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# Carbon castles vs. beetle-fungus armies

## Phenology of the spruce bark beetle and Norway spruce resistance in times of climate change

PETTER ÖHRN

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# Carbon castles vs. beetle-fungus armies. Phenology of the spruce bark beetle and Norway spruce resistance in times of climate change

## Abstract

In recent years, carbon sequestration in European forests has decreased due to extensive droughts and bark beetle outbreaks brought on by climate change. The Eurasian spruce bark beetle (*Ips typographus* (L.)) is a natural disturbance agent in forests where Norway spruce (*Picea abies* (Karst)) is dominant. Through mass attacks the beetle can overwhelm defences of healthy trees. Drought stress can further reduce tree resistance and predispose the trees to bark beetle attack.

The aim of this thesis was to generate knowledge and investigate both the region-specific seasonal phenology of the spruce bark beetle in southern Sweden and the resistance of its host, the Norway spruce. Pheromone traps and felled trees that were colonised were used to determine flight activity and required thermal sums (degree-days, dd >5°C) for flight start, re-emergence of parental beetles, and the emergence of filial beetles. The influence of weather, phenology, and soil moisture conditions, and lag effects following a severe drought event in 2018, on spruce tree resistance was quantified by inoculating a blue stain fungus associated with bark beetles on three sites in southern Sweden on four occasions during the 2019 and 2021 growing seasons. Fungal growth was expected to show susceptibility to bark beetle attack.

Flight activity started at the end of April (47 dd after 1 January). Re-emergence of parental beetles from the first brood started at the end of May (122 dd after colonisation), and continued flight showed that sister broods were frequent. The new generation started to emerge at the end of June (437 dd after colonisation) to initiate a second generation. Tree resistance was lower in the early season (June) and differed with both precipitation levels and local soil moisture conditions. Resistance was higher in 2021 than 2019, indicating a recovery. In both years, there was a significant correlation between lesion size (tree resistance) and water availability in the autumn of the previous year. In the current climate in southern Sweden, tree resistance is lowest during tree attacks from sister brood flights (June). Understanding thresholds in both bark beetle development and tree drought stress variables is crucial for predicting the impact of future bark beetle outbreaks in a climate change context.

Keywords: *Picea abies, Grosmannia europhioides*, blue stain fungi, *Ips typographus*, flight activity, drought, soil moisture

# Granens försvar mot granbarkborren i ett föränderligt klimat

Under senare år har kolinlagringen i europeiska skogar minskat på grund av ett förändrat klimat som medfört omfattande torka och utbrott av barkborrar. Angrepp av granbarkborren *Ips typographus* (L.) är en naturlig störningsfaktor i skogar dominerade av gran *Picea abies* (Karst). Genom massangrepp kan barkborren övervinna försvaret hos friska träd. Torkstress kan minska trädens försvarsförmåga och göra dem mer mottagliga för barkborreangrepp. Efter den extrema torkan 2018 som drabbade stora delar av Europa, inklusive Sverige, har granbarkborren angripit och dödat hundratals miljoner kubikmeter av gran. Syftet med denna avhandling var att öka kunskapen om regionala och säsongsmässiga variationer i granbarkborrens fenologi och granens försvarsförmåga mot angrepp i Södra Sverige.

Feromonfällor och fällda träd som koloniserats användes för att bestämma flygaktivitet och temperatursummor (daggrader (dd) >5°C) för första svärmning, syskonkull och kläckning av den nya generationen. Granens försvarsförmåga mot angrepp undersöktes på tre lokaler i södra Sverige vid fyra tillfällen under växtsäsongerna 2019 och 2021 genom att mäta tillväxten av en barkborreburen blånadssvamp, som inokulerades i träden. Svampens tillväxt jämfördes i relation till, granens fenologi (skottskjutning), markfuktighetsförhållanden och graden av torkstress under olika tidsperioder innan inokulering.

Granbarkborrarna började flyga i slutet av april (47 dd efter 1 januari) då en första kull anlades. Anläggning av en andra kull (syskonkull) började i slutet av maj (122 dd efter första kullens anläggning). Fortsatt hög flygaktivitet under säsongen indikerade att syskonkullar var vanliga. Den nya generationen började lämna sina värdträd i slutet av juni (437 dd efter kolonisering) för att initiera en andra generation.

Granens försvar var i genomsnitt lägre under tidiga säsongen (juni) än senare men varierade med både mängden nederbörd och lokala markfuktighetsförhållanden. Försvaret var högre under 2021 än 2019, vilket tyder på att granarna återhämtad sig efter den svåra torkan 2018, För båda åren fanns det en signifikant korrelation mellan nekrosstorlek (trädförsvar) och tillgänglighet på vatten föregående höst. I det nu rådande klimatet i södra Sverige är granens försvar lägst vid trädangrepp under tiden för syskonkullsvärmning av granbarkborren (juni).

Med pågående klimatförändringar är kunskap om tröskelvärden både i barkborreutveckling och trädens torkstress avgörande för att bättre förutsäga omfattningen av framtida barkborreutbrott.

Nyckelord: *Picea abies, Grosmannia europhioides*, blånadssvamp, *Ips typographus*, svärmning, torka, markfuktighet

# Preface

-Stå inte bara där och titta på träd, gubbe!

Allan, 7 år

*“The evolution’s on the run*
*We had our ways, now here’s a different song*
*A golden age to walk upon*
*So rise and shine for a brighter day*
*Just leave on sunset till you know the way”*
The Soundtrack of Our Lives

# Dedication

Till "granens susning vid vars rot mitt bo är fästat"

Zacharias Topelius

# Contents

# List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Öhrn, P., Långström, B., Lindelöw, Å. and Björklund, N., 2014. Seasonal flight patterns of *Ips typographus* in southern Sweden and thermal sums required for emergence. Agricultural and Forest Entomology, 16(2): 147-157. https://doi.org/10.1111/afe.12044.

II. Öhrn, P., Berlin, M., Elfstrand, M., Krokene, P. and Jönsson, A.-M., 2021. Seasonal variation in Norway spruce response to inoculation with bark beetle-associated bluestain fungi one year after a severe drought. Forest Ecology and Management 496. https://doi.org/10.1016/j.foreco.2021.

III. Öhrn, P., Berlin, M., Weslien, J.-O., Elfstrand, M., Krokene, P. Jönsson, A.-M., and Menkis, A. Site-specific and seasonal variation in Norway spruce response to inoculation with spruce bark beetle-associated bluestain fungus (submitted).

Papers I-II are reproduced with the permission of the publishers.

The contribution of Petter Öhrn to the papers included in this thesis was as follows:

I. Participated in designing the experiment. Performed fieldwork, laboratory work and data analysis. Main writer of the manuscript, correspondence with the journal.

II. Responsible for the initial ideas and contributed to the conceptualisation and the experimental design. Performed fieldwork, laboratory work and data analysis. Main writer of the manuscript together with AMJ, who was responsible correspondence with the journal.

III. Responsible for the initial ideas and contributed to the conceptualisation and the experimental design. Performed fieldwork, laboratory work and data analysis. Main writer of the manuscript, correspondence with the journal.

# 1. Introduction

## 1.1 Bioeconomy of Sweden and the importance of forestry

The bioeconomy in Sweden contributes approximately 11% to GDP, showing that there is a high demand for forest resources and various forest ecosystem services. The bioeconomy concept includes connecting decarbonisation, sustainability and green growth, producing a solution to the major societal and environmental challenges of our time (Holmgren et al., 2022). The transition to a bio-based economy, aiming to counteract the negative effects of climate change, poses a major challenge to society and requires maintenance of the function of forest ecosystems. A balancing act lies ahead in the “trilemma” of the diverging and sometimes disparate high-level targets in both increasing wood production to substitute raw fossil materials and forest carbon storage capacity, while increasing forest biodiversity (Högbom et al., 2021).

Various agents of damage represent a significant threat to a sustainable forest industry. A report on climate adaptation by the Swedish Forest Agency in 2019 examined how a future climate will affect forest pest damage, and the economic consequences (Ståhlberg, 2019). During a baseline period (1990-2010) the yearly cost due to spruce bark beetle was approximately SEK 100 million. With no mitigation measures, the cost in 2050 was estimated to increase to around SEK 600 million. The cost of the most recent outbreak (2018-2022) has been estimated by the Swedish forest agency to 14 billion SEK. Thus, we can conclude that, until current date, we are well within the range of those previous estimated costs.

Forest tree breeding is an effective tool for producing suitable plant material to increase tree growth at a relatively low cost (Myking et al., 2016; Rosvall & Lundström, 2011). Historically, the focus in tree breeding has been on increasing the production of wood, but recent drought stress events and insect outbreaks have increased calls for trees more resistant to biotic and abiotic stressors (Mageroy et al., 2023).

# 2. Background

## 2.1 Expanding empire of the bark beetle

Around 6000 species of bark beetles are described, but only a handful of them are considered as “tree-killing bark beetles,” defined by the ability to kill healthy trees that have an active defence (Raffa & Berryman, 1983). Tree-killing bark beetles pose the greatest insect threat to conifer forests around the globe. After more than 200 years of research, the drivers of outbreaks are still not fully known, so knowledge must be increased to tackle future challenges (Biedermann et al., 2019; Hlásny et al., 2019).

Tree-killing bark beetles, sometimes also referred to as “aggressive bark beetles,” have three main characteristics. First, the use of chemical communication, involving the release of both aggregation and anti-aggregation pheromones, which can regulate attack density and shift the attack to neighbouring bark surfaces or trees. Second, the intricate association with symbiotic blue stain fungi, which may involve degradation of host toxins, helps to exhaust tree defences, production of beetle semiochemicals for beetle pheromone production, and possibly provide nutrition (Netherer et al., 2021). Third, a high level of resistance to chemical tree defence reactions, such as high resin flow (Franceschi et al., 2005).

Among the most effective “tree-killers” is the mountain pine beetle (*Dendroctonus ponderosae*). In the past two decades alone, it has contributed to the devastation of 900 million m$^3$ of forest in North America, converting large areas of temperate and boreal forest from a small net carbon sink into a large carbon source (Ghimire et al., 2015; Kurz et al., 2008). Comparable disturbances are presently affecting carbon sequestration in Norway spruce (*Picea abies*) forests across Central Europe. The warm and dry summer of

2018 triggered a still ongoing outbreak of the Eurasian spruce bark beetle (*Ips typographus*) in both central Europe and southern Sweden (Fig. 1). This was an unprecedented outbreak, with more than hundred million m$^3$ of damaged Norway spruce in central Europe (Patacca et al., 2023) and an estimated 34 million m$^3$ of spruce forest killed in Sweden up to 2023 (Wulff & Roberge, 2023). For Sweden, this is nearly 80% of all the spruce killed by bark beetles since 1960 (Schroeder & Kärvemo, 2022). The spruce trees killed by bark beetles following the storm event “Gudrun” that struck southern Sweden in 2005 was only 10% of the magnitude of current outbreak.

Abiotic stress events such as storm damage or drought may reduce the tree defence, making trees more susceptible to attacks by insect pests and fungal pathogens. Ongoing climate change means that these events can occur more frequently. In recent years there is clear evidence of increasing impacts of such natural disturbance events on the European forest (Patacca et al., 2023).

**Figure 1 (next page)** a) The spruce bark beetle (*Ips typographus)* is the size of a mouse dropping (4-5 mm). Compensating size with numbers, these “beetle-fungus armies” are capable of overcoming the defences and killing living b) spruce trees, “carbon castles”. Underneath the bark, c) the spruce bark beetle breed in galleries, d) here with dens larval feeding on a windfallen tree. Notice the brown boring dust on the bark indicating beetle entrance holes. (Photos: Petter Öhrn and Göran Liljeberg (b)).


a)
b)
c)
d)

## 2.2 The Eurasian spruce bark beetle (*Ips typographus)*

The spruce bark beetle is present over most of its host range in the boreal and temperate forest in Europe and Asia (Fig. 2). Like other aggressive bark beetles the population dynamics of the spruce bark beetle are characterised by two separate stages – endemic and epidemic (Hlásny et al., 2019b; Raffa et al., 2008). At low, endemic population levels, these beetles typically breed in the phloem under the bark of recently dead or dying Norway spruce trees and in unbarked timber. The transition from low endemic population levels to epidemic outbreak levels is strongly affected by abiotic disturbance events such as windthrow or drought (Fig. 3), and can be described by a threshold model (Økland & Bjørnstad, 2006). The epidemic threshold is dependent on the beetle density and the susceptibility (resistance) of the host tree population (Fig. 3) (Berryman, 1978, 1982; Raffa & Berryman, 1983).

At epidemic outbreak levels, bark beetles can overcome the defences of living trees. Spruce bark beetle attacks on living spruces are often preceded by a good availability of storm-felled trees, with absent or reduced defence capacity. This has been the typical trigger for bark beetle outbreaks in Sweden (Marini et al., 2013). However, severe drought stress may also reduce the trees' defences and trigger bark beetle outbreaks (Raffa et al., 2008), as has been observed recently.

Like other ectothermic organisms where body temperature is controlled by external conditions, the development rate of the spruce bark beetle is directly dependent on the ambient temperature, which determines the number of generations (potential multivoltinism) and sister broods, and attack pressure in the current and subsequent seasons (Baier et al., 2007).

The spruce bark beetle initiates swarming in spring when temperatures rise above 16.5°C (Lobinger, 1994). Emerging from overwintering, they take flight in search of suitable breeding material within the forest. The male initiates gallery construction by emitting aggregation pheromones to attract females but also other males (Christiansen & Bakke, 1988). The male attracts between one and four females to create a polygamous gallery system. After mating, the females lay their eggs in niches along the mother gallery (Fig. 1c).

After approximately three weeks, once parent beetles have finished mating and constructing galleries, they move on to another tree to initiate a second brood, a so-called sister brood. On reaching full maturity, the first brood (new generation) has various options depending on thermal conditions and photoperiod. In central Europe, a fully developed second generation, along with multiple sister broods, is typical. Conversely, at higher altitudes and further north in Europe (e.g., Sweden), one generation with one or several sister broods is most common.

Spruce bark beetle overwinter in Fennoscandia in the adult stage, as larvae and pupae do not survive winter temperatures (Annila, 1969). Depending on climatic conditions, beetles may remain under bark or emerge from the tree during late autumn to overwinter in the soil. In the lowlands of central Europe, in multivoltine populations, the majority of beetles stay beneath the bark of standing or fallen trees during winter (Dworschak et al., 2014; Faccoli, 2002; Kasumović et al., 2019; Wermelinger et al., 2012). In the northernmost parts of Europe, where the spruce bark beetle is mainly univoltine (Annila, 1969; Fritscher & Schroeder, 2022), more than 70% of the new generation have been shown to leave the brood tree (Hedgren & Schroeder, 2004; Pettersen & Austerå, 1975; Weslien, 1992) to hibernate beneath snow cover in the ground litter (Austarå, 1993; Lindelöw & Weslien, 1986). These studies were conducted in the boreal zone (mainly north of latitude 60° N). A recent study, conducted mainly in the boreonemoral zone in southern Sweden (mainly south of latitude 60° N), found that nearly 50% of beetles stayed to overwinter beneath the bark of infested trees (Weslien et al., 2024).

The hot and dry weather in 2018 reduced the availability of water and weakened the spruce trees, while the heat led to the development of a second generation of spruce bark beetles during the summer (Fritscher & Schroeder, 2022a). This phenomenon (bivoltinism) is unusual in Sweden, because it is usually too cold for the second generation to develop and reproduce the same year (Lange et al., 2006). With climate change, increasing temperatures and longer growing season increase the probability that two generations of the spruce bark beetle will become a common phenomenon, which may lead to increased risks of bark beetle outbreak (Jönsson et al., 2009; Lange et al., 2006).

## 2.3 Bark beetles and blue stain fungi – warfare allies

A bark beetle can be likened to a small bus in that it acts as a vehicle, carrying and transporting other organisms (Nikiforuk, 2011) such as fungi, mites, nematodes, bacteria, or viruses, all part of a ectosymbiotic community. These organisms reside in pits on the beetles' exoskeleton, on the elytra, and in the gut (Hofstetter et al., 2022). The ectosymbyotic members may also interact with host tree, bark beetle competitors, and natural enemies within the trees. Natural enemies are attracted to volatiles emitted by fungi and bacteria that may help them to locate their prey within a tree (Boone et al., 2008).

The majority of fungal associates of bark beetles are *ophiostomatoid,* blue stain fungi. The European spruce bark beetle has several such fungal partners which may differ in space and time (Persson et al., 2009). The most studied and frequently found in association with the spruce bark beetle are *Endoconidiophora polonica*, *Grosmannia penicillata*, *Ophiostoma bicolor*, *Grosmannia europhioides*, and *Ophiostoma ainoae* (Netherer et al., 2021).

The blue stain fungi support the bark beetle in overcoming and exhausting host tree defence systems (Krokene, 2015). After successful host tree colonisation by the bark beetle, the fungi can access and inoculate the phloem tissue underneath the rigid bark. While entering, the fungi trigger hypersensitive wound reactions in the phloem with increasing levels of specialised metabolites, resulting in a necrotic lesion (Fig. 7) (Franceschi et al., 2005). After exhaustion of tree defences, symbiotic fungi can provide nutrition for both primary and secondary bark beetles, and can concentrate the nutrition to areas close to the feeding galleries in support of beetle larvae and callow adults (Six, 2020; Six & Elser, 2020).

Recent findings show that spruce bark beetle symbionts such as *G. europhioides* and *G. penicillate* can produce bark beetle aggregation pheromones, which indicates a cross-kingdom convergent evolution of chemical signals with mutual benefit to both beetle and fungi (Zhao et al., 2019).

The spruce bark beetle is associated with a large fungal diversity which also have different temperature optimum (Persson et al., 2009). It is likely that climate change may affect the assemblages of organisms associated with bark beetles. However, knowledge is poor about the individual contribution of each bark beetle symbiont, so climate-driven shifts in microbial associates may not have a significant impact on beetle fitness (Netherer & Hammerbacher, 2022).

Blue stain fungi are often used as proxies for bark beetle colonisation when studying conifer resistance, since fungal growth is easier to manipulate experimentally than bark beetle (personal experience in **Papers II-III** and unpublished work, Erbilgin et al., 2021; Krokene et al., 2012; Netherer et al., 2016; Zhao et al., 2011). Importantly, a correlation has been shown between tree resistance to blue stain fungi and resistance to bark beetle attack (Christiansen, 1985).

## 2.4 Norway spruce (*Picea abies*)

Norway spruce is an evergreen conifer tree native to northern, central and eastern Europe, and is one of the most planted spruces, both within and outside its natural range (Fig. 2). Norway spruce has an important role in the boreal ecosystem, along with the spruce bark beetle and its associated blue stain fungi. It harbours around 1100 different species of fungi, insects and lichens, and hosts the highest number of associated species, followed by the Scots pine (*Pinus sylvestris*) and oak (*Quercus* spp.) with 920 and 880 species, respectively (Sundberg et al., 2019).

Spruce forest provides ecosystem services such as $CO_2$ sequestration and carbon-neutral raw materials. Cultivated mainly for production of wood for the forest industry, the Norway spruce is one of the major economically important tree species in Europe, constituting 25% of the productive forest (Hlásny et al., 2021). In Sweden the spruce constitutes 40% of the total standing timber volume. Each year some 200 million spruce seedlings are planted for regeneration after harvest, of which 75% have been genetically improved in the Swedish tree breeding programme (www.skogsstyrelsen.se).

Norway spruce seeds and plants of non-native origin have been used for regeneration in Sweden. The need for reforestation grew after charcoal production for mining in the 19th century, initiating large-scale imports of seed sources. The first imports were from Germany, Denmark and Finland. With growing knowledge in preferred adaptation profiles of Norway spruce, central European seed sources were abandoned and replaced by seeds from eastern Europe, mainly Belarus but also Romania and the Baltic states. Seeds from Belarus have a high growth rate and their later bud burst makes them less affected by late spring frost. Consequently, the current gene pool of Norway spruce in Sweden has been strongly influenced by many different seed sources of non-native origin (Myking et al., 2016).

Norway spruce typically exhibits lower drought stress tolerance compared to other conifer species grown in Europe, such as Scots pine (*Pinus sylvestris*), Douglas fir (*Pseudotsuga menziesii*) and silver fir (*Abies alba*) (Vitali et al., 2017; Zang et al., 2014). This lower tolerance in spruce can be partially attributed to the later termination of shoot elongation and radial growth, which increases its vulnerability to late summer drought conditions (Pichler & Oberhuber, 2007).

The timing of bud burst in spring is a key phenological trait used to select trees with a high wood quality and vitality and that are under strong genetic control (Hannerz et al., 1999). Trees with late bud burst are preferred for deployment in southern Sweden due to their reduced susceptibility to frost damage (Persson & Persson, 1992; Werner & Karlsson, 1982). The longer spring in southern Sweden compared to further north increases the exposure of spruce seedlings to recurrent frost episodes (Jönsson et al., 2004). However, knowledge is limited on how shoot phenology impacts tree growth and resistance to pathogen or insect attack (Krokene et al., 2012). While tree resistance to bark beetle attacks has not been an explicit selection criterion in the Swedish breeding programme, recent research has identified genetic components of tree resistance to both attack by spruce bark beetle (Korecký et al., 2023) and infection by blue stain fungi associated with bark beetles (Steffenrem et al., 2016).

## 2.5 Spruce tree resistance and seasonality

The many defence mechanisms of conifer trees are sometimes compared to the fortification of a medieval castle. Nevertheless, large armies of bark beetles and their fungal partners may overcome these defences and kill millions of trees (Franceschi et al., 2005; Mageroy & Krokene, 2020; Nikiforuk, 2011).

Conifers have an integrated multiple defence system including both preformed, constitutive defence that forms the first tier of defence and inducible defence, forming the second tier (Franceschi et al., 2005; Krokene et al., 2012). The outer periderm is the first line of the preformed defence system.

There is a baseline of preformed resin ducts which the inducible defence system upregulates by forming traumatic resin ducts. This is a cost-effective response to insect or pathogen attack.

A seasonality in spruce tree defence to fungal infection has been demonstrated (Horntvedt, 1988; Krokene et al., 2012), which appears to be related to shoot phenology, and could be a result of tree physiological trade-offs between growth and defence processes. Several independent but similar hypotheses attempt to explain patterns in plant defence allocation. The expanded Growth-Differentiation Balance (GDB) hypothesis incorporates all the other hypotheses into its conceptual framework to predict how plants balance between growth, defence, and other differentiation on the cellular level (e.g. Herms & Mattson, 1992; Lorio, 1986). When plant resources are allocated to growth, less is available for investment in defences (Huang et al., 2019).

Severe drought leading to water deficit in trees is harmful for tree health. The stress that plants experience due to drought is affected by the amount of water present in the soil, which is determined by both precipitation levels and the soil's capacity to retain water. Any mild stress event may lead to either increased investments in constitutive defences, activation of the tree’s defence responses, or priming of defence responses (Mageroy et al., 2020).

