2000. *Ophiostoma europhioides* and *Ceratocystis pseudoeurophioides*, synonyms of *O. piceaperdum*. Mycological Research 104 : 238-243.

Jankowiak, R., Kacprzyk, M. & Mlynarczyk, M. 2009. Diversity of ophiostomatoid fungi associated with bark beetles (Cloeoptera: Scolytidae) colonizing branches of Norway spruce (Picea abies) in southern Poland. Biologia 64: 1170-1177. Doi: 10.2478/s11756-009-0188-2.

Jørgensen, C. A., Lund, A. & Treschow, C. 1939. Undersøgelser over rodfordærveren, *Fomes annosus* (Fr.) Cke. Summary in English: Studies on the heart rot fungus, *Fomes annosus* (Fr.*).* Den Kongelige Veterinær- og Landbohøjskoles aarskr. 71: 71-129.

Juurikäävän torjunta Keski-Suomessa 2012. Metsäkeskus. [Electronic source]. Available at: www.metsakeskus.fi/metsakeskus-ja-alueet/alueet/keski-suomi/uutiset. [Updated Sep 20th 2012]. [Cited Sep 27th 2012].

Jönsson, A. M., Harding, S., Bärring, L. & Ravn, H. P. 2007. Impact of climate change on the population dynamics of *Ips typographus* in southern Sweden. Agricultural and Forest Meteorology 146 : 70–81. Doi:10.1016/j.agrformet.2007.05.006.

Kadlec, Z., Stary, P. & Zumr, V. 1992. Field evidence for the large pine weevil, *Hylobius abietis* as a vector of *Heterobasidion annosum*. European Journal of Forest Pathology 22: 316-318.

Kajimura, H. & Hijii, N. 1992. Dynamics of the fungal symbionts in the gallery system and the mycangia of the ambrosia beetle, *Xylosandrus mutilatus* (Blandford) (Coleoptera: Scolytidae) in relation to its life history. Ecological Research 7: 107-117.

Kallio, T. 1970. Aerial Distribution of the Root-Rot Fungus *Fomes annosus* (Fr.) Cooke in Finland. Acta Forestalia Fennica 107: 1-55.

Kasanen, R. 2009. Metsäpuiden sienitaudit. Metsäkustannus Oy. 221 p.

Kasanen, R., Terhonen, E., Huuskonen, S., Sun, H. & Uotila, A. 2011. High infection rate of residual conifer stumps by *Heterobasidion* species in an area with assumed low infection pressure. Scandinavian Journal of Forest Research 26: 404-412. Doi: 10.1080/02827581.2011.586366.

Kausrud, K. L, Grégoire, J-C., Skarpaas, O., Erbilgin, N., Gilbert, M., Økland, B and Stenseth N. C. 2011. Trees Wanted—Dead or Alive! Host Selection and Population Dynamics in Tree-Killing Bark Beetles. PLoS ONE 6: e18274. Doi: 10.1371/journal.pone.0018274.

Kirjanpainajahyönteisten määrä on lisääntynyt paikoin epidemia-asteelle Etelä- ja Kaakkois-Suomessa, 2012. The Finnish Forest Research Institute. A notice. Available in Finnish at : http://www.metla.fi/tiedotteet/2012/2012-06-20-kirjanpainaja.htm. [Updated Jul 2nd 2012]. [Cited Aug 1st 2012].

Kirschner, R. 1998. Diversität mit Borkenkäfern assoziierten filamentöser Mikropilze. Dissertation, Eberhard-Karls-Universität Tübingen. Tübingen, Germany.

Klimetzek, D., Vité, J. P. & König, E. 1981. Über das Verhalten mitteleuropäischer Trypodendron-Arten gegenüber natürlichen und synthetischen Lockstoffen. Allgemeine Forst- ung Jagdzeitung 152: 64-70.

Korhonen, K. 1978. Intersterility groups of *Heterobasidion annosum*. Communicationes Instituti Forestalis Fenniae 94.6: 1-25.

