# Invasion potential and host suitability for selected bark- and wood-boring insect pests

Invasjonspotensiale og egnethet av ulike vertstrær for utvalgte
bark- og vedboende insektskadegjørere

Philosophiae Doctor (PhD) Thesis

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## Contents

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# Abstract

Introduction of nonnative species is often a by-product of increasing global trade. Some nonnative species may reach extremely high population densities, deplete resources and cause extensive economic and ecological damage in invaded areas. For example, nonnative bark- and wood-boring beetles may become extremely damaging pests due to their ability to attack living trees and cause large-scale ecosystem impacts. Pest risk analysis (PRA) is an essential tool that can help reduce the risk of new invasions by identifying phytosanitary measures against high risk nonnative species and commodities. The studies presented in this thesis deal with key PRA issues concerning bark- and wood-boring beetles, such as the probability that new pest species will arrive, establish, spread and expand within new areas, and the potential economic and environmental impact this may have. We focus on some of the most destructive bark- and wood-boring beetle species in temperate and boreal forests and their potential to invade European countries. In addition, we study whether access to novel host tree species may facilitate range expansion of a native pest species in Norway.

Some bark- and wood-boring beetles in the genus *Agrilus* from North America and Asia may be serious invasive pests on trees, but they have not been intercepted in Norway. Maximum Entropy (MaxEnt) analyses indicated a high probability of potential distribution for four *Agrilus* species in Europe, including Norway for some. Combined with import statistics of deciduous wood chips and the distribution of suitable host trees, we conclude that some *Agrilus* species have a high probability of establishment in northern Europe, including Norway. We argue that preemptive phytosanitary measures are important to prevent introduction of these species, since new species arriving by import are difficult to detect and eradication after establishment is often unsuccessful despite large efforts.

Maturing stands of introduced Sitka and Lutz spruce in western and northern Norway may represent a risk of invasions into new regions by the most damaging bark beetle in Europe, the European spruce bark beetle *Ips typographus*, especially if temperatures increase in the future. We demonstrate that these two North American spruce species are quite similar to Norway spruce in constitutive and induced terpene composition but that the terpene concentrations are somewhat lower in the North American spruces. We also show that *I. typographus* is fully capable of producing viable offspring in cut logs of the two North American spruces, as well as successfully producing its aggregation pheromone. However, the beetle's fungal associate *Endoconidiophora polonica* did not perform well in Sitka and

Lutz spruce. Overall, our results suggest that Sitka and Lutz spruce are suitable hosts for *I. typographus* in Norway and thus may facilitate beetle range expansion. If the beetle should establish in North America, terpenoid chemistry and pheromone production are not likely to be obstacles for beetle colonization of these species in their native range.

Keywords: invasive species; wood chips; phytosanitary risk; *Agrilus*; Sitka spruce; Lutz spruce; potential distribution; plant defense.

# Sammendrag

Introduksjon av fremmede arter er ofte en uheldig bieffekt av økende global handel. Noen fremmede arter kan bli svært tallrike og gjøre omfattende økonomisk og økologisk skade der de etablerer seg. Fremmede bark- og trelevende biller kan for eksempel gjøre stor skade på grunn av sin evne til å angripe levende trær, noe som kan påvirke hele skogøkosystemer. Arbeidet med å forhindre introduksjon av fremmede arter bør bygge på føre var-prinsippet, både fordi det kan være vanskelig å oppdage fremmede arter i tide, og fordi det er svært vanskelig og kostbart å utrydde fremmede arter etter de har etablert seg. Risikoanalyse (Pest Risk Analysis; PRA) er et sentralt verktøy for å identifisere plantesanitære tiltak mot skadelige fremmede arter eller handelsvarer som utgjør en uakseptabel risiko. Denne oppgaven tar for seg utvalgte bark- og trelevende biller med fokus på de sentrale spørsmålene i en risikoanalyse: sannsynligheten for innførsel, sannsynligheten for etablering, sannsynligheten for spredning, og den potensielle innvirkningen artene kan ha på økonomi og miljøet. Vi fokuserer på noen av de potensielt mest skadelige bark- og trelevende artene og deres invasjonspotensiale i Norge. I tillegg undersøker vi om tilgang til nye vertstrær fra Nord-Amerika kan legge til rette for at et naturlig hjemmehørende skadeinsekt i Norge kan utvide sitt utbredelsesområde.

Flere nordamerikanske og asiatiske biller i slekten *Agrilus* kan potensielt gjøre stor skade i Europas skoger. Gjennom analyser av importstatistikk for amerikansk og asiatisk løvtreflis til Europa viser vi at det er sannsynlig at fremmede *Agrilus*-arter kan bli innført til Europa med importert flis. Analyser av potensielt utbredelsesområde for utvalgte nordamerikanske *Agrilus*-arter i Europa kombinert med utbredelsen av deres potensielle vertstrær viser at det også er sannsynlig at disse artene kan etablere seg i Europa, inkludert Norge. Vi argumenterer derfor for viktigheten av forebyggende risikoreduserende plantesanitære tiltak for å unngå introduksjon av disse artene, siden artene er vanskelige å oppdage gjennom prøvetakning og fordi det det vil være svært kostbart og vanskelig å utrydde dem etter at de er etablerte.

Områder med sitka- og lutzgran langs nordvestkysten av Europa kan stå i fare for å bli angrepet av granbarkbillen (*Ips typographus*) hvis klimaet forandrer seg i fremtiden. Våre resultater viser at det kjemiske forsvaret til disse to nordamerikanske granartene er forholdsvis likt vanlig gran når det gjelder stående og indusert terpensammensetning, men at terpenvolumene er noe lavere i de nordamerikanske granartene. Vi viser også at granbarkbillen kan produsere levedyktig avkom i de to nordamerikanske vertstrærne, samt at billen er i stand til å produsere normale mengder av sitt aggregasjonsferomon. Granbarkbillens symbiotiske blåvedsopp *Endoconidiophora polonica*, som hjelper granbarkbillen med å drepe trærne, vokste derimot ikke like godt i de nordamerikanske grantrærne. Samlet sett tyder våre resultater på at sitka- og lutzgran vil være mottakelige for angrep av granbarkbillen i Norge. Heller ikke i Nord-Amerika ser terpenkjemi og feromonproduksjon ut til å ville være en hindring for angrep og etablering av granbarkbillen. Disse funnene er viktige for forvaltning av sitka- og lutzgran i Europa, men har også betydning dersom granbarkbillen skulle etablere seg i Nord-Amerika hvor sitka- og lutzgran har sitt naturlige utbredelsesområde.

Nøkkelord: invaderende arter; treflis; karanteneskadegjører; plantehelse; *Agrilus*; sitkagran; lutzgran; potensiell utbredelse; planteforsvar

## Glossary of phytosanitary terms

All definitions are from the international standards for phytosanitary measures, ISPM no. 5 Glossary of Phytosanitary Terms (IPPC, 2010).

**Commodity** - A type of plant, plant product, or other article being moved for trade or other purposes.

**Establishment** - Perpetuation, for the foreseeable future, of a pest within an area after entry.

**IPPC International Plant Protection Convention** - As deposited in 1951 with FAO in Rome and as subsequently amended.

**Introduction** - The entry of a pest resulting in its establishment.

**ISPM** - International Standard for Phytosanitary Measures.

**Nonnative** - Individuals of a species moved from their native range to a new location outside of their native range. Also called exotic species or introduced species.

**Pathway** - Any means that allows the entry or spread of a pest.

**Pest** - Any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products

**Pest categorization** - The process for determining whether a pest has or has not the characteristics of a quarantine pest or those of a regulated non-quarantine pest.

**Pest risk analysis (PRA)** - The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it.

**Phytosanitary measure** - Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests.

**Quarantine** - Official confinement of regulated articles for observation and research or for further inspection, testing and/or treatment.

**Regional plant protection organization** - An intergovernmental organization with the functions laid down by Article IX of the IPPC.

**Spread** - Expansion of the geographical distribution of a pest within an area.

## List of publications and contributors

***Article I*** Flø, D., Krokene, P., Økland, B. (2014). Importing deciduous wood chips from North America to northern Europe – the risk of introducing bark- and wood-boring insects. Scandinavian Journal of Forest Research 29(1): 77-89.

***Article II*** Flø, D., Krokene, P., Økland, B. (2015). Invasion potential of *Agrilus planipennis* and other *Agrilus* beetles in Europe: import pathways of deciduous wood chips and MaxEnt analyses of potential distribution areas. EPPO Bulletin 45(2): 259-268.

***Article III*** Flø, D., Økland, B., Norli, H. R., Krokene, P. (2016). Constitutive and induced terpenoid defenses of co-evolved and naïve host trees of the European spruce bark beetle *Ips typographus*. Unpublished manuscript

***Article IV*** Flø, D., Økland, B., Krokene, P. (2016). Reproduction of *Ips typographus* in co-evolved and naïve spruce hosts. Unpublished manuscript.

Table 1. Individual contributions to the articles included in this thesis.

| | Paper I | II | III | VI |
|---|---|---|---|---|
| Idea and planning | DF, BØ | DF, BØ | DF, PK, BØ | DF, PK, BØ |
| Fieldwork | | | DF, PK, BØ | DF, PK, BØ |
| Lab work | | | DF, TK, HRN | DF, TK |
| Analysis | DF, BØ | DF, BØ | DF, PK | DF |
| Discussions | DF, BØ, PK, OS | DF, BØ, PK, OS | DF, PK, BØ, OS | DF, PK, BØ |
| Manuscript preparation | DF, BØ, PK | DF, BØ, PK | DF, PK, BØ | DF, PK, BØ |

Contributors: DF: Daniel Flø, PK: Paal Krokene, BØ: Bjørn Økland, OS: Olav Skarpaas, TK: Torstein Kvamme, HRN: Hans Ragnar Nordli

# Introduction

## The invasive species problem

Introductions of nonnative species are often a by-product of increasing global trade. International trade provides important goods, such as food, live plants, and forest products. However, trade in forest products can also spread pests that cause serious damage to native species and forest ecosystems. Increasing human population and economic growth since the industrial revolution have broken down natural barriers to species movement trough global trade. Oceans that previously represented insuperable barriers for species can now be crossed in days. Today, nonnative species arrive at accelerating rates overland, by air or with ships to new areas throughout the world (Figure 1). Species may even be released into new environments deliberately, either to increase food supply, as with the introduction of African honey bees to Brazil in the 1950s to increase honey production (Winston 1992), or to control pests, as with the introduction of the Asian ladybug to USA to control aphids (Koch & Galvan 2008). Most nonnative species are unable to establish in new areas or if they do they rarely reach high population densities (Williamson & Fitter 1996). However, a few species become invasive, reach extremely high population densities, and cause extensive economic and ecological damage (Liebhold et al. 1995).

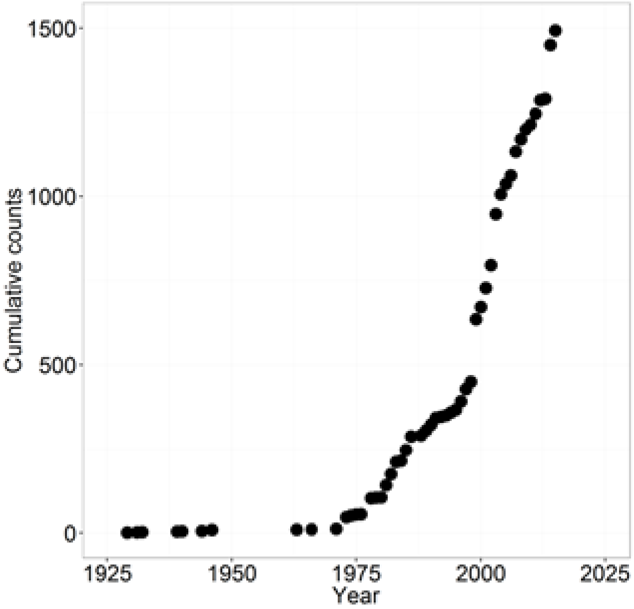

Figure 1. Cumulative detections of nonnative species throughout the world since the 1920’s. Data for introduced, naturalized, and invasive species from Gbif.org (1,911 records).

## International organizations and measures against invasive species

Efficient measures against the increasing numbers of invasive species require a set of tools, which are under constant improvement. These tools are organized at both international and national levels (Figure 2). Because regulations against nonnative species often interfere with international trade, all applied tools and methods must build on solid scientific principles and international consensus. At a global level, the common efforts to reach consensus are organized under the United Nations (UN) Food and Agriculture Organization (FAO). FAO is responsible for developing international standards under the International Plant Protection Convention (IPPC). These International Standards for Phytosanitary Measures (ISPMs) include several measures and international regulations aimed at preventing spread of harmful organisms associated with plants and plant products between countries and regions. These are legally binding by the World Trade Organization (WTO) through the sanitary and phytosanitary measures agreement (the SPS agreement). Currently, the burden of proof when it comes to risks of introducing nonnative species lies with the plant protection organizations, and this prevents countries from banning trade with other countries unless there is scientific proof of damage, as stated in the SPS agreement. At the regional level, FAO regulations are followed up in nine regional plant protection organizations (RPPO), including the North American Plant Protection Organization (NAPPO) and the European and Mediterranean Plant Protection Organization (EPPO). These organizations, together with the National Plant Protection Organizations (NPPOs, such as the Norwegian Food Safety Authority), facilitate and implement IPPC regulations at regional and

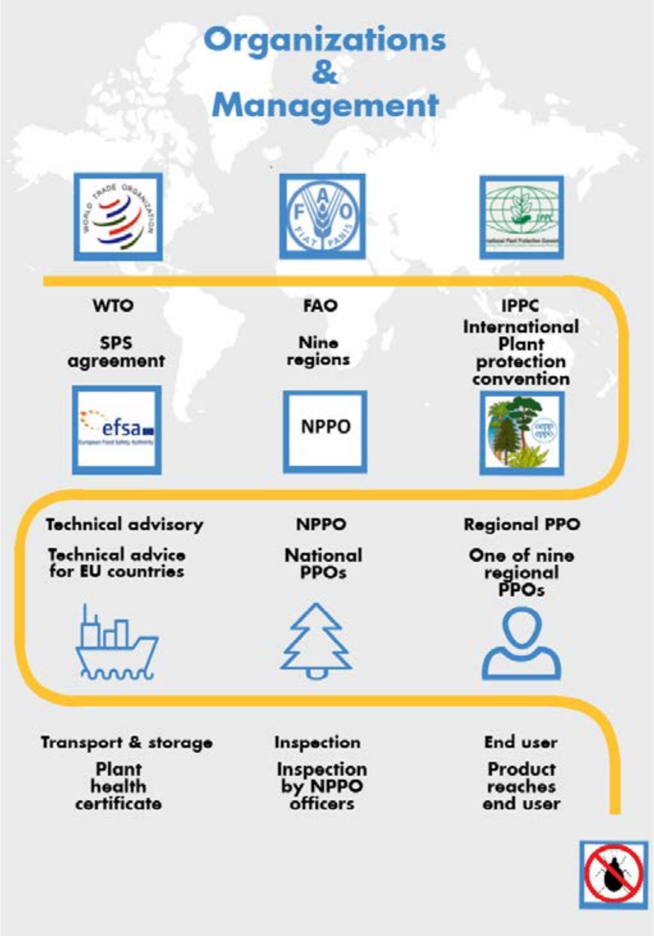

Figure 2. Schematic overview of the organization of phytosanitary measures and regulations at the international, regional and local level. PPO = Plant Protection Organization, SPS = Sanitary and PhytoSanitary measures.

national levels. For countries within the European Union (EU), phytosanitary regulations are also dealt with in a regional organization called the European Food Safety Authority (EFSA).

## Pest risk analysis (PRA) - the first step to counteract invasive species

The most important tool against invasive species is the so-called pest risk analysis (PRA). A PRA includes a set of questions to be answered regarding the species in question, and is the basis for determining whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it. A PRA consists of three parts: initiation, pest risk assessment, and pest risk management (PRM). Based on the questions in the initiation, it is decided whether the species qualifies for a full PRA, which involves more detailed questions. The key objectives of the full PRA are to determine (1) the probability of arrival (probability that the pest will enter a given area), (2) the probability of establishment (probability that the pest will establish in the area), (3) the probability of spread (probability of expansion of the pest within the area), and (4) the potential negative effects the pest is likely to have on the economy and the environment. The first three objectives address the key steps in the biological invasion process (Figure 3). Based on the results of the full PRA, it is concluded whether the species is a pest that should be recommended for regulation or not. If the conclusion is that a species should be regulated, the last phase (PRM) is executed. In the PRM section, information on every possible host plant and introduction pathway is analyzed to find appropriate measures that can reduce risks to an acceptable level. If the recommended regulation from the PRA is

Figure 3. Simplified schematic overview of the biological invasion process and corresponding management options.

accepted and adopted by the NPPO, these measures will be a part of the national regulation for import of goods and host materials that may contain this species. When a pathway includes several potential invasive pests, PRAs can be performed not only for a single species but for the whole pathway. A so-called pathway risk analysis is for example relevant for the import of deciduous wood chips, which is the topic of article I and II of this thesis.

## Invasive bark- and wood-boring beetles

Bark- and wood-boring beetles can be extremely damaging invasives because they may attack living trees and have large-scale impact on forest ecosystems (Gandhi & Herms 2010a; Kenis et al. 2009; Økland et al. 2011). Between 1980 and 2006, bark- and wood-borers accounted for 56% of all new insect species that became established in the US (Aukema et al. 2010), incurring annual costs of about 2 billion US$ (Aukema et al. 2011). Since 1900, the number of new tree-attacking species detected has been on average 3 per year in Canada and 2.5 per year in the US (Aukema et al. 2010; Nealis et al. 2015). In comparison, fewer nonnative bark- and wood-boring insects have been introduced in Europe, but also on this continent the economic and ecological impacts of invasive insect species have been significant, with about 1,300 recorded nonnative insect introductions in total (DAISIE 2009), with an estimated annual cost of €12 billion (Kettunen et al. 2009). The high costs and impacts of invasive species are expected to continue and even increase with further globalization and growth in international trade. Preemptive measures will usually be most cost-effective to prevent introduction and establishment of invasive bark- and wood-boring insects. Introductions of nonnative species are mostly accidental, and the species are usually not detected until they are firmly established. Eradication of established species is often difficult, and despite high expenditures detection and eradication programs often fail (Økland et al. 2010). In addition, a PRA has often not been performed before a problem occurs, and since the process of performing risk assessments and adopting new regulations takes time it can be too late to solve an immediate problem. Thus, valuable time may be saved by proactively collecting basic, PRA-related information about potentially harmful species that have not yet been intercepted. For species that are identified as having a high invasion potential further research on invasion potential and implementation of preemptive regulations and management programs may be warranted.

## Nonnative invasives and native expanding species: two closely related issues

Reducing the impacts of invasive species is not only about stopping species from being imported into new countries. National borders are human constructs, and invasions can also happen within countries, as demonstrated by the recent range expansion of the mountain pine beetle (*Dendroctonus ponderosae*) within Canada. This is an illustrative example of how a native species can invade new ecosystems, encounter novel host trees and have serious impacts. The mountain pine beetle is a naturally occurring outbreak species on lodgepole pine (*Pinus ponderosa*), but with its recent range expansion it has entered regions dominated by jack pine (*Pinus banksiana*), a species that has no co-evolutionary history with the mountain pine beetle (Erbilgin et al. 2014). These naïve trees are chemically different from lodgepole pine and are more vulnerable to beetle attacks. Like the mountain pine beetle, the European spruce bark beetle *Ips typographu*s may expand its geographic range and its host range within national borders. The spruce bark beetle is one of the most damaging pests in Europe’s boreal forests and among the few insects that can kill healthy spruce trees on a large scale. If the spruce bark beetle should expand its range in a future warmer climate it may encounter new host species*,* such as Sitka and Lutz spruce. These North American species have been extensively planted in costal northwestern Europe since the 1960s. Attacks on Sitka spruce have been reported from the UK and Sweden (Browne & Laurie 1968; Økland et al. 2011), but have been rare partly because the beetles and the trees do not fully overlap geographically. Spruce bark beetle interactions with Sitka and Lutz spruce are poorly documented, and we do not know whether the beetle will be able to attack and reproduce in plantations of Sitka and Lutz spruce. More knowledge about these issues is essential to inform future spruce bark beetle management.

## This thesis deals with key PRA questions for major forest pests

As explained above, the four key PRA questions address the probability of pest arrival, the probability of establishment, the probability of spread and expansion, and the potential impact an established pest will have on economy and environment. In the individual studies included in this thesis we addressed these PRA questions by investigating the probability of arrival and establishment in Europe of destructive bark- and wood-boring beetles in the genus *Agrilus* (article I and II). We also studied the potential of a native bark beetle (*Ips typographus*) to colonize introduced spruce species in Norway and thus expand its range within the country (article III and IV). In addition, we discussed the potential impact these bark- and wood-boring beetles may have on economy and the forest environment. Our main objective was to strengthen the scientific foundation for future phytosanitary regulations and policies for bark- and wood-boring insect pests that represent a high risk of forest damage in Norway. More specific objectives were explored in four individual articles:

**Objectives article I:** The objective of the first article was to identity species of bark- and wood-boring beetles that have the potential to become invasive in northern Europe through import of deciduous wood chips from North America. Deciduous wood chips are an emerging commodity pathway into Europe, and it is important to determine which species might be associated with the pathway and characterize risks associated with these species. This could provide the basis for further PRA of specific species. It is also important to identify any high risk import regions in Europe with suitable host trees and climate for invasive bark- and wood-boring beetles. Beetle species were identified from the literature based on the likelihood of them being imported with deciduous wood chips to northern Europe, and the presence in this region of suitable host trees and climatic conditions for establishment and spread. Emphasis was given to species with the potential to have serious economic and ecological impact.

**Objectives article II:** The objective of the second article was to identify the countries in Europe with the highest potential for introduction of the bark- and wood-boring beetles identified in article I (mainly *Agrilus* species in the family Buprestidae). We analyzed trade statistics of deciduous wood chips to evaluate the risk of arrival along this pathway from North America to Europe. Since climatic suitability is an important factor in establishment, Maximum entropy modeling (MaxEnt) was used to predict potential distribution of the selected North American *Agrilus* species in Europe. The potential distribution of *Agrilus* species and the distribution of potential host trees were then coupled with import statistics of wood chips to identify the countries with the highest potential of introducing *Agrilus* species.

**Objectives article III:** The objective of the third article was to determine whether the North American species Sitka and Lutz spruce are chemically suitable hosts for the spruce bark beetle *Ips typographus*, or if their terpenoid composition might be an obstacle for tree colonization and pheromone production by the beetle. We also compared the growth of the beetle's symbiotic blue-stain fungus *Endoconidiophora polonica* in the three spruce hosts. The destructive impact of bark and wood-boring beetles is largely determined by the trees' ability to defend themselves, and the naïve host theory (Lymbery et al. 2014) predicts that the beetle-fungus complex will have greater impact on host trees with which they have no co-evolutionary history. The spruce bark beetle is native to Europe but may become increasingly damaging by expanding into new regions with extensive plantations of nonnative spruce species. The suitability of such potentially novel host trees for the spruce bark beetle is crucial to determine the potential for future beetle expansion.

**Objectives article IV:** The objective of the fourth article was to determine the ability of the European spruce bark beetle to breed in the potentially novel host trees Sitka and Lutz spruce. Beetle reproductive performance in Sitka and Lutz spruce was compared with that in Norway spruce by determining beetle entry into the bark, gallery construction, offspring production, and offspring length and weight. Such data is crucial to determine if the beetle will be able to reproduce, spread and expand in nonnative spruce forests in Europe, and also in the native range of Sitka and Lutz spruce in North America if the beetle was to be introduced there.

# Methods

## Potential invasive bark- and wood-boring insects in deciduous wood chips (Article I)

A set of criteria was established to select species of bark- and wood-borers with a high likelihood of becoming invasive in northern Europe. Knowing that invasive pests usually adapt to congeneric hosts, we listed all deciduous tree genera that occur both in eastern North America and northern Europe, as well as all species within these genera that occur in northern Europe. We screened the literature and cross-checked databases for bark- and wood-boring beetles that are known pests of deciduous trees in eastern North America. Then we reduced the number of species by focusing on species that are known to be able to kill healthy deciduous trees, especially European species. Also, the species should be abundant and widely distributed, to ensure that we selected species that were predisposed to tolerate a wide range of climatic conditions. The species should also have traits that favor survival through one or several pathway processes, such as transport in raw materials for production of chipped wood, the chipping process itself, and transport in chipped wood.

## Analysis of trade statistics (Article II)

Import data for deciduous wood chips from 2004 to 2013 was collected for all 28 European Union member states using Eurostat's CN8-database (Eurostat 2016). This database employs the 8-digit combined nomenclature (CN) and data collection follows a harmonized methodology. Other European countries, such as Norway, collect import data using other methodologies that are difficult to harmonize with the EU system. Export data to the EU was collected for the United States and Canada, which harbor several potentially invasive *Agrilus* species identified in article I, including the highly damaging emerald ash borer *A. planipennis*. Since the emerald ash borer is native to Far East Asia (China, Taiwan, Japan, and Korea) and is introduced to European Russia (Haack et al. 2002), we also collected export data from these countries.