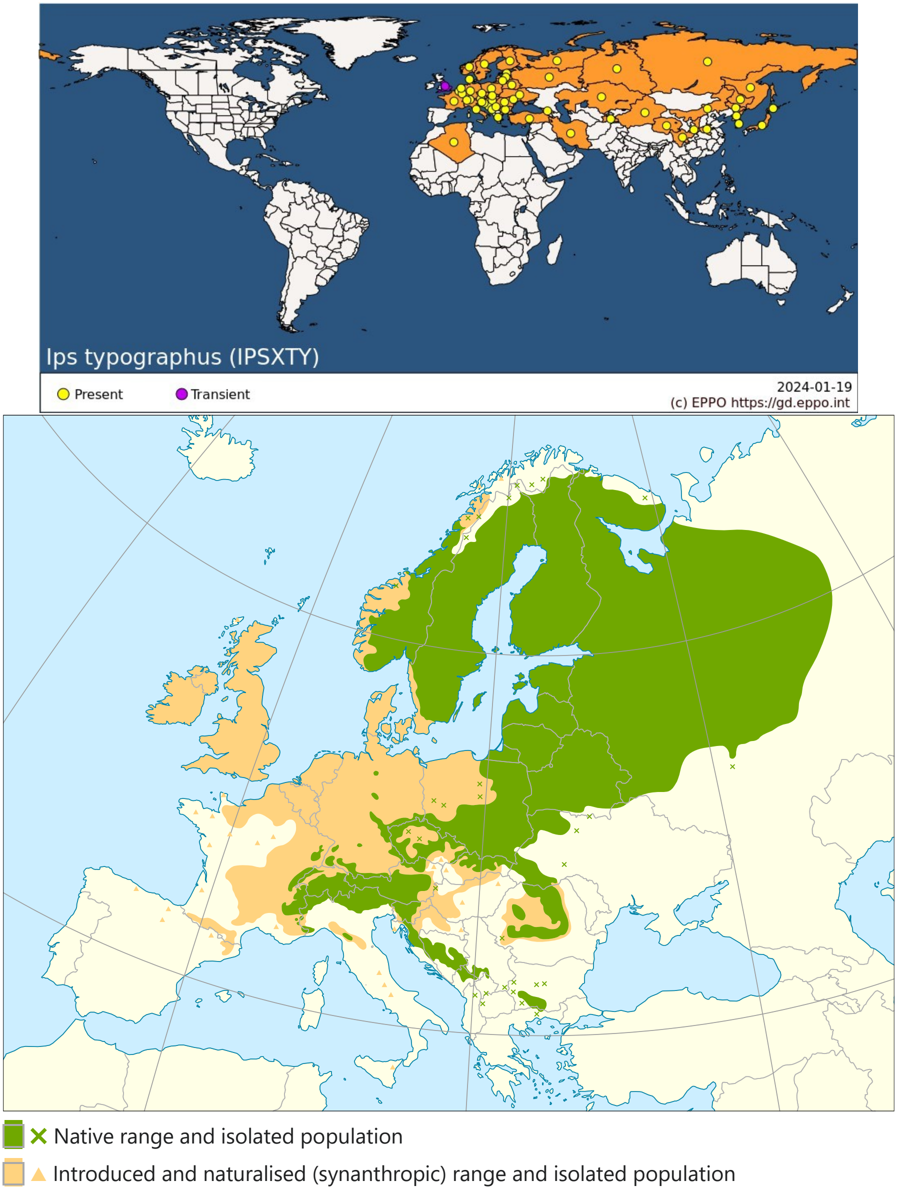


**Figure 2** Distribution map of the Eurasian spruce bark beetle *Ips typographus* (upper) and distribution map of its major host (in Europe), the Norway spruce *Picea abies* (lower) (Caudullo et al., 2017, Open access article distributed under the terms of the Creative Commons CC-BY license).

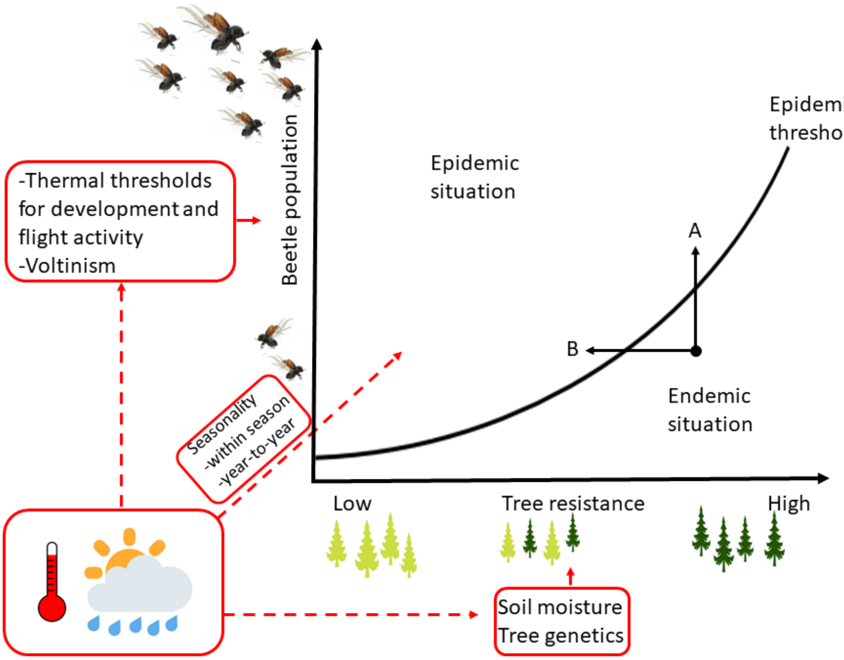


**Figure 3** A theoretical model for the relationship between host-tree resistance and threshold of successful bark beetle attack, modified after Berryman (1978, 1982). The epidemic threshold is determined by the beetle population density and the level of tree resistance. A transition from endemic (non-outbreak) to epidemic (outbreak) situation can be triggered by both the increase in beetle population (A) and lowered tree resistance (B). The epidemic threshold is dynamic and context-dependent, so there is no fixed bark beetle level that will result in an outbreak. Red boxes show the investigated variables in this thesis, with arrows indicating possible impacts on the model. (Spruce trees modified from File:Grangärde vapen.svg - Wikimedia Commons under https://creativecommons.org/licenses/by-sa/2.5/deed.en. Photo of flying beetle: Niklas Björklund.)

# 3. Aim of the thesis

The PhD project aimed to investigate both the phenology of the Eurasian spruce bark beetle (*Ips typographus*) and the resistance of its host, the Norway spruce (*Picea abies*). The specific aims and hypotheses were:

- The aim of **Paper I** was to analyse seasonal flight activity and thermal sums in the lifecycle of the spruce bark beetle.

    - Describe **flight pattern** and **voltinism** of *Ips typographus* in southern Sweden.
    - Determine **critical thermal sums** allowing the prediction of distinct phases **in the life cycle** of *Ips typographus*, such as the onset of the spring flight, re-emergence of parent beetles, and the development time of a new generation.

- The aim of **Paper II** was to study seasonal variation and potential lag-effects in defence mechanisms of Norway spruce one year after a severe drought.

    - **Site-specific** differences: Trees at the site with the most severe drought in 2018 have lower defence capacity in 2019 than trees at sites experiencing moderate drought.
    - **Seasonal** differences: Spruce trees differ in defence capacity over the season, with lower defence capacity during periods of active growth.

  - Differences between **provenances**: in spring, defence dynamics of Swedish and east European spruce provenances, with a generally early and late timing of bud burst, respectively, differ.

- The aim of **Paper III** was to quantify how the resistance of Norway spruce trees is influenced by weather, provenance, phenology, and soil moisture conditions.

  - **Site-specific** differences (environmental): for each provenance class, trees growing on sites with relatively low precipitation are less resistant than trees growing on sites with more precipitation.
  - **Seasonal** and **soil moisture** differences (environmental): within and between growing seasons for each provenance class, trees growing under dry soil conditions are less resistant than trees growing under moist soil conditions.
  - **Provenance** differences (genetic): tree resistance differs between trees with early and late bud burst in the spring, represented by Swedish and east European provenances, respectively.

# 4. Methods

This section presents an overview of the of the materials and methods used in this thesis. For more detailed descriptions and statistical analyses, see each paper.

## 4.1 Study sites (Papers I-III)

All study sites were situated in the southern part of Sweden (Fig. 4). All the sites of the spruce bark beetle flight activity and development study **(Paper I)** were within the area affected by the storm in 2005 (Anonymous, 2006) and the subsequent spruce bark beetle outbreak (Långström et al., 2009). The sites for the tree defence studies **(Papers II-III)** were all situated within the area affected by the severe drought event in 2018 (Lindroth et al., 2020) and the subsequent spruce bark beetle outbreak (Schroeder & Kärvemo, 2022; Wulff & Roberge, 2023).

## 4.2 Spruce bark beetle flight activity and development (Paper I)

At each of the four beetle study sites, two spruce trees were cut, baited with pheromone, and left on the ground before the first spruce bark beetle flight, repeatedly in 2008, 2009 and 2010. The trees were inspected weekly and when no more entrance holes (and new boring dust) were observed, it was considered fully colonised (Fig. 5). A 70-cm stem section was cut from the bole and suspended in a bag fitted with a collection bottle in a shaded position. Emerging beetles were collected weekly, counted and categorised, based on their colour: darker – parental beetles or lighter – filial beetles.

This procedure was repeated in June and July to study the seasonal progress of beetle colonisation and the thermal sums for development depending on the seasonal timing of colonisation. In June, the trees were expected to be colonised by parental beetles, mainly re-emerging parental beetles flying to initiate a sister brood. In July, both late flying re-emerging parental beetles and emerging beetles of the filial (new) generation were expected to colonise the trees (Fig. 6). At the end of the growing season all bark was removed, and the remaining live and dead beetles in each stem section were counted to estimate the mortality (number dead beetles / total number beetles (dead under bark + live emerged trapped beetles)).

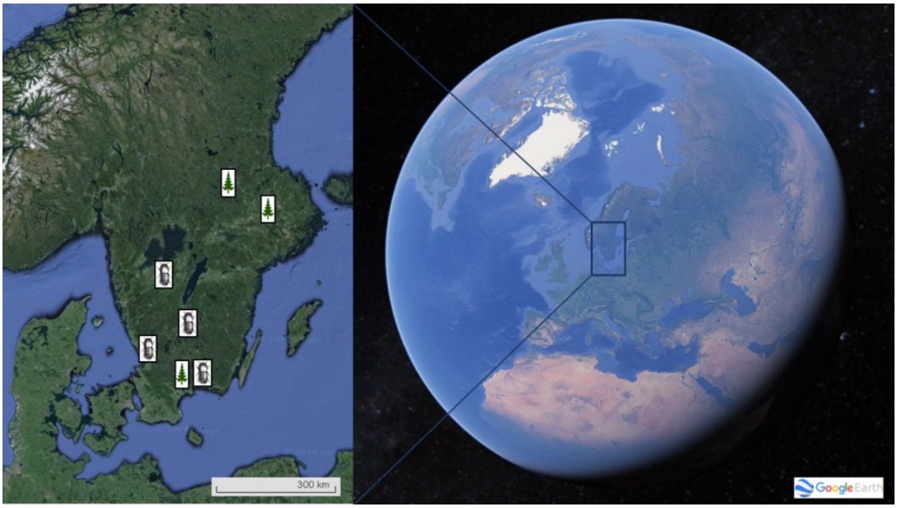


**Figure 4** Map of southern Sweden and all study sites in a global context. The "**beetles**" represents field sites of the study on spruce bark beetle flight activity and development (**Paper I**) and the "**spruces**" represents sites of Norway spruce tree defence to fungal inoculation with *Grosmannia europhioides* (**Papers II-III**). "Beetle" sites, from north to south, were Remningstorp, Asa, Tönnersjöheden and Gammalstorp and "spruce" sites, from north to south, were provenance trials in Norberg, Lugnet and Skärsnäs.

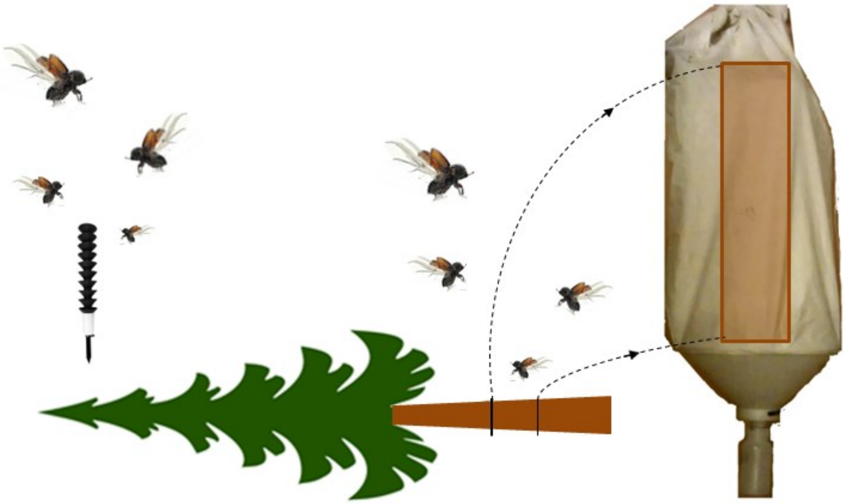
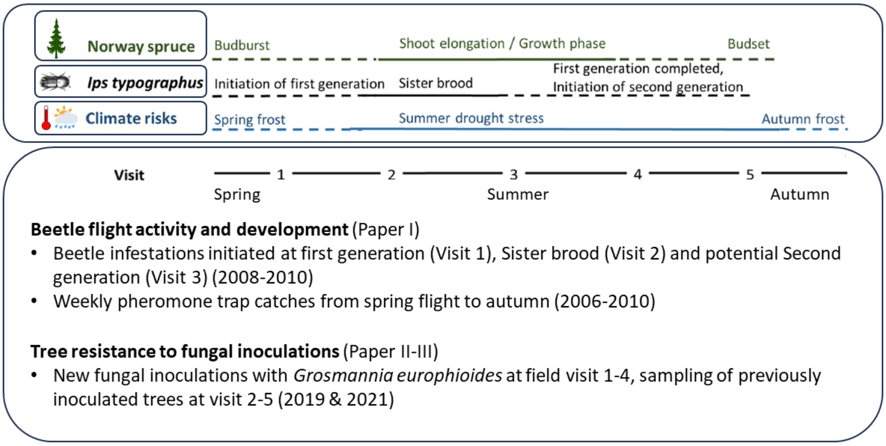


**Figure 5** Seasonal beetle flight activity was recorded using pheromone traps (left). Beetle development was recorded in cut trees baited with pheromone before swarming in spring. Fully infested stem sections were placed in emergence bags fitted with a collective bottle (right) in May, June, and July. Throughout the season both flight traps and emergence bag traps were emptied weekly. (Spruce trees modified from File:Grangärde vapen.svg - Wikimedia Commons under https://creativecommons.org/licenses/by-sa/2.5/deed.en; Photo of flying beetle: Niklas Björklund.)

## 4.3 Spruce tree resistance experimental trees (Papers II & III)

In 2002, study sites were set up to gather data for effective selection and deployment of breeding materials of Norway spruce (Lundströmer et al., 2020). These sites, part of a large test series in Sweden, included 64 provenances and seed orchard crops of south Swedish and east European origin. Seedlings were planted in an incomplete randomised block design, with ten blocks selected for study at each site. Each year (2019 and 2021), 20 trees from four provenances were chosen (two provenances with early and two provenances with late bud burst), in total 480 trees across three sites. At two sites, blocks were selected along a soil moisture gradient in 2021. At the third site, the same blocks that had been used in the 2019 were used again in 2021, due to the absence of a moisture gradient. Soil moisture levels in the blocks selected in 2021 were determined using LIDAR-derived terrain indices at sites with gradients (Ågren et al., 2021).

In this study, previously gathered data on tree phenology and growth were used. The trees had undergone assessments for growth, vitality, and damage six and 13 years post-planting as part of the tree breeding programme. Bud burst timing, shoot elongation, and lignification of wood cells were assessed in 2004, two years after planting. These phenological traits were found to be closely related to the geographical origin of the trees (Lundströmer et al., 2020). Bud burst and shoot elongation measurements were taken using a bud development score (Krutzsch, 1973) (Fig. 7) in late spring 2004 when the trees were four years old. Most trees were in active bud development during this time. Provenance classes with early or late spring phenology were selected based on these scores for further analysis of tree resistance (Fig. 7).



**Beetle flight activity and development** (Paper I)

- Beetle infestations initiated at first generation (Visit 1), Sister brood (Visit 2) and potential Second generation (Visit 3) (2008-2010)
- Weekly pheromone trap catches from spring flight to autumn (2006-2010)

**Tree resistance to fungal inoculations** (Paper II-III)

- New fungal inoculations with *Grosmannia europhioides* at field visit 1-4, sampling of previously inoculated trees at visit 2-5 (2019 & 2021)

**Figure 6** The upper box shows a schematic representation of Norway spruce shoot phenology (green line), in relation to spruce bark beetle (*Ips typographus*) activity (black line) and climate-related risks for tree damage (blue line). Dashed lines represent temporal variability, as influenced by provenance-specific tree phenology (growth rhythm) and inter-annual variation in temperature and precipitation conditions (modified from Öhrn et al., 2021). The lower box shows an overview of the timing of major field data acquisitions of this thesis, which was synchronised with key events in the life cycle of the spruce bark beetle *Ips typographus*.

## 4.4 Spruce tree resistance: fungal inoculations (Papers II & III)

In both studies, tree resistance was evaluated by introducing the bark beetle-associated blue stain fungus *Grosmannia europhioides* into the stem of each tree at three closely spaced inoculation points (Fig. 7). The fungal inoculum comprised malt agar plugs colonised by *G. europhioides*, which is known to be a relatively virulent and common associate of the spruce bark beetle in Sweden (Zhao et al., 2019). Trees were inoculated on four occasions between May and August in 2019 and 2021 (Fig. 6). A total of nearly 480 trees (240 each year) were inoculated and evaluated for lesion size.

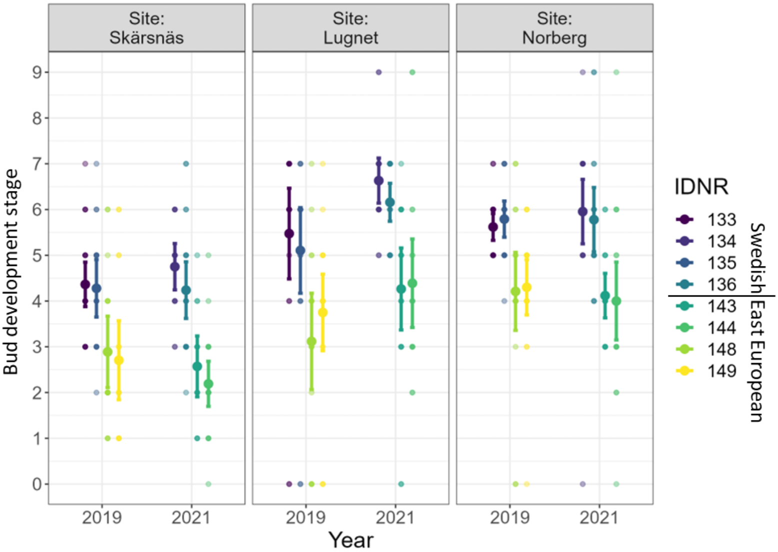


**Figure 7** Variation in shoot phenology in Norway spruce, provenances originating from Sweden or eastern Europe, that were selected for inoculation with *Grosmannia europhioides* in 2019 and 2021. For provenance-specific origin see IDNR codes in Lundströmer et al. (2020). Shoot phenology was assessed in spring 2004, when trees were four years old, using a bud burst scale ranging from 0 to 9, where 0 = 'dormant bud' and 9 = 'all needles more or less scattered, new buds begin to form' (Krutzsch, 1973).

The first inoculations were in late May, when overwintering beetles start to fly and initiate the main brood (Fig. 6). The second inoculations were in late June, when mated females re-emerge from the first tree, colonising to establish the first sister broods in new hosts. The third and fourth inoculations were in late July and late August, respectively, aligning with the flight periods of offspring from main broods (July) and sister broods (Aug).
The size of necrotic lesions on the stem was measured one month after fungal inoculation. To obtain a quantitative measure of tree resistance we removed the bark over the inoculated area to expose the necrotic lesions on the sapwood surface All lesions were then photographed (Fig. 7) to measure the total area of each lesion (using ImageJ software; Abràmoff et al., 2004). Size of lesion area was used as an indicator of tree resistance, i.e., the larger the area the lower the resistance (Krokene & Solheim, 1999).

**Figure 8** Inoculation of bark beetle associated with blue stain fungus *Grosmannia europhioid*es was performed by removing three bark plugs (using a 5 mm diameter cork borer) and injecting a fungal agar slush. One month later, the tree was revisited, and the bark peeled off to measure the area of the necrotic lesion (light brown zones) on the xylem surface. (Photo: Petter Öhrn.)

## 4.5 Weather and soil moisture data sources (Papers I-III)

Daily precipitation and temperature data for all field sites (Fig. 4) were taken from the closest stations of the Swedish Meteorological and Hydrological Institute (www.smhi.se) or a weather station at the research stations at Asa or Tönnersjöheden. Cumulative temperature sums for each site were calculated as growing degree days (dd) above a threshold of 5°C, starting from 1 January (**Papers II & III**) or timing of beetle colonisation (**Paper I**), to quantify thermal differences between sites throughout the season and thermal requirements for beetle flight and development.

We used monthly and cumulative standardized precipitation evaporation index (SPEI) values to analyse how necrotic lesion area was related to previous weather conditions **(Paper III)**. The SPEI values were derived from the SPEI Global Drought Monitor https://spei.csic.es/map (Beguería et al., 2014). SPEI is a standardized measure of drought that is expressed relative to the long-term average (zero-line), where positive values indicate wetter and negative values dryer than average conditions.

Data on soil moisture conditions at each site was taken from maps supplied by the Swedish Forest Agency (Kartor; skogsstyrelsen.se). In **Paper II** only a simple map with two classes (above or below 1-metre groundwater level) was available but in **Paper III** a more detailed map was used (the SLU soil moisture map; Ågren et al., 2021). For this map the soil moisture is given a value from 0-100 and is visualised in the map by a colour gradient.

# 5. Results and discussion

## 5.1 Spruce bark beetle flight activity and development (Paper I)

### 5.1.1 Seasonal flight patterns

During the study years of **Paper I** (2006-2010), there were long periods of spruce bark beetle flight in southern Sweden (Figs. 9 & 11). The flight activity started in mid-April and lasted until mid-August. On average, 10% of the flight activity occurred before the end of April and 50% before mid-June. This was earlier than previously reported from central Scandinavia and southern Finland (Annila, 1969; Austarå et al., 1977; Eidmann & Klingström, 1990). The length of the flight period was more in line with the flight period reported from Denmark (Harding & Ravn, 1985) or even central Europe (Baier et al., 2007; Faccoli & Stergulc, 2004).

After this study and after the most recent outbreak, knowledge of the flight period of the spruce bark beetle has increased after comprehensive monitoring by the Swedish Forest Agency and forest owner associations and companies, with pheromone traps emptied weekly throughout the flight period all over Sweden (Spruce bark beetle monitoring; (skogsstyrelsen.se).

The length of the flight period is very much dependent on temperature. Increasing spring temperatures due to climate change has extended the flight period; between 1980 and 2010 the period in which temperatures were suitable for spring flight of spruce bark beetle lengthened by about 5 days per decade (Öhrn, 2012). In response to the knowledge gained after **Paper I** was conducted, Swedish forest legislation on when fresh conifer timber should be removed from the forest was adjusted to an earlier timepoint. According to the current provisions in the Forest Protection Act for pest management of harmful insects, the wood must be removed from the forest and transported to industry before 1 July in the south of Sweden and 15 July in the north (www.skogsstyrelsen.se).