Korhonen, K. & Lipponen, K. 2001. Juurikääpä – lajit, levinneisyys ja torjunnan nykytilanne. Metsätieteen aikakauskirja 3/2001. [PDF-document]. Available at: http://www.metla.fi/aikakauskirja/full/ff01/ff013453.pdf. [Cited Jan 20th 2012].

Korhonen, K. & Stenlid, J. 1998. Biology of *Heterobasidion annosum*. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds). *Heterobasidion annosum*: biology, ecology, impact and control. CAB International. Wallingford. pp. 43-70.

Krokene, P. & Solheim, H. 1996. Fungal associates of five bark beetle species colonizing Norway spruce. Canadian Journal of Forest Research 26: 2115-2122.

Krokene, P. & Solheim, H. 1998a. Pathogenicitry of blue-stain fungi associated with aggressive and nonaggressive bark beetles. Phytopathology 88: 39-44.

Krokene, P. & Solheim, H. 1998b. Assessing the virulence of four bark beetle-associated bluestain fungi using Norway spruce seedlings. Plant Pathology 47: 537-540. Doi: 10.1046/j.1365-3059.1998.00268.x.

Kumaresan, V. & Suryanarayanan, T. S. 2002. Endophyte assemblages in young, mature and senescent leaves of *Rhizophora apiculata:* evidence for the role of endophytes in mangrove litter degradation. Fungal Diversity 9: 81-91.

Laki metsän hyönteis- ja sienituhojen torjunnasta 8.2.1991/263. 2001. [Electronic source]. Available at: http://www.finlex.fi/fi/laki/ajantasa/1991/19910263. [Updated Aug 23rd 2001]. [Cited Jan 25th 2012].

Lindelöw, Å., Risberg, B. & Sjödin, K. 1992. Attraction during flight of scolytids and other bark- and wood-dwelling beetles to volatiles from fresh and stored spruce wood. Canadian Journal of Forest Research 22: 224-228.

Lindgren, B. S. 1990. Ambrosia beetles. Journal of Forestry 88: 8-11.

Lindsey, J. K. 1995. Modelling frequency and count data. Clarendon Press. Oxford. 291 p. ISBN 0-19-852331-9.

Linnakoski, R., de Beer, Z. W., Ahtiainen, J., Sidorov, E., Niemelä, P. & Pappinen, A. & Wingfield, M. J. 2010. *Ophiostoma* spp. associated with pine- and spruce-infesting bark beetles in Finland and Russia. Persoonia 25: 72-93. Doi: 10.3767/003158510550845.

Linnakoski, R. 2011. Bark beetle-associated fungi in Fennoscandia with special emphasis on species of *Ophiostoma* and *Grosmannia*. Academic dissertation. Faculty of Science and Forestry, School of Forest Sciences, University of Eastern Finland. Dissertationes Forestales 119. 74 p. Available at: http://www.metla.fi/dissertationes/df119.pdf. [Cited Mar 15th 2012].

Lygis, V., Vasiliauskas, R. & Stenlid, J. 2004. Planting *Betula pendula* on pine sites infested by *Heterobasidion annosum*: disease transfer, silvicultural evaluation, and community of woodinhabiting fungi. Canadian Journal of Forest Research 34: 120–130.

Löyttyniemi, K. 1967. Tikaskuoriaisesta (*Trypodendron lineatum*, Oliv., Col., Scolytidae) kuorellisen havupuutavaran pilaajana. Silva Fennica 2.4: 49-57.

Martikainen, P. 2000. Flight period and ecology of *Trypodendron proximum* (Niijima) (Col., Scolytidae) in Finland. Journal of Applied Entomology 124: 57-62.

Mathiesen-Käärik, A., 1953. Eine Übersicht über die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden und einige neue Bläuepilze. Meddelanden från Statens Skogforskninginstitut, Stockholm 43: 1-74.

McKee, R. K. 1969. Effects of ultraviolet irradiation on zoospores of *Phytophthora infestans*. Transactions of the British Mycological Society 52: 281-291.

