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Testing host choice of *Ips typographus* in Norway spruce and two North American spruce species, using field studies and lab bioassays

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Master in Ecology

Preface

This thesis is submitted as the final part of my master degree in Ecology at the department of Ecology and Natural Resource Management, the Norwegian University of Life Sciences.

I would like to express my sincere thanks to my supervisor Paal krokene for his guidance and cooperation during the whole process. I am very fortunate to have someone as skilled and encouraging to supervise me. I also thank Rylee Issitt and Bjørn Økland for guiding me during the field experiment, and Gro Wollebæk and Inger Heldal for valuable help with the laboratory experiments.

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Abstract

The spruce bark beetle *Ips typographus* is one of the most damaging pests in Europe's boreal forests and cause catastrophic tree mortality during outbreaks. Norway spruce (*Picea abies*), an economically important forest tree, is the principal host for *I. typographus.* The beetle carries a number of fungal symbionts into the bark when they attack trees. These bluestain fungi help the beetles by metabolizing the tree's defense chemicals. *Ips typographus* has been intercepted more than 200 times in US ports but has never become established in North America. To test if the beetle can find suitable host trees in North America, I compared the preference of *I. typographus* for its historical host Norway spruce and two common North American spruce species, black spruce (*P. mariana*) and white spruce (*P. glauca*). I observed no notable differences in beetle preference for the historical European host and the two North American spruce species when the beetles were allowed to colonize cut spruce bolts in the field. The beetles also produced abundant offspring in all three spruce species. Different fungi, either accompanied by *Ips typographus* or the North American spruce beetle *Dendroctonus rufipennis*, were used to study the fungi's influence on beetle host choice. In lab bioassays with semi-natural spruce bark media *I. typographus* showed a clear preference for media colonized by fungi. This preference did not depend on whether the fungi were European or North American. In the absence of fungus, the beetles readily tunneled into medium from Norway spruce, black spruce or white spruce bark. Bluestain fungi emit volatile organic compounds and further bioassays demonstrated that *I. typographus* could select specific fungi using smell. In conclusion, *I. typographus* readily colonizes and produce offspring in North American spruce species, and fungal colonization increases beetle tunneling into spruce bark media. These results suggest that the beetle will be able to find suitable host trees if it should become established in North America.

Keywords: , Bioassays, black spruce, *Endoconidiophora polonica, E. rufipennis, Grosmannia penicillata* host choice, *Ips typographus, Leptographium abietinum*, Norway spruce, white spruce,

Contents

1 Introduction

Natural disturbances such as bark beetle outbreaks can have devastating economic impacts in commercial production forests (Franklin et al., 2000; Lehnert et al., 2013). During outbreaks bark beetles can kill enormous numbers of fresh and live trees (Paine et al., 1997), with catastrophic outcomes for forestry and wide-ranging effects on forest ecology. For example, in the Czech Republic bark beetle outbreaks have led to the felling of 5.3 million m^3 of salvaged timber in 2017, and as much as 18 million m^3 in 2018 (Hlásny et al., 2019).

Tree-killing bark beetles can conquer healthy trees through pheromone mediated mass attacks that destroy the inner bark and cambium (Kärvemo et al., 2014; Schroeder & Lindelöw, 2002; Six & Wingfield, 2011). For all aggressive bark beetles and their host trees the risk of bark beetle outbreaks increases as the beetle population density increases. Every tree has a critical threshold density of beetle attacks that it can resist (Berryman, 1982). As beetle reproduction increases, the beetle population may cross the so-called epidemic threshold and become epidemic. The most economically harmful bark beetles belong to the genera *Ips* and *Dendroctonus*, where some species are changing the species distribution of boreal forests at great geographical scales (Matthews et al., 2018). The Eurasian spruce bark beetle *Ips typographus* is considered to be the most aggressive tree-killing bark beetle in Europe. Its main hosts is Norway spruce, an economically valuable spruce species in Europe (Schroeder & Cocoş, 2018).

Conifers have evolved for millennia to defend themselves against bark beetle attack. Trees produce resin consisting of toxic terpenoids as a chemical defense against bark beetles (Keeling & Bohlmann, 2006). To overcome this co-evolved defense, beetles take advantage of other natural disturbances such as storm and drought. These disturbances can provide weakened or dying trees that the beetles can colonize without facing strong tree defenses (Marini et al., 2017). Another strategy the beetles use to overcome tree defenses is to vector phytopathogenic fungi. Bark beetles carry different bluestain fungi that can provide additional nutrients for the bark beetle brood (Bleiker & Six, 2014) and reduce tree defenses by metabolizing tree secondary metabolites such as terpenes and phenolics (DiGuistini et al., 2011; Hammerbacher et al., 2013).

Ips typographus carries different bluestain fungi from the genera *Endoconidiophora*, *Ophiostoma* and *Grosmannia* (Kirisits, 2004). *Endoconidiophora polonica* is considered to be

the most virulent fungus associated with *I. typographus* and can kill healthy trees when mass-inoculated in densities comparable to those during a bark beetle mass attack (Krokene & Solheim, 1998b; Krokene & Solheim, 2001). Another fungal associate of *I. typographus*, *Grosmannia penicillata*, produces longer necrotic lesions in Norway spruce bark than other fungal associates and also seems to be involved in overcoming tree defense (Zhao et al., 2019). As fungi appear to be important for *I. typographus* establishment in a tree, the beetles seem to prefer to tunnel into fungus-infected bark. Evidence for such behavior comes from a recent study showing that the beetles prefers to enter a semi-natural bark medium when the medium is colonized by specific fungi (Zhao et al., 2019). The beetles probably detect the fungi from the volatile organic compounds the fungi emit. This indicates that volatile organic compounds help beetle to recognize and pick up suitable fungal associates (Kandasamy et al., 2019).

Because of the powerful defenses in healthy trees, tree-killing bark beetles are usually relatively host-specific and normally colonize a single tree species or a few closely related species in the same genus. However, beetles can be exposed to novel host trees through human actions, such as when trees are introduced outside their natural range or when beetles become invasive in new areas. Novel hosts will often be favorable for bark beetles because they normally lack effective defenses against attackers they do not share a co-evolutionary history with (Cudmore et al., 2010). For example, the mountain pine beetle *Dendroctonus ponderosae* normally colonizes lodgepole pine (*Pinus contorta*) found at lower elevations. However, due to global warming the beetles now colonize whitebark pine (*Pinus albicaulis*) that grows at higher altitudes and historically has had little contact with the beetles (Raffa et al., 2013). Another example is the red turpentine beetle, *Dendroctonus valens,* that is native to North America but has successfully established in China where it is causing severe mortality in evolutionary naïve pine species (Sun et al., 2004; Yin, 2000).

Nowadays, different food packaging materials like pallets, carting etc. used in global trade provide important pathways for the spread of bark beetles worldwide (Økland et al., 2011). For example, bark beetles were intercepted about 6,825 times near U.S. ports between 1985 and 2000, and among the most frequently detected species were *I. typographus* with 286 interceptions (Haack, 2001). But the existence of a transport pathway to a new area is not the only factor needed for bark beetle establishment. The pathway must also be wide enough to

transport many individuals over an extended period of time. The presence of suitable host species is also a necessary prerequisite if a non-native bark beetle shall be able to become established in a new area (Cudmore et al., 2010). Even though novel hosts often are more favorable for bark beetles than the historical host this is not always true, and in some cases the beetles prefer the historical host over the novel host (Raffa et al., 2013). Thus, to predict the risk of beetle establishment in new areas it is important to understand if for example North American spruce species are suitable host to non-native bark beetles. Colonization experiments done under field conditions in Europe have shown that *I. typographus* can breed successfully in different North American hosts like Engelmann spruce (*P. engelmannii*), white spruce (*P. glauca*), Sitka spruce (*P. sitchensis*), Lutz spruce (*P. x lutzii*) black spruce (*P. mariana*) and red spruce (*P. rubens*) (Økland et al., 2011).

Because *I. typographus* can breed in several North American spruce species it is still unclear why the beetle is so successful in Europe but has never been able to establish populations in North America. In this thesis I studied beetle preference and performance in novel and historical hosts, both in the field and in the laboratory, and with and without the presence of bluestain fungi associated with bark beetles from Europe or North America. I also studied the response of *I. typographus* to fungal volatiles to understand if fungi produce volatiles that are attractive to the beetles. The overall goal of my study was to determine *Ips typographus*' host preference and how it is influenced by bluestain fungi. My specific research questions were: (1) are the North American black and white spruce suitable hosts for *Ips typographus* and key bluestain fungi vectored by the beetle?; (2) how is beetle host choice influenced by beetle-associated bluestain fungi?; and (3) does *I. typographus* differentiate between different fungal species, and how?

2 Methods

Six separate experiments were performed, including three experiments using cut spruce bolts and three experiments using bioassays with semi-natural spruce bark media (Figure 2.1). The aim of the experiments was to understand *Ips typographus'* preference for different spruce species and to learn how beetle host choice is influenced by the presence of different beetle-associated fungi.

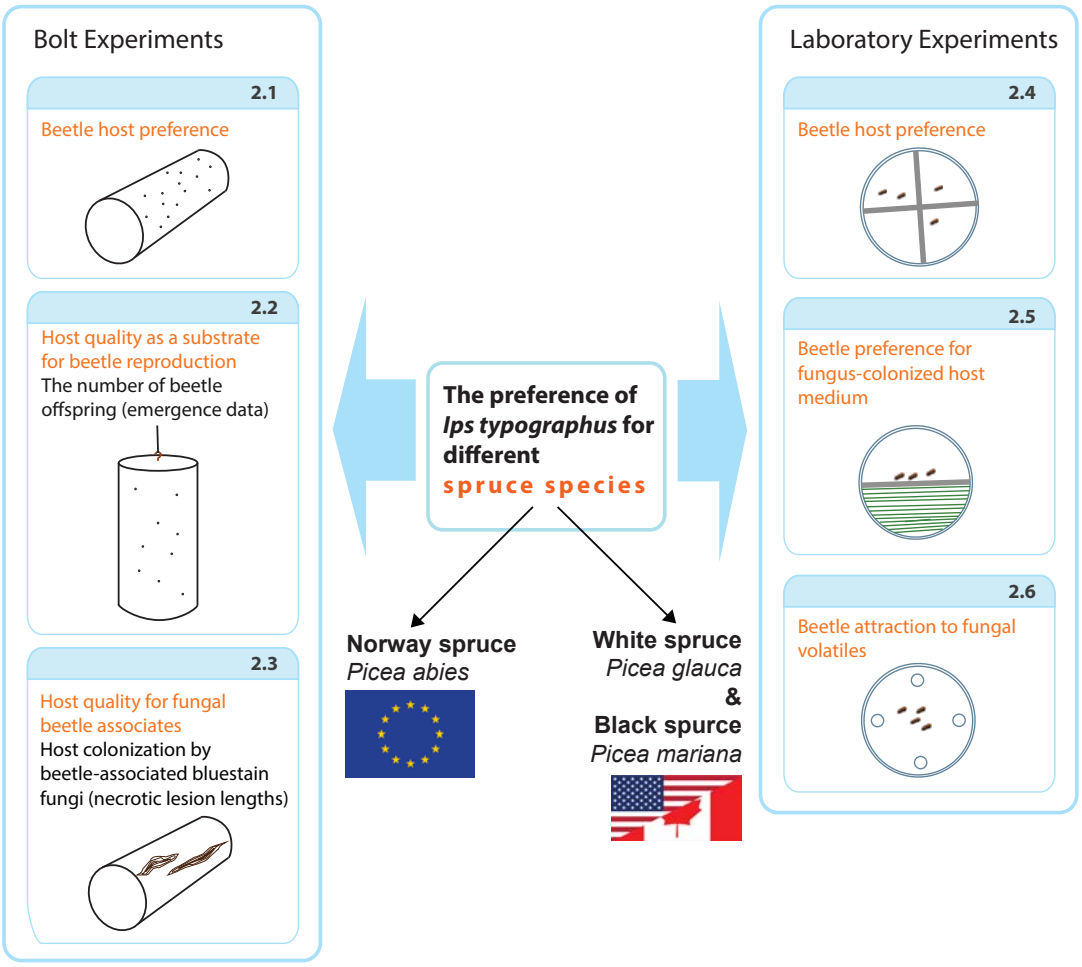

Figure 2.1: An overview of the experimental work that was done to understand the preference of *Ips typographus* for different spruce species originating from Europe or North America. Different fungal associates of bark beetles were included in some of the experiments to determine if the presence of fungi altered beetle host choice. 2.1, 2.2 etc. refers to different subchapters of the methods chapter.

2.1 Beetle host preference for different spruce species in the field

Ten individual trees from each of three different spruce species (Norway spruce, *Picea abies*; black spruce, *P. mariana;* white spruce, *P. glauca*) were felled on 22 April in a forest stand established in 1963 near Prestebakke in Halden (58.99°N, 11.54°E,) and transported to Ås. Three (two in the field + one for fungal inoculation) 40-45 cm long bolts were cut from each tree (90 bolts in total) and the cut ends were sealed with melted paraffin wax to reduce desiccation and loss of resin or other chemicals from the bolts. On 7 May 2018, I brought two bolts per tree to a fresh clear-cut area in a spruce forest in Ås (59.65°N, 10.81°E) and laid out 10 groups of bolts with about 10 m distance between groups. Each group consisted of two bolts from each spruce species placed in a circle with one end of the bolts facing towards the center of the circle (Figure 2.2). A dispenser releasing the aggregation pheromone of *I. typographus* (Ipslure) was placed at the center to attract beetles to the bolts. I left the bolts in the clear-cut for three weeks, until all bolts had visual evidence of *I. typographus* attack. On 28 May 2018, I brought the bolts back from the field to rear out the new generation of beetles (see chapter 2.2). To determine beetle preference for the different spruce species I inspected the bolts carefully and counted bark beetle entrance holes in the bark. These holes represent the number of beetles that had entered the bolts to breed. There bolts had holes of different sizes and I counted only the larger entrance holes that most likely were made by *I. typographus*. In cases where two entrance holes were less than 1 cm apart they were considered to represent a single attack and counted as one.

