Czech University of Life Sciences in Prague
The Faculty of Forestry and Wood Sciences
Department of Forest Protection and Entomology

Doctoral dissertation thesis

THE FACTORS BEHIND SPRUCE RESISTANCE TO BARK BEETLE ATTACK

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Scientific supervisor: **prof. Ing. Marek Turčáni, PhD.**
Study programme: **Forestry Engineering**
Field of study: **Forest Protection and Game Management**

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CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Forestry and Wood Sciences

Ph.D. THESIS ASSIGNMENT

Nataliya Korolyova

Forestry Engineering
Forest Protection and Game Management

Thesis title

The factors behind spruce resistance to bark beetle attack

Objectives of thesis

1. To develop the methods for Last Tree Standing (LTS) studies in Norway spruce (*Picea abies*) stands under the conditions of Central Europe.

2. To quantify impact of stochastic, external (environmental, stand) and internal factors in Norway spruce (*Picea abies*) survival during severe bark beetle (*Ips typographus*) outbreaks.

Methodology

To fulfil the objectives of the dissertation thesis, the research methodology includes several successive stages that incorporate theoretical argumentation of the methods to be used, remotely sensed and field data collection, and statistical analyses.

The first stage of the research implies compilation of a literature review that summarizes the current state of knowledge and existing evidence on the factors of Norway spruce resistance to *Ips typographus* mass attacks. The gaps in our understanding of mechanisms underpinning tree survival during severe bark beetle outbreaks will be identified. A methodological framework that embraces divergent approaches and inter-disciplinary methods to study extant trees' resistance to bark beetle outbreaks will be developed.

The second stage comprises the study of morphological, stand and environmental factors of tree survival, incorporating the following algorithm:

1) Identification of surviving trees, or LTS, located in severely disturbed area at the border of Czechia, Germany, and Austria using high-resolution aerial time-series imagery available in Google Earth Pro (GE) soft-ware spanning time period from 2000 to 2019.

2) Measuring or estimating morphological and stand parameters of surviving trees using GE imagery.

3) Obtaining satellite imaging data from Landsat 5 (Thematic Mapper sensor) for 1986 to estimate forest stand densities across the study region that were present prior to initiation of the bark beetle outbreak.

4) Defining the boundaries of disturbed study area using visual inspection and supervised classification that will be performed in Erdas Imagine software. The study area will be filtered for non-spruce areas, including roads, rocks, meadows, and water sources, such as rivers and lakes.

5) Extracting climate data from Copernicus and high-resolution CHELSA databases; compiling them to calculate 30-year means of precipitation, temperature and aridity index for the target trees.

6) Predicting pre-outbreak stand densities using Landsat 5 imagery and non-linear regression models fit in likelihood framework. Based on the formulated hypotheses, models with different permutations of spectral and environmental variables will be tested.

7) Stand density prediction model calibration using compiled dataset of reference and surviving trees. The calibration dataset will consist of GE-derived densities for LTS locations, as well as density records obtained from Remote Forest Inventory plots that overlap the study region. Additionally, density records will be obtained from field surveys conducted in 2020.

8) Calculating the values of spectral and environmental parameters such as slope, aspect, folded aspect, northness, Topographic wetness index, Heat load index, Topographic position indices for different radii, potential solar radiation, structural index, Normalized difference vegetation index, etc. for each of the LTS and reference trees.

9) Conducting ground-truth survey to prove surviving status of GE-identified trees and to test the accuracy of GE-derived measurements of LTS crown parameters. For ground-truthing survey, a subset of LTS positions that represents 20% of the dataset will be generated randomly.

10) Estimating diameter at breast height (DBH) for GE-identified LTS, including trees located in Germany and Austria, for which taking field measurements is constrained by administrative barriers for field work in publicly- and privately-owned forests in these countries. Allometric scaling models to estimate DBH from associated crown projection area will be developed and calibrated using data from field survey conducted within our study area in 2020 that measured tree DBH and crown dimensions for 99 trees. To ensure an adequate sample size, we will supplement field data with individual tree records from Remote Forests,a network of permanent forest monitoring plots.

11) Testing for non-random patterns of LTS spatial distribution. This analysis will be conducted based solely on surviving trees' relative spatial positions using a hot spot analysis conducted in ArcGIS software.

12) Random forests classification of dead (reference) and surviving trees to discern the relative importance of multiple environmental and spectral variables in predicting tree survival status. We will apply the random forests algorithm using randomized decision trees. Stratification of the predictor space into a number of simple non-overlapping regions will be based on greedy, recursive binary splitting that minimizes Gini index measuring the purity of the classifier.

13) Compilation of the final LTS and reference tree dataset comprising GE-identified surviving trees, and reference trees derived from Remote Forests database and field survey conducted in 2020.

14) Logistic regression models will be fit to a combined dataset in likelihood framework to test for the effects of the most potentially important environmental variables identified by the results of Random forests classification on survival probability. The final best model will include morphological, stand and environmental predictors of tree resistance during bark beetle outbreak.

At the third stage of the research logistic regression models to quantify the effects of chemical, morphological and stand factors on tree resistance to bark beetle attacks will be developed. The dataset represents the results of the experiment conducted in Vosges, a mountainous spruce-dominated forest region in eastern France, in 2003 during an ongoing bark beetle (*Ips typographus*) outbreak. Study plots were selected in close proximity to freshly formed bark beetle spots. To test the hypothesis that secondary defence compounds influence tree resistance capacity during bark beetle outbreak, agarose gel that mimics bark beetle attack was inoculated into each of 97 trees. Phloem was sampled twice, before treatment and three weeks after treatment. Multiple measurements of stand, tree morphology and vigour parameters were taken. Bark beetle activity and tree living status was then monitored during the following four months. The models will be formulated to account for non-liner patterns in chemical, morphological, and stand covariates distribution. A simulated annealing, a global optimization algorithm will be used to determine maximum likelihood-based estimates of unknown model parameters.

Keywords

Ips typographus, tree survival, tree resistance, survival markers, climate change, drought, disturbance, competition, carbon, self-shading, crown, tree vigour, insolation, spatial distribution

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Recommended information sources

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Expected date

2021/22 SS – FFWS – Doctoral Thesis Defence

The Dissertation Thesis Supervisor

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Supervising department

Excellent team for mitigation

Electronic approval: 2. 5. 2022

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Chairperson of Field of Study Board

Electronic approval: 31. 8. 2022

prof. Ing. Róbert Marušák, PhD. Dean

Prague on 29.05.2023

STATEMENT OF INDEPENDENCE

I hereby declare that this Ph.D. Thesis, titled “The factors behind spruce resistance to bark beetle attack”, was created independently and in an ethical manner. I declare all the information sources and literature have been indicated accordingly, and the Thesis was prepared under direct supervision of my scientific supervisor.

I agree with the disclosure of this Ph.D. Thesis according with Czech Law (Act No. 111/1998 Coll. Sb.) regardless of the Defence of Thesis results.

November 8, 2023

In Prague

"According to Darwin's Origin of Species, it is not the most intellectual of the species that survives; it is not the strongest that survives; but the species that survives is the one that is able best to adapt and adjust to the changing environment in which it finds itself."

Megginson, 'Lessons from Europe for
American Business', Southwestern
Social Science Quarterly (1963)
44(1): 3-13, at p. 4.

Table of contents

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Abstract

Forests in the Northern Hemisphere experience severe climate change intensified insect outbreaks. This study focuses on Norway spruce (*Picea abies*) resistance to *Ips typographus* attacks, aiming to unveil the factors influencing tree survival during bark beetle outbreak in Central Europe. To investigate tree survival mechanisms, we used a multidisciplinary approach based on analytical chemistry, dendroecology, and remote sensing methods. To quantify tree survival responses, machine learning techniques and non-linear logistic regression models fit in likelihood framework were used. Two beetle-disturbed areas located in the Bohemian Forest and Vosges mountains served as study sites, employing experimental and observational approaches to explore tree defence mechanisms and life-history trade-offs. We hypothesised that tree survival is a non-random process governed by external and internal factors. To study tree chemical defence mechanisms, we conducted a Methyl jasmonate (MJ) inoculation experiment and mechanical wounding experiment. To discern tree life-history trade-offs in the allocation of limited resources, the radial growth rates prior to disturbance were compared among surviving and reference trees. These rates were also used to reconstruct disturbance in the Bohemian Forest area. We used remotely sensed Landsat 5, Google Earth Pro historical imagery and field data to estimate stand density prior to the outbreak. We found that tree survival is a non-random process driven by multiple external and internal factors and their interactions. The probability of spruce survival is positively and non-linearly associated with the proximity to previously attacked hosts, catechin production, self-shading and long-term climatic water balance. Survival probability has negative non-linear relationships with tree size, stand density prior to an outbreak, stem sun-illumination and crown defoliation. Climatic water balance interacted with stand and tree-level factors, aggravating the negative effects of stand density and tree size, and enhancing the positive effects of self-shading and moisture availability on tree survival. Surviving trees exhibited slower radial growth than reference trees in decades preceding the outbreak ($p<0.05$). Although we did not identify any differences in the whole terpene profiles between LTS and reference trees, as shown by untargeted gas chromatography – mass spectrometry, surviving and references trees varied in the abundance of individual compounds prior to and after MJ inoculation. Our findings enhance understanding of Norway spruce survival mechanisms during bark beetle outbreaks, suggesting potential heritability of resistance traits. Maintaining surviving individuals in post-disturbance areas and incorporating them into tree breeding programs could improve forest resilience, but further research on genetic and chemical factors across diverse environments is crucial for a more comprehensive understanding.

Key words: tree resistance, tree survival, *Ips typographus*, *Picea abies*, chemical defence, bark beetle outbreak, forest disturbance, phenolics, terpenes, epicubenol, β-ylangene, self-shading, insolation, drought, heat, climate change, Last Tree Standing

The list of published papers

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9. **Korolyova**, N., Buechling, A., Ďuračiová, R., Jakuš, R. 2023. The Last Tree Standing: Climate, stand and tree level effects on spruce survival during *Ips typographus* outbreak. In: Mäkipää, R. & Berninger, K. (eds.). Climate resilient and sustainable forest management: IBFRA conference 28-31 August 2023. Book of abstracts. Natural Resources and Bioeconomy Studies 77/2023. Natural Resources Institute Finland. Helsinki. p. 144. https://jukuri.luke.fi/bitstream/handle/10024/553741/luke-luobio_77_2023.pdf?sequence=1&isAllowed=y

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The list of abbreviations used in the text

DBH – diameter at breast height

GCMS – gas chromatography–mass spectrometry

GE – Google Earth Pro

LTS – Last Trees Standing

mgAE g^{-1} of phloem – milligrams vanillyl alcohol equivalent per gram of freeze-dried phloem

NSC – non-structural carbohydrate

MJ – Methyl jasmonate

RF – Random Forests

SNP – single nucleotide polymorphism

VOC – volatile organic compounds

I. INTRODUCTION, CURRENT KNOWLEDGE AND THEORETICAL FRAMEWORK TO STUDY TREE SURVIVAL

In the Northern Hemisphere, large-scale severe bark beetle outbreaks modify landscapes, compromise forest functions, and degrade habitat for many dependent species. Extreme disturbances that disrupt the multitude of ecosystem services provided by forests, including erosion control, maintenance of hydraulic regimes, timber supply and carbon storage, have significant social and economic consequences (Kooperman et al., 2018). The forested area affected by tree mortality has increased twofold since 1984 (Senf and Seidl, 2018). In Europe, the volume of harvested wood has doubled, rising from 31 Mm^3/year in 2018 to 70 Mm^3/year in 2020 (Korolyova et al., 2022a). This augmentation that can be largely attributed to eruptive populations of the Eurasian bark beetle, *Ips typographus* – one of the most economically important forest pests in this part of the world (Jönsson et al., 2012). Increases in the severity and frequency of storm-induced and climate-change intensified beetle disturbances, as well as the expansion of insect populations into forests at northern latitudes (Kautz et al., 2017), actuate research questions related to host tree resistance and survival potential under strong herbivore pressure.

1.1 The mechanism of host selection and tree resistance

The mechanism of host selection and tree resistance in the “spruce – bark beetle” system is poorly elucidated. Despite a long history of study, a comprehensive understanding of tree defence mechanisms against *I. typographus* is still lacking (Lehmanski et al., 2023). Tree resistance, including induced defence responses, determines the ability of bark beetles to colonize living trees. Generally, a robust, multi-tiered system of tree defences maintain bark beetle populations at endemic levels and prevent beetles from reaching outbreak population densities (Krokene, 2015). Relationships between the defence capacity of trees and herbivory pressure from bark beetles underlies a conceptual model of tree survival that integrates life-history theory (Herms and Mattson, 1992). This theoretical model predicts a critical limit or threshold of beetle colonization that may be deterred given the internal physiological and resource constraints of a host tree (Christiansen et al., 1987).

Host trees have evolutionarily developed multiple defence mechanisms against bark beetles. The capacity of mass attacks to exhaust the defensive apparatus of host trees is reinforced by the activity of microbial species, which may be vectored by beetles and symbiotically impair tree physiological functions (Lieutier et al., 2009). Pheromone-coordinated mass attacks and phytopathogenic symbionts act synergistically to overwhelm tree defences. The first line of conifer defences consists of preformed (constitutive) defences exhibited by omnipresent mechanical formations and chemical composition of organic compounds.

Mechanical defences comprise structural elements that deter invaders by providing toughness or thickness of tissues, including impregnation of tissues with lignin and suberin polymers that enhance resistance to penetration, degradation, and ingestion. Chemical defences include substances causing toxic or inhibitory effects, such as specialized plant metabolites (Franceschi et al., 2005). Carbon demanding defence reactions drain resources away from other vital plant functions, such as growth and reproduction, thus, not all kinds of defences are expressed continuously. Plants have evolved the capacity to up-regulate additional, inducible reactions in response to biotic and abiotic stressors. The integration of preformed and inducible defence types provides a cost-effective and flexible response to bark beetle attacks. Preformed defences may inhibit initial insect attacks, while inducible defences ensure that potential invasions are recognized and dealt with timely and effectively. A vast majority of existing studies investigated tree responses to actual beetles' boring into the bark and to a beetle vectored fungal pathogen *Ceratocystis polonica (C. polonica)* and *Endoconidiophora polonica (E. polonica)*, while relatively fewer studies were dedicated to disentangling constitutive, including chemical, tree defences.

A successful tree defence reaction that integrates both preformed and inducible mechanisms typically progress through one to four successive stages (Franceschi et al., 2005). The first stage deters beetles by means of specialized preformed tissues as well as stored secondary metabolites. Although constitutive barriers may be sufficient in many circumstances, they may fail to inhibit attacks under extreme beetle densities (above some threshold) or when a host tree is stressed physiologically. The subsequent induced stage of defence aims to kill or compartmentalize individual insects that have overcome preformed barriers (Månsson, 2005). Commonly, the first two stages are realised sequentially, since inducible defences are triggered immediately after an attacking organism penetrates into the living bark and contacts preformed defensive structures. The third stage of defence involves sealing and compartmentalizing beetle-damaged areas to ensure uninterrupted physiological functioning and prevent the development of secondary infections vectored by opportunistic organisms. Induced defences may be displayed temporally and locally (in the vicinity of an attacked site), or systemically and globally (on a whole tree level) to increase the efficiency of dealing with future bark beetle colonisation attempts (Krokene et al., 2008).

1.2 Abiotic stress factors that predispose trees to bark beetle attacks

Tree defence capacity depends strongly on the physiological condition of a host, which is modified by a diverse array of biotic and abiotic stress factors. From the *Ips typographus* attack on Norway spruce point of view, we can distinguish two key types of abiotic challenges: a) thermal stress caused by abruptly elevated insolation levels (Kautz et al., 2013), and b) drought stress (Netherer et al., 2015). Despite the existence of other potential stress factors (e.g. fungal pathogens, air and water pollution) that may impact physiological responses to bark beetle attack, their importance in tree defence functions is less pronounced, compared to the factors mentioned above. The increasing frequency of global warming induced drought and heatwaves have actualized the importance of insights into a molecular mechanism of plant reaction to thermal and drought stresses. A number of recent studies have attempted to investigate the molecular basis of plant resistance to abiotic stresses. However, our current knowledge of the regulatory networks involved in tree response to drought and thermal stress is predominantly derived from experiments conducted on model plants, such as *Arabidopsis* and agricultural species. Genetic markers of *P. abies* resistance to drought and heat stress, which modify host survival capacity during bark beetle outbreaks, have not yet been identified. Despite strong empirical evidence for an aggregation of beetles on host trees with high sun exposure and elevated bark temperatures (Kautz et al., 2013), disentangling the relative roles of heat stress vs. water limitation is hindered by methodological challenges (Christiansen, E., Bakke, 1997; Kausrud et al., 2012).

Thermal stress. Plants experience detrimental effects when exposed to high temperatures, commonly known as heat stress (HS), including an impairment of phloem function and an enhancement of leaf transpiration rates (Kautz et al., 2013). The compounding impacts of persistent heat stress may eventuate in individual tree mortality (Park Williams et al., 2013). Associated selection pressures have led to a suite of coping mechanisms to facilitate tree survival. One of the key reactions involves an activation of specific genes, referred to as heat shock transcription factors (HSFs) and heat shock proteins (HSPs) (Ohama et al., 2017). HSFs are responsible for rapidly triggering the expression of HSPs, and both HSFs and HSPs play crucial roles in plant response to heat stress and thermotolerance induction. Under transient or mild heat stress, trees adjust their respiration and transpiration rates to balance water loss and heat dissipation rates. Heat stress can adversely affect the functioning of essential cellular components, such as DNA, RNA, proteins, and lipids (Zhao et al., 2021). Plant thermosensors may consist of a single or combination of protein molecules, enabling timely detection and adequate responses to temperature changes.

Drought stress. Persistent moisture deficit leads to stomatal closure by means of upregulated levels of endogenous abscisic acid, which consequently reduces the availability of CO_2 for photosynthesis (Iuchi et al., 2001). Gradual depletion of the stored non-structural carbon reserves (NSCs) (McDowell et al., 2008) reduces the carbohydrate pools required for the production of secondary metabolites, compromising tree defence capability (Bentz et al., 2010). Another consequence of water limitation on a molecular level is the upregulated synthesis of proteins associated with stress tolerance (Harfouche et al., 2014; Klápště et al., 2020; Trujillo-Moya et al., 2018). For example, late embryogenesis-abundant (LEA) proteins, including dehydrins, stabilise other proteins and cell membranes, preventing protein aggregation in water limited conditions (Stival Sena et al., 2018). HSPs that belong to the chaperones protein family enhance membrane stability and detoxify the reactive oxygen species (Harfouche et al., 2014) that otherwise can trigger irreversible degradation processes in basic cell structures, including DNA, protein, and lipids (Shields et al., 2021). Energy demanding production of stress tolerance proteins can be compromised under water and photosynthate constrained conditions.

1.3 The types of host trees

Based on our field observations and previously published findings (e.g. Jakuš et al., 2011; Korolyova et al., 2022a), from a host selection and tree resistance points of view, the following three types of trees can be distinguished: (1) primarily attacked trees; (2) secondarily attacked trees; and (3) resistant surviving trees, which we refer to as Last Trees Standing (LTS). LTS are intact living trees that have evaded or successfully deterred bark beetle colonization and feeding.

Primarily attacked trees are susceptible, physiologically-stressed hosts selected by pioneer beetles using olfactory and visual cues in the absence of insect-synthesised aggregation pheromones (Korolyova et al., 2022b). This process is termed “primary attraction”. Although primary attraction has not been convincingly proven experimentally in a natural forest environment (Lehmanski et al., 2023), it has been studied in the context of volatile monoterpene emissions in both field (Blaženec et al., 2021; Gossenauer-Marohn, 1988; Niemeyer and Watzek, 1996; Reddemann and Schopf, 1996) and laboratory (Schiebe et al., 2019a) conditions.

Secondarily attacked trees are killed by beetles subsequent to the successful initial colonisation of primarily targeted individuals. Augmented by insect immigration and reproduction, bark beetle behaviour “switches” to extant neighbouring trees using a pheromone mediated aggregation mechanism (Schlyter and Anderbrant, 1989).

Surviving trees or the Last Trees Standing. Forests disturbed by the bark beetle outbreaks retain a certain minor proportion of survivors – living trees that successfully evaded, tolerated or repelled feeding herbivores (Six et al., 2018a). Even in stands with high mortality levels, a few scattered, mature trees may persist (Balogh et al., 2018; Erbilgin et al., 2017; Jakuš et al., 2011a; Maclauchlan, Lorraine, Brooks, J. E., White, 2015; Six et al., 2018a). Typically, these survivors are large dominant or co-dominant individuals (Jakuš et al., 2011a). For the purpose of this research, we will refer to such trees as “Last Trees Standing” (LTS) (Korolyova et al., 2022a).

Our unpublished field observations conducted in Central Europe reveal different types of LTS. The classical type is a single surviving individual surrounded by a large number of beetle-killed trees. Survival may occur in small groups of trees comprising a few individuals that either avoided attacks, or successfully defended themselves against colonisation attempts. In areas affected by past bark beetle outbreaks, the LTS phenomenon is represented by scarce occurrences of alone-standing old surviving trees surrounded by saplings or young trees. Additionally, surviving individuals can be categorized according to canopy strata, life-history stage and host suitability: a) upper-canopy trees, b) suppressed trees, c) reproductively mature, and d) juvenile trees.

1.4 The drivers of tree survival

Tree survival can be explained by various ecological, stand, and individual tree biology factors, as well as by bark beetle related determinants (Jakuš et al., 2011a). Studies focused on tree survival factors during bark beetle disturbances are mostly recent and not numerous. In southern Sweden, the storms Gudrun and Per[1] preceded a beetle infestation of ~6.6 mln m^3 of standing and fallen timber in 2005-2008 (Långström et al., 2009; Nohrstedt and Parker, 2014; Valinger et al., 2019), of which the attacked standing wood comprised 3 mln m^3 (Laisi, 2017). This extreme wind-storm and subsequent beetle outbreak inspired an investigation aimed at elucidating why some spruce had survived herbivore pressure in the Nyteboda forest nature reserve (Laisi, 2017). Young trees with large relative crown lengths and relatively small stem diameters (at breast height, 1.3 m; DBH) were found to be more resistant to attacks. Out of all potential hosts with morphological characteristics beneficial for bark beetles, only a certain proportion was attacked, since habitat and microclimate also were important drivers of tree infestation (Laisi, 2017). Literature evidence further suggests that tree survival may be influenced by inherent differences in the chemistry or underlying genetic makeup of individual trees within a population. For example, Erbilgin et al. (2017) found that resistant and attacked lodgepole pine trees had different amounts of the same chemical compounds that can differentially affect *Dendroctonus ponderosae* foraging activity. Six et al. (2018) pioneered research into the genetic basis of tree resistance to bark beetles by having demonstrated that surviving individuals genetically differ from the so-called general population of trees. On a molecular level, under severe stress, Norway spruce clones were shown to downregulate photosynthesis and growth, and upregulate starch and sucrose pathways. It is hypothesized that a greater distribution or allocation of carbon to starch reserves serves as a survival

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[1] In January 2005, Storm Gudrun struck the west coast of southern Sweden, becoming the most devastating storm in modern Swedish history. Its hurricane-force winds caused widespread destruction, uprooting and breaking 70 million m^3 of trees (the country's annual volume of harvested timber), leading to blocked roads, disrupted rail traffic, and damage to infrastructure. Around 730,000 people were left without power. Two years later, in January 2007, Storm Per hit Sweden, affecting a larger area but causing less damage compared to Gudrun.

strategy, enhancing stored resources that may fuel the production of defensive compounds under herbivory pressure. (Huang et al., 2021). However, it remains unclear how carbon allocation affected by long-term stress modifies the production of constitutive and secondary metabolites in response to bark beetle attacks in mature trees growing in natural forests. Idiosyncratic physiological responses in trees (Kirisits and Offenthaler, 2002; Zhao et al., 2011), including those attributed to microclimate effects and high genetic variability in environmentally relevant traits in natural populations (Rosner and Hannrup, 2004), the scarcity of surviving trees, removal or natural decay of beetle-killed dead wood, logistic constraints associated with old-disturbed forest areas, challenges related to plot selection prior to an outbreak, among other factors, can explain the relatively low number of studies on tree survival during bark beetle outbreaks.

In general, the presence of surviving trees may be attributed to some combination of the following general factors:

a) chance: trees survival is a stochastic process (i.e. some proportion of available living trees, which may vary depending on the population density of bark beetles during a given outbreak, wholly escape attack and herbivory because insect dispersal patterns were random);

b) external factors: environmental or stand conditions (Jakuš et al., 2011a; Laisi, 2017);

c) internal factors: induced or constitutive defensive mechanisms (Brignolas et al., 1998, 1995b; Korolyova et al., 2022b; Lieutier et al., 2003a; Schiebe et al., 2012) that are potentially under genetic control (de la Mata et al., 2017; Six et al., 2018a).

On a landscape level, tree survival has been shown to be a non-random process (Korolyova et al., 2022a), suggesting that an investigation of the last two groups of survival factors may provide a wealth of information to improve our understanding of the environmental, morphological, chemical, and genetic bases for resistance. Currently, there is no general consensus among scientists about whether survivorship is driven predominantly by internal or external factors. On one hand, it has been found that tree survival is governed by external conditions, such as stand or environmental parameters (Jakuš et al., 2011a; Korolyova et al., 2022a; Laisi, 2017). However, a number of studies have also associated bark beetle resistance with internal (Schiebe et al., 2012), likely genetic control (Strom et al., 2002; Yanchuk et al., 2008), or even genetic predetermination (de la Mata et al., 2017; Six et al., 2018a). Research suggests that in an era of climate change, resistance will depend on the capacity of trees to tolerate warmer or drier environments without a concomitant reduction in defences (e.g., a chemical profile that prevents bark beetle attacks on a host tree, or on some other phenotypic trait that is potentially under genetic control) (Lindström et al., 1989). Identification of genetic markers responsible for resistance to beetle-vectored fungi and drought or heat stress tolerance could facilitate tree breeding programs. Retaining surviving trees that carry heritable resistance traits in their natural habitat as a seedling source could promote better adaptation of vulnerable stands to changing environments.

1.5 Approaches to studying bark beetle host selection and tree resistance

Tree resistance can be studied on a tree, stand, and landscape scale levels. A mechanistic understanding of tree persistence through bark beetle disturbance requires deep insights into tree-level metabolic processes associated with induced defensive responses and omnipresent constitutive defences. Both types of defensive reactions are strongly related to the physiological state of host trees, which may be influenced by a myriad of biotic and abiotic factors. A diverse array of effects associated with multiple stressors that are mediated by innate life history trade-offs shape the capacity of trees to deal with bark beetle mass attacks.

Depending on the research methodology, tree resistance can be investigated using phenomenological and experimental approaches, or a combination of these methods. Phenomenological studies involve analyses of observational data collected for forest trees in natural settings both prior and subsequent to bark beetle herbivory (Jakuš et al., 2011a; Korolyova et al., 2022a; Schiebe et al., 2012). Phenomenological studies may also be conducted exclusively in post-disturbance areas by surveying both surviving and reference (control) trees, where reference trees may include recently killed host trees (Jakuš et al., 2011a) or extant mature trees in intact stands located in close vicinity to a disturbed area (Korolyova et al., 2022a). Living reference trees are assumed to be susceptible to beetle herbivory. The main disadvantage of post-disturbance sampling is associated with the dynamics of tree chemical processes, which, due to environmental changes, may differ in trees located in intact stands adjacent to beetle-affected areas relative to isolated individuals that are surrounded by beetle-killed trees. However, according to Erbilgin et al. (2017), the post-disturbance sampling of surviving trees (termed LTS here) to study the mechanisms of tree resistance proves to be a better approach than sampling multiple trees before an outbreak. Several practical reasons underly this contention. Firstly, the scarcity of resistant trees in post-outbreak areas (i.e. reflecting the inherently low survival potential of individual trees in a host species) means that in a disturbed forest, surviving trees comprise less than 1% of the initial population size of the host tree species prior to disturbance (Hawkins et al., 2012). Further, a large proportion of the small number of surviving trees experience wind breakage, windfall, or are selectively harvested after an insect outbreak, magnifying associated field survey efforts required to locate any suitable survivors. Also, post-hoc chemical analyses of resistant tree tissues (from LTS) requires a significantly smaller investment of financial and logistical resources compared with a comprehensive survey of all trees in intact stands before outbreaks. Finally, an appropriate selection of intact tree stands for surveying prior to insect disturbance is impeded by the partially stochastic nature of insect dispersal processes during population outbreaks.

However, a post-disturbance field sampling approach may also be subject to potential biases. The main drawback is the potential unavailability of samples from a reference group due to wood decay in killed trees or the physical removal of infested trees by forest managers. Although sampling immediately after an outbreak when reference, insect-killed trees are still available can partially address this issue, uncertainty concerning the “purity” of the LTS samples arises. Severe bark beetle outbreaks may continue for several years during which time insects successive generations of offspring are produced, emerge from colonized trees, and disperse omnidirectionally from disturbance centres. This phenomenon may generate a landscape mosaic of beetle-killed trees interspersed with islands of living intact trees. Thus, sampling promptly after the first season or after several seasons of repeated dispersal events does not guarantee that extant trees which are alive at the time of field sampling will continue to successfully deter potential future herbivory. In other words, before the population outbreak has fully subsided, the identification of individual trees that are truly resistant as a consequence of a robust defensive capacity is ambiguous. As was already mentioned, one solution is to sample LTS in a site disturbed several decades ago, and then identifying living mature individuals of the same size and age as the LTS but located in adjacent undisturbed forest to serve as a reference tree group (Korolyova et al., 2022a; Six et al., 2018b). This approach relies on an assumption that the living reference trees are strongly susceptible to bark beetles and that a negligible proportion (<1%) are resistant to bark beetle colonization and feeding.