Metsälainsäädäntöön tulevia uudistuksia, 2012. [PDF-document]. Available in Finnish at: http://www.mmm.fi/attachments/metsat/newfolder/6CMjUdDfU/Metsalainsaadanto.pdf. [Cited Mar 18rd 2013].

Metsätuho-opas, 2010. The Finnish Forest Research Institute. [Electronic source]. Available at: http://www.metla.fi/metinfo/metsienterveys/lajit_kansi/trline-n.htm. [Updated Aug 5th 2010]. [Cited Apr 3rd 2012].

Mitchelson, K., & Korhonen, K. 1998. Diagnosis and differentiation of Intersterility groups. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds). *Heterobasidion annosum*: biology, ecology, impact and control. CAB International. Wallingford. pp. 71-92.

Müller, M. M., Varama, M., Heinonen, J. & Hallaksela, A-M. 2002. Influence of insects on the diversity of fungi in decaying spruce wood in managed and natural forests. Forest Ecology and Management 166: 165-181. Doi: http://dx.doi.org/10.1016/S0378-1127(01)00671-5.

Niemelä, T. & Korhonen, K. 1998. Taxonomy of the genus Heterobasidion. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds). *Heterobasidion annosum*: biology, ecology, impact and control. CAB International. Wallingford. pp. 27-33.

Nijholt, W. W. 1967. Moisture and fat content during the adult life of the ambrosia beetle, *Trypodendron lineatum* (Oliv.). Journal of the Entomological Society of British Columbia 64: 51-55.

Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K-H. 2008. Intraspecific ITS Variability in the Kingdom Fungi as Expressed in the International Sequence Databases and Its Implications for Molecular Species Identification. Evolutionary Bioinformatics 4: 193-201.

Nilsson, R. H., Ryberg, M., Abarenkov, K., Sjökvist, E. & Kristiansson, E. 2009. The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. FEMS Microbiology Letters 296: 97-101.

Nilsson, R. H., Veldre, V., Hartmann, M., Unterseher, M., Amend, A., Bergsten, J., Kristiansson, E., Ryberg, M., Jumpponen, A. & Abarenkov, K. 2010. An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecology 3: 284-287. Doi: 10.1016/j.funeco.2010.05.002.

Okuda, A., Yamato, M. & Iwase, K. 2011. The mycorrhiza of Schizocodon soldanelloides var. magnus (Diapensiaceae) is regarded as ericoid mycorrhiza from its structure and fungal identities. Mycoscience 52: 425–430. Doi: 10.1007/s10267-011-0121-8.

Økland, B. & Bjørnstad, O. N. 2006. A Resource-depletion model of Forest Insect Outbreaks. Ecology 87: 283–290.

Oliva, J., Thor, M. & Stenlid, J. 2010. Long-term effects of mechanized stump treatment against *Heterobasidion annosum* root rot in *Picea abies*. Canadian Journal of Forest Research 40: 1020-1033.

Orbay, L., McLean, J. A., Sauder, B. J. & Cottell, P. L. 1994. Economic losses resulting from ambrosia beetle infestation of sawlogs in coastal British Columbia, Canada. Canadian Journal of Forest Research 24: 1266-1276.

Otrosina, W. J. & Garbelotto, M. 2010. *Heterobasidion occidentale* sp. nov. and *Heterobasidion irregulare* nom. nov.: A disposition of North American *Heterobasidion* biological species. Fungal Biology 114: 16-25.

Park, J. & Reid, M. L. 2007. Distribution of a bark beetle, *Trypodendron lineatum*, in a harvested landscape. Forest Ecology and Management 242: 236-242.

Paulus, B., Nilsson, H. & Hallenberg, N. 2007. Phylogenetic studies in *Hypochnicium* (Basidiomycota), with special emphasis on species from New Zealand. New Zealand Journal of Botany 45: 139-150. Doi: 0028-825X/07/4501-0139.

Persson, Y., Rimvydas, V., Långström, B., Öhrn, P., Ihrmark, K. & Stenlid, J. 2009. Fungi vectored by the bark beetle *Ips typographus* following hibernation under the bark of standing trees and in the forest litter. Microbial ecology 58: 651-659. Doi: 10.1007/s00248-009-9520-1.