## Analysis of climatic similarities (Article II)

Because establishment and further spread of nonnative bark- and wood-boring beetles are extremely difficult to observe directly, the beetles' potential geographical range in the invaded region needs to be conceptualized and predicted. In article II, we modelled the potential distribution in Europe of selected *Agrilus* species identified in article I. Maximum entropy species habitat modeling (MaxEnt) (Phillips et al. 2004; Phillips et al. 2006) was chosen because it has been shown to outperform other species distribution modeling (SDM) methods, and because it will accept presence only data and a small number of presence points (Elith et al. 2006). MaxEnt takes a set of environmental variables (such as temperature and precipitation), as well as a set of species occurrence locations, and produces a model of the potential geographical range of the species. We used MaxEnt to estimate the relationship between *Agrilus* occurrence records in North America and the environmental characteristics of those sites to predict the distribution in Europe. All model input data was compiled from freely available sources. *Agrilus* presence data was downloaded from the GBIF database (GBIF 2016), and 19 meteorological bioclimatic variables from the WorldClim database (Hijmans et al. 2005) were used as predictors.

## Terpene and pheromone analyses (Article III)

To determine the chemical suitability of Sitka and Lutz spruce as hosts of the spruce bark beetle we compared constitutive and induced terpene levels in the bark of the two North American species with those in Norway spruce. Constitutive terpene levels were determined in bark samples collected from 10 trees per species. To determine induced terpene levels, the same trees were wounded and treated with the plant hormone methyl jasmonate, and one month later new induced bark samples were collected. Terpenes were extracted from the bark using hexane and quantified using gas chromatograph-mass spectrometry (GC-MS). Terpenes were identified using a customized automated process and quantified relative to an internal standard (pentadecane). The same GC-MS procedure was used to quantify gut contents of cis-verbenol and 2-methyl-3-buten-2-ol (methylbutenol), the two most important components in the aggregation pheromone of the spruce bark beetle. Pheromone sampling from bark beetles guts was done by introducing vigorous beetles into bolts cut from the experimental trees. After 48 hours, tunneling male beetles were removed from the bark, and their hind gut was removed and analyzed by GC-MS to identify and quantify pheromone compounds.

## *I. typographus* and *E. polonica* performance experiments (Article IV)

To assess beetle reproductive performance in the different spruce species, we introduced beetles into cut bolts at a controlled low attack density. Multiple beetles were allowed to colonize each bolt under the cover of glass vials, and after three days the glass vials were removed and each bolt was suspended inside an emergence net with a collection bottle underneath. The bottles were emptied every second week and the emerged beetles were counted and stored at 4 °C. At the end of the experiment the bark was cleared from the bolts, all living beetles remaining under the bark were collected and counted, and the number of beetle galleries was determined. All beetles were dried in an oven, and a random selection of beetles from each bolt were individually weighted and measured lengthwise. Another set of cut bolts from the same trees was inoculated with the beetles' phytopathogenic fungal associate, *Endoconidiophora polonica*. The fungus was left to colonize the bark for 90 days before the outer bark was removed and the length of the necrotic lesions in the inner bark was measured.

# Results and discussion of individual articles

## Potential invasive bark- and wood-boring beetles in deciduous wood chips (Article I)

Increasing import of wood chips to Europe, and the identification of wood chips as a potential pathway for introduction of bark- and wood-boring beetles (McCullouch et al. 2007), represent a challenge for phytosanitary authorities. We defined a procedure to identify beetle species with a potential to invade northern Europe through import of deciduous wood chips from North America. Ten *Agrilus* species where identified as having a potential to be transported with wood chips and become invasive. These species are known to attack European tree species planted in North America, and are small enough to survive chipping and transport. Northern European trees have not co-evolved with these herbivores and are thus likely to lack adequate defenses, providing most of the identified beetle species an opportunity to spread in "defense-free space" (Gandhi & Herms 2010b) in Europe. Beside tree defenses, several other factors may influence the risk of establishment of *Agrilus* species in Europe, such as climatic similarity between North America and northern Europe, propagule pressure and presence of the species along the pathway, Allee effects, interactions with natural enemies and competitors, and the species' capability of spread. More research on these factors could help mitigate risks of introduction. Introduction of *Agrilus* species to northern Europe is likely to incur huge economic costs and considerable ecological consequences. We therefore recommend that preemptive phytosanitary measures should be taken against these species, with PRA-assessments of individual species.

Figure 4. The bronze birch borer *Agrilus anxius* is native to North America but can attack and kill European birch species. Photo: Kent Loeffler, Cornell University.

## Trends in European imports of deciduous wood chips (Article II)

Our analysis of import data showed that over the last decade there has been a significant increase in EU imports of deciduous wood chips from countries where high-risk *Agrilus* species are present, and from the largest trade partner Russia the increase has been exponential (Figure 4). A further increase in import of wood chips to the EU may be expected, as EU aims to use more bioenergy to satisfy future energy demands (EU 2005; UNECEFAO 2009). The new information about potential import pathways and candidate pest species presented in article I and II calls for adjustments to current management procedures to reduce the risk of species introductions. Because eradication usually is impossible once a pest is established the best options are preemptive measures, such as adopting phytosanitary regulations that reduce the likelihood that *Agrilus* species will be present in wood chips. Establishment of new *Agrilus* species in Europe could potentially be extremely damaging and costly. In the United States, management of *A. planipennis,* which also is present in Russia, has an estimated annual cost of 1.7 billion USD (Aukema et al. 2011).

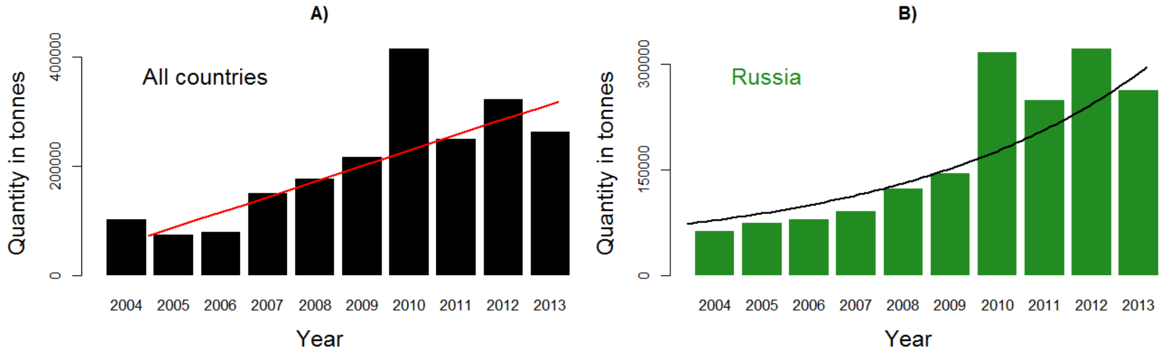

Figure 5**.** Annual quantities of deciduous wood chips exported from eight selected countries outside Europe to the EU over the 10-year period 2004– 2013. The trend line in (A) indicates a linear increase for all countries combined. Export from Russia (B) showed an exponential increase in the same period. Note that the y-axis is scaled differently between figure panels.

## Environmental suitability in Europe for selected *Agrilus* species (Article II)

Our MaxEnt models indicated that large areas in Europe provide high environmental suitability for four potentially very damaging North American *Agrilus* species, particularly in Eastern Europe and European Russia for *A. anxius*, *A. bilineatus* and *A. planipennis* and in southern Europe for *A. politus*. The model predictions of potential *Agrilus* distribution also overlap with the distribution of known and potential host trees in Europe, suggesting that these beetles would be able to establish in Europe if they were introduced with e.g.

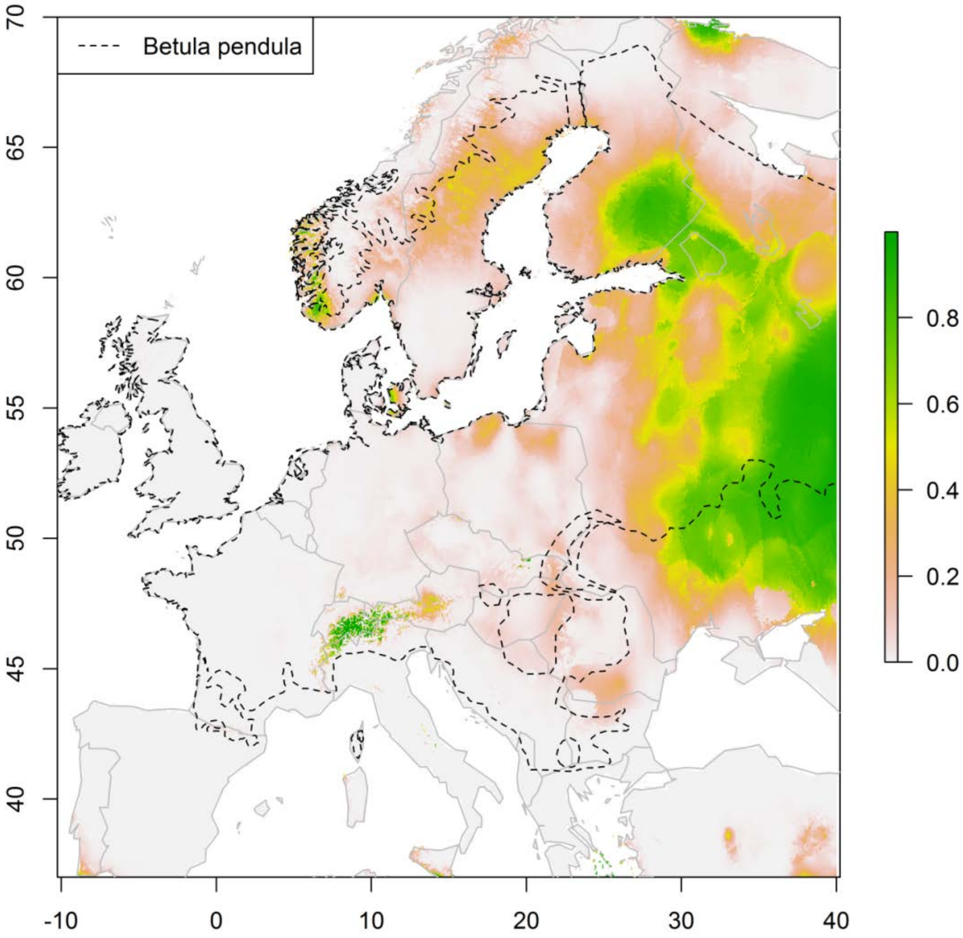

Figure 6. Potential distribution of the bronze birch borer *Agrilus anxius* in Europe predicted by Maximum entropy modeling (MaxEnt). Colors indicate probability of occurrence of the beetle (green = high, white = low) and dashed lines show the distribution of the host tree silver birch *Betula pendula*.

imported wood chips. *Agrilus anxius*, *A. bilineatus* and *A. planipennis* have a high potential distribution in Eastern Europe, including European Russia, Ukraine, Belarus and Moldova, but can also find suitable climate and host trees in parts of Western Europe. The MaxEnt model for the bronze birch borer *A. anxius* (Figure 6) does for example indicate areas of potential distribution along the west coast of Norway. This is an area that previously has received imports of several thousand tons of wood chips from North America containing birch and other tree species. The bronze birch borer, which has a documented ability to kill the two major birch species in Norway (*Betula pubescen* and *B. pendula*), could potentially cause considerable economic and ecological damage if it was introduced to western Norway (Muilenburg & Herms 2012).

## Terpene composition and pheromone production in native and nonnative spruce species (Article III)

Overall, we found relatively small differences in terpene composition between the spruce bark beetle's historic host Norway spruce and the two potentially novel hosts Sitka and Lutz spruce. This suggests that the two North American spruce species may be chemically suitable hosts for the spruce bark beetle. The NMDS ordination plot (Figure 7) illustrates the clear spatial pattern of similarity between the three spruce species, where points that are closer to each other in the ordination space are more similar in terpene chemistry. All spruce species showed a strong induced response in terpene concentrations. This induced response increases the trees' resistance to the spruce bark beetle, but since the response was weaker in Sitka and Lutz spruce than in Norway spruce the novel host trees are likely to be less resistant than the historical host. The spruce bark beetle produced its aggregation pheromone equally well on the two novel hosts as on the historical host. Even though Norway spruce contained more of the pheromone precursor (-)-α-pinene than the two novel hosts, we found no significant difference in beetle production of cis-verbenol between tree species. The production of methylbutenol was also equally high on the two novel hosts as on Norway spruce, indicating that the trees' chemical defenses had a similar effect on beetle physiologically in all tree species.

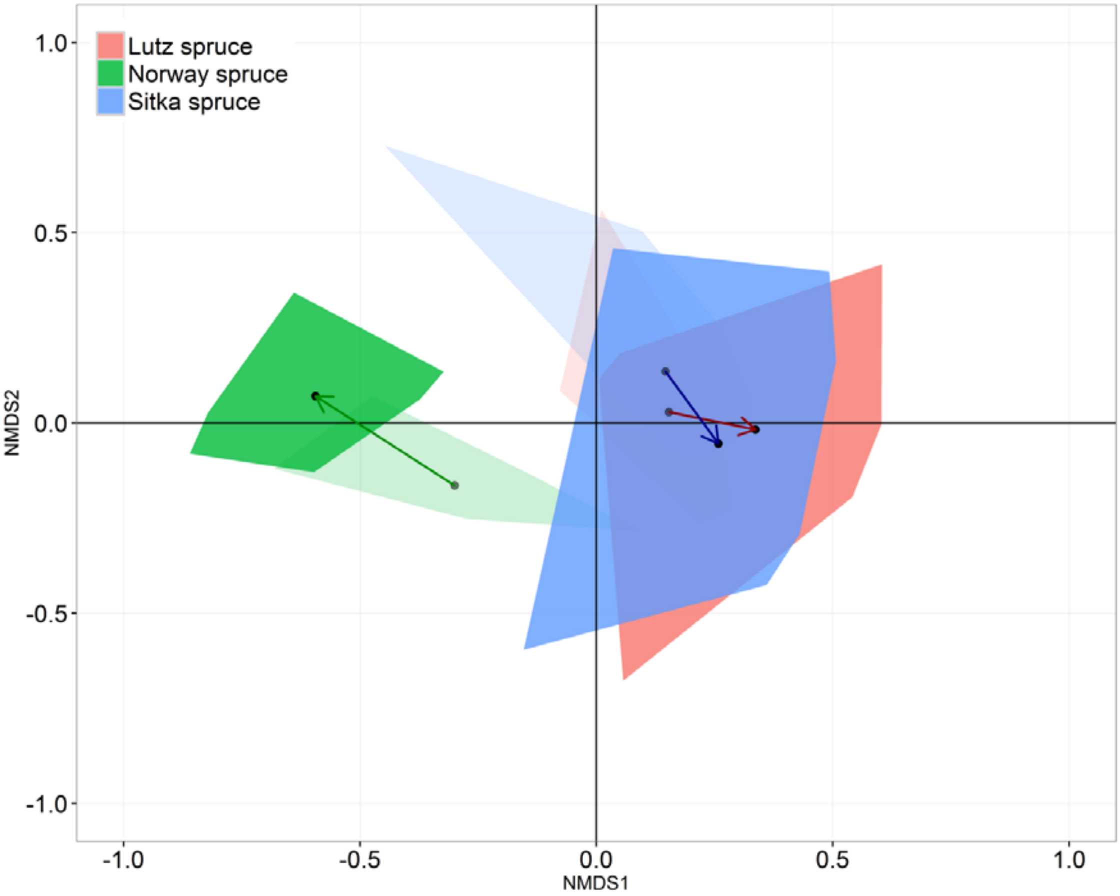

Figure 7. Non-metric multidimensional scaling (NMDS) showing the overall compositional similarity in terpenoids between Sitka, Lutz and Norway spruce in constitutive bark samples (tint color) and induced bark samples (darker color). Black dots indicate mean site scores, and arrows indicate direction of change from constitutive to induced samples.

### Reproduction of *Ips typographus* in native and nonnative spruces (Article IV)

Our controlled breeding experiment showed that the spruce bark beetle could breed successfully in the two novel host trees Sitka and Lutz spruce. Beetle offspring developed equally well in Sitka and Lutz spruce as in Norway spruce in terms of offspring length and weight. However, there was a significant difference between spruce species in number of galleries established and total number of offspring produced: beetles reared on Norway spruce established 5 and 3 times as many galleries as on Sitka and Lutz spruce, respectively, and total offspring production in Norway spruce was 2.7 and 4.7 times higher than in Sitka and Lutz spruce. The fact that offspring quality (i.e. length and weight) was similar across tree species, but that fewer beetles were produced on Sitka and Lutz spruce, indicates that the beetles were less willing or able to establish maternal galleries in the novel hosts. Overall, the results of our breeding experiment suggest that if the spruce bark beetle expands westwards in Norway and encounters plantations of Sitka and Lutz spruce it will be able to successfully reproduce and establish in these novel hosts. Our results also indicate that the beetle would be able to reproduce and establish in native populations of Sitka and Lutz spruce in North America if it was introduced there. However, it is uncertain how the lower offspring productivity in the novel hosts would affect further spread and expansion of the spruce bark beetle in these areas, since the spruce bark beetle seems to need hundreds of propagules to mass-attack trees and establish new viable populations (Liebhold & Tobin 2008).

## Performance of *E. polonica* in native and nonnative spruces (Article IV)

The spruce bark beetles' most phytopathogenic fungal associate, *Endoconidiophora polonica*, did not perform well in Sitka and Lutz spruce in our experiment. Mean phloem necrosis length induced by *E. polonica* in Sitka and Lutz spruce was 11 and 12.2 centimeters 90 days after inoculation, compared with 28.8 cm in Norway spruce. *Endoconidiophora polonica* is thought to assist the beetles in killing trees by exhausting the trees' constitutive and induced defenses. The fungus is a primary colonizer of fresh Norway spruce sapwood and phloem and has been demonstrated to kill trees in experimental mass-inoculations that simulate beetle mass-attacks (Krokene & Solheim 1996). Without the same assistance from *E. polonica* in breaking down tree resistance it might be more difficult for the spruce bark beetle to establish and spread in Sitka and Lutz forests. However, it is difficult to predict how critical this will be, since the beetle has many other fungal associates that may contribute to break down tree defenses in these hosts (Krokene & Solheim 1996; Linnakoski et al. 2016).

## Overall conclusions within a PRA framework

The main objective of the work included in this thesis was to strengthen the scientific foundation for future phytosanitary regulations and policies for bark- and wood-boring insect pests representing a high risk of forest damage in Norway. Our approach was to address this objective within the framework of the four key PRA questions: (1) the probability of pest arrival, (2) the probability of pest establishment, (3) the probability of spread and expansion of the pest, and (4) the potential ecological and economic impacts of the pest. The first three questions address the main steps in the biological invasion process, while the fourth addresses the outcome of interactions between the invasive organism and its new environment. In this concluding chapter I will show how our main findings relate to the four PRA questions, before I summarize the management implications of our findings.

To reduce the probability of arrival of nonnative species we must identify emerging import pathways like wood chips as possible threats and characterize the associated risks. Proper risk characterization is then the basis for further actions such as pest risk analysis of specific species. The probability of arrival of bark- and wood-boring insects is naturally limited by a series of filters the species needs to pass through, such as the ability to colonize the bark and sapwood of living trees (Liebhold et al. 2012). Invasive species must also have behavioral, physiological, and morphological traits that favor survival through transport (McCullouch et al. 2007). In article I we identified 10 *Agrilus* species or subspecies that should be risk assessed because they have the ability to colonize living trees and probably may enter northern Europe through import of deciduous wood chips from North America. These *Agrilus* species also have behavioral, physiological, and morphological traits that favor survival through transport of raw materials for production of chipped wood, the chipping process itself, and transport of chipped wood (article I).

The probability that nonnative species will establish once they have arrived in a new environment also depends on multiple factors, such as the ability to overcome abiotic environmental barriers at the site of introduction, as well as reproduction barriers and local dispersal barriers (Liebhold & Tobin 2008). The broad distribution in North America of the selected *Agrilus* species identified in article I suggests that they may be pre-adapted to establish across a wide geographic range in Europe as long as suitable hosts are present. This hypothesis is supported by our species distribution models in article II, showing that four *Agrilus* species may find suitable climatic conditions in large parts of Europe where they also will find potential hosts. In the case of the spruce bark beetle the suitability of widely planted nonnative conifer species as hosts for the beetle suggests that the beetle should be able to establish in new areas given the right climatic conditions.

The process of spread and range expansion of nonnative and native species into new areas can be considered in several ways. Spread is traditionally thought of as the continuous dispersal of a species coupled with population growth, a process called diffusion (Skellam 1951). However, transportation of *Agrilus* species in wood chips by humans between North America and Europe can also be considered as an extreme form of spread (Liebhold & Tobin 2008). Because the *Agrilus* species identified in article I has documented ability to attack and kill European host trees, and because their predicted geographic distribution in Europe overlaps with the distribution of the host trees, these *Agrilus* species will probably be able to spread within Europe. A different aspect of spread is the natural range expansion of native species into new areas in response to e.g. climate change. Such range expansion by the European spruce bark beetle into Sitka and Lutz dominated spruce forests in Europe appears likely, since the spruce bark beetle is able to reproduce (Article IV; Økland et al. 2011) and produce its aggregation pheromones in these North American species (Article III). Also, if the spruce bark beetle was to be introduced to native North American Sitka and Lutz forest it would probably be able to reproduce and spread through diffusion.

Novel insect-host tree interactions can be very damaging and cause severe ecological and economic impacts, as exemplified by the complete mortality caused by the North American *Agrilus anxius* when it attacks European birch species (Muilenburg et al. 2011). An important aspect of such severe ecological and economic impacts is that nonnative insects may have unpredictable and fatal effects on the host three populations. The trees will often be more vulnerable to attack because they do not share a co-evolutionary history with the attacking insect, i.e. they are evolutionary naïve (Lymbery et al. 2014). Because North American Sitka and Lutz spruce were not dramatically different from Norway spruce in terpene composition (Article III) but had significantly lower terpene volumes these novel host species may be more susceptible than Norway spruce to attack by the spruce bark beetle. In addition, the fact that the beetle successfully produces its aggregation pheromone in the North American hosts suggests that the spruce bark will be able to mass attack and kill these species (Article III), with possible severe negative economic and ecological impacts on Sitka and Lutz forests in both Europe and North America. These findings are important for future Lutz and Sitka spruce management in Europe, where a climate induced range shifts of the spruce bark beetle into Sitka and Lutz spruce dominated forests may be expected in the future. Also, if the spruce bark beetle becomes established in North America, where it has been intercepted repeatedly but has not yet established (Haack 2001), it will probably be able to mass attack these spruce species in their native ranges. However, the reduced offspring production we observed on Sitka and Lutz spruce might negatively affect spruce bark beetle establishment and expansion, since the beetle seems to need hundreds of propagules to establish a new population (Liebhold & Tobin 2008; Schlyter & Anderbrant 1989).

This thesis highlights the importance of and need for international phytosanitary management to prevent spread of bark- and wood-boring beetles between countries and continents. International trade in plants for planting and wood products is a major pathway for the introduction of invasive bark- and wood-boring beetles (Liebhold et al. 2012), resulting in extensive ecological and economic damage (Aukema et al. 2011). Many of the most damaging bark- and wood-boring beetles that have been introduced around the world were not known to be harmful and the pathways were unregulated before they invaded, highlighting the need for more basic ecological research on potentially harmful organisms and pathways. Because detection of bark- and wood-boring beetles is difficult and eradication of established pests is almost impossible (GAO 2006; Økland et al. 2012) the best management options are preemptive measures, such as reducing the risk of arrival of *Agrilus* species through phytosanitary regulations. For example, work by Økland et al. (2012) influenced European phytosanitary regulation requirements on imports of wood chips to the EU, and since 2014 imported wood chips must originate from countries known to be free of *Agrilus anxius*, i.e. excluding imports from North America (EU 2014). The phytosanitary systems must be continually improved, and there is a need for more international data sharing and research collaboration on actual and potential invasive species. This include efforts to develop tools for assessing the probability that a given species may become invasive, as well as improved methods for early detection of invasive species. Finally, because bark- and wood-boring beetles will expand their range in response to changing environmental conditions such as a warming climate, it is important to monitor latitudinal and altitudinal redistribution of species and changes in their outbreak dynamics within countries and continents.

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# Paper I

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REVIEW ARTICLE

# Importing deciduous wood chips from North America to northern Europe – the risk of introducing bark- and wood-boring insects

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Increasing inter-continental trade with wood chips represents a challenge for phytosanitary authorities as such trade may lead to pest introductions and invasions with huge impacts on forest ecosystems and economy. Predicting species invasions and their impacts in advance may be difficult, but improved information about potential invasive species ahead of any interceptions is an important precautionary step to reduce the probability of invasions. Here we identify bark- and wood-boring insects that have a potential to become invasive in northern Europe and that may be introduced by import of deciduous wood chips from North America. The potentially most damaging species belong to the beetle genus *Agrilus* (Buprestidae), which includes the highly damaging emerald ash borer *A. planipennis*. We give a brief presentation of this and seven other *Agrilus* species or subspecies and review factors of importance for the risk of establishment and potential economic and ecological impacts of these species. We also discuss one Scolytinae, *Hylurgopinus rufipes*. There are strong indications in the literature that some north European trees are highly susceptible to attack from the selected beetle species. We therefore conclude that because north European trees have not coevolved with these herbivores and thus may lack adequate defenses, most of the identified beetle species are likely to spread in "defense- and enemy-free space" if they are introduced to northern Europe, with considerable economic and ecological consequences.