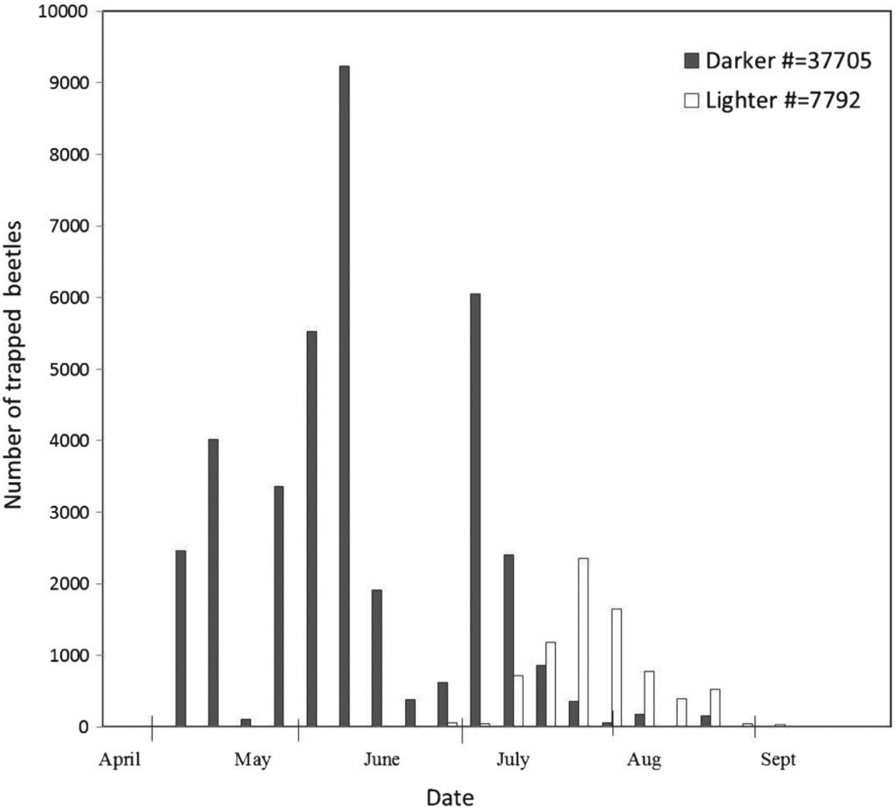


**Figure 9** Number of recently-emerged spruce bark beetles caught weekly in pheromone traps categorised according to colour (darker = parental beetles and lighter = new generation beetles). Data pooled for the Tönnersjöheden, Remningstorp and Gammalstorp sites in 2009-2010.

### 5.1.2 Thermal sums for spring flight

The spring flight of the spruce bark beetle started at an accumulated thermal sum of 47 ± 24 dd (>5°C), on average 27 April. This varied somewhat between study years and sites but corresponded well with previous studies from Denmark (Harding & Ravn, 1985) and southern Finland (Annila, 1969).

To find the best predictor for flight initiation, flight temperature thresholds and thermal sums were tested for their correspondence with the estimated first day of flight day using linear regression functions. Using only first day with daily maximum exceeding 16°C showed no correspondence with first flight ($R^2 = 0.0012$) A thermal sum was a better predictor than only using a flight temperature threshold for the initiation of the spring flight, but an even better predictor was to combine thermal sum and first day with daily maximum exceeding 16°C ($R^2 = 0.87$, $p < 0.001$). The first flight then occurred 3 days before the predicted flight date.

### 5.1.3 Thermal sums for re-emergence of parental beetles

The first re-emergence of parental beetles (from trees infested in May) occurred on 24 May, approximately three weeks after the first brood was initiated (Figs. 10 & 11). This occurred at an accumulated thermal sum (starting from colonisation) of 115 ± 46 dd. After another 2 weeks (10 June), 50% of the parental beetles had emerged at 256 ± 107 dd. Laboratory results verified under field conditions in southern Norway estimated the thermal sum to 168 dd >7.5°C for mean re-emergence from sun exposed trees (Anderbrant, 1986). From southern Finland, Annila (1969) reported 150-200 dd >5 °C for re-emergence. In a more recent study from the Czech Republic conducted at three elevations (600, 800 and 1100 m a.s.l.) in the Sumava mountains during two seasons, the first re-emergence ranged between 472 dd corresponding to 42 days (in shaded conditions at 600 m a.s.l.) and 103 dd corresponding to 22 days (in shaded conditions at 1100 m a.s.l.) (Davídková & Doležal, 2017). The large variation may be due to the timing and thermal sum of re-emergence depending on density, with beetles leaving the brood tree earlier when attack density is high (Anderbrant, 1986). Studies use thermal sums based on different thresholds but overall, my results are in line with other studies.

Re-emergence of parental beetles occurred at increasingly higher thermal sum in the trees that were colonized later in the season. In trees colonized in June, parental beetles started to emerge at nearly 300 dd and in July the thermal sum was around 450 dd (compared to 115 dd in May). Higher thermal sums in trees colonized by second and third sister brood was also reported from Czech Republic (Davídková & Doležal, 2017). The prolonged time before re-emergence may be due to that, the trees are attacked, to a larger extent, by older beetles that has exhausted their energy supplies after repeated oviposition.

### 5.1.4 Thermal sums for emergence of the new generation

The emergence of the new generation from trees colonised in May started on average on 29 June, around 8 weeks after the brood was initiated at a thermal sum of 449 ± 107 dd (>5°C) after colonisation (Figs. 10 & 11). After another 7 weeks (on 20 August), 50% had emerged at 1115 ± 294 dd. The initiation of emergence was in line with observations from Denmark (573 dd >5 °C) and Finland (442 dd >5°C) (Annila, 1969; Harding & Ravn, 1985).

In a more recent study classifying colours of beetles (lighter in new generation) in breeding substate and trap catches in Sweden, the onset of new generation (defined as 5% trap catch) occurred at 744 dd >5°C in southern and at 688 dd in northern Sweden (Fritscher & Schroeder, 2022). This higher thermal sum could partly be explained by a lag time of measurements between beetle emergence from tree (my work) and capture of beetles in pheromone traps. During this lag time the new generation of beetles may be involved in nutritional feeding required for pheromonal response or weather conditions for flight may be inadequate, e.g., too cold and/or rainy. A lower thermal sum for development in northern compared to southern Sweden, which has been further verified (Lindman et al., 2023), indicates that thermal adaptation is region-specific.

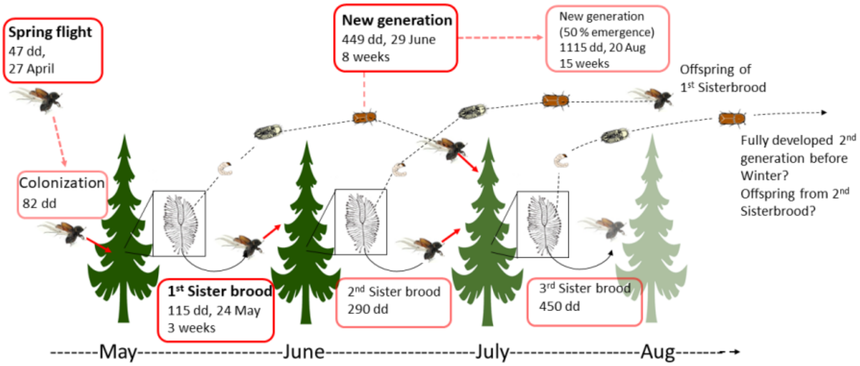


**Figure 10** Seasonal timing of critical thermal sums (day degrees >5°C (dd)) and average dates during the study years (2008-2010) and distinct phases in the life cycle of the spruce bark beetle. Starting with spring flight and colonisation (dd accumulated from 1 January), the thermal sums for sister brood and new generation emergence was accumulated from the time of colonisation.

### 5.1.5 Beetle development and mortality

The proportion of spruce bark beetles in southern Sweden that re-emerged decreased significantly in broods developed in trees colonised later in the season. Nearly 95% of parental beetles re-emerged from under the bark before the end of the season from the trees colonised in the first swarming in May (Fig. 11). The corresponding proportions in June and July were 58% and 20%, respectively. However, the proportion of those beetles contributing to sister brood flight is unclear. In the Czech Republic similar proportions have been reported, with 91% and 38% re-emerging once or twice, respectively (Martinek, 1956). In Scandinavia, both laboratory (Anderbrant & Löfqvist, 1988) and field studies (Bakke, 1983) have reported about 60% of the beetles re-emerging from the first brood. However, Anderbrant (1989) estimated, based on the number of attacked trees in a south Norway forest, that only one-third of the beetles produced a sister brood.

Both parental and filial beetles suffered significantly higher mortality in trees colonised later in the season. The parental mortality rate under the bark in trees colonised in May, June, and July was 3%, 38% and 59%, respectively. The corresponding mortality rate for filial beetles in trees colonised by their parents in May, June and July was 3%, 9% and 22%, respectively. The higher mortality late in the season might be due to an increase in pathogens (Wegensteiner et al., 2007).

In accordance with earlier work (Annila, 1969), filial beetles continued to emerge until October. Most likely, the beetles that emerged late hibernated in the vicinity of the brood tree, since trap catches ceased already by the end of August (Fig. 11). A more recent study showed that more than 50 percent of the spruce bark beetle population in southern Sweden was leaving the brood tree before winter, and that trees with greenish crowns possibly attacked later in the season had a lower proportion leaving the tree before winter than trees with totally brown crown (Weslien et al., 2024). The proportion of beetles overwintering in the tree is crucial for the efficiency of sanitation logging (in order to reduce spread of beetle infestations).

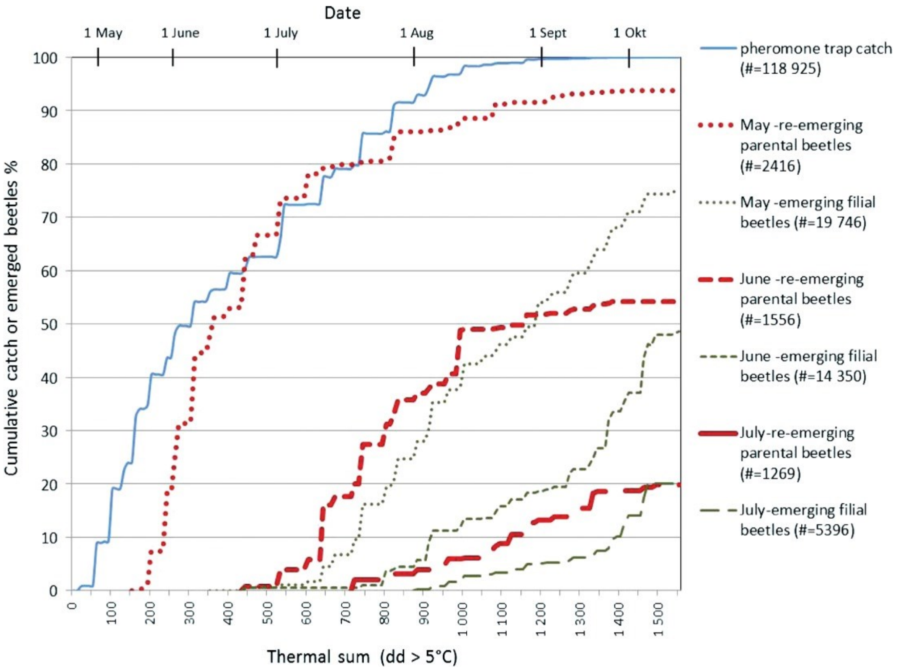


**Figure 11** Cumulative percentage of catch from pheromone traps, and percentage of emerged beetles from emergence logs, in relation to thermal sum accumulated from 1 January (lower *x*-axis). An estimation of approximately when these thermal sums occur is also provided (average of the recorded years; upper *x*-axis) in Asa, Tönnersjöheden, Remningstorp (2008–2010) and Gammalstorp (2008–2009). Numbers in the legend represent the total number of trapped beetles, emerged, and not emerged beetles (corresponding to 100%).

## 5.2 Spruce tree resistance (Papers II & III)

### 5.2.1 Site-specific differences

In **Paper II** differences in tree resistance were compared during 2019, between trees growing on a site that experienced the most severe drought in 2018 and two other sites experiencing moderate drought. The additional field data retrieved in 2021 presented in **Paper III** gave additional insights into the potential lag effects in resistance mechanisms in the years following severe drought.

The study sites showed a similar within seasonal pattern of tree resistance across the growing seasons in 2019 and 2021, which may indicate site-specific determinants of tree resistance (Fig. 13).

In **Paper II**, one hypothesis was that trees at the site most severely affected by the 2018 drought would be less resistant the following year than trees at sites that were moderately affected. This hypothesis was rejected when lesion size was correlated with the drought index for the entire previous growing season (SPEI for April-August; Fig. 12). However, when lesion data was re-analysed in **Paper III** against the individual month with the most severe drought (Lugnet field site, May 2018; Fig. 1), the hypothesis was confirmed. This indicates that there is a time lag effect of drought and that the relationship between water availability and tree resistance is dynamic and dependent on both environment (site characteristics) and year-to-year variability in weather conditions.

This was further validated by the initial hypothesis in **Paper III**: trees on sites experiencing drier weather conditions exhibited lower resistance compared to those on sites with higher precipitation levels (see Fig. 13). We observed indications of lag effects, with significant correlations between tree resistance and the degree of drought (lower SPEI values) in the months preceding fungal inoculation, spanning both the preceding growing season and specific months (Fig. 14). These findings suggest that, in 2019, tree resistance was still reduced due to the severe drought experienced the previous year. This impact was considerably less pronounced in 2021, a year lacking severe drought conditions. Nonetheless, in both years, tree resistance was associated with the drought severity during the preceding autumn and early winter (reflected in the 9-month SPEI value).

The presence of lag effects in Norway spruce responses to environmental conditions is in line with studies on both net ecosystem carbon uptake (Lagergren et al., 2019) and tree ring growth (Aldea et al., 2024; Matisons et al., 2024; Song et al., 2021). Negative correlations were shown between stem growth and weather conditions (SPEI or precipitation) during autumn-winter the previous year.

The availability of water at the onset of the vegetation period is determined by the quantity of winter precipitation and its adequacy in replenishing groundwater levels (Meier et al., 2022). Consequently, winter precipitation can impact both tree growth and biotic resistance in the following season, as shown in **Paper III** and supported by other research findings.

### 5.2.2 Seasonal differences

Both provenance classes displayed seasonal differences in tree resistance (**Papers II & III**). Tree resistance was generally lower during the period of most active growth in June. This is in line with the Growth-Differentiation Balance hypothesis, predicting that spruce trees will allocate less resources to defence when growth is at its maximum (Herms and Mattson, 1992). Tree resistance in Norway spruce follows the seasonal pattern of stem growth rate, which reaches its maximum in late June when seasonal solar radiation is also highest (Darenova et al., 2020; Rossi et al., 2006). Similar studies on seasonal differences in Norway spruce resistance to blue stain fungi found lowest tree defence to be in mid-June (Horntvedt, 1988; Krokene et al., 2012).

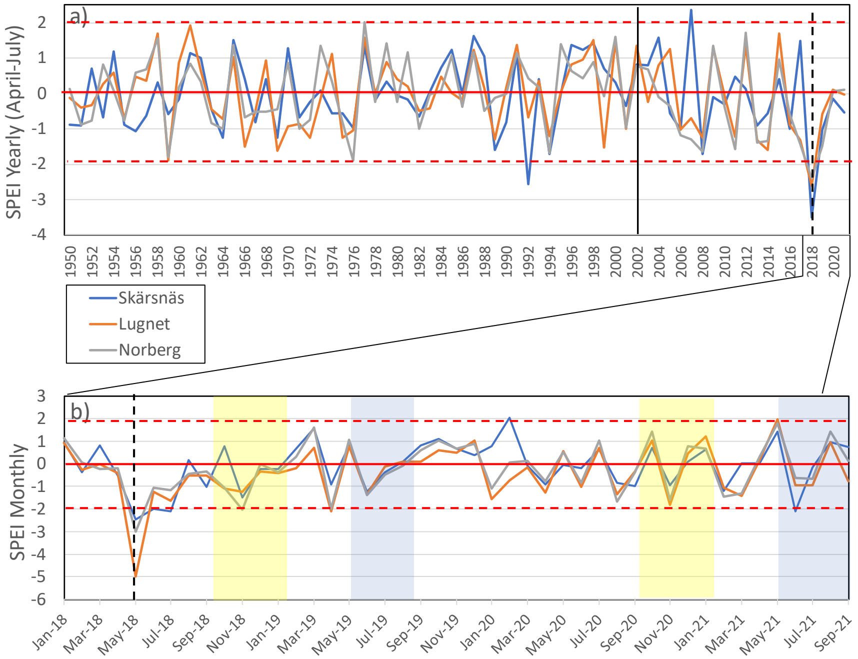


**Figure 12** Standardized Precipitation Evapotranspiration Index (SPEI) values for the period April-August from 1950 to 2019 for three sites in Sweden (a), and monthly SPEI values from January 2018 to September 2021 (b). The vertical solid line in 2002 shows the provenance trial establishment. The 72-year time series includes a severe drought event in 2018 [vertical dashed line in (a)], with the most severe drought month [vertical dashed line in (b)].Blue areas show periods for assessment of Norway spruce susceptibility to fungal inoculation, and yellow areas show periods that represent the best fit (according to an AIC model) of linear regressions of SPEI against tree susceptibility. SPEI was derived from http://spei.csic.es/map and is based on z-scores. Red solid lines represent the average condition, and values exceeding ± 1.96 (red dashed lines) are significantly drier or wetter than the average (95% confidence level). AIC: Akaike Information Criterion.

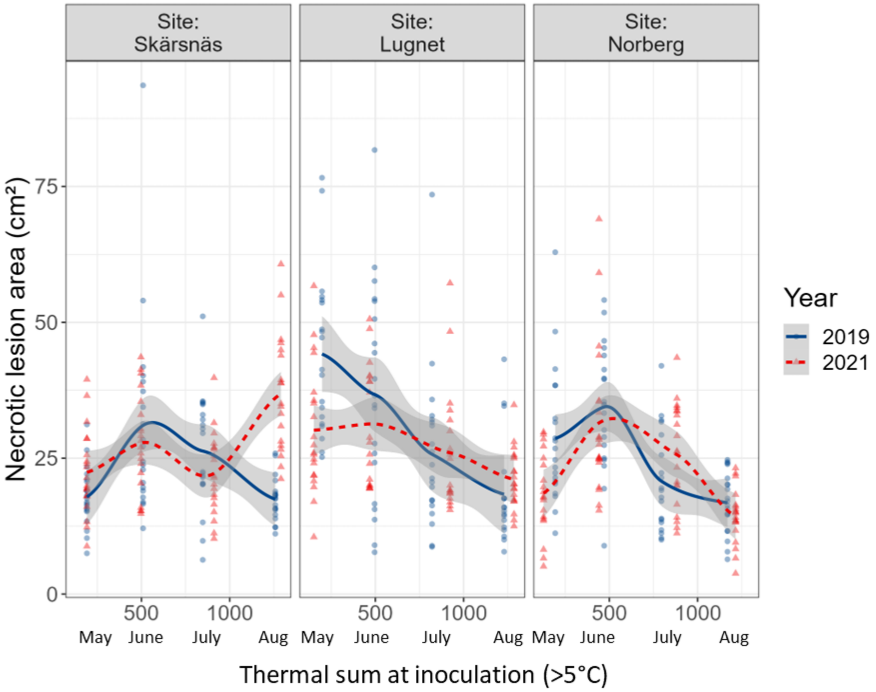


**Figure 13** Seasonal variability in susceptibility of Norway spruce trees to inoculation with *Grosmannia europhioides* (expressed as necrotic lesion area). Susceptibility of trees inoculated in 2019 and 2021 is fitted to the thermal sum accumulated up to the time of inoculation using Loess curves. Additional x-axis shows monthly timing of fungal inoculation.

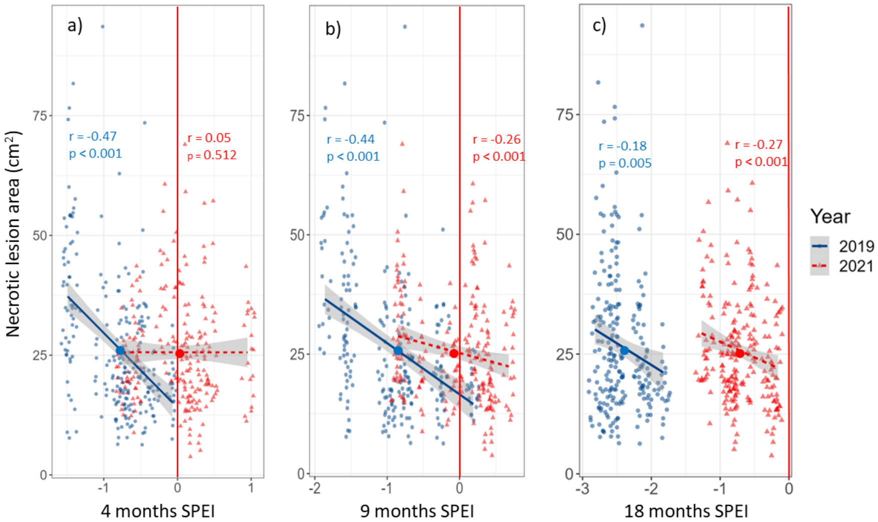


**Figure 14** Relationship between water availability and necrotic lesion area for each individual Norway spruce trees inoculated with *Grosmannia europhioides* in 2019 (one year after a very dry summer) or 2021 (a period with normal precipitation). In each year, at each of three sites, spruce trees were inoculated on four occasions yielding in total 452 observations. (blue and red dots). Water availability was measured as the cumulative Standardized Precipitation Evaporation Index (SPEI) for three periods preceding inoculation: 4 months (a), 9 months (b) or 18 months (c). The vertical red line represents the average SPEI value for each period, and values to the left and right are respectively drier or wetter. The yearly mean value is indicated by the blue (2019) and red (2021) dots centred on each regression line. Regression lines show Pearson's linear regression for 2019 (n= 236) and 2021 (n= 216) with representative r- and p-values.

### 5.2.3 Soil moisture differences

When a gradient was observed in the soil moisture condition after a field visit at Skärsnäs in 2019, the tree resistance was compared between dry and moist soil, defined by ground water level below or above 1 metre (www.skogsstyrelsen.se). The results showed that trees on moist soils had smaller lesions **(Paper II),** but those trees were smaller and therefore had less phloem available for fungal growth.

Still, this gave inspiration, and together with the new SLU soil moisture map (Ågren et al., 2021) a gradient could also be detected in Norberg before field measurements in 2021 **(Paper III)**. A new analysis with two sites (Skärsnäs & Norberg) for two growing seasons (2019 & 2021) gave additional results.

The hypothesis, stating that spruce trees growing on dry soil types show less resistance compared to those in moist soil types, was rejected. There was no consistent impact of soil type (dry, intermediate, moist) on tree resistance. This could be attributed to the relationships between soil moisture and tree resistance seldom being linear (Krokene et al., 2023). Notably, lesions were significantly larger, indicating reduced tree resistance in trees growing on intermediate soil moisture conditions (Fig. 15). However, when tree size was considered (lesion size relative to tree diameter), no significant effect was detected (Fig. 15). A lower tree resistance on intermediate soil moisture conditions than at sites with moist or dry conditions was most apparent at one of the sites in 2019 (Norberg, data not shown). This is in line with reports from Austria of lower tree mortality to spruce bark beetle attacks on chronically dry soils with low soil moisture than on wetter soils (Netherer et al., 2019). Trees situated in chronically dry locations might exhibit higher drought tolerance due to adaptation to such conditions, e.g., through the development of a deeper root system (Fan et al., 2017).

In 2021, lesions were overall significantly smaller than in 2019 (Fig. 13) which may indicate a recovery in tree resistance. This difference varied between sites and within the season, but was most evident on intermediate soil moisture conditions (data not shown).