Piri, T. 2003. Silvicultural control of *Heterobasidion* root rot in Norway spruce forests in southern Finland. Academic dissertation. Metsäntutkimuslaitoksen tiedoksiantoja 898. Finnish Forest Research Institute. Dark Oy. Vantaa.

Piri, T. 2011. Männyn tyvitervastauti, taudin torjunta ja eteneminen Pohjanmaalla. [PDF-document]. Available at: http://www.metla.fi/ohjelma/mkl/seminaarialustukset/seinajoki/Piri_Seinajoki.pdf. [Cited Feb 26th 2012].

Platt, J. L. 2000. Lichens, earth tongues, and endophytes: evolutionary patterns inferred from phylogenetic analyses of multiple loci. PhD thesis. Oregon State University, Corvallis, Oregon, USA. 196 p. Available at: http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/33058/PlattJamieL2000.pdf?sequence=1. [Cited Aug 10th 2012].

Ranger, C. M., Reding, M. E., Oliver, J. B., Schultz, P. B., Moyseenko, J. J. & Youssef, N. 2011. Comparative Efficacy of Plant-Derived Essential Oils for Managing Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae) and Their Corresponding Mass Spectral Characterization. Journal of Economic Entomology 104: 1665-1674. Doi: http://dx.doi.org/10.1603/EC11106.

Redfern, D. B., Pratt, J. E., Gregory, S. C. & MacAskill, G. A. 2001. Natural infection of Sitka spruce thinning stumps in Britain by spores of *Heterobasidion annosum* and long-term survival of the fungus. Forestry 74: 53 -71.

Rishbeth, J. 1948. *Fomes annosus* Fr. in pines in East Anglia. Forestry 22: 174-183.

Rishbeth, J. 1951. Observations on the biology of *Fomes annosus*, with particular reference to East Anglian pine plantations, II. Spore production, stump infection, and saprophytic activity in stumps. Annals of Botany 15: 1-21.

Rita, H. 2004. Vetosuhde (odds ratio) ei ole todennäköisyyksien suhde. Metsätieteen aikakauskirja 2: 207-212. Available at: http://metla.eu/aikakauskirja/full/ff04/ff042207.pdf.

Rita, H. & Komonen, A. 2008. Odds ratio: An ecologically sound tool to compare proportions. Annales Zoologici Fennici 45: 66-72.

Root, R. B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (*Brassica oleracea*). Ecological Monographs 43. The Duke University Press. Durham, USA. pp. 95-124.

Rossman, A. Y., Samuels, G. J., Rogerson, C. T. & Lowen, R. 1999. Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Studies in Mycology 42: 1-248.

Salom, S. A. & McLean, J. A. 1990. Flight and landing behavior of *Trypodendron lineatum* (Coleoptera: Scolytidae) in response to different semiochemicals. Journal of Chemical Ecology 16: 2589-2604.

Salom, S. M. & McLean, J. A. 1991. Environmental influences on dispersal of *Trypodendron lineatum* (Coleoptera: Scolytidae). Environmental Entomology 20: 565-576.

Schoch, C. L. & Hyde, K. D. 2011. Dothideomycetes. Fungal Diversity 51: 1. Doi: 10.1007/s13225-011-0148-3.

Schoch, C. L., Shoemaker, R. A., Seifert, K. A., Hambleton, S., Spatafora, J. W. and Crous, P. W. 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98: 1041–1052. Doi: 10.3852/mycologia.98.6.1041.

Schönhar, S. 1978. Infektionsversuche mit *Fomes annosus*-Sporen an vitalen und absterbenden Fichtenwurzeln. Allgemeine Forst- und Jagdzeitung 149: 73-74.

Schönhar, S. 1980. Untersuchungen über die Überlebensdauer von *Fomes annosus*-Sporen im Boden. Allgemeine Forst- und Jagdzeitung 151: 197-199.