2.2. Host quality as a substrate for beetle reproduction

As a continuation of the previous experiment, I used the same bolts to rear out the new beetle generation in order to determine the host quality of the three spruce species to *Ips typographus*. To count the entrance holes for the first experiment I hung all 60 bolts from the ceiling in an insectarium (a large shaded room with netted walls, providing close to ambient temperature, humidity and windflow and thus mimicking a natural shaded environment). After counting the entrance holes, I covered each bolt in an emergence net attached to a large plastic funnel with a collection bottle beneath, to ensure that all beetles emerging from the bolt would be collected (Flø et al., 2018). When I noticed that the new beetle generation was starting to emerge (26 June), I started to collect beetles from the emergence traps twice a week. I recorded the number of emerged beetles for each individual tree for further analysis. I kept on collecting beetles from

26 June to 25 October. I also recorded daily average temperature from the meteorological station of NMBU (1.3 km away), to determine how temperature affected beetle emergence.

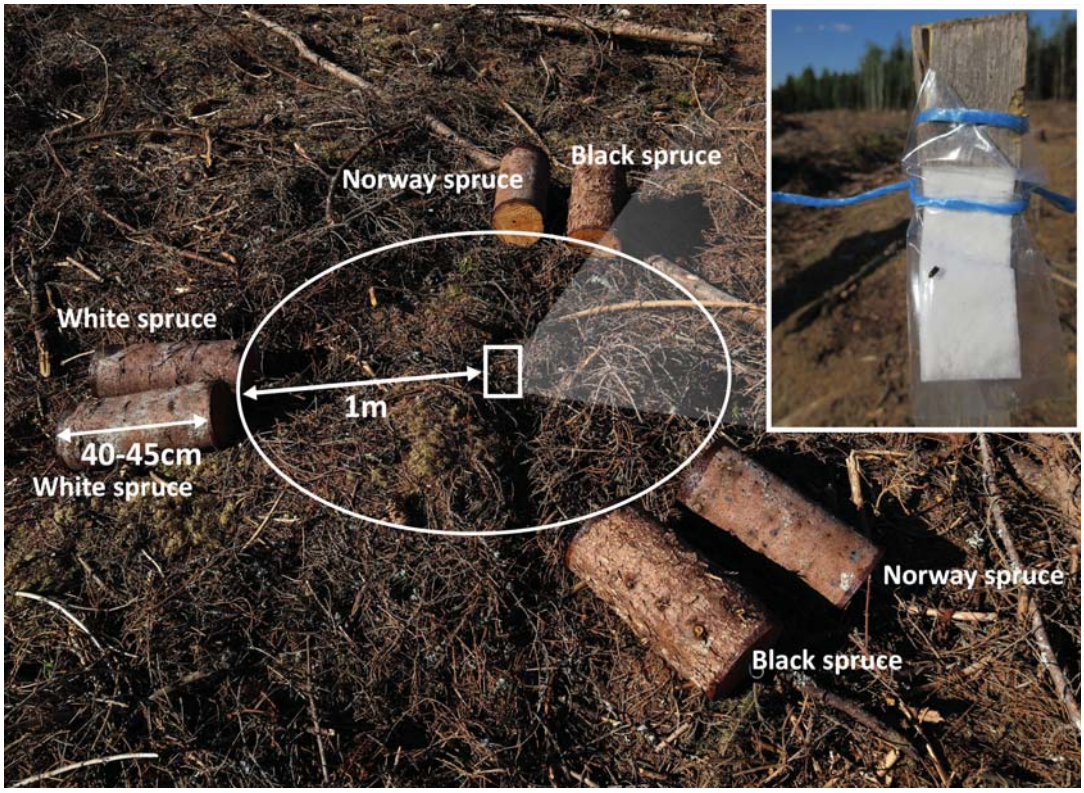

Figure 2.2: Bolts from individual trees of three different spruce species (Norway spruce, black spruce, white spruce) paired randomly to test *Ips typographus* host preference in the field. A dispenser (white rectangle) releasing the beetle's aggregation pheromone (inset) was placed on a pole in the center of each group of bolts to attract beetles to the area.

2.3. Infecting spruce bolts with beetle-associated bluestain fungi

I inoculated spruce bolts with four species of bluestain fungi that are common associates of either *I. typographus* or the North American spruce beetle *Dendroctonus rufipennis* (Table 2.1). Two different isolates were used for each fungus. For this experiment I used the third bolt from the 30 spruce trees that had been felled at Prestebakke on 22 April. The bolts, which were sealed with melted paraffin wax and had been stored at 4 °C since 7 May, were placed in the insectarium and allowed to warm up to ambient temperature for two days, before they were inoculated with fungus on 16 August.

Table 2.1: Species of bluestain fungi inoculated in the bark of three different spruce species (Norway spruce, black spruce, white spruce).

Fungus	Isolate number	Beetle vector	Geographic range
Endoconidiophora polonica	1993-208/115*	*Ips typographus*	Europe
	1997-770/9		
Grosmannia penicillata	2006-209/44/2*		
	1980-91/54		
Endoconidiophora rufipennnis	1993-403/463*	*Dendroctonus rufipennis*	North America
	1992-633/262/9		
Leptographium abietinum	1992-635/310/4*		
	1992-633/9/2		

* Isolate that was also used in the beetle choice study (see chapter 2.5)

I inoculated the bolts by removing a plug of bark using a 5 mm cork borer, inserting inoculum into the hole, and gently putting the bark plug back in place so that most of the inoculum stayed inside the bark. Each bolt was inoculated twice with a control (sterile malt agar: 2% agar, 1.4% malt) and once with each isolate from the four fungal species (Table 2.1). I made two bands of inoculations encircling the bolts about 10 cm from each end (Figure 2.3). This was done to ensure that the necrotic lesions formed in response to the inoculations would not extend to the very end of the bolt. To prevent fungal lesions from coalescing I distributed the inoculation sites in the upper and lower band in such a way that one site never was right below another. Three weeks after inoculation (6 September), I removed the outer bark over the inoculation sites and measured the full length of the necrotic lesion for all treatments. When I inoculated the bolts I also measured the phloem thickness on two bark plugs per bolt using a slide caliper.

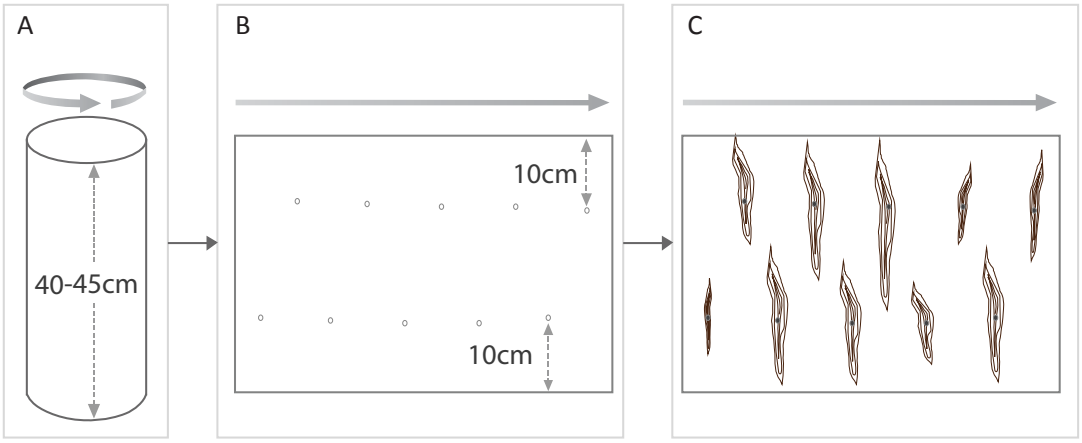

Figure 2.3: Pattern of fungal inoculations in spruce bolts. **A**. Spruce bolts were inoculated with two isolates of each of four species of bluestain fungi and two mock control inoculations (10 inoculations per bolt in total). **B**. Rectangular representation of the cylindrical bark area. Small circles show the inoculation sites. Upper inoculation sites were offset relative to the lower ones. **C.** This was done to prevent lesions from coalescing (illustration shows expected fungal growth three weeks after inoculation).

2.4. Beetle host preference in spruce bark agar

In addition to the host preference experiment in the field I did bioassays to test if beetle host preference could be determined in the laboratory. These were choice experiments where beetle could choose among semi-natural agar media amended with ground bark from each of the three spruce species. I used bark from nine individual trees from each tree species instead of 10, because there was a bolt missing from Norway spruce (thus, n = 9).

I peeled the bark from all 27 bolts, put the bark from each bolt in individual plastic bags, and stored it at – 24 °C. Bark powder was then prepared by freezing bark samples in liquid nitrogen and grinding the bark into a fine powder using a pestle and mortar. To make a semi-natural spruce bark medium, I followed the protocol described by Kandasamy and co-workers (Kandasamy et al., 2019). I heat-sterilized (121°C for 20 min) a mixture of water (25 ml), 1.75 g bark powder (7% of water by volume), and 1 g agar (4% of water by volume) and made one Petri dish (92 mm diameter) of spruce bark agar for each individual spruce bolt (i.e. tree individual). I

also made several Petri dishes of control medium by heat sterilizing a mixture of 4% agar and water.

When the medium had cooled down and solidified, I made choice arenas by combining spruce agar from three different spruce bolts and control medium in new Petri dishes (Figure 2.4). To physically separate the four different medium types in each arena, I used a strip of sterile agar. Bark agar from each of the 27 spruce bolts was thus combined with control medium and distributed among four replicated choice arenas, making a total of 36 arenas. The four arenas with bark from each spruce bolt are not true biological replicates, but rather technical replicates, since spruce bolts (i.e. individual trees) are the unit of replication in my experiments. Into each arena I released four live *I. typographus* that had emerged recently from Norway spruce bolts. I attempted to keep an equal ratio of male and female beetles by sexing the beetles using the characteristics described in Schlyter et al. (Schlyter & Cederholm, 1981). The Petri dishes were then closed and sealed using parafilm, to prevent the beetles from escaping. The choice arenas were left in the dark for 18 hours, before beetle choice was evaluated by counting the number of beetle entrance holes in the different quadrants of the arenas.

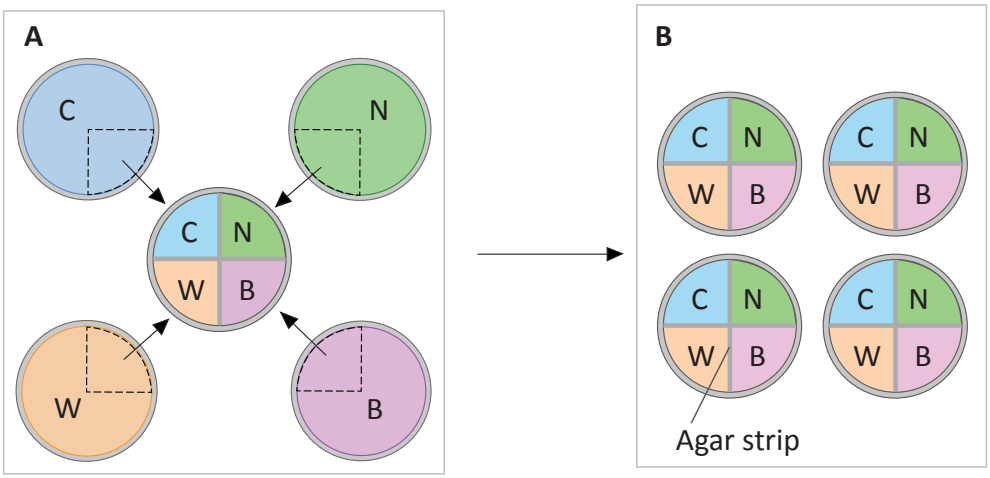

Figure 2.4: A. Procedure used to make choice arenas with sterile agar media (C) and three different kinds of spruce bark agar media (N = Norway spruce, W = White spruce, B = Black spruce). Quadrants were cut from Petri dishes with different media and placed together in a new Petri dish. **B**. Four choice arenas made from the four Petri dishes depicted in A. A thin strip of sterile agar was used to physically separate the different kinds of media.

2.5. Beetle preference for spruce bark agar with or without bluestain fungi

In a separate series of choice experiments I tested how beetle choice of Norway spruce, black spruce or white spruce was influenced by the presence of bluestain fungi. I cultured four different fungal species on bark medium from all three spruce species to test if *I. typographus* responded differently to fungi associated with European or North American bark beetles. I included only the most virulent isolate of each fungus in these experiments (see Table 2.1). I noticed that the different fungi grew at different rates in different spruce bark media. Because optimal growth rate for *E. rufipennis* and *L. abietinum* is 18 °C and 22 °C, respectively (Solheim & Krokene, 1998), I cultured these fungi at their optimal growth temperature in a climate chamber.