In contrast to the previously described observational studies, experimental research approaches attempt to modify or control natural conditions in order to isolate a biological response to a predefined driver. Experiments rely on specific predetermined hypotheses. A diverse array of techniques is employed to manage moisture conditions and bark beetle pressure within experimental environments. In the studies of tree resistance to bark beetles, the following experimental design tools are used:

1. to control **tree drought stress** and assess tree resistance capacity under controlled environmental conditions:
    1.1 trenching and snow removal (Erbilgin et al., 2021);
    1.2 installation of artificial roofing (Netherer et al., 2015);
    1.3 irrigation (Ježík et al., 2015; Vakula et al., 2014);
2. to account for **bark beetle attacks**:

- 2.1 in the presence of bark beetles:
    - 2.1.1 natural attacks (e.g. Jakuš et al., 2011; Korolyova et al., 2022b);
    - 2.1.2 attacks manipulated via bark beetle crushing on phloem surfaces (Erbilgin et al., 2021);
    - 2.1.3 pheromone baiting of trees (Erbilgin et al., 2021; Vakula et al., 2014);
    - 2.1.4 installation of “attack boxes” on tree trunks (Netherer et al., 2015).
- 2.2 in the absence of bark beetles, i.e. simulation of real attacks:
    - 2.2.1 MJ inoculation into the phloem (Schiebe et al., 2012);
    - 2.2.2 mechanical wounding of trees that can be conducted:
        - i) in the presence of beetle-vectored fungi (Brignolas et al., 1998, 1995b; Lieutier et al., 2003a; Viiri et al., 2001), or
        - ii) absence of beetle-vectored fungi (Korolyova et al., 2022b).

1.6 Methodological framework to studying the Last Trees Standing

Interactions among biotic and abiotic factors mediating “insect – host” relationships are non-linear and diverse. Numerous genes in a large spruce genome govern, both, life-history strategies and physiological stress responses, which in turn may regulate tree survival. Tree resistance to herbivory depends on a composite of quantitative traits that are dependent on several minor-effect genes (Plumb et al., 2020; Poland and Rutkoski, 2016; Wang et al., 2023). The complex nature of host tree resistance and the myriad of factors that determine tree outcomes during extreme levels of herbivory suggest that a comprehensive quantitative understanding of the bark beetle – spruce system requires a **multidisciplinary research approach that is coupled with both phenomenological and experimental methods.** A combined application of dendroecological, remote sensing, genomics and analytical chemistry based methods is key for advancing this field. In particular, molecular underpinnings of spruce resistance and survival have not been comprehensively quantified. For example, the specific functions of volatile organic compounds, such as terpenes and phenolics, are still poorly quantified, but are assumed to play an important role in tree defence chemistry on both molecular and stand levels. Additionally, from a molecular biology point of view, the study of genetic markers, such as SNPs, has the potential to illuminate the basis for tree resistance. Tracking the regulation of gene expression through RNA transcripts could help identify critical genes that are differentially expressed in response to environmental stimuli. From an ecological perspective, the study of morphological traits, competitive interactions, and environmental constraints, including stand composition and structure, sun exposure, climate, and topographical parameters, can improve our understanding of both endogenous and exogenous factors that potentially influence tree survival. The application of dendroecological methods facilitates an investigation of evidence for life-history trade offs and, thus, tree resistance potential against bark beetles, as the dynamics of stem growth reflect long-term resource allocation patterns in trees. Finally, remotely sensed datasets, such as satellite imagery, may be used to study the physical characteristics of host trees. For example, spectral signatures may be identified and used to discriminate host tree condition and their potential susceptibility to insects.

When deliberating on the selection of study and experimental design, it is imperative to acknowledge the temporal dynamics inherent in tree chemistry. There exists no categorical assurance that findings acquired years post-disturbance are unequivocally aligned with the tree's defensive responses during the historical outbreak. Nevertheless, a notable advantage intrinsic to this particular study design is that LTS constitute individuals that have withstood the disturbance, thus representing a cohort of inherently resilient specimens. Specifically, these trees represent "pure" resistant entities, characterized by an exceedingly diminished probability of succumbing to beetle infestations in the subsequent years of an ongoing outbreak. Conversely, conducting chemical analyses amidst an ongoing bark beetle outbreak ensures the scrutiny of tree chemistry precisely during the manifestation of the upregulated defence mechanisms. However, this approach lacks the guarantee that unattacked trees sampled in the current year will remain uncolonized in the subsequent years of disturbance. These methodological considerations are intrinsic to investigations concerning the chemical facets of tree resistance mechanisms against bark beetles, particularly when experiments are conducted on mature trees within natural environments. In an ideal scenario, trees should be sampled during an ongoing outbreak, with continuous monitoring of their statuses for multiple years until the conclusive resolution of the disturbance. Due to temporal constraints and the unavailability of multiple years to implement the "ideal" design, a pragmatic approach was adopted. Both sampling methodologies for chemical analysis were employed to strike a balance and achieve a trade-off between the likelihood of a tree being a genuine resistant survivor and the temporal proximity to the actual disturbance event. In the Bohemian Forest study area, our sampling focused on "pure" resistant LTS that had endured a bark beetle outbreak 15-20 years prior. Conversely, in the Vosges mountains, spruces were sampled amid an ongoing *Ips typographus* outbreak, with the monitoring of tree statuses extended until the conclusion of the growing season (end of September).

The various methods belonging to each of the above-mentioned fields can be utilized in both experimental and phenomenological based LTS studies. Remotely sensed data are used to monitor the physiological condition of host trees, to detect the locations of surviving and killed trees, and to identify the environmental correlates of mortality patterns. Combining phenomenological and experimental methods will attenuate limitations associated with studies performed in purely controlled environments, allow insights to be extrapolated to un-sampled natural populations, and, consequently, advance general ecological theory.

The application of an integrated approach is particularly important in studies that attempt to uncover the genomic markers of physiological responses to stress and the associated ability of trees to deter herbivores. The comparatively large Norway spruce genome, coupled with high genetic variability among disjunct populations, creates a significant research challenge. For example, preliminary unpublished results from analyses of SNPs acquired from phloem tissues of surviving trees located in the Šumava mountains in the Czech Republic have so far failed to identify a common, definitive genetic signal of herbivore resistance. Genomic differences across disjunct trees and subpopulations in different environmental settings appear to be obfuscating our ability to detect an unambiguous marker of survival potential. Small sample sizes, due to the scarcity of surviving trees, is a major limitation.

To address the many research challenges, including high genetic variability among natural populations, LTS scarcity, and the presence of complex interactions among diverse drivers, it is important to combine studies in both controlled (greenhouse) and in natural environments where bark beetle outbreaks are ongoing. Previously, the majority of experiments testing for spruce resistance to bark beetles and their fungal symbionts has been conducted in controlled settings using seedlings and saplings (Axelsson et al., 2020; Biedermann et al., 2019; Nybakken et al., 2021; Öhrn et al., 2021; Rosner and Hannrup, 2004; Wilkinson et al., 2022). Greenhouse experiments are low-risk and cost-effective, and facilitate a targeted investigation of specific regions in a plant genome, while simultaneously controlling for confounding factors (e.g. microclimate variation, drought stress). Thus, transcriptome profiling in conifers has been limited exclusively to seedlings, while relatively few studies have attempted to uncover the transcriptome-based regulatory mechanism for defensive responses in mature trees (Krokene et al., 2008). Consequently, the genes/gene families that are upregulated in response to drought or thermal extremes have not been identified in adult *P. abies*.

Thus, an integrated, multi-dimensional research paradigm, as proposed here, is universal and can be applied to studies of tree survival regardless of study area location. In contrast, experimental designs and procedures are typically location- and species-specific, since terrain, climate, microclimates, inter-tree competition, forest structure and composition, etc. shape interactions between hosts and insect herbivores.

Hypotheses

Our overarching hypothesis is that Norway spruce survival depends on a complex combination of external and internal factors, which modify the physiological responses of hosts to different stressors and environmental changes prior and during bark beetle disturbance. The underlying theoretical premise is that plant performance depends on limited pools of energy, water, and nutrients, resulting in life history trade-offs in resource allocation between primary (transpiration, growth) and secondary (defence, reproduction) metabolic functions. In short, available resource supplies accumulated in plant organs determine tree defence potential against eruptive insect species.

Another theoretical underpinning for our hypotheses is the so-called “switching” mechanism of bark beetle mass attack (Schlyter and Anderbrant, 1989). This mechanism, which is mediated by bark beetle pheromones, plays an important role in the complex interactions between *P. abies* and *I. typographus* (Schlyter and Anderbrant, 1989). We assumed that the first targets in the first stages of a bark beetle outbreak are highly susceptible trees, whose physiological condition and defence capacities have been diminished by environmental extremes (e.g. high levels of solar radiation that cause heat or drought stress). We expected that initially or primarily attacked trees are selected by beetles in the absence of aggregation pheromones. Instead, insect choice is purportedly driven by kairomones emitted by acutely stressed trees. Subsequent secondary targets are comparatively resistant neighbouring trees. Presumably, mass attacks on secondary trees are facilitated by the presence of insect-produced pheromones. Pheromone-mediated tree selection is referred to as a “switching” mechanism. We anticipated that a third category of trees is comprised of a minor proportion of resistant mature individuals that remain intact during an outbreak.

Based on these underlying assumptions and available literature evidence, we formulated a set of a priori hypotheses about the effects of multiple divergent factors on spruce survival. We postulated that physiological variables underlying survival are shaped by tree level parameters (e.g. tree size and relative crown length) and environmental factors (stand competition and moisture availability). Previously published studies have identified a strong preference for large diameter hosts among beetles under eruptive population densities. Simultaneously, for mechanical reasons, hydraulic pathways transporting water from roots to foliage are limited in taller trees, presumably compromising photosynthetic activity and defence capacity in larger spruce. Thus, we hypothesised that survivorship would decrease in larger diameter trees. Conversely, we expected a stronger defence potential in individuals with more extensive and vigorous (minimally defoliated) crowns, as total leaf area is positively associated with photosynthetic activity (Honkanen and Haukioja, 1994; Wiley, 2020). We posited that high competition for resources in dense stands compromises tree physiological functions, constraining resistance capacity against bark beetles. We also assumed that soil moisture deficit exacerbates the negative effects of other factors (resource competition from neighbouring trees and / or tree height dependent hydraulic constraints (Koch et al., 2004; Ryan and Yoder, 1997), aggravating beetle caused tree mortality risk. We hypothesized that more resistant trees have larger stores of secondary metabolites (terpenes and phenolics) that fuel the production of defensive compounds and consequently increase survival probability. Also, we expected that sun exposure has a significant effect on survival, due to concomitant effects on bark temperature, tree physiology, as well as the life cycle and behaviour of the bark beetle. Following the “growth – defence trade-off” hypothesis (Herms and Mattson, 1992), we posited that surviving trees exhibit slower long-term radial growth rates relative to beetle-killed trees. Finally, we expected that survival probability increases exponentially with distance from a bark beetle population source.

Thus, we tested the following hypotheses:

1. H0.01: Spruce survival during bark beetle outbreak is a non-random process.
2. H0.02: Larger diameter trees have lower survival probability.
3. H0.03: Trees with longer crowns have a higher chance to survive.
4. H0.04: Higher stand density prior to an outbreak negatively effects tree survival.
5. H0.05: Trees experiencing more severe long-term moisture deficit have lower survival probability.
6. H0.06: Trees located closer to the previously attacked hosts have smaller chances to survive.
7. H0.07: Stem sun exposure negatively effects tree chances to survival.
8. H0.08: More defoliated tees have lower chances to survive.
9. H0.09: Elevated induced production of phenolic catechin increases tree survival probability.
10. H0.10: Concentrations of phenolics in spruce phloem increase after mechanical wounding.
11. H0.11: Initial and induced concentrations of phenolic compounds are higher in surviving than beetle-killed trees.
12. H0.12: Phenolic concentrations are higher in uninoculated phloem of secondarily attacked than in the uninoculated phloem of primarily attacked trees.
13. H0.13: Concentrations of terpene compounds increase in response to MJ inoculation.
14. H0.14: Surviving trees exhibit higher production of terpene compounds in response to MJ inoculation than reference trees.

15. H0.15: Surviving trees have higher initial and MJ-induced concentrations of terpene compounds than reference trees.
16. H0.16: Total and primary crown defoliation is more pronounced in primarily attacked than surviving and secondarily attacked trees.
17. H0.17: Juvenile crown ratio is higher in secondarily attacked than primarily attacked trees.
18. H0.18: Surviving trees are located farther away from the previously attacked hosts than primarily and secondarily attacked trees.
19. H0.19: Surviving trees demonstrate slower long-term growth prior to an outbreak than reference trees.

Study aim and objectives

The main aim of the dissertation is to investigate the mechanism of Norway spruce (*Picea abies*) survival and the effects of stochastic, external (environmental, stand), and internal factors on tree resistance during severe bark beetle *Ips typographus* outbreaks in Central Europe using the methods of analytical chemistry, dendroecology, and remote sensing. We aimed to conduct our research in two study areas affected by bark beetle disturbances, one located in the Bohemian Forest region, the other one in the Vosges mountains (France).

This dissertation integrates both phenomenological and experimental tools. Our study region consists of two separate areas. The phenomenological study was conducted in the Bohemian Forest region (located at the border of Czechia, Germany, and Austria) in protected, previously-disturbed, monodominant spruce forests. Analyses were conducted using a combination of satellite and aerial imagery, as well as field survey data. In this study area, we cored surviving and reference trees for dendroecological analyses of radial growth rates to investigate evidence for life-history trade-offs that could potentially influence tree survival outcomes. We also conducted a MJ inoculation experiment on LTS and reference trees to investigate the role of chemical-based defence mechanisms. In particular, we evaluated terpene responses to a mimicked bark beetle attack using untargeted metabolomics (GCMS). Our second study area is located in the Vosges Mountains of eastern France in a strictly-protected biological reserve (La Chatte Pendue). In 2003, we conducted a mechanical wounding experiment to compare tree phenolic defence responses among primarily attacked, secondarily attacked and non-attacked spruce trees during an ongoing bark beetle outbreak.

To test *a priori* hypotheses and realize thesis goals, we designed the following surveys, experiments and analyses:

- to develop a general methodological framework for studying tree survival;

- to identify individual Norway spruce trees that survived a severe multi-year bark beetle outbreak in the Bohemian Forest study area, which includes Šumava NP, Bayerisher Wald NP, and adjacent stands in Germany and Austria; the detection method uses a high-resolution time-series of aerial imagery in Google Earth Pro (GE) for the years 2000 to 2019; results are validated using datasets acquired from field surveys;
- to compile a reference tree dataset from field surveys of extant living trees to serve as proxy data for bark beetle susceptible trees (since actual beetle-killed trees have decayed or been removed from our study site by managers);
- to delineate the boundaries of the bark beetle disturbed area via visual inspection of high-resolution imagery, and to discriminate spruce vs non-spruce dominated forest areas using supervised image classification;
- in the Bohemian Forest portion of the study area, to reconstruct stand densities prior to the insect outbreak using non-linear regression models fit with a multi-year time series of spectral signatures of forest canopy characteristics (from Landsat satellite imagery); field surveys are used to validate reconstructions;
- to test for a random spatial pattern in the distribution of surviving trees in the Bohemian Forest study area;
- to measure the crown dimensions of surviving trees using high resolution GE aerial imagery and to validate results with field survey data;
- to develop allometric models that estimate stem diameter at breast height (DBH) as a function of crown projection area; crown dimensions are derived from aerial photography; results are validated with field survey data;
- for all LTS and reference tree locations, to extract spectral properties from Landsat imagery and environmental parameters (topographic and climatic) from gridded climate datasets (E-OBS and CHELSA);
- in the study area located in the Bohemian Forest region, to collect increment cores and phloem samples from all LTS and reference trees for subsequent dendroecological and chemical analyses;
- in the Bohemian Forest region study area, to test for differences in long-term radial growth rates of surviving vs reference trees;
- in the study area located in the Bohemian Forest region, to test for differences in the phloem terpene content of surviving and reference trees based on samples from a MJ experiment;
- in the Vosges mountains in France, to test for differences in phloem phenolic content, as well as morphological characteristics (e.g., crown condition), among primarily attacked, secondarily attacked, and non-attacked host trees using data acquired from a mechanical wounding experiment;

- to conduct exploratory analyses using machine learning (Random Forest classification) to compile a multivariate set of explanatory variables potentially important for tree survival during disturbance; the most important factors are then used to fit regression models;
- to develop non-linear logistic regression models to draw inferences regarding relationships between chemical, morphological and environmental factors and potential spruce survival during extreme bark beetle outbreaks in Central Europe; models are fit with field-collected, laboratory-generated, and remotely-sensed datasets.

II. METHODS AND DESCRIPTION OF STUDY SITES

2.1 Study areas

2.1.1 Study area in the Bohemian Forest region

We investigated the factors of tree survival in the Bohemian Forest study area, which encompasses one of the largest areas of contiguous forest in Central Europe. The area continues to be affected by a severe, ongoing outbreak of *I. typographus* that first began in the mid-1990's and is still ongoing (Knížek and Liška, 2020). Our analyses were restricted to montane stands of Norway spruce in the border region of Germany, Austria, and the Czech Republic. The disturbed forest area currently spans ~12,400 ha (Fig. 1). Elevations range from 950 to 1400 m. The continental climate exhibits four distinct seasons with moisture being evenly distributed throughout the year. Total annual precipitation ranges from ~740 to 1455 mm. Mean annual temperatures range from 4.6 to 7.4°C. The study region has experienced a discernible warming trend and intermittent periods of abnormally dry conditions since the 1980's (Fig. 2), consistent with patterns across Europe generally (Moravec et al., 2021). Forest composition is dominated by *P. abies* with scarce occurrences of *Sorbus aucuparia* L. (European mountain ash) and *Fagus sylvatica* L. (European beech). Mixed-severity natural disturbances, such as windstorms and bark beetle outbreaks, have shaped forest composition and structure (Emborg et al., 2000; Svoboda et al., 2012). Specifically, at local scales, wind disturbances resulting in tree fall and gap formation recur at an average frequency of approximately 40 years (Janda et al., 2017). Landscape-scale storm or insect events that cause significant tree mortality (i.e., affecting over 50% of canopy trees) are also prevalent in the region. The average rotation period, defined as the mean interval between such high-severity events, is 170 years (Čada et al., 2016). The current development of a considerable portion of the Bohemian Forest can be attributed to a period of intense natural disturbance activity in the early 1800's. Importantly, most forest stands in the region have remained relatively unaffected by anthropogenic land use as a consequence of limited timber extraction in the 19th century and the establishment of conservation areas in the 20th century (Brůna et al., 2013; Čada et al., 2016; Svoboda et al., 2012). At present, a significant proportion of the study area is protected under the jurisdiction of the two reserves, namely the Bavarian Forest National Park in Germany and the Šumava National Park in Czechia. However, to mitigate the risk of beetle dispersal to adjacent commercial forests, ongoing insect disturbances are partially managed near the political boundaries between Austria and Czech Republic

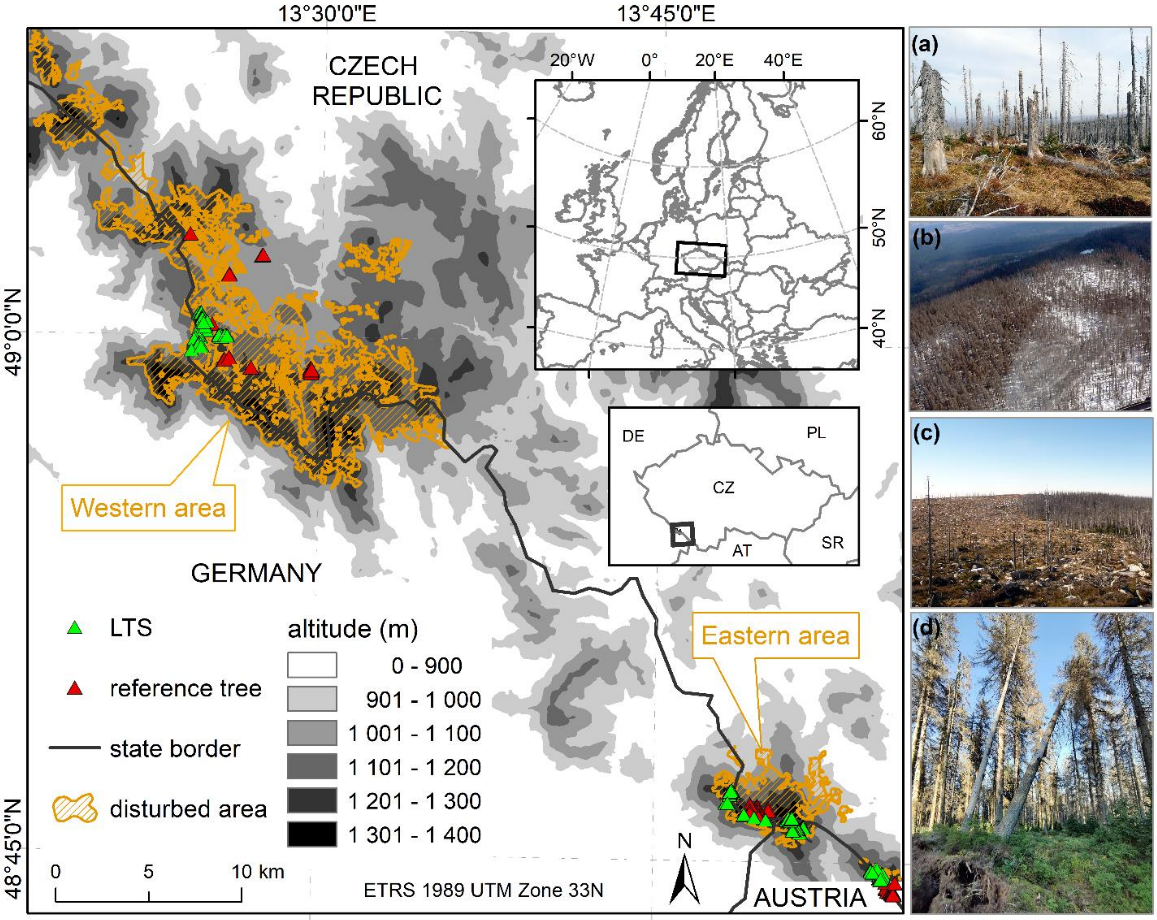

Fig. 1. The study area in the Bohemian Forest region representing spruce forest severely disturbed by a prolonged bark beetle *I. typographus* outbreak and the locations of 184 surviving (LTSs) and 414 reference trees used in the regression analyses. Reference trees were living mature spruces sampled from the general *P. abies* population in the study area. Samples collected from the western and eastern parts of the study area were combined in the analyses. The photos in panels (a) and (c) illustrate beetle-affected landscapes in close proximity to LTS. Photo in panel (c) was taken during a helicopter flight over the disturbed area. The photo in panel (d) shows reference trees that were infested by the beetles in July 2021 but were alive in 2020 at the time of sampling.

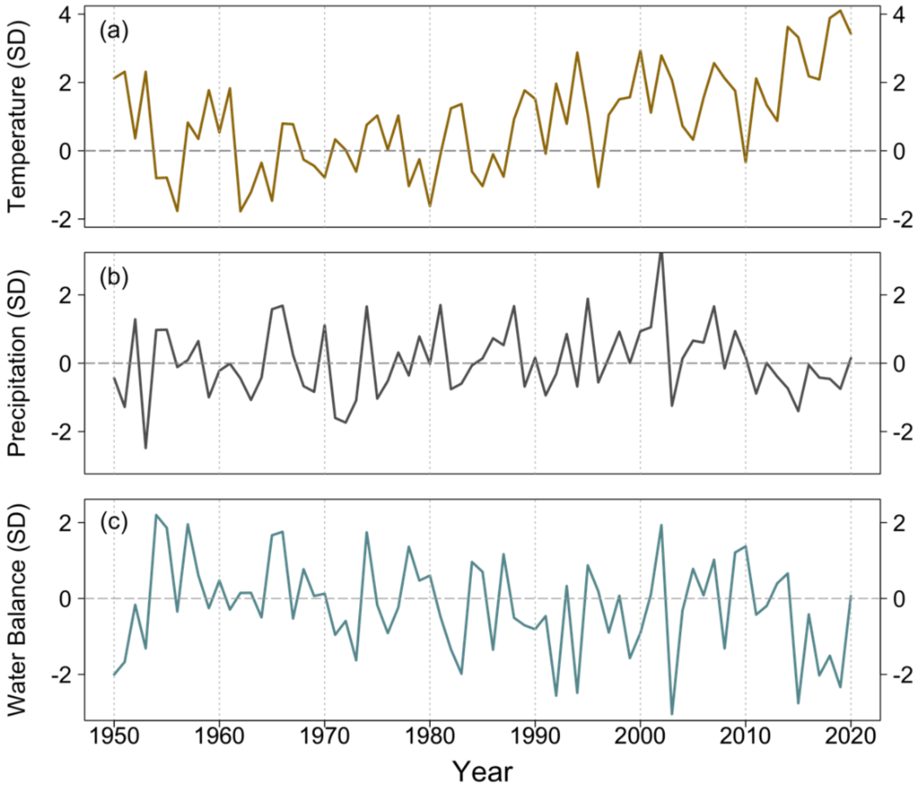

Fig. 2. Mean annual temperature (a), precipitation (b), and growing season climatic water balance (c) dynamics in the Bohemian Forest study region during a 30-year period preceding the outbreak (1960-1989). The values are shown as standard deviations from the mean. Climate data were acquired from a gridded climate dataset (E-Obs; Cornes et al., 2018) for each of 598 trees used in the regression models.

2.1.2 Study area in the Vosges mountains

The second study site is located in the Vosges Mountains in the Alsace-Lorraine region of eastern France. In 2003, a mechanical wounding experiment was conducted during an ongoing bark beetle outbreak in a Norway spruce dominated stand in the strictly protected biological reserve of La Chatte Pendue (48.44222◦ N, 7.131667◦ E). The reserve covers a total area of 271 ha. Elevations range from 600 to 900 m a.s.l. Both Atlantic and continental air masses shape local climate. Significant variation in temperature and precipitation also result from complex topography and steep elevational gradients. Annually, total precipitation ranges from 692 to 1200 mm. Mean annual temperatures vary from between 8.1 and 11 °C (Abdullah et al., 2019) (CRU TS4.04, 2021). To mitigate browsing impacts from dense ungulate populations, protective fences were installed. The experimental plots were established in close vicinity to a bark beetle source (trees that were previously attacked following wind damage). In our study sites, host colonisation by bark beetles began a week after the completion of experimental bark treatments. We monitored bark beetle activity and the progression of the outbreak for a period of five months.

2.2 Google Earth LTS identification

A partial preliminary field survey identified a few existing occurrences of isolated surviving spruce trees in Šumava NP. Logistic and administrative constraints prevented field identification of a larger sample of surviving trees in an old disturbed forest in Šumava NP and within the Austrian portion of the Bohemian Forest adjacent to Trojmezna. Using a high-resolution time-series of aerial imagery from GE that spanned a period from 2000 to 2019, we carefully searched for surviving trees and recorded their coordinates. The detection of surviving trees relied on manual methods – visually but systematically scanning the images. We excluded surviving trees growing at established forest edges, in mixed forests, and at the banks of rivers and streams. This filtering aimed to account for potential acclimation to thermal and radiation stressors, repellent effects of non-host broad-leaf volatiles on beetle behaviour, and water abundance on tree defence potential. We verified tree detection accuracy using data collected from a subsequent field survey conducted in the fall of 2020.

2.3 Tree and stand level parameters

2.3.1 Field measurements of tree crown and site parameters

In the Bohemian Forest study plots, we derived the crown dimensions of LTS based on their crown projection area, as captured in GE photography. We calculated the relative crown length, or self-shading, as a ratio of crown length from apex to the lowest branch to the total tree height, which was also measured using GE projection areas of separately standing extant trees. We checked the accuracy of GE-derived self-shading values using field survey or ground truth data (which was also used to verify LTS detection accuracy). During the field survey, the crown length and tree height were measured using a laser rangefinder Nikon Laser Forestry Pro. The widest and the narrowest parts of the crown width at the crown base were measured using a forestry measuring tape, and the values were averaged.

In the La Chatte Pendue biological reserve, we measured the size and crown parameters of 97 spruce trees, characterized by a wide range of stem sizes. Specifically, DBH ranged from 15 to 83 cm. Proxies of tree physiological condition, such as juvenile, productive, and saturated crown ratios, crown transparency, proportion of secondary shoots, and primary and total crown defoliation ratios, were estimated via careful visual inspection by trained field experts. For each experimental tree, we recorded sun illumination (i.e., stem proportion exposed to the sun during a diurnal solar cycle) and site characteristics, such as azimuth, slope, aspect, and distance to the nearest beetle-attacked tree.

2.3.2 LTS DBH prediction using allometric models

To estimate the DBH of surviving trees (LTS), an allometric model was developed based on associated crown projection area, which as described above, was derived from GE imagery. The diameters of the reference trees, in contrast, were directly measured in the field. However, due to the scattered nature of scarce LTS occurrences in remote and often difficult to access terrain, a significant portion of surviving trees were identified through analyses of remotely-sensed imagery rather than field surveys. Consequently, image-based estimates of crown dimensions were used to determine the corresponding stem diameters for these trees.

The utilization of mathematical rules that describe relationships between different plant organs has become common practice in ecology (Lehnebach et al., 2018). Previous research has demonstrated the efficacy of using allometric equations to estimate the structural dimensions of plants, provided that adequate sample sizes are available (Enquist et al., 1999). In this study, three alternative functional forms were tested for the allometric model: power, modified exponential, and sigmoidal formulations (Table S2.1 in Korolyova et al., 2022a; Sit and Poulin-Costello, 1994).

To maximize the sample size available for model development, data from three different sources were compiled. The first source was a ground-truth survey conducted in 2020, which involved the measurement of attributes for 37 LTS. The purpose of this survey was to evaluate the accuracy of image-based tree detection, as detailed in subsection 2.2 of the Methods. The second dataset was acquired from a separate field survey, also performed in 2020, which involved the measurement of 99 non-LTS (i.e. living mature spruce trees) within the Bohemian Forest study area. The final data source was an existing inventory of primary forests compiled by the Remote Forests Project (https://www.remoteforests.org/project.php). The Remote Project has established a network of permanent inventory plots in remnant primary forest stands across Central Europe. For this study, we acquired Remote data for 2367 Norway spruce trees collected in spruce-dominated stands at high elevations in the Czech Republic and neighbouring Slovakia.