Schönhar, S. 1995. Untersuchungen über die Infektion der Fichte (*Picea abies* Karst.) durch *Heterobasidion annosum* (Fr.) Bref.. Allgemeine Forst- und Jagdzeitung 166: 14-16.

Shoemaker, R. A., Hambleton, S., Lacroix, M., Tesolin, M. & Coulombe, J. 2002. *Rhexocercosporidium carotae*. Canadian Journal of Plant Pathology 24: 359–362.

Shore, T. L., McLean, J. A. & Zanuncio, J. C. 1987. Reproduction and survival of the ambrosia beetle *Trypodendron lineatum* (Oliv.) (Coleoptera: Scolytidae) in Douglas-fir and Western hemlock logs. The Canadian Entomologist 119: 131-139.

Six, D. L. 2012. Ecological and Evolutionary Determinants of Bark Beetle - Fungus Symbioses. Insects 3: 339-366. Doi: 10.3390/insects3010339.

Six, D. L. & Wingfield, M. J. 2011. The Role of Phytopathogenicity in Bark Beetle–Fungus Symbioses: A Challenge to the Classic Paradigm. Annual Rewiev of Entomology 56: 255-272.

Stenlid, J. & Johansson, M. 1987. Infection of roots of Norway spruce (*Picea abies*) by *Heterobasidion annosum*, II. Early changes in phenolic content and toxicity. European Journal of Forest Pathology 17: 217-226.

Stenlid, J. & Redfern, D. B. 1998. Spread within the tree and stand. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.). *Heterobasidion annosum*: biology, ecology, impact and control. CAB International, Wallingford. pp. 125-141.

Suh, S-O, Blackwell, M., Kurtzman, C. P. & Lachance, M-A. 2006. Phylogenetics of Saccharomycetales, the ascomycete yeasts. Mycologia 98: 1006–1017. Doi: 10.3852/mycologia.98.6.1006.

Terhonen, E., Marco, T., Sun, H., Jalkanen, R., Kasanen, R., Vuorinen, M. & Asiegbu, F. 2011. The effect of latitude, season and needle-age to the mycobiota of Scots pine (*Pinus sylvestris*) in Finland. Silva Fennica 45: 301-317. Available at: http://www.metla.fi/silvafennica/full/sf45/sf453301.pdf.

Viiri, H. 2004. Fungi associated with *Hylobius abietis* and other weevils. In: Lieutier, F., Day, K. R., Battisti, A., Grégoire, J-C. & Evans, H. F. (eds). Bark and wood boring insects in living trees in Europe, a synthesis. Kluwer Academic Publishers, Dordrecht, the Netherlands. pp. 381-393. ISBN 1-4020-2241-7.

Viiri, H. & Lieutier, F. 2004. Ophiostomatoid fungi associated with the spruce bark beetle, *Ips typographus*, in three areas in France. Annals of Forest Science 61: 215-219. Doi: 10.1051/forest:2004013.

Wang, Z., Binder, M., Schoch, C. L., Johnston.P. R., Spatafora, J. W., & Hibbett D. S. 2006a. Evolution of helotialean fungi (Leotiomycetes, Pezizomycotina): A nuclear rDNA phylogeny. Molecular Phylogenetics and Evolution 41: 295–312. Doi:10.1016/j.ympev.2006.05.031.

Wang, Z., Johnston, P. R., Takamatsu, S., Spatafora, J. W. & Hibbett, D. S. 2006b. Toward a phylogenetic classification of the Leotiomycetes based on rDNA data. Mycologia 98: 1065–1075. Doi: 10.3852/mycologia.98.6.1065.

Wermelinger, B. 2004. Ecology and management of the spruce bark beetle *Ips typographus* - a review of recent research. Forest Ecology and Management 202: 67–82.

Whitney, H. S. 1982. Relationships between Bark Beetles and Symbiotic Organisms. In: Mitton, J. B. & Sturgeon, K. B. (eds.). Bark Beetles in North American Conifers. A System for the Study of Evolutionary Biology. University of Texas Press. pp. 183-211.

Witzell, J., Berglund., M. & Rönnberg, J. 2011. Does temperature regime govern the establishment of *Heterobasidion annosum* in Scandinavia? International Journal of Biometeorology 55: 275-284.