Eight Petri dishes with spruce agar media were made from each individual tree following the method described in chapter 2.4. Choice arenas were then made by combining sections of spruce agar media with or without fungal colonization, as outlined in Figure 2.5. For this experiment the beetles had a choice between bark agar only and bark agar colonized by a specific fungus. For each of the four fungal species I had two choice arenas (technical replicates) from each tree individual (Figure 2.5). I used three *Ips typographus* individuals in each choice arena and tried to include at least one female per arena.

The arenas were closed, sealed with parafilm, and left in the dark. I counted beetle entrance holes in the bark agar once after 24 hours and again after 48 hours. I also photographed any beetle tunnels made in the media after 5 days. Total tunnel length in each media type was measured in each choice arena using the image processing software ImageJ (Schneider et al., 2012).

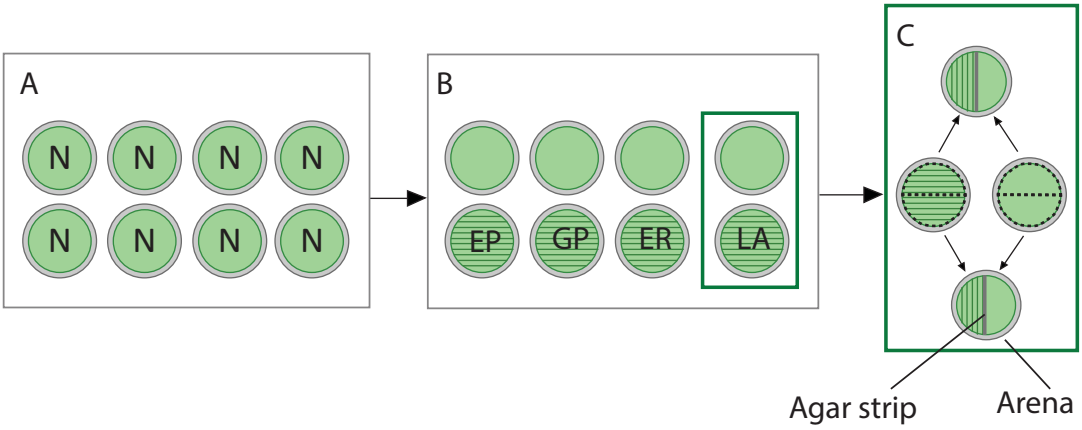

Figure 2.5: Petri dishes with spruce bark agar media were made using bark powder from each of 27 individual spruce trees (nine trees each of Norway spruce, black spruce and white spruce). **A**. As an example, eight Petri dishes from one Norway spruce tree (N) are shown. **B**. Four of these were left as they were (upper circles) and the other four were colonized with either *Endoconidiophora polonica* (EP)*, Grosmannia penicillata* (GP)*, E. rufipennis* (ER)*,* or *Leptographium abietinum* (LA)*.* For each fungus separately, two-choice arenas were made by combining bark agar from Petri dishes with or without fungus, as shown in the green rectangle for *L. abietinum*. **C.** The bark agar in each Petri dish was cut in halves and combined to make two choice arenas. A thin strip of sterile agar was used to physically separate the two halves. A total of 216 choice arenas were made for the 27 trees.

2.6. Fungal volatile choice bioassay

In a final series of bioassays I tested if *I. typographus* could select media colonized by bluestain fungi based on smell. Large Petri dishes (Ø 13 cm) were used as bioassay arenas, based on a method developed by Kandasamy and co-workers (2019) (Figure 2.6). I attached four circular plastic cups to the base of each Petri dish so that the cups were kept at an equal distance from each other. The distance between the center of each plastic cup and the wall of the Petri dish was 2 cm (Kandasamy et al., 2019). The opening of the cups faced upwards. In each cup I made four equidistant holes (Ø 4 mm) 0.9 cm above the base of the Petri dishes to allow beetles to walk into the cup (Figure 2.6). I also made eight small holes (Ø 1 mm) were made in the Petri dish wall to allow some air flow inside the Petri dishes. Filter paper was placed in the bottom of the arena to provide a rough surface to ensure easy movement by the beetles inside the arena. The

plastic cups were loaded with different odor sources. A 10 mm diameter plug from each of three fungi (*E. polonica*, *E. rufipennis*, *G. penicillata*) grown on malt agar for 15 days was placed in three of the cups. The fourth cup was loaded with a 10 mm control plug of sterile malt agar. I released four beetles into each of 20 replicated arena and sealed the arenas using a rubber band that filled the space between the lid and the lower part of the Petri dish. All arenas were kept in darkness at room temperature (20°C). Two large fans were used to ensure continuous wind flow throughout the experiment. After 16 hours I inspected the arenas and counted the number of beetles that had entered each plastic cup as well as any no-choice beetles that remained on the filter paper.

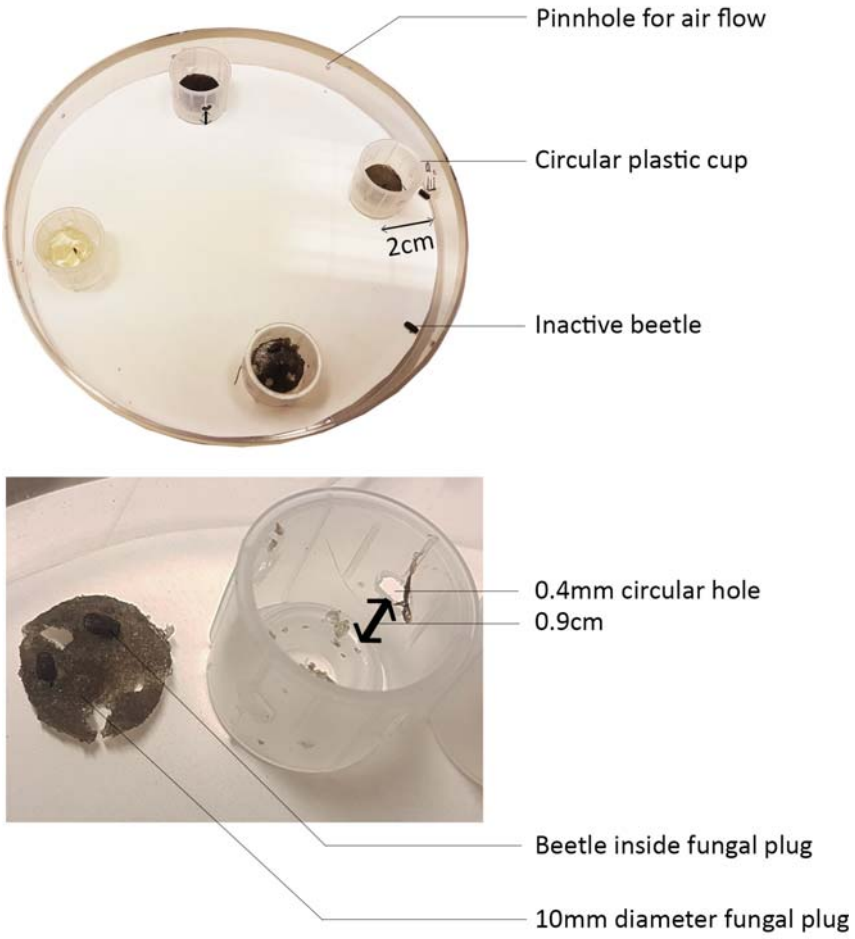

Figure 2.6: Arenas used to test if *Ips typographus* is attracted to volatiles emitted by bluestain fungi. The test arenas consisted of large Petri dishes with four perforated plastic cups loaded with agar media colonized by fungi or control media (from Kandasamy et al. 2019).

2.7 Statistical analysis

All statistical tests were conducted in the R language using R studio (R studio, USA). Before analysis I checked whether the data were normally distributed or not using the Shapiro-Wilk normality test. For normally distributed data, I ran one-way analysis of variance (ANOVA) using R's aov function followed by Tukey's HSD to test for significant differences between individual treatments. For data that were not normally distributed, I used the non-parametric Kruskal-Wallis test using Kruska.test funcion in R. To test for differences between individual treatments, I ran Dunn's test after Kruskal-Wallis. I used the ggpolt2 library in R studio to visualize data and to make all figures.

3 Results

3.1 Spruce bolt experiments: beetle host preference in the field, beetle brood production, and fungal colonization success

3.1.1 Beetle preference for different spruce species

Host preference of *Ips typographus* was measured by counting the number of beetle entrance holes in bolts of different spruce species. More beetles entered white spruce than black spruce and Norway spruce (Figure 3.1), but there were no significant differences between species (p = 0.362, Kruskal-Wallis test). Attack density was more variable in the novel hosts, white and black spruce than in the native host Norway spruce (Figure 3.1).

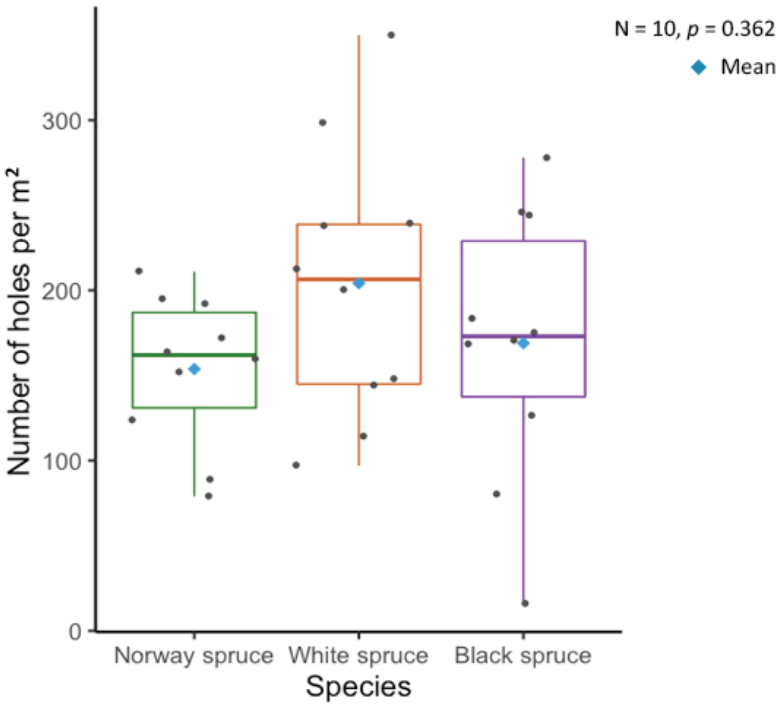

Figure 3.1: Densities of entrance holes made by *Ips typographus* in cut bolts of Norway spruce, white spruce and black spruce placed in a fresh clear-cut in Ås, Norway on 7 May 2018. The thick line inside the boxes represents the median, boxes show the middle quartiles representing 50% of the data, and error bars span the rest of the data (the lower and upper quartiles). Black dots show data for the 10 individual bolts used for each spruce species.

3.1.2 Beetle entrance and emergence from spruce bolts

The reproductive success of *Ips typographus* in different host species was measured by quantifying beetle emergence from the bolts. For all spruce species, significantly more beetles emerged from the bolts compared to the number of beetles that entered the bolts ($p < 0.001$, Kruskal-Wallis test; Figure 3.2). There was no significant effect of different spruce species on the number of emerging beetles after controlling for phloem thickness ($F_{2,24} = 0.197$, $p = 0.822$; 1-way ANCOVA). Although the attack density was high in white spurce, higher number of offspring were found in Norway spruce and black spruce. There were no consistancy of offspring production in all species. Among them black spruce had the most variability.

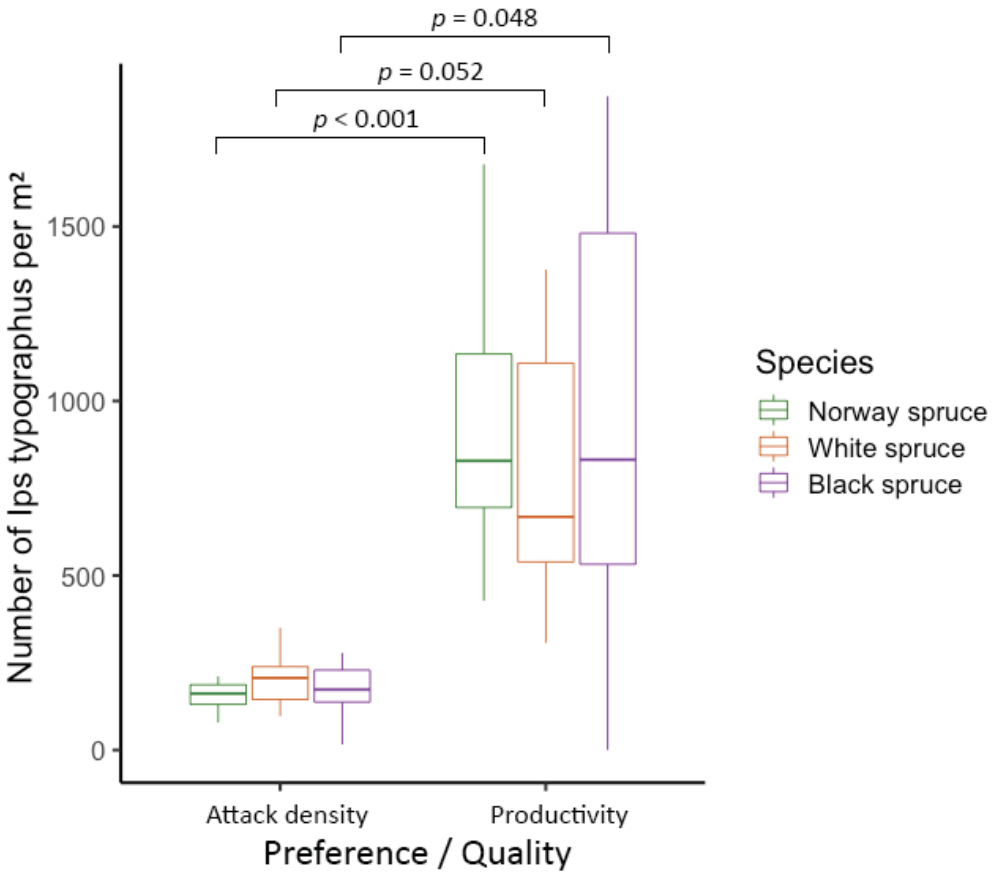

Figure 3.2: Densities of *Ips typographus* entrance and emergence holes in bolts of Norway spruce, black spruce and white spruce placed in a fresh clearcut in Ås, Norway on 7 May 2018. P-values show a significant difference in entrance and emergence density for each spruce

species (Kruskal-Wallis test). The thick line inside the boxes represents the median; boxes represents middle 50% score of all data. Error bars represent the rest of the data except the outlier (black dots). Outliers represent the data that is 1.5 times the interquartile range.