All three datasets provided field-based measurements of stem DBH and crown dimensions. To ensure robust model development, we filtered records to exclude uncertain measurements and missing data. We additionally excluded data from damaged trees, suppressed or shaded understory stems, and juvenile trees (<10 cm DBH or < 7 m in height). After applying these filters, for each tree we computed the mean of maximum and minimum crown width in order to derive an estimate of average crown radius. Estimates of crown radius were then used to calculate crown area, assuming a circular cross-section and using the formula π * radius^2.

The filtered and quality-controlled records were used to fit and evaluate competing allometric regression models. The first dataset, comprised of 30 LTS after filtering, was used exclusively as an independent test dataset. The test data therefore included information from the target LTS population. The records from the second and third datasets were combined and randomly subdivided into independent calibration (2175 trees) and testing groups (222 individuals).

Model specification

We fitted and compared three alternative regression models as presented in Table S2.1 in Korolyova et al. (2022a). The estimated model coefficients (see α parameter in Table S2.1) were locality-specific. Unknown model parameters were estimated using a global optimisation algorithm (simulated annealing, Goffe et al., 1994), and likelihood calculations were performed using a normal probability density function (PDF). Given the presence of heteroscedastic residuals in the model, the error term for the ith (tree) observation was computed using a power function with an intercept, represented by equation (2.1):

$$\varepsilon_i = a + X_i^b \qquad (2.1),$$

where *a* and *b* are annealing-estimated parameters, and *X* is a vector of predicted DBH for the *i*th observation. Model selection was done based on Akaike information criterion (AICc) corrected for a model complexity. All analyses were performed using the R programming language (R Development Core team, 2023) and “likelihood” package (Murphy, 2015).

2.3.3 Pre-outbreak stand density estimation

A cloudless and snow-free Landsat 5 (https://landsat.gsfc.nasa.gov/landsat-4-5/tm) Thematic Mapper (TM) image from the 17th of July 1986 (i.e. before emergence of the first infestation signs in the Bohemian Forest study area) was used to delineate the spatial extent of bark beetle disturbed stands. The TM image had seven spectral bands and a spatial resolution of 30 m. The same Landsat 5 image was also used for stand density prediction prior to the outbreak.

To begin with, in GE, we digitized the perimeters of the beetle affected spruce stands, as well as non-spruce areas, such as meadows, water sources, roads, bedrock, and broad-leaf or mixed stands. This process was aided with input from local field experts. The total extent of the digitized disturbed area was ~13890 ha. We then used supervised image classification with likelihood estimation to attribute all TM image pixels within the digitized area with a land cover type. The resulting land cover types were then re-classified to produce binary single-attribute classes: spruce versus non-spruce. After filtering non-spruce areas, the final extent of monodominant spruce forest affected by the *I. typographus* outbreak was ~12400 ha. Analyses were conducted in ERDAS Imagine (Version 16.5.0).

We developed non-linear regression models to predict stand density from various spectral-based indices. We assumed, consistent with abundant literature evidence (Cohen and Goward, 2004; Freitas et al., 2005; Sivanpillai et al., 2006), that forest canopies varying in terms of physical structure and leaf chemistry (moisture and chlorophyll content) produce characteristic spectral signatures. In other words, they absorb and reflect light in different wavelength regions of the electromagnetic spectrum, providing quantitative information that can be analysed to determine various attributes of the system. We therefore formulated a set of regression models to estimate forest stand density (i.e., trees ha^{-1}) in disturbed areas prior to the onset of the insect outbreak. The predictive performance of the models was carefully validated using GE-derived pre-outbreak estimates of stand density within 30 m radii of all LTS, augmented with stand density records from the Remote Forests Inventory database.

We incorporated several spectral metrics (wavelength band ratios) in our forest density analyses. Specifically, we computed a normalized difference vegetation index (NDVI), Tasseled Cap and a structural index. NDVI is a widely used proxy for vegetation cover. NDVI is quantified as the difference between near-infrared spectral band, which is reflected by a vegetation cover, and red light, absorbed by green plants. We hypothesized that NDVI increases with stand density but saturates at full canopy cover (Coppin and Bauer, 1994). Tasseled Cap metrics included separate wetness, greenness, and brightness indices (Crist and Cicone, 1984). The wetness index was hypothesized to increase with increasing stand density and tree age, where NDVI levels off (Cohen and Spies, 1992; Fiorella and Ripple, 1993). We also quantified a structural index from the ratio of Landsat TM band 4 to band 5. The structural index has been used in similar applications with high predictive performance (Danson and Curran, 1993; Fiorella and Ripple, 1993). To account for topographical drivers, we estimated values of terrain aspect using a 25 m resolution DEM. Estimates of aspect were then converted to so-called folded aspect values, which are symmetrically distributed (McCune, 2007; McCune and Keon, 2002).

2.3.4 Dendroecological data collection and processing

To investigate evidence for life history trade-offs and potential relations with tree defence against bark beetles, we collected and analysed tree core data. In the Bohemian Forest study area, from each of 229 trees (110 LTS and 119 reference trees), we extracted two cores at breast height from opposite sides of a stem using a 5 mm increment borer. If an extracted core was decayed or a stem had an irregular shape, we collected an additional, third core. In total, we collected 471 cores.

The cores were processed using standard dendroecological procedures. We first glued all cores onto wooden mounts. The samples were then sanded using industrial sanding machines. We manually polished the sanded cores to remove scratches, producing a clean surface with an unobstructed view of the tracheid cells (under magnification). Radial growth rates (ring-widths) were measured using a sliding platform, a microscope and WinTsap software. Statistical and visual cross-dating were conducted with CDendro software. To validate measurements, we selected 3 to 5 of the longest high-quality cores, cross-dated those cores, and then created a mean curve for each of four field survey localities (Modrava, Smrčina, Hraničnik, Dreissessel). The overall mean correlation coefficient among the growth measurements was 57.7%, indicating high accuracy of radial growth measurements. The mean correlation coefficient was quantified as a mean of correlation coefficients calculated for each tree relative to the corresponding mean curves identified for each locality.

2.4 Climate data

To account for long-term moisture conditions, we developed a seasonal climatic water balance averaged over a 30-year period (Senf et al., 2020). The water balance was computed as the difference between seasonal precipitation and potential evapotranspiration (PET) (Dyer, 2009). The method to estimate PET was sourced from Turc (1961), integrating solar radiation and temperature for monthly intervals. The monthly global solar radiation for each year in the period from 1990 to 2019 was calculated using the Area Solar Radiation function in ArcGIS 10.8 (“ESRI 2021. ArcGIS Desktop,” 2021). We also used a DEM (25 m resolution) from the European Union’s Earth observation programme (Copernicus; https://www.copernicus.eu/en). For our calculations, we assumed that the ratio of direct and diffuse radiation did not exceed 50%. We obtained high-resolution gridded climate data (temperature and precipitation) from two sources, CHELSA V1.2 (Karger et al., 2017) and E-OBS (Cornes et al., 2018). The CHELSA dataset had a finer spatial resolution (30 arcsec or ~900 m) relative to E-OBS (0.1 °), but was only available for years before 2014. Combining the two datasets provided an continuous temporal sequence of climate values spanning our 30-year analysis period. From the monthly climate data, we calculated precipitation sums for growing season periods (May to September), and then averaged growing season totals over a 30-year interval (see Fig. 2 for average long-term climate patterns).

2.5 Exploratory analyses

2.5.1 Random Forests analysis (Bohemian Forest and Vosges mountains)

To inform the selection of predictor variables subsequently used to fit statistical survival models, we conducted exploratory analyses using machine learning based approaches. Specifically, we used Random Forest (RF, Breiman, 2001) to estimate the strength and relative importance of relations between tree survival and a suite of biological and environmental covariates. We fit RF models separately with datasets from the two study areas (i.e., the Bohemian Forest and the Vosges mountains).

In the Bohemian Forest study area, the RF classifier was fit with datasets corresponding to the locations of surviving and beetle-killed trees from across the entire disturbed portion of the region, as was reconstructed from Landsat TM spectral data (see 2.3.3 *Pre-outbreak stand density estimation*). Based on our hypothesis that moisture and thermal limitations compromise the physiological condition of trees and their ability to survive, we developed several explanatory variables (N=13) to serve as proxies for climatic stress. Specifically, we quantified terrain characteristics, including shape and orientation, that could influence site water availability. Additionally, we estimated levels of incident solar radiation for each tree, as insolation plays a crucial role in evapotranspiration rates. We employed a Tasselled Cap transformation of the spectral data to derive reflectance-based indicators (e.g., wetness, brightness, and greenness indices) of soil and vegetation properties. We also used NDVI as an additional proxy for relative plant vigour. We hypothesized that low NDVI values are indicative of moisture limitation and associated physiological stress in trees.

In the Vosges mountains of France, our second study site, we again used RF as an initial exploratory tool. Our goal was to identify the most important morphological, chemical, and environmental covariates of tree survival from among a large set of potential predictors. We tested a total of 26 covariate terms. Specifically, we fit RF with variables quantifying tree anatomy, including stem size (DBH), relative crown length and shape, and measures of bark exposure to solar radiation. We also parameterized RF models with measures of phloem chemical content (e.g., catechin, astringin, taxifolin, piceid, and isorhapontin) measured before and after experimental treatments. Another chemical variable tested was the absolute change, termed production rate (difference between post- and pre-treatment concentration), of all detected phenolic compounds. We assumed that a successfully colonized host tree would serve as future beetle source. We therefore fit models with the measured distance between an experimental tree and the nearest observed beetle-killed tree.

In all analyses, individual RF models were fit with 500 bootstrapped sub-samples of the training datasets. At each node in an individual tree, three variables were randomly chosen from the available pool of predictors (N=26 for France data and N=13 for the Bohemian Forest dataset) as potential candidates for splitting the training data (Liaw and Wiener, 2002). The contribution of each predictor to a mean Gini coefficient was used as a quantitative measure of the relative importance of each model covariate. Consequently, the Random Forest approach served as a valuable tool for data reduction, enabling a selection of the most influential predictors of survival, which were then evaluated within a more formal hypothesis-driven statistical framework (Lucas, 2020). The Random Forest classification was performed in R using the package “randomForest”.

2.5.2 Testing for random spatial distribution of LTS in the Bohemian Forest

We postulated that the survival of trees was not a random process but rather the one governed by complex interactions among various biotic and environmental factors. To detect evidence for non-random patterns in the locations of surviving trees (LTS), we examined their spatial distribution. Specifically, we used the Optimized Hot Spot function in ArcGIS to analyse the density and spatial arrangement of LTS and beetle-killed trees. For dead trees, we used the spatial coordinates for a total of 6.23 mln trees predicted to occupy the overall disturbed forest area (see section 2.3.3 for methods of stand density reconstruction).

2.6 Experimental design for chemical defence analyses

We investigated the potential roles of both terpene and phenolic based compounds in tree defence against bark beetles. To address this question, we conducted two separate field experiments. The contribution of terpenes to tree survival was analysed using an inoculation experiment in the Bohemian Forest. The influence of phenolics was explored using a wounding experiment in the Vosges Mountains site. The following subsections describe these experiments.

2.7 Field sampling of LTS and reference trees

The nature of a terpene-based defence function was investigated in the Bohemian Forest study area. We collected an independent dataset for this experiment. Specifically, we identified 110 surviving and 119 reference trees. We classified LTS as living, mature, often solitary individuals surrounded by standing decaying beetle-killed trees and/or dead wood laying on the ground.

The minimum threshold for LTS DBH (35 cm) was set based on our field observations regarding the size range of mature host trees across the study site.

Due to logistical, managerial (partial removal of beetle-infested wood) and wood decay reasons, the beetle-killed trees were unavailable for the direct sampling, thus we adopted methods in Six et al. (2018). This approach assumes that living mature trees in close proximity to an outbreak and of a similar size and age as resistant trees (LTS), are, for the most part, strongly susceptible to beetle herbivory and therefore provide proxy data for beetle-killed trees. High tree mortality rates (98-99%) during severe bark beetle outbreak in our study area support the possibility to use this approach. The refence trees grow in intact forest patches comprising the fragments of mature spruce stands unaffected by wind and bark beetle caused disturbances, i.e. without any visible signs of bark beetle attacks on host trees. We selected the reference trees from the interior of the intact stands, avoiding individuals growing at the stand edges or at the margins of the forest gaps. We therefore selected 119 living mature spruce trees to serve as reference trees. Thus, 110 LTS and 119 reference individuals were sampled for genetic (the results are not shown here) and dendroecological analyses. We also used the data for 119 reference trees in our remote sensing analyses, as was described in section 2.3.2. However, considering human and financial resource constraints, out of 229 field-identified trees (110 LTS and 119 reference trees), only 81 individuals (40 LTS and 41 reference trees) were sampled for chemical analyses of defence mechanism in the experiment described in subsection 2.9.1.

The mean LTS age was 144 years, while the reference trees were 135 years old on average. Age differences between groups were insignificant ($p=0.191$). We measured morphological parameters (DBH, height, crown parameters) and recorded site characteristics (e.g., slope, aspect, forest floor composition) according to strictly-defined protocols. Using a 5 mm increment borer, we extracted two increment core samples from both LTS and reference trees at breast height (1.3 m above the ground).

2.8 Experimental design for chemical defence analyses

2.8.1 MJ experiment in the Šumava NP for terpene analysis

To investigate potential differences in chemical-based defence mechanisms between surviving and reference trees, between the 30th of June and 2nd of July 2021, we conducted an inoculation experiment. Out of 229 field-identified trees, 81 trees (40 LTS and 41 reference trees) located in Germany and Czechia were used in the experiment. Methyl-jasmonate (MJ) was inoculated into each stem at 1.3 m above the ground height. MJ has been shown to provoke the chemical responses in spruce only locally, i.e. ~7 cm around the inoculation area during 4 weeks following the treatment (Franceschi et al., 2005). Additionally, our field observations and communication with experienced researchers involved in similar fungal inoculation field experiments confirmed that a localized tree defence reaction expands sideways from the inoculation zone rather than vertically along the stem. Thus, following the experimental design described in Schiebe et al. (2012), we anticipated that MJ would not substantially change the chemical defence potential of a whole tree. Three weeks later, between the 19th and 22nd of July, 2021, we extracted treatment samples of phloem from around the inoculation spot (original treatment site). We collected an additional control sample, presumably unaffected by the treatment, from a location 50 cm higher on the tree stem. Samples were collected using a 0.5 mm diameter hollow leather punch. All samples we submerged in liquid nitrogen immediately after field extraction for transport to the laboratory. In the lab, all samples (N=162, one “control” and one “treatment” sample for each of 81 trees) were stored at –80 °C for subsequent gas chromatography-mass spectrometry (GCMS) and ultra-high-performance liquid chromatography (UHPLC) analyses.

2.8.2 Mechanical wounding experiment in the Vosges mountains for analyses of phenolic based defences

To analyse the composition and production of particular phenolic compounds in response to the agarose gel inoculation, during the third week of April 2003 (2003-04-13 to 2003-04-19), from each of 97 experimental trees located in the Vosges mountains study area, we collected a 2 cm wide by 8 cm long sample of bark and phloem tissue. On the same day, we inoculated each tree with sterile malt-agar from opposite sides of a stem at 1.3 m above the ground. Agarose treatment simulated conditions experienced by a tree during bark beetle boring attempts, and presumably triggering defensive responses, including changes in tree chemistry. We used a sterile 5 mm leather punch to extract the samples of phloem and outer bark. After introducing a 5 mm diameter disk of malt-agar into the hole, we returned the bark plug to its original position. Two weeks after agar treatment (between 2003-04-27 and 2003-05-03), we collected a 6 cm by 1 cm strip of phloem and bark from each experimental tree from the point of prior inoculation and oriented longitudinally (parallel with the stem). After extraction, all samples were immediately stored in liquid nitrogen. After transport to the laboratory, all samples were preserved using lyophilization.

2.9 Laboratory processing of samples for chemical analyses

2.9.1 Untargeted terpene resin acid extraction and analysis

In the laboratory, the field-collected phloem samples were prepared for analyses. The outer bark was first carefully removed. The remaining phloem was then ground to a fine powder and dissolved in liquid nitrogen to produce a homogeneous liquid sample. Chemical extraction was performed by subjecting the homogenized phloem to a 10-minute treatment with a 2 mL hexane solution. This hexane solution contained 5 µg/mL of the internal standard 1-bromododecane. The extraction process was carried out in an ultrasonic bath for a duration of 10 minutes. The resulting extracts were carefully filtered into 2 mL vials, which were then stored at a temperature of -80 °C prior to analysis. For the separation, identification, and quantification of constituent compounds, a gas chromatograph (Agilent 7890B, Agilent, USA) coupled with a mass spectrometer equipped with a time-of-flight mass analyser, namely Pegasus 4D (LECO, USA), was employed. Chemical separation was achieved using an HP-5MS UI capillary column (manufactured by Agilent), 30 m in length, 0.25 mm in internal diameter and with a film thickness of 0.25 µm. During the analysis, a 2-minute spitless period in a hot programmed temperature vaporizer (PTV) set at 265 °C was implemented for solid phase microextraction (SPME) analysis. The gas chromatograph (GC) oven was programmed as follows: an initial temperature of 40 °C was maintained for 1 minute, followed by gradual warming at a rate of 20 °C/min until an upper temperature of 280 °C was reached. To analyse the phloem extracts, 1 µL of the extract was injected into a cold PTV injector at a temperature of 20 °C, using the spitless mode. After injection, the inlet temperature was rapidly increased to 265 °C at a rate of 8 °C/s. The GC oven temperature program consisted of an initial temperature of 40 °C for 1 minute, followed by a ramping process at a rate of 5 °C/min until 210 °C was reached. The temperature was then further increased at a rate of 20 °C/min to 320 °C, which was maintained for 6 minutes. During the analysis, ions with an ionization energy of 70 eV were collected within a mass range of 35 to 500 Da at a frequency of 10 Hz. The obtained data were processed using ChromaTOF software (LECO), which used automated spectral deconvolution and peak finding algorithms. Additionally, a built-in peak alignment tool known as Statistical Compare was utilized to align all chromatographic signals with a signal-to-noise ratio (S/N) higher than 50. The identification of the compounds was confirmed by comparing their retention times with those of respective standards and matching the obtained mass spectra with entries in the NIST mass spectral library (The National Institute of Standards and Technology; 2017).

2.9.2 Phenolics extraction and analysis

Before extraction of phenolics, we removed the outer bark and edges of each sample. The samples were lyophilised prior to this manipulation. For those samples collected subsequent to inoculation, a 2.5 mm buffer surrounding the inoculation area and an additional 2.5 mm edge at the longitudinal ends of the material were also separated and excluded from analyses. No evidence of necrotic tissue was observed in any of the phloem samples.

The phloem samples were grounded into fine powder and 30 mg of the powder were taken from each sample for phenolic extraction. Following methods in Brignolas et al. (1995a), we separated and removed resinous compounds using a pentane wash. Specifically, we mixed the ground phloem with 2 mL of sodium metabisulfite and pentane. This solution was placed in a water bath for 30 minutes and then centrifuged at -5 °C and 16,350 g (relative centrifugal force) for 20 minutes. After removing the supernatant, we washed the residue twice. The washed powder was then added to a 1.9 mL solution of 80% methyl alcohol and 2.5 mM vanillyl alcohol (an internal standard for phenolic analyses). This mixture was added to a sonicating water bath for 15 minutes and again centrifuged for 20 minutes at -5 °C and 16,350 g. The supernatant was separated for further analyses. Detection and identification of phenolics was performed using a high-performance liquid chromatography (HPLC). To measure adequate light intensity during chromatographic runs, we employed a photodiode array detector 991 Waters 600E (an optical device used to record how much light is absorbed by a substance). The photodiode array was equipped with a silica column (Merck, Lichrospher RP 18). The liquid was comprised of acetonitrile, acetic acid and water in a proportion of 1:99 by volume. The share of acetonitrile varied from 5 to 70% (Lieutier et al., 2003b). The composition of phenolic compounds was identified based on the absorption spectra peaks that coincide with the previously published results from similar studies conducted on *P. abies* (e.g., Brignolas et al., 1995a, 1995b; Lieutier et al., 2003b).

2.10 Disturbance reconstruction using a dendrochronological approach

We expected to observe a relationship between tree growth rates prior to an insect outbreak and the potential of those trees to defend against herbivory and survive the disturbance. We used the increment core samples to quantify stem growth. However, we lacked quantitative site-specific information regarding the onset of a given outbreak. Historical records were imprecise in this regard. GE imagery for the study region in the Bohemian Forest had not yet been acquired at the time of the first bark beetle outbreaks (late 1980s to early 1990s). We therefore used a dendrochronological approach to reconstruct the onset of bark beetle disturbances in different localities.

To compare the growth rates of surviving and susceptible trees prior to the outbreak, we had to reconstruct the years when the disturbance had begun, since the onset of the beetle infestation was locality specific, and high-resolution GE imagery from the 1990’s was unavailable. One of the most reliable sources of information for disturbance reconstruction were the cores collected from LTS and the reference population of spruce trees in the Bohemian Forest study area. Thus, the reconstruction of past disturbances was based on the identification of so-called release events, which are anomalous increases in annual growth due to some major change in the local environment (e.g., death of neighbouring trees due to bark beetle or other disturbance). Mortality events may benefit survivors by decreasing competition for available resources at a site (Lorimer and Frelich, 1989; Rubino and McCarthy, 2004). We calculated release years using three different dendrochronological methods: namely, (1) the absolute increase method, (2) the growth averaging method, and (3) the boundary line (BL) approach. For each approach, the number of years to be averaged, including the target year, for a period prior to a potential release was set alternately to 5 and 10 years. The minimum number of years between identified consecutive releases was 2 years. The number of years during which the threshold value of a given criteria (growth rate) should be exceeded for an event to be deemed a release was two years. The thresholds for detection of moderate and major releases were 25 and 50%, respectively. In other words, growth rates following a major disturbance were 50% higher relative to average pre-disturbance growth, while moderate releases appertained to growth increase rate ranging from 25 to 49.9%. For disturbance reconstruction, we used the raw, unstandardized growth rates. Removal of trends associated with age and geometry is more critical in dendroclimatic studies or analysis of release events at juvenile ontogenic stages of tree development (Lynch et al., 2019). Common detrending techniques, such as fitting the observed ring-width series with a cubic spline or polynomial functions, that eliminate the “biological growth trend” to reveal the climatic trends (Fritts, 1976), were shown to confound the identification of growth deviations from the average growth rate, decreasing the accuracy of bark beetle disturbance reconstruction (Veblen et al., 1991). Thus, we used non-detrended ring-width series to identify the years of the bark beetle outbreak experienced by spruce trees at a mature ontogenic stage. Disturbance reconstruction analyses were performed using the “TRADER” package in the R programming environment.

The results of the retrospective outbreak onset years obtained using dendroecological methods were checked for compatibility with the results of GIS-based disturbance reconstruction. We analysed the time-series historical Landsat imagery spanning the beetle-affected forest in our study area for the period 1985 – 2020 in ArcGIS 10.5 software to identify the onset of disturbance in each locality using spectral vegetation indices (NDVI and EVI) (not shown). We used the proportional agreement between the four methods as a measure of reconstruction performance. In other words, we counted the number of times when disturbance year coincided across all methods (one GIS-based and three dendroecological techniques), and then calculated the percentage of coincidences in the total number of identified release years for a corresponding method. This value served as a proxy of method reliability, with the highest value (40%) obtained for the BL method (not shown), thus we chose this method as the one most effectively identifying disturbance events. Another reason for choosing the BL approach was the possibility to standardise the growth rates accounting for the relationships among tree age, size, and canopy class, the factors affecting tree development (Black and Abrams, 2004). The BL method is a running mean method for release detection (Rubino and McCarthy, 2004). This method stands out of other dendroecological approaches used for disturbance reconstruction in a way that it scales the release event based on a boundary line condition. The boundary line is a maximum expected growth augmentation, quantified according to radial growth patterns preceding a release (Splechtna et al., 2005). The function “boundaryFit” from the “TRADER” R package scales percent growth change (Nowacki and Abrams, 1997) by prior growth. The BL, a negative exponential curve, exhibits a pattern denoting the correlation between the preceding growth and the subsequent response to a release event. This relationship remains consistent irrespective of tree age, size, and social status (Black and Abrams, 2003). Black (2009) proposes that a minimum of ~50,000 measurements of species specific ring widths are required for precise boundary line quantification. However, our dataset spanning the years 1865 to 2021 only comprises 13,862 ring width measurements. To overcome the limitation of small sample size, we employed the regional boundary line proposed by Splechtna et al. (2005). Splechtna et al. (2005) analysed 147,949 growth increments of *P. abies* collected from 51 Alpine sites. These data were included in the International Tree Ring Data Bank (https://www.ncei.noaa.gov/products/paleoclimatology/tree-ring) and in the dataset compiled by Grabner et al. (2004) . Thus, we used the already existing BL equation derived for a large-scale Norway spruce dataset in Europe. The same boundary line was used in the disturbance reconstruction conducted by Svoboda et al. (2012).

A moderate release refers to the partial removal of a canopy tree or the removal of the neighbouring trees, while a major release reflects catastrophic mortality of all overtopping canopy trees (Svoboda et al., 2012). Thus, to account for both neighbouring sub-canopy and overtopping canopy tree mortality caused by beetles, the growth release years were identified for two types of releases: 1) using only major releases, and 2) all (major and moderate) releases. Only percentage changes in growth rate (%GC) values between 50 and 100% of the boundary line were regarded as major releases. Values of %GC between 20% and 49.9% were classified as moderate releases (Black and Abrams, 2003). Trees can temporarily maintain physiological functions even after disruption of conductive vessels in the xylem and phloem using carbohydrates and water stored in roots, heartwood, and branch tissues. Thus, we subtracted one year from identified release years to account for a time lag between bark beetle colonization and subsequent growth responses.

The described method of disturbance reconstruction yields disturbance year for each individual tree, but our aim was to identify locality specific disturbance years. Quantification of site specific disturbance years is justified by the fact that growth releases in each individual tree do not necessarily occur simultaneously, as beetles do not kill 100% of host trees in the same year in a given locality (Eisenhart and Veblen, 2000). Commonly, locality specific disturbance years are considered to be those in which at least 20 trees per site (Veblen et al., 1991), or, on a landscape level, 25% of sites (Lynch et al., 2019) exhibit growth releases. In this study, the scarcity of surviving trees and the associated limited sample size, coupled with heterogeneity in the presence of growth release events across trees (i.e. not all trees in each locality exhibited releases), prevented us from considering the year in which the majority of trees in a given locality showed releases as a site specific disturbance year. For example, in Hraničnik, out of 14 sampled extant LTS, a substantial growth acceleration was recorded in only two individuals. Despite the low number of released trees, site specific disturbance reconstruction is possible even if two or more trees per site initiate releases (Lynch et al., 2019). Thus, to identify disturbance year in each locality, we used the weighted mean, which accounted for the frequency of a specific disturbance year occurrences in the respective site. The published disturbance chronologies reconstructed using the related methods commonly have temporal resolutions that span decades (e.g. see Alfaro, R. I., Campbell, R., Vera, P., Hawkes, B., & Shore, 2003; Eisenhart and Veblen, 2000; Svoboda et al., 2012). Hence, precision of bark beetle disturbance reconstruction exceeding several years is quite common.

2.11 Statistical analysis of tree, stand, environmental chemical, and dendroecological data

2.11.1 Untargeted terpene profiling of LTS and reference trees

To investigate tree chemical defence mechanism against bark beetles, in the Bohemian Forest study area, we conducted a MJ inoculation experiment described in 2.9.1. We aimed to test our hypothesis positing that surviving trees have different induced and/or constitutive chemical defence mechanisms than the reference trees. Particularly, we expected to find the differences in the whole terpene profiles of LTS vs. reference trees prior to or after MJ inoculation. We also anticipated to observe the differences in the content of individual terpene compounds and their production rates identified in the samples of inoculated and uninoculated phloem of surviving vs. reference trees.

Out of 81 experimental trees sampled in the Bohemian Forest study area, 69 trees (36 LTS, 33 reference trees) yielded high-quality data after completion of laboratory processing and gas chromatography – mass spectrometry (GCMS) analyses. For each LTS and reference tree, the measurements of “control” (C) and “treatment” (T) samples were available. Terpene concentrations in some phloem samples were measured multiple times during chromatographer operation cycles. Associated values were averaged. Thus, the dataset comprised the samples of 69 trees, with a corresponding “treatment” and “control” sample values for each experimental tree (69*2=138 samples in total). For statistical comparisons of terpene responses among C and T samples, we used the abundance of specific mass from the mass spectra of a variable divided by the abundance of specific mass from the mass spectrum of internal standard (hereafter referred as “mass abundance ratio”). To identify terpene compounds, a unique mass spectrum of each peak produced by a sample in the gas chromatogram was used.

To discern potentially divergent patterns in the terpene profiles of surviving versus reference trees, we performed PCA analyses using the raw terpene responses and field data. Continuous field parameters, such as DBH, tree height, self-shading ratio, and elevation, were converted to categorical covariates. Categorical tree parameters, such as decay classes (eight) of coarse woody debris defined by Holeksa et al. (2008), canopy layer and survival statuses, were directly input to PCA performed using SIMCA 15 (Sartorius Stedim Data Analytics AB, Malmö, Sweden).