**Keywords**: naïve hosts; wood-boring insects; *Agrilus*; invasive species; phytosanitary risk

## Introduction

Introduction and establishment of species beyond their native range can lead to high economic costs and severe ecological damage (Brockerhoff et al. 2006b; Kettunen et al. 2008; Liebhold & Tobin 2008; Pimentel et al. 2000). Insects that bore into the bark and wood of living trees may severely impact ecosystem structure and function due to the ability of some species to kill healthy trees (Kenis et al. 2009; Gandhi & Herms 2010; Økland et al. 2011). Between 1980 and 2006, bark- and wood-borers accounted for 56% of all new insect species detected in the United States (Aukema et al. 2010). There are now more than 400 introduced species attacking woody plants in the United States alone (Liebhold et al. 1995; Mattson et al. 2007; Langor et al. 2008; Aukema et al. 2010), and a new high-impact forest pest is predicted to establish every 5 to 6 years (Koch et al. 2010). Europe has 109 nonindigenous insect species established on woody plants, including 57 species from North America and 52 from Asia (Mattson et al. 2007). A complicating factor is that species introductions often go undetected for a long time, with a lag phase that can last for years until the population suddenly grows rapidly (Mack et al. 2000).

The major pathways for introduction of bark- and wood-boring insects are trade with living plants and various commodities of non-squared wood, including wood chips (EPPO 2009; Liebhold et al. 2012). The import of wood chips to Europe is expected to increase due to growing demands for energy production (EPPO 2011b). Wood chips may be large enough to allow survival of several insect species (McCullough et al. 2007) and represent a challenge for inspectors. Økland et al. (2012) did for example show that insect detection with 90% certainty from a ship load of ~21,000 metric tons of wood chips requires a sampling volume of 27 million liters. Furthermore, phytosanitary regulations have not been updated to accommodate the recent increase in imports of biomass for bioenergy production (Kopinga et al. 2010). Import of coniferous wood chips is regulated by the European Union and several European countries to prevent the spread of the pinewood nematode, *Bursaphelenchus xylophilus*. However, import of deciduous wood chips to Europe is still largely unregulated, and except for a few species (EPPO 2005; EPPO 2011a), little is known about forest pests that could be transported from North America to Europe in these large import volumes.

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So far, no major tree-killing insects on deciduous trees have been introduced to northern Europe, but, for example, the bark beetles *Xyleborinus alni*, feeding on recently dead or dying *Salix* spp., and *Cyclorhipidion bodoanus*, feeding in hollow *Quercus* trees, have been recorded on deciduous trees in Sweden (Lindelöw et al. 2006; Lindelöw 2009). Bark- and wood-boring beetles are regularly intercepted all over the world (Humble & Allen 1998; Lindelöw 2000; Haack 2001, 2006; Brockerhoff et al. 2006a), and the EPPO (2012) reporting service frequently reports interceptions of non-indigenous bark- and wood-boring beetles from different wood commodities in Europe. The arrival and establishment of insect pests capable of killing healthy deciduous trees in Europe seem likely, due to increasing international trade volumes. One illustrative case is the recent establishment of the East-Asian emerald ash borer *Agrilus planipennis* in the Moscow region (Baranchikov et al. 2008). This species has also been introduced in North America where it exclusively attacks ash, has killed millions of trees during the last 10 years, and is threatening several endemic ash species and the functioning of whole forest ecosystems (Poland & McCullough 2006; Gandhi & Herms 2010).

In this paper, we followed a defined procedure to identify bark- and wood-boring insects with a potential to invade northern Europe (Fennoscandia, the Baltic states, and northern provinces of European Russia) through import of deciduous wood chips from North America. Wood packaging material (WPM) was not considered here, because it is already regulated through ISPM 15 (Food and Agriculture Organization 2009). We restricted our focus to bark- and wood-boring beetles, since beetles are a major and relatively well-studied group of tree-boring insects. We give a brief presentation of the biology of each identified species and review factors that may influence the risk of establishment and potential economic and ecological impacts.

## Identification of high-risk species

We used the following criteria to identify bark- and wood-boring beetles to be included:

(1) The species should not be present in Europe, so only species that are endemic to North America or are introduced to North America from areas other than Europe were included.
(2) The species should be abundant, widely distributed, and have a northerly distribution within North America to increase the likelihood that they will be adapted to the climatic conditions in northern Europe. The geographic area of origin for potential invaders was further narrowed down to eastern North America (east of the 100th meridian, as defined by Baker 1972), since this is the most important area for export of wood chips to northern Europe.
(3) The species should be associated with a host tree in a genus with representatives in northern Europe, since insects that are invading new areas usually colonize hosts within the same genus as in their native range (Mattson et al. 1994; Niemela & Mattson 1996; Roques et al. 2006; Mattson et al. 2007). We included all North-American deciduous tree genera that occur in Fennoscandia, which is a representative region for the tree species composition in northern Europe (Table 1).
(4) The insect species should be a pest in its native range and be able to colonize the bark and sapwood of the trunk of living trees. To find bark- and wood-borers that are pests on the tree genera selected in (3), we searched the literature (Craighead 1950; Browne 1968; Baker 1972; Johnson & Lyon 1976; Ives & Wong 1988; Mattson et al. 1994; Solomon 1995), cross-checked with relevant databases (ISPI 2009; GISD 2012; NAFC-ExFor 2012; NAPIS 2012; U.S. Forest Service 2012; Bugwood.org 2013; EPPO 2013) and consulted experts on North American bark- and wood-borers.
(5) The species should have behavioral, physiological, and morphological traits that favor survival through transport of raw materials for production of chipped wood, the chipping process itself, and transport of chipped wood. We searched the literature for information about survival rates in wood chips and physical dimensions of egg, larva, pupa, and imago.

After the first screening of the literature using criteria 1 to 3, the initial list contained 50 beetle species. Following criteria 4 and 5, we ended up with nine bark- and wood-boring beetle species or subspecies satisfying the selection criteria. The most important reasons for excluding species were that they were not regarded as pests, had a southerly distribution, were not considered established in North America, or most importantly, were too large to likely survive in wood chips. Eight of the selected species belong to the genus *Agrilus* (Buprestidae) and one to Scolytinae (*Hylurgopinus rufipes*). Except for the East-Asian species *A. planipennis*, all are native to North America. In *Agrilus*, the size of prepupae and pupae and the timing of pupation in the outer sapwood facilitate survival through the wood chipping process. Furthermore, survival through chipping has been experimentally documented for one of the species; McCullough et al. (2007) showed that *A. planipennis* prepupae can survive the chipping process with a 10-cm screen. The dimension of the different life stages of *A. planipennis*, including the

Table 1. Bark- and wood-boring beetles discussed in this paper, their tree hosts, distribution area in North America, and length of their life stages.

| Species name | Genera prone to attack | Host tree species in north America | Known host tree species appearing in Fennoscandia | Other potential host tree species appearing in Fennoscandia | Pest distribution | Egg mm | Larva mm | Pupa mm | Imago mm |
|---|---|---|---|---|---|---|---|---|---|
| *Agrilus anxius* | *Betula* | *B. alleghaniensis*<br>*B. lenta*<br>*B. occidentalis*<br>*B. papyrifera*<br>*B. populifolia* | *B. maximowicziana*<br>*B. pendula*<br>*B. pubescence*<br>*B. szechuanica* |  | All southern Canadian provinces, Alaska and Contiguous US except 12 southern states (Muilenburg & Herms 2012). | 1.3–1.5 | 25 | NA | 6–12 |
| *A. bilineatus* | *Castanea*<br>*Quercus* | *C. dentata*<br>*Q. alba*<br>*Q. coccinea*<br>*Q. ellipsoidalis*<br>*Q. macrocarpa*<br>*Q. prinus*<br>*Q. stellate*<br>*Q. velutina* | *Q. rubra* | *Q. petraea*<br>*Q. robur* | Southeastern Canada. Eastern and Central US westwards to Texas and the Rocky Mountains (Haack & Acciavatti 1992). | 1 | 25 | NA | 6–12 |
| *A. g. granulatus* | *Populus* | *P. deltoids*<br>*P. trichocarpa* | *P. nigra* | *P. alba*<br>*P. balsamifera*<br>*P. tremula* | From New York south to North Carolina and Louisiana, westwards to Colorado, Montana and southern Alberta, Canada (Solomon 1995). | NA | 27–40 | NA | 7–11 |
| *A. g. liragus* | *Populus* | *P. deltoides.*<br>*P. grandidentata*<br>*P. tremuloides*<br>*P. trichocarpa* | *P. balsamifera* | *P. alba*<br>*P. nigra*<br>*P. tremula* | New Brunswick to British Columbia. South to Pennsylvania and Arizona (Bright 1987). | 1.2 | 30–40 | NA | 7.2–10.3 |
| *A. horni* | *Populus* | *P. grandidentata*<br>*P. tremuloides* | *P. alba*<br>*P. tremula* | *P. balsamifera*<br>*P. nigra* | From Massachusetts to Arizona in the US, north to Ontario and Aweme, Manitoba in Canada (Bright 1987). |  |  |  |  |
| *A. planipennis* | *Fraxinus* | *F. americana*<br>*F. nigra*<br>*F. pensylvanica* | *F. angustifolia*<br>*F. excelsior* |  | Invasive in 19 US states (Kansas, Minnesota, Iowa, Missouri, Wisconsin, Michigan, Illinois, Indiana, Kentucky, Tennessee, Ohio, West-Virginia, Virginia, Pennsylvania, New York, Connecticut, Maryland, Massachusetts, New Hampshire; USDA 2013), and the Canadian provinces of Ontario and Quebec (CFIA 2013). | 1 | 30–36 | 13–17.5 | 13 |
| *A. politus* | *Acer*<br>*Salix* | *A. glabrum*<br>*A. pensylvanicum* |  | *A. campestre*<br>*A. platanoides* | Transcontinental across Canada and throughout the United States (Bright 1987). | NA | NA | NA | 5–8.5 |

Table 1 (*Continued*)

| Species name | Genera prone to attack | Host tree species in north America | Known host tree species appearing in Fennoscandia | Other potential host tree species appearing in Fennoscandia | Pest distribution | Egg mm | Larva mm | Pupa mm | Imago mm |
|---|---|---|---|---|---|---|---|---|---|
| | | *S. babylonica*<br>*S. lasiolepsis*<br>*S. lucida*<br>*S. scouleriana* | | *A. pseudoplatanus*<br>*A. rubrum*<br>*S. caprea* | | | | | |
| *A. pensus* | *Alnus*<br>*Betula* | *A. rugosa*<br>*B. nigra* | | *A. glutinosa*<br>*A. incana*<br>*B. maximowicziana*<br>*B. pendula*<br>*B. pubescence* | Manitoba to Nova Scotia, south to Pennsylvania and New Jersey (Bright 1987). | 1.2 | NA | NA | 7.5 |
| *Hylurgopinus rufipes* | *Ulmus* | *U. americana*<br>*U. pumila*<br>*Fraxinus sp.* | | *U. glabra* | Throughout the eastern US north of Mississippi, west to North Dakota. In Canada, from New Brunswick to Manitoba (Solomon 1995) | NA | 3–5 | NA | 2–3.5 |

pupal stage, was summarized by Chamorro et al. (2012), and is presented in Table 1. Maximum larval and imago lengths for the selected species are 25–40 mm and 7–11 mm, respectively, with *A. planipennis* being the longest species. Pupal size is not available for the other *Agrilus* species, but as they have smaller larvae, imagines, they probably also have shorter pupae than *A. planipennis* and should thus be well suited to survive chipping.

Besides *H. rufipes*, there are other candidates of small bark and ambrosia beetles in the curculionid subfamilies Scolytinae and Platypodinae (Wood 1982; Wood et al. 1992) that may be imported by wood chips and that has been encountered in other wood materials at ports of entry (Brockerhoff et al. 2006a; Haack 2006). Due to the extensive number of species, their complex biology, and the lack of documentation on survival in wood chips and attack on European tree species, we limited our selection to *Agrilus*, where survival in wood chips has been documented (McCullough et al. 2007), and the one candidate among the small scolytids that is known to kill deciduous trees in eastern North America (Baker 1972).

## Presentation of the selected wood-borers

The selected *Agrilus* species share many morphological characteristics and have similar life cycles. The larvae make typical zig-zag–shaped tunnels in the phloem and need to feed on living or dying phloem to develop (Bright 1987; Anderson 1944). Before pupation, the larvae usually enter the outer sapwood, and the larvae of the alder birch borer *Agrilus pensus* can even bore through the stem from one side to the other (Carlson & Knight 1969). All the selected *Agrilus* species make a D-shaped exit hole on the stem and feed on various tree species for a period during their adult life. During mating, the males hover around leaves of host trees in their visual search for females and use contact pheromones to determine species and sex of potential partners (Lelito et al. 2007; Domingue et al. 2011; Lelito et al. 2011). Many *Agrilus* species have high fecundity; *A. planipennis* can lay 200 eggs (Rutledge & Keena 2012), and females of the bronze birch borer *Agrilus anxius* mate several times and may lay 375 eggs (Claire Rutledge, personal communication). The morphology and biology of the native elm bark beetle, *H. rufipes*, differs from the *Agrilus* species. *H. rufipes* constructs egg galleries in the bark, in which the larvae also pupate (Baker 1972). Winter is spent under the bark either as adults or larvae. *H. rufipes* is the primary vector in North America of the devastating Dutch elm disease (DED), which is caused by the fungus *Ophiostoma novo-ulmi* (McLeod et al. 2005). Adults can feed on healthy elms and inoculate them with DED. All the species presented below are widely distributed throughout eastern North America and occur in a wide range of forest environments.

Bronze birch borer *Agrilus anxius* (Gory 1841) is a major pest of *Betula* spp. and can have expansive periodic outbreaks (Muilenburg & Herms 2012). The larvae need living phloem to develop (Anderson 1944), and field tests have shown that European birch species may suffer 100% mortality if attacked by *A. anxius* (Nielsen et al. 2011). The life cycle is completed in one or two years, depending on climate and host condition. Fourth instar larvae construct pupal cells in the outer sapwood, where they must overwinter and be exposed to freezing temperatures before they pupate in April to May. Adults emerge from the stems between May and July, at about 305 degree-days (base temperature 10°C, starting date January 1) in Ohio and Michigan (EPPO 2011a).

*A. anxius* was added to the EPPO alert list in 2010 (EPPO 2010), and a pest risk analysis was completed in 2011 (EPPO 2011a). A pest risk assessment of *A. anxius* was performed by The Norwegian Scientific Committee for Food Safety (VKM 2012) in 2012, which supported the EPPO (2011a) pest risk analysis. The EPPO (2011b) pest risk analysis concluded that there was a moderate probability of entry of *A. anxius* into Europe, a high probability of establishment, a risk of spread, and high ensuing mortality of birch throughout the PRA area with major economic consequences. The main pathway of concern for introduction into EU and Scandinavia is chipped deciduous wood. Ornamental trees, lumber, and firewood also represent a risk (EPPO 2011b).

Emerald ash borer *Agrilus planipennis* (Fairmaire 1888) is native to Far East Asia, where it is not considered a major pest on native trees (Rebek et al. 2008). The species has been introduced to North America and to the Moscow region in Russia (Baranchikov 2007; Baranchikov et al. 2008). *Agrilus planipennis* mainly kills *Fraxinus nigra*, *F. pennsylvanica*, and *F. americana* in North America, and mainly *F. pennsylvanica* in the Moscow region, but *F. excelsior* is also very susceptible (Baranchikov et al. 2008, 2009). The abundance of *Fraxinus* increases towards southern Europe, where two other potential hosts are present, *Fraxinus angustifolia* and *F. ornus*. The life cycle of *A. planipennis* is completed in one or two years depending on climate and host condition, and adults are active between May and July (Wang et al. 2010). Attacks start in the canopy and trees usually die within three years (Poland & McCullough 2006; Wessels-Berk & Scholte 2008).

*Agrilus planipennis* is on the NAPPO list of quarantine pests for both USA and Canada (NAPPO 2013). It was added to the EPPO alert list in 2004, and a pest risk analysis was performed in 2003 (EPPO 2005). The main pathways for introduction are plants for planting (including bonsai), untreated wood, and wood packing material EPPO (2003a, 2003b). Firewood and wood chips are also possible pathways (Haack et al. 2010).

Twolined chestnut borer *Agrilus bilineatus* Weber, 1801 is the principal pest of *Quercus* spp. and *Castanea dentate* in North America and is reported to kill trees (Muzika et al. 2000). It primarily hastens the death of stressed trees, but may kill apparently healthy trees when population densities are high. The larval tunnels in the phloem may girdle the trunk and disrupt nutrient transport (Dunbar & Stephens 1976; Bright 1987). Attacks start in the canopy and trees may die within two to three years (Haack & Acciavatti 1992).

Granulated poplar borer *Agrilus granulatus* Say, 1823 is common in stressed native and planted *Populus* species in North America (Bright 1987; Solomon 1995). It attacks trees that are severely weakened by drought, disease, or winter injury and prefers trees growing in poor site conditions. The larva bore in the phloem and sometimes into the outer sapwood. The lifecycle is completed in one to two years. Morphologically this subspecies may be confused with the subspecies *A. granulatus liragus* (see below), but they have slightly different host preferences and are usually treated separately in the literature.

Bronze poplar borer *Agrilus granulatus liragus* Barter & Brown, 1929 attacks stressed, injured, and dying *Populus* species in North America. It prefers living phloem (Bright 1987) and causes decline and frequently death of the host (Barter 1965; Ives & Wong 1988). Attacks may spill over from weakened to healthy trees when population densities are high (Bright 1987). The life cycle is completed in one to two years, depending on temperatures and host vigor.

Aspen root girdler *Agrilus horni* Kerremans, 1900 attacks stressed and apparently healthy suckers of *Populus* spp. According to Nord et al. (1965), infestation by *A. horni* leads to the certain death of attacked suckers, especially in hybrid aspen plantations. On the experimental plots reported in Nord et al. (1965), the Eurasian species *Populus alba*, *P. tremula*, and various hybrids were most susceptible. Unlike other *Agrilus* species, *A. horni* larvae first bore down into the root phloem before they turn around and spiral upwards in the stem phloem. They pupate in the stem. The life cycle is assumed to be completed in two or more years.

Common willow agrilus *Agrilus politus* Say, 1825 mainly attacks *Salix* spp. and *Acer* spp. but is reported from several other genera as well, such as *Quercus* spp., *Corylus* spp., and *Alnus* spp. (Bright 1987; Solomon 1995). The damage inflicted on the hosts may be significant, but *A. politus* is considered to be of little economic importance in North America (Bright 1987). *A. politus* is the most widespread and polyphagous of the *Agrilus* species treated here, suggesting that it has substantial ecological plasticity and adaptability to a wide range of forest environments.

Alder birch borer *Agrilus pensus* Horn, 1891 attacks stressed *Alnus rugosa* and *Betula nigra* trees. According to Solomon (1995), *A. pensus* plays a more primary role in tree killing than its near *Agrilus* relatives. Similarly, Carlson and Knight (1969) state that *A. pensus* is a more significant tree killer than *A. g. liragus* and *A. anxius*. *Agrilus pensus* oviposits only in living trees or branches, although it seems likely that the host is under some type of stress (Bright 1987).

Native elm bark beetle *H. rufipes* Eichhoff, 1868 prefers *Ulmus* spp., mainly American elm (*Ulmus americana*) and Siberian elm (*Ulmus pumila*) (Anderson & Holliday 1999; McLeod et al. 2005), but is also registered as a minor pest on common elm (*Ulmus glabra*) (EPPO 2013). It has been reported attacking *Fraxinus* spp. (Baker 1972), but species-specific information on *Fraxinus* is lacking in the literature. This species attacks stressed trees, but summer-emergent adults are attracted to and feed on healthy elm trees (Swedenborg et al. 1988). *H. rufipes* populations can grow large during drought periods, when the beetles aggressively attack healthy trees.

## Factors that may influence the risk of establishment

For a species to become invasive it needs to overcome a sequence of biotic and abiotic barriers limiting the probability that it will survive and establish a new population (Richardson et al. 2000). Biological characteristics of the species and the biotic and abiotic nature of its new environment will determine how likely it is to pass successfully through all these barriers. For most of the selected species we do not have sufficient information to do a thorough assessment of the prospects of successful establishment, so we will instead apply the general literature on species invasion to discuss the importance of the different barriers for our selected species.

### *Climatic similarity*

Differences in climate are unlikely to stop the selected species from becoming established in northern Europe. There are many similarities between the climates of eastern North America and Europe, particularly within the continental and oceanic climate regions. As an example, both *A. anxius* and *A. planipennis* have expanded their ranges vastly within the continental climate zones. The two areas where *A. planipennis* first established in North America (Michigan) and Europe (Moscow region) have similar climates (precipitation and temperature) according to Köppen-Geiger climate maps (Peel et al. 2007). The very broad distribution of many of the selected species, especially *A. anxius*, *A. politus*, and now also *A. planipennis*, suggests that they may be adapted to colonize a wide geographic range in Europe and perhaps Asia, as long as suitable host species are present. Northern Europe lies further north than most of the source areas in North America and will thus have a different photoperiodic regime. This could influence the establishment success of species and might be one of several factors explaining why there are relatively few invasive bark- and wood-borers in northern Europe.

### *Presence of the species along the pathway*

Several characteristics of the selected species make it likely that living individuals will be present in the commodity pathway all the way up to arrival of wood chips in northern Europe. *Agrilus* beetles may be present in the outer sapwood of their host trees at any time of the year and are difficult to detect by visual inspection. Bark- and wood-boring beetles are usually more abundant in stressed and weakened standing trees and are thus likely to be present in wood typically used for wood chip production. Wood chips are often produced from low-quality wood such as damaged trees, salvage harvesting, or logging residues that do not meet the quality demands for lumber (Hall 2002). Even if better qualities were to be used it would be difficult to avoid the presence of bark- and wood-boring species, because they may attack apparently healthy trees and because of the large volumes that are logged by forest harvesters.

After harvesting, the wood is either chipped on site or transported to a chipping facility or port, where chips are either stored in piles or shipped directly. The survival rate of beetle pupa during chipping is influenced by the chipping screen size. The size of wood chips exported from North America corresponds well with a rather coarse screen size of 10 cm (Økland et al. 2012), which is known to allow survival of *Agrilus* pupae (McCullough et al. 2007). Simulation modeling performed by Økland et al. (2012) indicates that the chipping screen must be reduced to 6 mm or less to ensure that no *Agrilus* species will survive the chipping process. There is no ISPM standard for deciduous wood chips, and every national regulation of wood chips allows for larger than 6 mm chip thickness, including the regulations of more restrictive countries such as New Zealand (MAF 2003).

During storage and transport of wood chips, many organisms will usually die due to heat development resulting from fermentation. However, individuals may still survive in parts of the piles where temperatures stay below lethal levels or in cases where excessive heat development does not occur. On arrival to the production site, the wood chips are unloaded and stored in the open or in silos for up to several months. Most propagules, especially eggs and larva, usually die along the commodity pathway, but some prepupa, pupa, and adults may survive chipping, storage, and transport (McCullough et al. 2007). Even with a low survival rate the numbers of survivors may be significant in large shiploads.

### *Propagule pressure and Allee effect*

The number of individuals required to establish a new population varies from species to species and with environmental conditions. For instance, the spruce bark beetle *Ips typographus* seems to need hundreds of propagules (Liebhold & Tobin 2008), while it is assumed that the citrus longhorn beetle *Anoplophora chinensis* only needs a couple of individuals (van der Gaag et al. 2008). High propagule pressure is undoubtedly an advantage for successful establishment since it lowers the probability of extinction from stochastic events and reduces the effects of inbreeding depression and inverse density-dependent (e.g. Allee) effects (Colautti, Grigorovich et al. 2007; Lockwood et al. 2005). The propagule pressure of bark- and wood-boring insects is difficult to estimate directly because the propagules are hidden inside the host tree. However, the volume of the commodity pathway can be considered as a proxy for propagule pressure (Levine & D'Antonio 2003). The import volumes of chipped deciduous wood into Europe are increasing to meet the EU's target of obtaining 20% of its energy consumption from renewable sources by 2020 (Lins 2004). In addition to the large overall import volumes involved, wood materials usually also harbor large amounts of insects per unit volume.

In small populations resulting from low propagule pressure mate finding may be difficult, resulting in a low probability of establishment (Drake 2004; Haack 2006; Brockerhoff et al. 2006a, 2006b). The importance of Allee effects for invasive species appears to differ significantly between insect taxa. For example, *Ips typographus* has been frequently intercepted in US ports but has consistently failed to establish, probably due to a combination of low propagule pressure and Allee effects related to mate finding and the need to mass-attack host trees (Liebhold & Tobin 2008). For *A. planipennis*, there are indications that Allee effects are not important, since the species has established itself efficiently in both Michigan, USA and the Moscow region. Furthermore, since its introduction to North-America in 2002, it has spread quickly to 19 US states (USDA 2013) and two Canadian provinces (CFIA 2013). We lack information about establishment and spread of other *Agrilus* species in new environments, but based on the similarities in their biology we may assume that they, like *A. planipennis*, may be weakly influenced by Allee effects.