3.1.3 Temporal patterns of beetle emergence from spruce bolts

Early in the collection period (July), the emergence of *Ips typographus* from Norway spruce was very high and then it slowed down (Figure 3.3). Beetle emergence from white spruce, on the other hand, was slow initially but picked up from late August. Emergence from black spruce was relatively high and constant until the temperature went below 5 °C in early october. Degree-day sum data shows gradual increase of temperature until September 25 and decrease afterward till the end of the collection. It also verify the reduction of beetle emergence at the end of the collection period.

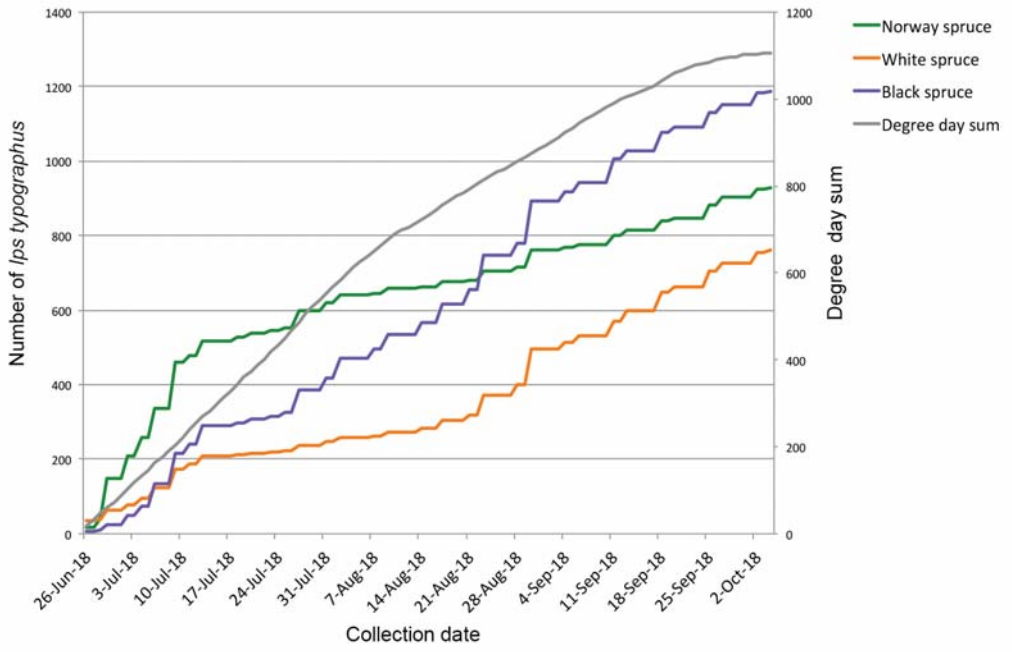

Figure 3.3: Cumulative number of *Ips typographus* emerging from cut bolts of Norway spruce, black spruce and white spruce plotted against cumulative degree-day sums (mean daily temperature, base temperature 5 °C). The start date for calculating degree-days was set to 26 June, the first day of beetle collection.

I collected emerging beetles until early October, when the temperature dropped below 5 °C. After October 4, I peeled the bark from the two bolts from each spruce species that had the highest number of emerging beetles during the collection period. Most of the peeled bolts had no live beetles left under the bark. The few bolts that contained live beetles had relatively few beetles (<5% of the total number of beetles that had emerged from the bolts until then).

3.1.4 Colonization success of different bluestain fungi in spruce bolts

Overall, *Grosmannia penicillata* had the longest lesions, but there were relatively few significant differences between treatments because there were too many variables within treatments.

In Norway spruce, one isolate (GP1) of *Grosmannia penicillata*, made significantly longer lesions than the other isolates including all three fungi (Figure 3.4.A). The other isolate (GP2) of *G. penicillata*, were longer than other fungi but not significantly longer than EP1, ER2 and LA2 (Figure 3.4.A). Lesions in the control were significantly shorter than all fungal isolates except EP2 and ER1 (Figure 3.4.A). (Figure 3.4.A).

In white spruce, lesions in the control treatment were significantly shorter than the others (Figure 3.4.B). One isolate (EP2) from *E. polonica* and one isolate (ER1) from *E. rufipennis* made significantly shorter lesion than other fungi isolate and significantly longer lesion than control (Figure 3.4.B). Both isolates from G. penicillata (GP1 and GP2) made significanly longer lesion than all other isolates. One *E. polonica* isolate (EP1) made long lesions in some bolts and was significantly different from lesions in all other fungi (Figure 3.4.B).

In black spruce, lesion lengths from *G. penicillata* (GP1 and GP2) were longer but not significantly longer than LA2 from *L. abietinum* (Figure 3.4.C). Lesions in the control was significantly short except one isolate (ER1) form *E. rufipennis* (Figure 3.4.C).

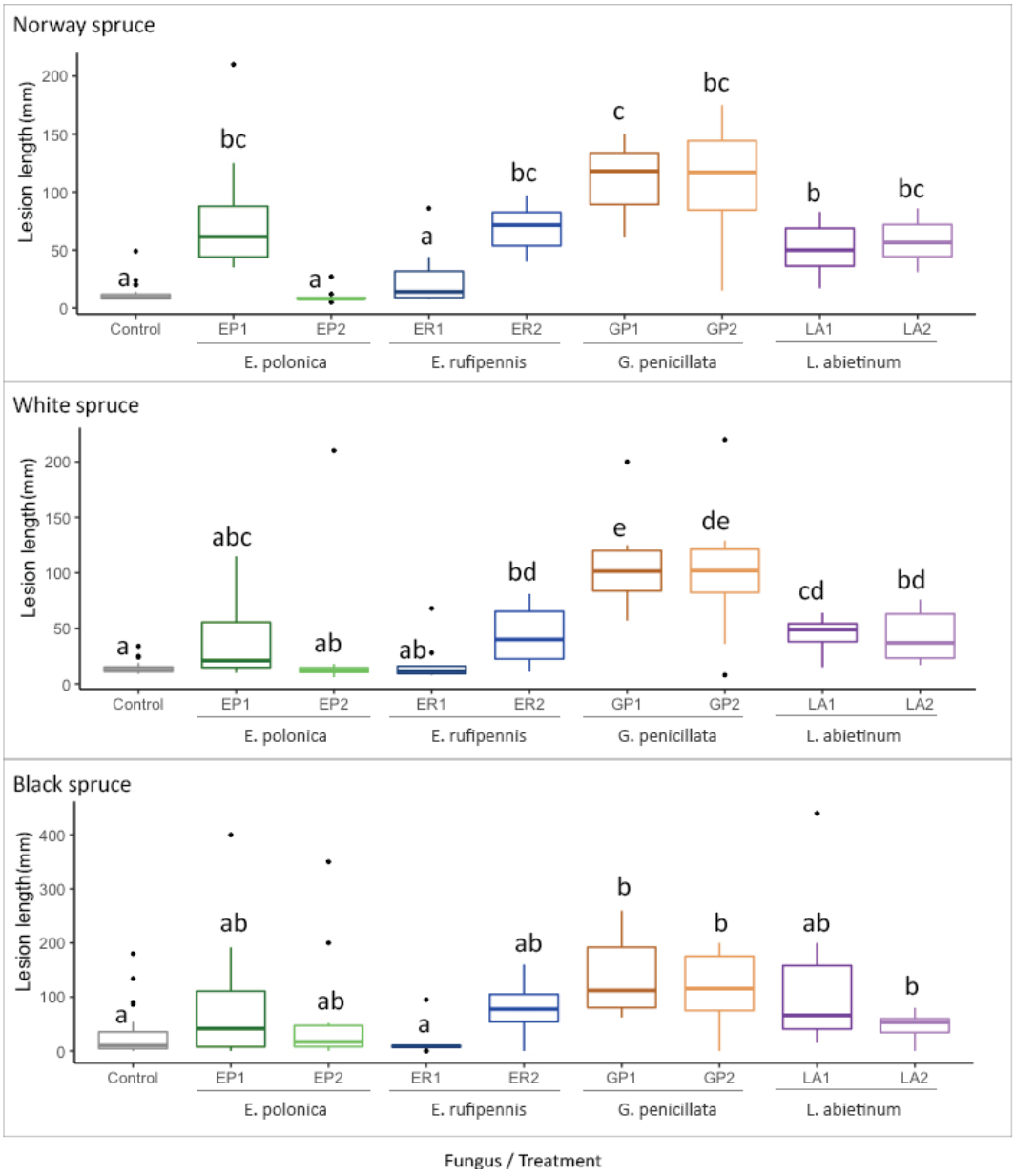

Figure 3.4: Lengths of necrotic lesions in the inner bark 3 weeks after inoculation with four bluestain fungi in cut bolts of Norway spruce, white spruce and black spruce. For each fungus, two isolates were inoculated (e.g. EP1 and EP2). Only agar (without fungus) was inoculated for the control treatment. Treatments with different letters (a-e) differed significantly (ANOVA and Tukey's HSD test with $p < 0.05$). E = *Endoconidiophora*, G = *Grosmannia*, L = *Leptographium*. The thick line inside the box represents the median. For an explanation of boxplot see figure 3.2.

3.2 Laboratory tests of beetle host preference in spruce bark agar

3.2.1 Beetle preference in spruce bark agar without bluestain fungi

Beetle host preference was tested by quantifying beetle entry and tunneling in choice arenas with semi-natural bark media. Beetles were released in Petri dish arenas with agar media amended with bark powder from Norway spruce, white spruce or black spruce, in addition to a medium control without bark powder (Figure 3.5.A). *Ips typographus* did not show any significant preference for media with bark powder from Norway spruce, white spruce or black spruce but beetle tunneled high in Norway spruce. However, beetles tunneled less in the control than in the three spruce bark agar media. The difference was significant between control and Norway spruce ($p = 0.006$, Kruskal-Wallis with Dunn's post hoc test) but not between control and white and black spruce ($p = 0.807$ and $p = 0.277$, respectively). A third of the beetles (32%) made no choice in the experiments and did not tunnel into any medium. After 3-4 days every media grew several fungus in it.

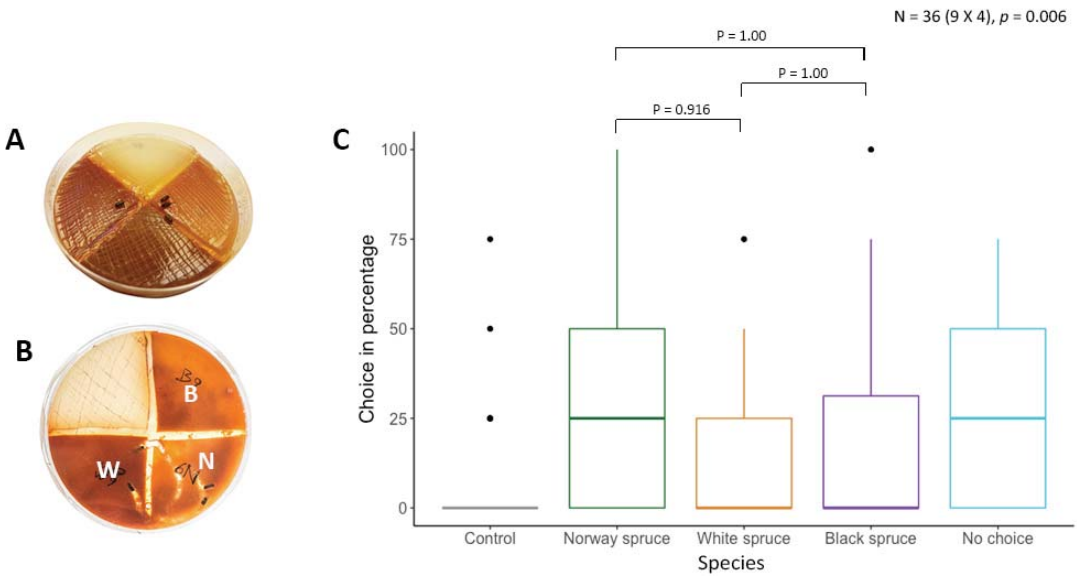

Figure 3.5: **A.** Four *Ips typographus* beetles released in a spruce agar media with three kinds of bark agar medium and a control. **B.** Beetles entering and tunneling in the medium. N, B and W represent agar medium amended with bark powder from Norway spruce, black spruce and white spruce, respectively. C represents the medium control with no bark powder. **C**. Boxplot showing beetle preference (i.e. entering rates) for different media. For an explanation of boxplot see figure 3.2.