Wood, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behaviour of bark beetles. Annual Review of Entomology 27: 411-446.

Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. 1998. *Heterobasidion annosum*. Biology, ecology, impact and control. CAB International, Wallingford. 589 p.

Yuceer, C., Hsu, C-Y, Erbilgin, N. & Klepzig, K. D. 2011. Ultrastructure of the mycangium of the southern pine beetle, *Dendroctonus frontalis* (Coleoptera: Curculionidae, Scolytinae): complex morphology for complex interactions. Acta Zoologica 92: 216–224. Doi: 10.1111/j.1463-6395.2011.00500.x.

Zhang, N., Castlebury, L. A., Miller, A. N., Huhndorf, S. M., Schoch, C. L., Seifert, K. A., Rossman, A. Y., Rogers, J. D., Kohlmeyer, J., Volkmann-Kohlmeyer, B. &

Sung, G-H. 2006. An overview of the systematic of the Sordariomycetes based on a four-gene phylogeny. Mycologia 98: 1076-1087. Doi: 10.3852/mycologia.98.6.1076.

FIGURES

Figure 1. Piri, T. 2011. Männyn tyvitervastauti, taudin torjunta ja eteneminen Pohjanmaalla. [PDF-document]. Available at: http://www.metla.fi/ohjelma/mkl/seminaarialustukset/seinajoki/Piri_Seinajoki.pdf. [Cited Feb 26th 2012].

Figure 2. Kasanen, R. 2010.

Figure 5-6. Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA, 2012. [Electronic source]. Available at: http://www.biology.duke.edu/fungi/mycolab/primers.htm. [Cited Mar 6th 2012].

APPENDIX

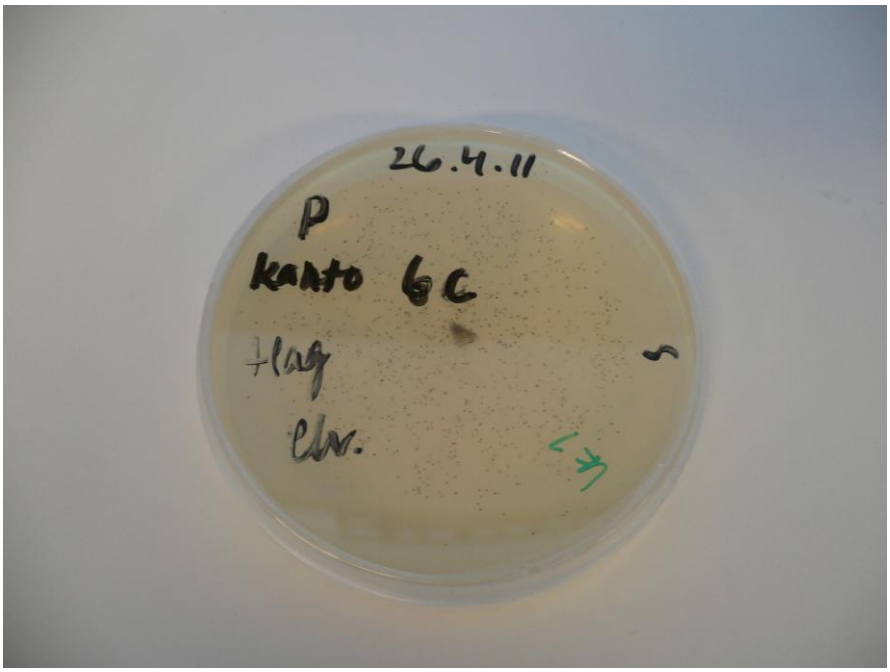

Figure 12. Isolate 10 (P stump 6c ster.) was designated to the Dothideomycetes sp..