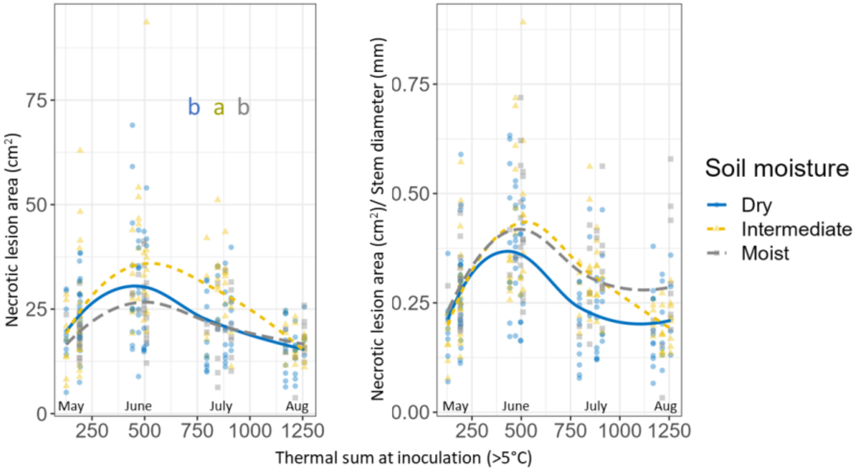


**Figure 15** Seasonal variability in susceptibility of Norway spruce trees to inoculation with *Grosmannia europhioides* expressed as necrotic lesion (left) area and necrotic lesion area relative to stem diameter (right) of trees growing in dry, intermediate, or moist soil moisture conditions. Susceptibility of trees is fitted to the thermal sum accumulated up to the time of inoculation using Loess curves. Data is pooled for trees inoculated at two sites in 2019 and 2021. Different coloured letters indicate significant differences in susceptibility between soil conditions (Tukey's test following 3-way ANOVA, $\alpha = 0.05$). Trees inoculated at Skärsnäs in August 2021 were excluded, see Results section in **Paper III** for description. Additional x-axis shows monthly timing of fungal inoculation.

### 5.2.4 Differences between provenances

There was no difference in tree resistance between Swedish and east European provenance classes in terms of lesion size (**Papers II & III**).

In Lugnet in 2019, a subsample of 40 trees was analysed for the amount of resin ducts (**Paper II**). A positive relationship was found between lesion area and resin ducts area, indicating that a stronger local reaction to fungal inoculation triggers a stronger induction of traumatic resin duct formation (data not shown). In addition, there were significantly more resin ducts produced in east European compared to Swedish provenance classes (total area resin duct ($\mu m^2$/ µm tangential annual ring): 10.0±2.3 for Swedish and 16.0±2.3 for east European). However, the number of resin ducts from all trees subjected to fungal inoculations in 2019 and 2021 (n = 472) were quantified, and no differences between provenance classes were found (data not shown).

The considerable variability within- and overlap between the timing of budburst of provenances may have made it more difficult to detect differences in tree resistance in this study, but this does not exclude the possibility that there is a genetic component to timing of bud burst and tree resistance in spruce (Korecký et al., 2023; Steffenrem et al., 2016). In order to further investigate the influence of growth rhythm, an experiment was conducted where spruce clones with distinctly different timing of bud burst were inoculated with *Grosmannia europhioides*. The study revealed that trees with early bud burst exhibited greater resistance to inoculation during early summer compared to trees with late bud burst (Öhrn, unpublished data).

## 5.3 Spruce tree resistance in relation to beetle flight activity (Papers I-III)

Norway spruce tree resistance, measured as lesion formation in the phloem, followed a seasonal pattern, reaching a minimum in early season, mainly in June (**Papers II & III**), and coincides with a period of high bark beetle flight and tree colonisation activity (Paper I). However, in **Paper III,** beetle flight activity sometimes peaked a few weeks before or after minimum tree resistance; Fig. 16). At this time, the accumulated thermal sum at the field sites was around 500 dd (>5°C), which is sufficient for the new bark beetle generation to start to emerge (**Paper I**) and possibly initiate a second generation. However, start of emergence is slow (Figs. 10 & 11) and, based on flight trap catches, the onset of the new generation in southern Sweden has been reported to occur at a thermal sum of 744 dd (Fritscher & Schroeder, 2022). At this time, during the season (thermal sum) the beetle infestations consist mainly of sister broods by re-emerging parent beetles, but also partly by the first generation initiating a second generation (**Paper I**).

In Sweden, the spruce bark beetle mainly produces one generation per year (univoltine). In most years, the new generation does not fly, but stays in the tree over the winter or exits in the autumn to overwinter in the ground. In 2018 and 2019 (first year of fungal inoculations), the second-generation beetles amounted to 18 and 15% of the total seasonal trap catch (in Tönnersjöheden) in southern Sweden, respectively (Fritscher & Schroeder, 2022b). In 2021 (second year of fungal inoculations), this proportion is unknown. In the current climate in southern Sweden, tree resistance is at its lowest level during tree attacks from mainly sister brood flight, and the importance of the second generation will obviously increase in a future climate. In a warming climate, this lowest level of tree resistance will probably coincide more with the emergence of a second generation of spruce bark beetle. In this future climate, two generations will be common in most years (Bentz et al., 2019), leading to an increase in tree mortality (Bentz et al., 2019; Jönsson & Lagergren, 2018).

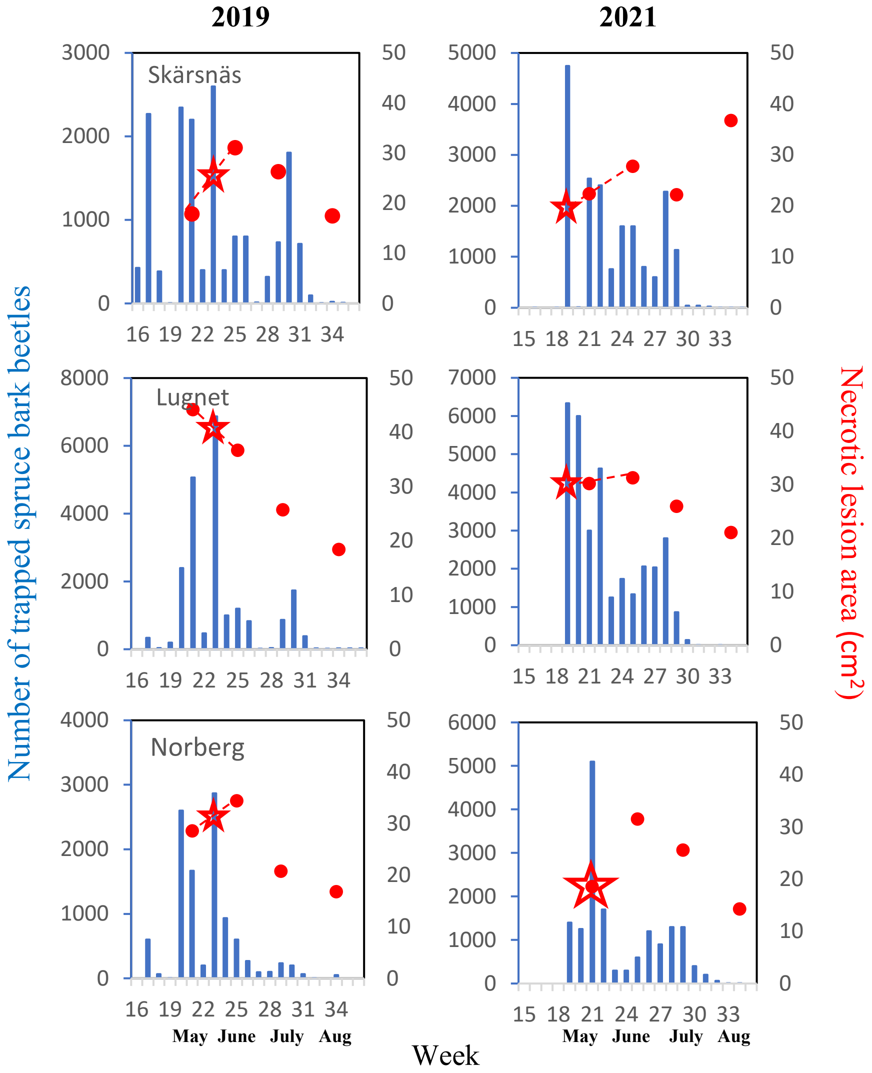


**Figure 16** Seasonal variability in susceptibility of Norway spruce trees to inoculation with *Grosmannia europhioides* in 2019 and 2021 (expressed as necrotic lesion area relative to stem diameter) (dots) and weekly trap catches of the spruce bark beetle in pheromone-baited traps (bars). Trap data comes from the closest traps managed by the Swedish Forest Agency: Lagan (80 km from Skärsnäs), Vallentuna (35 km from Lugnet), and Hedemora (18 km from Norberg), (pxweb.skogsstyrelsen.se). Red stars indicate inter- or extrapolated tree susceptibility levels at the time of maximum weekly beetle trap catch each season. Of the beetle trap catches from individual trapping sites, 82-89% corresponded with the average regional trap catch (from the four closest trap sites in 2021, representing the regional level of beetle flight activity).

# 6. Conclusions and perspectives

This thesis has provided new knowledge on both the region-specific seasonal phenology of the spruce bark beetle in southern Sweden and the resistance of its host, the Norway spruce. Using pheromone insect traps and felled trees that were colonised, flight activity and required thermal sums for the re-emergence of parental beetles and the emergence of filial beetles could be determined. The influence of weather, phenology, and soil moisture conditions on spruce tree resistance, and lag effects following a severe drought event, was quantified by inoculating a blue stain fungus associated with bark beetles on three sites in southern Sweden. This was done on four occasions during two growing seasons.

In this thesis, I showed how phenology of the spruce bark beetle and Norway spruce resistance varies within and between seasons. The main conclusions were:

- Considerable spruce bark beetle flight activity in July and nearly complete re-emergence of parental beetles from trees colonised during spring flight indicates that sister broods are frequent (**Paper I**).

- The high flight activity in July and the emergence of filial (new generation) beetles indicates that an initiation of a second generation is frequent (**Paper I**).

- In the current climate with limited bivoltinism, the effect of temperature on the number of sister broods may be important for the population build-up during outbreaks in southern Sweden (**Paper I**).

- Trees growing on sites with drier weather conditions displayed a lower level of resistance than trees on sites that received more precipitation (**Paper III**).

- The higher levels of detected tree resistance in 2021 than 2019 indicate that Norway spruce trees were slowly recovering from the 2018 drought (**Paper III)**.

- Tree resistance in Norway spruce differed over the growing season, with lower resistance during the period of most active tree growth (**Papers II & III**). This period mainly coincides with tree attacks by re-emerged beetles flying to initiate sister broods (**Paper I**).

- There was no consistent effect of soil moisture (dry, intermediate or moist) on tree resistance. Tree resistance levels were lower on intermediate soils, but when tree size was considered, there was no difference (**Paper III**).

- There was no difference in Norway spruce resistance in relation to phenology, i.e., early (Swedish) or late flushing (east European) provenances (**Papers II & III**).

Knowledge generated in this thesis specifically, and from the ongoing bark beetle outbreak in general, could be used to mitigate the effects of future outbreaks. **Paper I** helped to increase knowledge of how temperature affects development, re-emergence and flight patterns of the spruce bark beetle in southern Sweden. Information about these factors is important in population management of the species.

Timely removal of potential breeding material (log piles, wind-felled or standing weakened trees) is a key method for successful pest management. Generally, the recommendation is to remove trees before the beetle flight in the spring, or at least before the new generation starts to emerge around the beginning of July. The large proportion (95%) of beetles re-emerging to potentially initiate a sister brood in new trees shows that the best pest management would be achieved if trees are already removed before parent beetle re-emergence (in the end of May during the study years), about three weeks after initial colonisation (Fig. 10).

Knowledge is still lacking on how tree resistance and flight activity of beetles vary over the season, and how this affects tree mortality. **Paper III** highlights the early season, and specifically June, as a time when the resistance of spruce trees is low. This often coincides with a period of high bark beetle flight and tree colonisation activity, as shown in **Paper I**. A relatively low tree resistance and high beetle activity is likely to increase the probability of successful attack. In the case of severe winter-spring drought, special attention must be taken early in the season to monitor beetle activity and remove infested trees, to prevent transition to outbreak levels.

The results in **Paper III** indicate that Norway spruce resistance was higher in 2021 than in 2019 and that trees were gradually recovering from the 2018 drought. However, further research is necessary to understand how spruce forests will adapt to climate change, particularly regarding how phenology influences tree resistance to spruce bark beetle attacks following extreme weather events. The findings from **Paper III** also offer input to modelling how increasing drought stress may affect the risk of bark beetle outbreaks. Studying the interactive effect of beetle flight intensity and seasonal tree drought stress variables to identify thresholds for transition from endemic to epidemic is essential for forecasting the effects of future bark beetle outbreaks.

**Papers II** and **III** did not reveal any differences in tree resistance between Swedish and east European provenance classes with generally early and late bud burst. However, in the more recent study, Norway spruce was showing greater resistance to fungal inoculation in early summer in clones with early bud burst compared to clones with late bud burst (Öhrn, unpublished). Different soil types (dry, intermediate or moist) did not show any consistent effect on tree resistance. This could be because the relationship between soil moisture and tree resistance is rarely linear (Krokene et al., 2023). More research is needed on how Norway spruce resistance is affected by both phenology (timing of bud burst) and soil type, especially during drier weather conditions.

Norway spruce is still the most important conifer for timber production in Europe, despite many forest areas being exposed to acute mortality risks due to predisposing factors (susceptible age, structure, climate-related stress) to bark beetle attack (Seidl et al., 2011). A large volume of mature spruce together with long clearcut edges in the forest are the most important biotic factors for spruce bark beetle population build-up, and it is advised that this be considered when developing adaptive management methods to reduce future risk of bark beetle outbreak (Gohli et al., 2024). In Sweden, the standing volume of Norway spruce has increased dramatically, from about 150 million $m^3$ in 1930 to around 500 million $m^3$ today (Lindbladh et al., 2014). However, in the most recent years, more pine than spruce has been planted, especially on drier soil conditions (www.skogsstyrelsen.se).

My overall conclusion is that timing of sanitation measures is crucial for efficient control of bark beetle outbreaks. For the future, knowledge on thresholds in both bark beetle development and tree drought stress variables is crucial for predicting the impact of future bark beetle outbreaks in a climate change context. I suggest more diverse forest management with different tree species in different forest structures. This, coupled with higher genetic diversity, partly through genetically improved plants will contribute to climate change mitigation. This will hopefully increase forest resistance and resilience to bark beetle outbreaks, as well as other expected or unforeseen insect pest and pathogen disturbances.

Time is running short, but let’s “rise and shine for a better day”.

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# Popular science summary

*This thesis shows that the sister broods of the spruce bark beetle are often established when the trees are most susceptible to attack, specifically when the spruce is growing most actively in June. This makes it particularly important for the forest owner to detect new attacks during that period. The thesis also demonstrates that the resistance of the spruce is reduced by drought, and it takes a long time for the spruce to recover from a period of poor water availability. Drought in the previous year also results in lower resistance the following year. With ongoing climate change, knowledge of threshold values for both the development of the spruce bark beetle and the tree drought stress is crucial for better predicting the extent of spruce bark beetle attacks.*

In recent years, carbon storage in European forests has decreased as a result of climate change, leading to extensive drought and outbreaks of bark beetles. The spruce bark beetle, approximately 5-mm long, bores into the bark of Norway spruce (*Picea abies*) to reproduce. Mass attacks, meaning many boreholes within a short period, are facilitated by pheromones that allow the bark beetle to overcome the defence of living trees. When trees are stressed by drought, their resistance decreases, and fewer boreholes are needed to kill the tree, a pre-requisite for the spruce bark beetle to reproduce in the bark. The spruce bark beetle (*Ips typographus*) is also aided by fungi that attack the tree's defence system. Since the extreme drought of 2018 that affected large parts of Europe, including Sweden, the spruce bark beetle has attacked and killed hundreds of millions of cubic metres of spruce.

Removing infested trees before the new generation leaves the trees is the best-known method for reducing the number of spruce bark beetles. Knowledge of when the spruce bark beetle attacks trees and the extent to which the trees can defend themselves is important for practical forestry. The spruce's defence capacity likely varies over the summer depending on water availability and the growth phase of the spruce, as some phases are very energy-demanding and leave less energy for defence against bark beetle attacks.

The aim of this thesis was to gain more knowledge about when the spruce bark beetle flies and when it attacks spruce in southern Sweden, and how the spruce susceptibility to bark beetle attack varies over the growing season depending on water availability and growth rhythm.

Traps baited with pheromones were used to determine the flight activity of spruce bark beetles during the summer. Fallen trees attacked by spruce bark beetles were used to determine when they start flying in the spring, when they fly to establish a new brood of spruce bark beetles (sister brood), and when the new generation starts to fly and how this is affected by temperature. The spruce susceptibility to attack was investigated by measuring the growth of a fungus carried by the spruce bark beetle. A culture of the fungus was injected into the bark, and the growth of the fungus was measured during different periods throughout the growing season at three sites during 2019 and 2021. Large growth of the fungus in the outer wood is expected to correspond to low defence against the spruce bark beetle.

The spruce bark beetle began to fly at the end of April to establish a first brood. The establishment of a second brood (sister brood) began at the end of May. Continued high flight activity later in the season indicated that sister broods were common. The new generation started to leave the infested trees at the end of June to initiate a second-generation brood.

The spruce resistance was on average lower during the early season (June) than later,but varied with both the amount of precipitation and local soil moisture conditions. Resistance was higher in 2021 than in 2019, indicating a recovery after the severe drought of 2018. For both years, there was a clear relationship between tree resistance and the availability of water in the previous autumn. In the current climate in southern Sweden, spruce resistance is lowest during the season in June, when attacks from sister broods of spruce bark beetles are most common. This makes it particularly important for the forest owner to identify new attacks during this period.

# Populärvetenskaplig sammanfattning

*Avhandlingen visar att granbarkborrens syskonkullar ofta anläggs då träden har sämst försvarsförmåga mot angrepp, nämligen då granen växter som mest i juni. Det gör det särskilt viktig för skogsägaren att hitta färska angrepp under den perioden. Avhandlingen visar även att granens försvar försämras av torka och att det tar lång tid för granen att återhämta sig från en period med dålig vattentillgång. Torka under föregående år ger sämre försvar också året efter. Med pågående klimatförändringar är kunskap om tröskelvärden för både granbarkborrens utveckling och trädens torkstress avgörande för att bättre kunna förutsäga omfattningen av granbarkborreutbrott.*

Under senare år har kolinlagringen i europeiska skogar minskat som en följd av ett förändrat klimat som medfört omfattande torka och utbrott av barkborrar. Granbarkborren är en ca 5 mm lång skalbagge som borrar sig in genom barken på granar *Picea abies* för att där förökar sig. Massangrepp, dvs många inborrningar under kort tid, möjliggörs av doftämnen som gör att barkborren kan övervinna försvaret även hos levande träd. När träden stressas av torka kan trädens motståndskraft minska och det behövs färre inborrningar för att döda trädet vilket är nödvändigt för att granbarkborren ska kunna föröka sig i barken. Till sin hjälp har granbarkborren svampar som angriper trädets försvarssystem. Efter den extrema torkan 2018 som drabbade stora delar av Europa, inklusive Sverige, har granbarkborren angripit och dödat hundratals miljoner kubikmeter av gran.

Att ta ut angripna träd innan den nya generationen lämnar träden är den bästa kända metoden för att minska antalet granbarkborrar. Kännedom om när granbarkborren angriper träd och i vilken grad träden då kan försvara sig är viktig kunskap för det praktiska skogsbruket. Granens försvarsförmåga kan sannolikt variera över sommaren beroende på vattentillgång men även på vilken tillväxtfas granen befinner sig i, eftersom vissa faser är mycket energikrävande och lämnar mindre energi över till försvar mot barkborreangrepp.

Syftet med denna avhandling var att tillföra mera kunskap om när granbarkborren flyger och när den angriper granar i Södra Sverige och hur granens försvarsförmåga mot angrepp varierar över tillväxtsäsongen beroende på vattentillgång och tillväxtrytm.

Fångstfällor betade med doftämnen (feromoner) användes för att bestämma granbarkborrarnas flygaktivitet under sommaren. Fällda träd som angripits av granbarkborrar användes för att bestämma när de börjar flyga på våren, när de flyger för att anlägga en ny kull granbarkborrar (syskonkull) och när kläckning av den nya generationen startar och hur detta påverkas av temperaturen.

Granens försvarsförmåga mot angrepp undersöktes genom att mäta tillväxten på en svamp som granbarkborren bär med sig. En odlingskultur av svampen sprutades in i barken och hur mycket svampen växte mättes under olika perioder under växtsäsongen på tre platser under 2019 och 2021. En stor tillväxt av svampen i ytterveden förväntas motsvara ett lågt försvar mot granbarkborren.

Granbarkborren började flyga i slutet av april då en första kull anlades. Anläggning av en andra kull (syskonkull) började i slutet av maj. Fortsatt hög flygaktivitet senare under säsongen visade att syskonkullar var vanliga. Den nya generationen började lämna de angripna träden i slutet av juni för att påbörja en andra generation.

Granens försvar var i genomsnitt lägre under tidiga säsongen (juni) än senare men varierade med både mängden nederbörd och lokala markfuktighetsförhållanden. Försvaret var högre under 2021 än 2019, vilket tyder på en återhämtning efter den svåra torkan 2018. För båda åren fanns det ett tydligt samband mellan trädens försvarsförmåga och tillgänglighet på vatten föregående höst. I det rådande klimatet i södra Sverige är granens försvar som lägst under säsongen i juni, då angrepp från syskonkullsvärmande granbarkborrar är vanligast. Det gör att det under denna period är särskilt viktig för skogsbrukaren att identifiera färska angrepp.

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Färden har gått genom två barkborreutbrott, tiotusentals fångade och räknade barkborrar och hundratals träd som alla överlevde plågsamma trädförsök. Uppkomsten av denna avhandling har tagit sin tid. Men under livets gång har den fått gro och växa fram. Desto större tacksamhet känner jag inför alla som stöttat och bidragit under färden. Jag har många att tacka för att den äntligen är klar. Jag vill börja med att rikta ett stort tack till alla mina handledare som genom sina olika karaktärer och kunskaper och med tålamod burit och stöttat upp mig så att denna avhandling blivit möjlig. Tack Anna-Maria, att du höll mig ovan ytan i glappet mellan SLU och Skogforsk och var en grundbult och ett stort stöd i att sy ihop de forskningsansökningar som ledde fram till denna avhandling. Thanks till mina SLU-handledare, Audrius och Malin och all other mykopats att ni välkomnade mig till institutionen där jag länge varit hangaround och hört så mycket om. Tack till Mats och Janne på Skogforsk, för förädlade och barkborriga tankar. Även om du inte varit en officiell handledare Paal, tack för din våldsamma men konstruktiva behandling.

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Jag minns tydligt vad som var min första kontakt med skogsforskning. Det var ett studiebesök vid Skogshögskolan i Garpenberg i lågstadiet. Där fick jag bevittna mina klasskompisars mammor sortera barr. Det avskräckte inte, men det kom ju att dröja ett tag innan jag övertygades om vilken väg jag skulle välja. Eller möjligen hur en Gudrun och en ovanligt torr sommar valde vägen åt mig.

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# Appendix

Agricultural and Forest Entomology

*Agricultural and Forest Entomology* (2015), **17**, 223 DOI: 10.1111/afe.12107

# CORRIGENDUM

In the paper by Öhrn *et al.* (2014), some errors have been found in the two tables. In Table 1, there was a mismatch between two felling dates. In Table 2, there were incorrect dates for onset of colonization. Both tables are printed again below.

## Reference

Öhrn, P., Långström, B., Lindelöw, Å. & Björklund, N. (2014) Seasonal flight patterns of *Ips typographus* in southern Sweden and thermal sums required for emergence. *Agricultural and Forest Entomology*, **16**, 147–157.