### *Species interactions*

Positive or negative interactions with other species are not believed to be crucial for establishment of North American *Agrilus* species in northern Europe. Parasitism and other negative species interactions, such as competition, predation, and disease, tend to be highly species specific, and insects that are freed from these negative interactions can be said to experience "enemy-free space" in their new environment. This will usually increase their fitness and can result in uncontrolled population growth (Keane & Crawley 2002; Colautti, Ricciardi et al. 2004). There is little information on parasitism for our selected species, but it seems likely that they will benefit from the loss of negative interactions (i.e. the enemy release hypothesis). For example, in its native range in Asia *A. planipennis* has several known potential competitors (e.g. the bark beetles *Hylesinus cholodkovskyi*, *H. laticollis* and *H. fraxini*) and parasitoids (e.g. *Oobius agrili*, *Tetrastichus planipennisi*, *Spathius depressithorax*, and *S. generosus*) (Liu et al. 2007), whereas in North America it has only one known parasitoid, *Atanycolus cappaerti* (Cappaert & McCullough 2009) and in Russia there are so far no known parasites. Generalist predators like woodpeckers are known to forage for *A. planipennis* and other *Agrilus* species but are probably not efficient enough to hinder establishment.

Invasive species may be promoted by positive species interactions in new environments (Økland et al. 2009, 2011; Lu et al. 2011). Although this may be less important for the selected *Agrilus* species, which do not engage in group attacks and do not have fungal mutualists involved in host tree colonization, *H. rufipes* has a mutualistic relationship with DED in North America and will also benefit from this in areas where DED is present in northern Europe. *H. rufipes* did little damage in North America before DED was introduced but picked up the pathogen and became one of the main vectors of the disease. *H. rufipes* is attracted to and oviposits in trees infected by fungi and especially trees infected by DED (McLeod et al. 2005). When adults that have emerged from infected trees feed on healthy elms they inoculate the trees with the disease. If introduced, *H. rufipes* may become an efficient vector of DED in Europe, and particularly in northern Europe were the current DED vectors seem to be relatively inefficient (Solheim et al. 2011).

### *Naïve hosts*

Local tree species tend to be adapted to attacks from local insects, but if the same insects attack so-called naïve hosts (i.e. hosts without a co-adapted history with the insect), the trees may succumb. Because naïve hosts often lack effective defenses against novel herbivores, they can be said to represent "defense-free space" for the herbivores (Gandhi & Herms 2010; Raupp et al. 2010). There are many examples showing that naïve hosts are preferred by invasive insects (Bryant et al. 1994; EPPO 1999; Glynn & Herms 2004; Bertheau et al. 2010;

Cudmore et al. 2010; Desurmont et al. 2011; Hulcr & Dunn 2011), and polyphagous insects that can colonize, feed, and develop on a range of naïve hosts can achieve rapid population growth (Bertheau et al. 2010).

In *Agrilus*, there are many examples of extensive tree killing when the insects have been introduced to new areas and encountered naïve host trees. Asian ash species within the native range of *A. planipennis* are much more resistant to attack than the North American white ash (*F. americana*), green ash (*F. pennsylvanica*), and black ash (*F. nigra*), which are readily killed by the insect (Gandhi & Herms 2010; Raupp et al. 2010). Also in Russia *A. planipennis* is killing green ash (*F. pennsylvanica*), which has been introduced from North America, as well as native European ash (*F. excelsior*) (Baranchikov et al. 2009). Similarly, native North American birch species, which have coevolved with *A. anxius*, are less susceptible to attack than introduced birch species such as Eurasian silver birch (*B. pendula*), white birch (*B. pubescence*), Sichuan birch (*B. szechuanica*), and monarch birch (*B. maximowicziana*), which all suffer 100% mortality (Nielsen et al. 2011). *Agrilus horni* attacks and kills Eurasian *Populus alba*, *P. tremula*, and various aspen hybrids (Nord et al. 1965). Bright (1987) noted that *A. horni* is "especially troublesome in orchard-like experimental plots of various *Populus* species" and was perhaps referring to attacks on trials with Eurasian *Populus* species. Introduced *Populus* species in North America are also attacked by *Agrilus g. granulatus* and *A. g. ligarius*, which attacks *P. nigra* and *P. balsamifera*, respectively (Barter 1965; Solomon 1995). The Wych elm (*Ulmus glabr*a) in northern Europe lacks effective defenses against DED, but the disease has not spread as fast in northern Europe as in, for example, Great Britain. This is believed to be due to the lack of an effective vector for the disease. However, if *H. rufipes* was to be introduced to northern Europe, it could be a more effective vector than the native *Scolytus laevis*. If *U. glabra* proves to be a naïve host for *H. rufipes*, this could lead to a vicious cycle with extensive elm mortality.

The numerous examples given above suggest that naïve hosts are very likely to be an important factor promoting establishment and spread of bark- and wood-borers in northern Europe if they should become introduced. *Agrilus politus* and *A. pensus* are the only of our selected species without a documented history of causing mortality in European tree species. However, this does not necessarily mean that they are not harmful, as there are numerous examples of bark- and wood-boring insects that are not considered primary pests in their native range but are killing naïve trees in new environments (e.g. *Agrilus planipennis*) (Allen & Humble 2002; Haugen & Hoebeke 2005). It may therefore be prudent to expect that also *A. politus* and *A. pensus* may be capable of primary attacks and benefit from naïve hosts should they be introduced.

### *Capability of spread*

*Agrilus* wood borers appear to have considerable flight capacity and maneuverability, and this is likely to promote their ability to establish and spread if introduced into northern Europe. Most *Agrilus* species seem to fly only the distance needed to encounter a suitable host (Carlson & Knight 1969), and field tests have for example shown that most *A. planipennis* females fly only a few hundred meters (Mercader et al. 2009; Siegert et al. 2010). However, recent flight mill experiments demonstrate that *A. planipennis* has a considerable flight potential that even may exceed 20 km within a 24 hour period and that mated females fly almost 2.5 times further than unmated females (Taylor et al. 2010). The high flight capacity indicated by these experiments is supported by field observations. In Russia, the yearly diffusion of *A. planipennis* has been estimated to be at least 9 km (Y. Baranchikov, personal communication). From 2009 to 2012, *A. planipennis* dispersed 130 km westwards in Russia from Mozhaisk to Vyazma, and it has now spread a total distance of 230 km since it was first discovered in Moscow in 2002 (Y. Baranchikov, personal communication). *A. anxius* is thought to be an equally good flyer as *A. planipennis* and is capable of a yearly diffusion of 16–32 km (Federal Register 2003). *A. anxius'* ability to follow its primary hosts through North America demonstrates good dispersal capabilities. Historically, *A. anxius* was restricted to the natural distribution area of its primary hosts, but due to extensive planting of ornamental birch species it has now dispersed southward in the United States and expanded its range vastly (Muilenburg & Herms 2012). Little is known about the flight capacity of other *Agrilus* species.

According to Kaston (1939), *H. rufipes* can fly considerable distances to locate elm trees, although it normally probably only fly far enough to locate the next suitable tree (Anderson & Holliday 2003). The beetles use visual cues to seek out suitable host trees (Anderson & Holliday 2003), but it has also been shown that DED infected trees emit semiochemicals that attract *H. rufipes* (McLeod et al. 2005).

In addition to biological dispersal, human-mediated dispersal may create new satellite populations and increase the overall rate of spread of bark- and wood-borers. The spread of *A. planipennis* in the United States since its introduction around 2000 has for example been facilitated by movement of plants for planting non-squared wood (Muirhead et al. 2006) and possibly firewood (Haack et al. 2010). The relative importance of biological versus human-mediated dispersal is not known.

## Potential economic and ecological impacts

### *Ecosystem effects*

Together with climate change and habitat loss, biological invasions are considered the most important driving force of global environmental change. In the United States alone, 49% of all species listed as threatened are thought to be at risk due to competition or predation by non-indigenous species (Wilcove et al. 1998). Invasive, tree-killing bark- and wood-borers have the capacity to change tree species composition and forest structure, and thereby indirectly affect whole ecosystems. Direct and indirect ecosystem effects have been documented for several invasive forest insects, such as hemlock woolly adelgid *Adelges tsugae*, balsam woolly adelgid *A. picea*, gypsy moth *Lymantria dispar*, green spruce aphid *Elatobium abietinum*, and *A. planipennis* (Ellison et al. 2005; Lovett et al. 2006; Kenis et al. 2009; Gandhi & Herms 2010). Severe ecosystem effects are also expected if some of the most aggressive bark beetles should become introduced to new continents (Økland et al. 2011).

The current widespread killing of ash trees by *A. planipennis* in North America may affect the species communities associated with North America's 16 native ash species. By 2004, *A. planipennis* had killed approximately 15 million ash trees in eastern United States and was threatening an estimated 850 million ash trees in Michigan alone (Poland & McCullough 2006). Of the 282 plant and animal species that are estimated to be dependent on ash in North America, 43 species are clearly threatened if their host tree should be eliminated (Gandhi & Herms 2009). Tree mortality caused by *A. planipennis* leads to canopy gaps and changes in the microenvironment and understory succession (Gandhi & Herms 2010). This may in turn facilitate the establishment of invasive plants and lead to an "invasion meltdown" – the process by which one invasive species facilitates for other invasives (Simberloff & Von Holle 1999).

The introduction of *A. planipennis* in the Moscow region may pose an ecological and economic threat to European ash forests (Baranchikov 2010). Large-scale tree killing by *A. planipennis* is disrupting carbon fluxes and storage and can have global effects (Flower et al. 2012). Extensive tree killing and severe ecological consequences are also expected if *A. anxius* is introduced to northern Europe (EPPO 2011b; Nielsen et al. 2011), as potentially susceptible birch species are important and widely distributed throughout Eurasia (Hultén & Fries 1986). Effects of widespread killing of ash and birch forest in Eurasia by *A. planipennis* and *A. anxius* may include changes in the composition of the fauna and flora associated with these forests due to altered tree species composition, extinction of species dependent on ash or birch, and extensive erosion and mobilization of carbon stores in the soil (Gandhi & Herms 2010; Økland et al. 2012). Introduction of *H. rufipes* to northern Europe could increase the spread of DED, one of the most destructive forest pathogens of all times, which has killed hundreds of millions elms in Europe and North America over the past decades (Brasier & Buck 2001). The loss of elms by DED in North America has changed the forest structure, bird densities, and diversity (Crooks 2002).

### *Economic impact*

Introduction of *Agrilus* species and *H. rufipes* to northern Europe is likely to incur huge economic costs. The ~11,000 non-indigenous plant and animal species that are present in Europe today (DAISIE 2009) incur an estimated annual cost of €12.5 billion (Kettunen et al. 2008), and the more than 50,000 non-indigenous species in the United States carry an estimated annual cost of $137 billion (Pimentel et al. 2000). Bark- and wood-borers are the most costly guild of non-native forest insects, with yearly costs in the United States alone estimated to $3.5 billion in private and government expenditures (Aukema et al. 2010). *Agrilus planipennis* is the most costly of all the introduced wood-borers in the United States, incurring an estimated annual cost of $1.7 billion. The largest expense is related to removal and replacement of dead trees. Eradication of *A. planipennis* is no longer considered a feasible strategy in North America, and land owners are instead advised to spread losses over time by protecting trees with insecticide treatments for a period or to cut losses by doing nothing (Vannatta et al. 2012).

The European ash *Fraxinus excelsior* is an important tree species throughout Europe, and its density increases southwards towards the Mediterranean region, where its range overlaps with *F. angustifolia* and *F. ornus* (Hultén & Fries 1986; FRAXIGEN 2005). *Fraxinus* is widely used as an ornamental tree in parks, graveyards, along roads, and in city streets throughout Europe. It is also a valuable timber species used for carpentry, furniture, house interiors, tools, and various sports equipment (FRAXIGEN 2005).

EPPO performed a pest risk analysis of *A. anxius* for Europe in 2011 and concluded that an introduction would cause high mortality of birch throughout the EPPO region, with major economic and environmental impacts (EPPO 2011b). This probably also applies to regions in Asia where susceptible birch species are abundant and widely distributed (Hultén & Fries 1986; Nielsen et al. 2011).

## Concluding remarks

We have identified nine insects that probably have great potential to invade northern Europe through import of deciduous wood chips from North America. The potentially most damaging species belong to the beetle genus *Agrilus*, which includes a species with a record of massive tree killing following introduction to new areas (*A. planipennis*). There are also other candidate insect groups that could be introduced via import of wood chips, but in this paper we have emphasized a group with a documented ability to survive in wood chips and a high damaging potential.

Screening for potential invaders by identifying common traits and preforming risk assessments is not without difficulties. Only a few of the species that are introduced to a new region actually become established invasives, so there is always a risk of identifying false positives (Smith et al. 1999). On the other hand, the next great threat may very well be a seemingly innocuous species that does not appear in any risk assessments. Still, although it may be difficult to predict species invasions and their impacts in advance, improved information about potential invaders ahead of any interceptions remains an important preventive step to reduce the probability of invasions. It is well known that the time from the first interception of a species until effective phytosanitary regulations are in place usually is too long to prevent irreversible invasion and damage.

The most effective way of stopping biological invasions is to prevent arrival. Once an invasive species has become established, eradication is at best very difficult and costly. Presently there are no regulations of import of deciduous wood chips from North America to Europe, even though there are several North American beetle species with a clear potential to become invasive. It is probably impossible to single out trees infested by *Agrilus* during harvesting, since *Agrilus* species attack different tree species and may be found in both healthy looking and declining trees. Furthermore, since wood chips are produced from mixed forests, phytosanitary regulations of individual tree species may be impractical, and a better alternative may be to regulate the deciduous wood chip commodity as a whole.

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# Paper II

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# Invasion potential of *Agrilus planipennis* and other *Agrilus* beetles in Europe: import pathways of deciduous wood chips and MaxEnt analyses of potential distribution areas*

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Bark- and wood-boring beetles in the genus *Agrilus* (Coleoptera: Buprestidae) can survive wood-chipping, and *Agrilus planipennis* has established in North America and European Russia with devastating impacts on forest ecosystems. The work presented in this paper combined import statistics of deciduous wood chips, Maximum entropy modelling (MaxEnt) of climatic similarities, and the distribution of potential tree hosts to predict the likelihood of four selected North American *Agrilus* species to become introduced and established in Europe. In agreement with the EU's energy-policy target of increased use of wood chips, there was a linear or exponential increase in European imports of deciduous wood chips during the past 10 years from countries harbouring potentially harmful *Agrilus* species. MaxEnt showed high environmental suitability in Europe for the four selected *Agrilus* species, particularly in Eastern Europe and European Russia for *A. anxius*, *A. bilineatus* and *A. planipennis* and in southern Europe for *A. politus*. Documented susceptible host trees are widely distributed in the predicted areas of *Agrilus* distribution in Europe, and these areas receive large quantities of deciduous wood chips from countries where these and other *Agrilus* species are present. Thus, it was concluded that the fundamental conditions for introduction and establishment of *Agrilus* species in Europe are in place.

## Introduction

Currently, EU-countries import deciduous wood chips from North America, European Russia and Asia. These wood chips can harbour tree-killing bark- and wood-boring beetles in the genus *Agrilus* (Coleoptera: Buprestidae) which are present in the area(s) of origin. The volume of such imports is expected to increase to satisfy future demands for renewable energy production in Europe (EU, 2005; UNECEFAO, 2009). A recent review (Flø *et al.*, 2013) of the literature on potentially invasive insect pests that may be introduced with wood chips from North America to northern Europe identified eight *Agrilus* species or subspecies that are likely to attack European trees. These *Agrilus* spp. have a broad distribution in North America, suggesting that they are pre-adapted to a wide range of climatic conditions and therefore may be able to colonize a wide geographic range in Europe if suitable host trees are present. High-risk *Agrilus* species that may be imported with deciduous wood chips were identified by considering (i) the likelihood that the species could arrive with the pathway of deciduous wood chips; (ii) the presence of susceptible host trees in Northern Europe; (iii) the climatic similarity between Northern Europe and the species' native range in North America; and (iv) the severity of the damage the species may cause in Northern Europe (Flø *et al.*, 2013).

Because deciduous wood chips may bring new and potentially very harmful *Agrilus* species into European forests this pathway needs to be assessed in more detail. Import of deciduous wood chips is mostly unregulated, even though wood chips are often large enough to allow survival of *Agrilus* and other potential pests (McCullough *et al.*, 2007). *Agrilus* is a hyperdiverse genus with nearly 3000 valid species and subspecies (Bellamy, 2008), but only three species appear on EPPO's Lists of pests recommended for regulation as quarantine pests or on the EPPO Alert list: *Agrilus anxius* (Gory 1841) is on the A1 List of pests absent from the EPPO region, *A. planipennis* (Fairmaire 1888) is on the A2 List of pests locally present in the EPPO region, and *A. auroguttatus* (Schaeffer 1905) is on the EPPO Alert list. Experience has shown that many high-impact invasive insects were not on phytosanitary lists prior to becoming invasive pests, and some were not even known to be significant pests in advance, such as the emerald ash borer *A. planipennis* that was detected in the USA in 2002 (Haack *et al.*, 2002). When pre-emptive quarantine measures fail and new species are introduced it is often too late to prevent further spread, and one must attempt to limit economic losses and there may be no choice but to accept the often devastating ecological impacts. Improved

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information about potential import pathways and candidate pest species ahead of any interceptions is essential to develop programs for early detection and eradication.

The goal of the present study was to determine if the fundamental conditions are in place to allow introduction and establishment of selected *Agrilus* species in Europe. The authors analysed import volumes of deciduous wood chips to 28 EU countries from North America, a region that harbours several *Agrilus* species that are documented to kill European trees (Flø *et al.*, 2013). Other areas in European Russia and Asia, where *A. planipennis* is present as an invasive (European Russia) or is native (Asia), were also considered. The potential distribution in Europe was explored using Maximum entropy modelling (MaxEnt) for three North American *Agrilus* species that are not yet present in Europe [*A. anxius* (bronze birch borer), *A. bilineatus* (two-lined chestnut borer), and *A. politus* (common willow agrilus)], as well as *A. planipennis* (emerald ash borer), which is present in North America, Asia and European Russia.

## Methods

### Data sources

*Export/import data for deciduous wood chips*
Import data for deciduous wood chips from 2004 until 2013 was collected for all 28 European Union member states using Eurostat's CN8-database (Eurostat, 2014). This database employs the 8-digit combined nomenclature (CN) and data collection follows a harmonized methodology. Other European countries, such as Norway, collect import data using other methodologies that are difficult to harmonize with the EU system. Export data to the EU was collected for the United States and Canada, which harbour several potentially invasive *Agrilus* species (Flø *et al.*, 2013), as well as European Russia, where *A. planipennis* has been introduced, and other countries in *A. planipennis*' native range (China, Taiwan, Mongolia, Japan, South Korea, North Korea) (Haack *et al.*, 2002). The target commodity was located under chapter '44 wood and articles of wood' in the World Customs Organization's harmonized system, more specifically under heading 44.01, code 44.01.2200 including non-coniferous wood in chips or particles (World Customs Organization, 2015). Data was converted from 100 kg units to tonnes (t). Mongolia was excluded from further analyses since the trade data showed that no wood chips were exported from Mongolia to the EU during the 10-year trade period under study.

*Species presence data*
Presence data for *Agrilus* species in North America was downloaded from the GBIF database (GBIF, 2014). Additional presence data for *A. planipennis* in European Russia was added from the literature (Orlova-Bienkowskaja, 2013). All synonym species names were included and all named presence locations and addresses were geo-referenced to acquire a sufficient number of presence points (Hijmans *et al.*, 2014). Only *Agrilus* species with more than 50 presence points were used for further analyses. These included the twolined chestnut borer *Agrilus bilineatus* (Weber 1801), the common willow agrilus *Agrilus politus* (Say 1825), the bronze birch borer *Agrilus anxius*, and the emerald ash borer *Agrilus planipennis* (Fairmaire). Distribution maps for potential host trees in Europe were downloaded from the EUFORGEN website (EUFORGEN, 2014). No GIS-data were available for *Betula pubescence*, but according to Hulte´n & Fries (1986) its distribution is similar to that of *B. pendula*, except that *B. pubescens* has a more northern distribution.

*Meteorological data*
High resolution meteorological data based on interpolations of observed data from 1950 to 2000 were downloaded from the WorldClim database (WorldClim, 2014). The data covered Europe (−10 to 40° longitude, 37 to 70° latitude) and North America (−170 to −55° longitude, 30 to 70° latitude) at a resolution of 30 arc-second grid cells (approximately 1 km$^2$). The 19 downloaded bioclimatic variables represented annual trends in temperature, precipitation and climate ranges. Further details on the meteorological data are reported by Hijmans *et al.* (2005).

*Maximum entropy modelling (MaxEnt)*
To explore the potential distribution in Europe species distribution models (SDMs) were built for the four selected *Agrilus* species. Maximum entropy modelling (MaxEnt) (Phillips *et al.*, 2004, 2006) was chosen as the SDM tool because it has been shown to outperform other SDM methods, and because it will accept presence only data and a small number of presence points (Elith *et al.*, 2006).

Since the primary interest was not in pinpointing which environmental variables were most important in determining species distributions, MaxEnt was treated as a machine learning process. All 19 bioclimatic variables from the WorldClim database were used as predictors and the algorithm chose the most important variables through the default regularization settings. For replicate runs of the SDMs the default setting 'cross-validation' was chosen, as it utilizes all the available data and thus makes better use of the limited data (Phillips, 2014).

*Model performance*
Model performance was measured using the area under the curve (AUC) parameter of the receiver operator characteristic (ROC). AUC can be interpreted as the probability of correctly predicting species presence in a randomly selected geographic grid cell. In the ROC analysis, each grid cell in the predictor data set receives scores from the independent testing data set, the relationship between the true positive rate and the false positive rate is plotted, and the AUC is calculated. If the AUC of the test data run is close to 0.5

the model performs no better than a random model, and if AUC is 1.0 the model provides a perfect fit with no false negatives (van Erkel & Pattynama, 1998; Phillips, 2014). The average AUC value of 10 independent cross-validation runs is presented for each *Agrilus* species.

*Background point selection*

MaxEnt was not run entirely in its default mode (Table 1). For two of the selected species (*A. anxius* and *A. bilineatus*), background points (or pseudo-absence points = the absence of observations) were not selected from the entire geographical area covered by the North American bioclimatic variables, as is default. This was done to avoid creating an artificially high AUC caused by a large number of true negatives (grid cells where both the predicted and the actual value are negative). Instead, background points were selected randomly from within circles of a given radius created around presence points (Table 1). The circle radius was set to fall within the limits of the known distribution of the host tree, because the targeted *Agrilus* species were assumed to be uniformly distributed throughout the range of its host tree (or trees). This approach ensured that the background points included in the MaxEnt analyses only included the range of environmental conditions the authors wanted MaxEnt to distinguish between. The penalty function (the beta multiplier or regularization multiplier) was downsized in order to restrict the distribution predictions and to get a more conservative, localized prediction map, even though this may cause a somewhat over-fitted model (Phillips, 2014). The SDMs were fitted to the known North American distribution of each *Agrilus* species and the prediction maps were inspected visually to ensure that the predicted species distribution approximately matched the distribution of the native host tree, before projecting the model onto Europe. If the SDM did not match the host tree distribution in North America the model settings were adjusted to improve the match. The settings used in the MaxEnt models and summary statistics for the tree *Agrilus* species are given in Table 1. For all three species omission rates showed a close fit to predicted omissions, indicating that the test and training data sets were independent.

*Analysis software*

All data was downloaded and handled in R version 3.1.1 (R Core Team, 2014). MaxEnt version 3.3.3k (Phillips *et al.*, 2004) was run through R using the dismo-package version 1.0-5 (Hijmans *et al.*, 2014).

## Results

### Trade data on deciduous wood chips

Trade data for the period 2004–2013 indicated a linear increase in total yearly quantities of deciduous wood chips exported to the EU from eight selected countries where potentially invasive *Agrilus* species were present (Fig. 1A). The total yearly amount of wood chips exported from the eight countries to the EU (Table 2) showed a gross increase of 64% from 2004 to 2013, with a mean yearly increase of 9.8%. However, although the overall analysis suggested a linear increase in total yearly trade volumes individual countries showed considerable year-to-year variation in exported quantities (Fig. 1).