3.2.2 Beetle preference in spruce bark agar with bluestain fungi

I also tested beetle host preference by quantifying beetle entry in arenas with a choice between spruce bark media with or without fungal colonization. Beetles could choose between spruce bark media colonized by fungus and control spruce bark agar without any fungus.

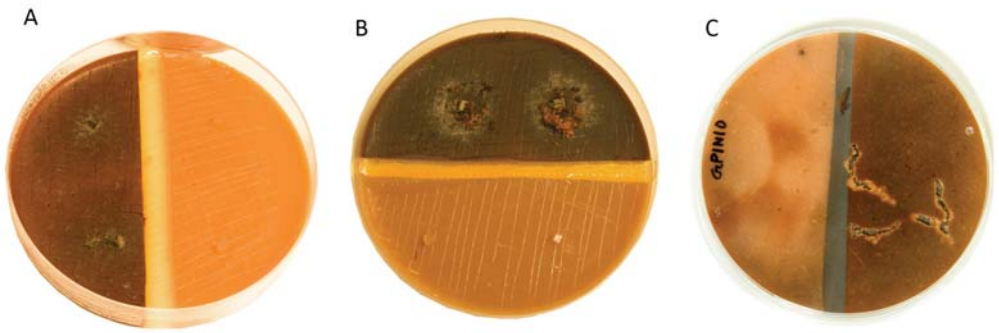

Figure 3.6: **A.** Spruce bark agar medium colonized by fungus (left) and un-colonized bark agar medium (right). **B.** Beetles usually entered the fungus-colonized medium just beside the inoculation plug. **C**. Beetle tunneling inside the medium (viewed from the bottom of the Petri dish).

3.2.2.1 Norway spruce

After 24 hours, *Ips typographus* showed higher preference for Norway spruce bark agar medium colonized by fungi associated with North American bark beetles (*E. rufipennis* and *L. abietinum*) compared to medium colonized by the beetles' own fungi (*E. polonica and G. penicillata*) (Figure 3.7.A-D) and it remained the same after 48 hours (Figure 3.7.E-H). After 48 hours, *Ips typographus* showed a much stronger preference for all fungi (Figure 3.7.E-H). After 24 hours, there were significant differences in preference between *E. rufipennis* and *E. polonica* ($p = 0.012$, Kruskal-Wallis with Dunn's post hoc test) and *E. rufipennis* and *G. penicillata* ($p = 0.012$, Kruskal-Wallis with Dunn's post hoc test). But after 48 hours, significant difference remained only between *E. rufipennis* and *G. penicillata* ($p = 0.032$, Kruskal-Wallis with Dunn's post hoc test). There were significant differences between the controls and fungus-colonized spruce bark media for all four fungi, both after 24 and 48 hours ($p < 0.01$, Kruskal-Wallis with Dunn's post hoc test). Tests had been done for each fungus separately except the comparison among all four fungi. Relative to inactive beetles, the preference for fungus colonized spruce bark media were significant for *E. rufipennis* ($p = 0.001$, Kruskal-Wallis with Dunn's post hoc test) after 24 hours

but after 48 hours beetles' preference were significant also for *E. polonica* (p = 0.033, Kruskal-Wallis with Dunn's post hoc test) and for L. abietinum (p < 0.001, Kruskal-Wallis with Dunn's post hoc test). After 48 hours, preference for *E. polonica* and *E. rufipennis* were consistently higher than the highly varied preference for *G. penicillata* and *L. abietinum*. Almost half (48.2%) of the beetles did not show any preferences after 24 hours, but this was reduced to 23.6% after 48 hours.

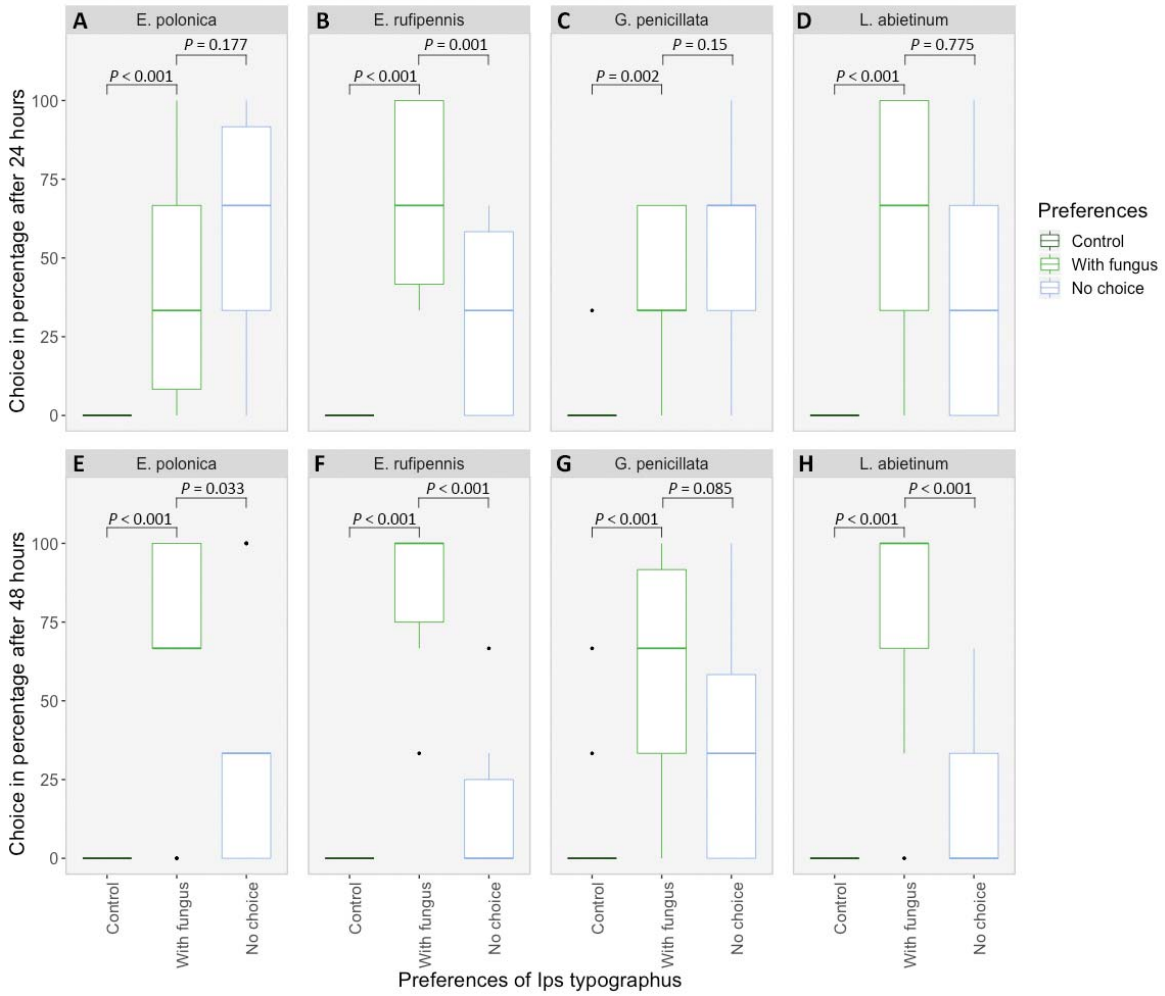

Figure 3.7: Tunneling of *Ips typographus* in Norway spruce bark media colonized by four different bluestain fungi (*Endoconidiophora polonica, E. rufipennis, Grosmannia penicillata, Leptographium abietinum*). P-values represent comparisons by Dunn's posthoc test after Kruskal-Wallis test. For an explanation of boxplot see figure 3.2. E = Endoconidiophora, G = Grosmannia, L = Leptographium.

3.2.2.2 White spruce

There were no significant differences among the preferences for all four fungi both after 24 and 48 hours ($p = 1$, Kruskal-Wallis with Dunn's post hoc test). There were significant differences between the control and fungus-colonized white spruce media after 24 and 48 hours ($p < 0.001$, Kruskal-Wallis with Dunn's post hoc test) (Figure 3.8). Tests had been done for each fungus separately except the comparison among all four fungi. After 24 hours, relative to inactive beetles, *Ips typographus* did not show any strong attraction towards any of the four fungi ($p = 1$, Kruskal-Wallis with Dunn's post hoc test) in white spruce agar mediam. But after 48 hours, relative to inactive beetles, *Ips typographus* showed higher attraction for *E. rufipennis*, *G. penicillata* and *L. abietinum* ($p < 0.01$, Kruskal-Wallis with Dunn's post hoc test) than *E. polonica* ($p = 0.08$, Kruskal-Wallis with Dunn's post hoc test). After 24 hours, beetles' preference for *G. penicillata* was more varied than all other fungi (Figure 3.8.A-D) but the preference remained consistently high after 48 hours than that of after 24 hours (Figure 3.9.G). Half (50%) of the beetles did not show any preferences after 24 hours. After 48 hours, the percentage was reduced to 26.2%. The percentage indicated a similar activity as in Norway spruce (see 3.2.2.1).

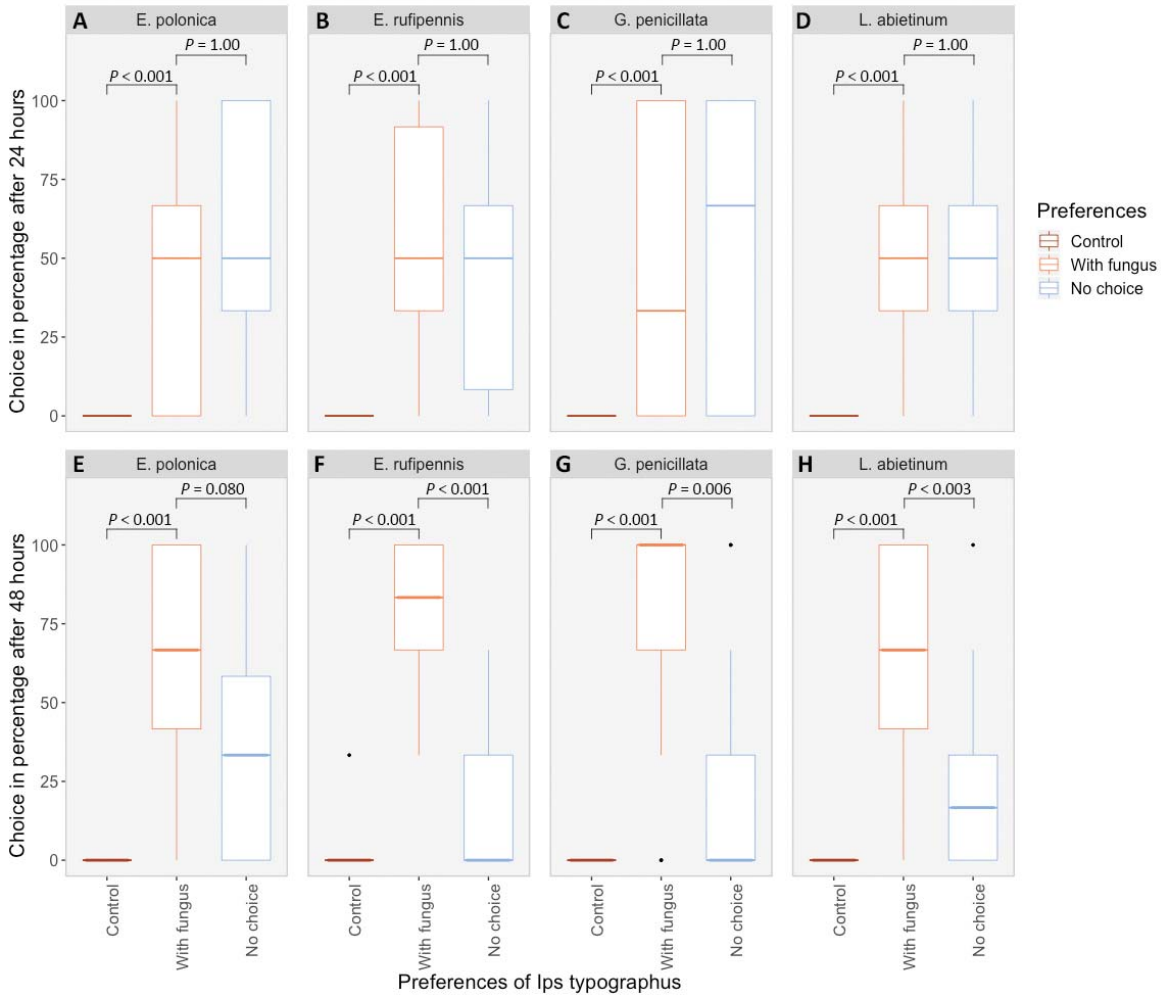

Figure 3.8: Tunneling of *Ips typographus* in white spruce bark media colonized by four different bluestain fungi (*Endoconidiophora polonica, E. rufipennis, Grosmannia penicillata, Leptographium abietinum*). P-values represent comparisons by Dunn's posthoc test after Kruskal-Wallis test. For an explanation of boxplot see figure 3.2. E = Endoconidiophora, G = Grosmannia, L = Leptographium.