**Table 1** Dates when trees were felled and stem sections placed in emergence bags at different locations in southern Sweden from 2008–2010. In all years stems were removed from the emergence bags for examination at the end of the season (Oct-Dec).

| | | | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|---|---|
| Location | Felling | Cohort | Felled | Bagged | Felled | Bagged | Felled | Bagged |
| Asa | 1st | May | winter | 20 May | 14 Mar | 8 May | 11 Mar | 1 Jun |
| | 2nd | June | 30 May | 25 Jun | 28 May | 29 Jun | 28 May | 29 Jun |
| | 3rd | July | 1 Jul | * | 29 Jun | 30 Jul | 8 Jul | 27 Jul |
| Tönnersjöheden | 1st | May | winter | 20 May | winter | 12 May | 23 Apr | 1 Jun |
| | 2nd | June | 4 Jun | 25 Jun | 25 May | * | 1 Jun | 23 Jun |
| | 3rd | July | 1 Jul | 30 Jul | 23 Jun | 7 Jul | * | 27 Jul |
| Remningstorp | 1st | May | 14 Apr | 19 May | 6 Apr | 11 May | 6 Apr | 2 Jun |
| | 2nd | June | 2 Jun | 16 Jun | 1 Jun | 29 Jun | 3 Jun | 21 Jun |
| | 3rd | July | 27 Jun | 4 Aug | 29 Jun | 27 Jul | 12 Jul | 2 Aug |
| Gammalstorp | 1st | May | 21 Apr | 21 May | 27 Apr | 11 May | * | * |
| | 2nd | June | 5 Jun | 12 Jul | 26 May | 13 Jul | * | * |
| | 3rd | July | 12 Jul | * | 13 Jul | 27 Jul | * | * |

*Data missing.

**Table 2** The recorded dates and thermal sums (dd > 5 °C) at flight initiation at the four locations in 2006–2010. Flight was considered to have started when the weekly pheromone trap catch consisted of more than 10 beetles. The start date of first trap catch was considered to be the first day, during that week, when the maximum temperature exceeded 16 °C. The onset of colonization was considered to occur when the first mother galleries could be observed.

| | | First pheromone trap catch | | Onset of colonization of cut trees | |
|---|---|---|---|---|---|
| Location | Year | Date | Thermal sum | Date | Thermal sum |
| Asa | 2006 | 4 May | 32 | * | * |
| | 2007 | 13 Apr | 34 | * | * |
| | 2008 | 21 Apr | 10 | 28 Apr | 33 |
| | 2009 | 23 Apr | 35 | 28 Apr | 65 |
| | 2010 | 19 May | 91 | 20 May | 103 |
| Tönnersjöheden | 2006 | 3 May | 36 | * | * |
| | 2007 | 13 Apr | 62 | * | * |
| | 2008 | 23 Apr | 28 | 28 Apr | 55 |
| | 2009 | 10 Apr | 19 | 28 Apr | 103 |
| | 2010 | 14 May | 68 | 20 May | 113 |
| Gammalstorp** | 2008 | 25 Apr | 38 | 28 Apr | 55 |
| | 2009 | 24 Apr | 53 | 27 Apr | 71 |
| | 2010 | 18 May | 101 | 20 May | 125 |
| Remningstorp** | 2008 | 23 Apr | 26 | 28 Apr | 58 |
| | 2009 | 23 Apr | 59 | 27 Apr | 92 |
| | 2010 | 7 May*** | 52 | 19 May | 108 |
| Average (Julian day number) | | 27 Apr (118) | 47 | 5 May | 82 |
| st dev | | 12 | 24 | 10 | 28 |

*Data missing.

**Data missing 2006–2007.

***First beetles were trapped at 13 °C.

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Corrigendum

# Corrigendum to “Seasonal variation in Norway spruce response to inoculation with bark beetle-associated bluestain fungi one year after a severe drought” [For. Ecol. Manag. 496 (2021) 119443]

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The authors regret following:

## 2. Material and methods

### 2.1. Study sites and experimental trees

The correct name of the Latvian provenance is Malta. (Administratively Malta parish belongs to Rezekne municipality.)

## 3. Results

**Figure 5.** the tabular description of scale (Krutzsch class) should be the changed, as scale 1–4 corresponds to late budburst and scale 5–7 to early budburst. Correct description: Swe late, scale 1–4; Swe early, scale 5–7; and East late, scale 1–4; East early, scale 5–7.

Following this, the last sentence in third paragraph in the result section will be replaced by: “Only at Skärsnäs **early**-flushing trees tended to develop smaller lesions but the differences was not statistically significant.”

**Table 3.** df-values should be changed for Site*Time of inoculation, Site* Provenance class and Time of inoculation* Provenance class to **6, 2 and 3**, respectively.

The authors would like to apologise for any inconvenience caused.

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# Seasonal flight patterns of *Ips typographus* in southern Sweden and thermal sums required for emergence

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**Abstract**

1. The spruce bark beetle *Ips typographus* is the major tree-killing bark beetle in Eurasia. To increase knowledge about its seasonal flight patterns and about the thermal sums required for emergence, a study was conducted in southern Sweden from 2006 to 2010.
2. Seasonal flight patterns were recorded by pheromone traps and development was recorded by felling trees three times during each season.
3. Flight began, on average, on 27 April [after 47 degree-days (dd) > 5 °C]. More than 50% of flight activity occurred after mid-June, and this continued to mid-August.
4. Re-emergence of parental beetles after they had produced the first brood started, on average, on 24 May (after 122 dd). Continued flight and oviposition demonstrated that sister broods were frequent. A higher proportion of parental beetles re-emerged from trees colonized in May (95%) than in July (20%). Beetles that colonized trees late had to accumulate a higher thermal sum before re-emerging (400 dd).
5. Filial beetles began to emerge, on average, on 29 June (after 437 dd) and started to fly in early July, giving rise to at least a partial second generation in each year. A higher proportion of filial beetles had emerged from trees colonized in May (75%) than in July (15%).
6. Knowledge of these region-specific flight patterns and the associated thermal sums required for emergence will facilitate efficient pest management by enabling timely removal of fallen and standing weakened host trees. The obtained data will also be useful for improving models that predict the population dynamics in a warmer climate.

**Keywords** Degree-days, flight activity, pest management, phenology, phytosanitary measures, *Picea abies*, sister brood, spruce bark beetle, voltinism.

## Introduction

The European spruce bark beetle *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) is one of the most destructive pests of Norway spruce (*Picea abies* L. Karst.) forests in Europe and Asia (Christiansen & Bakke, 1988). During outbreaks of *I. typographus*, millions of Norway spruce trees have been killed. As a result of salvage logging and a reduced quality of the wood as a resource for timber or pulp production, there have been large economic losses (Schelhaas *et al.*, 2003). In the 1990s, approximately 30 million $m^3$ of spruce were killed by *I. typographus* after several severe storm-fellings in Central Europe (Grégoire & Evans, 2004). In Sweden, approximately 9 million $m^3$ of Norway spruce have been killed subsequent to 1960 (Kärvemo & Schroeder, 2010), of which the most recent outbreak in southern Sweden, between 2006 and 2010, damaged approximately 3.5 million $m^3$ (Swedish Forest Agency, unpublished data). The outbreak was triggered by a storm in January 2005 that blew down 75 million $m^3$ of wood (Långström *et al.*, 2009). This is the most severe storm felling in Swedish history, and necessitated efficient efforts to handle the large volumes of damaged spruce in a short period of time, aiming to prevent the loss of timber value and a build-up of bark beetle populations.

The extensive damage caused by the most recent outbreak in southern Sweden has highlighted the need for more efficient control of bark beetle populations, involving mainly well-timed removal of potential host trees with reduced defence capacity

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(e.g. fallen or weakened trees) (Christiansen *et al.*, 1987; Christiansen & Bakke, 1988). This requires good knowledge of the regionally specific seasonal flight patterns and the temperature-dependent development of the beetle (Netherer & Pennerstorfer, 2001).

*Ips typographus* starts to fly in spring after a specific thermal sum is reached (Doležal & Sehnal, 2007a). During flight, the beetles disperse in search of trees suitable for breeding. After initial excavation of a nuptial gallery, the male frequently attracts up to three females to create a polygamous gallery system. After mating, the females lay eggs in niches along their gallery (Bombosch, 1954). Thereafter, the parental beetles may leave the gallery and initiate one or more sister broods (Martinek, 1956). The time until re-emergence and the occurrence of sister brood flights depends upon both intraspecific competition (Anderbrant *et al.*, 1985) and temperature (Anderbrant, 1986). Thus, higher colonization densities result in earlier re-emergence (Anderbrant *et al.*, 1985) and, similarly, higher temperatures results in earlier re-emergence and an increased reproductive success (Wermelinger & Seifert, 1999). When the beetles of the first brood are fully developed, they may emerge and initiate a second generation within the same season (bivoltine) or they may hibernate (univoltine), depending on the thermal conditions and the photoperiod (Doležal & Sehnal, 2007b). The development of filial beetles is temperature-dependent, which affects the time of emergence (Wermelinger & Seifert, 1998). *Ips typographus* in northern Europe are primarily univoltine (Anderbrant, 1989), whereas, at lower elevations in Central Europe, beetles are primarily bivoltine and occasionally trivoltine (Wermelinger *et al.*, 2011).

A key factor in determining the extent of *I. typographus* outbreak is how large proportion of the parent beetles that initiate a second and a subsequent third brood the same year (Wermelinger & Seifert, 1999); also important is knowledge of the proportion of the second generation that develops fully before winter. It has been shown that the initiation of a second generation is dependent on both temperature and day length (Schopf, 1985), which are factors that vary within the geographical range of *I. typographus*.

Knowledge of the region-specific thermal sums required for certain phases in the life of *I. typographus* is therefore necessary for efficient pest management, involving planning for the timely removal of fallen and standing weakened host trees. Colonization and reproductive success of *I. typographus* are generally higher in such trees and it is therefore recommended that they are removed before spring flight (i.e. before the first attack) to prevent reproduction and avoid the consequent deterioration in wood quality (Mulock & Christiansen, 1986; Furuta, 1989).

Thermal sums, the cumulative temperature requirement for development above a lower threshold [measured in degree-days (dd)], can forecast the initiation of spring flight, the time of development from egg to imago, and the subsequent emergence of the new generations, as well as the re-emergence of parental beetles (Annila, 1969; Anderbrant *et al.*, 1985). The thermal sums required for flight, development and emergence have been shown to differ between regions as an adaptation to local conditions (Bentz *et al.*, 2001, 2011; Netherer & Pennerstorfer, 2001). Thermal sums for the different phases in the life of *I. typographus* in southern Sweden are, however, unavailable, and knowledge about seasonal flight patterns in this region is also sparse (Trägårdh, 1939; Eidmann & Klingström, 1990).

The present study aimed: (i) to describe the flight pattern and voltinism of *I. typographus* in southern Sweden and (ii) to determine critical thermal sums allowing the prediction of distinct phases in the life cycle, such as the onset of the spring flight, re-emergence of parent beetles, and the development time of a new generation.

## Materials and methods

### *Study sites*

The flight activity and development of *I. typographus* was studied in southern Sweden (Götaland) in 2006–2010 at the two research stations, Asa (57°10′N, 14°47′E) and Tönnersjöheden (56°41′N, 13°06′E), and, in 2008–2010, at two additional locations, Gammalstorp (56°19′N, 15°19′E) and Remningstorp (58°27′N, 13°40′E). All of the study locations were within the area affected by the storm in 2005 and the subsequent *I. typographus* outbreak. All locations were also affected by another storm in 2007 (Anonymous, 2006; Bergqvist, 2009).

### *Seasonal flight patterns and thermal requirements*

Flight activity was monitored at each location using two flight interception traps baited with a pheromone (Ipslure®; KjemiKonsult ANS, Norway) that attracts *I. typographus* from long distances (Helland *et al.*, 1984). The traps were emptied weekly from mid-April to September. The pheromone dispensers were renewed in late June or early July. Trap catches were stored in a freezer (−18 °C) before quantification in the laboratory. The number of beetles caught was determined either by direct counting (if less than 100) or measured by volume (100 mL corresponding to 4400 individuals, $r^2 = 0.984$; $P < 0.001$, $n = 341$; Å. Lindelöw, unpublished data). All beetles trapped in 2009 and 2010 (45 497 in total), with the exception of the beetles trapped in Asa, were categorized, according to elytra colour, into parental beetles (darker) or filial beetles (lighter) (Merker & Wild, 1954).

Daily air mean and maximum temperature were recorded in an open area by a weather station at 1.7 m height in Asa and Tönnersjöheden. The corresponding temperature data relating to Remningstorp and Gammalstorp were obtained from the Swedish Meteorological and Hydrological Institute (http://www.smhi.se/en) weather stations in Remningstorp and Bredåkra, respectively.

To avoid effects arising from single individuals flying unusually early, spring flight was considered to have started when the weekly trap catch exceeded 10 individuals. The start date was considered to be the first day during that week when the maximum temperature exceeded 16 °C [i.e. the lowest temperature when flight was initiated in the present study, which is also close to the flight initiation threshold of 16.5 °C reported by Lobinger (1994)] or, if there were no such days, the day with the highest daily maximum temperature during that week. The correlation between expected flight date according to thermal requirements (flight threshold temperature, thermal

**Table 1** Dates when trees were felled and stem sections placed in emergence bags at different locations in southern Sweden from 2008–2010

| | | | 2008 | | 2009 | | 2010 | |
|---|---|---|---|---|---|---|---|---|
| Location | Felling | Cohort | Felled | Bagged | Felled | Bagged | Felled | Bagged |
| Asa | First | May | Winter | 20 May | 28 May | 8 May | 11 March | 1 June |
| | Second | June | 30 May | 25 June | 14 March | 29 June | 28 May | 29 June |
| | Third | July | 1 July | * | 29 June | 30 July | 8 July | 27 July |
| Tönnersjöheden | First | May | Winter | 20 May | Winter | 12 May | 23 April | 1 June |
| | Second | June | 4 June | 25 June | 25 May | * | 1 June | 23 June |
| | Third | July | 1 July | 30 July | 23 June | 7 July | * | 27 July |
| Remningstorp | First | May | 14 April | 19 May | 6 April | 11 May | 6 April | 2 June |
| | Second | June | 2 June | 16 June | 1 June | 29 June | 3 June | 21 June |
| | Third | July | 27 June | 4 August | 29 June | 27 July | 12 July | 2 August |
| Gammalstorp | First | May | 21 April | 21 May | 27 April | 11 May | * | * |
| | Second | June | 5 June | 12 July | 26 May | 13 July | * | * |
| | Third | July | 12 July | * | 13 July | 27 July | * | * |

*Missing data.

In all years, stems were removed from the emergence bags for examination at the end of the season (October to December).

sums and a combination of both) and measured date for flight initiation was tested using regression analyses run in MINITAB, version 15 (Minitab Inc., State College, Pennsylvania).

The thermal sums for parental and filial beetle emergence were calculated using 5 °C as the lower developmental threshold. A threshold of 5 °C was used because it has been reported as the lower temperature threshold for preimaginal development in a study conducted with beetles originating from Finland (Annila, 1969). A threshold of 5 °C has also been applied in later studies (Harding & Ravn, 1985; Jönsson *et al.*, 2011). Thermal sums expressed as dd were calculated by summing the daily mean temperature above the specified threshold. Differences in thermal sum requirements for emergence between cohorts of beetles from trees felled at different dates were tested using one-way analysis of variance (ANOVA) in MINITAB. When differences were significant ($P < 0.05$), they were further examined using Tukey's test.

### *Development and mortality*

At all four locations, two Norway spruce trees were cut and left on the ground, before the first flight of *I. typographus* in 2008, 2009 and 2010 (Table 1). All trees were initially baited with pheromone to ensure beetle colonization and to decrease the initial colonization time, thereby potentially avoiding a protracted emergence and less precision in the determination of the critical thermal sums. Each tree was inspected weekly for entrance holes made by *I. typographus*. When the tree was avoided and no additional entrance holes were observed, despite beetles being caught in the flight interception traps (thus indicating flight activity), a tree was considered fully colonized. A 70-cm stem section was cut from the middle part of the bole and suspended in an emergence bag fitted with a collection bottle, in a nearby shaded position. Emerging beetles were collected weekly, counted and categorized into parental beetles or filial beetles (Merker & Wild, 1954).

The procedure was repeated at the beginning of June and July (Table 1) to enable monitoring of the seasonal progress of beetle colonization and to enable study of the thermal sums necessary for complete development depending on when during the season the tree was colonized. In June, trees are expected to be colonized by flying parental beetles and by re-emerging parental beetles. In July, both late-flying, re-emerging parental beetles and emerging beetles of the new generation are expected to colonize the trees. The beetles associated with trees felled on different dates may thus be considered to approximately represent biologically distinct cohorts and are referred to as such (e.g. May cohort) hereafter. At the end of the season, the number of exit holes and mother galleries, as well as living and dead adult beetles, on each stem was counted. For each cohort, colonization density was estimated by counting the number of egg galleries/m$^2$.

Differences in colonization density were tested using one-way ANOVA in MINITAB. Differences in percentage emergence and mortality between cohorts were tested using the Kruskal–Wallis test in MINITAB. When significant differences occurred, Nemenyi's method was used to separate significantly different means.

## Results

### *Seasonal flight patterns and thermal requirements for spring flight*

Flight of *I. typographus* began, on average, on 27 April, when a mean ± SD thermal sum of 47 ± 24 dd (>5 °C) was exceeded (Table 2). Colonization of cut trees was found to occur 1 week later, on average, on 5 May, at a mean ± SD thermal sum of 82 ± 28 dd (Table 2).

To find the best predictor for flight initiation, flight temperature thresholds and thermal sum were tested for their correspondence with the estimated first day of flight using linear regression functions. The first day of flight showed no correlation with the first day, with a daily maximum temperature exceeding 16 °C ($y = -0.0297x + 120.92$; $r^2 = 0.0012$; $P = 0.899$), although it did significantly correlate with the calculated critical thermal sum threshold (47 dd) ($y = 0.9186x + 11.324$; $r^2 = 0.5797$; $P < 0.001$). An improved

**Table 2** The recorded dates and thermal sums (degree days > 5 °C) at flight initiation at the four locations in 2006–2010

| | | First pheromone trap catch | | Onset of colonization of cut trees | |
|---|---|---|---|---|---|
| Location | Year | Date | Thermal sum | Date | Thermal sum |
| Asa | 2006 | 4 May | 32 | * | * |
| | 2007 | 13 April | 34 | * | * |
| | 2008 | 21 April | 10 | 21 April | 33 |
| | 2009 | 23 April | 35 | 22 April | 65 |
| | 2010 | 19 May | 91 | 20 May | 103 |
| Tönnersjöheden | 2006 | 3 May | 36 | * | * |
| | 2007 | 13 April | 62 | * | * |
| | 2008 | 23 April | 28 | 23 April | 55 |
| | 2009 | 10 April | 19 | 13 April | 103 |
| | 2010 | 14 May | 68 | 15 May | 113 |
| Gammalstorp** | 2008 | 25 April | 38 | 28 April | 55 |
| | 2009 | 24 April | 53 | 24 April | 71 |
| | 2010 | 18 May | 101 | 20 May | 125 |
| Remningstorp** | 2008 | 23 April | 26 | 28 April | 58 |
| | 2009 | 23 April | 59 | 24 April | 92 |
| | 2010 | 7 May[a] | 52 | 19 May | 108 |
| Average (Julian day number) | | 27 April (118) | 47 | 5 May | 82 |
| SD | | 12 | 24 | 10 | 28 |

[a]First beetles were trapped at 13 °C.

*Missing data.

**Data missing 2006–2007.

Flight was considered to have started when the weekly pheromone trap catch exceeded 10 beetles. The start date of the first trap catch was considered to be the first day, during that week, when the maximum temperature exceeded 16 °C. The onset of colonization was considered to occur when the first mother galleries could be observed.

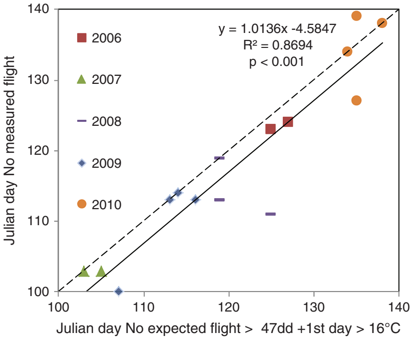

**Figure 1** Correlation between Julian day number of measured first flight of *Ips typographus* in spring and Julian day number of expected first flight according to average thermal sum and the first day with temperature exceeding 16 °C (solid line) recorded 2006–2010 in Asa and Tönnersjöheden and 2008–2010 in Gammalstorp and Remningstorp ($n = 16$) (for a definition of start date, see Table 2). Dashed line indicates a 1 : 1 ratio.

prediction of first flight was obtained when both the critical thermal sum of 47 dd and a subsequent daily maximum temperature above 16 °C had been reached (Fig. 1). On average, the first flight occurred 3 days before the predicted date.

Almost 50% of the observed flight activity (trapped beetles) occurred after mid-June, and flight activity continued until mid-August (Fig. 2). Beetles from at least seven distinct life stages (defined by sister brood- or new-generation flight) could be distinguished during the season (Fig. 2). The first beetles flying, from the end of April until the end of May, were brood offspring beetles from the previous year (Fig. 2). On average, the first 35% of the yearly catch consisted of individuals flying for the first time that season, and re-emerging parental beetles started to contribute to the catch at the end of May (Fig. 2 and Table 3). Between the end of May and the end of June, the trap catch consisted of both parental beetles flying for the first time and re-emerged parental beetles that were flying to initiate a second brood (Table 3). The filial beetles (the new generation, lighter in colour than the parents), and also parental beetles that potentially had re-emerged for a second time to lay a third brood, started to contribute to the catch in early July, by which time approximately 70% of the total catch of beetles for the year had occurred (Fig. 2). Thus, the new-generation beetles and parents re-emerging for a second time together consisted of less than 30% to the total catch. In mid-July, by when approximately 80% of the beetles had been caught, beetles from the third brood may have been present together with parental beetles re-emerging for a third time (Fig. 2). Based on the colour of the elytra, at least 17% of the total catch consisted of filial beetles from the first and second generation (Fig. 3); thus, beetles re-emerging from beginning of July onwards consisted of approximately 13% [100 − (70 + 17)] of the total catch.