To investigate the presumable differences in individual defensive metabolites involved in MJ-induced chemical defence reaction, we compared terpene responses observed in MJ inoculated (T) and uninoculated (C) phloem of surviving and reference trees. Additionally, we tested the differences between LTS and reference trees in the residuals between T and C mass abundance ratios recorded for each individual compound. We used simple statistical tests to investigate the differences in terpene content between inoculated and uninoculated samples (T – C). This difference proxies the production rate of each terpene compound in response to MJ treatment, which mimics bark beetle attacks. Terpene concentration comparisons were performed using a two-sample T-test for normally distributed data with equal variances. In instances where the data exhibited normal distribution but with heterogeneous group variances, Welch's T-test, also known as an unpaired two-sample T-test, was applied due to its enhanced reliability for disparate sample sizes and unequal variances (Derrick et al., 2016; Ruxton, 2006). For non-normally distributed data, a two-sample Wilcoxon test, commonly referred to as a rank sum test, was utilized (Bridge and Sawilowsky, 1999).

2.11.2 Comparisons of phenolic concentrations in inoculated vs. uninoculated samples in the Vosges mountains

To test for the differences between the mean phenolic compound levels observed in the phloem of uninoculated trees and those treated with malt-agar (collected 14 days after inoculation), we employed a paired two-sample Wilcoxon test. The Wilcoxon test, also referred to as the Wilcoxon signed-rank test, is a non-parametric statistical test that facilitates comparisons between paired data sets that do not follow a normal distribution (Woolson, 2008). The test was performed on a dataset of 96 trees, including 25 beetle-infested trees and 71 trees that survived without evidence of attack. Outliers were previously removed. We defined outlier data as having values that differed from mean concentration levels by at least 5 standard deviations.

2.11.3 Phenolic profiles of beetle attacked vs. non-attacked spruces

We compared the phenolic content of phloem in surviving versus beetle-killed trees from the Vosges experiment in France. First, we employed a two-sample Wilcoxon test to compare phenolic concentrations in cases where the data followed a normal distribution and had equal variances. This test assumes that the data points are independently and evenly distributed within each group. In situations where the data exhibited a normal distribution but had heterogeneous group variances, we utilized Welch's T-test, which is also known as an unpaired two-sample T-test. This test is particularly advantageous when dealing with the unbalanced sample sizes and unequal variances. The use of Welch's T-test enhances the reliability of the statistical analysis (Derrick et al., 2016; Ruxton, 2006). For cases where the data did not conform to a normal distribution, we employed a two-sample Wilcoxon test, also referred to as a rank sum test. This non-parametric test does not assume a normal distribution (Bridge and Sawilowsky, 1999).

2.11.4 Differences in phenolic, crown, and site characteristics among surviving, primarily attacked, and secondarily attacked trees

To examine whether there were any statistically significant variations in the mean values of site, physiological, and chemical parameters among surviving trees, primarily attacked trees, and secondarily attacked trees, we conducted a one-way analysis of variance (ANOVA). Subsequently, to assess the differences between primarily attacked trees and secondarily attacked trees, a two-sample T-test was employed. To ensure that the data adhered to the assumptions of normality required for ANOVA and T-test analyses, we performed a Shapiro-Wilk test using the "stat" package in R. Homogeneity of variance was evaluated using Levene's test (the “car” package in R). If the normality assumption was violated for either the one-way ANOVA or the two-sample T-test, we employed the Box-Cox transformation method, utilizing the “MASS” package in R. This transformation technique aims to improve the normality of non-normally distributed data (Osborne, 2010; Sakia, 1992). If heterogeneity of variance was detected, we employed a non-parametric approach, specifically the Kruskal-Wallis test based on data point rankings. This test, available through the “stat” package in R, quantifies whether the medians of two or more groups with unequal sample sizes differ. The Kruskal-Wallis test serves as a viable non-parametric alternative to the two-sample Wilcoxon test, offering an extension to comparisons involving multiple groups (Breslow, 1970).

In instances where the one-way ANOVA test revealed significant differences between group mean values, we proceeded with a post-hoc Dunn’s test, which was conducted using the “FSA” package in R. This test is suitable for making comparisons between group means with varying levels of observations (Zar, 2010). To account for multiple comparisons, we applied the Benjamini-Hochberg adjustment, a step-down procedure suitable for independent or positively dependent tests (Benjamini and Hochberg, 1995). If the Kruskal-Wallis test indicated significant differences in median values, we performed a post-hoc pairwise Wilcoxon’s test to identify specific pairs of tree groups that exhibited variations in the mean parameter values.

2.11.5 Analysis of surviving and reference tree radial growth rates collected in the Bohemian Forest study area

We compared the mean growth rates of 110 surviving and 119 reference trees. We used one-way ANOVA for normally distributed data with homogeneous variances, and alternately, Wilcoxon rank sum tests with continuity correction for non-normally distributed growth rates or ring widths with unequal variances. We compared mean growth rates for different time intervals. Specifically, we considered average annual growth for 20, 40, 60, 80, 90, and 100 year intervals that preceded each reconstructed locality-specific disturbance event.

2.12 Survival modelling

2.12.1 Modelling of tree survival factors in the Bohemian Forest

Logistic regression analyses were used to investigate the nature and strength of selected factors assumed to influence host survival probability. Alternate models were formulated to test each of four *a priori* hypotheses: (1) that survival may be negatively affected by competition stress, (2) that the degree of stem self-shading by canopy leaves and relative crown depth may moderate environmental stress effects on tree physiology and enhance survival, (3) that survival potential decreases with increasing tree size, and (4) that water stress compromises tree defence mechanisms and negatively impacts survival.

Regression models were multiplicative and non-linear. A fully parameterized model with three factors had the following form:

$$s = s_m^* \times \mathrm{size} \times \delta \times L \times \mathrm{CWB} \qquad (2.2),$$

where *s* is a predicted survival probability, s^*_m is a maximum potential survival probability, *size* is a tree DBH, δ is pre-outbreak stand density, L is a self-shading index (ratio of crown length to tree height), *CWB* is a climatic water balance calculated as the difference between total precipitation and PET. No collinearity issues among the covariates were observed (variance inflation factors (VIF) < 1.25 for all terms). VIF was derived from an equivalent general linear model (Canham and Murphy, 2017). To mitigate parameter trade-offs during model fitting, all predictor variables were scalar terms with values that ranged from zero to one (Canham and Murphy, 2017). The scalar covariates fractionally reduce, by multiplication, the maximum potential survival term (s^*_m), which represents expected survival under optimal levels of all other factors. To account for potential geographical variation in predicted survival probability, due to contrasting edaphic conditions or other local factors, unique values of potential survival (N=3) were estimated by country (Czech Republic, Germany, and Austria) (Canham and Murphy, 2017).

We fit each covariate in eqn. 2.2 with different functional forms depending on the data distribution patterns and the expected shape of survival in response to tree, stand, and environmental drivers. Size and density terms were fit with exponential functions that are able to accommodate monotonically increasing or decreasing survival responses:

$$\mathrm{size}\ or\ \delta = e^{(-a \times X^b)} \qquad (2.3),$$

where a and b were shape parameters estimated by the annealing algorithm, and X was a vector of observed DBH or stand density values, respectively.

We hypothesized that the relative depth of a tree crown protects the corresponding stem from overheating and dampens temperature-mediated VOC emissions that potentially modify bark beetle behaviour. Our models therefore incorporated a self-shading index, modelled using a logistic function that saturates at high values:

$$L = L_a + \frac{1 - L_a}{\left(1 + \left(\frac{S}{L_b}\right)^{L_c}\right)} \quad (2.4),$$

where L_a , L_b , and L_c are estimated parameters governing the shape of a survival response curve, and S is a vector of observed self-shading ratios.

We expected that soil moisture limitation degrades spruce defensive capacity, decreasing survival probability during a bark beetle attack. We quantified levels of growing season moisture availability, termed a seasonal climatic water balance (CWB), from the difference of precipitation and PET. The CWB was modelled using a flexible Gaussian function that is able to fit monotonically increasing, decreasing, or unimodal survival responses:

$$\mathrm{CWB} = e^{-0.5 \times \left(\frac{C - C_a}{C_b}\right)^2} \quad (2.5),$$

where C is a vector of observed seasonal CWB, and C_a and C_b are parameters quantified by the annealing algorithm. A long-term CWB was calculated for growing season months, i.e. from May to September, over a 30-year period preceding the outbreak.

The likelihood function was defined as follows:

$$\text{log likelihood} = \sum_{i=1}^{n} \begin{cases} \log(p_i) & \text{if tree } i \text{ survived} \\ \log(1 - p_i) & \text{if tree } i \text{ died} \end{cases} \quad (2.6)$$

In eqn. 2.6, p_i stands for the probability that a tree survived, and $(1 - p_i)$ is the probability that a tree was beetle-killed. The model was fit using a dataset of 598 trees, including 184 LTS and 414 reference trees. We designed several models with different permutations of covariates (eqn. 2.2) and selected the model that maximized fit and parsimony (Burnham and Anderson, 2004). Specifically, AIC corrected for small sample size was used to rank the competing models (Johnson and Omland, 2004). The likelihood modelling approach and AIC-based model selection are robust to temporal and spatial autocorrelation issues (Buechling et al., 2017; Hubbell, 2001; Uriarte et al., 2004a). As already mentioned, we did not detect evidence for significant collinearity between covariates.

Support intervals, which approximate more traditional 95% confidence intervals, were used to quantify prediction uncertainty (Edwards, 1992; Uriarte et al., 2004b). To evaluate the goodness of fit of the models, we employed a pseudo-R^2 approach (Canham and Murphy, 2017; Uriarte et al., 2004a). The probability of survival for each tree was computed based on the best-fitting model. We subsequently categorized these predictions into distinct 5% survival classes, (e.g., 0-5%, 5-10%, etc). Subsequently, we compared the mean predicted probability of survival within each class to the observed proportion of individuals that actually survived within that class. This comparison provided an estimate of overall explained variance. To calculate bias, we examined the slope of the regression line between observed and predicted survival across the binned classes. Unbiased predictions were represented by slopes approaching 1.0.

2.12.2 Modelling of tree survival factors in the Vosges mountains

Similar to our survival analysis conducted for the Bohemian Forest, we used the non-linear logistic regression to investigate the factors of spruce resistance to bark beetles. Based on our hypotheses and RF classification results, we fit models of the following general form:

$$s = s_m^* \times D \times I \times CD \times Cat \qquad (2.7)$$

where s is predicted survival probability, s^*_m is a maximum potential survival probability, D represents the linear distance to a bark beetle source, I refers to stem illumination, CD designates the extent of crown defoliation, and Cat is catechin production. Cat was calculated as the difference between catechin concentration in the phloem after mechanical wounding and the concentration measured in untreated samples. Model complexity was constrained by a relatively small sample size of the fitting dataset (96 spruce trees). Thus, we did not incorporate any additional terms. Our model was multiplicative in form, accounting for interactions between covariates. All covariates were dimensionless scalars ranging from 0 to 1.

Each term was modelled using a different nonlinear function that best captures the fit between the variable and the survival response. We hypothesised that survival probability will increase with increasing distance between a previously killed tree and a target tree. The distance effect was fit with a two-parameter exponential function that asymptotes at large values:

$$D = 1 - \frac{D_b}{e^{D_a \times X}} \qquad (2.8),$$

where D_a and D_b are shape parameters calculated by the annealing algorithm, and X is a vector of length measurements quantifying the separation distance between an experimental tree and the nearest beetle-colonized tree.

We expected that enhanced catechin production will be associated with elevated survival capacity. Thus, this covariate was modelled with an asymmetrical lognormal function that is able to fit monotonically increasing, decreasing, or unimodal data distributions:

$$Cat = e^{-0.5 \times \left(\frac{\ln \left(\frac{X}{Cat_a} \right)}{Cat_b} \right)^2} \quad (2.9),$$

where Cat_a and Cat_b are unknown shape parameters, and X corresponds to the experimentally determined values of catechin production (from section 2.9.2).

The effects of crown defoliation (i.e. crown proportion with senescent, damaged or chlorotic foliage) as well as the degree of stem sun illumination (0 representing a fully shaded stem and 1 designating fully illuminated bark) were both modelled using exponential functions:

$$CD \text{ } or \text{ } I = e^{(-a \times X)} \quad (2.10),$$

where the shape parameter a is estimated by the likelihood algorithm, and X is a vector of observations for either crown defoliation, or sun illumination.

The model fitting dataset was comprised of information from 71 surviving and 25 beetle-killed reference trees. Analyses were conducted in a likelihood framework. Maximum likelihood values of all model parameters were estimated using simulated annealing, a global optimisation algorithm (Goffe et al., 1994). The most parsimonious models were selected based on AIC, corrected to account for a small sample size (96 trees) (Johnson and Omland, 2004). Survival probability was quantified using eqn. 2.6. The log likelihood was computed suggesting that observations were independent. In contrast to the conventional parametric methodologies, the estimation of parameters and comparisons among different permutations of the terms incorporated in eqn. 2.7 using likelihood and parsimony criteria are usually not influenced by the autocorrelated data structures (Hubbell, 2001; Uriarte et al., 2004b). Additionally, it should be mentioned that no correlation among the predictors was identified using the variance inflation factors, which did not exceed 1.05 for all covariates included in a similar general linear model.

The goodness of fit of the models was calculated using a pseudo-R^2 described in the last paragraph of the subsection *2.11.1*. All statistical analyses were performed in the R programming language (R Development Core team, 2023). To fit logistic regression models, we used the “likelihood” package (Version 1.7; Murphy, 2015).

III. RESULTS

3.1 Spruce survival during bark beetle outbreak in the Bohemian Forest study area

3.1.1 LTS identification using a combination of remotely sensed and field survey data

Inspection of remotely sensed GE imagery resulted in the detection of a total of 184 occurrences of LTS distributed across three bordering countries – Czechia, Austria, and Germany. Distances between adjacent LTS ranged from 3 to 640 m. Stand conditions associated with surviving trees were relatively open, ranging from ~30 to 365 trees·ha^{-1}. The mean stand density for LTS and reference trees was 288 trees·ha^{-1} (for the values of other covariates incorporated in the regression models see Table 1 in Korolyova et al. 2022a). The mean LTS self-shading ratio was generally high and reached 76%. The LTS occurrences fell into a relatively narrow altitudinal band that ranged from 1110 to 1370 m. Results of an independent field survey conducted in the fall of 2020 for 37 randomly selected surviving spruce individuals (20% of the total sample size) revealed high GE identification accuracy. Field data indicated that all LTS were correctly identified in image analyses. We did not assess omission error.

3.1.2 Estimation of tree size and crown parameters

GE-derived self-shading indices evaluated during the fall 2020 field survey were accurately estimated and unbiased, as indicated in Fig 3. The mean absolute error was 15%, and the root mean square error reached 19%.

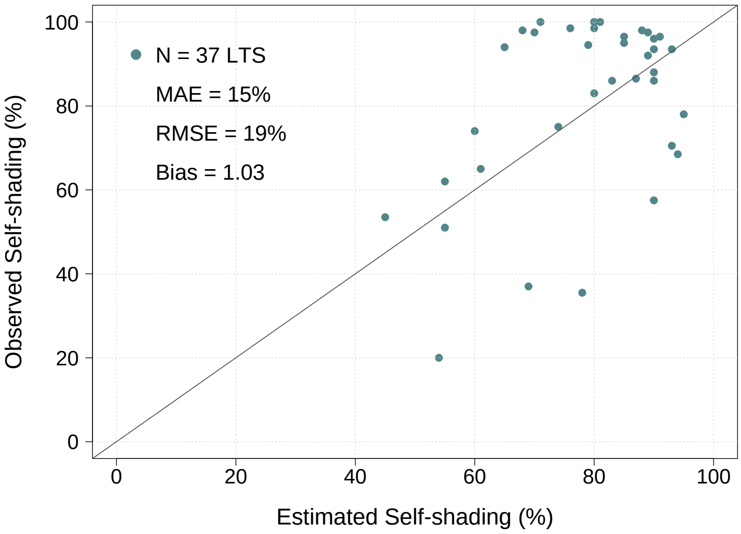

Fig. 3. Observed vs. GE-estimated self-shading ratios for 37 ground-truthed LTS. A diagonal slope (1:1) demonstrates a perfect accuracy of remote sensing-based measurements.

We used the allometric models to predict DBH from the crown projection area of surviving trees. The allometric model performance, assessed by AICc, indicated that the power function optimization yielded marginal improvements in comparison to a modified exponential function (see Table S2.1 in Korolyova et al., 2022a). DBH values predicted using a power function demonstrated a mean absolute error of 8.8 cm and a root mean square error (RMSE) of 10.4 cm when evaluated against the partitioned test data consisting of 222 samples. Similarly, when applied to a sample of 30 field measurements of surviving trees, the power model exhibited a mean absolute error of 8.5 cm and a RMSE of 10.6 cm. The slope of the regression model fit with a test dataset comprising 222 individuals revealed unbiased DBH predictions (the slope of observed vs predicted values ~ 1.0) (Fig. 4, Uriarte et al., 2004).

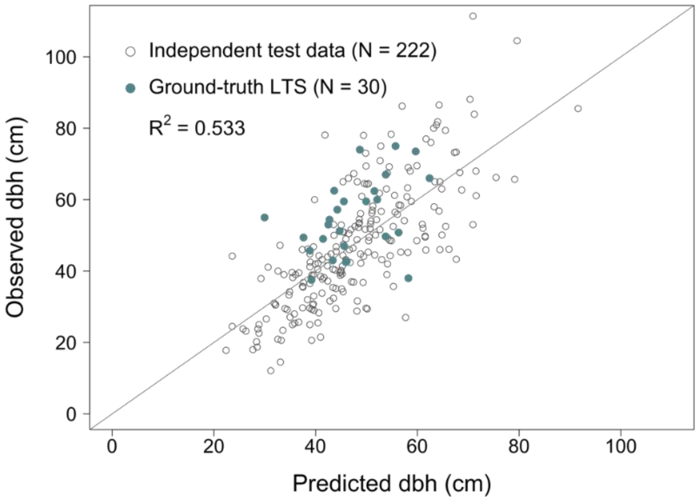

Fig. 4. Observed vs. predicted DBH for the test data (open circles, 222 trees) and field-surveyed surviving trees (filled circles, 30 trees).

3.1.3 Pre-outbreak stand density reconstruction in an old-disturbed natural forest

The best model for the spectral (Landsat 5) band-based reconstruction of stand densities prior to the outbreak comprised six, accounting for 47% of the variance observed in the calibration dataset (see Table S4.1 in Korolyova et al., 2022a). The residuals were unbiased, as evidenced by the slope of the relationship between observed and predicted values (~0.99) (Fig. 5), with a mean absolute error being 48.6 trees·ha^{-1}.

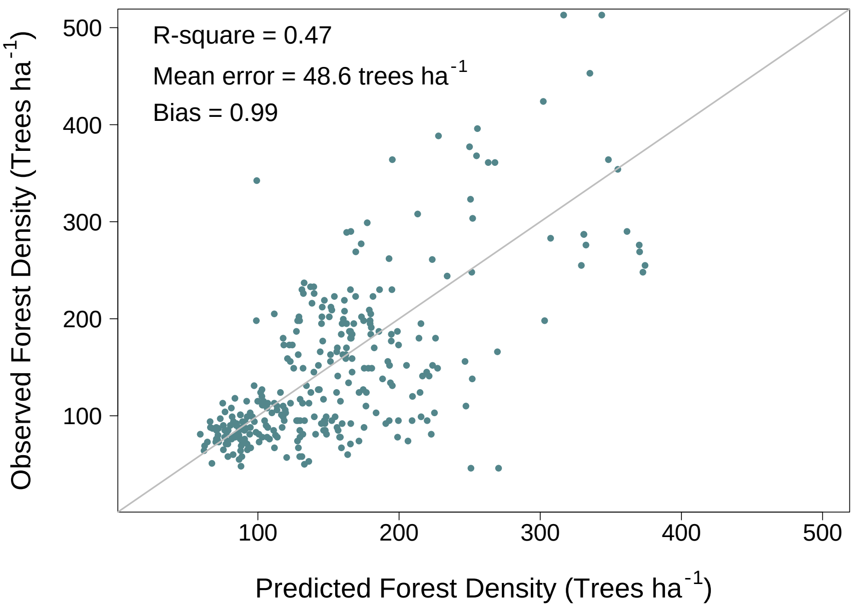

Fig. 5. Observed vs. predicted pre-outbreak stand density. The diagonal line delineates a ratio between observed and estimated values (1:1 stands for a complete correspondence).

The best model included Thematic mapper (TM) bands 3, 4, and 5, Tasselled Cap wetness, the structural index, and scaled (folded) aspect. Contrary to our assumptions, NDVI did not improve the model performance, thus we replaced it with the bands TM3 and TM 4. The number of estimated beetle-killed tree positions reconstructed using the best performing model and covariate values derived from component image pixels yielded ~6.4 mln dead trees in severely disturbed spruce forest study area.

3.1.4 The pattern of spatial distribution of surviving trees in the Šumava NP

The Optimised Hot Spot analysis detected statistically significant spatial clusters of surviving trees in the study area (Fig. 6), indicating that LTS distribution was not random and that spruce trees survive bark beetle outbreaks not by mere chance. Instead, survival is driven by multiple internal and external factors and their complex interactions.

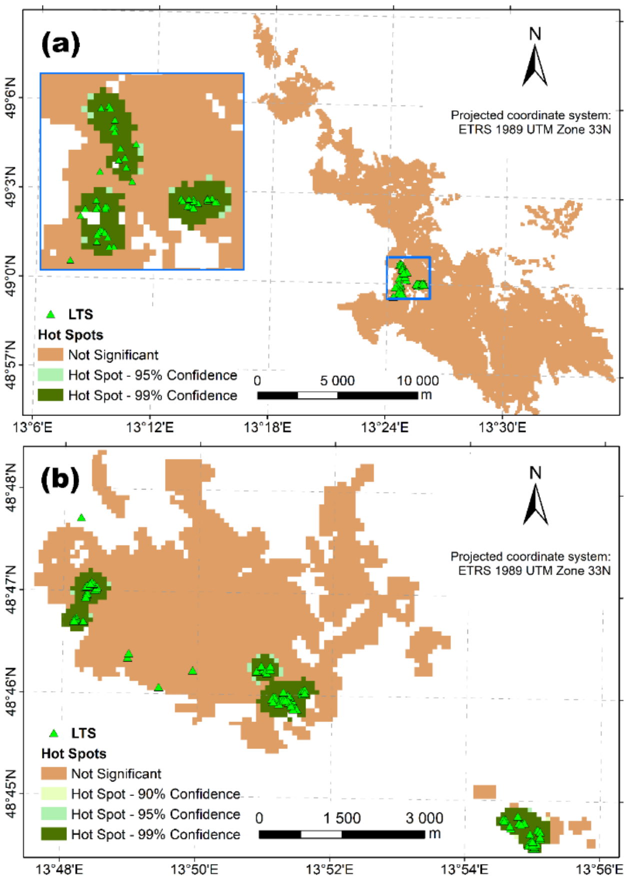

Fig. 6. Clustering of surviving trees in the western (panel a) and eastern (panel b) portions of the study area. Testing for statistically significant spatial clustering was performed using the Optimized Hot Spot analysis tool in ArcGIS.

3.1.5 The key drivers of tree survival in the Bohemian Forest study area

The Random Forest classification served as an exploratory approach to differentiate between environmental conditions, stand and tree level factors that either fostered or hindered tree survival during the bark beetle outbreak. In the Bohemian Forest study area, the classification model exhibited a notable predictive power, as indicated by a mean misclassification rate of 6.3% for LTS and 5.7% for trees affected by beetle infestation. The site-specific solar radiation intensity, along with the vegetation index derived from the spectral data (NDVI), revealed the most influential factors that determined survival outcomes (Fig. 7). The topographical characteristics, such as slope, aspect, and the shape of the terrain surface demonstrated lesser importance once the effects of insolation and NDVI were considered. The obtained results were subsequently used to inform the selection of explanatory variables in the regression models, which encompassed a climatic water balance designed to incorporate insolation effects.

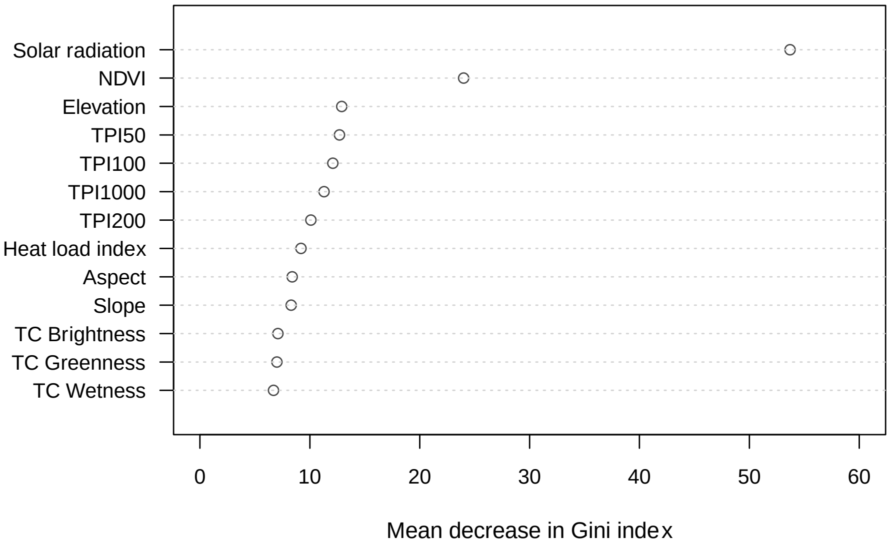

Fig. 7. Results of the Random Forest classification performed using 13 environmental and spectral predicting variables and a dataset comprising 6.4 mln beetle-colonised trees and 184 surviving trees in the Bohemian Forest study area. The importance of explanatory factors was quantified using the Gini index that measures the purity of classified groups. Thus, the predictor variable exhibiting the largest mean reduction in the Gini index was considered the most influential. The analysis included topographic position indices (TPI50, TPI100, TPI200, and TPI1000), representing the spatial attributes within the radii of 50 m, 100 m, 200 m, and 1000 m, correspondingly. NDVI stands for a normalised difference vegetation index.

3.1.6 Comparisons of radial growth rates between LTS and reference trees in the Šumava NP

Statistical comparisons showed that surviving trees grew significantly slower than reference trees during 20, 40, 60, 80, and 90 years prior to the disturbance reconstructed using all (major and moderate) releases (Fig. 8, panels b, d, f, h, j). If only the major releases were accounted for in disturbance reconstruction, LTS exhibited slower growth than reference trees during 20, 40, and 80 years preceding the bark beetle epidemics (Fig. 8, panels a, c, g). No significant differences were identified between LTS and reference tree growth rates 100 years prior to the outbreak regardless of major or all releases were used for the disturbance reconstruction (Fig. 8, panels l, k). Ninety years prior to the outbreak, when disturbance was reconstructed using only the major releases, dead trees grew faster than surviving individuals (Fig. 8, panel i) but the difference was on the margin of a significance threshold (p=0.05).

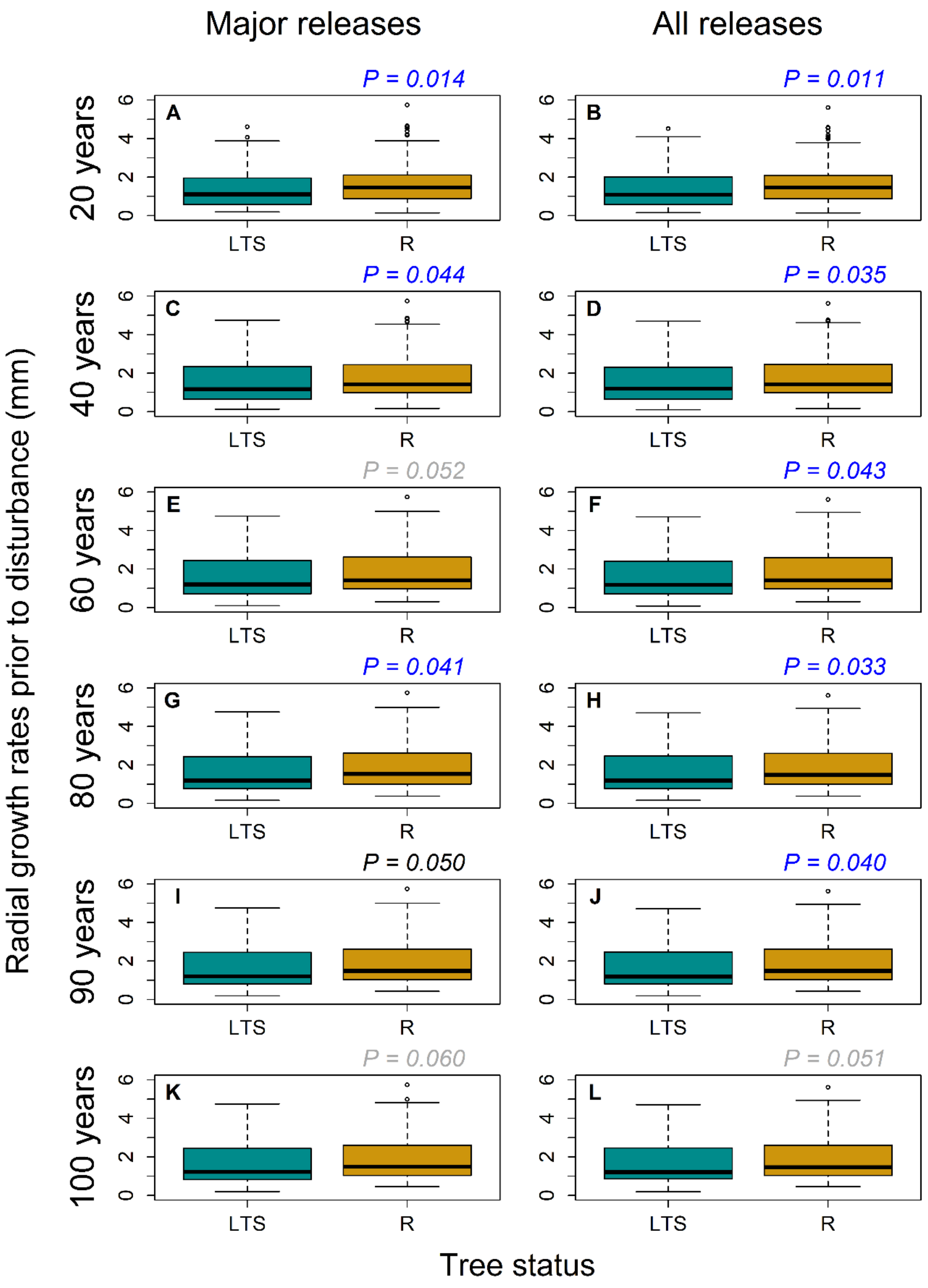

Fig. 8 Boxplots showing mean radial growth rates of surviving (LTS) vs. reference (R) trees 20, 40, 60, 80, 90, and 100 years prior to the outbreak in the Bohemian Forest study area. Reference trees are living mature trees growing in intact stands in a proximity to bark beetle disturbed areas. Assuming high mortality rates (up to 98-99%) during severe bark beetle outbreaks, these trees are assumed to be representative of bark beetle killed individuals. Locality-specific disturbance years were reconstructed using major (Major releases) and all (All releases) growth releases (see *2.11 Disturbance reconstruction using dendrochronological approach* in the Methods). Statistically significant differences were identified for all periods not exceeding 90 years prior to disturbance.