*Export volumes from individual countries*

The Russian Federation, Canada, the USA and China exported wood chips to the EU in all of the 10 years under study (Table 2). Russia was consistently the largest exporter and shipped 84% of the total quantity of wood chips from the selected countries to the EU. Russian exports to the EU increased exponentially from 2004 to 2013 (Fig. 1B). Export from Canada to the EU was generally low, except for the years 2007–2010, while USA had a relatively low export all years except 2004 (Fig. 1C). Export from Asia to the EU was very low: the Republic of Korea exported small quantities of wood chips most years, Japan exported small quantities for 4 years, whereas exports from China increased rapidly until 2011 and then dropped to very low levels

**Table 1** Maximum entropy modelling (MaxEnt) settings and summary statistics for analysis of potential distribution areas in Europe for four North American *Agrilus* species

| MaxEnt settings and results | *A. politus* | *A. anxius* | *A. bilineatus* | *A. planipennis* |
|---|---|---|---|---|
| Beta/Regularization multiplier | 0.5 | 0.5 | 0.5 | 1 |
| Doclamp | TRUE | TRUE | TRUE | TRUE |
| Circle radius for background point selection | FALSE | 700 km | 300 km | FALSE |
| Mean no. of training points | 111.6 | 40.5 | 43.2 | 33.3 |
| Regularized training gain | 1.951 | 1.884 | 1.200 | 2.145 |
| Unregularized training gain | 2.777 | 2.627 | 1.887 | 2.833 |
| Iterations | 500 | 500 | 500 | 500 |
| Training AUC | 0.975 | 0.968 | 0.932 | 0.981 |
| Mean test sample | 12.4 | 4.5 | 4.8 | 3.7 |
| Test gain | 1.235 | 1.561 | 1.073 | 1.709 |
| Mean no. of background points | 10111.6 | 7934.5 | 9189.2 | 10033.3 |

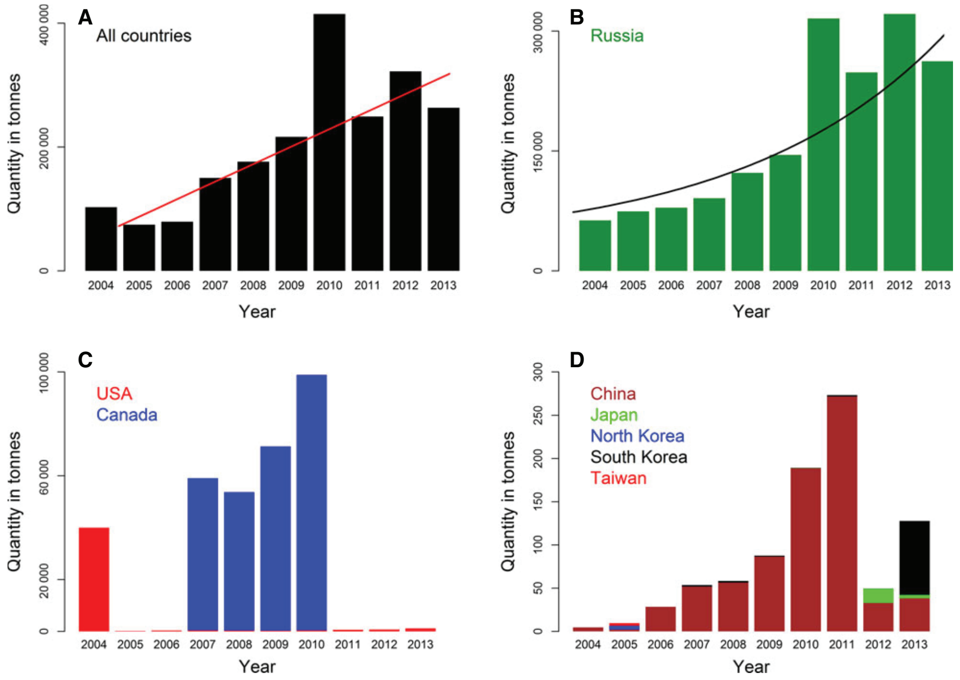

**Fig. 1** Annual quantities of deciduous wood chips exported from eight selected countries outside Europe to the EU over the 10-year period 2004–2013. The trend line in (A) indicates a linear increase for all countries combined (Import Quantity = 42 860 + 29 472 (YEAR), $R^2 = 0.64$). Export from Russia (B) showed an exponential increase in the same period (Import Quantity = 31 488 + $a$ ($b^{\mathrm{YEAR}}$); where $a = 631\,610$ with SE 17 930, $t = 2.34$ and $P < 0.048$; and $b = 1.217$ with SE 0.062, $t = 19.61$ and $P < 0.0001$). Note that the *y*-axis is scaled differently between figure panels.

**Table 2** Export of deciduous wood chips, from countries outside Europe and European Russia that harbour *Agrilus* spp., into EU in the 10-year period 2004–2013. All trade data are given in tonnes. *N* denotes the number of years with export from each country during 2004–2013. The total exported volume was 2 049 538 t

| Country | *N* | Min | Mean | Max | Sum | SD | % of total |
|---|---|---|---|---|---|---|---|
| Russia | 10 | 0.3 | 14 355 | 314 057 | 1 722 559 | 54 214 | 84.05 |
| Canada | 10 | 0.5 | 2817.4 | 98 511 | 281 740 | 13 779 | 13.75 |
| USA | 10 | 0.1 | 193 | 39 909 | 44 357 | 21 | 2.16 |
| China | 10 | 0.1 | 5 | 221 | 761.3 | 87 | 0.04 |
| South Korea | 7 | 0.2 | 3 | 85.6 | 92.6 | 16 | 0.005 |
| Japan | 4 | 0.1 | 0.5 | 16.5 | 20.6 | 2.6 | 0.001 |
| North Korea | 1 | 0 | 0.4 | 4.4 | 4.4 | 1.4 | 0.0003 |
| Taiwan | 1 | 0 | 0.3 | 3 | 3 | 0.9 | 0.0001 |
| Total | | | | | 2 049 538 | | 100 |

(Fig. 1D). The Democratic People's Republic of Korea and Taiwan exported only a single shipment each during the 10-year period (Table 2), to Denmark and Austria, respectively.

### *Import volumes into individual EU countries*

Of the 28 EU countries, Finland, Germany, Italy, Sweden, and Estonia imported 99.5% of all deciduous wood chips imported into the EU (Table 3). Finland alone imported

**Table 3** Import of deciduous wood chips to the five EU countries with the largest imports during the 10-year period 2004–2013. All import data are given in tonnes. *N* denotes the number of years with import to each country during 2004–2013

| Country | *N* | Min | Mean | Max | Sum | SD | % of EU total |
|---|---|---|---|---|---|---|---|
| Finland | 10 | 0.5 | 48 100 | 314 057 | 1 923 987 | 87 642 | 93.90 |
| Germany | 10 | 0.1 | 1052 | 41 123 | 42 099 | 6498 | 2.05 |
| Italy | 9 | 0.2 | 2018 | 39 909 | 40 362 | 8919 | 1.97 |
| Sweden | 9 | 0.1 | 447 | 14 347 | 22 368 | 2083 | 1.10 |
| Estonia | 5 | 0.1 | 474 | 7334 | 9479 | 1651 | 0.50 |
| Total | | | | | 2 038 295 | | 99.52 |

93.9% of all deciduous wood chips to the EU and was the only country that had a consistently high import every year. Most of Finland's import (1 683 530 t; 88%) came from Russia. Finland also imported 240 443 t from Canada between 2005 and 2010 and small amounts from China (0.9 t), and USA (12.9 t). Germany, the second largest importer, imported 41 124 t of wood chips from Canada over three years, 886 t from the USA over nine years, and small quantities from China (74 t over eight years) and Russia (14 t in 2010). Italy imported 40 203 t over nine years from the USA, and 159 t from China over seven years. Sweden imported 22 286 t over five years from Russia, 79.4 t over six years from the USA, 2.4 t over six years from China, 0.2 t from Canada in 2011, and 0.1 t from Japan in 2011. Estonia imported 9479 t from Russia over four years and 0.1 t from the USA in 2007.

## Potential distribution estimated by Maximum entropy modelling

### *Agrilus anxius*

MaxEnt modelling of potential distribution of *A. anxius* in Europe showed a high probability of distribution in European part of Russia, Finland, Belarus and Ukraine, especially in areas with a humid continental climate (Fig. 2). In addition, the probability of distribution was high in the Alps, in Eastern Denmark, and in coastal areas of Western Norway. MaxEnt predicted the potential distribution of *A. anxius* with excellent accuracy (in terms of prediction of true positives and true negatives), giving an average test AUC value of 0.903, with a standard deviation of 0.085. Over the 10 cross-validation runs the total contribution of the four most important environmental variables to the MaxEnt model was on average 57.7%, with mean temperature of the wettest quarter of the year contributing 21.2%, mean temperature of the coldest quarter 13.2%, minimum temperature of the coldest month 12.1%, and mean temperature of the driest quarter 11.2%. The mean temperature of the wettest quarter was also the strongest contributor in the test of individual variables, based on jackknife resampling of training and test results (i.e. leaving out one variable at a time and re-estimating bias and standard error).

### *Agrilus bilineatus*

Similar to *A. anxius*, the MaxEnt model prediction of potential distribution of *A. bilineatus* in Europe showed a high probability of distribution in areas with a humid continental climate in Finland, European Russia, Estonia, Latvia, Moldova, Belarus, Ukraine, and Poland (Fig. 3). In Scandinavia and other parts of Western Europe, the probability of distribution was higher in some coastal areas than further inland. MaxEnt predicted the potential distribution of *A. bilineatus* with moderate model accuracy, giving an average test AUC value of 0.829, and a standard deviation of 0.115. Over the 10 cross-validation runs five variables contributed on average >10% to the MaxEnt model, and their total contribution to the model averaged 59.3%. Annual mean temperature contributed on average 15.3%, and mean temperature of the warmest quarter of the year,

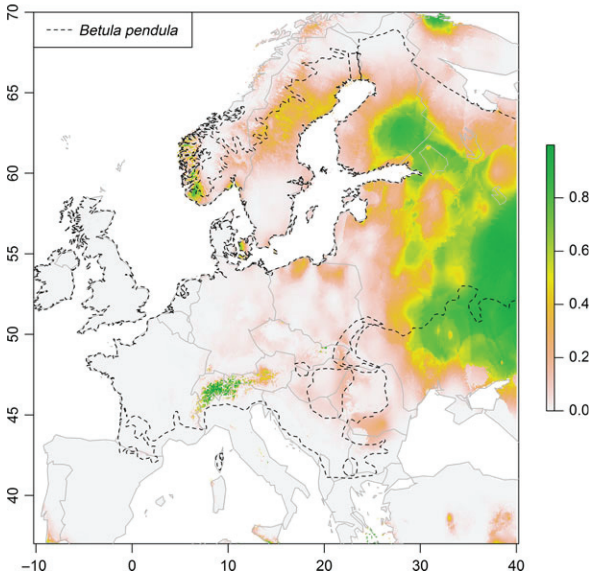

**Fig. 2** Potential distribution of the bronze birch borer *Agrilus anxius* in Europe predicted by Maximum entropy modelling (MaxEnt). Colours indicate probability of occurrence of *A. anxius* (green = high, white = low) and dashed lines show the distribution of the host tree silver birch *Betula pendula.*

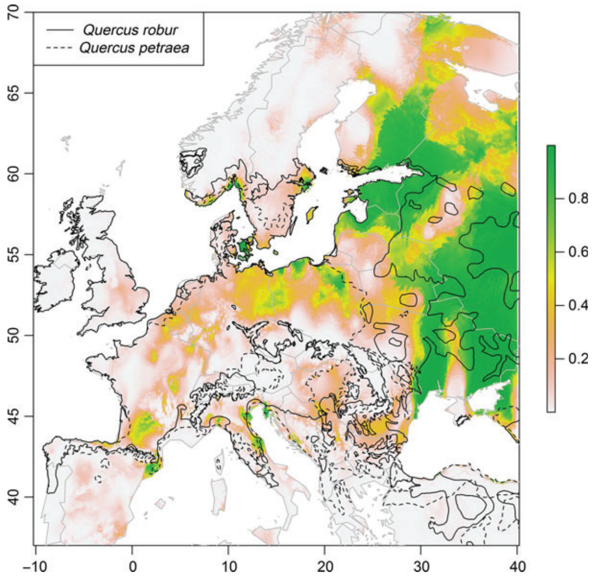

**Fig. 3** Potential distribution of the twolined chestnut borer *Agrilus bilineatus* in Europe predicted by Maximum entropy modelling (MaxEnt). Colours indicate probability of occurrence of *A. bilineatus* (green = high, white = low). The solid and dashed lines show the distribution of two potential host trees (pedunculate oak *Quercus robur* and sessile oak *Q. petraea*) respectively.

temperature seasonality (the difference between annual maximum and annual minimum temperature), mean temperature of the coldest quarter, and isothermality (mean diurnal temperature range divided by the annual temperature range) all contributed between 10.8% and 11.2%.

*Agrilus politus*

In contrast to the other three species, the MaxEnt model prediction for *A. politus* showed a high probability of potential distribution in southern areas of Europe around the Mediterranean Sea and elsewhere in areas dominated by a temperate climate. MaxEnt predicted the potential distribution of *A. politus* with moderate model accuracy, giving an average test AUC value of 0.872, and a standard deviation of 0.062. Over the 10 cross-validation runs four variables contributed on average >10% to the MaxEnt model, and their total contribution to the model averaged 52.2%. Precipitation of the coldest quarter of the year contributed 16.1% to the model, annual mean temperature contributed 12.5%, maximum temperature of warmest month 11.9%, and mean temperature of warmest quarter 11.7%. Precipitation of coldest quarter of the year had a permutation importance in the jackknife resampling test of 22.9%, which indicates that the model depends heavily on this variable.

*Agrilus planipennis*

The MaxEnt model prediction for *A. planipennis* showed a high probability of potential distribution immediately surrounding its current distribution in European Russia, as well as in Finland and Belarus, areas dominated by a continental climate. MaxEnt predicted the potential distribution of *A. planipennis* with excellent model accuracy, giving an average test AUC value of 0.933, and a standard deviation of 0.088. Over the 10 cross-validation runs three variables contributed on average >10% to the MaxEnt model, and their total contribution to the model averaged 73%. Precipitation of the driest month of the year contributed 45.9% to the model, minimum temperature of coldest month contributed 16.7%, and temperature seasonality 10.4%. Precipitation of the driest month of the year had a permutation importance in the jackknife resampling test of 16%, which indicates that the model depends heavily on this variable.

## Discussion

The species distribution models presented in this paper show that large areas in Europe have high environmental suitability for the four selected *Agrilus* species, mainly in the continental climate zone. The model predictions of potential *Agrilus* distribution in Europe also overlap with the distribution of known and potential host trees in Europe, suggesting that these beetles would be able to establish in Europe if they were introduced with e.g. imported wood chips. *Agrilus anxius, A. bilineatus* and *A. planipennis* have a high potential distribution in Eastern Europe including European Russia, Ukraine, Belarus and Moldova, but can also find suitable climate and host trees in parts of Western Europe. The risk of accidentally introducing *A. anxius* and *A. bilineatus* is increased by the substantial import of deciduous wood chips to the EU from Canada and USA (326 000 t in total from 2004 to 2013, 15.9% of total EU import of deciduous wood chips; Table 2). Import of deciduous wood chips from North America could also bring *A. planipennis* to Europe, as this species is well established in the eastern parts of USA and Canada. Because most deciduous wood chips imported to the EU come from Russia, there is also a risk of importing *A. planipennis* from the east, as this species expands westwards from its present distribution around Moscow. The greatest risk of importing *A. planipennis* with wood chips to the EU is probably associated with the high volumes of deciduous wood chips shipped from Russia to Finland, and to a lesser degree to Germany, Italy, Sweden, and Estonia (Table 3). Finland's high import volumes probably reflect the country's role as a leading pulp and paper producer in Europe (Lamers *et al.*, 2012).

### Trade statistics and risk of introduction

The risk of introducing new and harmful *Agrilus* species to Europe may continue to increase in the coming years. Over the last decade, there has been a significant increase in EU imports of deciduous wood chips from countries where high-risk *Agrilus* species are present, and from Russia, the largest trade partner, the increase has been exponential (Fig. 1). A further increase in import of wood chips to the EU may be expected, as the EU aims to use more bioenergy to satisfy future energy demands (EU, 2005; UNECEFAO, 2009). One fundamental problem with using trade data to identify high-risk imports and quantify risks is that the biological resolution of the Eurostat data tends to be low. Wood chip consignments often include a mixture of tree species with different probabilities of containing harmful *Agrilus* species, but unfortunately there is no information about tree species composition in the trade data and it is difficult and time-consuming to obtain such data upon inspection (Økland *et al.*, 2012). The biological state of the wood used for chipping may also vary much between consignments, but again it is impossible to separate high- and low-quality wood chips based on the trade data alone. Wood chips for bioenergy purposes are typically made from low-quality wood from damaged trees, salvage harvesting, or logging residues that do not meet the quality demands for sawn wood and are more likely to contain bark- and wood-boring insects (Hall, 2002). Wood chips intended for pulp and paper production are generally of better quality, but most consignments may still include some low-quality wood chips. Wood chip consignments may also contain wood chips of varying sizes, including fractions that exceed maximum size limits (Roberts & Kuchera, 2006; Kopinga *et al.*, 2010) and thus have a larger probability of harbouring live *Agrilus* beetles (McCullough *et al.*, 2007).

The geographical resolution of the trade data is usually quite crude, and insufficient to determine the location of the trees used for wood chip production. Russia, the USA and Canada are vast countries, but the trade data does not give the precise area of origin of the wood chips or the trees used in wood chip production.

## Potential distribution area and climate

### Agrilus anxius, A. bilineatus A. politus, *and* A. planipennis

According to the species distribution models presented in this paper *A. anxius, A. bilineatus* and *A. planipennis* have the highest probability for potential distribution in the European part of Russia and neighbouring areas, in areas with a humid continental climate. *Agrilus politus* was predicted to have the highest potential distribution in southern parts of Europe around the Mediterranean, in areas dominated by a more temperate climate.

The climatic niche of *A. anxius* seems to encompass dry and cold winters and warm and wet summers (Fig. 2). The potential distribution of *A. anxius* in Europe was largely determined by temperature. Low temperatures are not believed to be a limiting factor for *A. anxius* in Europe, since *A. anxius* needs to be subjected to freezing temperatures to complete its life cycle (Barter, 1957).

The MaxEnt model for potential distribution of *A. bilineatus* (Fig. 3) was determined by winter and summer temperatures, but also by temperature seasonality and isothermality. High temperature seasonality and isothermality is characteristic for the humid continental climate type.

*Agrilus planipennis* (Fig. 4) is already established in European Russia and is spreading towards the eastern parts of Europe (Orlova-Bienkowskaja, 2013, 2014b). The MaxEnt model for potential distribution of *A. planipennis* is localized around its current distribution area in European Russia, but also shows a potential distribution northward to Finland and Sweden, westward to the Baltic countries, and southward towards Belarus and Ukraine – mainly areas within a continental climate type. This coincides with the climate in other parts of *A. planipennis'* invasive range: the Northern United States and Southern Canada has a humid continental climate, according to the Köppen-Geiger climate classification maps (Peel *et al.*, 2007). A similar climate is also found in *A. planipennis'* native range in Far East Asia (Peel *et al.*, 2007).

In the MaxEnt model for potential distribution of *A. politus* (Fig. 5), precipitation of the coldest quarter was the most important variable, together with annual mean temperature of warmest month and mean temperature of warmest quarter. In *A. politus'* potential distribution area in Europe the coldest quarter (i.e. the winter months) are normally also the driest months. *Agrilus politus* seems to prefer a considerably drier and warmer climate than *A. anxius*, *A. bilineatus*, and *A. planipennis.*

### *Pest significance of* Agrilus anxius, A. bilineatus A. politus, *and* A. planipennis

*Agrilus anxius* is documented to kill the two most important birch species in Europe, *Betula pendula* and *B. pubescens*, as well as the two Asian species *B. maximowicziana*, and *B. szechuanica* (Nielsen *et al.*, 2011). *Betula pendula* and *B. pubescens* are widely distributed in the modelled distribution area of *A. anxius* in Europe. At present *B. pendula* and *B. pubescens* have very few insect pests that can kill

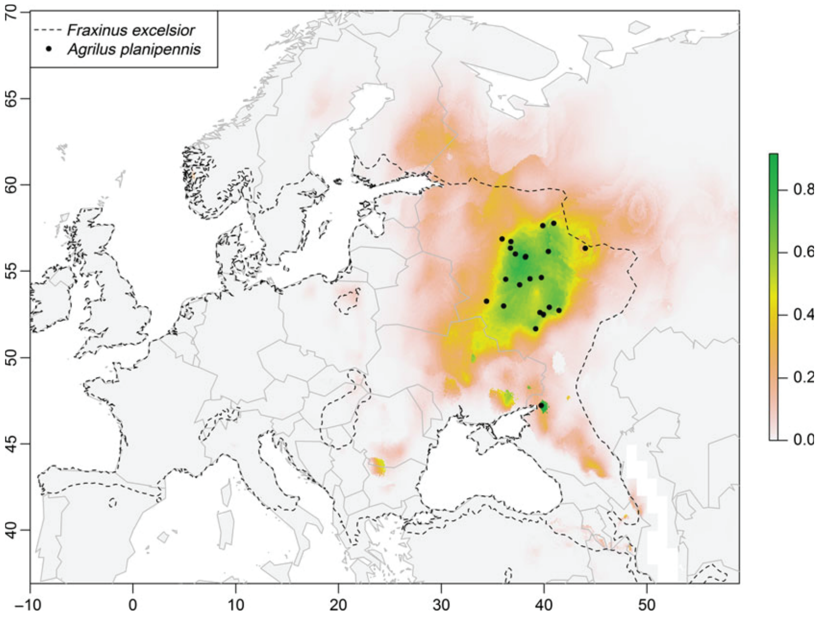

**Fig. 4** Potential distribution of the emerald ash borer *Agrilus planipennis* in Europe predicted by Maximum entropy modelling (MaxEnt). Colours indicate probability of occurrence of *A. planipennis* (green = high, white = low). Dashed lines show the distribution of the host tree European ash (or common ash) *Fraxinus excelsior*. Black dots show the presence of *A. planipennis* according to Orlova-Bienkowskaja (2014a).

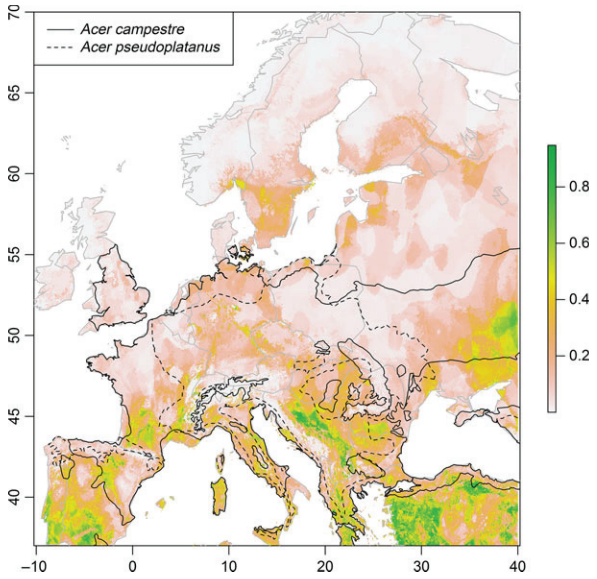

**Fig. 5** Potential distribution of the common willow agrilus *Agrilus politus* in Europe predicted by Maximum entropy modelling (MaxEnt). Colours indicate probability of occurrence of *A. politus* (green = high, white = low). The solid and dashed lines show the distribution of two potential host trees (field maple *Acer campestre* and sycamore maple *A. pseudoplatanus*) respectively.

them, and introduction of *A. anxius* could have dramatic effects on extensive areas of birch forests in Eurasia.

*Agrilus bilineatus* has been documented to kill the European species *Quercus robur* in North America (Muzika *et al.*, 2000), and it has many other potential host species in Europe, including the common and widespread *Q. petraea*. The predicted distribution area of *A. bilineatus* in Europe overlaps to a large extent with the distribution of *Q. robur*. Introduction to Europe, and subsequent attack on the highly valued *Q. robur* could be costly, as this species is used in forestry and is highly valued as an ornamental tree. *Quercus robur* is also ecologically important because of its high number of associated species (Southwood *et al.*, 2004).

*Agrilus politus* is distributed throughout the United States (including Alaska) and most Canadian provinces (Bright, 1986) and attacks several species of maple (*Acer*) and willow (*Salix*) in this extensive distribution area. An ability to kill European host trees has not been documented for *A. politus*, but several potential host species are available in Europe. These include *Acer campestre, A. platanoides, A. pseudoplatanus*, *Salix alba, and S. caprea*, which are some of the most common maple and willow species across Europe.

*Agrilus planipennis* was detected in North America 13 years ago (Haack *et al.*, 2002), and has become one of the most devastating tree-killing insect pests ever introduced. North American ash species show very little documented resistance (Whitehill *et al.*, 2014) and mortality rates approach 100% in some areas. European ash species are also expected to be highly susceptible to *A. planipennis*, and the beetle appears to thrive in its invasive range in Russia (Orlova-Bienkowskaja, 2014a). The Eurostat trade data suggests that Finland, Germany, Italy, Sweden and Estonia run the highest risk of introducing this *Agrilus* species with imported wood chips from Russia (Table 3). The main European host of *A. planipennis*, *Fraxinus excelsior*, is present in all these countries. On the other hand, the model prediction for potential distribution of *A. planipennis* is centred within the continental climate area, suggesting a low probability of establishment in countries located in the oceanic climate zones, such as Great Britain, parts of France and Germany, or countries in the Mediterranean climate zone, for example Italy and Spain.

## Management implications

The analyses of wood chip import pathways reported in this paper and the modelled distribution areas of selected *Agrilus* species in Europe strongly suggest that the fundamental conditions are in place for introduction and establishment of *Agrilus* species in Europe. This new information about potential import pathways and candidate pest species calls for adjustments to current management procedures to reduce the risk of species introductions. These include efforts to reduce the likelihood that *Agrilus* will be present in wood chips. Establishment of new *Agrilus* species in Europe could potentially be extremely damaging and costly. Eradication will probably be impossible, or if possible, the management costs would be very high. In the United States, management of *A. planipennis* alone has an estimated annual cost of 1.7 billion USD (Aukema *et al.*, 2010). Most of these costs are related to removal and replacement of dead trees.