### *Thermal sums for re-emergence of parental beetles and emergence of filial beetles*

The first re-emergence of parental beetles from the May cohort was observed, on average, on 24 May, approximately 3 weeks

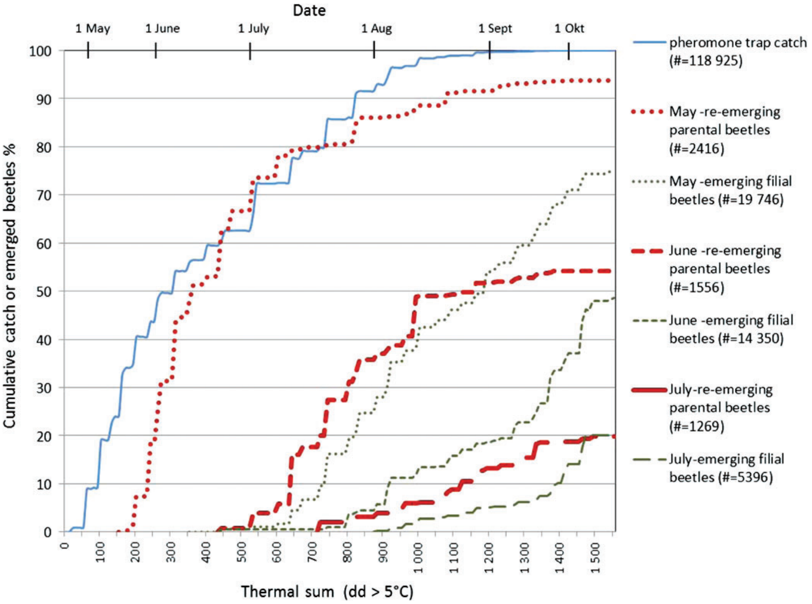

**Figure 2** Cumulative percentage of catch from pheromone traps, and percentage of emerged beetles from emergence logs, in relation to thermal sum accumulated from 1 January (lower *x*-axis). An estimation of approximately when these thermal sums occur is also provided (average of the recorded years; upper *x*-axis) in Asa, Tönnersjöheden, Remningstorp (2008–2010) and Gammalstorp (2008–2009). Numbers in the legend represent the total number of trapped beetles, emerged, and not emerged beetles (corresponding to 100%). For individual years and locations, see Supporting information, Appendix S1.

after the first brood was initiated (Table 3). This occurred at a thermal sum, accumulated after colonization, of 115 ± 46 dd. After little more than another 2 weeks, on average, on 10 June, 50% of the parental beetles had re-emerged, at a thermal sum after colonization of 256 ± 107 dd. The corresponding thermal sums if calculated from 1 January were 195 ± 58 dd for the start of re-emergence and 334 ± 110 dd for the time when 50% of the parental beetles had re-emerged.

The emergence of filial beetles (the new generation) from the May cohort started, on average, on 29 June, approximately 8 weeks after the brood was initiated (Fig. 2 and Table 3). This occurred at a thermal sum accumulated after colonization of 449 ± 107 dd. After another 7 weeks, on average, on 20 August, 50% of the filial beetles had emerged, at a thermal sum after colonization of 1019 ± 280 dd. The corresponding thermal sums calculated from 1 January were 527 ± 100 dd for the start of emergence and 1115 ± 294 dd for the time when 50% of the new generation had emerged.

There were no differences in the thermal sum requirements of filial beetles, regardless of the time of colonization. A lower proportion of parental beetles had re-emerged at the end of the season from trees colonized later in the season, and they re-emerged after accumulating a higher thermal sum (Fig. 4). There was an increasing delay in parental re-emergence in trees colonized later in the season (Fig. 5). However, filial beetles emerged at the same thermal sum, regardless of when the tree was colonized. There was no correlation between colonization density and the timing of re-emergence (data not shown),

Because the yearly thermal sum calculated from the start of the colonization was approximately 1420 dd for all locations and years (data not shown), subtracting the thermal requirement for filial beetle emergence (i.e. 449 dd) (Table 3) gave a thermal window of 971 dd that would have allowed time for almost 50% of the new generation to produce a second generation, from which at least some individuals should have time to be fully developed adults before winter (Fig. 2).

### Development and mortality

The proportion of beetles that re-emerged was significantly lower in trees colonized later in the season. Almost 95% of the parental beetles of *I. typographus* from the May cohort re-emerged from under bark before the end of the season (Fig. 2). The corresponding values for the June and July cohorts were 60% and 20%, respectively. The mortality of parental beetles under bark was significantly higher in trees colonized later in the season (Table 4). Only 3% of the May cohort was found dead under bark. In the June and July cohorts, 40% and 60%, respectively, of the parental beetles were found dead.

In the May cohort, 75% of the offspring left the trees before the end of the season, 3% were found dead under bark, and the remaining 22% stayed to overwinter underneath the bark

**Table 3** The recorded dates and thermal sums (degree-days > 5 °C) at the start, when 50% of the parental beetles had re-emerged, and when the filial beetles had emerged from stem sections (May cohort) that were colonized during the first swarming in spring and then put into emergence bags (for a definition of start date, see Table 2)

| | | Re-emergence of parental beetles | | | | Emergence of filial beetles | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Date | | Thermal sum | | Date | | Thermal sum | |
| Location | Year | Start | 50% | Start | 50% | Start | 50% | Start | 50% |
| Asa | 2008 | 17 May | 31 May | 130 | 201 | 25 June | 27 July | 436 | 766 |
| | 2009 | 8 May | 26 May | 43 | 127 | 25 June | 9 August | 360 | 870 |
| | 2010 | 2 June | 24 June | 95 | 256 | 7 July | 29 July | 427 | 721 |
| Tönnersjöheden | 2008 | 22 May | 29 May | 148 | 207 | 9 July | 24 October | 616 | 1482 |
| | 2009 | 14 May | 31 May | 83 | 197 | 1 July | 28 August | 489 | 1154 |
| | 2010 | 21 June | 29 June | 218 | 291 | 1 July | < 50% | 318 | 1315[a] |
| Gammalstorp | 2008 | * | 17 June | * | 394 | 19 June | 18 July | 407 | 711 |
| | 2009 | 12 May | 23 May | 78 | 148 | 30 June | 19 July | 489 | 726 |
| | 2010 | * | * | * | * | * | * | * | * |
| Remningstorp | 2008 | 22 May | 10 June | 157 | 382 | 1 July | 19 August | 553 | 1126 |
| | 2009 | 19 May | 28 June | 111 | 442 | 7 July | 15 August | 573 | 1010 |
| | 2010 | 31 May | 17 June | 91 | 176 | 22 June | < 50% | 278 | 1333[a] |
| Date (Julian day number)/thermal sum from colonization | Average | 23 May (143) | 10 June (161) | 115 | 256 | 29 June (180) | 20 August[b] (232) | 449 | 1019 |
| | st dev | 13 | 14 | 46 | 107 | 6 | N/A | 107 | 280 |
| Thermal sum from 1 January | Average | N/A | N/A | 195 | 334 | N/A | N/A | 527 | 1115 |
| | st dev | N/A | N/A | 58 | 110 | N/A | N/A | 100 | 294 |

[a] The thermal sum at the end of the season was used because less than 50% of the beetles had emerged by the end of the season. This was carried out so that an underestimated average value was not obtained.

[b] Date when the average thermal sum for 50% emergence (1019 degree-days) was reached on average during the years investigated.

*Missing data.

N/A: Not available.

**Table 4** Percentage emergence and mortality of parental and filial beetles (mean ± SD) from emergence logs in Asa, Tönnersjöheden, Remningstorp (2008–2010) and Gammalstorp (2008–2009)

| | May ($n = 11$) | June ($n = 10$) | July ($n = 10$) | *Kruskal–Wallis* *H* | *P* |
|---|---|---|---|---|---|
| Parental – re-emerged | $94.8 \pm 9.7^{a}$ | $57.9 \pm 33.3^{b}$ | $20.1 \pm 21.5^{b}$ | 18.9 | < 0.001 |
| Parental – alive under bark | $2.7 \pm 8.8^{a}$ | $3.8 \pm 12.1^{a}$ | $20.4 \pm 21.2^{b}$ | 9.2 | 0.01 |
| Parental – dead under bark | $2.6 \pm 3.9^{a}$ | $38.3 \pm 31.5^{b}$ | $59.6 \pm 22.6^{c}$ | 18.4 | < 0.001 |
| Total number of trapped parental beetles | 6130 | 1556 | 1269 | — | — |
| Filial – emerged | $75.3 \pm 26.8^{a}$ | $46.4 \pm 27.9^{ab}$ | $14.7 \pm 11.5^{b}$ | 16.6 | < 0.001 |
| Filial – alive under bark | $21.6 \pm 2.9^{a}$ | $44.7 \pm 29.0^{ab}$ | $63.9 \pm 31.9^{b}$ | 8.3 | 0.016 |
| Filial – dead under bark | $3.1 \pm 4.2^{a}$ | $8.9 \pm 8.5^{b}$ | $21.5 \pm 28.0^{c}$ | 8.8 | 0.012 |
| Total number of trapped filial beetles | 24 925 | 15 121 | 6046 | – | – |

Means followed by a different superscript lowercase letter within a row are significantly different ($\alpha = 0.05$).

(Table 4). In the other cohorts, a decreasing proportion of filial beetles left the trees: approximately 50% from the June cohorts and 15% from the July cohorts (Fig. 4 and Table 4). Also, offspring from trees that were colonized later showed higher mortality: approximately 9% from the June cohorts and 22% from the July cohorts (Table 4). The difference between cohorts was significant with respect to both proportion of emerged beetles and mortality (Table 4). By the end of the season, all beetles from all cohorts (May, June and July) had developed to adults, except for a very small fraction of the offspring (0.03%) found as pupae under bark in the July cohort (Table 4).

The colonization density (mother galleries/m$^2$) did not significantly differ between cohorts [May (mean ± SD): 263 ± 141; June: 173 ± 124; July: 132 ± 127. one-way ANOVA: $F = 1.43$; $P = 0.255$], and neither did the reproductive success (adult daughters/mother gallery) (May: 4,1 ± 3,2; June: 4,9 ± 5,1; July: 5,6 ± 8,1, one-way ANOVA: $F = 0{,}36$; $P = 0.702$).

## Discussion

### *Seasonal flight patterns*

*Ips typographus* usually has a long period of flight activity in southern Sweden, starting in mid-April and lasting into mid-August (Fig. 2). Over the whole study period, 10% of the flight activity occurred before the end of April and 50%

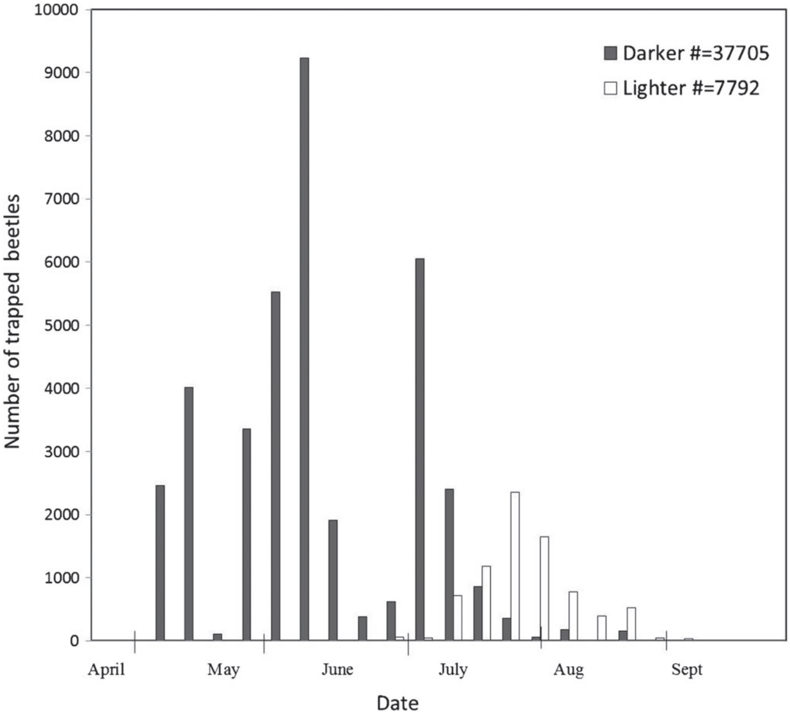

**Figure 3** Number of recently-emerged beetles cought weekly in pheromone traps categorized according to elytral colour (darker and lighter) in Tönnersjöheden, Remningstorp and Gammalstorp (2009–2010).

after mid-June. Previously, it had generally been assumed that, in Sweden, the flight period started in mid-May and lasted until the beginning of July (Trägårdh, 1939; Eidmann & Klingström, 1990). However, the long flight period shown here is more in line with findings from Denmark (i.e. flight activity between early May and mid-August) (Harding & Ravn, 1985) or the flight period in Central Europe, which is even longer (i.e. between April and September) (Faccoli & Stergulc, 2004; Baier *et al.*, 2007). Such a long flight period has also been recorded further north (i.e. in southern Norway) but only during extremely warm summers (Bakke *et al.*, 1977). Thus, the length of the flight period appears to be explained to a large extent by temperature, and southern Sweden appears to be sufficiently warm for a long flight period. The current relatively long flight period may be an effect of climate change. Öhrn (2012) found that temperatures suitable for spring flight of *I. typographus* occur almost 3 weeks earlier now than 30 years ago in this region, indicating a trend towards longer flight periods.

We also found that the flight period consisted of the main spring flight followed by one or several, sister brood flights (Fig. 3). In addition, a second generation was initiated every year (Fig. 3). However, a completed second generation appears to be a rare phenomenon (Fig. 3).

### Thermal sums for emergence

The spring flight of *I. typographus* was initiated when the accumulated thermal sum had reached an average of approximately 47 ± 24 dd (>5 °C) (Fig. 2 and Table 2). We interpret the large variation mainly as a result of that day degrees may frequently accumulate for long periods without reaching the flight threshold temperature (Fig. 1). The spring flight initiation in 2010 was later than in the other years investigated in the present study but it was not considered to be an outlier because such late flight initiations have previously been observed both in Sweden and in other Nordic studies (Regnander, 1976; Bakke *et al.*, 1977; Harding & Ravn, 1983). The thermal sum for when the spring flight was initiated in the presemt study corresponds well with results from Denmark (Harding & Ravn, 1983) and southern Finland (Annila, 1969), where it is initiated at approximately 45 dd.

A thermal sum appears to be a better predictor than using a flight threshold temperature of 16 °C for the initiation of the spring flight. An even better prediction was obtained when both the calculated required thermal sum and a subsequent temperature above the flight threshold temperature was included in the analysis. Based on the later calculation, the first flight occurred before the predicted flight date (3 days), which was expected because the thermal threshold temperature was calculated based

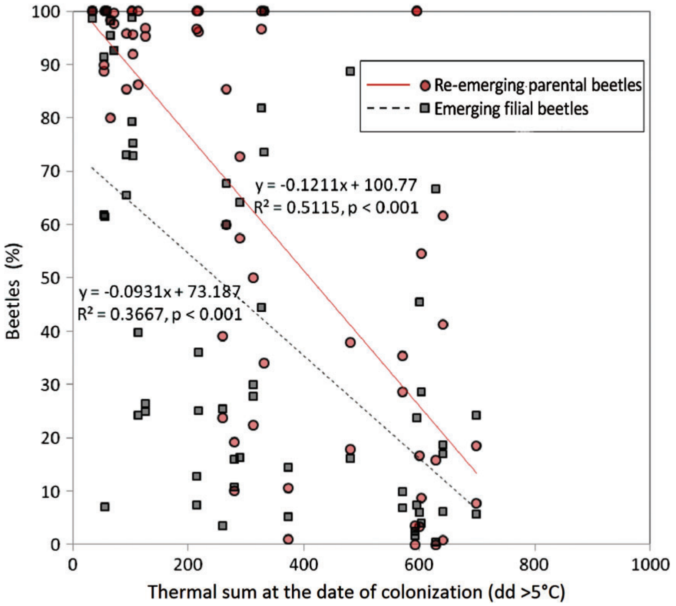

**Figure 4** Correlation between the thermal sum at colonization and the percentage of re-emerged parental (solid line) and emerged filial (dotted line) *Ips typographus* collected from emergence bags at the end of the season in Asa, Tönnersjöheden, Remningstorp (2008–2010) and Gammalstorp (2008–2009) ($n = 61$).

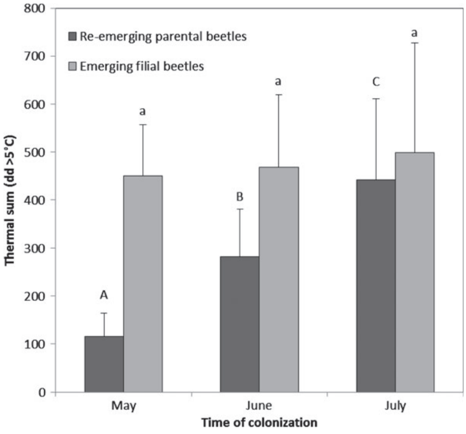

**Figure 5** The start of parental beetle re-emergence ($F = 19.25$; $P < 0.001$) and filial beetle emergence ($F = 0.22$; $P = 0.807$) expressed as the thermal sum > 5 °C accumulated after colonization of the tree (mean ± SD) for different cohorts (month) in Asa, Tönnersjöheden, Remningstorp (2008–2010) and Gammalstorp (2008–2009). Cohorts with different letters are significantly different ($\alpha = 0.05$).

on when the flight occurred (i.e. any potential lag until the flight threshold temperature occurred was included in the calculation of the thermal threshold temperature). This result requires further verification because it was made on the same dataset as that used to calculate the thermal sum for spring flight.

The re-emergence of parental beetles in the present study required a thermal sum of 122 dd > 5 °C (Fig. 2 and Table 3). Laboratory results, which also were verified in a field experiment in southern Norway, estimated the thermal sum to be 168 dd > 7.5 °C for mean re-emergence from sun-exposed trees (Anderbrant, 1986). Field studies in southern Finland suggested 150–200 dd > 5 °C for re-emergence (Annila, 1969). Thus, the estimated thermal sum in the present study was slightly lower; however, comparisons between studies should be interpreted with caution because the thermal sums are based on different thresholds.

There was an increasing delay in parental re-emergence in trees colonized later in the season. This may be because June and July are warmer than May, which could lead to faster deterioration of the under-bark environment, leading to suboptimal conditions for both parental beetles and their offspring. However, this is not likely because the reproductive success did not differ between cohorts. Also, higher temperatures not only mean a faster drying of wood, but also faster beetle development (Wermelinger & Seifert, 1998). A more likely explanation is that the prolonged time until re-emergence in trees colonized late in the season is because they are attacked, to a larger extent, by older beetles that have already exhausted their energy supplies after repeated oviposition. This is also supported by an increased mortality of parental beetles in later colonizations.

The thermal sum required for the emergence of filial beetles (new generation) was 449 dd > 5 °C in southern Sweden. This is similar to observations made in Finland (442 dd > 5 °C) and Denmark (573 dd > 5 °C at exposed positions) (Annila, 1969; Harding & Ravn, 1985).

### *Voltinism*

The calculation of percentages of the seven distinct life stages that was based on trap catches during certain periods needs to be treated carefully because it depends strongly on factors such as temperature conditions in the current year, as well as population history and resource availabilty. As with many insect species, *I. typographus* voltinism depends both on thermal and photoperiodic thresholds and therefore varies over latitude and altitude with respect to the number of days needed to complete development to the stage that can survive hibernation (imago). The considerable flight activity late in the season and the appearance of newly-emerged individuals in July shows that sister brood flights and partial second-generation flight occur frequently (Fig. 3).

Confirmed records of bivoltinism in Fennoscandia are rare and the phenomenon has only been recorded in years with extremely warm summers. In Sweden, initiation of a second generation was recorded in 1932, 1935 and 1937 (Trägårdh & Butovitsch, 1935; Butovitsch, 1938). A completed second generation has only been recorded in 1975 (in southern Norway; Austarå *et al.*, 1977), in 2006 (in southern Sweden; Långström *et al.*, 2009) and in 2010 (in southern Finland; Pouttu & Annila, 2010). A completed second generation was recorded in the present study in 2010 from a tree that was felled on 5 August and subsequently attacked, presumably by filial beetles that had already emerged from other trees attacked earlier during the season. By the end of the season, more than 90% of the offspring had reached the adult stage and more than 10% of them had emerged from the tree. Calculations performed by Jönsson *et al.* (2009) indicated that a fully developed second generation should be expected in some years each decade. Nonetheless, the present study showed that the thermal conditions in southern Sweden during each of the study years would have allowed the development of a second generation. Emergence and initiation of a second generation may have been inhibited by a shorter day length by the end of the season (Doležal & Sehnal, 2007b). As an adaption to climate change, we can expect a second generation of *I. typographus* to occur more frequently in Fennoscandia (Lange *et al.*, 2006; Schlyter *et al.*, 2006; Jönsson *et al.*, 2007, 2009). The thermal sums that we have reported in the present study could be used to improve simulation models in southern Sweden because they are both more region specific and based on a higher number of replicates of localities and years than the previous data sets that have been used for simulations of bark beetle population dynamics in this region in a future warmer climate (Harding & Ravn, 1985; Jönsson *et al.*, 2007, 2011).

### *Development and mortality*

The proportion of parental beetles in southern Sweden that re-emerge and produce a sister brood is unclear. However, almost 95% of the parental beetles re-emerged at least once before the end of the season (Fig. 2). The probability of re-emergence was significantly reduced for colonizations that occurred later in the season (Table 4). This result is in line with findings from Czech Republic, where the proportion of beetles re-emerging at least once was 91%, whereas 38% were reported to re-emerge a second time (Martinek, 1956). In the Nordic countries, both laboratory studies (Anderbrant, 1988) and field studies (Bakke, 1983) have indicated that approximately 60% of the beetles re-emerge. However, based on calculations based of the number of attacked trees and the estimated re-emergence in a south Norway forest, it has been estimated that only one third of the beetles actually produced a second brood (Anderbrant, 1989). Thus, we should expect that much less than 95% of the parental beetles in the present study produced a second brood.

Filial beetles continued to emerge until October, which is in accordance with previous results (Annila, 1969). The beetles that emerged late probably hibernated close to where they emerged because the pheromone trap catches ceased already by the end of August (Fig. 2). The filial beetle mortality increased in latter colonizations (almost 30% in July) compared with less than 5% in the May cohort. A possible explanation is that pathogens (e.g. viruses and microsporidia) are more frequent in late emerging *I. typographus* than those that emerge early (Wegensteiner *et al.*, 2007).

## Conclusions

Considerable flight activity of *I. typographus* in July, almost complete re-emergence of parental beetles from trees colonized during spring flight, and newly-emerged beetles that fly in early July indicate that sister broods and at least an initiation of second-generation brood are frequent. The effect of temperature on the number of sister broods may, under current climatic conditions with limited bivoltinism, be one of the most important factors influencing the rate of propagation of *I. typographus* during outbreaks in southern Sweden.

### *Practical implications*

Timely removal of weakened host trees is a key method for successful pest management and the general recommendation is that cut or fallen trees should be removed before first colonization in spring. However, an alternative pest management strategy, which is more efficient but risky, is to remove trees once they have already been colonized; this method requires that infected trees are removed before the new bark beetle generation emerges, and is even more effective if they are removed before the parent beetles leave the tree to establish a sister brood. The later alternative is motivated by the large proportion (95%) of re-emerging beetles. An early removal also benefits natural enemies because a lower number of them will have time to colonize the bark beetle-infested tree.

The data obtained in the present study may be used to help predict: (i) when parental beetles and filial beetles will start to emerge; (ii) the proportion of beetles that will re-emerge; and (iii) the extent of mortality by the end of the season. This information should be useful for pest management purposes because it allows the timely removal of colonized trees before sister brood and new-generation flights. The results should also prove useful for improving simulation models of the propagation potential for *I. typographus* in a warmer climate.

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## Supporting information

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**Doc. S1.** Yearly flight patterns and thermal sums per study site (2008–2010).

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# Seasonal variation in Norway spruce response to inoculation with bark beetle-associated bluestain fungi one year after a severe drought

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## ABSTRACT

In 2018, up to 4 million m$^3$ Norway spruce was killed by the spruce bark beetle *Ips typographus* in Sweden. The event was unique for Sweden, in terms of both affected volume and the fact that it was triggered by severe drought stress, not by ample availability of relatively defenseless storm-felled trees. The outbreak continued in 2019 and 2020, each year with twice as many trees killed as in 2018. The aim of this study was to quantify seasonal variation and potential lag-effects in tree defense capacity the year after a severe drought stress. Inoculation with a bark beetle-associated bluestain fungus, repeated four times with one-month-intervals between May and August 2019, were carried out at three field sites with spruce provenances of Swedish and East European origin representing early and late bud burst, respectively.