3.1.7 Chemical composition of terpenes in the phloem of LTS vs. reference trees in response to Methyl jasmonate inoculation

To investigate the chemical basis of spruce survival mechanisms, we performed GCMS analysis using the phloem samples of 40 LTS and 41 reference trees (81 trees in total) collected during a Methyl jasmonate inoculation experiment in the Bohemian Forest study area. After laboratory processing, only 69 trees (40 LTS, 41 reference trees) yielded pre- and post-treatment terpene data. Untargeted GCMS analysis detected 98 terpene compounds in the phloem of surviving and reference tree samples. Out of the 98 compounds, 32 VOCs belonging to six chemical classes could be identified: 13 monoterpenes (tricyclene, α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-phellandrene, 3-carene, α-terpinene, limonene, β-phellandrene, γ-terpinene, terpinolene), 5 oxygenated monoterpenes (eucalyptol, cis-4-thujanol, isopinocamphone, α-terpineol, trans-pinocarvyl acetate), 10 sesquiterpenes (α-cubebene, longipinene, α-ylangene, β-elemene, α-gurjunene, β-ylangene, caryophyllene, germacrene d, humulene, unidentifiable sesquiterpene), one oxygenated sesquiterpene epicubenol, two benzene derivatives (p-cymene, methylthymol), and one terpene ester, bornyl acetate (Table 3). The overwhelming majority of monoterpene content is represented by α- and β-pinene, followed by β-Phellandrene (Table 3). The structure of oxygenated monoterpene responses was dominated by eucalyptol. Among the sesquiterpenes, caryophyllene, humulene, and longipinene were the most represented compounds.

In all trees, regardless of tree status (LTS or reference tree), the total amount of VOCs increased almost three times after MJ treatment (Table 3, columns 8, 9). The most significant increment was observed in the content of oxygenated monoterpenes (10-fold) and terpene esters (6-fold). Monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and benzene derivatives followed the general pattern for all VOCs, having increased by 2-3 times on average. Two compounds that exhibited the largest increases were eucalyptol (49-fold increase, $p<0.001$) and 3-carene (14-fold increase, $p>0.05$).

We did not find any significant differences between the whole terpene profiles of surviving and reference trees, as revealed by the results of PCA (not shown). Instead, we identified significant differences in the content of individual terpene compounds and chemical classes. For instance, the production of two sesquiterpenes and one oxygenated sesquiterpene was significantly lower in LTS vs beetle-killed trees (Table 3, columns 2, 3). In the uninoculated phloem samples, we observed a higher content of four monoterpenes in LTS than in reference trees (Table 3, columns 4, 5), while in the inoculated phloem, the contents of one sesquiterpene and one oxygenated monoterpene were smaller in surviving than in beetle-killed spruces (Table 3, columns 6, 7).

The content of all 14 identified monoterpenes increased by 2-3 times in response to MJ treatment three weeks after inoculation (Table 3). Statistically significant increases were observed for tricyclene, α-pinene, camphene, sabinene, β-pinene, α-terpinene, limonene, γ-terpinene, and the sum of all monoterpene responses (Table 3). Similarly, in all trees, five identified oxygenated monoterpenes (eucalyptol, cis-4-thujanol, isopinocamphone, α-terpineol, and trans-pinocarvyl acetate), two benzene derivatives (p-cymene and methylthymol), terpene ester bornyl acetate, and all sesquiterpenes, except α-gurjunene and germacrene D, for which the changes were statistically insignificant, exhibited two- to three-fold increments in response to MJ treatment (Table 3). The content of the one identified oxygenated sesquiterpene epicubenol also increased, but insignificantly.

The total amount of volatile compounds was higher in surviving than in reference trees both before and after MJ inoculation, but the difference was insignificant (Table 3, columns 4–7). The initial content of monoterpenes before MJ treatment exhibited substantial diversity between surviving and reference trees, with the majority of compounds found in larger amounts in LTS (Table 3). Tricyclene, camphene, α-terpinene and sabinene were significantly higher in surviving than in reference individuals. All differences between LTS and reference trees in monoterpene content, whose responses were more pronounced in dead than surviving trees, were insignificant. Similarly, no significant differences between LTS and reference trees were found in the rest of the chemical classes prior to MJ treatment. The initial content of all oxygenated monoterpenes differed only slightly or did not vary among LTS and reference tree samples. The total initial amount of all sesquiterpenes was slightly larger in the phloem of surviving trees, but the difference was insignificant. The majority of individual sesquiterpenes were the same or marginally and insignificantly higher in the reference trees.

Two significant differences in the after-treatment VOC content were identified in trans-pinocarvyl acetate and one unidentifiable sesquiterpene, whose amounts were lower in LTS than in the reference killed trees (Table 3, columns 6, 7). The majority of the identified monoterpenes represented more pronounced responses in surviving than in reference trees; however, the differences were insignificant (Table 3).

Interestingly, the overall MJ-induced production of VOCs was more intensive in reference trees, despite revealing lower levels of pre- and after-treatment VOC contents than LTS. However, the difference was statistically insignificant (p=0.46) (Table 3, columns 2, 3). We found three significant differences between the phloem samples of LTS and reference individuals in MJ-induced production (Treatment minus Control) of volatiles. In all three cases, reference trees generated higher amounts of monoterpenes (β-ylangene, epicubenol, and one unidentifiable sesquiterpene) than surviving trees in response to MJ treatment. The production of the majority of monoterpenes was 1.5–2 times higher in LTS than in beetle-killed reference trees, but the differences were insignificant. Production of the prevailing monoterpenes α- and β-pinene was only slightly lower in LTS than in reference trees and did not exceed 9% and 4%, respectively (Table 3).

Production of three out of four identified oxygenated monoterpenes was insignificantly lower in LTS than in reference trees (Table 3, columns 2, 3). Two sesquiterpenes mentioned earlier (β-ylangene and unidentifiable sesquiterpene) demonstrated significantly lower absolute change values in LTS than in reference trees. Out of the remaining 8 sesquiterpenes, 6 were synthesized in larger amounts in surviving than in reference trees, but the differences were insignificant. Similarly, the production of the only one identified terpene ester, bornyl acetate, and one benzene derivative, p-cymene, was higher in LTS than in reference trees, while the other benzene derivative, methylthymol, was synthesized in smaller amounts in surviving individuals in response to MJ ($p>0.05$) (Table 3).

Table 3. Comparisons of raw mean responses of volatile compounds to Methyl jasmonate treatment by chemical class for surviving vs. reference Norway spruce trees in the Bohemian Forest study area

VOC name	**Production (T – C)**		**Control**		**Treatment**		**All trees**	
	LTS	**Reference**	**LTS**	**Reference**	**LTS**	**Reference**	**Control**	**Treatment**
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Monoterpenes								
Tricyclene	0.94 (-0.81; 2.69)	0.58 (-0.14; 1.30)	0.47 (0.20; 0.73)*	0.36 (0.12; 0.59)*	1.40 (-0.34; 3.16)	0.94 (0.13; 1.75)	0.42 (0.16; 0.67)***	1.18 (-0.21; 2.58)***
α-pinene	53.39 (-37.67; 144.46)	58.18 (-0.97; 117.33)	41.53 (22.39; 60.68)	35.77 (16.59; 54.93)	94.93 (0.93; 188.93)	93.95 (30.55; 157;34)	38.78 (19.53; 58.01)***	94.46 (14.21; 174.71)***
Camphene	1.0 (-0.71; 2.70)	0.68 (0.02; 1.35)	0.48 (0.24; 0.72)*	0.38 (0.11; 0.65)*	1.48 (-0.28; 3.23)	1.06 (0.25; 1.88)	0.43 (0.17; 0.69)***	1.28 (-0.11; 2.67)***
Sabinene	2.14 (-1.56; 5.83)*	1.08 (-1.71; 3.87)*	0.89 (0.36; 1.42)	1.04 (-1.12; 3.21)	3.03 (-0.69; 6.76)	2.12 (0.32; 3.92)	0.97 (-0.57; 2.50)***	2.60 (-0.38; 5.58)***
β-pinene	48.49 (-2.06; 99.05)	49.88 (-5.73; 105.49)	22.93 (12.5; 33.36)	22.48 (11.29; 33.67)	71.43 (18.21; 124.64)	72.36 (14.90; 129.83)	22.72 (11.99; 33.44)***	71.87 (16.99; 126.75)***
β-Myrcene	0.01 (-0.04; 0.07)	0.02 (-0.06; 0.10)	0.03 ($3\cdot10^{-3}$; 0.06)	0.04 ($-6\cdot10^{-3}$; 0.08)	0.04 ($-7\cdot10^{-4}$; 0.09)	0.05 (-0.02; 0.13)	0.03 ($2\cdot10^{-3}$; 0.07)	0.05 (-0.01; 0.11)
α-Phellandrene	0.03 (-0.11; 0.18)	0.03 (-0.07; 0.13)	0.04 ($-2\cdot10^{-3}$; 0.08)	0.04 ($3\cdot10^{-3}$; 0.07)	0.07 (-0.06; 0.20)	0.07 (-0.03; 0.17)	0.04 ($4\cdot10^{-4}$; 0.07)	0.07 (-0.05; 0.19)
3-Carene	2.64 (-2.58; 7.87)	1.58 (-1.60; 4.76)	0.10 (-0.02; 0.23)	0.22 (-0.19; 0.64)	2.74 (-2.5; 7.99)	1.8 (-1.33; 4.92)	0.16 (-0.15; 0.46)*	2.29 (-2.06; 6.65)*
α-Terpinene	0.08 (-0.08; 0.24)	0.06 (-0.03; 0.15)	0.05 (0.01; 0.08)*	0.03 ($8\cdot10^{-4}$; 0.06)*	0.13 (-0.03; 0.29)	0.09 (-0.01; 0.20)	0.04 ($6\cdot10^{-3}$; 0.07)***	0.11 (-0.03; 0.25)***
limonene	2.30 (-1.50; 6.10)	1.86 (-0.47; 4.18)	1.01 (0.45; 1.57)	0.79 (0.27; 1.31)	2.65 (0.20; 5.10)	2.65 (0.20; 5.10)	0.90 (0.36; 1.45)***	2.99 (-0.27; 6.26)***
β-Phellandrene	9.38 (-0.83; 19.59)	9.76 (-4.07; 23.58)	3.94 (2.04; 5.84)	3.99 (1.95; 6.03)	13.32 (2.50; 24.14)	13.74 (-0.31; 27.80)	3.96 (2.01; 5.92)***	13.52 (1.14; 25.90)***
γ-Terpinene	0.22 (-0.14; 0.59)	0.14 (-0.01; 0.29)	0.11 (0.04; 0.17)	0.09 (0.01; 0.16)	0.33 (-0.04; 0.70)	0.23 (0.03; 0.42)	0.19 (0.07; 0.30)***	0.72 (0.21; 1.24)***
Terpinolene	0.63 (0.08; 1.18)	0.44 (0.05; 0.83)	0.20 (0.09; 0.32)	0.16 (0.06; 0.26)	0.83 (0.26; 1.41)	0.61 (0.18; 1.03)	0.19 (0.07; 0.30)	0.72 (0.21; 1.24)
Total	*121.26 (-20.68; 263.20)*	*124.30 (-4.88; 253.47)*	*71.79 (41.13; 102.44)*	*65.38 (35.11; 95.66)*	*121.69 (14.84; 228.54)*	*117.35 (37.57; 197.13)*	*68.72 (38.30; 99.14)****	*191.44 (51.78; 331.10)****
Oxygenated monoterpenes								
Eucalyptol	0.46 ($-4\cdot10^{-4}$; 0.92)	0.49 (-0.09; 1.06)	0.01 ($5\cdot10^{-3}$; 0.02)	0.01 ($4\cdot10^{-3}$; 0.02)	0.47 (0.01; 0.93)	0.50 (-0.08; 1.08)	0.01 ($5\cdot10^{-3}$; 0.02)***	0.49 (-0.03; 1.00)***
cis-4-Thujanol	0.05 (-0.04; 0.14)	0.05 (-0.02; 0.12)	0.02 ($3\cdot10^{-3}$; 0.04)	0.02 ($3\cdot10^{-3}$; 0.04)	0.07 (-0.02; 0.16)	0.07 ($-6\cdot10^{-3}$; 0.15)	0.02 ($5\cdot10^{-3}$; 0.04)***	0.07 (-0.01; 0.16)***
isopinocamphone	0.03 (-0.01; 0.07)	0.06 (-0.02; 0.12)	0.01 ($5\cdot10^{-3}$; 0.02)	0.01 ($4\cdot10^{-3}$; 0.02)	0.04 ($4\cdot10^{-3}$; 0.08)	0.07 ($-2\cdot10^{-4}$; 0.14)	0.01 ($5\cdot10^{-3}$; 0.02)***	0.06 ($-9\cdot10^{-4}$; 0.11)***

VOC name	**Production (T – C)**		**Control**		**Treatment**		**All trees**	
	LTS	**Reference**	**LTS**	**Reference**	**LTS**	**Reference**	**Control**	**Treatment**
_1_	_2_	_3_	_4_	_5_	_6_	_7_	_8_	_9_
α-Terpineol	0.17 (-0.27; 0.61)	0.07 (-0.08; 0.22)	0.02 ($7\cdot10^{-3}$; 0.02)	0.03 (-0.07; 0.14)	0.19 (-0.25; 0.62)	0.10 (-0.01; 0.21)	0.02 (-0.05; 0.1)***	0.14 (-0.18; 0.47)***
trans-Pinocarvyl acetate	0.02 (0.03; 0.07)	0.04 (-0.03; 0.10)	0.01 ($-1\cdot10^{-4}$; 0.04)	0.01 ($5\cdot10^{-3}$; 0.02)	0.03 (-0.01; 0.08)*	0.05 (-0.01; 0.11)*	0.01 ($4\cdot10^{-3}$; 0.02)***	0.04 (-0.01; 0.10)***
Total	_0.73 (-0.04; 1.51)_	_0.70 (-0.05; 1.46)_	_0.07 (0.04; 0.11)_	_0.09 (-0.03; 0.20)_	_0.81 (0.04; 1.58)_	_0.79 (0.05; 1.52)_	_0.08 ($-2\cdot10^{-3}$; 0.16)_***	_0.79 (0.05; 1.55)_***
Sesquiterpenes								
α-Cubebene	0.17 (-0.27; 0.61)	0.07 (-0.08; 0.22)	0.04 ($7\cdot10^{-3}$; 0.08)	0.04 (-0.01; 0.01)	0.09 (-0.06; 0.24)	0.12 (-0.05; 0.28)	0.04 (-0.01; 0.09)***	0.1 (-0.05; 0.09)***
Longipinene	0.3 (-0.45; 1.05)	0.17 (-0.03; 0.38)	0.09 ($7\cdot10^{-3}$; 0.18)	0.11 (-0.01; 0.22)	0.39 (-0.38; 1.17)	0.28 (0.02; 0.54)	0.1 ($-2\cdot10^{-3}$; 0.2)***	0.34 (-0.25; 0.93)***
α-Ylangene	0.11 (-0.10; 0.31)	0.17 (-0.10; 0.43)	0.05 ($-8\cdot10^{-4}$; 0.10)	0.07 ($-7\cdot10^{-4}$; 0.14)	0.15 (-0.07; 0.37)	0.23 (-0.06; 0.53)	0.06 ($-2\cdot10^{-3}$; 0.12)***	0.19 (-0.07; 0.45)***
β-Elemene	0.05 (-0.10; 0.20)	0.03 (-0.02; 0.08)	0.02 ($6\cdot10^{-3}$; 0.04)	0.02 ($6\cdot10^{-4}$; 0.05)	0.07 (-0.08; 022)	0.05 ($-9\cdot10^{-3}$; 0.11)	0.02 ($4\cdot10^{-3}$;0.04)*	0.06 (-0.06; 0.18)*
α-Gurjunene	0.08 (-0.15; 0.32)	0.21 (-0.19; 0.62)	0.07 (-0.03; 0.18)	0.10 (-0.03; 0.23)	0.15 (-0.13; 0.45)	0.31 (-0.16; 0.78)	0.09 (-0.03; 0.20)	0.23 (-0.16; 0.62)
β-Ylangene	0.12 (-0.16; 0.40)*	0.28 (-0.15; 0.70)*	0.10 ($4\cdot10^{-3}$; 0.19)	0.12 (-0.02; 0.26)	0.22 (-0.11; 0.90)	0.40 (-0.11; 0.90)	0.11 (-0.01; 0.22)**	0.30 (-0.11; 0.71)**
Caryophyllene	0.69 (-0.87; 2.25)	0.55 (-0.26; 1.36)	0.41 (0.06; 0.76)	0.34 (0.04; 0.64)	1.09 (-0.53; 2.72)	0.89 (-0.03; 1.80)	0.38 (0.05; 0.7)***	1.0 (-0.34; 2.3)***
Germacrene D	0.08 (-0.23; 0.38)	0.01 (-0.04; 0.05)	0.02 ($-2\cdot10^{-3}$; 0.05)	0.02 ($-5\cdot10^{-3}$; 0.05)	0.10 (-0.21; 0.41)	0.03 (-0.01; 0.07)	0.02 ($-3\cdot10^{-3}$; 0.05)	0.07 (-0.16; 0.29)
Humulene	0.31 (-0.30; 0.92)	0.19 (-0.1; 0.47)	0.19 (-0.04; 0.42)	0.14 (0.02; 0.26)	0.50 (-0.16; 1.15)	0.33 ($-2\cdot10^{-3}$; 0.65)	0.16 (-0.02; 0.35)***	0.42 (-0.11; 0.94)***
X46 – unidentifiable	$5\cdot10^{-3}$ (-0.01; 0.03)*	0.01 (-0.01; 0.04)*	0.01 ($5\cdot10^{-3}$; 0.02)	0.01 ($6\cdot10^{-3}$; 0.02)	0.02 ($-2\cdot10^{-3}$; 0.04)*	0.03 ($3\cdot10^{-3}$; 0.05)*	0.01 ($6\cdot10^{-3}$; 0.02)**	0.02 ($2\cdot10^{-4}$; 0.04)**
Total	_1.79 (-1.46; 5.04)_	_1.69 (-0.47; 3.84)_	_1.00 (0.38; 1.62)_	_0.98 (0.27; 1.69)_	_2.79 (-0.45; 6.03)_	_2.66 (0.29; 5.04)_	_0.99 (0.33; 1.65)_***	_2.73 (-0.11; 5.57)_***
Oxygenated sesquiterpenes								
epicubenol	0.04 (-0.07; 0.15)*	0.08 (-0.1; 0.26)*	0.04 (-0.01; 0.09)	0.05 (-0.04; 0.15)	0.08 (-0.05; 0.21)	0.13 (-0.06; 0.32)	0.05 (-0.03; 0.12)	0.1 (-0.06; 0.27)
Total	_0.04 (-0.07; 0.15)_*	_0.08 (-0.1; 0.26)_*	_0.04 (-0.01; 0.09)_	_0.05 (-0.04; 0.15)_	_0.08 (-0.05; 0.21)_	_0.13 (-0.06; 0.32)_	_0.05 (-0.03; 0.12)_	_0.1 (-0.06; 0.27)_
Benzene derivate								
p-Cymene	0.29 (-0.27; 0.85)	0.20 (-0.06; 0.46)	0.06 (0.02; 0.11)	0.06 (-0.02; 0.14)	0.35 (-0.20; 0.91)	0.26 (-0.03; 0.55)	0.06 ($-3\cdot10^{-3}$; 0.13)***	0.31 (-0.14; 0.76)***
Methylthymol	0.02 (-0.02; 0.06)	0.03 (-0.02; 0.08)	0.02 ($8\cdot10^{-3}$; 0.03)	0.02 ($6\cdot10^{-3}$; 0.03)	0.04 ($-3\cdot10^{-3}$; 0.08)	0.05 ($-2\cdot10^{-3}$; 0.10)	0.02 (0.01; 0.03)***	0.04 ($-2\cdot10^{-3}$;0.09)***

VOC name	**Production (T – C)**		**Control**		**Treatment**		**All trees**	
	LTS	**Reference**	**LTS**	**Reference**	**LTS**	**Reference**	**Control**	**Treatment**
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Total	*0.31 (-0.26; 0.89)*	*0.23 (-0.07; 0.53)*	*0.08 (0.03; 0.14)*	*0.08 (-0.01; 0.17)*	*0.40 (-0.17; 0.96)*	*0.31 (-0.02; 0.64)*	*0.08 (0.01; 0.15)******	*0.15 (-0.11; 0.82)******
Terpene ester								
Bornyl acetate	0.26 (-0.43; 0.94)	0.11 (-0.02; 0.24)	0.05 ($-9\cdot10^{-3}$; 0.11)	0.04 (0.01; 0.06)	0.31 (-0.39; 1.01)	0.14 ($7\cdot10^{-3}$; 0.28)	0.04 (-0.005; 0.09)***	0.23 (-0.29; 0.74)***
Total	*0.26 (-0.43; 0.94)*	*0.11 (-0.02; 0.24)*	*0.05 ($-9\cdot10^{-3}$; 0.11)*	*0.04 (0.01; 0.06)*	*0.31 (-0.39; 1.01)*	*0.14 ($7\cdot10^{-3}$; 0.28)*	*0.04 (-0.005; 0.09)******	*0.23 (-0.29; 0.74)******
Total VOCs	***124.39 (-21.14; 269.92)***	***127.10 (-4.87; 259.07)***	***73.04 (41.91; 104.16)***	***66.62 (35.77; 97.47)***	***197.43 (46.27; 348.59)***	***193.71 (58.29; 329.15)***	***69.97 (39.03; 100.90)******	***195.65 (52.83; 338.47)******
Unknown compound (X94)	0.29 (-0.12; 0.71)	0.30 (-0.16; 0.78)	0.06 ($4\cdot10^{-3}$; 0.11)**	0.12 (-0.02; 0.25)**	0.35 (-0.06; 0.76)	0.43 (-0.04; 0.89)	0.09 (-0.02; 0.19)***	0.39 (-0.05; 0.82)***

Note: Compound values are presented by the abundance of specific mass from mass spectra of variable divided by the abundance of specific mass from the mass spectrum of an internal standard (1-Bromodecane). The numbers in brackets show ± SD from the mean. *** p<0.001; ** p<0.01; * p<0.05.

To better inform our understanding of chemical tree survival factors, we fitted a RF model with the absolute differences between C and T sample responses (production). The model yielded nine terpenes (not shown) that best predicted survival status according to the mean decrease in the Gini coefficient, estimating classification purity. The best-ranking predictor was an absolute change in epicubenol. RF run on the normalized sums of VOCs aggregated by a chemical class showed that the most important covariate was again the normalized sum of oxygenated sesquiterpenes, a class presented by only one compound (epicubenol) that ranked first in the above-mentioned RF model.

3.1.8 Tree survival modelling results in the Šumava NP

We used the non-linear logistic regression models to quantify the effects of the most influential tree, stand and environmental factors on spruce survival. The best regression model incorporated the non-linear effects of tree size, stand density, self-shading, and long-term climatic water balance (see Table 2 in Korolyova et al., 2022a). The predicted survival probabilities were unbiased and accounted for approximately 78% of the variation observed in the dataset, as measured by a pseudo-R^2 statistic (Fig. 10).

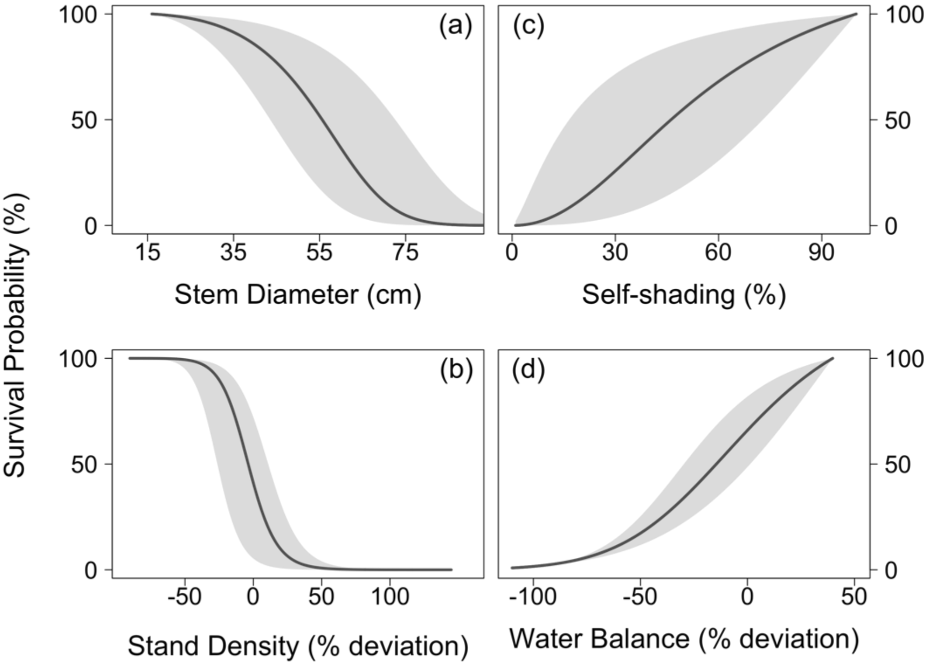

Fig. 9 Predicted tree survival probability during severe bark beetle outbreak in the Bohemian Forest study area. Survival probability was calculated using non-linear logistic regression models fitted in a likelihood framework. The models were fitted with tree size (DBH, panel a), stand (stand density, panel b), crown architecture (self-shading, panel c), and environmental (seasonal 30-year climatic water

balance (CWB), panel d) data for 184 surviving (LTS) and 414 reference trees. Self-shading is a proportion of the crown length in the total tree height. CWB was quantified as the difference between precipitation and PET.

Larger diameter trees were more prone to infestation (Fig. 9, panel a). Severe stand competition for limited resources prior to the outbreak was associated with a steep decline in survival probability (Fig. 9, panel b). In contrast, survival increased monotonically in response to growing self-shading and seasonal water availability (Fig. 9, panels c and d, respectively), indicating the buffering effects of these covariates on tree resistance to herbivores. The model predictions were unbiased, with a slope of ~1.0 between the predicted and observed values (Fig. 10).

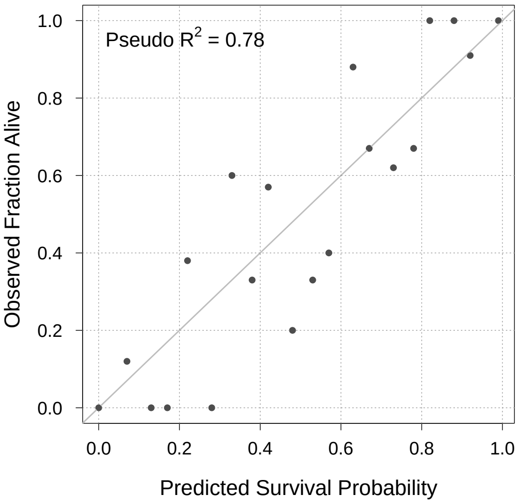

Fig. 10. The goodness of fit of the logistic regression models of tree survival during severe bark beetle outbreak in the Bohemian Forest study area. The diagonal reference line represents the perfect model fit corresponding to a 1:1 ratio of observed vs. predicted values of survival probability.

Interactions among predictor variables demonstrate that moisture limitation modifies survival responses, aggravating the negative effects of larger tree size (Fig. 11, panel a) and more severe resource competition (Fig. 11, panel b) on survival capacity, and amplifying the positive effects of self-shading on tree resistance under high water availability (Fig. 11, panel c). However, with the exception of the stand density effects, the support intervals exhibited substantial overlap among the covariates.

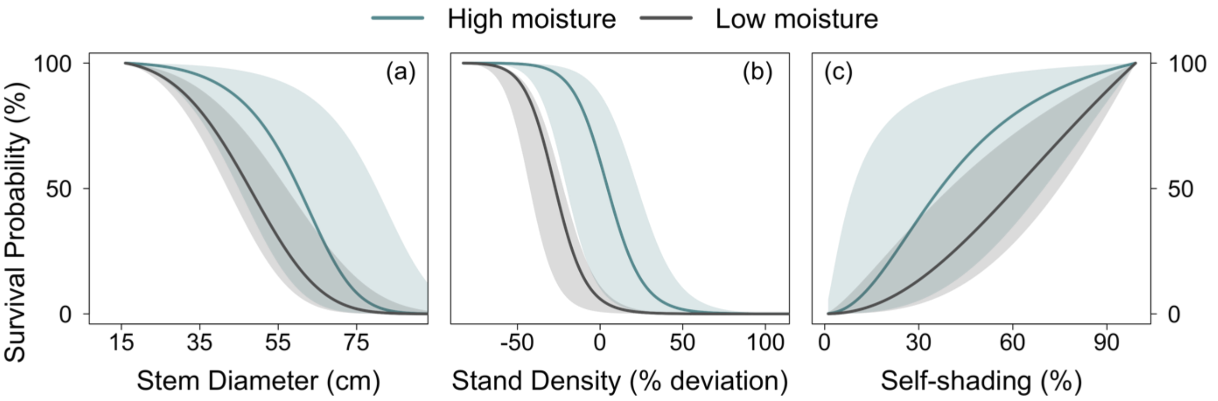

Fig. 11. Interactions between tree- and stand-level factors of tree survival during a bark beetle outbreak in the Bohemian Forest study area. Survival responses are quantified using non-linear logistic regression models. Survival potential influenced by tree size (panel a), competition (panel b), and self-shading (panel c) is shown for conditions of high (light turquoise) and low (grey) moisture availability. The shaded regions delineate support intervals that are similar to 95% confidence intervals, showing the degree of result reliability.