Because eradication is usually impossible the best options are pre-emptive measures, such as reducing the risk of entry of *Agrilus* species through phytosanitary regulations. *Agrilus planipennis* may survive the wood chipping process (McCullough *et al.*, 2007), and since it is the largest of the *Agrilus* species discussed in this paper it is very likely that other *Agrilus* species also may survive chipping. *Agrilus planipennis* is believed to have been introduced to North America with wood packaging material (Herms & McCullough, 2014), and to Russia through plants for planting or wood packaging material (EPPO, 2007; Orlova-Bienkowskaja, 2013). Reducing the maximum permitted diameter of wood chips is not alone considered a sufficient pre-emptive measure, for two main reasons. First, the actual chip dimensions tend to be highly variable and to exceed the dimensions specified by producers and regulators (EPPO, 2013). Second, very little is known about the survival of pests in wood chips of different sizes and qualities. Alternative pre-emptive measures to reduce the occurrence of viable beetles in wood chips could be disinfection of wood chips with ionizing radiation or heat treatment. However, there is currently no information about the effectiveness of these measures (EPPO, 2013).

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## Potentiel d'invasion d'*Agrilus planipennis* et d'autres *Agrilus* en Europe: filières d'importation de copeaux de bois de feuillus et analyses MaxEnt des zones de répartition potentielles

Les coléoptères de l'écorce et du bois du genre *Agrilus* (Coleoptera: Buprestidae) peuvent survivre aux processus de fabrication des copeaux de bois, et *Agrilus planipennis* s'est établi en Amérique du Nord et dans la partie européenne de la Russie avec des impacts dévastateurs sur les écosystèmes forestiers. Le travail présenté dans cet article combine des statistiques sur l'importation des copeaux de bois de feuillus, la modélisation du maximum d'entropie (MaxEnt) des similitudes climatiques, et la répartition des arbres-hôtes potentiels pour prévoir la probabilité d'introduction et d'établissement en Europe de quatre espèces d'*Agrilus* d'Amérique du Nord. L'objectif de la politique énergétique de l'UE d'accroissement de l'utilisation des copeaux de bois s'est accompagné d'une augmentation linéaire ou exponentielle des importations européennes de copeaux de bois de feuillus au cours des 10 dernières années en provenance de pays dans lesquels des espèces d'*Agrilus* potentiellement nuisibles sont présentes. La modélisation MaxEnt a montré que les conditions environnementales en Europe sont très favorables aux quatre espèces d'*Agrilus* étudiées, notamment en Europe de l'Est et dans la partie européenne de la Russie pour *A. anxius*, *A. bilineatus* et *A. planipennis*, et dans le sud de l'Europe pour *A. politus*. Les arbres-hôtes sensibles connus sont largement répandus dans les zones de répartition potentielle de ces *Agrilus* en Europe, et ces zones reçoivent de grandes quantités de copeaux de bois de feuillus provenant de pays où ces espèces et d'autres espèces d'*Agrilus* sont présentes. Ainsi, il est conclu que les conditions fondamentales de l'introduction et de l'établissement d'espèces d'*Agrilus* en Europe sont réunies.

## Инвазивный потенциал *Agrilus planipennis* и других жуков рода *Agrilus* в Европе: пути распространения с импортом щепы древесины лиственных пород и анализы MaxEnt зон потенциального распространения

Развивающиеся под корой и в древесине жуки рода *Agrilus* (Coleptera: Buprestidae) способны выжить при переработке древесины в щепу. Златка *Agrilus planipennis* уже акклиматизировалась в Северной Америке и Европейской части России с разрушительным воздействием на лесные экосистемы. Работа, представленная в статье, сочетает статистику импорта щепы древесины лиственных пород, моделирование максимальной энтропии (MaxEnt) климатических аналогий и распространенность потенциальных деревьев-хозяев для спрогнозирования вероятности завоза и акклиматизации в Европе выбранных четырех североамериканских видов *Agrilus*. В соответствии с задачей увеличения использования древесной щепы, в рамках энергетической политики ЕС, на протяжении последних 10 лет отмечается линейное или экспоненциальное увеличение импорта в ЕС щепы лиственных пород из стран, в которых имеются потенциально вредные виды рода *Agrilus*. Анализ MaxEnt показал высокую экологическую пригодность среды в Европе для четырех видов *Agrilus*, особенно в Восточной Европе и в Европейской части России, для *A. anxius*, *A. bilineatus* и *A. planipennis* и, в южной части Европы, для *A. politus*. Зарегистрированные восприимчивые деревья-хозяева широко распространены в спрогнозированных зонах потенциального распространения видов рода *Agrilus* в Европе, и именно в эти зоны завозятся большие объемы щепы лиственных пород из стран, где присутствуют эти и другие виды *Agrilus*. Таким образом, авторы приходят к выводу, что основные условия для интродукции видов рода *Agrilus* в Европу уже существуют.

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# Paper III

# Constitutive and induced terpenoid defenses of co-evolved and naïve host trees of the European spruce bark beetle *Ips typographus*

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# Abstract

The European spruce bark beetle *Ips typographus* (L.) is one of the most damaging pests in Europe's boreal forests and among the few insects that can kill healthy trees over large areas. The beetle's relationship with its historic co-evolved host Norway spruce (*Picea abies* (L.) H. Karst) is well described, and involves complex interactions with the trees' terpenoid defenses. However, spruce bark beetle interactions with potential host trees originating outside the beetles' natural range are little known and may be unpredictable, since trees without a co-evolutionary history with the beetle may lack effective defenses. In this study we compared the chemical suitability to the spruce bark beetle of the historic host Norway spruce with Sitka spruce *Picea sitchensis* Bong. Carrière and Lutz spruce *Picea glauca x lutzii* Little, two evolutionary naïve hosts of North American origin that are extensively planted in Northwestern Europe. The bark of the three spruce species had a similar chemical composition and contained comparable amounts of terpenoids, although Norway spruce had significantly higher total terpenoid levels than Sitka and Lutz spruce. Terpenoid levels increased in all three spruce species one month after application of the defense hormone methyl jasmonate, with a 25- to 35-fold increase in total terpenoid levels but modest qualitative changes in terpenoid composition. Beetles tunneling in the three spruce species produced similar amounts of cis-verbenol and 2-methyl-3-buten-2-ol, the two main components of the beetles' aggregation pheromone. The fact that Norway spruce bark contained significantly more precursor for cis-verbenol ((-)-α-pinene) than Sitka and Lutz spruce did not influence cis-verbenol production. Our results suggest that the terpenoid chemistry of Sitka and Lutz spruce bark is conducive to attack by the spruce bark beetle. If the spruce bark beetle should establish in North America, terpenoid chemistry and pheromone production are not likely to be obstacles for beetle colonization of these species in their native range.

Keywords: *Picea sitchensis*, *Picea abies*, *Picea lutzii*, Pinaceae, terpenoids, naïve host, α-pinene, cis-verbenol, methylbutenol

## 1. Introduction

The European spruce bark beetle *Ips typographus* (L.) is one of the most damaging pests in Europe's boreal forests and one of the few insects that can attack and kill healthy trees over large areas. The beetles enter the bark of timber, windthrows or healthy trees, where they mate and oviposit in characteristic egg galleries. The larvae tunnel through the bark as they develop, and the full development from egg to adult takes one to several months, depending on local climatic conditions. The main host of the spruce bark beetle in Europe is Norway spruce (*Picea abies* (L.) H. Karst.), but the beetle may occasionally reproduce in other spruce species (Økland et al. 2011). The relationship between the spruce bark beetle and its main host is probably shaped by a complex arms race of chemical warfare during their long co-evolutionary history (Franceschi et al. 2005). The trees defend themselves by constitutive defenses, which include storage of toxic terpenoids in premade resin ducts, and inducible defenses, such as mobilization of stored and induced terpenoids when the beetles tunnel into the bark (Keeling & Bohlmann 2006). The beetles use specific host terpenoids as precursors to produce the aggregation pheromones that coordinate their deadly mass-attacks (Blomquist et al. 2010). Because terpenoids have this dual role of defending trees against attack and synergizing beetle mass-attacks, quantitative and qualitative aspects of the trees' terpenoid defenses are important determinants of tree suitability as a host for the spruce bark beetle.

Much empirical evidence supports the importance of terpenes and other secondary compounds in conifer resistance against bark beetle attack (Franceschi et al. 2005; Keeling & Bohlmann 2006). Tree resistance is determined both by the chemical composition of the trees' constitutive defenses and by the ability to launch effective induced defenses. Studies have shown that a tree's ability to effectively mobilize terpenoid defenses can be a reliable resistance marker, as trees with strong induced defenses are more resistant to bark beetle attack than trees with a weaker or slower response (Boone et al. 2011; Schiebe et al. 2012; Zhao et al. 2011b). The plant defense hormone methyl jasmonate (MeJA) can be used as a tool to experimentally induce tree defenses (Krokene 2015; Martin et al. 2002). Spraying Norway spruce stems with MeJA induces and/or primes the terpenoid defenses and reduces tree colonization by the spruce bark beetle (Erbilgin et al. 2006). Trees sprayed with MeJA had fewer beetle attacks, shorter egg galleries and lower beetle reproduction than untreated trees. Methyl jasmonate treatment also reduces tree colonization by the symbiotic phytopathogenic fungi associated with the spruce bark beetle (Franceschi et al. 2005; Zeneli et al. 2006).

Bark beetle interactions with novel host trees may be unpredictable, since the novel hosts do not share a co-evolutionary history with the beetles and thus are evolutionary naïve and may lack effective defenses (Burke & Carroll 2016; Cudmore et al. 2010). Novel bark beetle-host tree associations arise when trees are planted outside their native range and when beetles become invasive in other continents or undergo local range expansion, like the mountain pine beetle *Dendroctonus ponderosae* Hopkins in western Canada (Cudmore et al. 2010; Erbilgin et al. 2014). The range of the spruce bark beetle currently overlaps with several evolutionary naïve spruce species of North American origin that are widely planted in Europe, but we know little about the suitability of these species as hosts of the spruce bark beetle. The spruce bark beetle also presents a high invasive risk in North America, where it was intercepted 286 times between 1985 and 2000 (Haack 2001). It is thus of great practical interest to determine if the spruce bark beetle is likely to thrive in other spruce species than Norway spruce.

To assess the chemical suitability of potential new hosts of the spruce bark beetle we compared the terpenoid composition of three different spruce species: the co-evolved historic host Norway spruce, and two potentially novel hosts of North American origin; Sitka spruce *Picea sitchensis* Bong. Carrière and Lutz spruce *Picea glauca x lutzii* Little. Sitka spruce is abundant in Northwestern Europe, where it is planted over more than 1.2 million hectares (Mason & Perks 2011). Spruce bark beetle attacks on Sitka spruce have been reported from the UK and Sweden (Browne & Laurie 1968; Økland et al. 2011), but there is no detailed information about the chemical suitability of Sitka spruce as a host of the spruce bark beetle. Lutz spruce, a natural hybrid between Sitka spruce and white spruce *Picea glauca* (Moench) Voss (Lockwood et al. 2013), is less widely planted in Europe than Sitka spruce but occurs within the range of the spruce bark beetle. Our main goal was to determine whether Sitka and Lutz spruce are chemically suitable hosts for the spruce bark beetle, or if their terpenoid composition might be an obstacle for tree colonization and pheromone production by the beetle. To achieve this we determined the constitutive and induced terpenoid profile of the three spruce species, as well as the beetles' ability to produce aggregation pheromones in the bark of each species.

## 2. Material and methods

### 2.1 Study area and sampling of trees

This study was carried out in a 0.75 ha experimental stand established in 1963 near Prestebakke in Halden, SE Norway (N 58.9990 E 11.5219). The stand was planted with Norway spruce and different North American conifer species, with blocks of 200-400 trees per species planted in parallel rows. On June 1 2014, 10 trees each of Norway, Sitka and Lutz spruce were selected, diameter at breast height (DBH) was measured, and debris was removed from the bark surface of the lower stem using a plastic brush. To determine constitutive terpene levels in the trees, four bark samples equally spaced around the stem circumference were taken from each tree at 0.5 meter height using a 9 mm cork borer. These constitutive samples were pooled, wrapped in aluminum foil and flash frozen in liquid nitrogen. To determine induced terpene levels, the same trees were treated with the plant hormone methyl jasmonate (MeJA) by placing a filter paper (5 x 5 mm) soaked in a 50 mM MeJA solution in each cork borer hole. The holes were sealed with a 9 mm bark plug taken from a neighboring "donor-tree" of the same species that was not used for terpene sampling. One month later (July 1, 2014), new bark samples were collected immediately above the original sampling positions using a 9 mm cork borer, the four samples were pooled, wrapped in aluminum foil, and flash frozen in liquid nitrogen for later analysis of induced terpene levels ("induced samples"). All constitutive and induced samples were stored at -80 $^{\circ}$C until terpene analysis.

About 10 months after the final bark sampling (May 13, 2015), all experimental trees were felled. A 1.2 m long stem section was cut from each tree between 1.0 and 2.2 m above ground and brought to the lab. The cut ends were sealed with melted paraffin wax to reduce desiccation and the stem sections were stored outdoors. Four days later each stem section was divided into three 0.4 m long bolts, the freshly cut ends were sealed with melted paraffin wax, and the bolts were stored at 4 ˚C. One bolt per tree was used for quantification of pheromone production by the spruce bark beetle (see below), whereas the remaining bolts were used in a separate study of beetle reproductive performance and fungal colonization success (Flø et al. manuscript).

## 2.2 Analysis of terpenes

### *2.2.1 Sample preparation and extraction*

For each sample type (constitutive and induced) the four bark plugs from each tree were split in two, one half was returned to -80 °C as a backup, and the other half was processed for terpene analysis. First the cork bark was removed, and the remaining phloem was submerged in 1 ml hexane (≥95% Sigma-Aldrich) containing 0.20 mg pentadecane (≥99% Sigma-Aldrich) as internal standard, and 3-tert-butyl-4-hydroxyanisole (Sigma-Aldrich) as antioxidant. All samples were extracted in hexane at room temperature for 48 hours before the extracts were filtered, transferred to Agilent MS vials with crimp top, and stored at -80 °C. The phloem was dried at 80 °C for 24 hours and weighted for absolute amount calculation.

### *2.2.2 Terpene analysis*

Terpene analysis was carried out on an Agilent 6890 N gas chromatograph (GC) connected to an Agilent 5973 mass spectrometer (MS) and fitted with an autosampler. The GC was operated in splitless mode at 250 °C with an injection volume of 1 µL, using a 30-m fused silica Agilent J &W Scientific DB-Wax separation column (Agilent Technologies) with an inner diameter of 0.25 mm and film thickness of 0.25 µm. A 2.5-ml methyl-deactivated pre-column (Varian Inc., Lake Forest, CA, USA) with the same inner diameter was coupled to the analytical column via a press-fit connector (BGB Analytik AG, Boeckten, Switzerland). After sample injection, the temperature was held at 40 °C for 2 min and subsequently raised 6.9 °C/min to 160 °C and then 21.5 °C/min to 250 °C. The temperature was then held constant at 250 °C for 3.6 min, giving a total running time of 27.18 min. The MS was operated in scan mode from m/z 40 to 550 with a threshold of 50 and 2.86 scans/s. The transfer line temperatures were set at 280 °C, the ion source temperature at 230 °C, and the quadrupole temperature at 150 °C. Volatile compounds were identified using a Deconvolution Reporting System (DRS, version A.02.00, Agilent Technologies), which combines an automatic mass spectral deconvolution and identification software (AMDIS version 2.62, NIST) with a mass spectral library (NIST08 database) and GC–MS software (ChemStation version D.03.00, Agilent Technologies). The AMDIS database contained 1100 mass spectra of volatile compounds, 180 of which were connected to Kovats retention indexes (Kováts, 1958). To obtain comparable retention times for all samples, the retention time was locked and referenced according to the internal standard heptyl acetate at 10.748 min by use of the ChemStation retention time-locking program. Peaks that were present in the chromatogram but not identified by the DRS were manually interpreted using the NIST08 database. To ensure reliable identification, a match factor of at least 70 was used (Stein 1999). Compound identification was verified by comparing mass spectra and retention times with those obtained for synthetic standards on the same column. Terpenes were quantified as pentadecane equivalents by dividing the peak areas from the total ion chromatogram of single terpenes by the peak area of the internal standard pentadecane. The monoterpene α-pinene was provided by Yngve H. Stenstrøm (Norwegian University of Life Sciences, Ås, Norway), whereas all other compounds were acquired as standards from Aldrich, Fluka, Chiron AS, Supelco, and SAFC.

### *2.2.3 Chiral terpene analysis*

The separation was performed on a 30-m Cyclodex-B column (Agilent Technologies) with an inner diameter of 0.25 mm and film thickness of 0.25 µm. After sample injection, the column temperature was held at 40 °C for 0.5 min and raised by 2 °C/min to 80 °C, then raised by 10 °C/min to 220 °C and held constant for 1 min, giving a total running time of 36 min. The injection volume, injector temperature and the mass-spectrometric parameters were the same as for the non-chiral analysis.

## 2.3 Beetle pheromone quantification

On July 10, 2015, spruce bark beetles were introduced into the 0.4 m bolts cut from the 30 experimental trees to determine the beetles' ability to produce pheromones in the different spruce species. The beetles had been collected in pheromone baited traps in early June 2015 and stored at 4 ˚C. Ten vigorous beetles were placed individually on the bark surface of each bolt. Each beetle was covered by a glass vial held tightly against the bark by a rubber band extending around the bolt. To facilitate beetle entry into the bark a superficial wound was made through the cork bark at each position. After 48 hours, tunneling beetles were removed from the bark, their hind gut was removed, and the guts were analyzed by GC-MS to quantify pheromone compounds, following the procedure described above. Since males are the pheromone producing sex in the spruce bark beetle only male beetles were analyzed. The spruce bark beetle can only be reliably sexed by removing the subgenital plate and aedeagus, and during this process the hindgut of males was removed and pooled by bolt (n=1 to 6 males per bolt) in 100 µl hexane containing 0.1 µg/ml pentadecane. To compare pheromone quantity on a per male basis the total amount of each pheromone compound detected in a sample was divided by the number of males in the sample.

### 2.4 Data analyses

All statistical analyses were carried out in R (v.3.3.1) (R Core Team 2015) using the packages Vegan (v 2.3-5) for Detrended Correspondence Analysis (DCA), Nonmetric Multidimensional Scaling (NMDS) and Procrustes analysis (Oksanen et al. 2016) and ggplot2 (Wickham 2009) for plotting. NMDS was used to visualize differences in chemical composition among tree species and treatments (constitutive vs. induced samples) based on Bray-Curtis dissimilarities of square-root-transformed concentration data for 107 compounds from constitutive samples and 127 compounds from induced samples. The NMDS model was set to two dimensions, plots were centered, rotated to principal components, and axes were rescaled to half-change units. For both constitutive and induced samples convergence was found after 20 iterations with a goodness-of-fit measure (called 'stress') of 0.09, which indicates a good fit (Kruskal & Wish 1978). To find the optimal configuration we used NMDS in parallel with DCA and employed Procrustes analysis to see if the two different methods revealed the same structure. Since no structural differences were found between NMDS and DCA, and since NMDS gave an acceptable representation of the data structure, only the NMDS results are presented here.

Many terpenoids only occurred in a few samples from each tree species and in trace amounts, and were assumed to be biologically less important. For further comparisons between tree species we therefore included only the most abundant terpenoids, i.e. compounds making up one percent or more of the total volume. In the constitutive samples these terpenoids made up at least 93% of the total terpenoid volume in each tree species, while in the induced samples they made up at least 89%. Mean constitutive and inducible terpenoid amounts were compared between tree species, both for individual compounds and for total terpenoids and total mono-, sesqui-, and diterpenes. Treatments were compared using one-way ANOVA and pairwise comparisons based on Tukey and Kramer (Nemenyi) test with Tukey-Dist approximation for independent samples (chi-square).

Because (-)-α-pinene in the host tree is the main precursor for the beetle pheromone component cis-verbenol (Birgersson et al. 1988), we tested four hypotheses for how cis-verbenol production might vary with host terpene levels. Data from all three spruce species were considered together, as we assumed that effects of terpene levels on pheromone production are independent of other host tree qualities. $H_0$: cis-verbenol production is independent of the amount of (-)-α-pinene in the bark and there is no correlation between cis-verbenol and (-)-α-pinene within the range of (-)-α-pinene concentrations found in our samples. $H_1$: cis-verbenol production increases with the amount of (-)-α-pinene in the bark (positive correlation). $H_2$: cis-verbenol production varies with the relative amount of (-)-α-pinene in the bark (amount of (-)-α-pinene divided by the total amount of all other terpenes). $H_3$: cis-verbenol production varies with the amount of (-)-α-pinene minus the total amount of all other terpenes. $H_2$ and $H_3$ takes into consideration that the beetles must detoxify other host terpenoids in addition to (-)-α-pinene. $H_2$ assumes a linear effect of other terpenes, whereas $H_3$ assumes that other terpenes must be detoxified first before the beetles can use (-)-α-pinene to produce cis-verbenol. The function cor.test in R was used to calculate Spearman's rho statistics between cis-verbenol and terpene amounts when testing hypotheses $H_0$-$H_3$.

## 3. Results

### 3.1 Overall differences in terpenoid composition between Sitka, Lutz and Norway spruce

The NMDS plot (Figure 1) shows the overall chemical similarity between tree species, i.e. the closer the tree species are in the plot, the more similar is their terpenoid composition. The axis range spanned by the data (ca. 1.5 half-change units), indicates that there are only moderate differences between tree species in terpenoid composition, i.e. the tree species mostly share the same compounds. The data for Sitka and Lutz spruce (blue and red areas) and their mean site scores (black dots) move in the same direction from constitutive to induced samples, indicating that the terpenoid composition changes similarly between these species after MeJA treatment. Norway spruce (green areas and arrow) moves away from Sitka and Lutz following induction, becoming slightly more different in terpene composition after MeJA treatment.

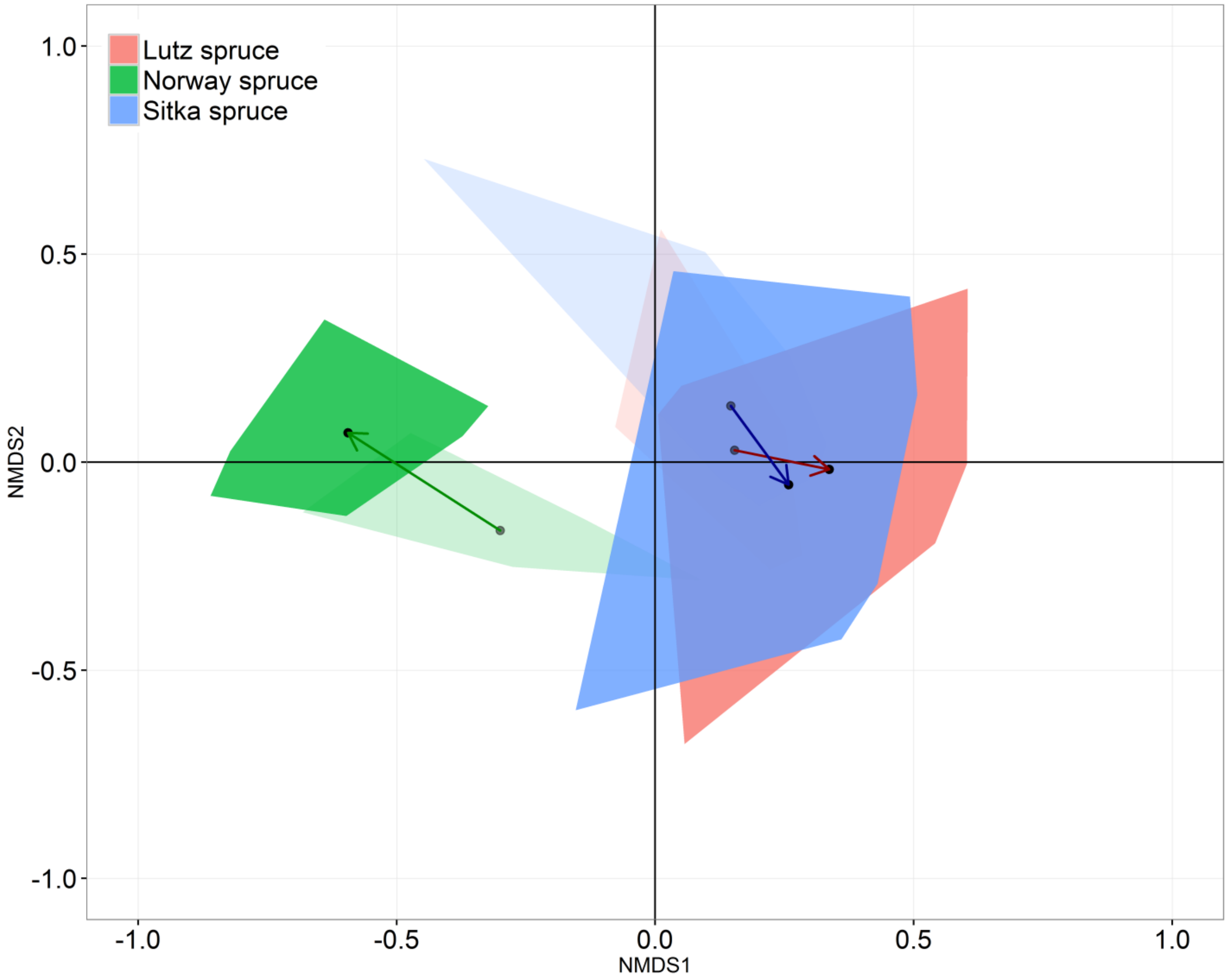

Figure 1. Non-metric multidimensional scaling (NMDS) showing the overall compositional terpenoid similarity between Sitka, Lutz and Norway spruce in constitutive bark samples (tint color) and induced bark samples (darker color). Black dots indicate mean site scores and arrows indicate direction of change from constitutive to induced samples.