All sites had experienced moderate to severe drought stress in 2018, and site-specific defense capacity correlated positively with the cumulative precipitation two months before inoculation. Sites with two-month precipitation levels <100 mm had larger necrotic lesions in the phloem following inoculation, an indication of lower tree defense capacity. Lesion size did not differ between provenances, and all trees were able to confine fungal infection successfully.

There were some seasonal differences in necrotic lesion size, with the sites Skärsnäs and Norberg having significantly larger lesions in June than in May, and site Lugnet having large lesions also in May. Lesions were generally smaller in July and August than in June.

The cross-sectional area and number of traumatic resin ducts was measured in sapwood samples from one site, Lugnet, to quantify an additional aspect of tree defenses. The area of resin ducts produced in May and June were larger than that in July and August. This is in line with a positive correlation between lesion area and resin duct area, indicating that a stronger fungal infection following inoculation in spring triggered a stronger induced defense response. The East European provenances had more resin ducts than Swedish provenances, but the area of resin ducts did not differ significantly between provenances.

## 1. Introduction

The spruce bark beetle (*Ips typographus*) is a major forest pest in Europe and the risk of beetle outbreaks is strongly influenced by climatic factors. Beetle-induced mortality of Norway spruce (*Picea abies* (L.) H. Karst) trees averaged 150 000 m$^3$ per year in south Sweden for the period 1990–2010 (Marini et al., 2017). However, in 2018 the beetles killed 3–4 million m$^3$ spruce, and 7 million m$^3$ in both 2019 and 2020 (Wulff and Roberge, 2021). This extreme mortality was triggered by the warm and dry summer in 2018, leading to the completion of two beetle generations and rapid population build-up. Historically, bivoltine development of the spruce bark beetle has rarely been observed in Sweden (Öhrn et al. 2014), but is expected to occur much more frequently in a warmer climate (Jönsson et al., 2012).

Norway spruce has evolved multiple defenses to resist insect and pathogen attack. These include preformed constitutive defenses and induced defenses that are activated in response to attack (Franceschi et al., 2005). Anatomical structures such as resin ducts in the phloem and sapwood are important in both lines of defense. Resin ducts are filled with terpenoid resin, a key chemical defense in many conifers

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which dose-dependently inhibits bark beetle colonization (Zhao et al., 2011a). Traumatic resin ducts in the sapwood are induced in response to insect attack, pathogen infection or abiotic stress (Krokene, 2015). To overcome tree defenses the spruce bark beetle engages in mass-attacks synchronized by aggregation pheromones (Bakke et al., 1977; Franceschi et al., 2005). If successful, the aggregated attack leads to massive inoculation of beetle-vectored bluestain fungi forming necrotic lesions in the bark, invading the sapwood and gradually overwhelming the tree's defense capacity (Krokene, 2015).

The 2018 spruce bark beetle outbreak in Sweden was unique, as it was triggered by drought stress, and not by a large supply of storm-felled trees with little to no defense capacity, which is the usual trigger of outbreaks in Sweden (Marini et al., 2013). Severe drought stress has been shown to reduce tree resistance to fungal infection and to trigger bark beetle outbreaks (Netherer et al. 2021, Raffa et al. 2008). The continuation of the outbreak into 2019 may indicate carry-over effects of the 2018 drought stress. Such lag-effects contribute to uncertainties in assessments of how climate change may affect the risk of spruce bark beetle attacks (Jönsson et al., 2012). Quantitative information on lag-effects is lacking because severe natural drought stress events are rare and it is expensive to carry out realistic drought stress experiments in the field.

Norway spruce makes up 42% of the forest volume in Sweden and about 200 million seedlings are planted each year. To promote vigorous trees with high wood quality, selection of suitable seed sources (provenances) has been carried out for>100 years (Myking et al., 2016). Tree phenology is a key selection character, as the timing of shoot elongation and wood formation determines the risk of frost damage, since provenances with later bud burst are less exposed to spring frost damage (Hannerz, 1999). East European provenances are commonly selected, as they in general have later bud burst and increased growth compared to local provenances in southern Sweden (Persson and Perssson, 1992; Werner and Karlsson, 1982). Tree defense capacity has not been selected for *per se*, even though there is a genetic component to tree resistance to bluestain fungi (Steffenrem et al., 2016). A key concern about selecting for resistance is that physiological tradeoffs between growth and defense may lead to a negative correlation between growth and tree defense capacity (Herms and Mattson, 1992; Krokene et al., 2012). The timing of budburst in relation to the flight activity of the spruce bark beetles may thus influence tree defense capacity in spring.

A general, seasonal variation in resistance to fungal infection has been demonstrated in Norway spruce previously (Horntvedt 1988; Krokene et al. 2012). This variation appears to be related to shoot phenology and could be a result of physiological trade-offs between growth and defense processes (Herms and Mattson 1992; Krokene et al. 2012). Several independent but partially overlapping hypotheses attempt to explain patterns in plant defense allocation. The expanded Growth-Differentiation Balance (GDB) hypothesis incorporates all the other hypotheses into its conceptual framework to predict how plants balance resource allocation to growth-related processes versus defense and other differentiation-related processes (Mattson and Herms, 1992). Since the GDB hypothesis predicts that there is a trade-off in allocation to growth and defense, actively growing trees are expected to be less resistant to attack. When plant resources are allocated to growth, less are available for investment in physical and chemical defenses.

The aim of this study was to quantify potential lag-effects of drought on tree resistance, as 2019 provided a unique opportunity to better understand and predict how climate change may affect spruce vulnerability to bark beetle attacks. We inoculated Norway spruce trees with bluestain fungi on four different occasions throughout the growing season at three field sites. Spruce provenances with different phenology that experienced severe or moderate drought stress in 2018 were inoculated. The size of necrotic lesions in the phloem and traumatic resin duct formation in the sapwood following fungal inoculation provided measures of tree defense capacity. We tested three hypotheses for how environmental and genetic factors influence tree defense capacity:

H1. Site-specific differences: Trees at the site with the most severe drought in 2018 have lower defense capacity in 2019 than trees at sites experiencing moderate drought.

H2. Differences between provenances: Swedish and East European spruce provenances, with a generally early and late timing of budburst, respectively, differ in defense dynamics in spring.

H3. Seasonal differences: Spruce trees differ in defense capacity over the season, with lower defense capacity during periods of active growth.

## 2. Material and methods

### 2.1. Study sites and experimental trees

Tree defense capacity was assessed at three field sites in S-Sweden; Skärsnäs [56.2°N, 14.3°E], Lugnet [59.6°N, 17.5°E], and Norberg [60.1°N, 15.8°E] (Fig. 1a). All three sites had been influenced by the warm and dry summer in 2018, with total precipitation during April – August being>100 mm below average at Skärsnäs and about 50 mm below average at Lugnet and Norberg (Fig. 1b). The 2018 annual temperature sums were around 1800°days (dd) (>5 °C) at Norberg and 2000 dd at Skärsnäs and Lugnet, which is about 50% above the 1961–1990 average (Fig. 1c). The Standardized Precipitation Evapotranspiration Index (SPEI) (Begueria et al., 2014) was significantly below normal at all sites and indicated that the drought was more severe at Skärsnäs (SPEI −3.5) than at Lugnet and Norberg (SPEI −2.0) (Fig. 1d).

The field sites were established in 2002 as part of one of Sweden's largest test series of Norway spruce seed sources, with 64 provenances and seed orchard crops of south Swedish and Eastern European origin (Lundströmer et al., 2020). At each site, 50 replicates (2 trees × 25 blocks) of each provenance were planted in a randomized block design. Each block, measuring ca. 20 × 24 m, was planted with 2-year-old seedlings in a 2 × 2 m formation in May-June 2002. We randomly selected 10 out of the 25 blocks at each site for this study (i.e. 20 trees per provenance).

Previously collected data on tree phenology, growth, vitality and damage were used in the present study. All trees had been assessed for growth, vitality and damages six and 13 years after planting. In addition, assessments of phenological characters (timing of bud burst and lignification of wood cells indicating growth cessation) made in 2004 has been shown to correlate with the geographical origin of the trees (Lundströmer et al., 2020). Budburst and shoot elongation measurements were scored on all trees according to the Krutzsch-scale (Krutzsch, 1973) in early summer 2004, when the trees were 4 years old (in mid-May at Skärsnäs, in late May at Lugnet, and in early June at Norberg). At the time of scoring, virtually all provenances had active bud development, i.e. were above class 0 ('dormant bud') but below class 8 ('all needles more or less scattered, new buds begin to form'). Based on these scores we selected provenances with an early or late spring phenology for comparisons of defense capacity. We selected the provenances Ängelsfors and Bollebygd as representative Swedish provenances with an early spring phenology, and the provenances Istra and Rezekne (both from Latvia) as representative Eastern European provenances with a late spring phenology. The selected provenances were inspected to ensure that they did not have more damages than the average for all provenances.

### 2.2. Experiments and sampling

Tree defense reactions were assessed by inoculation with a bark beetle-associated fungus, a common method to elicit and evaluate conifer defense responses (Steffenrem et al., 2016). As inoculum we used malt agar colonized by *Grosmannia europhioides* (isolate 7.206.1 sampled August 16 2007 from a Norway spruce tree at Asa experimental forest and reseach station [57.2°N, 14.8°E], gifted by Dr. Ylva Strid, SVA), as this relatively virulent bluestain fungus is a frequent associate of the spruce bark beetle in Sweden (Zhao et al. 2018). Inoculum was prepared

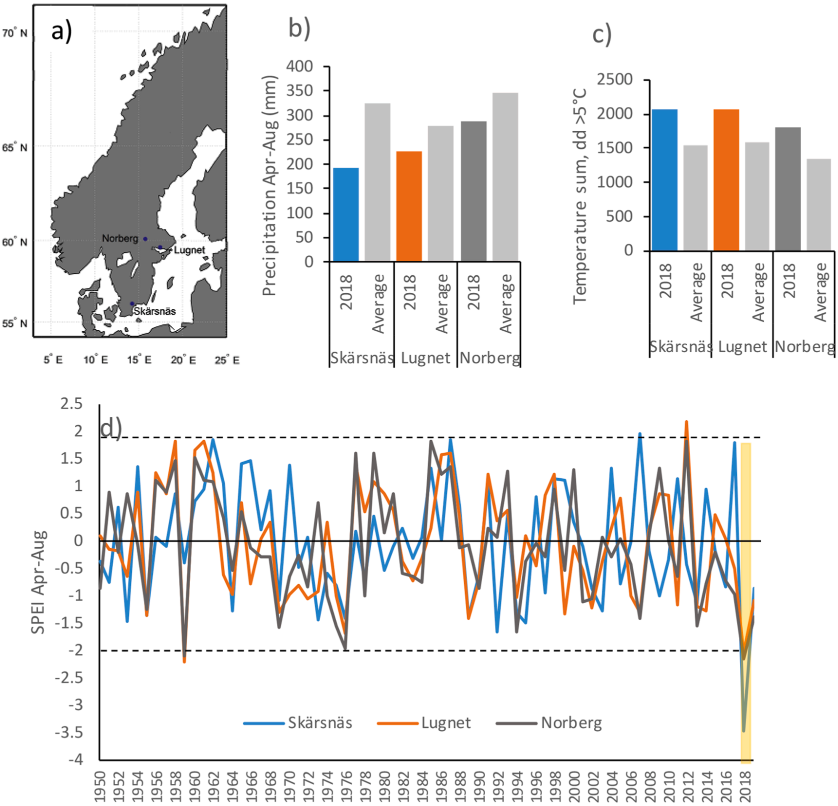


**Fig. 1.** (a) Map displaying the three sites. (b) Precipitation during April-August (main tree growth period) and (c) total annual degree days (>5 °C) for 2018 and the long time average (1961–1990). (d) Standardized Precipitation Evapotranspiration Index (SPEI) during April-August 1950–2019 (2018 and 2019 highlighted). Gridded precipitation and temperature data are from SMHI http://luftweb.smhi.se/. SPEI was derived from http://spei.csic.es/map and is based on z-scores.Black solid line represent the average condition, and values exceeding ± 1.96 (dashed lines) are significantly drier or wetter than the average (95% confidence level).

by growing *G. europhioides* on malt extract agar (MEA, 2%) for approximately three weeks before inoculation into Norway spruce. On the day of inoculation, MEA colonized by *G. europhioides* was mashed into a slush and loaded into syringes without needles for ease of inoculation.

Trees were inoculated on four occasions from May to August (Fig. 2, Table 1). At each field site, we inoculated five trees from each of four provenances every month, in total 240 trees (except four trees that could not be recovered after inoculation) (Table 2). The north facing side of the tree was inoculated three times about 1.3 m above ground, with 10 cm between inoculation sites (see Fig. 3). Bark plugs were removed with a 4 mm cork-borer and malt agar slush with living *G. europhioides* was injected into the holes. The bark plugs were then put back and biofilm was wrapped around the tree to keep the plugs in place. The first inoculations were made in mid-May, when overwintering spruce bark beetles normally initiate their main brood. The second inoculations in

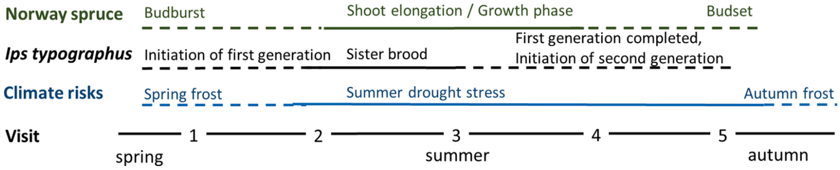


**Fig. 2.** Schematic representation of Norway spruce shoot phenology (green line), in relation to spruce bark beetle (*Ips typographus*) activity (black line), and climate-related risks for tree damage (blue line). New fungal inoculations were performed at field visit 1–4, and the sampling of previously inoculated trees were carried out at visit 2–5, i.e. inoculation and sampling were coordinated during visit 2–4. Dashed lines represent temporal variability, as influenced by provenance-specific tree phenology and inter-annual variation in temperature conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**
Timing of field visits, accumulated temperature sums (degree days calculated from January 1, 2019) and monthly precipitation at the field sites used in this study. Meteorological data are from nearby stations of the Swedish Meteorological and Hydrological Institute.

| Site | Date of field visit | Degree days (dd, > 5 °C) | | Precipitation between visits (mm) |
|---|---|---|---|---|
| | | From Jan 1st | Accumulation between visits | |
| Skärsnäs | 17-May | 191 | – | 58* |
| | 17-Jun | 509 | 318 | 67 |
| | 17-Jul | 847 | 338 | 37 |
| | 19-Aug | 1258 | 411 | 26 |
| | 17-Sep | 1554 | 296 | 47 |
| Lugnet | 20-May | 198 | – | 19* |
| | 18-Jun | 496 | 298 | 57 |
| | 19-Jul | 821 | 325 | 56 |
| | 20-Aug | 1229 | 408 | 39 |
| | 18-Sep | 1528 | 299 | 48 |
| Norberg | 21-May | 194 | – | 19* |
| | 19-Jun | 471 | 277 | 155 |
| | 20-Jul | 795 | 324 | 66 |
| | 21-Aug | 1169 | 374 | 59 |
| | 19-Sep | 1406 | 237 | 99 |

* The value for May is the sum for the previous 30-day-period.

**Table 2**
Number of inoculated Norway spruce trees per field visit, average stem diameters and seasonal variability in necrotic lesion area (mean ± SD) following inoculation with *Grosmannia europhioides*. Means followed by different letters are significantly different (Tukey's test following ANOVA, $\alpha = 0.05$; small letters: comparisons within sites between inoculation times; capital letters: comparisons between sites).

| Site | Visit | Number of inoculated trees (n) | Stem diameter (mm) | | Necrotic lesion area ($cm^2$) | Necrotic lesion area ($cm^2$)/ tree diameter (mm) |
|---|---|---|---|---|---|---|
| | | | Total | Growth last 5 years | | |
| Skärsnäs | May | 20 | 79 ± 25 | 22 ± 6 | 17.9 ± 5.5 a | 0.24 ± 0.08 a |
| | June | 20 | 73 ± 25 | 24 ± 7 | 31.1 ± 18.2b | 0.43 ± 0.15b |
| | July | 20 | 83 ± 22 | 26 ± 7 | 26.3 ± 10.5 ab | 0.32 ± 0.11 a |
| | August | 20 | 73 ± 23 | 25 ± 11 | 17.5 ± 4.0 a | 0.27 ± 0.12 a |
| | Total | 80 | 77 ± 24B | 23 ± 8B | 23.2 ± 12.3 A | 0.31 ± 0.14 A |
| Lugnet | May | 19 | 96 ± 16 | 44 ± 10 | 44.2 ± 15.0c | 0.47 ± 0.16b |
| | June | 20 | 87 ± 17 | 42 ± 8 | 36.7 ± 19.6 cb | 0.43 ± 0.21b |
| | July | 20 | 92 ± 15 | 40 ± 10 | 25.7 ± 14.5b | 0.27 ± 0.13 a |
| | August | 18 | 93 ± 12 | 41 ± 7 | 18.4 ± 9.6 a | 0.20 ± 0.11 a |
| | Total | 77 | 92 ± 15 A | 40 ± 11 A | 31.4 ± 17.9B | 0.34 ± 0.19 A |
| Norberg | May | 20 | 93 ± 33 | 32 ± 10 | 28.6 ± 12.4 bcd | 0.33 ± 0.13b |
| | June | 20 | 80 ± 22 | 29 ± 7 | 34.4 ± 11.9 dc | 0.44 ± 0.13c |
| | July | 19 | 99 ± 24 | 36 ± 7 | 20.8 ± 9.2 ab | 0.21 ± 0.08 a |
| | August | 20 | 88 ± 23 | 30 ± 6 | 16.8 ± 5.5 a | 0.20 ± 0.07 a |
| | Total | 79 | 90 ± 26 A | 30 ± 9 A | 25.2 ± 12.0 AB | 0.30 ± 0.14 A |

**Fig. 3.** Three necrotic lesions on a Norway spruce tree with the bark pulled aside. The area of the necrotic lesions following *Grosmannia europhioides* inoculation (light brown zones) was measured on the xylem surface. The white pin in the center of the stem measures 10 mm in diameter.

mid-June represented the timing of any sister broods, and the third and fourth inoculations in mid-July and mid-August represented the timing of a second beetle generation (Fig. 1). The size of necrotic lesions in the bark following fungal infection was measured one month after inoculation. To obtain a quantitative measure of tree defense capacity, the bark on the inoculated part of the stem was removed to expose the necrotic lesions in the inner bark and on the sapwood surface. All lesions were photographed (Fig. 3) and the total area of each lesion on the sapwood surface was measured using ImageJ software (Abràmoff et al., 2004).

### 2.3. *Analysis of traumatic resin ducts and 5-year sapwood growth increment*

The number and area of traumatic resin ducts (Kane and Kolb, 2010; Rosner and Hannrup, 2004) were quantified from sapwood samples collected in October 2019 at the Lugnet site from a subset of 40 trees (five trees, two provenance classes, and four inoculation times). These trees were representative for all trees and provenances sampled at Lugnet in terms of lesion area. A single sapwood sample was collected from each tree 0.5 m below the inoculation area and 90° counterclockwise using a 4 mm diameter cork-borer. Sapwood cross-sections were prepared from the cork-borer samples using a sliding freezing microtome and photographed at 50 × magnification (Leica Microsystems CMS GmbH). Traumatic resin ducts were counted over ~2.5 mm tangential width of the cross-sections and total resin duct area was quantified using ImageJ software (Abràmoff et al., 2004).

### 2.4. *Data sources, calculations and statistical analysis*

Precipitation and temperature data were derived from the closest stations of the Swedish Meteorological and Hydrological Institute (SMHI). Precipitation data came from Olofström (8 km from Skärsnäs), Skjörby (13 km from Lugnet) and Norberg (8 km from Norberg), and temperature data from Hästveda Mo (27 km from Skärsnäs), Enköping Mo (29 km from Lugnet) and Avesta (23 km from Norberg). Temperature data from Avesta was corrected for the difference in elevation between Avesta and Norberg [corrected mean $\text{temp}_{\text{Norberg}}$ = mean

$\text{temp}_{\text{Avesta}} - (0.065\ ^{\circ}\text{C} * 91\ \text{m}_{\text{elevation difference [Norberg –Avesta]}})]$. Cumulative temperature sums were calculated as growing degree days (dd) above a threshold of 5 °C, starting from 1 January (Hannerz, 1999), to quantify thermal differences between sites throughout the season.

Variation in soil moisture conditions at the study sites was determined from data from the Swedish Forestry Commission (https://kartor.skogsstyrelsen.se/kartor/?startapp=skogligagrunddata). The experimental blocks within sites were classified as relatively moist (ground water level $< 1$ m deep) or dry (ground water level $> 1$ m deep). At Skärsnäs, four out of 10 experimental blocks were situated on a plain surrounded by ditched bogs and had moist soil. Six blocks with dry soil were situated on sloping terrain above the moist blocks. Due to the clear difference between blocks, the effect of soil conditions was analysed for this site. At Lugnet, seven out of 10 blocks were classified as having moist soil conditions. However, since this site was on flat former agricultural land with clay, it had no obvious soil moisture gradient and there were no clear difference between dryer and moister blocks. All 10 blocks at Norberg had relatively dry soil conditions.

To quantify potential differences in stem increment of individual trees over the last 5 years, we calculated the difference between stem diameter measured in September 2019 and stem diameter measured in 2014. To capture genetic components we compared trees from early-flushing Swedish provenances with trees from late-flushing Eastern European provenances. To capture phenotypical components and variation among individuals, budburst data for individual trees were included in the analysis.

Necrotic lesion areas of inoculated trees were analyzed using a three-way ANOVA to compare the three field sites, the two provenance classes with predominately early or late phenology, and the four inoculation times. Two different lesion measures were analyzed: total lesion area per tree (in $\text{cm}^2$) and total lesion area relative to stem diameter (in $\text{cm}^2\text{mm}^{-1}$). By being normalized to tree size the latter measure accounts for the potential importance of tree size for tree defense capacity.

We did a logarithmic regression analysis between necrotic lesion area and precipitation at each study site to assess the effect of cumulative precipitation one to 12 months prior to inoculation. The interval with the best correlation (highest $r^2$-value) was selected for further analysis to separate the effects of timing of inoculation and tree defense capacity. Another logarithmic regression was done to compare tree defense responses between sites with low and high precipitation.

Data on traumatic resin ducts from Lugnet were subjected to a two-way ANOVA to determine if the quantity (number or area) of traumatic resin ducts differed between provenances with early and late phenology or between inoculation times. For comparison between the smaller subset of trees used for resin duct analyses and all trees sampled at Lugnet, necrotic lesion data was tested with a similar ANOVA, to ensure that the subsample was representative of the larger sample. All statistical analyses were performed using JASP Team (2019), JASP (Version 0.9.2).

## 3. Results

The study sites differed in bioclimatological conditions (Table 1). The temperature sums accumulated between fungal inoculation and measurement of necrotic lesions were in general 20–30 dd lower at Norberg than at the other two sites. The average temperature sums across sites were 298 dd for the period May to June, 329 dd for June to July, 396 dd for July to August, and 277 for August to September. Monthly precipitation in 2019 differed between sites, with Skärsnäs receiving more rain than the other sites in the spring, and Norberg having a wetter summer than Skärsnäs and Lugnet (Table 1).

Trees inoculated at Skärsnäs had significantly smaller stem diameter than trees at Lugnet and Norberg (Table 2). Furthermore, the stem diameter increment during the last 5 years was significantly smaller at Skärsnäs. At all sites, larger trees tended to have larger necrotic lesions, and the same differences in response were detected between sites, time of inoculation and provenance classes when tree defense capacity was analyzed in terms of absolute lesion area or normalized lesion area per stem diameter (Table 2 and 3). In the following we therefore focus on absolute lesion areas. At Skärsnäs and Norberg, lesion size peaked in June (Table 2), whereas lesion size was smaller and about the same in May, July and August. At Lugnet, lesions were largest in May and June, and successively smaller thereafter. The differences in lesion size between Swedish and East European provenances were not statistically significant, and were small in comparison with the differences between inoculation times. At Skärsnäs, there was a temporal difference in defense capacity between trees growing under different soil moisture conditions (Table 3).