3.2 Spruce survival during bark beetle outbreak in the Vosges mountains

3.2.1 Changes in phenolic content in response to mechanical wounding in the Vosges mountains

To investigate the chemical factors of Norway spruce resistance to *I. typographus* in the Vosges mountains study area, we conducted a mechanical wounding experiment during an ongoing bark beetle outbreak in a mature natural stand. As revealed by High Performance Liquid Chromatography (HPLC), the phenolic compound contents in the phloem responded differently to the agar inoculation. In response to the treatment, piceid and astringin contents significantly decreased compared to their initial concentrations observed 14 days prior to the treatment (Fig. 12, panels b and c). In contrast, the content of (+)-catechin exhibited a substantial increase from 9.6 to 18.2 mgAE g^{-1} of phloem (Fig. 12, panel a). However, taxifolin and isorhapontin did not demonstrate pronounced dynamics in response to the treatment.

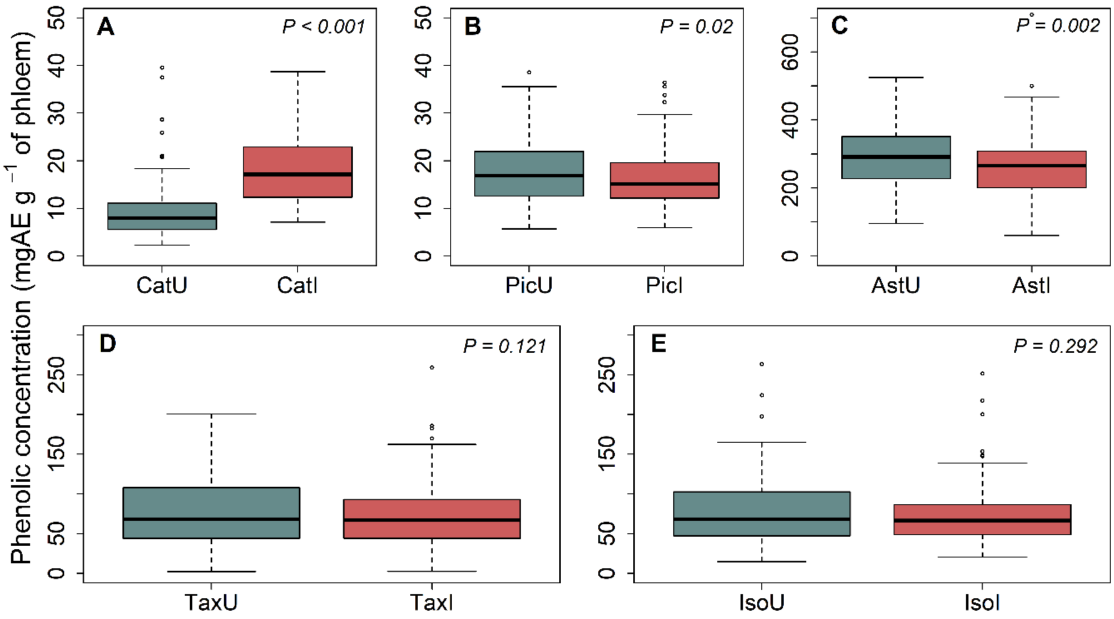

Fig. 12 Phenolic responses of Norway spruce trees to agar gel inoculation conducted in the Vosges mountains study area. The letter “U” stands for uninoculated samples, while the letter “I” refers to inoculated phloem. (+)-catechin (Cat), piceid (Pic), astringin (Ast), taxifolin (Tax), and isorhapontin (Iso) are five phenolics identified using HPLC analysis.

3.2.2 Phenolic profiling of non-attacked vs beetle-attacked Norway spruce trees

Interestingly, phenolic concentrations and dynamics of phenolic production did not differ between attacked and non-attacked Norway spruce trees (Fig. 13), except for catechin content measured 14 days after the agar treatment, which was significantly lower (p = 0.006) in individuals that escaped herbivore (mean = 17 mgAE g^{-1} of phloem) than in beetle-killed trees (mean = 21.5 mgAE g^{-1} of phloem) (Fig. 13, panel c). The variance of catechin content change in response to inoculation was significantly larger in dead than in surviving trees (p < 0.001) (Fig. 13, panel a).

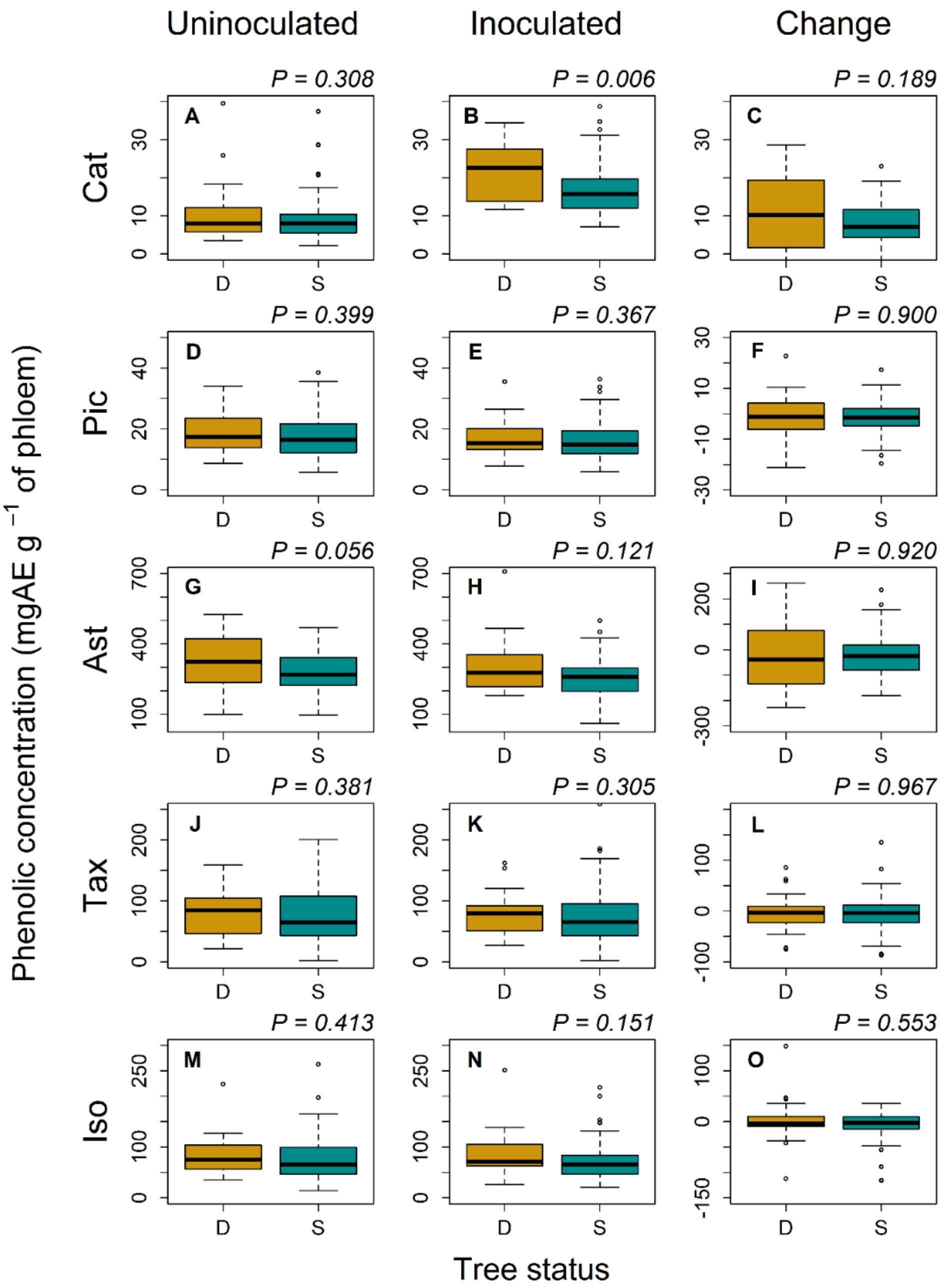

Fig. 13 Boxplots showing phenolic responses in beetle-killed vs surviving trees. The concentrations of five identified phenolic compounds (three stilbenes: piceid (Pic), astringin (Ast), and isorhapontin (Iso), and two flavonoids: (+)-catechin (Cat) and taxifolin (Tax)) were measured in untreated (Uninoculated) and treated (Inoculated) phloem samples. Additionally, the production of phenolic compounds in response to agar gel inoculation is shown (Change). Statistically significant differences were manifested only in catechin content in inoculated phloem (panel b).

3.2.3 The differences in crown and site characteristics among primarily attacked, secondarily attacked and surviving trees in the Vosges mountains

To gain deeper insights into spruce resistance factors and bark beetle host selection, we examined differences in crown and site characteristics among trees with varying statuses (surviving, primarily, and secondarily beetle-attacked). Significant differences in spatial locations were found among surviving, primarily, and secondarily attacked trees ($p < 0.001$) (Fig. 14, panel a). The mean distance to the nearest infested individual for surviving trees (30 m) was threefold the mean distance measured for beetle-killed individuals (Table 1 in Korolyova et al., 2022b). Mean distances to the nearest infested tree significantly differed between primarily and secondarily colonized trees ($p = 0.03$) (Fig. 14, panel a). Primarily attacked trees were, on average, five times farther (16 m) from the nearest colonized tree than secondarily attacked individuals (5 m) (Table 1 in Korolyova et al., 2022b). Post-hoc Dunn's test indicated significant differences in the distance from a target tree to the nearest attacked individual among all groups of trees (Fig. 15, panel a). Surviving trees were located farther from the previously attacked trees than primarily ($p = 0.01$) and secondarily ($p < 0.001$) attacked individuals. Primarily attacked trees grew closer to the previously infested spruces than the secondarily attacked individuals ($p = 0.03$). A considerable range of crown defoliation was observed when comparing non-attacked trees (mean = 0.19) to primarily attacked trees (mean = 0.46), indicating significant variability ($p = 0.002$). Similarly, trees that were the primary beetles' targets had higher total defoliation ratios than secondarily infested (mean = 0.20) individuals ($p = 0.02$) (Fig. 14, panel b). The ratio of primary crown defoliation varied significantly between surviving trees and those primarily attacked ($p = 0.022$) (Fig. 14, panel c).

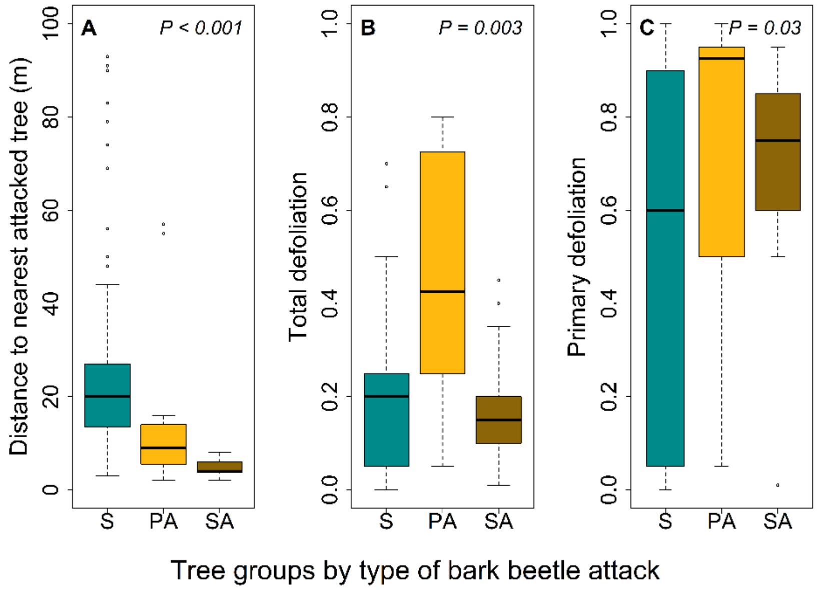

Fig. 14 Comparisons of site and tree morphological characteristics were conducted among surviving (S, non-attacked), primarily attacked (PA), and secondarily attacked (SA) individuals. Tree groups categorized by the type of bark beetle attack are depicted in brown, yellow, and turquoise. Primary attack is defined as the successful beetle colonization of a host tree in the absence of previously attacked neighbouring trees. Secondarily attacked trees were infested subsequent to the primary attack between 10 and 23 weeks after the mechanical wounding treatment. Distance (panel a) was measured from each target tree to the nearest colonized neighbouring tree. Crown defoliation refers to a missing portion of a productive crown, with primary defoliation indicating missing primary shoots in the productive part of the tree crown (for more details, see Table A1 in Korolyova et al., 2022b). P-values in panels (a) and (b) were obtained using non-parametric Kruskal-Wallis tests used to analyse groups with unequal numbers of observations. The significance reported in panel (c) was quantified using a one-way ANOVA applied to the Box-Cox-transformed data.

Contrary to our expectation that larger trees would be more susceptible to beetle mass attacks, tree size (DBH) did not emerge as a relatively strong survival predictor. The mean DBH of resistant trees was smaller than that of beetle-killed trees, but the difference was statistically insignificant. Our preliminary models indicated that the sun-illuminated stem ratio was a better survival predictor compared to self-shading, calculated as a ratio of crown length to total tree height.

3.2.4 Variations in chemical and morphological parameters among primarily and secondarily attacked trees

We observed significant differences in uninoculated phloem taxifolin concentration between primarily attacked (mean = 101 mgAE g^{-1} of phloem) and secondarily attacked (mean = 64 mgAE g^{-1} of phloem) trees (p = 0.013) (Fig. 15, panel a). The juvenile crown ratio was found to be significantly lower in primarily attacked trees (mean = 0.07) compared to secondarily attacked individuals (mean = 0.10) (p = 0.023) (Fig. 15, panel b).

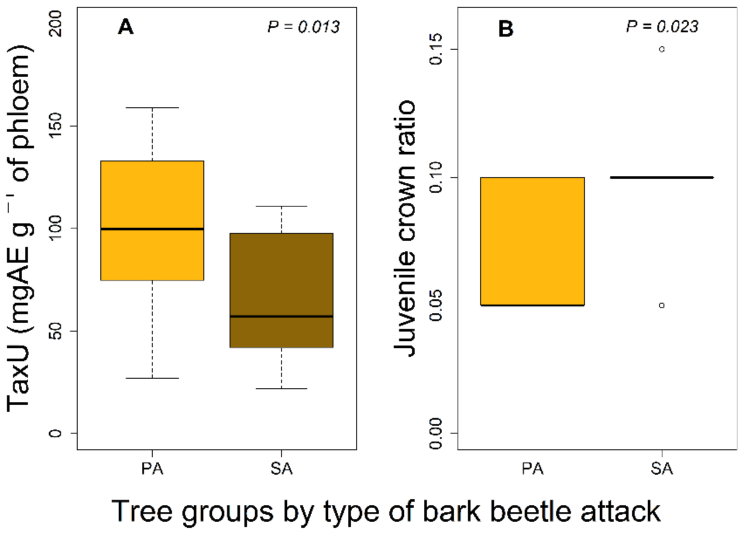

Fig. 15 The differences in phenolic profile (panel a) and crown architecture (panel b) between primarily attacked and secondarily bark beetle attacked spruce trees. TaxU stands for taxifolin concentration in the uninoculated phloem. Juvenile crown ratio refers to the proportion of a young growing part of a crown located at the top of a tree (Cudlín, 2015).

3.2.5 The key drivers of tree survival in the Vosges mountains

To better inform our selection of best predictors of spruce survival in the Vosges mountains study area, we performed the Random Forests classification, similarly as we did for the Bohemian Forest study area. In the Vosges mountains study area, the RF analysis yielded a mean out-of-bag error rate of 19.9%, indicating the overall accuracy of the classification model. The classification error rates for LTS and beetle-infested trees were found to be below 14.7% and 23.8%, correspondingly. Among the variables considered in the analysis, the proximity of a target tree to the nearest attacked tree was classified as the most informative predictor of tree survival (Fig. 16). To select the most relevant predictors of tree survival probability, we used the mean decrease in the Gini coefficient. The first nine covariates ranked as potentially informative predictors included DBH, proportion of illuminated stem, production (absolute change) of (+)-catechin concentration, (+)-catechin content in inoculated phloem, self-shading ratio, primary crown defoliation ratio, crown defoliation ratio, and isorhapontin content in inoculated phloem, as shown in the variable importance plot (Fig. 16). According to the RF model, these predictors exhibited the highest associations with tree survival responses.

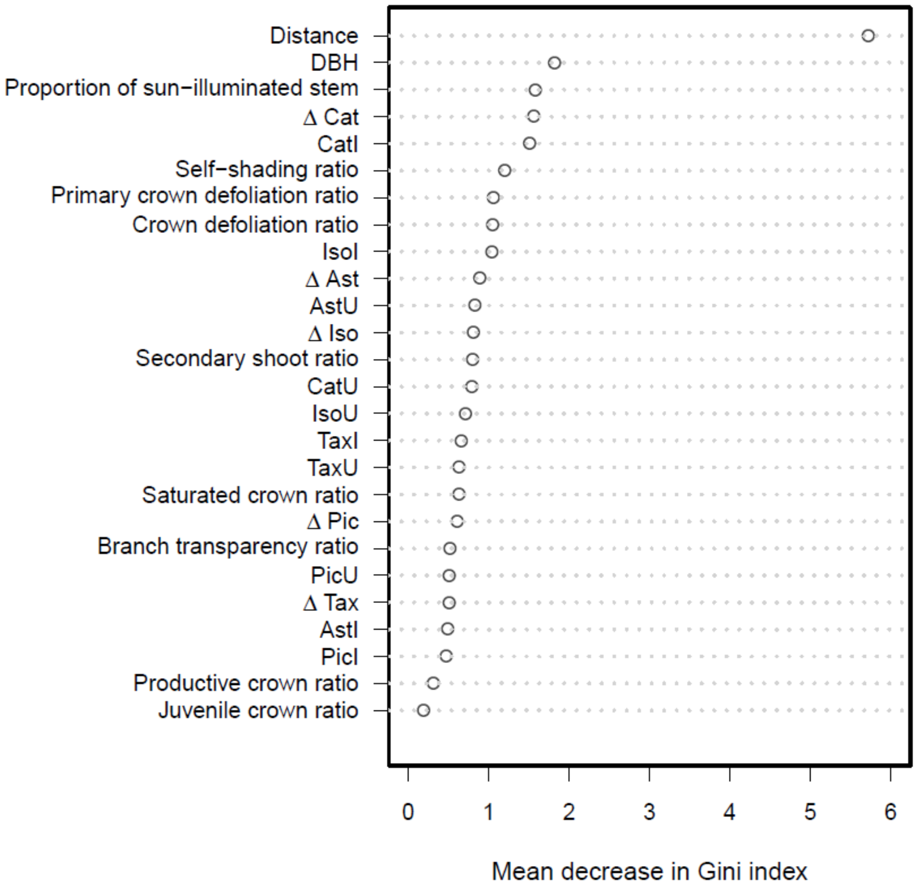

Fig. 16 Random Forests classification results showing comparative importance of 26 explanatory variables in predicting tree survival in the Vosges mountains study area. Variable importance was determined based on the influence of each variable on the resulting class purity, quantified using the Gini index. The predictor variables included in the analysis comprised the concentrations of phenolic compounds (catechin (Cat), astringin (Ast), isorhapontin (Iso), piceid (Pic), and taxifolin (Tax)), site characteristics, and tree morphological and crown architecture parameters. The letters “U” and “I” stand for uninoculated and inoculated phloem, respectively; Δ denotes production of the respective compound in response to mechanical wounding (post-treatment minus pre-treatment concentration).

3.2.6 Tree survival modelling results in the Vosges mountains

We observed the non-linear effects of site, morphological, and chemical factors of Norway spruce survival during the bark beetle outbreak in the Vosges mountains study area. The best regression model explained 71.1% of variability of survival responses and was unbiased (Fig. 17). Contrary to our expectations, we did not detect any evidence for lower resistance to bark beetle attacks in larger trees, as incorporating DBH into a regression model did not improve its predictive power (not shown).

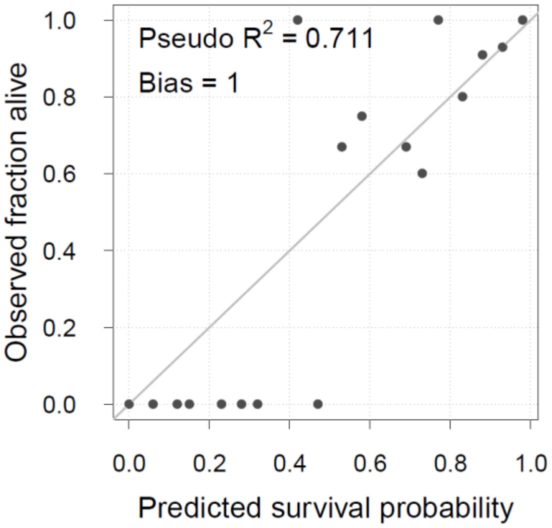

Fig. 17 The goodness of fit of tree survival logistic regression models fit for 96 experimental Norway spruce trees located in the Vosges mountains study area. The diagonal line represents the perfect model fit corresponding to 1:1 ratio of observed vs. predicted values of tree survival probability. Observed survival probability was calculated using the pseudo-R^2 approach (see the Methods).

Survival responses to tree-level and stand effects were non-linear and varied in shape of the corresponding curves (Fig. 18). According to our expectations, decreasing proximity to bark beetle source substantially constrained survival potential. If the distance from a target tree to a previously attacked individual was below 25 m, survival probability of a target tree steeply declined (Fig. 18, panel a). Elevated levels of stem sun exposure and crown defoliation negatively affected tree resistance capacity (Fig. 18, panels b and c, respectively), supporting our physiological stress-based survival hypotheses. As anticipated, increasing production of (+)-catechin was associated with significant positive shift in survival probability (Fig. 18, panel d) that levelled off and showed downward trend after the absolute change in catechin concentration crossed a certain threshold. However, decline in survival probability when catechin production continued to increase was accompanied by the wide support limits, and is therefore unclear.

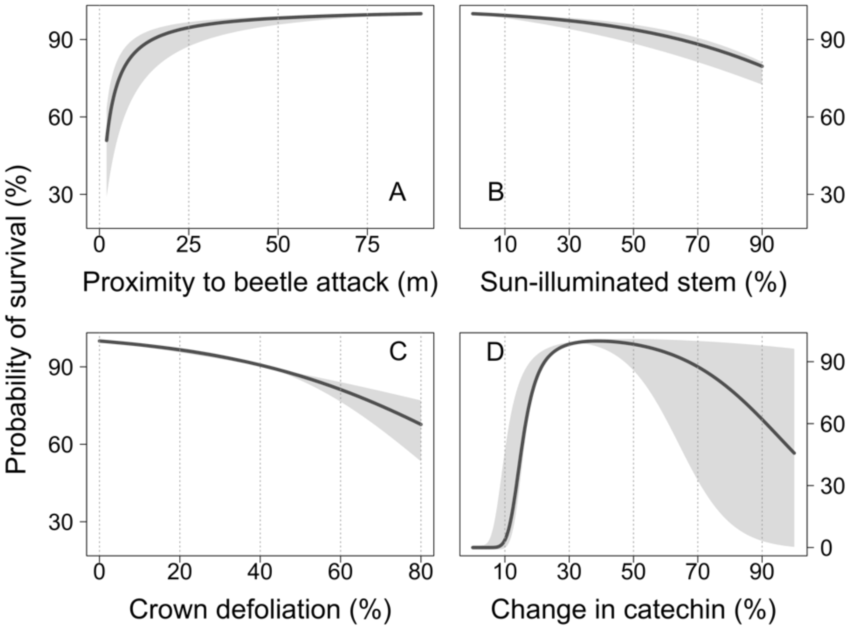

Fig. 18 Predicted tree survival probability during severe bark beetle outbreak in the Vosges mountains study area. Survival responses were derived from the non-linear logistic regression models fit using annealing, a global optimisation algorithm (Goffe et al., 1994). Survival probability is a maximum potential survival decreased by the potential effect of each predictor covariate, which were formulated in a unitless scalar forms: distance to the previously attacked neighbouring tree (panel a), proportion of sun-illuminated stem (panel b), crown defoliation (panel c), and production of (+)-catechin in response to agar gel inoculation (panel d). The model was fit with the data for 71 surviving and 25 bark beetle-killed trees. The shaded regions delineate support intervals that correspond to 95% confidence intervals in the parametric models.

IV. DISCUSSION

Our research has shown that spruce survival during bark beetle outbreak is a non-random process governed by a wide range of multiscale ecological and ontogenic factors, and their numerous diverse interactions. The results of the MJ inoculation experiment identified the differences in the concentrations of individual chemical compounds between surviving and beetle-killed trees, and among primarily attacked, secondarily attacked, and surviving spruces. We found significant variability between LTS and reference trees in individual volatile compounds omnipresent (constitutive defence) or synthesized in trees in response to mechanical wounding or MJ treatment (induced defence). Production rates of phenolic catechin turned out to be important factor in tree survival mechanism. However, overall chemical responses to manipulated stressors were diversified. The total and primary crown defoliation was indicative of tree physiological condition that modified tree defence capacities. Self-shading and stem sun exposure were strong insolation-related predictors of spruce resistance to *I. typographus*. Our modelling results provided evidence for stand and environmental effects on tree survival, such as stand density prior to the outbreak, proximity to a previously attacked host, and a long-term water availability. Sustained drought aggravated the negative competition and tree size effects. In contrast, water availability enhanced the positive effects of self-shading on tree survival. Historical radial growth rates differentiated beetle-killed dead and surviving trees, indicating that life-history trade-offs in allocation of limited resources modify tree defence potential.

4.1 Stand competition

The regression analyses confirm our hypothesis that competition among neighbouring trees has a detrimental impact on the host tree survival. An increase in pre-disturbance stand density is associated with a decrease in tree survival rates (Fig. 12, panel b). These findings are consistent with previous studies (Buonanduci et al., 2020), although not all previous research (Koontz et al., 2021) aligns with these results. Stand competition for limited resources may have a negative effect on photosynthetic performance and the availability of non-structural carbohydrates (NCS), other factors held constant. NCS compounds are essential for the synthesis of secondary metabolites associated with both constitutive and induced defence mechanisms (Huang et al., 2020a). Carbon limitation has been reported as a potential underlying factor of drought-related tree mortality and may also influence tree susceptibility to bark beetles (McDowell, 2011). However, the factors that regulate NSC allocation to different physiological functions, such as growth and defence, during periods of environmental stress are not well quantified for mature forest trees. Thus, the use of experimental approaches is necessary to better understand the underlying physiological processes and their effects on tree defence capacity (Hartmann and Trumbore, 2016; Huang et al., 2020a).

Tree survival partly governed by neighbourhood competition can be influenced by disturbance dynamics. Previous studies have identified significant correlations between beetle colonization rates of trees and the presence of wind-damaged hosts and blowdown events (Komonen et al., 2011; Schroeder and Lindelöw, 2002). These findings agree with the carbon limitation hypothesis, suggesting that beetles may preferentially select hosts with acute structural damage, indicating compromised defence mechanisms (Kausrud et al., 2012). Disturbances such as small-scale wind events, insect infestations, or forest management activities that create canopy gaps within forest interiors can increase the availability of potential host substrates by generating deadwood and causing injuries to the neighbouring undamaged trees located in close proximity to the gap edges. In addition to the direct effects of physical injuries, the formation of canopy gaps can alter the carbon-based defence capacity of undamaged trees by modifying the microclimate conditions. Canopy removal leads to increased solar radiation levels, resulting in elevated local temperatures and decreased humidity levels (Hais and Kučera, 2008). These altered microclimates can negatively impact the physiological processes of trees adapted to shaded environments (Schroeder and Lindelöw, 2002). The temperature-induced physiological stress experienced by trees adjacent to or within disturbance gaps may facilitate the successful tree colonization by the beetles. Thus, disturbance gaps and their associated edges can act as focal points that enhance beetle proliferation (Kautz et al., 2013).

As NDVI did not significantly improve the predictive power of the model, we replaced it with the bands TM3 and TM4, which yielded stronger models based on AICc, disagreeing with prior findings reported in the existing literature (e.g., Sivanpillai et al., 2006). Incorporating TM5 band (mid-infrared) substantially improved productive power of the model thanks to the increased energy absorption in TM band 5, which becomes more pronounced as stands develop complex architectural features, such as shadows, gaps as suggested by Jakubauskas (1996).

4.2 Tree size

A carbon allocation based hypothesis can partially explain the observed by us in the Šumava NP and previously reported (e.g., Jakuš et al., 2011; Six et al., 2018b) patterns of increased survival potential in dominant, large-diameter trees within forest ecosystems. Previous studies have found associations between tree canopy dominance, competition hierarchies, and the light use efficiency (Gspaltl et al., 2013). Particularly, it has been documented that large upper-canopy trees outcompete smaller neighbouring trees for light and soil moisture resources. The dominant trees demonstrate a higher resource use efficiency, indicated by higher biomass production per unit of absorbed photosynthetically active radiation (Gspaltl et al., 2013). Thus, it can be expected that dominant trees, which effectively acquire and use light resources for carbon assimilation, would exhibit an enhanced defence capacity, assuming other factors such as beetle population densities remain constant (Waring and Pitman, 1985).