3.2.2.3 Black spruce

There were no significant differences among the preferences for all four fungi both after 24 hours and 48 hours ($p = 1$, Kruskal-Wallis with Dunn's post hoc test). After 24 hours, relative to the inactive beetles *Ips typographus*' preference for *E. polonica*, *G. penicillata* and *L. abietinum* ($p = 0.006$, $p = 0.009$ and $p < 0.001$ respectively, Kruskal-Wallis with Dunn's post hoc test) were significantly low compared to *E. rufipennis* ($p = 1$, Kruskal-Wallis with Dunn's post hoc test). Tests have been done for each fungus separately except the comparison among all four fungi. But the preferences increase for all fungi after 48 hours. *E. rufipennis* was the most attractive to *Ips typographus* for tunneling compared to other three fungi (Figure 3.9). After 48 hours, more variability had been noticed in the preference for G. penicillata and L. abietinum than for E. polonica and E. rufipennnis (Figure 3.9). After 24 hours, more than half (67.6%) of the beetles didn't show any preferences and it reduced to 44.4% after 48 hours. Results from the inactive beetles indicated that Norway spruce and white spruce were more attractive than black spruce (see 3.2.2.1, 3.2.2.2).

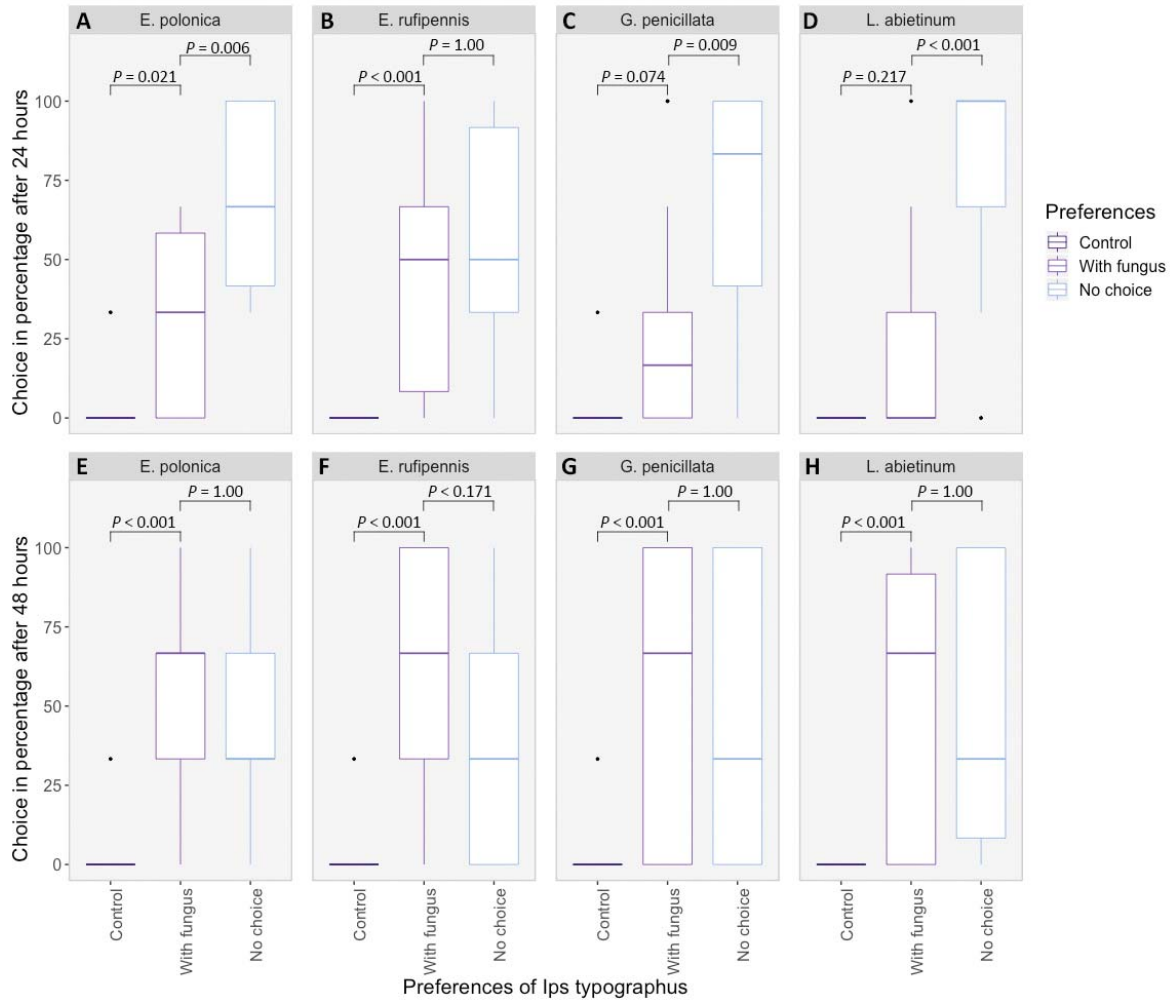

Figure 3.9: Tunneling of *Ips typographus* in black spruce bark media colonized by four different bluestain fungi (*Endoconidiophora polonica, E. rufipennis, Grosmannia penicillata, Leptographium abietinum*). P-values represent comparisons by Dunn's posthoc test after Kruskal-Wallis test. The thick line inside the boxe represents the median; For an explanation of boxplot see figure 3.2. E = Endoconidiophora, G = Grosmannia, L = Leptographium.

3.2.2.4 Beetle preference for specific fungi across spruce species

After 24 hours, more beetles entered the medium colonized by *E. polonica* for white spruce than for Norway spruce or black spruce. (Figure 3.7A, 3.8A and 3.9A). After 48 hours beetles' preferences for *E. polonica* were consistently high in Norway spruce (Figure 3.7E, 3.8E and 3.9E). But there were no significant preference for *E. polonica* in three spruce species both after 24 and 48 hours.

Media colonized by *G. penicillata* were less attractive to *I. typographus* for black spruce than for Norway spruce and white spruce after 24 hours (Figure 3.7C, 3.7C and 3.9C). After 48 hours *G. penicillata* were most attractive in white spruce bark agar media (Figure 3.7G, 3.8G and 3.9G). But there were no significant preference for *G. penicillata* in the three spruce species both after 24 and 48 hours.

For *E. rufipennis* more beetles entered fungus-colonized bark media in Norway spruce than in white spruce and black spruce both after 24 hours and 48 hours. (Figure 3.7, 3.8 and 3.9). But there were no significant preference for *E. rufipennis* in three spruce species both after 24 and 48 hours.

For *Ips typographus*, *Leptographium abietinum* was less attractive in black spruce compared to Norway spruce and white spruce bark agar media after 24 hours (Figure 3.7D, 3.8D and 3.9D). After 48 hours the preference for black spruce colonized by *L. abietinum* increase significantly (Figure 3.9D and 3.9H).

3.2.3 Beetle tunneling length in different spruce bark media

After registering beetle entry at 48 hours in the choice experiment with fungus-colonized and un-colonized spruce bark media (see 3.2.2), I left the Petri dishes an extra 3 days to allow *Ips typographus* to tunnel in the medium. Tunnel length differed significantly between the control medium and spruce bark media colonized by different fungi ($n = 9$, $p < 0.05$; ANOVA, Tukey's post-hoc test). The beetles tunneled most in media colonized by *G. penicillata* (mean 101.2 mm). *Ips typographus* tunneled on average 76.7 mm, 77.5 mm and 78.7 mm in spruce bark medium colonized by *E. polonica*, *E. rufipennis* and *L. abietinum*, respectively. Tunneling did not differ significantly among the four fungi colonized by Norway spruce. The same result was found for white spruce and black spruce.

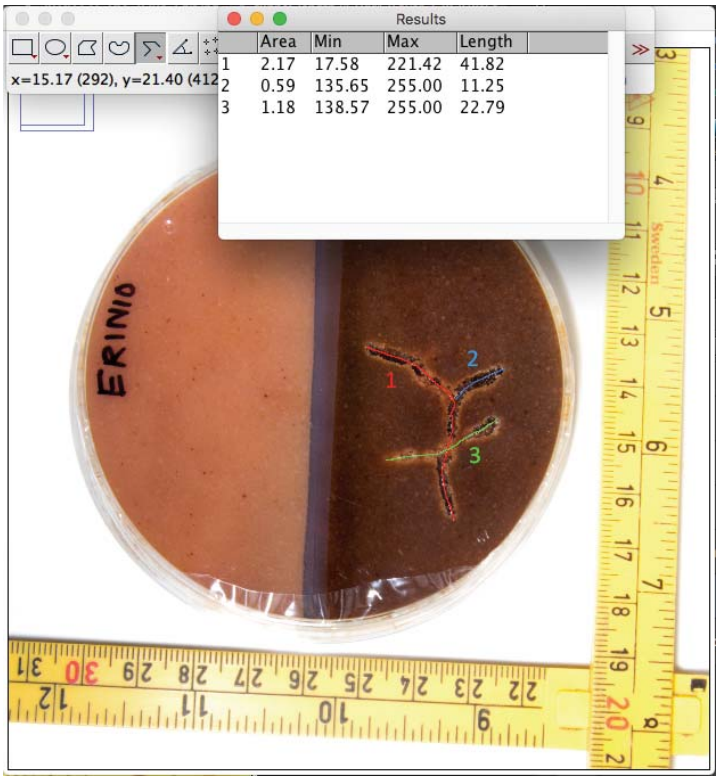

Figure 3.10: Beetles were released in the spruce bark agar media to observe their tunneling behavior. They chose between two types of spruce bark agar media where right side is colonized by fungus and left side is not. Tunnel length measured using ImageJ software. Three separate segmented tunnel line have identified. Lengths were measured in mm (see the results dialog box) by calibrating with the yellow scale in the picture. Total tunnel length were calculated and stored for statistical analysis.

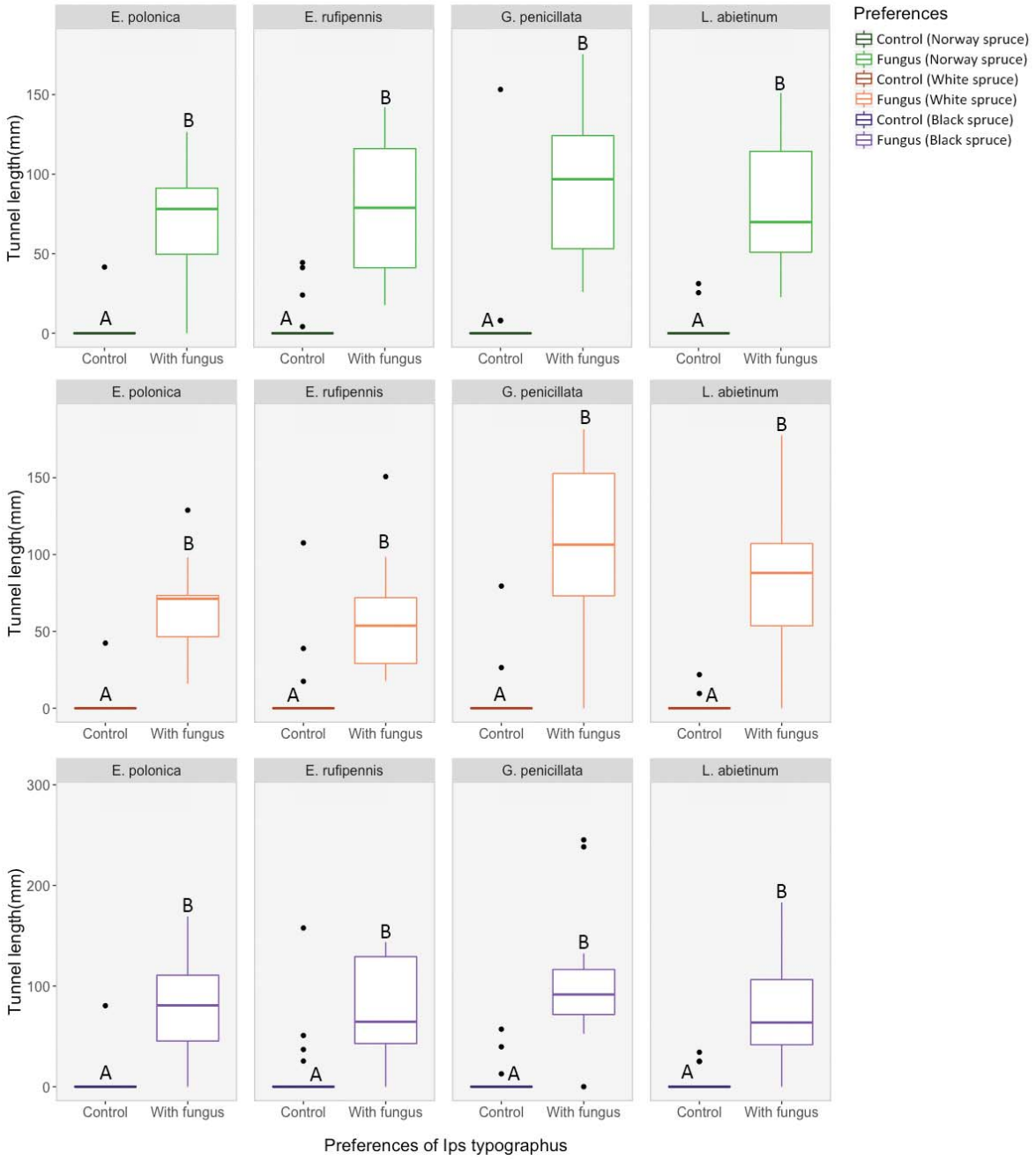

Figure 3.11: Tunneling length by *Ips typographus* after 5 days in spruce bark agar medium colonized by different bluestain fungi and in un-colonized bark agar (Control). Treatments with different letters (A, B) differed significantly (ANOVA and Tukey's HSD test with $p < 0.05$). The thick line inside the box represents the median; For an explanation of boxplot see figure 3.2. E = Endoconidiophora, G = Grosmannia, L = Leptographium.