The bud burst data from 2004 confirmed that Swedish provenances on average had earlier bud burst than East European provenances (Fig. 4). There was, however, considerable variation in timing of bud burst among individuals within each provenance class. The proportion of trees categorized as Krutzsch class 1–4 (i.e. having a later bud burst) was 27% for trees of Swedish origin and 39% for trees of East European origin. Early- and late-flushing individuals did not differ in lesion size (Figs. 4 and 5). Only at Skärsnäs, late-flushing trees tended to develop smaller lesions but the differences were not statistically significant.

The relationship between lesion size and total precipitation during 1 to 12 month periods prior to the inoculation was strongest for the 2-month precipitation sum ($r^2 = 0.63$, Fig. 6). The degree of explanation ($r^2$) for the other precipitation sums was between 0.30 and 0.55, but dropped below 0.30 for the 10- to 12-month sums. The 2-month precipitation sum was further analyzed to separate effects of inoculation time and provenance class (Fig. 6c). The analysis indicated that lesions were larger when total precipitation the preceding two months was below 100 mm. In general, the relationship was similar between provenances. There was no correlation between average lesion area for a site and the thermal sum that had accumulated between visits.

At Skärsnäs, the trees in blocks with moist soil were smaller (mean stem diameter 65 mm ± 3.5, $n = 32$) than trees in dry-soil blocks (85 mm ± 3.3, $n = 48$) ($t$-test, $p < 0.001$) and they also developed smaller lesions in June and July (Fig. 7). The difference between dry and moist soils was significant when tree defense capacity was calculated in terms of lesion area, but not when defense capacity was expressed as lesion area per stem diameter (Table 3).

In the wood samples collected at Lugnet in October, resin ducts were significantly larger and more numerous in trees inoculated in May and June compare to July and August (Table 4 and 5). East European provenances had more resin ducts than Swedish provenances, with

**Table 3**
p-values from ANOVA of stem growth and necrotic lesion data from Norway spruce trees inoculated with *Grosmannia europhioides* at three field sites. Data were analyzed in terms of site differences. For one site, Skärsnäs, a separate analysis was carried out to explore within-site differences in soil moisture conditions. P-values $< 0.05$ are shown in bold ($n = 236$).

| Variables | df | Lesion area ($\text{cm}^2$) | Lesion area/stem diameter ($\text{cm}^2\ \text{mm}^{-1}$) |
|---|---|---|---|
| **Sites (Skärsnäs, Lugnet, Norberg)** | 2 | **<0.001** | 0.088 |
| Time of inoculation | 3 | **<0.001** | **<0.001** |
| Provenance class | 1 | 0.869 | 0.823 |
| Site * Time of inoculation | 2 | **<0.001** | **<0.001** |
| Site * Provenance class | 27 | 0.974 | 0.438 |
| Time of inoculation * Provenance class | 18 | 0.902 | 0.289 |
| **Soil moisture (Skärsnäs)** | 1 | **0.015** | 0.708 |
| Time of inoculation | 3 | **0.002** | **<0.001** |
| Provenance class | 1 | 0.939 | 0.823 |
| Soil moisture * Time of inoculation | 3 | **0.013** | **0.032** |
| Soil moisture * Provenance class | 1 | 0.993 | 0.274 |
| Soil moisture *Time of inoculation * Provenance class | 3 | 0.895 | 0.911 |

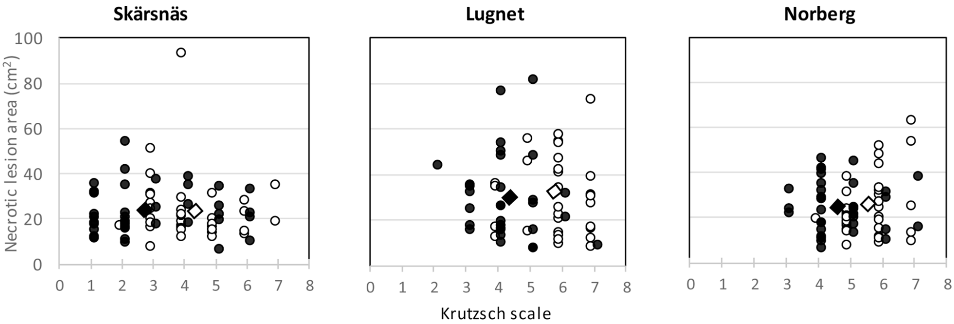
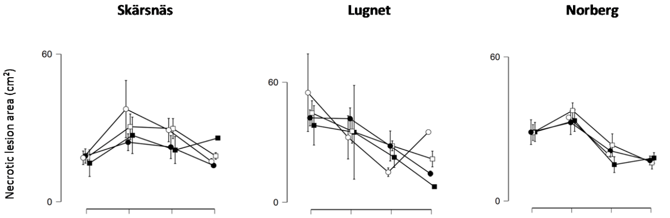


**Fig. 4.** Individual variation in shoot phenology and necrotic lesion area in Norway spruce trees following inoculation with *Grosmannia europhioides*. The shoot phenology of Swedish (○, predominantly early flushing) and East European (●, predominantly late flushing) provenances was assessed in spring 2004, when trees were 4 years old, using a bud burst scale ranging from 0 to 8, where 0 = 'dormant bud' and 8 = 'all needles more or less scattered, new buds begin to form' (Krutzsch,1973). Rhombi indicate mean value of necrotic lesion area and mean Krutzsch-value for Swedish (◇) and East European (◆) provenances.



| Prov. class | Scale | 17 May | 17 Jun | 17 Jul | 19 Aug | 20 May | 18 Jun | 19 Jul | 20 Aug | 21 May | 19 Jun | 20 Jul | 21 Aug |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Swe early | ○1-4 | 7 | 6 | 6 | 5 | 2 | 4 | 2 | 1 | 0 | 1 | 1 | 0 |
| Swe late | ●5-7 | 3 | 4 | 4 | 5 | 7 | 6 | 8 | 8 | 10 | 9 | 8 | 10 |
| East early | □1-4 | 8 | 8 | 6 | 9 | 8 | 7 | 6 | 8 | 6 | 6 | 7 | 6 |
| East late | ■5-7 | 2 | 2 | 4 | 1 | 2 | 3 | 4 | 1 | 4 | 4 | 3 | 4 |

**Fig. 5.** Seasonal variability in necrotic lesion area in Norway spruce following inoculation with *Grosmannia europhioides* (mean ± SE). The figure panels show the seasonal variation within Swedish (circles) and East European (squares) provenances for both early-flushing (Krutzsch class 1–4) and late-flushing (Krutzsch class 5–7) individuals. The number of sampled trees for each measurement is indicated in the table below the figure panels. See Fig. 4 for a definition of Krutzsch classes.

significant differences between provenance classes for trees inoculated in May and June (Table 5). The relationship between lesion area and resin duct area in individual trees was positive for both provenance classes, but statistically significant only for the East European provenances (Fig. 8).

## 4. Discussion

In Sweden, the 2018 drought stress event predisposed spruce trees to attack by the spruce bark beetle, with massive tree killing occurring in 2018, 2019 and 2020 (Schroeder and Fritscher, 2020; Wulff and Roberge, 2021). In this field study carried out one year after the drought stress event, we inoculated Norway spruce trees with the bark beetle-associated blue stain fungus *G. europhioides* to study carry-over effects of drought stress on tree defence capacity. Larger necrotic lesions in response to fungal infection are generally considered to be a symptom of lower tree defense capacity. Necrotic lesions differed in size both between sites and between inoculation times in our study. We identified a threshold in tree defense capacity at a precipitation sum of about 100 mm during the last two months before inoculation, with small lesions (about 20 $cm^2$ lesion area per tree) at sites with>100 mm precipitation and exponentially increasing lesion sizes at sites with lower precipitation levels (up to 45 $cm^2$ lesion area at 50 mm precipitation). We will discuss our results relative to three sets of factors that can influence both tree defense capacity and level of water stress: (1) site-specific conditions, (2) tree genetics, and (3) seasonal/phenological variability.

### 4.1. Site-specific differences

Previous studies in Norway spruce have shown that low water availability can alter the strain-specific severity of fungal infestation in spruce trees, leading to larger lesions and increased risk of seedling mortality at dryer sites (Linnakoski et al., 2017; Terhonen et al., 2019). In this study, we detected significant differences in tree defense capacity between sites. However, our first hypothesis of lower defense capacity in trees experiencing the most severe drought was rejected: trees at Lugnet developed the largest lesions following fungal inoculation in May, even though the 2018 drought was most severe at Skärsnäs (Fig. 2). In June, lesion size was more similar across the three sites. Total precipitation over the last two months before inoculation was the best predictor of lesion size ($R^2 = 0.63$), whereas the 12-month cumulative precipitation sum, which included the actual drought period, performed substantially poorer ($R^2 = 0.26$). This indicates that Norway spruce trees have a potential to recover after drought stress, at least up to a base defense level defined by water-dependent defense properties (i.e. resin viscosity and flow). Full recovery of tree defense capacity after drought, including replenishment of non-structural carbohydrate (NSC) stores and repair of drought-damaged xylem, may take substantially longer (Trugman et al., 2018).

NSC availability is important for the production of chemical

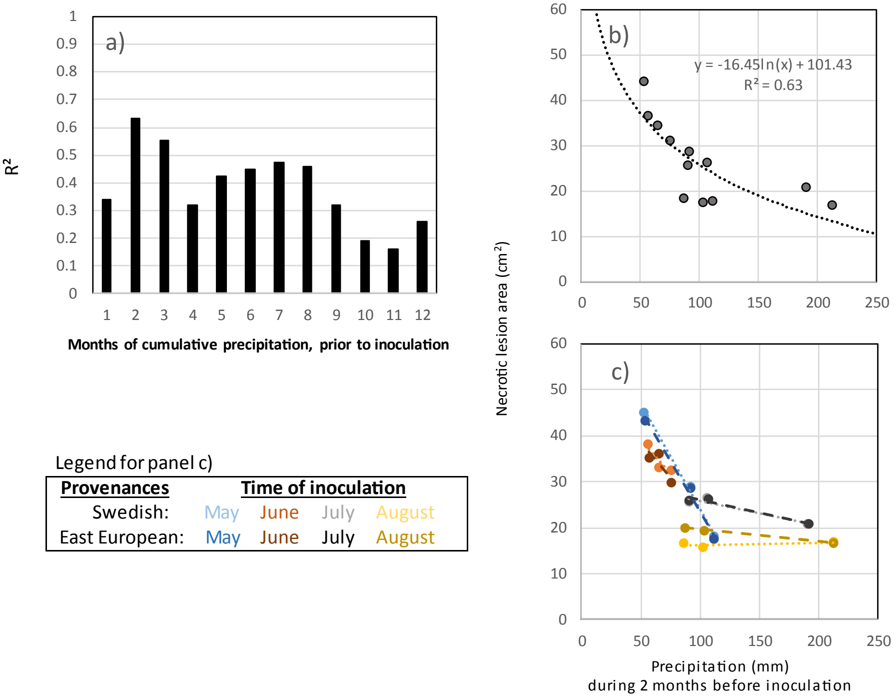


**Fig. 6.** (a) $R^2$-values of logaritmic regressions between necrotic lesion area in Norway spruce following inoculation with *Grosmannia europhioides* and cumulative precipitation over one to 12 month periods prior to inoculation. (b) The logaritmic regression with the highest $R^2$-value, corresponding to 2 months cumulative precipitation. Each circle represents the mean lesion area per study site and time of inoculation; n = 12 (3 study sites × 4 inoculation times). (c) Monthly linear regressions of lesion area versus precipitation data from nearby meteorological stations. Lesion data in May was correlated with total precipitation in March-April, lesion data in June was correlated with total precipitation in April-May, etc.. Each circle represents the mean value per study site and time of inoculation, and dotted or dashed lines show the time-dependent regression lines between the three study sites.

defenses. NSCs provide a resource buffer between growing seasons. Severe drought stress can reduce NSC stores because carbohydrates are used to support autotrophic respiration also when photosynthesis is restricted by water limitations (Hartmann and Trumbore, 2016). Such a loss of NSC stores has been indicated by ecosystem modelling (Jönsson and Lagergren, 2018). It has also been observed empirically: the biomass increment of Norway spruce and Scots pine in one growing season is correlated with the net ecosystem exchange of carbon in the previous growing season. Thus, reduced carbon uptake in one season leads to reduced biomass increment the following year (Lagergren et al., 2019). Spruce trees with low NSC stores have been found to prioritize defense, in the form of production of secondary metabolites, over growth and respiration (Huang et al., 2019). The correlation we observed between spruce defense capacity and short-term precipitation may thus rather indicate that water-stressed trees have impaired induction of resin flow (Kolb et al., 2019).

Acute drought leading to tree transpiration deficits has been associated with increased risk of spruce bark beetle attacks, with 35% of Norway spruce stands with a transpiration deficit above 40 mm being attacked (Netherer et al., 2019). Two measurement stations associated with the Integrated Carbon Observation System research infrastructure (ICOS), the Hyltemossa station and the Norunda station, are situated in southern and central Sweden, and trees at both stations were attacked by spruce bark beetles following the 2018 drought. In 2018, both stations showed a decrease in annual net ecosystem productivity (NEP) and annual evapotranspiration (Lindroth et al., 2020). At Hyltemossa, situated 80 km from Skärsnäs, the relative precipitation deficit was 0.52 and NEP came close to zero, due to a relatively large decrease in gross primary production (GPP) accompanied by an increase in ecosystem respiration. Norunda, 120 km from Norberg and 170 km from Lugnet, had a relative precipitation deficit of 0.36 and an increase in GPP relative to a normal year. Both the decrease in NEP and increase in ecosystem respiration were thus more pronounced at Hyltemossa than at Norunda, and this is in line with the observation that the 2018 drought stress was more pronounced in southern Sweden (including Skärsnäs) than in central Sweden (including Norberg and Lugnet).

### 4.2. Differences between spruce provenances classes

All trees inoculated with the blue stain fungus *G. europhioides* in this study were able to confine the infections and there was no significant difference in lesion size between Swedish and East European provenances. However, we found a general positive relationship between lesion area and resin duct area (Fig. 8), indicating that a stronger local reaction to fungal inoculation triggered a stronger induction of traumatic resin duct formation. This relationship was significant for East European provenances, which also produced more numerous traumatic resin ducts in May and June than the Swedish provenances. The resin duct area, however, did not differ significantly between provenances. Our second hypothesis that Swedish and East European spruce

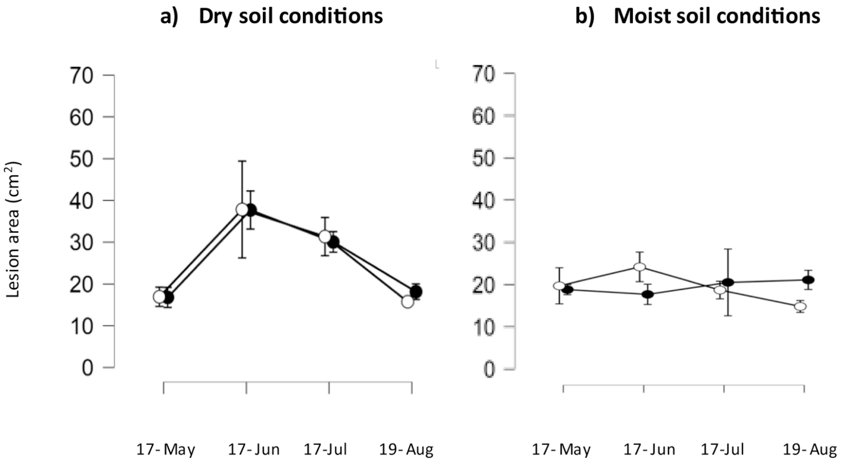


**Fig. 7.** Seasonal variability in necrotic lesions (mean ± SE) in Swedish (○) and East European (●) provenances of Norway spruce inoculated with *Grosmannia europhioides* at sites with dry and moist soil conditions. (a) Trees growing on dry soil (ground water level < 1 m depth) or on (b) moist soil conditions (ground water level > 1 m depth) in Skärsnäs (see Table 3b for statistical details).

**Table 4**
Seasonal variability in area of traumatic resin ducts formed in the last annual ring (2019) of Norway spruce trees following inoculation with *Grosmannia europhioides* (five blocks at site Lugnet, 20 trees per provenance class). For each provenance class, means followed by different letters are significantly different (Tukey's test following ANOVA, $p < 0.05$).

| Time of inoculation | Area resin ducts ($\mu m^2/\mu m$ tangential annual ring) Provenance class | |
|---|---|---|
| | Swedish | East European |
| May (n = 5) | 18.1 ± 5.8 a | 29.8 ± 6.7 a |
| June (n = 5) | 14.8 ± 2.2 ab | 25.4 ± 6.1 a |
| July (n = 5) | 4.4 ± 2.5 ab | 1.9 ± 0.9b |
| August (n = 5) | 3.4 ± 1.5b | 2.6 ± 2.2b |
| Total n = 20 | 10.0 ± 2.3 | 16.0 ± 3.8 |

**Table 5**
p-values from ANOVA of number and area of axial resin ducts and necrotic lesions in Norway spruce trees inoculated with *Grosmannia europhioides* (five blocks at site Lugnet, $n_{total} = 40$). p-values < 0.05 are shown in bold.

| | | Resin ducts | | Lesion area |
|---|---|---|---|---|
| Variables | df | number | area | |
| Block | 4 | 0.194 | 0.209 | **0.027** |
| Time of inoculation | 3 | **<0.001** | **<0.001** | **0.007** |
| Provenance class | 1 | **0.048** | 0.102 | 0.324 |
| Time of inoculation *Provenance class | 3 | 0.352 | 0.180 | 0.787 |

provenances differ in defense capacity was thus partly rejected and partly supported. The capacity of Norway spruce trees to respond to infection by increasing the number of resin ducts in the sapwood may be linked to the inherent growth rhythm of the trees. The pattern we observed in May and June may thus reflect differences in growth rhythm between the two provenance classes: East European provenances have a later onset of growth than Swedish provenances (Karlsson, 2009) and produce more traumatic resin ducts as they have a larger degree of freedom to form new structures.

Formation of traumatic resin ducts is part of the induced defense responses of conifer trees. For both Engelmann spruce *Picea engelmannii* (DeRose et al., 2017) and ponderosa pine *Pinus ponderosa* (Kane and Kolb, 2010) the likelihood that individual trees will survive bark beetle outbreaks has been found to correlate with their capacity to produce traumatic resin ducts. In whitebark pine *Pinus albicaulis*, individuals with large resin ducts relative to sapwood growth had lower mortality, which indicates a trade-off between growth and defense (Kichas et al., 2020). To predict tree growth responses and resistance in a climate change-context it is important to further disentangle how the interplay between tree genetics and environmental factors forms phenotypic responses (Vazquez-Gonzalez et al., 2020). While traditional tree breeding with both Swedish and East European provenances has focused on rapid growth and high wood quality, there might be reasons to also breed for tree resistance to biotic attacks.

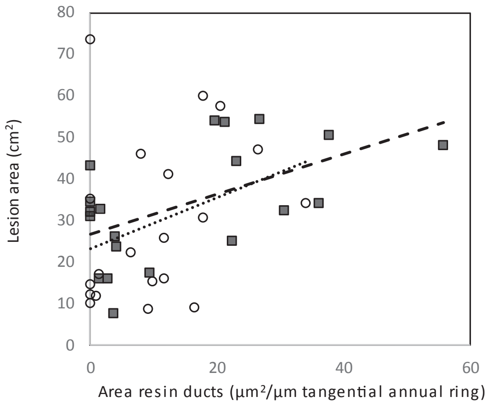


**Fig. 8.** Correlation between necrotic lesion area in Norway spruce following inoculation with *Grosmannia europhioides* and number and area of traumatic resin ducts in Swedish provenances (○, …., $r^2 = 0.10$, $p = 0.18$) and East European (■, ——, $r^2 = 0.31$, $p = 0.01$) provenances. Data are from trees in five blocks at site Lugnet, $n_{total} = 40$.

### 4.3. *Seasonal differences in tree defense capacity*

Both provenance classes displayed seasonal differences in defense capacity. Trees at Skärsnäs and Norberg provided support for our third hypothesis, stating that lesions would be larger due to a lower tree defense capacity during the period with most active growth, which is June. At Lugnet, the largest lesions were observed in May, and Swedish provenances in particular produced larger lesions in May than in June. This may be explained by the contributing effect of May being dryer, and hence more stressful for the trees, than June at Lugnet. Comparable studies of seasonal defense capacity of Norway spruce to bluestain fungi are sparse, but the few available studies show similar results. Horntvedt (1988), studying 25-year-old trees, and Krokene et al. (2012), studying 2- and 8-year old trees, found tree defense capacity to be lowest in mid-June. Low tree defenses in June, coinciding with the peak of sister brood swarming by the spruce bark beetle (Öhrn et al., 2014), indicate that sister broods may be important for the build-up of univoltine spruce bark beetle populations at higher latitudes and altitudes (Davídková and Doležal, 2017; Wermelinger and Seifert, 1999).

Trees growing on dry soils at Skärsnäs had lower defense capacity than trees growing on moister soils. It is, however, worth noting that in our study the trees growing on moister soils were also generally smaller. Moreover, the observed difference was not significant when expressed as lesion area per diameter. Lower resistance due to water stress has been observed in earlier studies (Matthews et al., 2018; Netherer et al., 2016; 2015). However, the relationship between tree resistance and drought stress is complex and non-linear, since there is also evidence that mild drought may enhance tree resistance (Christiansen and Glosli, 1996; Zhao et al., 2011a; 2011b).

## 5. Conclusion

In this field study we found evidence for a threshold in the sensitivity of Norway spruce to drought stress, where trees receiving less than about 100 mm precipitation in the two months preceding fungal infection had lower defense capacity than other trees. The relationship between accumulated precipitation and defense capacity was consistent across three study sites in southern Sweden, all being affected by a severe drought stress event the year before. The observed differences in spruce defense capacity between sites and throughout the growing season were likely caused by site-specific combinations of water- and NSC-limitations, influencing the trees' ability to produce resin-based defenses during their recovery phase following a severe drought. However, because this study included data on traumatic resin ducts from one site only, additional analysis is needed to further elucidate the relationship between resin-based defenses and drought stress. While 2018 was the first example of a drought-induced spruce bark beetle outbreak in southern Sweden, warm and dry summers are projected to become more frequent in this region in response to climate change. An increased awareness about provenance-specific predisposing factors in combination with triggering weather and climate events is therefore needed to identify timely countermeasures to limit the size and consequences of spruce bark beetle outbreaks.

## Author statement

All authors contributed to the conceptualization and writing of the manuscript. PÖ carried out the field work, and the data analysis. Financial support was provided by FORMAS project 2019-00097 granted to AMJ.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Doctoral Thesis No. 2024:35

The aim of this thesis was to investigate both the region-specific seasonal phenology of the spruce bark beetle in southern Sweden and the resistance of its host, the Norway spruce. Beetle flight activity was determined, thereby facilitating efficient pest management by enabling timely removal of infested trees. Tree resistance was lower in the early season (June) and differed with both precipitation levels and local soil moisture conditions. The findings underline the importance of understanding thresholds in both bark beetle development and tree drought stress variables for predicting the impact of future bark beetle outbreaks in the context of climate change.

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