### 3.2 Constitutive and induced terpenoid chemistry

Norway spruce had significantly higher total terpenoid levels than Sitka and Lutz spruce in both constitutive and induced samples (Table 1). All three spruce species had much higher concentration of mono-, sesqui-, and diterpenes in induced samples compared to constitutive samples. A total of 148 unique terpenoids were identified with >70% certainty in one or more of the three spruce species, of which 107 compounds were detected in constitutive samples and 127 in induced samples.

Table 1. Pairwise comparisons of means of total amount of terpenes (mg $g^{-1}$ dry weight phloem) in Sitka, Lutz and Norway spruce using Tukey and Kramer (Nemenyi) test with Tukey-Dist approximation for independent samples (chi-square) following ANOVA.

| | Norway-Lutz | Norway-Sitka | Lutz-Sitka |
|---|---|---|---|
| Constitutive | | | |
| Mean values | 0.07 – 0.04 | 0.07 –0.04 | 0.04-0.04 |
| Kruskal-Wallis χ2 | 37.7 | 38.5 | 0.005 |
| P-value | < 0.0001 | < 0.0001 | 1.00 |
| Induced | | | |
| Mean values | 2.6-1.1 | 2.6-1.5 | 1.1-1.5 |
| Kruskal-Wallis χ2 | 59.6 | 65.0 | 0.1 |
| P-value | < 0.0001 | < 0.0001 | 0.94 |

### *3.2.1 Constitutive terpenoid chemistry*

Total constitutive terpenoid levels in Norway spruce were 31 and 47% higher than in Sitka and Lutz spruce, respectively (Table 1). Sitka and Lutz spruce did not differ significantly from each other. The abundance of individual terpenoids was quite similar in the three spruce species, but Norway spruce had significantly higher concentrations of many terpenoids than the two other species (Figure 2). Fourteen of the 16 most abundant terpenoids in each spruce species were shared between all the species. The 16 most abundant compounds made up 93, 95 and 95% of total terpenoids in Norway, Sitka and Lutz spruce, respectively.

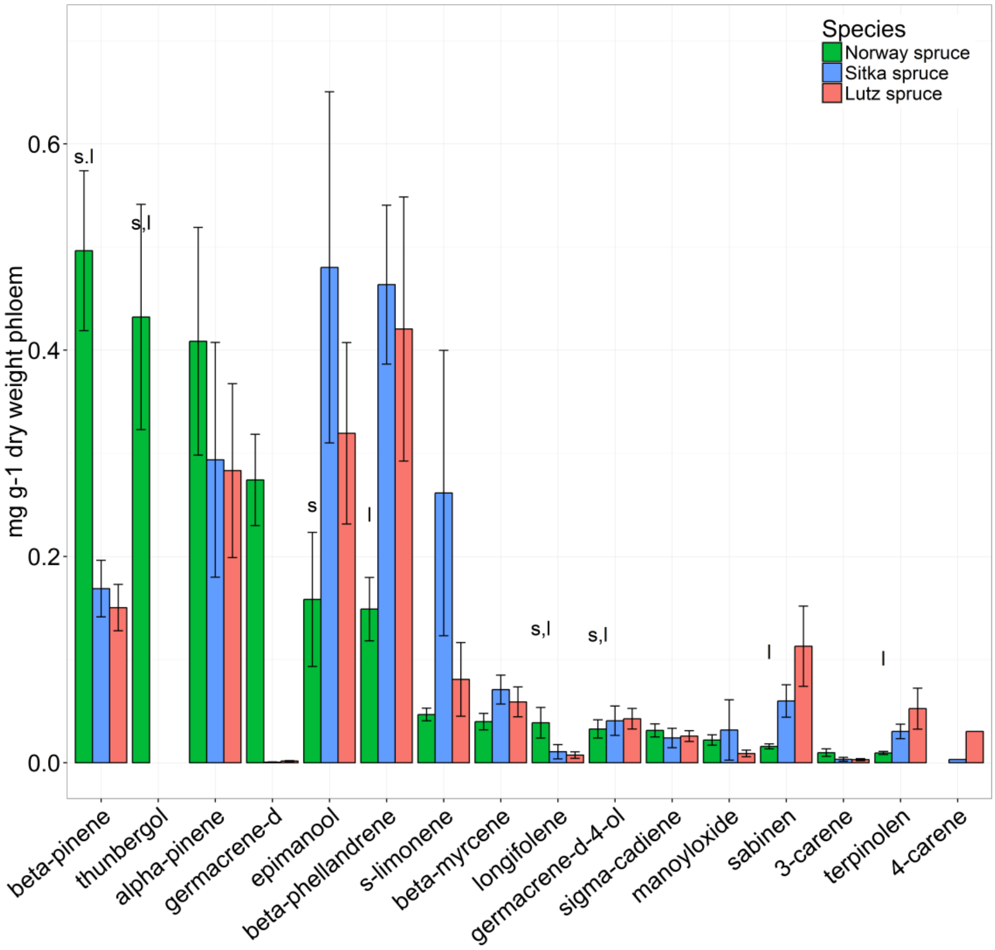

Figure 2. Mean and standard error (SE) of the 16 most abundant terpenoids in constitutive bark of Norway, Sitka and Lutz spruce. 's' indicates significant difference between Norway and Sitka spruce and 'l' indicates significant difference between Norway and Lutz spruce following ANOVA and Tukey HSD ($p < 0.05$).

### *3.2.2 Induced terpenoid chemistry*

Terpenoid levels increased in all three spruce species in response to MeJA treatment, with 25- to 35-fold increases in total terpenoids in the different species. Total induced terpenoid levels in Norway spruce were significantly higher than in Sitka (56%) and Lutz spruce (107%). There was no significant difference between the two North American species (Table 1). As with the constitutive chemistry, the abundance of individual terpenoids was quite similar in the three spruce species, but Norway spruce had significantly higher concentrations of many terpenoids than the two other species (Figure 3). The 16 most common compounds made up 89, 91 and 91% of total induced terpenoids in Norway, Sitka and Lutz spruce, respectively. Concentrations of the cis-verbenol precursor (-)-α-pinene differed significantly between

Norway spruce and the two North American spruce species in induced samples but not in constitutive samples.

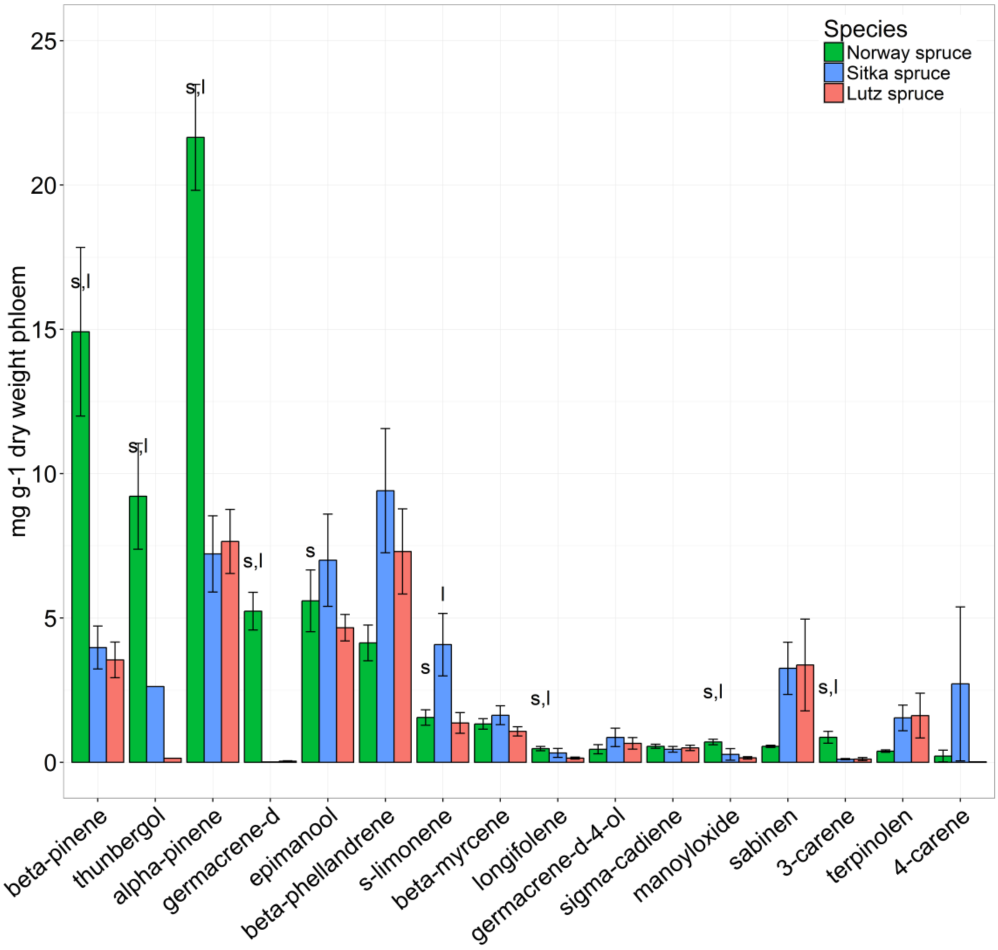

Figure 3. Mean and standard error (SE) of the 16 most common terpenoids in induced bark of Norway, Sitka and Lutz spruce. ‘s’ indicates significant difference between Norway and Sitka spruce and ‘l’ indicates significant difference between Norway or Lutz spruce and Sitka spruce following ANOVA and Tukey HSD ($p < 0.05$).

### 3.3 Pheromone production

Male spruce bark beetles produced comparable amounts of their two main pheromone components cis-verbenol and 2-methyl-3-buten-2-ol (methylbutenol) in all three spruce species, with no statistically significant differences in pheromone production between species ($F_{2, 27} = 0.45$, $P = 0.64$ for cis-verbenol; $F_{2, 27} = 0.72$, $P = 0.50$ for methylbutenol). The ratio between methylbutenol and cis-verbenol was 8:1 in Norway spruce, 10:1 in Sitka spruce, and 17:1 in Lutz spruce. Because there were no significant differences in cis-verbenol production between tree species, the influence of terpenes on the production of cis-verbenol was analyzed using terpene values from all tree species in the same analysis. We found no significant correlations between the amount of cis-verbenol produced by the beetles and any of the combinations of terpene amounts tested in hypotheses $H_1$-$H_3$ (Table 2). Thus, our results support the null hypothesis $H_0$, stating that the amount (-)-α-pinene in the host does not influence beetle cis-verbenol production within the range of (-)-α-pinene concentrations that occurred in our samples.

Table 2. Spearman's rho and P-value for rank correlations between the amount of cis-verbenol produced and H1: the amount of (-)-α-pinene, H2: the amount (-)-α-pinene divided by the amount of other terpenes, and H3: the amount (-)-α-pinene minus the amount of other terpenes.

| Hypotheses | Rho | P |
|---|---|---|
| $H_1$: (-)-α-pinene concentration influences cis-verbenol production | 0.06 | 0.75 |
| $H_2$: (-)-α-pinene/other terpenes influences cis-verbenol production | -0.31 | 0.10 |
| $H_3$: (-)-α-pinene - other terpenes influences cis-verbenol production | 0.02 | 0.92 |

## 4. Discussion

### 4.1 Constitutive terpenoid chemistry and host suitability

Norway spruce had significantly higher total constitutive terpene quantities than either Sitka or Lutz spruce, although the differences were quite small (< 2-fold). The somewhat lower terpenoid quantities in Sitka and Lutz spruce may make these exotic spruce species more suitable hosts for the spruce bark beetle, since the beetles will face less severe terpenoid defenses. The NMDS analysis of all detected terpenoids revealed relatively small differences in terpenoid composition between the three spruce species. The composition of individual terpenoids in constitutive bark also suggests that Sitka and Lutz spruce should be equally suitable hosts for the spruce bark beetle as Norway spruce. Sitka and Lutz spruce had very similar terpenoid compositions, as was expected since Lutz spruce is a natural hybrid between Sitka spruce and white spruce. Norway spruce, with its different terpenoid composition, belongs to a different clade in the spruce phylogeny (Lockwood et al. 2013). Five of the 16 most abundant constitutive compounds in all spruce species [(-)-α-pinene, ß-pinene, ß-phellandrene, myrcene] have been shown to elicit strong antennal responses in the spruce bark beetle, suggesting that they are important in the host selection process (Andersson et al. 2009; Kalinova et al. 2014). The high concentrations of these compounds also in Sitka and Lutz spruce suggests that the spruce bark beetle should be able to detect and colonize these North American spruce species under natural conditions. However, we cannot rule out that other physical or chemical defenses we did not quantify, such as phenylpropanoids and other phenolics, may influence the relative suitability of Norway spruce versus Sitka and Lutz spruce.

### 4.2 Induced terpenoid chemistry and host suitability

All three spruce species showed a strong induced response to MeJA treatment, with 25- to 35-fold increases in total terpenoid levels one month after treatment. This massive accumulation of terpenoids is expected to strongly reduce the suitability of the trees to the spruce bark beetle. Previous studies of the spruce bark beetle and other tree-killing bark beetles have shown that the beetles tend to avoid trees with very strong terpene accumulation (Boone et al. 2011; Schiebe et al. 2012; Zhao et al. 2011a). As with constitutive terpenoids, Norway spruce had significantly higher total terpene volumes than Sitka and Lutz spruce (56% and 107%), which probably would make Norway spruce less attractive than the two novel hosts. Thunbergene and thunbergol, which only appeared constitutively in Norway spruce, was also present in induced Lutz spruce but was still missing in Sitka spruce.

### 4.3 Possible roles of individual terpenoids

Spruce oleoresin is a complex mixture of terpenoids, where most terpenes have an unknown behavioral effect on the bark beetles (Andersson et al. 2009), and many terpenoids probably act in synergy (Phillips & Croteau 1999). Thunbergol, which was much more abundant in constitutive and induced Norway spruce bark than in the two North American spruces, has been shown to be toxic to both the spruce bark beetle and its symbiotic ally *E. polonica* (Zhao et al. 2011a). Other terpenes, like limonene, myrcene, and 3-carene, have also been shown to act as both attractants and repellents (Erbilgin et al. 2007a; Miller & Borden 2000; Wallin & Raffa 2000), and to be toxic to bark beetles (Raffa & Berryman 1982). Specific host terpenes may also elicit different responses in beetle populations from different regions (Erbilgin et al. 2007b). Research on the congeneric *Ips pini* demonstrated that increased quantities of the monoterpenes α-pinene, β-pinene and limonene reduces beetle establishment (Wallin & Raffa 2000). It is challenging to understand what effects qualitative differences in terpene chemistry have on the colonization biology of bark beetles, since the relationship between the beetles and most host terpenes are unknown. However, a quantitative increase in terpene volume is generally thought to be negative for attacking beetles (Erbilgin et al. 2006; Wallin & Raffa 2000; Zeneli et al. 2006; Zhao et al. 2011b), and this should make Sitka and Lutz more attractive to the spruce bark beetle. Also, the criteria by which bark beetles select their host trees are complex and involve visual and olfactory cues (Campbell & Borden 2006a; Campbell & Borden 2006b) from both hosts and non-hosts (Andersson et al. 2009).

### 4.4 Pheromone production

Our results demonstrate that the spruce bark beetle produced the two main components of its aggregation pheromone, methylbutenol and cis-verbenol, equally well in the novel hosts Sitka and Lutz spruce, as in the historical host Norway spruce. Cis-verbenol production in the beetles is induced by exposure to (-)-α-pinene in the host tree (Birgersson et al. 1988). Even though Norway spruce contained more (-)-α-pinene than the two novel hosts, we found no significant difference in cis-verbenol production between tree species. None of the three different hypotheses we tested of how concentrations of precursor (-)-α-pinene and other terpenes in the host bark might affect cis-verbenol production were supported by our data. The lack of any correlation between pheromone production and host tree chemistry contrasts with the results of Burke and Carroll (2016), who found a significant correlation between concentrations of α-pinene and pheromone production in the mountain pine beetle. Thus, Burke and Carroll (2016) found support for the equivalent of our hypothesis H2, using the ratio of (-)-α-pinene to other terpenes, which was at best marginally significant in our study ($p = 0.10$). Our data on the spruce bark beetle only supported the null hypothesis that (-)-α-pinene concentrations in the host do not influence cis-verbenol production. However, we expect that terpene levels might be a factor for cis-verbenol production when (-)-α-pinene levels are either very low or very high, and thus beyond the levels detected in our experimental trees.

The beetles' most abundant aggregation pheromone component, methylbutenol, is produced *de novo* and production is stimulated by beetle tunneling and feeding in the phloem (Birgersson et al. 1988). Males release substantial amounts of methylbutenol in the early phase of tree colonization, equaling >3% of their body weight over the first week of an attack (Birgersson and Bergström 1989). This has a substantial metabolic cost and only physiologically fit males are able to produce these amounts of methylbutenol. The fact that we did not find any significant differences in the amount of methylbutenol released by spruce bark beetles colonizing the different spruce species is another indication that the chemical defenses of Sitka, Lutz and Norway spruce represent a comparable physiological challenge for the spruce bark beetle.

The blend of methylbutenol, cis-verbenol and ipsdienol produced by male spruce bark beetles is known to be a potent attractant for the spruce bark beetle (Bakke et al. 1977), and the commercial pheromone blend Ipslure contains 1500 mg methylbutenol, 70 mg cis-verbenol and 15 mg ipsdienol (kjemikonsult.no, 2016). This gives a ratio between methylbutenol and cis-verbenol of about 20:1. In our experiment beetles feeding on Lutz spruce produced methylbutenol to cis-verbenol ratio of 17:1, compared to a ratio of 10:1 on Sitka spruce, and 8:1 on Norway spruce. These ratios are much lower than those found by Zhao et al. (2011a) for spruce bark beetles colonizing untreated Norway spruce bark (from 22:1 to 93:1 over the first six days after beetle entry), and are more similar to the ratios produced by beetles colonizing bark that had been treated with MeJA one month before beetle entry (16:1 to 39:1). Cis-verbenol and methylbutenol play different roles in beetle aggregation: the former is used for long-range orientation towards attacked trees, whereas the latter is as short-range orientation or arrestment stimulus which concentrates beetles to an attacked tree. These differences in ratios are believed to effect beetle orientation, attraction and ability to mass attack (Erbilgin et al. 2006; Zhao et al. 2011a).

### 4.5 Conclusion

Our results show that the three spruce species had a similar terpenoid composition, and that the majority of the terpene volume was made up of important host compounds in Norway spruce that elicit strong antennal responses in the spruce bark beetle. However, since Sitka and Lutz spruce had significantly lower terpene concentrations than Norway spruce they may be more prone to beetle attack than Norway spruce, especially since the beetle produced equal amounts of its pheromone components on all three spruce species, regardless of differences in (-)-α-pinene concentrations. No-choice conditions such as those used in our experiments can be problematic because beetles will tend to enter different hosts in equal amounts (Raffa et al. 2013). However, from other studies we know that the spruce bark beetle can complete its lifecycle in Sitka and Lutz spruce and produce offspring of comparable size as in Norway spruce (Økland et al. 2011; Flø et al. manuscript).

The data presented in this paper, including the successful production of the beetle aggregation pheromone components cis-verbenol and methylbutenol, suggests that the spruce bark will be able to mass attack and reproduce in Sitka and Lutz spruce. However, beetle host selection may be influenced by host attributes that we did no measure, such as potentially toxic or repellent phenolics or carbohydrate and lipid content. Sitka and Lutz spruce are extensively planted in maritime climates in Western Europe, and climate change-induced range shifts of the spruce bark beetle into these habitats may cause severe economic damage. Also, if the spruce bark beetle becomes established in North America, where it has been intercepted repeatedly (Haack 2001) but has not yet established, the beetle will probably be able to mass attack Sitka and Lutz spruce within their native ranges (Økland et al. 2011).

## 5. Acknowledgment

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# Paper IV

# Reproduction of *Ips typographus* in co-evolved and naïve spruce hosts

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# Abstract

The two North American spruce species Sitka spruce (*Picea sitchensis* (Bongard) Carrière) and Lutz spruce (*Picea x lutzii* Little), are extensively planted in northwestern Europe, but have not yet been subjected to outbreaks by the European spruce bark beetle, *Ips typographus* (L.). The spruce bark beetle is one of the most economically important forest pests in Europe's boreal forests with the ability to attack and kill large areas of Norway spruce (*Picea abies* (L.) H. Karst). We compared the reproductive performance of the spruce bark beetle on the co-evolved host Norway spruce and the two novel North American hosts Sitka and Lutz spruce, by a controlled breeding experiment. We also assessed the growth performance of the spruce bark beetle-associated blue-stain fungus *Endoconidiophora polonica* (Siemaszko) C. Moreau in the three spruce species. Our experiments showed that the spruce bark beetle can breed successfully in Sitka and Lutz spruce. Offspring length and weight was similar across spruce species, indicating that offspring quality or fitness was similar. However, total offspring production and total number of galleries were lower on Sitka and Lutz spruce. Since fewer females established galleries on Sitka and Lutz spruce compared to Norway spruce but offspring production per female was almost equal across spruce species, our results suggest that the beetles had difficulties starting attacks and establishing maternal galleries on the novel hosts. The symbiotic fungus *E. polonica* performed poorly on the two novel spruce hosts. Overall, our experiments suggest that the spruce bark beetle will be able to colonize Sitka and Lutz spruce in European plantations and in native spruce forests in North America. However, more research is needed on the importance of factors that reduce beetle colonization success and on the potential of the beetles to overcome such obstacles by adaptation.

Key words: Naïve host, Norway spruce, Sitka spruce, Lutz spruce, *Picea abies*, *Picea sitchensis*, *Picea lutzii,* the European spruce bark beetle

## Introduction

The European spruce bark beetle, *Ips typographus* (L), is one of the most economically important forest pests in Europe's boreal forests. During a 50-year period (1950–2000), the beetle killed about 150 million $m^3$ of its main host Norway spruce (*Picea abies* (L.) H. Karst.) in extensive outbreaks (Schelhaas et al. 2003). Several factors are involved when the spruce bark beetles select and attack a host tree, such as visual cues, chemical olfactory signatures of healthy versus stressed host trees, and pheromones from beetles that already have colonized the tree (Andersson et al. 2009; Krokene 2015). Abiotic factors like windthrows and drought are important drivers for bark beetle outbreaks (Netherer et al. 2015; Økland et al. 2015), and bark beetle-host tree interactions are directly and indirectly influenced by climate change (Bentz et al. 2010) and global trade (Haack 2001). All of these factors may be important for the beetle's ability to colonize and kill novel spruce hosts.

Since the 1960s, two North American spruce species, Sitka spruce (*Picea sitchensis* (Bongard) Carrière) and the Sitka/white spruce (*P. glauca*) hybrid Lutz spruce (*Picea × lutzii* Little), have been extensively planted in northern and western Europe. Plantations of Sitka spruce now exceeds 1.2 million hectare (Mason & Perks 2011), mainly in coastal regions of the British Isles (1.07 million ha), Norway (50 000 ha), France (50 000 ha), and Denmark (35 000 ha). In addition to possible future attacks on Sitka and Lutz spruce in Europe, the spruce bark beetle may become introduced in the native ranges of Sitka and Lutz spruce in North America. Due to the risk of future attacks by the spruce bark beetle more information is needed about beetle colonization success on these two potential host trees. Attacks by the spruce bark beetle on individual Sitka spruces have been observed in the British Isles and Sweden (Sean Murphy pers. comm.; Ulf Johansson pers. comm.), but no major outbreaks on Sitka spruce are known from Europe.

Breeding experiments by Økland et al. (2011) in Norway and Sweden showed that the spruce bark beetle can develop successfully in several North American spruce species, including Sitka and Lutz spruce. However, because these experiments did not control for attack density they could not distinguish beetle reproductive performance in each tree species from competitive effects due to different attack densities of colonizing beetles. In the present study our aim is to more precisely assess beetle reproductive performance in the different spruce species by doing controlled breeding experiments. Norway, Sitka and Lutz spruce have comparable terpene composition, but Sitka and Lutz spruce have significantly lower terpene concentrations and may thus be more prone to beetle attack (Flø et al. manuscript). The successful production of the beetles' main aggregation pheromone components in the novel hosts also suggests that the spruce bark beetle may thrive in Sitka and Lutz spruce (Flø et al. manuscript).