3.2.4 Fungal volatile choice bioassay

To test if *I. typographus* can select media colonized by bluestain fungi based on smell I let the beetles chose among malt agar plugs colonized by three different fungal isolates and a sterile malt agar plug as control. Three quarters (74%) of the beetles responded to different fungi by selecting and entering cups containing fungus-colonized medium (Figure 3.12). The rest of the beetles either did not make a choice (17%), or they chose the medium control (9%) (Figure 3.12.A). Significantly more beetles chose *E. rufipennis* (37%) rather than the control (p = 0.0017, Dunn's posthoc test after Kruskal Wallis test) (Figure 3.12.C). Also, more beetles chose *E. rufipennis* (37%) than *E. polonica* (9%) (p = 0.0013, Dunn's posthoc test after Kruskal Wallis test) and *G. penicillata* (28%) (Figure 3.12.C).

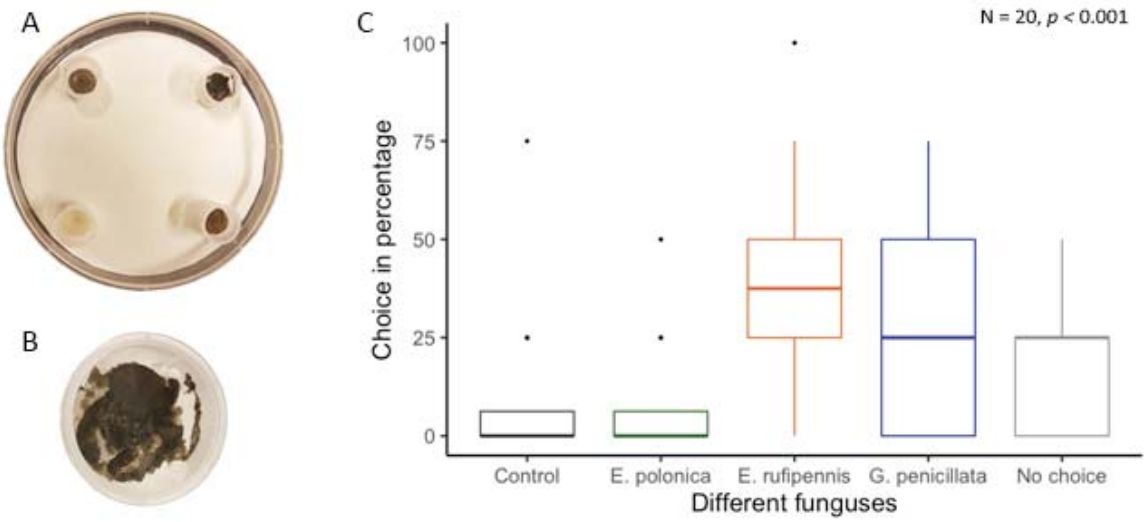

Figure 3.12: A. Bioassay setup for testing beetle detection of fungal volatiles. **B**. A beetle (arrow) that has entered a circular cup baited with fungus-colonized malt agar. **C**. Beetle preference for different baits in a four-choice bioassay. Sterile malt agar as a control was the fourth choice. The box plot (thick line inside the box = median; box = first quartiles; error bar = third quartile; dots = outliers that are 1.5 times larger than the third quartile) represents choices for fungal volatiles from three different fungi (p < 0.001, Kruskal-Wallis test). E = Endoconidiophora, G = Grosmannia, L = Leptographium.

4 Discussions

My results show that white spruce and black spruce are suitable for *Ips typographus* brood production. Thus, available host trees should not be an obstacle to beetle establishment in North America, but variability in host preference and reproductive quality could make their breeding success uncertain (Figure 3.1 and 3.2). Association with bluestain fungus could be an important additional factor for establishment of *Ips typographus* (Figure 3.7, 3.8, 3.9) but beetles might colonize trees even without any presence of fungus (Figure 3.7). However, if bluestain fungi are present, the beetles seem to always prefer bark medium colonized by fungi (Figure 3.7, 3.8, 3.9). In this case, beetle can differentiate between fungal species by detecting volatile organic compounds as a chemical cue (Figure 3.12).

I start this discussion by evaluating the beetles' host preference and reproductive output in their historical European host and in two novel North American spruce hosts. I also discuss how different fungi associated with European or North American bark beetles perform in these spruce species. Then I discuss how beetle host selection behavior is affected by the presence of these fungi. And finally, I briefly explore how the beetles detect bluestain fungi present in host tree tissues.

4.1. Beetle host preference and reproductive success

How do the beetles define a species as a suitable host when they first encounter a tree? Hosts can be recognized by volatile chemical cues when the beetles are outside the tree, or they can be recognized by secondary metabolites, which are released or produced by the hosts during/after initial tunneling. I observed no significant preferences by *Ips typographus* for the historical host Norway spruce over the novel hosts white and black spruce (see 3.1.1). This could indicate that the beetles did not detect any major differences in chemical defenses betweenthe three spruce species. This suggestion is also supported by a previous study that tested *Ips typographus'* preference for Norway spruce and six North American spruce species (Engelmann spruce, white spruce, Sitka spruce, Lutz spruce, black spruce and red spruce) (Økland et al., 2011). *Ips typographus* did not show any significant difference in breeding among novel and native species (Økland et al., 2011).

All the trees used in my experiments were collected from the same area, so climatic condition, an important co-factor for the host preference of beetles, did not shape the results by creating bias towards any specific spruce species. However, North American hosts are growing far from their natural habitat and that could become a stress factor for them. To ensure a good number (>50) of initial entrances, pheromone dispensers have been used (Figure 2.1). Aggression pheromone brought high number of beetles close to hosts and they chose preferable hosts from there. The production of the aggregation pheromone by *Ips typogrpahus* required chemical precursor from the host tree and it was provided manually in this experiment (Erbilgin & Raffa, 2000). Using this method could be a weakness of the study but it has been practiced before (Økland et al., 2011). I found higher variability in preference of novel host than native host (Figure 3.1). The reason could be the unfamiliarity of North American hosts to *Ips typographus.* When beetles find any host without a co-evolutionary history with it, their reaction varies against unknown resistance of the plant. In case of Norway spruce, *Ips typographus* has co-evolved with it for a long time. So the variation of plant secondary metabolites did not affect the *Ips typographus*' choice as much as it did with novel hosts (white spruce and black spruce).

I used cut bolts for the experiment. The bolts were sealed with paraffin wax immediately after cutting in order to keep bark cell fresh and alive (see 2.1). Bolts were even capable of induced defense, as seen by the necrotic lesions that formed in response to the fungal inoculations.

There were no significant differences in *Ips typographus*' offspring production in historical and novel species (see 3.1.2). Usually beetles take advantage of novel hosts. The reason for this was explained as the lack of co-evolutionary defense in novel hosts (Raffa et al., 2008). For example, mountain pine beetle is more successful in jack pine, a novel host than lodgepole pine, the historical host (Erbilgin et al., 2014). The result in my experiment could be explained through intraspecific competition. Female *Ips typographus* can reproduce 33 times more offspring in lowest density (♀♀0.5/100cm^2) than in higher density (♀♀31/100cm2) (Anderbrant et al., 1985). Because of using aggregation pheromone, the attack density might have been the same and offspring production did not differ afterwards.

Bark thickness could be an important factor for Ips typographus reproduction since thick phloem could provide better resources for larval development (Hedgren, 2004)(Hedgren, 2004). But it is not a factor in all cases (Reid & Robb, 1999). There were no significant differences of beetle

emergence from different spruce species after controlling the phloem thickness (see 3.1.2). There was high emergence of *Ips typographus* from Norway spruce during the beginning of the collection period (Figure 3.3). Temperature is always an important factor for bark beetle development (Wermelinger & Seifert, 1999). Since all the bolts have been left in ambient temperature, variability of temperature did not factor in. Another factor could be the availability of suitable nutrient from the Norway spruce, which leads to faster growth compared to novel host. Lack of growth even after a successful entrance into it could be the unfamiliar concentration of monoterpene and phenolics used by the novel hosts (Franceschi et al., 2005).

Therefore *Ips typographus* can easily establish themselves with North American spruce species, black spruce and white spruce as hosts.

4.2. Colonization success of beetle-associated fungi in different spruce species

In case of understanding the invasiveness of *Ips typographus* on new host in North America, I also need to understand the strength of accompanied fungi from Europe or any new potential fungi as symbiont from North America. Although there is a controversy in the scientific community about the importance for fungi for tree killing and host colonization (Kirisits, 2007), fungi help beetle by destroying cambium tissue and depleting tree defense (Lieutier et al., 2009). *Ips typographus* carry fungi from different genera like Endoconidiophora, Ophiostoma, Grosmannia etc. and their usage vary depending on geographical location and timing of attack etc (Zhao et al., 2019). Among them, *Endoconidiophora polonica* is a well-known fungus for its virulence on historical host Norway spruce and *G. penicillata* have been discussed for its high capability of degrading phenolics (Zhao et al., 2019). These two fungi and two other North American beetle (*Dendroctonus rufipennis*) associated fungi (E. rufipennis and L. abietinum) have been used for the experiment to understand how well-equipped *Ips typographus* would be while it attacks new host in North America.

G. penicillata was found to be the most suitable fungus among all other fungi used in the experiment (Figure 3.4). E. polinica, which is considered as the most virulent fungi associated with *Ips typographus,* was very successful in some bolts for every tree species. This result indicates sensitiveness of *E. polonica* against host defense since the host could have a highly variable resistance because of its number of parenchyma cell or the differences in phenolic composition (Evensen et al., 2000; Ganthaler et al., 2017). As *E. rufipennis* belongs to the same

genera as *E. polonica* and similar lesions found from both of them in all host species except white spruce, it could be a suitable candidate for building a new symbiotic relationship with *Ips typographus. L. abietinum* was the least successful fungus among the ones used for the study but made significantly longer lesions than control in all spruce species. So, instead of being the least successful fungi, *L. abietinum* could be an important symbiont of *Ips typographus*.

These results suggest that *Ips typographus* can degrade spruce defense with the help of different fungal associates and can colonize novel host like white spruce and black spruce as they do in Norway spruce.

4.3 Influence of beetle-associated fungi on beetle host choice

Beetles carry a number of fungi with them that tends to vary depending on geographical region and the time of attack on the hosts (Kirisits, 2007). It was evident here how important fungus is to *Ips typographus* by the fact that more than 95% of them chose to tunnel into fungus-colonized bark agar medium, instead of un-colonized bark agar medium (Figure 3.7, 3.8 and 3.9). In this regard, they also chose the fungi associated with North american beetles instead of un-colonized spruce bark agar medium even though those fungi were unknown to them. Another study also came to the same conclusion about the proclivity of beetles towards tunneling into fungal colonized medium (Zhao et al., 2019).

E. polonica, E. rufipennis and *L. abietinum* were the most preferable in Norway spruce although the difference with preference of other spruce species were not significant. This proves *E. polonica'*s ability to metabolize defense chemical with strong defense reaction in Norway spruce (Hammerbacher et al., 2013). It is known from a study that the *E. polonica* and *G. penicillata* are the most suitable fungi in Norway spruce for *Ips typograohus* (Zhao et al., 2019). But *E. rufipennis* and *L. abietinum* were surprisingly more preferable to *Ips typographus* than *E. polonica* in Norway spruce. It might be because *E. rufipennis* and *L. abietinum* have the ability to degrade phenolics from Norway spruce and even provide nutrient to beetles like *E. plonica*. There was a significant difference in beetles' preference between *E. rufipennis* and *G. penicillata* in Norway spruce. But the variety of tree defense from Norway spruce against G. penicillata could also be the answer to the preference variability of beetle for *G. penicillata* (Ganthaler et al., 2017). Another reason could be that this specific isolate of *G. penicillata* from this region was less adapted to the defense chemical in Norway spruce. However, since I found longer

lesions by *G. penicillata* than other fungi (Figure 3.4), it could indicate stronger defense reaction in the host (Krokene & Solheim, 1998a; Solheim, 1992). Another study proves *G. penicillata*'s higher adaptability in Norway spruce than *E. polonica* (Zhao et al., 2019).

On the other hand, *G. penicillata* was the most preferable in white spruce without any significant difference with other spruce species. I found irregular response of beetles in white spruce colonized by *E. polonica.* It could indicate *E. polonica*'s lower capability to degrade phenolics than *G. penicillata* in white spruce. A study found the same result where G. penicillata were stronger than E. polonica in Norway spruce (Zhao et al., 2019).