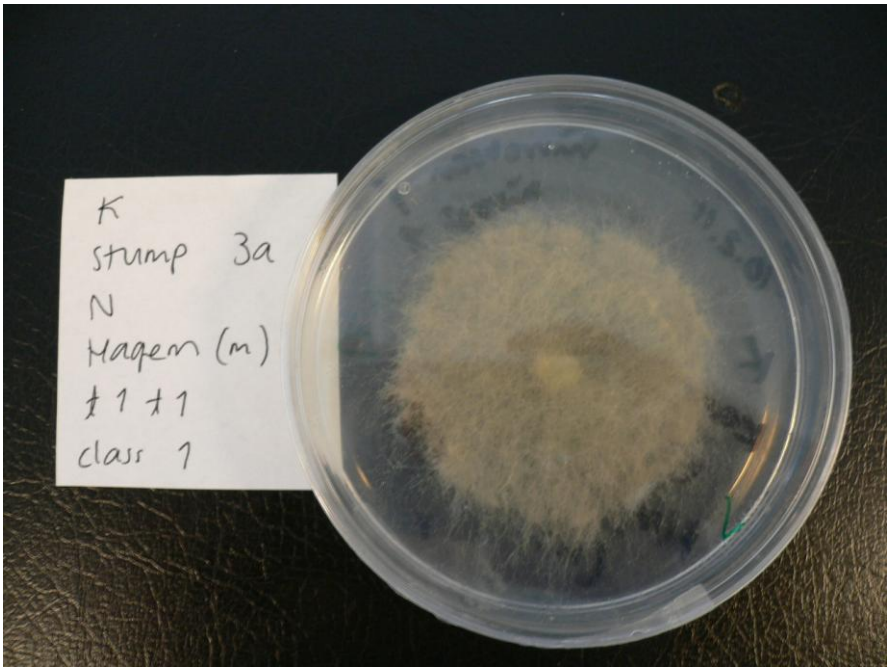

Figure 13. Isolate 34 (K stump 3a N) was designated to *Mucor* sp. (as a member of the Zygomycota).