The spruce bark beetle carries a multitude of fungi (Linnakoski et al. 2016) that may aid in breaking down spruce defenses (Krokene 2015). One of the most virulent fungal associates is the blue-stain fungus *Endoconidiophora polonica* (Siemaszko) C. Moreau, which has the ability to kill Norway spruce alone when experimentally inoculated into trees (Horntvedt et al. 1983). The relationship between the spruce bark beetle, *E. polonica* and its historic host Norway spruce is well documented (Krokene & Solheim 1996; Krokene & Solheim 1998; Krokene et al. 2003), but the interaction between the beetle, *E. polonica* and the novel hosts Sitka and Lutz spruce is not known.

In this article we report on the breeding success of the spruce bark beetle on Norway spruce and the two novel hosts Sitka and Lutz spruce. In breeding experiments where we control the colonization density we compare (1) the number of galleries, number of offspring per gallery, and size and weight of beetle offspring in the different spruce species, and (2) the ability of the symbiotic blue-stain fungus *E. polonica* to colonize and kill spruce phloem.

## Material and methods

### Study area and sampling of trees

On May 13 2015, 1.2 meter stem sections were cut from 10 trees of Sitka, Lutz and Norway spruce growing on a 0.75 hectare experimental plot established in 1963 near Prestebakke in Halden, SE Norway (N 58.9990 E 11.5219). All trees were growing in plots of approximately 60 × 20 meters and at similar densities and growth conditions (see Flø et al. manuscript for more details on the study site). The following day, each stem section was divided into three approximately 40 cm long bolts and the ends were coated with melted paraffin wax (VWR Chemicals) to minimize desiccation and nutrient loss. Ten bolts per tree species were chosen for either inoculation with *E. polonica* or for determination of beetle reproductive performance. The remaining 10 bolts were used in a study of the chemical composition of the different tree species (Flø et al. manuscript). All bolt and beetle measurements are presented in tables 1 and 2.

### Beetle reproductive performance experiment

To assess beetle reproductive performance we introduced beetles into 10 bolts per tree species at a controlled low density (~0.25 colonization sites per $dm^2$ bark surface) to minimize competition for breeding substrate. On the morning of May 27, the bolts were hung from the ceiling in the institute's insectarium and a superficial wound was made through the outer bark to facilitate entry of colonizing beetles. Each wound was then covered by a glass vial containing four beetles. The vials were held in place by rubber bands extending around the bolt. Depending on the size of the bolt, 3-4 glass vials were evenly spaced around the upper and lower part of the bolt, distributed to minimize competition between adjacent galley systems. The following morning, additional beetles were introduced into each glass, if necessary. The spruce bark beetle cannot be accurately sexed without dissecting the genitalia, and more beetles were added to ensure that there were enough females at each colonization site (every gallery system initiated by a pioneering male can accommodate up to four females). Additional beetles were sometimes also needed to replace beetles that were feeble or had died overnight. After three days, all glass vials were removed and each bolt was covered by an emergence net with a collection bottle underneath. The bottles were emptied biweekly for emerging offspring that were counted and stored at 4 °C. On October 12, all bark was cleared from the bolts, all remaining live beetles under the bark were collected and counted, and the number of gallery systems was recorded. All beetles were dried in an oven at 70 °C for 50 hours to determine beetle dry weight, before a random selection of beetles were individually weighted, and measured lengthwise (table 2).

## *Endoconidiophora polonica* inoculation

Ten bolts per tree species were inoculated with the blue-stain fungus *Endoconidiophora polonica* by removing a 5-mm diameter bark plug at each of four cardinal directions and injecting fungal inoculum (mycelium on malt agar) into the holes using a syringe. Bark thickness was measured on all four bark plugs using a caliper, before the holes were covered with the same bark plugs. The fungus was left to colonize the bolts for 90 days before the cork bark was removed and the length of the necrotic lesions in the inner bark was measured. Two measures were taken: (1) the length of the outer necrosis, which measures the full extent of fungal colonization, and (2) the length of the shorter inner necrosis, which represents the active host defense area. The *E. polonica* isolate used (no. 193-208/115 in the culture collection of the Norwegian Institute of Bioeconomy Research) had been growing on malt agar for 21 days at a constant temperature of 20 °C before inoculation.

## Continentality indices across spruce and spruce bark beetle distributions

To determine the distribution of Sitka spruce, Norway spruce, and the spruce bark beetle relative to climatic continentiality (i.e. the mean annual temperature range) we downloaded the coordinates of all records of these species from the database of the Global Biodiversity Information Facility (GBIF 2016) using the package dismo (Hijmans et al. 2016). For Lutz spruce not enough records were available to obtain meaningful distribution data. For Norway spruce we downloaded 131 993 coordinates, for Sitka spruce 9 583 coordinates, and for the spruce bark beetle 2 452 coordinates. To determine the climatic continentiality across the geographical distribution of the three species we downloaded climate data from Worldclim.org (Hijmans et al. 2005), and continentality indices coinciding with the coordinates of the records of the different species were extracted from the raster layers. Mean distance of all species records from the nearest coastline was calculated from all records of the spruce bark beetle and for a subset of 5 000 randomly selected records for each of the spruce species.

## Statistical treatment

All statistical analyses were carried out in R (v.3.3.1) (R Core Team 2016) using the packages sp (Bivand et al. 2013), rgeos (Bivand & Rundel 2016) and rgdal (Bivand et al. 2016) for spatial calculations, and ggplot2 (Wickham 2009) for plotting. Correlations between beetle offspring production and terpenoid quantities were calculated using the function cor, while differences between spruce species in length and weight of beetle offspring and in *E.*

*polonica* lesion lengths were tested by analysis of variance (aov) followed by Tukey honest significant difference tests (TukeyHSD) (R Core Team 2016).

## Results

### Beetle performance experiment

Many more spruce bark beetle offspring developed successfully in Norway spruce than in Sitka spruce (~3-fold more) and Lutz spruce (~5-fold more) (Table 1). The first offspring emerged from the bolts on July 27, but only a small proportion of the total brood had emerged from the bolts when the breeding experiment ended October 12 (14% of the total in Norway spruce and 22% in Sitka and Lutz spruce). The rest of the living offspring were recovered from under the bark of the bolts. The higher offspring production in Norway spruce than in Sitka and Lutz spruce corresponded with a higher number of breeding galleries (Table 1). Thus, the number of offspring produced per breeding gallery was very similar across spruce species; 26.8 in Norway spruce, 28.6 in Sitka spruce, and 22.4 in Lutz spruce (Table 1). There was no statistically significant difference in means of offspring weight ($F_{2,556}$ = 0.562, p = 0.60) or length ($F_{2,556}$ = 2.623, p = 0.07) between the different tree species (Table 2).

Table 1. Breeding substrate properties and reproductive performance of the spruce bark beetle in cut bolts from three different spruce species. All values are given as mean per bolt (n = 10 bolts per spruce species).

| Species | Bolt length (cm) | DBH (cm) | Surface area (dm2) | Bark thickness (mm) | No. beetles introduced | No. entrance holes | No. offspring | No. galleries | No. offspring per gallery | No. offspring per surface area |
|---|---|---|---|---|---|---|---|---|---|---|
| Norway spruce | 41.2 | 67.7 | 29.4 | 5.3 | 32.5 | 7.2 | 179.8 | 6.7 | 26.8 | 6.1 |
| Sitka spruce | 41.5 | 62 | 26.8 | 4.8 | 25.6 | 6.6 | 66.0 | 2.3 | 28.6 | 2.5 |
| Lutz spruce | 40.7 | 61.2 | 27.1 | 4.8 | 27.0 | 6.8 | 37.2 | 1.3 | 22.4 | 1.4 |

Table 2. Length and weight of spruce bark beetle offspring developing in cut bolts (n = 10) from three different spruce species. N = total number of beetle offspring measured per spruce species.

| Spruce species | Length (mm) mean ± SE | Weight (mg) mean ± SE | N |
|---|---|---|---|
| Norway spruce | 4.85±0.02 | 5.0±0.1 | 266 |
| Sitka spruce | 4.79±0.03 | 5.0±0.1 | 112 |
| Lutz spruce | 4.79±0.02 | 5.0±0.1 | 181 |

To test if beetle offspring production varied with spruce defense chemistry we investigated the relationship between offspring production and terpenoid levels in the bark of each bolt, using chemical data from Flø et al. (manuscript). Out of the 30 most abundant terpenoids in the different spruce species, offspring production was positively correlated only with absolute levels of thunbergene and thunbergol. Offspring production was also positively correlated with the inducibility of thunbergene, thunbergol and α-longipinen, i.e. with the difference in terpenoid concentration between bark where defenses had been induced by methyl jasmonate application and non-induced control bark (Flø et al. manuscript) (Figure 1). No other significant relationships between offspring production and terpene chemistry were found.

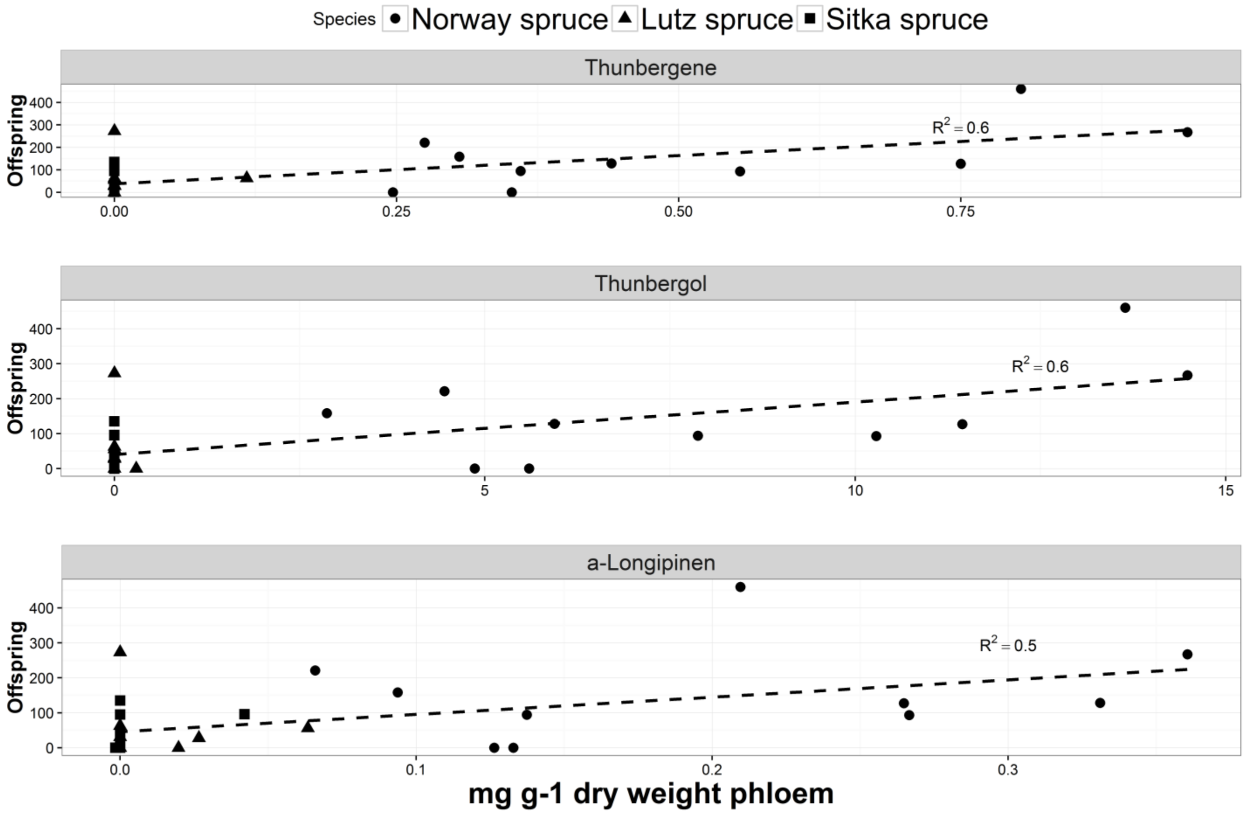

Figure 1. Relationship between offspring production by the spruce bark beetle and inducibility of the terpenoids thunbergene, thunbergol, and α-longipinene in the bark of Sitka, Lutz and Norway spruce. Inducibility = terpene concentration in the bark after defense induction by methyl jasmonate minus terpene concentration in non-induced control bark. Dotted lines are regression lines for the relationship between the dependent variable (number of offspring) and terpene inducibility. n = 10 trees per spruce species.

### Performance of *Endoconidiophora polonica*

There was a statistically significant difference between spruce species in inner necrosis lengths produced in response to *E. polonica* inoculation (one-way ANOVA: $F_{2,112} = 5.072$, $p = 0.008$). There was no significant difference between Sitka and Lutz spruce ($p = 0.98$), but *E. polonica* induced significantly longer inner necrosis lengths in Norway spruce than in the two North American spruce species ($p = 0.02$ for both Sitka and Lutz spruce). Also for outer necrosis lengths there were statistically significant differences between spruce species ($F_{2,117} = 18.096$, $p < 0.001$). As for inner necrosis there was no significant difference between Lutz and Sitka spruce ($p = 0.92$), but *E. polonica* induced significantly longer outer necrosis lengths in Norway spruce than in Sitka and Lutz spruce ($p < 0.001$ for both species).

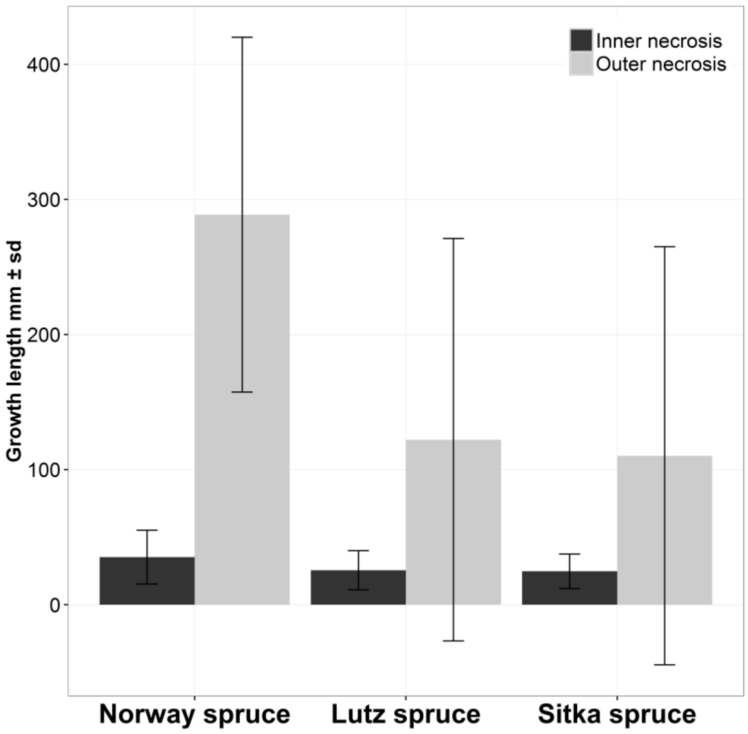

Figure 2. Necrosis lengths 90 days after inoculation with the blue-stain fungus *Endoconidiophora polonica* in cut bolts from three different spruce species. Inner necrosis represents the maximum extent of active host defenses, whereas outer necrosis represents the full extent of fungal colonization. Bars are ±1 standard deviation, n = 10 bolts per spruce species.

## Continentality indices across spruce and spruce bark beetle distributions

Based on their geographic distribution Norway spruce and the spruce bark beetle occur in more continental climates than Sitka spruce. The mean distance between the coastline and the coordinates for spruce bark beetle records was 151 km, compared with 148 km for the beetles' historic host Norway spruce and only 31 km for Sitka spruce. The temperature range between the warmest and coldest month of the year (i.e. the continentality) averaged 27.7 ˚C for records for the spruce bark beetle, 26.8 ˚C for Norway spruce records, and 20.8 ˚C for Sitka spruce records (Figure 3).

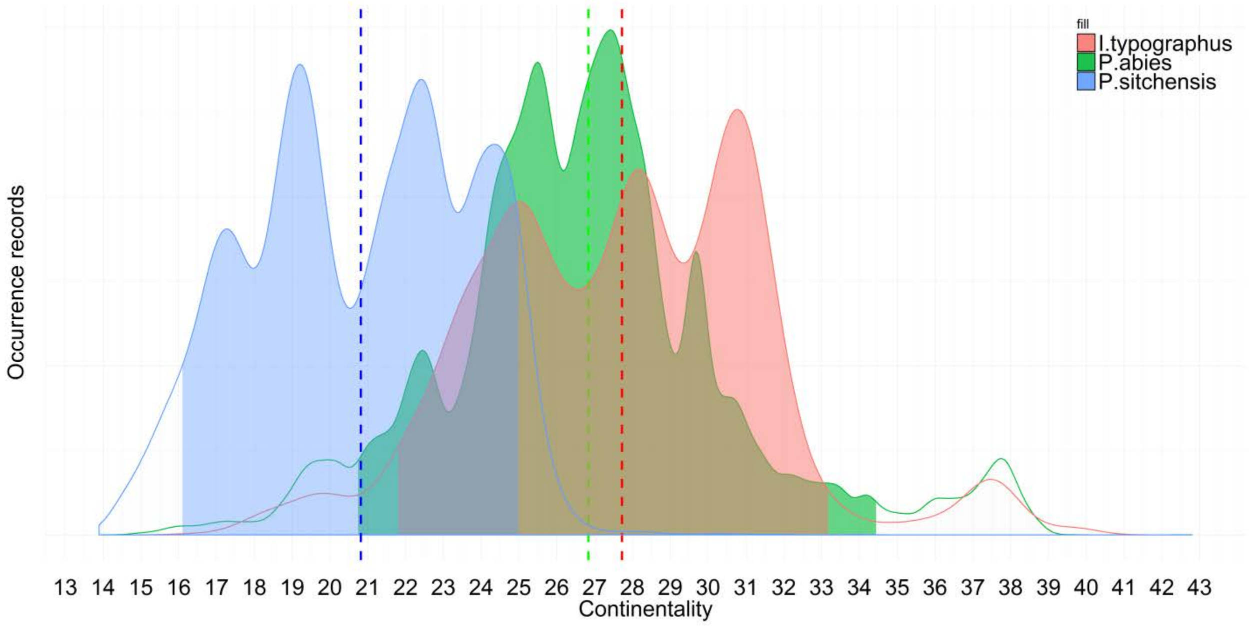

Figure 3. Normal distribution curves for the spruce bark beetle (red curve), its historical host Norway spruce (green curve), and the novel host Sitka spruce (blue curve). The colored area under each curve delineates the 95% of observations that fall within two standard deviations of the mean. X-axis represents climatic continentality, i.e. the annual temperature range (maximum temperature of warmest month - minimum temperature of coldest month). Dotted vertical lines represent the mean continentality value for each species.

## Discussion

### Beetle reproductive performance

Økland et al. (2011) showed that the spruce bark beetle can develop in Sitka and Lutz spruce, and attacks on single trees of these species have been reported from Sweden and Great Britain. However, it was unknown how well the spruce bark beetle performed on the two novel spruce species in terms of offspring quality and quantity. We show that offspring quality was equally good in the two novel North American hosts Sitka spruce and Lutz spruce as in the beetle's historic host Norway spruce in regard to length and weight of the offspring (Table 2). The fact that emerging offspring were almost identical in weight and length across spruce species and that their development time was the same indicate that the bark of the different spruce species offered equal nutrient quality. However, much fewer offspring were produced in the novel hosts than in Norway spruce, and this seemed to be because much fewer of the beetles entering Sitka and Lutz spruce were able to establish successful breeding galleries (Table 1). In Norway spruce almost every entrance hole led to successful breeding, but in Sitka and Lutz spruce the ratio of breeding galleries to entrance holes were as low as 1:2.9 and 1:5.2, respectively. The fact that beetles entered each tree species at similar rates could be a product of our no-choice conditions, since we caged beetles onto the bark for 3 days to encourage entry (Raffa et al. 2013). However, this was necessary to ensure that the density of attacking beetles was similar in all bolts.

We found significant correlations between the number of offspring produced and the inducibility of three terpenes (thunbergene, thunbergol and α-longipinene) which occur primarily in Norway spruce bark (Flø et al. manuscript). The effect of thunbergene and α-longipinene on the spruce bark beetle is unknown, while thunbergol has been shown to inhibit *E. polonica* growth in Norway spruce (Zhao et al. 2010). Establishment success of adult beetles and further brood development are likely to depend on the vigor of each individual tree, including qualitative and quantitative aspects of terpene-based defenses in the bark. Flø et al. (manuscript) found little variation between Sitka, Lutz and Norway spruce in constitutive and induced terpene composition. However, Norway spruce had significantly higher constitutive and induced terpene volumes than Sitka and Lutz spruce. In spruce bark where chemical defenses had been induced by application of the wound hormone methyl jasmonate, thunbergene and thunbergol were not detected in Sitka spruce but was present in Norway and Lutz spruce (Flø et al. manuscript). It is possible that lack of host terpenes like thunbergene and thunbergol from Sitka and Lutz spruce are interpreted as a non-host signal by the spruce bark beetle, which therefore tend not to enter the bark.

The fungus *Endoconidiophora polonica* is an important associate of the spruce bark beetle and can colonize fresh bark and sapwood and kill Norway spruce when experimentally inoculated into the stem (Krokene & Solheim 1996). However, this normally virulent fungus did not perform well on the two novel spruce species compared to Norway spruce. In our experiments the mean growth of *E. polonica* in the bark of Sitka and Lutz spruce was less than half of that in Norway spruce (Figure 2). The explanation for the low colonization success of *E. polonica* in the novel host trees could be that the defenses of the North American spruces efficiently confined the attacks, or that the fungus was unable to recognize these exotic species as potential hosts (non-host reaction). If the spruce bark beetle gets little assistance from *E. polonica* in breaking down tree resistance in Sitka and Lutz spruce the beetles may have a handicap if it attacks these species. However, the spruce bark beetle has many other fungal associates that may be important in breaking down host tree defenses (Krokene & Solheim 1996; Linnakoski et al. 2016) and we do not know how these perform in Sitka and Lutz spruce.

## Geographical distribution of host trees and management considerations

The main distribution area of the novel host species Sitka and Lutz spruce is in a narrow band along the coast, with Sitka spruce occurring on average 31 km from the ocean. Within this distribution area the climate is more oceanic, ocean currents warm the coastal regions in the winter and the trees are exposed to a smaller annual temperature range. The main distribution area of Norway spruce on the other hand is further inland (mean distance from ocean 148 km) where the trees are exposed to higher summer temperatures and lower winter temperatures. This is also were the spruce bark beetle primarily occurs (mean distance from ocean 151 km), and from Figure 3 we see that the range of the beetles overlap almost completely with the range of Norway spruce, while there is only a small overlap with Sitka spruce. Performance of the spruce bark beetle is expected to be optimal around the mean of its distribution area and to be lower towards the oceanic and continental extremes. The fact that the current overlap in distributions of Sitka spruce and the spruce bark beetle is so far from the spruce bark beetle's climatic optimum could explain why we see so little attack on Sitka spruce under current climatic conditions.

Climate change-driven geographic range shifts have already led to new and unpredictable bark beetle-host tree interactions in North America (Creeden et al. 2014). In Norway, the spruce bark beetle is already present in the northern range of Norway spruce in Trøndelag and Helgeland, but low temperatures may be the reason why no large beetle outbreaks have occurred here. However, a future warmer climate may promote outbreaks also in the northern range of spruce in Norway. More frequent extreme climatic events, like severe droughts and storms, could also increase the likelihood of bark beetle outbreaks in these areas (Benestad 2005). Plantations of Sitka and Lutz spruce are common in Trøndelag and Helgeland, making up about 10% of all planted spruce in Helgeland, but most of these plantations are not yet mature and are therefore for the moment less susceptible to bark beetle attacks (Astrup et al. 2009). However, as more stands of Sitka and Lutz spruce reach maturity we may expect bark beetle outbreaks to spill over from neighboring Norway spruce stands and increase the likelihood of attacks also on Sitka and Lutz spruce.

## Conclusions

We have shown here that the spruce bark beetle is capable of breeding successfully in both Sitka and Lutz spruce and that offspring quality in these novel hosts was similar to that in the historic host Norway spruce. However, the beetles established more galleries and produced more offspring in Norway spruce than in the other spruce species. It is possible that the spruce bark beetle and its symbiotic fungal associate *E. polonica* had problems recognizing Sitka and Lutz spruce as good hosts due to a lack of positive host signals. Thus, we might speculate that the beetles and the fungus were "naïve" in their interaction with these species. Sitka and Lutz spruce appear to be favorable hosts for the spruce bark beetle, since they have lower terpene levels in the bark (Flø et al. manuscript) and support the production of beetles of equal length and weight as those produced in Norway spruce. Because of the apparent fitness benefit to beetles that colonize Sitka and Lutz spruce it is possible that the beetles will adapt to these hosts over time in areas where the species co-occur. In such areas traditional forest management practices to mitigate the risk of spruce bark beetle outbreaks should also be applied to Sitka and Lutz plantations. Our results support the claims of Økland et al. (2011) that the spruce bark beetle is a great risk to spruce forests in North America, where the beetle has been intercepted many times (Haack 2006) but has not yet established. The spruce bark beetle may cause extensive ecological and economic damage to Sitka and Lutz spruce if it is introduced to North America.

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