In black spruce all the fungi came up with the most variable result and were similarly preferred by *Ips typographus*. More than half (55.6%) of the beetles were inactive in black spruce, whereas in Norway spruce and white spruce the percentages of inactive beetle were 23.6% and 23.2%. Percentage of inactive beetle in black spruce clearly indicates that black spruce is not as preferable to beetles as Norway spruce and white spruce (see 3.2.2.3). This suggests that the black spruce had a variety of defense and its combination with fungi was not attractive to beetles. This also indicates that in case of establishing in North America, beetle would chose white spruce rather than black spruce.

Many beetles were inactive during experiment. There could be several reasons for that. The spruce bark agar medium might not have been appealing to them or the beetles might not have been physically fit to tunnel into the spruce bark agar medium. However, beetle have been collected from the same log on the same day and only the most active beetle have been selected for the experiments. On the other hand, the experiments have been done late summer (September). Beetle usually start tunneling at the beginning of the summer. Maybe they were not so interested to tunnel during that period.

Using autoclave to sterilize the bark agar can degrade the quality of the defense chemistry, which could be a weakness of the experiment. So beetles choose Norway spruce as the most suitable host but the other spruces from North America have been closely chosen as well.

It is unclear if and how bark beetles choose their fungal associates in nature. I found that the beetles' preference for specific fungi varied much in European and North American spruce species. But the beetles always preferred fungus-colonized medium over un-colonized medium. This indicates that the presence of fungus change the beetles' tunneling behavior.

4.4 Detection of fungal species by the beetles

Many insects use volatile organic compound from fungi to orient towards breeding substrates or food sources. For example, vinegar flies, larvae of walnut twig beetles (*Pityophthorus juglandis*), red bay ambrosia beetle (*Xyleborous glabratus*) are attracted to volatile organic compounds from *Saccharomyces cerevisiae*, *Geosmithia morbida* and *Raffaelea lauricola* respectively (Christiaens et al., 2014; Luna et al., 2014; Saerens et al., 2010). Interestingly, red bay ambrosia beetle are not attracted to non-symbiotic fungi (Hulcr et al., 2011). *Ips typographus* were more attracted to volatile organic compound from *E. rufipennis,* which was a non-symbiotic fungi. The response of beetles was significantly different towards *E. polonica* from *E. rufipennis* but not from *G. penicillata,* suggesting that *E. rufipennis* could emit similar volatile organic compounds as *G. penicillata*.

Beetle in nature react to the combination of volatile organic compounds from fungus and hosts. But they did not experience such combination during the experiment because I did not grow fungus in spruce bark agar medium. Facing a combination of volatile organic compounds from fungus and spruce tree could have changed the results. Response of beetle to the fungal volatiles could have an important usage in outbreak control. For example attractive fungal volatiles can be used with pheromone for mass trapping of beetles. This could open a new window for controlling these devastating forest pests.

So *Ips typographus* have the capability of establishing in North America given that they are aided by their associated fungi or North American beetle associated fungi.

5 Conclusion

During bolt experiments, no significant difference found between North American and European spruce species either in attack density or in breeding success. Results indicate North American novel hosts are as suitable as European historical host. In case of beetle associated fungi, *G. penicillata* was more successful than North American beetle associated fungi in all three spruces. But the other important fungi *E. polonica* had a variable success in all spruces. *E. polonica* and *G. penicillata* are considered to be the most virulent fungi associated with *Ips typographus* in Europe (Zhao et al., 2019). Using *E. rufipennis* and *L. abietinum*, two fungi associated with North American bark beetles, -I found that the beetles do not rely on any specific fungi to colonize different spruce species. But when beetle has no fungus to choose from in a spruce agar media they tunneled into the host in the absence of fungi. *Ips typographus* can spot the presence of fungus by detecting volatile organic compounds. It could be a way of suitable fungal association during new host colonization.

References

Anderbrant, O., Schlyter, F. & Birgersson, G. (1985). Intraspecific competition affecting parents and offspring in the bark beetle Ips typographus. *Oikos*: 89-98.

Berryman, A. A. (1982). Biological control, thresholds, and pest outbreaks. *Environmental Entomology*, 11 (3): 544-549.

Bleiker, K. & Six, D. (2014). Dietary benefits of fungal associates to an eruptive herbivore: potential implications of multiple associates on host population dynamics. *Environmental Entomology*, 36 (6): 1384-1396.

Christiaens, J. F., Franco, L. M., Cools, T. L., De Meester, L., Michiels, J., Wenseleers, T., Hassan, B. A., Yaksi, E. & Verstrepen, K. J. (2014). The fungal aroma gene ATF1 promotes dispersal of yeast cells through insect vectors. *Cell Reports*, 9 (2): 425-432.

Cudmore, T. J., Björklund, N., Carroll, A. L. & Staffan Lindgren, B. (2010). Climate change and range expansion of an aggressive bark beetle: evidence of higher beetle reproduction in naïve host tree populations. *Journal of Applied Ecology*, 47 (5): 1036-1043.

DiGuistini, S., Wang, Y., Liao, N. Y., Taylor, G., Tanguay, P., Feau, N., Henrissat, B., Chan, S. K., Hesse-Orce, U. & Alamouti, S. M. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont Grosmannia clavigera, a lodgepole pine pathogen. *Proceedings of the National Academy of Sciences*, 108 (6): 2504-2509.

Erbilgin, N. & Raffa, K. F. (2000). Effects of host tree species on attractiveness of tunneling pine engravers, Ips pini, to conspecifics and insect predators. *Journal of Chemical Ecology*, 26 (4): 823-840.

Erbilgin, N., Ma, C., Whitehouse, C., Shan, B., Najar, A. & Evenden, M. (2014). Chemical similarity between historical and novel host plants promotes range and host expansion of the mountain pine beetle in a naïve host ecosystem. *New Phytologist*, 201 (3): 940-950.

Evensen, P., Solheim, H., Høiland, K. & Stenersen, J. (2000). Induced resistance of Norway spruce, variation of phenolic compounds and their effects on fungal pathogens. *Forest Pathology*, 30 (2): 97-108.

Flø, D., Norli, H. R., Økland, B. & Krokene, P. (2018). Successful reproduction and pheromone production by the spruce bark beetle in evolutionary naïve spruce hosts with familiar terpenoid defences. *Agricultural and Forest Entomology*, 20 (4): 476-486.

Franceschi, V. R., Krokene, P., Christiansen, E. & Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist*, 167 (2): 353-376.

Franklin, J. F., Lindenmayer, D., MacMahon, J. A., McKee, A., Magnuson, J., Perry, D. A., Waide, R. & Foster, D. (2000). Threads of continuity. *Conservation biology in practice*, 1 (1): 8-16.

Ganthaler, A., Stöggl, W., Kranner, I. & Mayr, S. (2017). Foliar phenolic compounds in norway spruce with varying susceptibility to Chrysomyxa rhododendri: analyses of seasonal and infection-induced accumulation patterns. *Frontiers in plant science*, 8: 1173.

Haack, R. A. (2001). Intercepted Scolytidae (Coleoptera) at US ports of entry: 1985–2000. *Integrated Pest Management Reviews*, 6 (3-4): 253-282.

Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L. P., Schneider, B., Bohlmann, J., Brand, W. A., Fenning, T. M., Gershenzon, J. & Paetz, C. (2013). A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. *Plant physiology*, 162 (3): 1324-1336.

Hedgren, P. (2004). The bark beetle Pityogenes chalcographus (L.)(Scolytidae) in living trees: reproductive success, tree mortality and interaction with Ips typographus. *Journal of Applied Entomology*, 128 (3): 161-166.

Hlásny, T., Krokene, P., Liebhold, A., Montagné-Huck, C., Müller, J., Qin, H., Raffa, K., Schelhaas, M.-J., Seidl, R. & Svoboda, M. (2019). *Living with bark beetles: impacts, outlook and management options*, 9525980758: European Forest Institute.

Hulcr, J., Mann, R. & Stelinski, L. L. (2011). The scent of a partner: ambrosia beetles are attracted to volatiles from their fungal symbionts. *Journal of Chemical Ecology*, 37 (12): 1374-1377.

Kandasamy, D., Gershenzon, J., Andersson, M. N. & Hammerbacher, A. (2019). Volatile organic compounds influence the interaction of the Eurasian spruce bark beetle (Ips typographus) with its fungal symbionts. *The ISME journal*: 1.

Kärvemo, S., Rogell, B. & Schroeder, M. (2014). Dynamics of spruce bark beetle infestation spots: Importance of local population size and landscape characteristics after a storm disturbance. *Forest ecology and management*, 334: 232-240.

Keeling, C. I. & Bohlmann, J. (2006). Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytologist*, 170 (4): 657-675.

Kirisits, T. (2007). Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In *Bark and wood boring insects in living trees in Europe, a synthesis*, pp. 181-236: Springer.

Krokene, P. & Solheim, H. (1998a). Assessing the virulence of four bark beetle‐associated bluestain fungi using Norway spruce seedlings. *Plant Pathology*, 47 (4): 537-540.

Krokene, P. & Solheim, H. (1998b). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. *Phytopathology*, 88 (1): 39-44.

Krokene, P. & Solheim, H. (2001). Loss of pathogenicity in the blue‐stain fungus Ceratocystis polonica. *Plant Pathology*, 50 (4): 497-502.

Lehnert, L. W., Bässler, C., Brandl, R., Burton, P. J. & Müller, J. (2013). Conservation value of forests attacked by bark beetles: Highest number of indicator species is found in early successional stages. *Journal for Nature Conservation*, 21 (2): 97-104.

Lieutier, F., Yart, A. & Salle, A. (2009). Stimulation of tree defenses by Ophiostomatoid fungi can explain attack success of bark beetles on conifers. *Annals of Forest Science*, 66 (8): 801-801.

Luna, E., Cranshaw, W. & Tisserat, N. (2014). Attraction of walnut twig beetle Pityophthorus juglandis (Coleoptera: Curculionidae) to the fungus Geosmithia morbida. *Plant Health Progress*, 15 (3): 135-140.

Økland, B., Erbilgin, N., Skarpaas, O., Christiansen, E. & Långström, B. (2011). Inter-species interactions and ecosystem effects of non-indigenous invasive and native tree-killing bark beetles. *Biological Invasions*, 13 (5): 1151-1164.

Paine, T., Raffa, K. & Harrington, T. (1997). Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual review of entomology*, 42 (1): 179-206.

Raffa, K. F., Aukema, B. H., Bentz, B. J., Carroll, A. L., Hicke, J. A., Turner, M. G. & Romme, W. H. (2008). Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. *Bioscience*, 58 (6): 501-517.

Raffa, K. F., Powell, E. N. & Townsend, P. A. (2013). Temperature-driven range expansion of an irruptive insect heightened by weakly coevolved plant defenses. *Proceedings of the National Academy of Sciences*, 110 (6): 2193-2198.

Reid, M. & Robb, T. (1999). Death of vigorous trees benefits bark beetles. *Oecologia*, 120 (4): 555-562.

Saerens, S. M., Delvaux, F. R., Verstrepen, K. J. & Thevelein, J. M. (2010). Production and biological function of volatile esters in Saccharomyces cerevisiae. *Microbial biotechnology*, 3 (2): 165-177.

Schlyter, F. & Cederholm, I. (1981). Separation of the sexes of living spruce bark beetles, Ips typographus (L.),(Coleoptera: Scolytidae) 1. *Zeitschrift für angewandte Entomologie*, 92 (1‐5): 42-47.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, 9 (7): 671.

Schroeder, L. M. & Lindelöw, Å. (2002). Attacks on living spruce trees by the bark beetle Ips typographus (Col. Scolytidae) following a storm‐felling: a comparison between stands with and without removal of wind‐felled trees. *Agricultural and forest entomology*, 4 (1): 47-56.

Schroeder, M. & Cocoş, D. (2018). Performance of the tree‐killing bark beetles Ips typographus and Pityogenes chalcographus in non‐indigenous lodgepole pine and their historical host Norway spruce. *Agricultural and forest entomology*, 20 (3): 347-357.

Six, D. L. & Wingfield, M. J. (2011). The role of phytopathogenicity in bark beetle–fungus symbioses: a challenge to the classic paradigm. *Annual review of entomology*, 56: 255-272.

Solheim, H. (1992). Fungal succession in sapwood of Norway spruce infested by the bark beetle Ips typographus. *European Journal of Forest Pathology*, 22 (3): 136-148.

Solheim, H. & Krokene, P. (1998). Growth and virulence of Ceratocystis rufipenni and three blue-stain fungi isolated from the Douglas-fir beetle. *Canadian journal of botany*, 76 (10): 1763-1769.

Sun, J., Miao, Z., Zhang, Z., Zhang, Z. & Gillette, N. E. (2004). Red turpentine beetle, Dendroctonus valens LeConte (Coleoptera: Scolytidae), response to host semiochemicals in China. *Environmental Entomology*, 33 (2): 206-212.

Wermelinger, B. & Seifert, M. (1999). Temperature‐dependent reproduction of the spruce bark beetle Ips typographus, and analysis of the potential population growth. *Ecological Entomology*, 24 (1): 103-110.

Yin, H. (2000). The synopsis on morphological and biological characters of Dendroctonus valens LeConte. *Acta Zootaxonomica Sinica*, 25 (1): 120-43.

Zhao, T., Kandasamy, D., Krokene, P., Chen, J., Gershenzon, J. & Hammerbacher, A. (2019). Fungal associates of the tree-killing bark beetle, Ips typographus, vary in virulence,

ability to degrade conifer phenolics and influence bark beetle tunneling behavior. *Fungal ecology*, 38: 71-79.

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