In our survival analysis conducted in the Vosges mountains, we anticipated to detect a higher vulnerability of larger diameter trees to beetle attacks. This expectation was based on substantial previous evidence indicating that larger host trees provide more abundant food resources for expanding beetle populations, rendering them more susceptible to beetle-caused mortality (e.g., Buonanduci et al., 2020; Chisholm et al., 2021; Koontz et al., 2021; Mezei et al., 2019, 2014; Morris et al., 2022; Seidl et al., 2011). However, in our study, we found that the mean DBH of resistant trees was smaller than that of trees killed by beetles. The lack of statistical significance in this difference, along with the small sample size and the limited range of DBH values among the dead trees, may explain why incorporating tree size did not enhance the informative power of the regression model.

4.3 Proximity to a bark beetle source

Our study provides compelling evidence of a clear inverse relationship between tree survival probability and the proximity to the nearest bark beetle source (previously attacked trees), confirming the hypothesis that distance plays a prominent role in shaping survival outcomes. The regression model (Fig. 18, panel a) and statistical comparisons (Fig. 14, panel a) demonstrate the distinguishable survival patterns between trees located in close proximity to beetle-infested individuals and non-infested trees. The survival rates decline exponentially for trees growing within a 25-meter radius of another beetle-killed tree, controlling for other factors (Fig. 18, panel a). These results agree with previous research (Jakuš et al., 2011a; Kautz et al., 2014, 2011; Lausch et al., 2011; Seidl et al., 2011) that provide evidence for an inverse pattern between distance to a beetle source and colonization probability.

The distance covered by beetles before selecting a potential host is influenced by their energy level, the attractiveness of specific host, and the beetles' host selection choice (Kautz et al., 2014). Various factors, such as tree physiology, ontogeny, stand composition, spatial distribution, and site-level characteristics, including proximity to forest edges or areas of recent disturbances, can influence the dispersal patterns and distances that beetles are able to overcome (Jakuš et al., 2011a). The flight capacity of *I. typographus* is constrained by the rapid depletion of proline, an essential energy source for flight activity, which may explain their tendency to select hosts in closer proximity to the previously colonized individuals (Schilling et al., 2012). As population density increases, beetles can afford to travel shorter distances, targeting more vigorous neighbouring hosts and potentially switching to adjacent trees to reduce competition for feeding substrate (Johnson and Coster, 1978; Schlyter and Anderbrant, 1989). These findings contribute to our understanding of the complex dynamics between tree survival, beetle infestation, and spatial factors, emphasizing the importance of distance as one of the key determinants in the “host tree – bark beetle” interactions.

4.4 Environmental factors of tree survival during bark beetle outbreak

Projected increases in mean global temperatures, climate extremes, and frequency of drought compromise host plant defence physiology and predispose ecosystems to progressively more intense herbivory pressure (Bentz et al., 2019; Janda et al., 2014; Schiebe et al., 2011; Thom et al., 2017). Warming temperatures have been shown to amplify the voltinism of bark beetles, leading to increased reproductive rates and range expansion (Jakoby et al., 2019). Recent research indicates an anticipated increase in the frequency of prolonged drought periods in Central Europe in the future (Hari et al., 2020). The new environmental trends can impair tree defence capacities by limiting water conductance, photosynthesized carbon, and the production of defensive chemicals. However, our research indicates that the specific traits of trees play a crucial role in shaping the outcomes of wind and drought induced bark beetle disturbances. For instance, as future droughts surge in prevalence, average potential height growth may be constrained due to hydraulic limitations (McDowell et al., 2020). The reduction in height may confer advantages by dampening susceptibility to drought-induced xylem damage (Koch et al., 2004) and enhancing the ability to maintain metabolic and defence functions. Conversely, recent studies have shown accelerated growth rates in Norway spruce in response to environmental changes (Cienciala et al., 2018). Persistence of these growth trends could lead to an increase in insect-driven tree mortality, given that larger trees are generally more vulnerable to such disturbances (Fig. 9, panel a).

The dynamics of future insect outbreaks are expected to be governed by complex feedback mechanisms. Natural disturbances play a crucial role in generating structural and compositional diversity within ecosystems (Turner, 2010). Model simulations have demonstrated that disturbance-driven heterogeneity in forest structure can mitigate the severity of subsequent insect herbivory (Sommerfeld et al., 2021). Consequently, historical processes shape developmental pathways in forested landscapes, establishing a template of varying stand conditions that, alongside climate factors, mediate bark beetle activity (Thom and Seidl, 2021). Additionally, dendroecological evidence suggests that the recent bark beetle outbreaks in Central Europe, in terms of their duration and severity, are not unprecedented historically (Čada et al., 2016). According to Čada et al. (2016), the previous disturbance patterns observed in the 19th and 20th centuries have primed the forests in this particular area, enabling them to support herbivore populations during periods of outbreaks. A period of relatively benign disturbance activity has promoted the development of a sufficient density and distribution of large host trees, thereby facilitating epidemic populations of bark beetles. Also, land use activities outside of forest reserves, such as timber harvesting or post-disturbance logging, have the potential to homogenize landscapes and promote the expansion of insect populations.

Environmental factors interact with tree level drivers of tree resistance modifying survival outcomes (Korolyova et al., 2022a). The stem sun exposure that proved to be a good predictor of spruce survival in the Vosges mountains is influenced by both environmental and tree morphology factors. Stem sun exposure indicates the extent to which a tree's own branches and the crowns of neighbouring trees protect its trunk against direct insolation. Our findings support the hypothesis that there is a positive relationship between the proportion of a stem exposed to sunlight and tree survival, which can be explained by carbon- and tree vigour-based resistance. Crown structure characteristics, such as total leaf area (Bazzaz, 1979; Enquist et al., 1999; Horn, 1971), as well as leave spatial distribution within the tree crown, play a crucial role in radiation absorption, photosynthesis, and transpiration processes (Wang and Jarvis, 1990). The rates of net carbon assimilation (Bauerle et al., 2007) influence the allocation of energy between growth and defence metabolic processes (e.g., Huang et al., 2020, 2019). Stem sun exposure can modify microclimatic conditions and consequently affect host tree suitability for the beetles. Higher temperatures in the bark and phloem intensify VOC emissions attracting beetles to their hosts (Esposito et al., 2016; Filella et al., 2007; Hietz et al., 2005; Janson, 1993; Marešová et al., 2020). Although not explicitly demonstrated, temperature gradients in the bark and phloem could theoretically influence bark beetle behaviour by affecting their spreading (Aukema et al., 2008) and dispersal performance (Hinze and John, 2020). It has been suggested that relative emission rates of monoterpenes could have more pronounced synergistic effects on the olfactory reactions of beetles than insect-produced pheromones (Jakuš and Blaženec, 2003). For instance, enhanced attraction of *I. typographus* to Norway spruce has been observed when elevated release rates of (–)-α-pinene were added in the baiting mixture compared to using the pheromones alone (Erbilgin et al., 2007). Our results support previous findings that reported an increase in the mortality of trees with lower crown ratios (Chisholm et al., 2021) and an aggravated predisposition to infestation documented for south-facing, sun-illuminated forest stand edges (Kautz et al., 2013).

The long-term consequences of climate warming for disturbance-driven trajectories of change remain uncertain, as they will be determined by complex interactions among climate variables, the metabolic and demographic responses of trees to environmental changes, and the existing and emerging patterns of landscape structure. The intricate feedbacks between natural disturbances, climate, and landscape conditions shape the dynamics of future insect outbreaks and underscore the importance of considering historical processes and landscape heterogeneity in understanding and managing these disturbances.

4.5 Crown morphology effects on survival

Our study presents significant findings concerning the influence of tree morphology factors on tree survival and their interactions with bark beetle dynamics. We observed a positive association between the relative crown height, or self-shading, and tree survival (Fig. 9, panel c). Longer crowns have the potential to obstruct visual cues that insects, including bark beetles, use to locate host trees (Goyer et al., 2004; Sanders, 1984). Additionally, larger crowns contribute to a more favourable carbon balance in trees by increasing the cumulative leaf area, which governs net carbon uptake and influences carbohydrate allocation to reserve pools fuelling defence functions (Bazzaz, 1979; West et al., 1999). The crown structure also influences microclimate conditions, with vertical gradients in air temperature and humidity within individual tree crowns affecting photosynthetic rates (Bauerle et al., 2007; Zweifel et al., 2002). It is important to note that photosynthetic responses to temperature are nonlinear, peaking at an optimum temperature level, beyond which assimilation rates decline (Smith and Dukes, 2013). Thus, variations in crown environments that cause leaf temperatures to exceed metabolic optima may reduce net photosynthesis (Gunderson et al., 2010). Long-term warming trends may have contrasting consequences for tree survival if warming rates are higher in sparser crowns and exceed optimum conditions for leaf photosynthesis. Nevertheless, the plant physiological acclimation capacity may compensate for temperature variability within tree crown (Smith and Dukes, 2013).

Our investigation provides evidence supporting the hypothesis that canopy-regulated temperatures may play a significant role in modulating the performance of bark beetles through their effects on insect aggregation behaviour (Kautz et al., 2013). While the exact mechanisms are not yet definitively explained, it is widely accepted that bark beetles respond to the emission rates of organic compounds, particularly monoterpenes, released by the bark tissues of host trees (Hulcr et al., 2006). The collective influence of monoterpene emission rates in relation to insect synthesized pheromones is believed to have a synergistic effect on beetle olfactory responses (Erbilgin et al., 2007; Hulcr et al., 2006; Jakuš and Blaženec, 2003). Particularly, higher terpene-to-pheromone ratios have been correlated with an increased attraction of bark beetles to host trees (Erbilgin et al., 2007). The production and release rates of monoterpenes are known to be influenced by temperature, implying that variations in microclimates mediated by the crown structure may influence the selection of host trees by insects (Esposito et al., 2016; Hietz et al., 2005; Marešová et al., 2020). Additionally, the cooling effect associated with xylem sap flow in trees may further influence these dynamics (Hietz et al., 2005). However, to gain a comprehensive understanding of bark beetle responses to plant-emitted organic compounds and the precise extent to which temperature-regulated volatile emissions can affect beetle behaviour, additional experimental studies are necessary.

Our findings on self-shading effects on spruce survival reveal the complex interplay between crown characteristics, microclimate variation, and bark beetle dynamics. The positive association between self-shading and tree survival highlights the importance of crown structure in influencing tree defence mechanisms and carbon balance. The potential role of canopy-regulated temperatures in modulating bark beetle behaviour emphasizes the intricate relationship between tree physiology, volatile emissions, and insect responses. These insights contribute to our understanding of the mechanisms underlying tree survival during bark beetle outbreaks and can inform forest management strategies aimed at mitigating the impacts of these interactions. From a forest management perspective, our findings suggest that sparser stands, allowing for the development of more extensive tree crowns (Dean and Baldwin, 1996), may enhance spruce stand resistance to bark beetles.

4.6 Tree physiological condition proxied by crown related parameters

Our study found that the overall ratio of crown defoliation serves as a reliable predictor of tree vulnerability to *I. typographus* attacks. This beetle species tends to target trees that are experiencing acute or chronic stress, particularly when their population densities are low. Previous research has shown that stressed trees exhibit a reduction in crown size (Christiansen et al., 1987; Økland and Bjørnstad, 2006; Wermelinger, 2004a) and increased crown transparency resulting from drought and competition effects (Jacobs et al., 2021). The inverse relationship between a host tree vigour and beetle-caused mortality arises from the fact that a healthy and vigorous tree, functioning at its maximum defence capacity (Seidl et al., 2007), requires 6.7 times more tunnelling beetles to successfully overcome its defences compared to a weakened and stressed tree (Kautz et al., 2014). Thus, the outcome of a colonization attempt is largely influenced by the balance between the host tree vigour and the density of beetle attacks (Christiansen et al., 1987; Lewis et al., 2010; Lieutier et al., 2003b; Mulock and Christiansen, 1986; Raffa and Berryman, 1983; Waring and Pitman, 1985).

The reliability of crown defoliation as an indicator of the current tree vitality has been questioned though, since it does not account for the early stages of foliage recovery (Cudlín et al., 2001; Dobbertin and Brang, 2001; Salemaa and Jukola-Sulonen, 1990). However, our findings suggest that both the total crown defoliation and defoliation of primary structures can serve as effective predictors of spruce tree susceptibility to bark beetle attacks. The source-sink concept (Herms and Mattson, 1992; Pacala et al., 1996) suggests that heavily defoliated and stressed coniferous individuals experience carbohydrate deprivation. Hence they prioritize the allocation of photosynthates to carbon-assimilating organs, such as apical meristems, over secondary metabolic processes like the production or storage of defence compounds (Jones and Hartley, 1999; Schreuder and Brewer, 2001). Previous studies have demonstrated that under conditions of low biomass in assimilative organs, trees that cannot sufficiently provide energy to all sinks reallocate carbohydrates from stem cambial activity and tree rings towards the intensive formation of secondary (epicormic) shoots (Nicolini et al., 2001; Polák et al., 2007). Thus, the lower survival potential observed in severely defoliated trees in our study could be explained by a trade-off between primary and secondary carbon sinks. However, the mechanistic understanding of carbon translocation strategies employed by *P. abies* in favourable and unfavourable environments remains an open question. Recent research has shown that in situations of negative carbon balance, stressed trees adopt conservative allocation strategies by prioritizing storage and mobilization of reserves for respiration rather than growth, ensuring their current and future survival (Huang et al., 2021).

4.7 Tree chemical defences

Our results show that chemical defences play an important role in tree survival during bark beetle outbreaks, which supports the previous findings (Cale et al., 2019; Nybakken et al., 2021; Schiebe et al., 2012). Our evidence suggests that different classes of chemical compounds seem to be associated with different modes of chemical defence mechanism. Particularly, the positive relationship between catechin production and tree survival probability (Fig. 16, panel d) indicates that phenolics play a pronounced role in induced tree defences against bark beetles, confirming the previous reports (Hammerbacher et al., 2011; Jyske et al., 2020; Laoué et al., 2021). Simultaneously, untargeted GCMS analysis following a MJ treatment experiment demonstrated that the initial content of individual monoterpenes was higher in LTS than beetle-killed trees (Table 3). This finding supports the inference that constitutive rather than inducible defence could play a more crucial role in tree survival in the Bohemian Forest study area, as beetles could be averted from attacking surviving trees by the olfactory cues signalling initial host unsuitability for herbivore. Additionally, our findings could indicate that terpenes could play a more crucial role in constitutive defence, while phenolics may be more important for induced chemical defence mechanism. The known attractive effects of monoterpenes on bark beetle host selection behaviour may vary with the concentrations of VOCs, which in high amounts can be toxic to *I. typographus* (Jakuš and Blaženec, 2003). The same pattern is representative of other bark beetle species. For example, experimental studies showed that monoterpene influences on pheromone attraction of *Ips pini* and other bark beetle species colonizing pines were dose-dependent, ranging from attractive and synergistic to inhibitory effects (Billings et al., 1976; Erbilgin et al., 2003; Erbilgin and Kenneth F. Raffa, 2000; Miller and Borden, 2000, 1990).

4.7.1 Phenolic response to sterile inoculation

Our study demonstrates an observed increase in catechin concentrations in response to mechanical wounding, which corresponds to the previous studies indicating a significant enhancement of this flavonoid following sterile malt-agar inoculation (Brignolas et al., 1995a, 1995b; Lieutier et al., 2003a), bark beetle-vectored fungi inoculation (Brignolas et al., 1998, 1995a, 1995b; Almuth Hammerbacher et al., 2014; Hammerbacher et al., 2019; Lieutier et al., 2003a), or natural *I. typographus* attacks (Rohde et al., 1996).

Significant reduction in stilbenoids astringin and piceid concentrations (Fig. 12, panels b, c) was previously reported in Brignolas et al. (1995b) and Lieutier et al. (2003a) (for piceid). By contrast, Brignolas et al. (1998) showed that piceid and astringin contents did not change over time in both sterile- and fungus-inoculated phloem. In mature Norway spruce trees (Brignolas et al., 1995b; Krajnc et al., 2014; Li et al., 2012; Viiri et al., 2001) and white spruce saplings (Hammerbacher et al., 2011), stilbenes decreased in response to *E. polonica* inoculation and *Heterobasidion* spp. infection (Danielsson et al., 2011), while aglucones (Viiri et al., 2001) and piceasides (Danielsson et al., 2011; Li et al., 2012) increased after fungal inoculation of mature spruce bark. The decline in piceid and astringin is commonly attributed to stilbene glucosides degradation caused by bark beetle vectored fungi (Zhao et al., 2019), especially *E. polonica*, during the processes like oxidation, deglycosylation, and dimerization (Hammerbacher et al., 2013), and/or utilization as carbon sources for fungal development (Wadke et al., 2016).

In agreement with Brignolas et al. (1998) and Lieutier et al. (2003a), significant changes in taxifolin and isorhapontin concentrations were not identified in this study (Fig. 12, panels d, e). Our evidence contrasts the results obtained by Brignolas et al. (1995a, 1995b) suggesting the fungal-induced decrease in all glucosides and increase in taxifolin regardless of inoculation mode (sterile or fungal). Our experimental data also differs from the inference reported in Hammerbacher et al. (2019) suggesting that the gene encoding enzyme Flavanone-3-Hydroxylase responsible for *E. polonica*-generated taxifolin synthesis was highly expressed in Norway spruce saplings. Constant taxifolin and isorhapontin contents observed in this study could result from the use of mechanical wounding as an external stress agent, instead of beetle-associated fungi, which is a stronger aggravator that causes, for example, larger reaction zones in inoculated phloem than sterile agarose gel (Brignolas et al., 1995b).

The divergent responses of stilbene glucosides and flavonoids to either agarose gel or fungi inoculation, as reported in the literature and in this study, may be explained by variations in the sample sizes and the monitoring periods used in different experiments. For instance, the lower stability of catechin compared to that of isorhapontin requires a larger sample size (Brignolas et al., 1998). Additionally, the time between inoculation and sampling should be adapted to local conditions, such as temperature (Brignolas et al., 1998).

4.7.2 Phenolic profiles of beetle-killed vs. surviving trees

Our study revealed that non-attacked trees displayed significantly lower catechin concentrations after treatment (Fig. 13, panel b). In contrast, previous research indicated that resistant clones exhibited higher catechin content than susceptible ones, as measured 12 days after inoculation with agarose gel and fungus (Brignolas et al., 1995a). Brignolas et al. (1998) concluded that more resistant clones were associated with elevated catechin and reduced isorhapontin contents in uninoculated phloem, as well as higher mean astringin values averaged over pre- and post-treatment concentrations – evidence that was not observed in our study. The relatively small sample size used in this study may have contributed to the lack of significance in the differences between most flavonoid and stilbene glucoside concentrations in treated and untreated phloem, as well as the insignificant wounding-induced absolute changes in phenolic contents among surviving and beetle-killed trees (refer to Fig. 13). Additionally, inherent intra-tree random variations in phenolic contents may influence the relationship between chemical markers and the survival status of trees (Brignolas et al., 1998). Thus, to identify chemical markers or assess tree resistance to bark beetles accurately, it is crucial to ensure an adequate sample size for each tree group based on the specific phenolic predictor and predefined precision rate.

4.7.3 Phenolic and morphological differences among surviving, primarily, and secondarily attacked trees

The stand-level spatial distribution of *I. typographus* attacked trees indicates the beetles' ability to distinguish between suitable and unsuitable hosts (Schiebe et al., 2012). It has been hypothesised that the success of attack and the primary attraction of beetles to a host tree are influenced by tree vigour (Lewis et al., 2010). According to our expectations, we observed differences in the crown characteristics and distances from previously attacked hosts between primarily and secondarily attacked trees. Primarily attacked trees displayed lower juvenile crown ratios (Fig. 15, panel b), which are influenced by past environmental conditions and may indicate the level of physiological stress experienced by the trees in the years preceding the experiment (Cudlín, 2015; Cudlín et al., 2001). This finding is supported by existing evidence suggesting that at the beginning of an outbreak, beetles are likely drawn by kairomone-induced primary host attractiveness (Kautz et al., 2014) and select downed or severely stressed trees (e.g., Wermelinger, 2004b). As population densities increase, behavioural plasticity (Boone et al., 2011) and Allee effects (Nelson and Lewis, 2008) enable beetles to switch from weakened first-targeted trees to more vigorous, resource-abundant hosts, even if these hosts possess better defence capacities (Kausrud et al., 2012; Wermelinger, 2004b).

Another crown characteristic reflecting the host resistance threshold (Lewis et al., 2010; Nelson and Lewis, 2008) and distinguishing surviving, primarily, and secondarily attacked trees is crown defoliation. Primarily attacked trees exhibited significantly higher total and primary structure defoliation ratios than surviving trees (Fig. 14, panels b, c), supporting our hypothesis of bark beetle primary host selection based on tree physiology. We argue that crown defoliation ratio (Fig. 14, panel b), especially defoliation of the primary crown structure (Fig. 14, panel c), can serve as an important marker of tree resistance (Cudlín, 2015; Jakuš et al., 2011a), since living needles play a crucial role in assimilating carbon through photosynthetic uptake of atmospheric CO_2, providing carbohydrates used as energy sources for the production of secondary metabolites functioning as defence compounds (e.g., Huang et al., 2020b). The loss of needles in the productive part of the crown robustly indicates tree physiological stress (Dobbertin, 2005; Jacobs et al., 2021). The total crown defoliation ratio in primarily attacked trees significantly surpassed that in secondarily targeted individuals, indicating beetles' preference for more susceptible, stressed, or downed trees with presumably diminished resistance potential. Likewise, the productive crown defoliation in secondarily attacked trees was lower compared to that in primarily attacked trees, supporting our vigour-based resistance hypothesis. However, this difference proved to be statistically insignificant, potentially due to an insufficient sample size.

We observed significantly lower amounts of taxifolin glucoside in uninoculated phloem in secondarily attacked trees compared to primarily attacked trees (Fig. 15, panel a). This contrasts with previous findings indicating that more resistant clones were associated with higher taxifolin concentrations averaged over treated and untreated phloem (Brignolas et al., 1998). As our experimental design involved beetle selection of host trees after mechanical wounding, post-treatment taxifolin concentrations may have potentially influenced the bark beetles’ choices, rather than the initial content of this phenolic compound. We argue that since *I. typographus* olfactory neurons respond to a diverse array of odours present in phloem and bark of a potential host (Andersson et al., 2009), other chemical compounds could modify the attractiveness of primarily attacked trees for beetles.

4.7.4 Change in catechin production

An increase in catechin production subsequent to mechanical wounding was found to be positively correlated with elevated tree survival probability (Fig. 18, panel d), providing support for our hypothesis that wounding may trigger an inducible defence system known as immune response ‘priming’ (Mageroy et al., 2020). Trees with higher resistance levels are presumed to emit less attractive olfactory stimuli, such as an increase in catechin, to beetles. Consequently, the chemical profile of the tree may play a beneficial role in preventing mass herbivore attacks. Catechin, a phenolic compound, has been known to exert inhibitory, antifeedant, and toxic effects on *I. typographus* (Faccoli and Schlyter, 2007; Almuth Hammerbacher et al., 2014; Hammerbacher et al., 2019, 2011; Huang et al., 2020b; Raffa et al., 2005). In contrast, trees with low levels of wounding-induced change in catechin content were susceptible to beetles (Fig. 18, panel d). This observation aligns with previous studies suggesting that stress-related insufficient, delayed, or impaired tree defence reactions may diminish the potential for tree survival (Hammerbacher et al., 2014; Kolb et al., 2019). However, a decrease in resistance capacity is observed when catechin production surpasses a certain threshold, resulting in high uncertainty of survival outcomes, as indicated by wide support intervals (Fig. 18, panel d). This uncertainty is likely attributed to the relatively small sample size and the high variability of this parameter among attacked trees (Fig. 13, panel c).

4.7.5 Terpene-based tree survival mechanisms

4.7.5.1 Terpene composition and reaction to MJ inoculation

To enhance our understanding of the terpene-based tree defence mechanism, we conducted a MJ inoculation experiment on LTS and reference Norway spruce trees in the Bohemian Forest study area. The experimental results revealed induced changes in the contents of the majority of identified terpene compounds and their chemical classes (Table 3, columns 8, 9). Consistent with previous findings (Martin et al., 2003; Nagel et al., 2022; Schiebe et al., 2012; Zaman, 2019), we observed substantial (3-fold) increases in the concentrations of mono-, sesquiterpenes, and their oxygenated derivatives in response to MJ (but see Martin et al., 2002 for sesquiterpenes), a phytohormone eliciting host defence. Benzene derivatives, p-cymene, and methylthymol, as well as the terpene ester bornyl acetate content measured three weeks after the inoculation, also increased, supporting the previous findings (Martin et al., 2003) and indicating the general pattern of VOC accumulation in the newly formed traumatic resin ducts (Celedon and Bohlmann, 2019; Franceschi et al., 2002). We observed a 3-fold increase in the total amount of all identified VOCs, with increases generally larger for oxygenated monoterpenes (10-fold) and terpene esters (6-fold). Our results are slightly different from those obtained by Nagel et al., (2022) who reported more than a 5-fold increase in the total concentrations of all VOCs identified in spruce samples in response to *E. polonica* inoculation. Additionally, in contrast to the mentioned study, monoterpenes and sesquiterpenes in this experiment demonstrated equally pronounced (3-fold) increments in their contents in response to MJ, while in Nagel et al., (2022) sesquiterpenes increased 3-7 fold, and monoterpenes increased by more than 4-fold 14 days after *E. polonica* inoculation.

The major terpenes detected in this study correspond to the compounds previously identified in Norway spruce phloem (Martin et al., 2003; Nagel et al., 2022; Schiebe et al., 2012; Schoss et al., 2023; Sousa et al., 2023). The most significant MJ-induced augmentation observed was in eucalyptol (49 times), which could be attributed to the synthesis of this compound during the catalysation of α-terpineol by 1,8-cineole synthase (Eisenbrand et al., 2021). α-Terpineol could be produced through fungi- or microorganism-mediated biotransformations of α-, β-pinene, or (+)-limonene (Román-Aguirre et al., 2005; Sales et al., 2020), the monoterpenes which were found in the largest quantities in our phloem samples (Table 3).

4.7.5.2 Differences in terpene profiles between LTS and reference trees

The PCA results (not shown) did not reveal any major differences in the entire terpene profiles between surviving and reference trees. This result agrees with the recent study showing that all spruce trees, regardless of bark beetle attack history, exhibited a similar magnitude of terpene and phenolic production in response to the inoculation of *E. polonica*, the *I. typographus* associated fungus (Nagel et al., 2022). Nagel et al. (2022) reported that the induction was accompanied by the same levels of upregulation in genes, enzymes and other chemical intermediates involved in the synthesis of secondary defensive metabolites among surviving, currently attacked and non-attacked mature spruces. The lack of distinction can be attributed to the high genetic variability in the chemical composition of forest trees in natural stands. The compositional ratios of terpenes within terpenoid blends are influenced by the unique genetic expression patterns inherent in trees, leading to significant variations in individual terpenes and phenolics within a comprehensive chemical profile. For example, *P. abies* harbours an extensive family of monoterpene synthase genes, whose transcriptional activity governs the intricacies and variances observed in terpene profiles. This gene expression is subject to inter-individual variation among trees (Martin et al., 2004). Within an individual tree, terpene profiles may change in response to bark beetle infestation due to modulations in inducible expression (Miller et al., 2005). These genes can be associated with the production of multiple distinct monoterpenes within a tree, contributing significantly to the divergences in chemical profiles among natural tree populations.

The lack of dissimilarities in the whole chemical profiles between surviving and reference groups of trees and the complexity in the observed individual terpene responses could also relate to the fact that in this experiment we imposed one stress factor – MJ mimicking the bark beetle boring attempts. Prior research demonstrated that plants uniquely respond to a combination of different abiotic and biotic stressors (Zandalinas et al., 2018). It was found that each combination of more than one stress factor creates distinct conditions, necessitating unique tree responses (Allen et al., 2010; Desprez-Loustau et al., 2006; Jactel et al., 2019; Littell et al., 2016; McDowell et al., 2019; Rennenberg et al., 2006; Temperli et al., 2013; Wolf and Paul-Limoges, 2023). Converging signals activated in plants in response to stress stimuli govern the regulation of stress-inducible genes, which encode proteins and enzymes directly implicated in stress metabolism, suggesting a specific acclimation reaction to a given stress stimulus (Casaretto et al., 2016). Thus, tree defence mechanisms cannot be directly explored by studying the effects of each individual stressor alone. The above-mentioned studies have additionally discerned regulatory transcripts, physiological reactions, and metabolite and protein complexes distinctive to particular stress combinations, underscoring the significance of investigating combined abiotic and biotic stress effects on tree defence capacity (Niinemets, 2010).

4.7.5.3 Differences in individual monoterpene compounds between LTS and reference individuals

The surviving trees exhibited significantly smaller amounts of trans-pinocarvyl-acetate after MJ treatment. This finding could partially explain the survival of resistant individuals and the stronger attractiveness of the reference group of trees to herbivorous insects. The observed lower post-inoculation concentrations of trans-pinocarvyl-acetate demonstrated by resistant trees are supported by previous studies that indicted attractiveness of oxygenated monoterpenes to beetles (Kandasamy et al., 2023; Schiebe et al., 2019b). Bark beetle-vectored fungi metabolize α- and β-pinene to trans-4-thujanol, an oxygenated monoterpene attractive to beetles (Kandasamy et al., 2023). *I. typographus* has evolutionarily developed the specialized olfactory neurons for the detection of oxygenated monoterpenes. Walking olfactometer experiments have shown that camphor and trans-4-thujanol attract beetles at specific doses, and the presence of symbiotic fungi enhances females' attraction to *I. typographus* pheromones (Kandasamy et al., 2023). Similar argumentation underpins our observation of a more intensive (though insignificantly, presumably due to an insufficient sample size) MJ-induced production of α- and β-pinene in the reference trees than in LTS (Table 3, columns 2, 3). In the reference trees, higher rates of α- and β-pinene production could subsequently lead to a greater abundance of oxygenated monoterpenes that are attractive to beetles and can be synthesized by fungi, arise from inherent biochemical processes, or stem from physical damage.