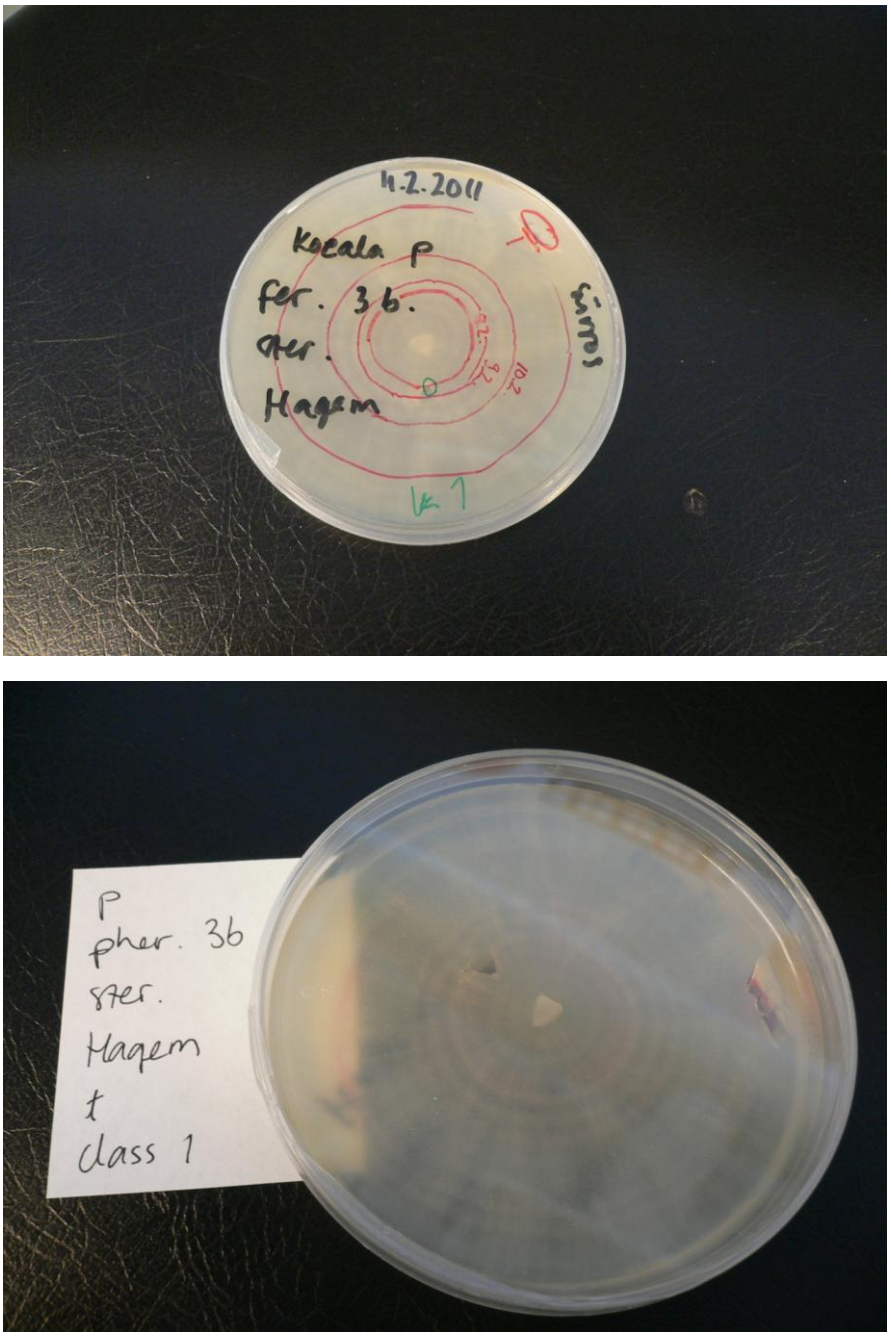

Figure 14-15. Sample 59 that remained unidentified was isolated from a sterilized beetle collected by pheromone trap from site P. The isolate was designated to quality class 1 of pure cultures. Growth could be roughly evaluated from the upper picture starting from the date of inoculation on 4th February 2011.

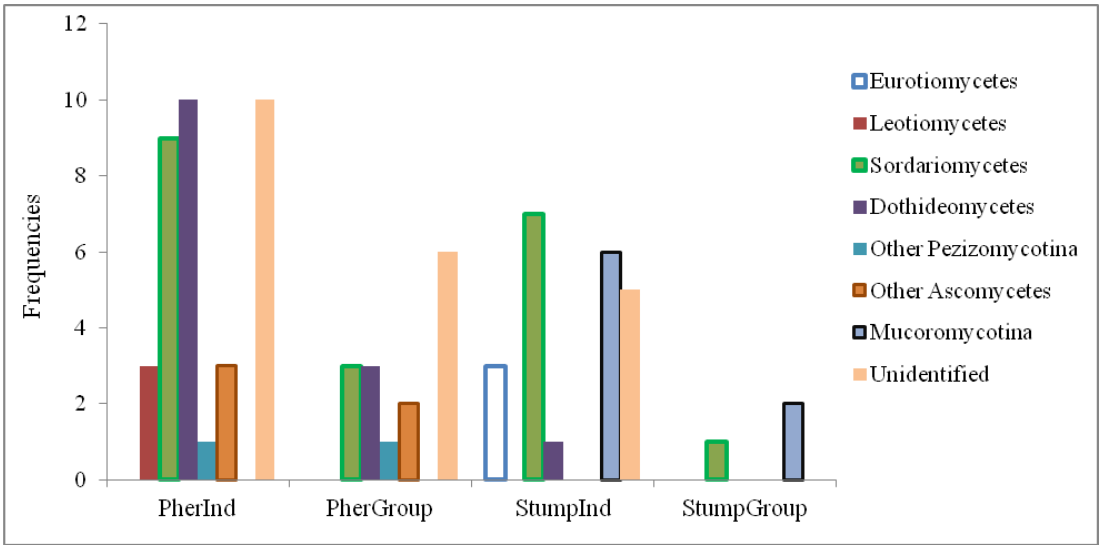

Figure 16. Frequencies of fungal isolates between combinations of trap types and treatement methods. (Pheromone traps (Pher), individual treatment (Ind.).)