In contrast to the post-treatment concentrations, we observed significantly larger monoterpene (tricyclene, camphene, α-terpinene, sabinene) amounts in LTS phloem samples prior to MJ inoculation. This finding suggests that constitutive rather than induced defence could be the key driving force behind tree survival. At first glance, it might seem that higher monoterpene amounts would be associated with stronger attraction of beetles and higher tree susceptibility to mass attacks. *I. typographus* attraction to the aggregation pheromone was shown to increase with the supplementary inclusion of a composite blend of monoterpenic tree volatiles (Hulcr et al., 2006). It was shown that two highest concentrations of (–)-α-pinene in the (–)-α-pinene-pheromone blend doubled *I. typographus* attraction to a host tree compared to the pheromone alone (Erbilgin et al., 2007). However, previous studies have reported that monoterpene effects on bark beetle behaviour may vary, being either attractive or repellent, depending on monoterpene concentrations (Jakuš and Blaženec, 2003). Scolytids' behaviour has been demonstrated to be influenced by the release rates and the ratios at which α-pinene and ethanol are emitted (Schroeder and Lindelöw, 1989). Consequently, beetles could avoid landing on the extant resistant trees, initially deterred by the olfactory blend of secondary metabolites comprising omnipresent constitutive chemical defences – defences available before the beetles attempt to bore under the bark. The toxicity of monoterpenes and their blends for *I. typographus* has been confirmed in laboratory bioassays. Myrcene, (+)- and (–)-limonene, and 3-carene revealed middle to high toxicity, while (+)-, (–)-α-pinene, and (–)-β-pinene demonstrated low toxicity to *I. typographus* (Everaerts et al., 1988; Zaman, 2019). Myrcene and limonene have been reported to be highly toxic to the mountain pine beetle (*Dendroctonus ponderosae*), the spruce beetle (*Dendroctonus rufipennis*), and the eastern larch beetle (*Dendroctonus simplex*) (Chiu et al., 2017; Werner, 1995).

We show that most of the post-treatment responses of the oxygenated mono- and sesquiterpenes were higher in reference than in surviving trees, although the differences were insignificant (Table 3). Our findings align with the results of previous field experiments coupled with gas chromatography-electroantennographic detection. Bark beetles tend to choose host trees emitting high amounts of oxygenated monoterpenes (Schiebe, 2012). Additionally, strong *I. typographus* antennal responses to camphor, pinocarvone, and trans-thujan-4-ol have been reported (Schiebe, 2012). It was shown that the relative proportions of oxygenated terpenoids notably increased in MJ-treated bark samples and continued to escalate over time in aerial samples collected from felled trees (Schiebe, 2012). These findings suggest that oxygenated host compounds have the potential to serve as discernible indicators of host physiological stress. Consequently, these compounds assume a significant role as putative biomarkers within the intricate framework of *I. typographus* host selection mechanisms and markers of spruce resistance to the pest insect.

4.7.5.4 Differences in individual sesquiterpene compounds between surviving and reference trees

Although we were unable to detect dissimilarities in the entire terpene profiles, statistically significant differences were found in a few VOCs between LTS and reference trees before or after MJ inoculation (Table 3). This finding suggests that individual compounds or their blends, rather than the entire orchestra of terpenoids, may play a role in tree survival outcomes. Contrary to expectations, we observed lower production rates of β-ylangene (sesquiterpene), epicubenol (oxygenated sesquiterpene), and one unidentifiable sesquiterpene in LTS compared to the reference trees. The surviving trees exhibited significantly smaller amounts of the same unidentifiable sesquiterpene after MJ treatment, most likely due to its less pronounced production rates in LTS compared to the reference group of trees.

To the best of our knowledge, scientific evidence on β-ylangene effects on Norway spruce resistance to bark beetles has not yet been documented. β-ylangene is a sesquiterpene found in grapefruit and orange essential oils and abundant among the sesquiterpenic components of cubeb oil. It has been postulated that the mechanistic trajectory traversed by both ylangene and its stereoisomeric counterpart, copaene, in the process of isomerization on silica gel mirrors the hypothetical pathway previously delineated for α-pinene (Hunter and Brogden, 1964). Earlier investigations documented considerable inhibitory effects exerted by two novel ylangene-type sesquiterpenoids against protein tyrosine phosphatase 1B and SH2-containing cytoplasmic tyrosine phosphatase-1 and -2 (Fan et al., 2014). The mechanism of tyrosine phosphorylation in plants has received very limited attention in research. Recent advancements in chemical analytical methodologies have supported the viewpoint that tyrosine phosphorylation is equally pervasive in plants as in animals. A variety of approaches have suggested that plants rely on changes in protein tyrosine phosphorylation to respond to stress (Shankar et al., 2015). Concomitantly, prior studies have substantiated the involvement of Protein Tyrosine Phosphatases in the dephosphorylation and inactivation of mitogen activated protein kinases (MAPKs), specifically contributing to the regulation of MAPK signalling cascades crucial for plant growth, development, and defence responses (Jiang et al. 2022). MAPKs serve as signalling modules that integrate multiple signalling pathways. One of the regulatory mechanisms governing the activity of MAPKs involves their negative regulators, known as MAPK phosphatases (MKPs). MKPs function as the integral components in the plants' responses to a myriad of environmental stimuli. Nonetheless, the specific mechanisms through which these phosphatases contribute to distinct signals remain largely unexplored (Jiang et al. 2018). Thus, the lower production rates of β-ylangene in LTS found by us might suggest that under stressful conditions (MJ inoculation), inhibition of the activity of Protein Tyrosine Phosphatases, which inactivate MAPKs and MKPs, is dampened in LTS. This implies that surviving trees may have a higher performance and efficiency of stress-signalling pathways than susceptible trees. Additionally, many dual specificity protein phosphatases (DSPs), a subclass of MAPK phosphatases, are known to bind starch and regulate starch metabolism through reversible phosphorylation (Shankar et al., 2015). Thus, LTS lower production rates of β-ylangene, which might be associated with less pronounced inhibition of tyrosine phosphatases inactivating stress signalling modules, may contribute to a more effective mobilisation of carbon pools for the energy-demanding synthesis of secondary defence compounds in surviving trees. However, the proposed mechanisms require further investigation.

Another compound that demonstrated lower production rates in surviving than reference trees was epicubenol. To the best of our knowledge, direct evidence for the antifeedant or anti-attractant effects of epicubenol on bark beetles' behaviour does not yet exist. Epicubenol is an oxygenated sesquiterpene detected in *P. abies* (Schoss et al., 2023; Wajs et al., 2007; Zorić et al., 2021), *P. glehnii* (Kurvyakov et al., 1979), *Pinus* spp. (Wajs et al., 2007; Zorić et al., 2021) and *Larix decidua* (Wajs et al., 2007). In *P. abies*, epicubenol was shown to vary with the season, with the highest abundance of sesquiterpenes observed in August (Schoss et al., 2023). A recent study reported its anti-fungal efficiency (Ljunggren et al., 2020). Epicubenol extracted using fractional distillation from Norway spruce turpentine exhibited anti-fungal effects on the economically important wood damaging brown-rot fungus *Coniophora puteana*. Antibacterial effects of epicubenol were documented for other woody species. Benabdesslem et al. (2022) observed antibacterial properties of cubenol and 1,10-di-epi-cubenol identified in *Aralia spinosa* essential oils.

Sesquiterpenes were shown to act as stress markers in conifers. Wounding of *Abies grandis* upregulated two synthases (delta-selinene and gamma-humulene) used for production of sesquiterpenes, namely delta-cadinene, a prominent constitutive defence compound (Steele et al., 1998). 1-epi-cubenol proved to be a stress indicator in industrially polluted areas in Lithuania, with epicubenol content being the highest in *Pinus sylvestris* growing in the most heavily polluted sites (Kupcinskiene et al., 2008). Epicubenol performed well as a stress marker in a perennial shrub *Salvia dolomitica*, but the plant stress reaction varied with different epicubenol stereoisomeric forms. Particularly, 1-epi-cubenol concentration was higher in moisture-abundant than in drought-stressed sites, while 1,10-di-epi-cubenol content was lower in mesic conditions and increased with aggravating water constraints (Caser et al., 2019). Terpene abundance was shown to be a marker of environmental stress in *Pinus nigra* (Supuka et al., 1997). Assuming a stress marker function of epicubenol, β-ylangene, and the unidentifiable sesquiterpene upregulation, it is possible to suggest that the lower production rates of these compounds observed in LTS compared to the reference group might indicate that MJ inoculation was a much stronger stressor for the beetle-susceptible individuals than for LTS. This, coupled with the observed larger amounts of monoterpenes in LTS prior to inoculation, might also mean that surviving trees relied more on constitutive rather than induced defence mechanisms. In other words, LTS might have evolutionarily developed more effective constitutive defences against beetles than the susceptible reference trees. Our findings agree with the previous results indicating that spruce clones that initially possessed high constitutive resistance, did not exhibit large changes in MJ-induced defence capacity, measured by the fungal lesion lengths (Puentes et al., 2021). Alternatively, if tree chemical profiles and defence reactions differ from generation to generation (Verma and Shukla, 2015), or even from tree to tree (Puentes et al., 2021), then the explanation for the lower production rates of β-ylangene, epicubenol and one unidentifiable sesquiterpene in surviving trees could be much simpler. Induced LTS chemical responses might have changed over time since the previous bark beetle outbreak, which, depending on the locality, occurred 15-25 years ago. We argue that an in-depth investigation into the stress response pathways involving spruce sesquiterpenes is essential to elucidate the impact of sesquiterpene upregulation, particularly with respect to their various stereoisomeric forms, on the defensive mechanisms of trees.

Divergent responses of epicubenol content to stress factors reported in the literature indicate the complexity of the chemical defence mechanism that is partly governed by the genetic architecture, gene expression and their intricate interactions with the environments. While the biochemical community is significantly advancing in the investigation of the genetic and chemical mechanisms involved the synthesis of secondary metabolites, commensurate progress in understanding the developmental mechanisms pivotal to their defensive efficiency is constrained (Kliebenstein, 2013). Progress is impeded by idiosyncratic responses demonstrated across and among tree populations and individuals. While the terpene composition remains characteristic across all coniferous species, the presence, concentrations, and stress-induced production of individual compounds exhibit species- and tree-specific traits (Kopaczyk et al., 2020). The recent findings of SNPs potentially associated with Norway spruce resistance to the bark beetle *I. typographus* showed that further investigation of genes associated with spruce survival are necessary to get insights into the underpinning molecular level processes (Korecký et al., 2023).

4.8 Limitations of chemical defence analysis

Physiological and chemical processes within a tree constitute a complex system influenced by dynamic interactions among various biotic and abiotic factors. Consequently, investigations into the spruce chemical defence system should ideally occur during an active bark beetle outbreak. This necessitates sampling surviving trees in a manner that closely aligns with peak local bark beetle population density levels. Sampling spruce trees that endured severe beetle disturbance 15-25 years ago, as was the case in this study, may complicate inferences about tree chemical defence mechanisms against bark beetles. However, due to reasons outlined in the Methods section, the availability of LTS and beetle-attacked trees may not coincide with the disturbance peak or immediately afterward. We argue that employing a combination of diverse methodological approaches to study tree survival and adopting alternative study designs, such as sampling living mature trees from the broader population to represent beetle-killed individuals, can provide an efficient solution to address the methodological challenges associated with LTS studies. In addition to temporal effects on tree chemical defence capacity, local microclimate and edaphic factors may introduce variability, potentially obscuring results and causing substantial differences in the responses of individual phenolic and terpene compounds between surviving and beetle-killed trees. Furthermore, factors such as insufficient sample size and the high genomic variability intrinsic to large sessile organisms, like trees, could also significantly contribute to the diverse chemical profiles observed in resistant and reference groups of trees growing in spatially disjunct localities. We also argue that the inclusion of a combination of biotic and abiotic stress factors experienced by trees prior to and during natural bark beetle outbreaks in the study/experimental design is essential for revealing the chemical markers of tree resistance to bark beetles.

4.9 Tree life-history trade-offs in resource allocation

While chemical defence mechanisms play a pivotal role in tree survival during a bark beetle outbreak, the study of tree life-history trade-offs in the allocation of limited resources is of no lesser importance. Drawing from a meta-analysis, Carmona et al. (2011) proposed that plant resistance to insect herbivory is more significantly influenced by physical defence mechanisms like latex and trichomes, alongside life history traits involving growth rate and phenology, than by the contribution of secondary chemistry. Our comparison of the long-term radial growth rates between LTS and reference trees showed that surviving spruces grew slower than reference individuals 20, 40, 60, and 80 years prior to bark beetle outbreak (Fig. 8). Our results support the “growth – defence trade-off” hypothesis (Herms and Mattson, 1992) and are consistent with the previous findings reporting that bark beetles were less likely to infest slower growing lodgepole (*Pinus contorta*) (Buonanduci et al., 2020; Cooper et al., 2018; Yanchuk et al., 2008), ponderosa (*Pinus ponderosa*) (de la Mata et al., 2017; Reed and Hood, 2021), and limber pines (*Pinus flexilis*) (Ferrenberg, 2014). Our findings align with those of a cross-continental study that involved over 7000 dead and living trees, representing 36 species across 190 sites. This meta-analysis demonstrated that angiosperms and trees that experienced mortality induced by biotic factors, mainly bark-beetles, were associated with small and short-term reductions in radial growth (Cailleret et al., 2017).

A number of studies provide contradictory evidence regarding the growth rates preceding bark beetle caused tree mortality. Faster radial growth in resistant trees prior to bark beetle disturbance was found in ponderosa pine (Knapp et al., 2013), whitebark pine (*Pinus albicaulis*) (Millar et al., 2012), lodgepole pine (Ferrenberg, 2014), incense cedar (*Calocedrus decurrens*) (Reed and Hood, 2021), and white spruce (*P. glauca*) (Hard, 1985; Hard et al., 1983; Hard and Holsten, 1985). Bark beetle selection for slower growing hosts observed in the aforementioned studies agrees with the plant vigour / plant stress hypothesis. This hypothesis postulates that drought and other abiotic stress factors compromise tree defence mechanisms under deficient resources, thus, amplifying the vulnerability of plants to herbivore (Jactel et al., 2012; Koricheva et al., 1998; Mattson and Haack, 1987; Rhoades, 1983; Waring and Pitman, 1985; White, 1984, 1974). Accordingly, diminished growth rates reflective of tree stress could indicate increased susceptibility to insects in beetle-killed trees, while faster growing, more vigorous individuals possessed sufficient resources to persist through disturbance.

Contrasting these two opposing logical considerations, some studies did not find any differences in the growth rate dynamics of beetle-resistant and susceptible coniferous trees prior to the outbreaks (e.g. see Reed and Hood (2021) for pinyon pine (*Pinus monophylla*) and white fir (*Abies concolor*); Sangüesa-Barreda et al. (2015) for Aleppo pine (*Pinus halepensis*)). We argue that the controversial evidence existing in the literature can be attributed to the differences in bark beetle population densities and stand and environmental conditions modifying tree physiological processes in years preceding disturbances, the premises greatly varying across the published studies. Additionally, the comparisons among existing evidence are obfuscated by their focus on different species of trees and bark beetles, attacking their hosts at varying ontogenic stages characterised by different growth rates. For example, it has been shown that *P. abies* is colonised by bark beetle species depending on its developmental stage and growth rates. Particularly, *I. typographus* attacks Norway spruce at the phase of declining growth (Stolina, 1982).

As was already emphasised in subsection *4.4 Environmental factors of tree survival during bark beetle outbreak*, despite the projected negative effects of climate change on forests ecosystems, including the consequences of bark beetle disturbances, the actual outcomes of such disturbances can be expected to depend on tree specific traits. If radial growth is constrained through water limitation (McDowell et al., 2020), then smaller trees on average will become less prone to drought-caused cavitation of conductive tissues (Koch et al., 2004). Also, smaller diameter, supressed trees provide less feeding substrate for the growing insect densities and thus are less suitable hosts for beetles (Koontz et al., 2021; Meddens et al., 2015). Though, substantially less selective bark beetle choices were reported under the peaking herbivore population densities (Reed and Hood, 2021). Previous observations indicate that insects tend to attack progressively smaller individuals once the largest, most vigorous trees have already been infested (Gibson et al., 2009). Thus, an interplay among tree traits, site and environmental parameters, landscape disturbance dynamics and bark beetle population densities will define the future of coniferous forests in the changing climate.

V. CONCLUSIONS

In the context of climate change, the extensive outbreak of pest insects, particularly the European spruce bark beetle *Ips typographus*, poses a significant threat to Northern Hemisphere forests. The economic and ecological impacts of these disturbances are profound, necessitating a thorough understanding of tree resistance mechanisms. This study focused on Norway spruce and aimed to unravel the factors contributing to its resistance against *I. typographus* attacks. Through a **multidisciplinary approach** encompassing analytical chemistry, dendroecology, remote sensing, and statistical methods, we explored internal and external factors influencing host tree survival.

In agreement with our expectations, we found that **spruce survival during a bark beetle outbreak is a non-random process** driven by evolutionarily developed host tree resistance mechanisms. **Smaller diameter trees tend to have higher survival probability** reflecting both tree life-history trade-offs in resource allocation and bark beetle population density dynamics. **More extensive crowns appeared to buffer the trees against herbivory pressure**, preventing the bark overheating and the associated enhanced emission of volatile compounds potentially attractive to the beetles. Accordingly, **host trees more exposed to solar radiation, are more prone to infestation**. **Increased competition diminishes survival capacities** presumably by compromising energy-demanding tree chemical defence mechanisms. **Long-term moisture deficit** proxied by the climatic water balance **dampens tree survival probability** through downregulation of photosynthetic activity and overall decline in tree physiological condition that affects chemical defence capacities against bark beetles. **Water constraints interact with the individual factors, aggravating the negative effects of stand density and tree size, and strengthening the positive buffering effects of self-shading on tree survival**.

The experimental outcomes align with existing studies and ecological theory, emphasizing the role of the physiological condition of trees in determining survival outcomes during bark beetle outbreaks. We demonstrate that **LTS were growing slower than the reference group** of living mature trees representing beetle-susceptible individuals, during 20, 40, 60, 80 and 90 years prior to the outbreak. This finding supports the “growth-defence trade-off” hypothesis positing that trees during their lifetimes allocate the limited resources between primary (respiration, growth, reproduction, etc.) and secondary (survival, defence, stress responses, etc.) metabolic functions. Therefore, LTS seem to have persuaded a relatively more conservative resource allocation strategy, investing more energy in future potential defence at the expense of a current growth. Unsurprisingly, we found that **increasing distance from the previously colonised host decreases tree survival probability**. The **crown defoliation** indicative of tree physiological condition **is a good predictor of tree survival** and can be used, apart from self-shading, as one of the morphological tree resistance markers. We show that **elevated production rates of phenolic catechin are non-linearly associated with increasing tree survival** capacities.

Our study supports the hypothesis that the **production of phenolics is positively associated with tree survival probability**. The results of the untargeted gas chromatography – mass spectrometry analysis did not reveal significant differences in the whole terpene profiles between LTS and reference trees. However, **we found the differences in individual terpene compounds between surviving and reference trees**. The higher amounts of monoterpene hydrocarbons tricyclene, camphene and α-terpinene observed in LTS, could indicate that constitutive rather than induced defence played a more important role in spruce survival, as monoterpene toxicity to beetles has been reported in previous studies. Prior research has demonstrated that terpene effects on bark beetle behaviour are dose-dependent and non-linear, thus, we should interpret our results with care.

We found that after MJ inoculation the contents of trans-pinocarvyl-acetate, an oxygenated monoterpene potentially attractive to beetles, and an unidentifiable sesquiterpene were lower in LTS compared to the reference group, contributing to our better understanding of tree survival mechanisms. LTS exhibited lower production rates of an oxygenated sesquiterpene epicubenol, a sesquiterpene β-ylangene, and an unidentifiable sesquiterpene than the reference individuals. Considering the existing evidence for epicubenol as a stress marker in woody plants, our findings could point to the fact that MJ inoculation that mimics bark beetle attacks induced less pronounced stress signals in LTS than reference trees. This may indicate that surviving trees, less sensitive to MJ, are in general more vigorous individuals that have larger resources for the synthesis of secondary defence compounds to defend themselves, or stronger constitutive defensive barriers eliminating the need for upregulated production of secondary metabolites in response to stress.

β-ylangene, a compound studied even less than epicubenol in terms of tree chemical defence mechanisms, might be involved in inhibition of the protein tyrosine phosphates responsible for inactivation of MAPKs, the modules signalling about environmental stresses in plants. Smaller β-ylangene production rates in LTS in response to MJ could indicate that in surviving trees the stress signalling modules might work more effectively than in the reference trees, since in LTS the stress signalling pathways are not supressed as much as in the reference group of trees. However, these mechanisms remain unknown and further research is needed to uncover the underlying processes on the molecular and genetic levels.

In this study we attempted to unveil the factors behind Norway spruce resistance to bark beetle *I. typographus*. Based on the classical knowledge of the ecological interactions, our field observations and inferences from our analyses, we developed a multidisciplinary research methodology that can be used to identify and study the phenomenon of trees that manage to persist through bark beetle disturbances. We investigated the tree-level effects of morphological, stand, environmental, chemical and dendroecological factors on Norway spruce resistance to bark beetle *I. typographus*. The assessment of the **landscape-scale temperature effects on spruce stand susceptibility** to bark beetle infestation (Kozhoridze et al., 2023) and the first results of the analysis of **genomic markers of spruce resistance** to bark beetles using a 50k SNP genotyping (Korecký et al., 2023) extend the results reported here. However, due to the complexity of the studied phenomenon, we argue that further research is required to better understand tree resistance and tree survival responses to myriads of factors modifying physiological condition of mature trees growing in the natural forests.

In conclusion, this research provides valuable insights into Norway spruce resistance to *I. typographus*, offering possible practical implications for informed forest management strategies. While short-term forest protection and longer-term CO_2 reduction are mandatory in the light of the ongoing bark beetle outbreaks, the intermediate mitigation measures suggesting increasing resilience on stand and tree level are less considered. A better understanding of complex multifactorial spruce resistance mechanisms against bark beetles and keeping resistant trees in the forest will facilitate tree breeding programs and ecological and genetic improvements on stand and tree levels. Climate change is anticipated to exacerbate the impacts of insect-driven disturbances. Our results show that particular outcomes of wind and drought induced bark beetle disturbances for forest ecosystems largely depend on individual tree characteristics. Therefore, it is crucial to consider the interplay between climate change, insect-driven disturbances, stand composition and structure, and tree traits when assessing the potential impacts on forest ecosystems. Understanding the complex dynamics and responses of trees to changing environmental conditions can provide insights into the vulnerability and resilience of tree populations in the face of climate change and insect outbreaks.

Potential practical applications of the findings

While climate change is extensively anticipated to impact future disturbances, our study demonstrates that the responses of tree resistance and survival are intricate and subject to the influence of local drivers, including tree size and stand structure. Our findings in Norway spruce survival factors during severe bark beetle *I. typographus* disturbance could have useful applications in practical forestry and adjacent sectors. Potential applications range from the identification and mapping of resistant trees and stands prior to and after disturbances to improvement of silvicultural methods.

1) Identification and mapping of trees and stands resistant/susceptible to bark beetles prior to a disturbance.

We show that spruce survival during severe large-scale *I. typographus* outbreaks is not a random process. Our results indicate that tree survival is influenced by tree phenotype, forest structure and climatic conditions. Tree survival varies with host tree size and stand density, a proxy of the level of competition for limited resources. An important finding in terms of stand management is that LTS are clustered, i.e. occur in groups (Korolyova et al., 2022a). Previous studies have shown that trees that survived bark beetle disturbance had significantly longer crowns than the neighbouring trees infested by *I. typographus* (Jakuš et al., 2011b). Our comprehensive analyses confirmed these findings (Korolyova et al., 2022b, 2022a). Our results are in agreement with earlier concept of forest stand resistance potential (Stolina, 1976). When the concept was established by Stolina in 1976, crown length was recognized as a significant characteristic for evaluating stand resistance to abiotic and biotic damages. Thus, self-shading ratio can be considered an important diagnostic indicator of the physiological state of trees and their ability to resist phytophagous beetles (Jakuš et al., 2023; Korolyova et al., 2022b, 2022a). In addition to relative crown length, trees that have survived *I. typographus* gradation differ from infested individuals in the level of crown transformation (Jakuš et al., 2015, 2011b; Korolyova et al., 2022b), chemical composition (Korolyova et al., 2022b; Schiebe, 2012) and genetic information (Korecký et al., 2023).

Based on our findings, we developed the **Methodology for the identification of spruce trees resistant to bark beetle infestation** (Jakuš et al., 2023). The methodology relies on crown characteristics of resistant individuals (the main criterion – self-shading ratio, and additional criteria – degrees of total and primary crown defoliation) and vegetation index EVI, derived from remote sensing data, to delineate resistant stands or sections within stands. For practical purposes, we established that a tree is considered resistant if the self-shading ratio is above 60%, total crown defoliation does not exceed 35%, and the maximum primary crown defoliation is set at 55%. These values are derived from the self-shading ratio, total and primary crown defoliation levels averaged over surviving trees and adjusted by the standard deviation (Korolyova et al., 2022b, 2022a). Thus, spruces with self-shading ratios above 60%, total and primary defoliation levels below 35% and 55%, correspondingly, can be confidently regarded as resistant. For practical purposes, for resistant spruce trees, we determined an indicative minimal EVI index value quantified using PlanetScope satellite data. This value corresponds to the mean EVI value calculated for intact spruce stand fragments and adjusted by the standard deviation (Trubin et al. 2022). Stand fragments for which EVI exceeds 2.24 can be considered relatively resistant when using the PlanetScope data. The Methodology is applicable at the local, medium, or national levels for identifying sites where bark beetle gradation could potentially occur in the future. Conversely, it serves as a valuable guide for identifying locations with spruce trees comparatively resistant to bark beetle infestation.

The robust defence mechanisms exhibited by clusters of resistant trees can effectively suppress pioneer beetle populations, thereby mitigating initial infestations. Information pertaining to identified resistant trees, stands, or stand sections holds direct applicability in practical forest protection. These locations possess the potential to withstand anticipated gradation, ensuring the efficacy of defensive measures. It is imperative to thoroughly survey these sites for newly infested trees and promptly administer treatments. The anticipated positive impact of precise sanitary cutting and the use of protective methods, such as barriers of pheromone traps or trap trees, would be significantly more pronounced in more resistant stands, given their higher likelihood of surviving a bark beetle outbreak. Beyond tree survival, the identification of resistant trees/stands can diminish the severity of the gradation wave, positively influencing adjacent, less resistant stands. LTS and, to a limited extent, resistant stands, should be meticulously documented, integrated into forest management planning and regional forest development planning records with precise geolocation/mapping, and acknowledged as integral components of forest management.

In the long term, applying criteria for the identification of resistant tree principles can significantly contribute to increasing the share of spruce plantations based on more resistant and resilient trees. This approach, in turn, helps mitigate the adverse effects of climate change on the forestry sector. Managing spruce stands before the next potential bark beetle disturbance could involve incorporating information about resistant and susceptible stands into harvesting planning. The aim would be to retain resistant stands, which have a higher survival probability, and subsequently establish age-structured spruce stands.

2) Identification and mapping of trees and stands resistant to bark beetles after disturbance.

Based on our results, we developed the certified **Maps of resistant trees (LTS) in the Šumava mountains** (Korolyova N., Kozhoridze G., Jakuš R., Löwe R., Turčáni, 2022). The Maps comprise two groups of LTS located in the Šumava NP (Czechia), Bayerischer Wald National Park (Germany), Bavarian State Forests (Germany) and Schlägl Monastery Forests (Austria). The first group (LTS I) incorporates resistant trees that persisted through disturbance and were identified according to the methodology described in Korolyova et al. (2022a) using visual inspection of high-resolution Google Earth (GE) time series imagery spanning the period from 2000 to 2019. Out of 184 GE-identified LTS, the randomly selected subsample of 37 LTS representing 20% of the whole sample size were ground-truthed during field surveys. The second group (LTS II) comprises 110 single extant spruce trees that survived the bark beetle outbreak and were identified during field surveys (as of 2021). The maps together with GPS coordinates can be used to locate resistant trees in the field. As LTS are prone to wind damage, their actual presence in the forest can be altered over time. For LTS I, the surveyed status is limited to the year 2019, whereas for LTS II, the presence was verified up to the year 2021. The LTS Maps can be used in forestry sector (a) as a direct source of seed material (cone collection), assuming that their offspring will be relatively resistant to *I. typographus* infestations, and (b) as a source of vegetative material for the establishment of seed orchards; by public administration and nature conservation sector (c) as the starting point to identify the areas of special value; by the scientific community (d) for further research.

3) Improvement of silvicultural methods.
    A) Planning of tree and stand planting accounting for larger inter-tree distances and uneven stand age.

The findings from previous research and the results of this study clearly indicate that trees with more extensive crowns are more likely to survive (Jakuš et al., 2011b; Korolyova et al., 2022a). The lower threshold of the relative crown length (self-shading ratio, or the ratio of crown length to the total tree height) associated with a higher probability of tree survival is 60% (Jakuš et al., 2023; Korolyova et al., 2022a). In other words, spruces with a relative crown length exceeding this value can be considered resistant to bark beetle attacks. This suggests that silvicultural methods should take into account a sufficient inter-tree distance to retain the lower branches and facilitate the development of long tree crowns.

    B) Using bark-beetle resistant seed sources in tree breeding programs.

The recent bark beetle disturbance has unequivocally demonstrated that the presently cultivated genotypes of Norway spruce are highly susceptible to concentrated bark beetle attacks. Two potential strategies emerge: altering the species composition of our forests or cultivating spruces resistant to bark beetle infestations. The latter approach offers dual benefits: firstly, resilient stands persist as forests in the post-disturbance period, and secondly, resistant trees serve as highly efficient tools for immediate forest protection.

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