

Česká zemědělská univerzita v Praze

Fakulta lesnická a dřevařská

Katedra ochrany lesa a entomologie



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zemědělská
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v Praze

**Vliv klimatických změn a provenience
na obranyschopnost lesních dřevin proti hmyzím
herbivorům**

Disertační práce

Biologie lesa

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**Effect of climate change and provenance on plant
defense against insect herbivores**

Dissertation thesis

Forest Biology

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ZADÁNÍ DISERTAČNÍ PRÁCE

Ing. Barbora Dvořáková

Biologie lesa

Název práce

Vliv klimatických změn a provenience na obranyschopnost lesních dřevin proti hmyzím herbivorům

Název anglicky

Effect of climate change and provenance on plant defense against insect herbivores

Cíle práce

1. Nalezení vztahů mezi klimatem, fyziologickými změnami rostlin a herbivory vázaných na tyto rostliny. Tyto vztahy ověřit u herbivorů živících se jak na listnatých, tak jehličnatých dřevinách.
2. Stanovit, zda environmentální podmínky prostředí, druh dřeviny a jejich provenience ovlivňují výskyt klikoroha borového a fyziologické reakce sazenic na jeho žír.

Metodika

První cíl, nalezení vztahů změn klimatu, fyziologických změn rostlin a vliv těchto změn na lesní škůdce bude ověřen dvěma experimenty.

První experiment se zaměřuje na pilatky *Pristiphora abietina* (Christ, 1791), *Pikonema scutellata* (Hartig, 1837) a *Pristiphora leucopodia* (Hartig, 1837). Tyto vztahy lze ověřovat na ekosystémové stanici na Bílém Kříži. Na stanici se nachází dvě sféry (skleněné dómky), ve kterých jsou pěstovány experimentální porosty smrku. Jedna sféra obsahuje normální atmosféru, druhá je obohacovaná plynným CO₂. Na tyto sazenice se nasadí punčošky, do kterých se umístí housenice pilatek. U nasazených housenic je nutno zaznamenat počáteční váhu před žírem a konečnou váhu po skončení žíru. Kontrolu je nutno provádět každý třetí den a pravidelně odebírat a shromažďovat trus pro následující analýzy (poměr C:N). U každé housenice se zaznamená celková doba vývoje.

Druhý experiment studuje vývoj housenek bekyně velkohlavé (*Lymantria dispar* Linnaeus, 1758) na sazenicích smrku a dubu pěstovaných v laboratorních podmínkách. Sazenice se umístí do fytokomor s regulovaným obsahem CO₂ a teplotou. Sazenice budou pěstovány v komorách po dobu jednoho roku, poté se jejich asimilační orgány odeberou a připraví se z nich umělá potrava, kterou se budou krmit housenky. U housenic se zaznamená počáteční hmotnost, množství zkonsumované potravy, množství vyprodukovaného trusu a konečná hmotnost housenek. Zároveň bude provedena chemická analýza asimilačních orgánů sazenic.

Výsledky působení různých podmínek klimatu na sazenice a následně na larvy lze ověřit použitím nutričních indexů (larval performance index). Zároveň bude provedena chemická analýza asimilačních orgánů sazenic.

Druhý cíl, bude naplněn několika experimenty. V prvním experimentu, budou důsledky působení environmentálních faktorů a druhu sazenic na výskyt klikoroha studovány v terénu. Na minimálně deseti lokalitách budou odchytávány brouci klikoroha do zemních pastí ve dvouúdobných intervalech a bude kvantifikován žír klikorohů na různých dřevinách. Vliv druhu dřevin, podmínek prostředí a provenience na žír klikoroha borového bude studován pomocí více experimentů. Sadební materiál dřevin bude získán z různých klimatických podmínek a řady zemí Evropy. Sazenice budou pěstovány v různých klimatických podmínkách. Posléze bude vyvolán stres žírem klikorohů. Zároveň bude provedena chemická analýza asimilačních orgánů sazenic.

Harmonogram řešení:

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Doporučený rozsah práce

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Klíčová slovaCO₂, herbivor, metabolity, provenience, teplota**Doporučené zdroje informací**

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"Prohlašuji, že jsem disertační práci na téma Vliv klimatických změn a provenience na obranyschopnost lesních dřevin proti hmyzím herbivorům vypracovala samostatně s použitím uvedené literatury a na základě konzultací a doporučení školitele.

Souhlasím se zveřejněním disertační práce dle zákona č. 111/1998 Sb. o vysokých školách v platném znění, a to bez ohledu na výsledek její obhajoby."

V Praze dne

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Všem, kteří byli součástí mé cesty, patří mé upřímné díky.

Abstrakt

Disertační práce se zabývá nepřímými účinky klimatické změny na interakce mezi lesními dřevinami a hmyzími herbivory prostřednictvím změn chemického složení hostitelských rostlin. Hodnotí vztah mezi těmito změnami a odezvou herbivorů napříč různými experimentálními úrovněmi. Prvním cílem bylo identifikovat vztahy mezi klimatem, fyziologickými změnami rostlin a odezvou herbivorů živících se na listnatých i jehličnatých dřevinách. Druhým cílem bylo posoudit vliv stanovištních podmínek, druhu dřeviny a provenience na výskyt klikoroha borového a reakci sazenic na jeho žír.

Studované vztahy mezi chemií hostitelských rostlin a odezvou herbivorů byly ověřovány v laboratorních a semi-field podmínkách, zatímco terénní část práce hodnotila vliv stanovištních a managementových faktorů na výskyt herbivorů a míru poškození sazenic.

V laboratorních podmínkách byl testován vliv kombinovaného působení zvýšené koncentrace atmosférického CO₂ a teploty na chemické složení dubu letního (*Quercus robur*) a smrku ztepilého (*Picea abies*) a na prospívání listožravých herbivorů (*Lymantria dispar* a *Lymantria monacha*). V semi-field podmínkách byl hodnocen vliv zvýšené koncentrace CO₂ v interakci s dostupností vody, dusíku a proveniencí smrku na chemické složení jehlic a růst tří druhů pilatek (*Pristiphora abietina*, *P. leucopodia* a *Euura scutellata*). Další experiment se zaměřil na chemickou odezvu dvou proveniencí borovice lesní (*Pinus sylvestris*) a jejich poškození klikorohem borovým (*Hylobius abietis*). Terénní studie hodnotily vliv stanovištních podmínek, stáří pasek a způsobu jejich managementu na výskyt klikorohů a míru poškození sazenic.

Výsledky prokázaly, že různé kombinace klimatických faktorů vedly ke změnám chemického složení rostlin, zejména v základních stechiometrických charakteristikách, především v poměru C:N, který je významným ukazatelem nutriční kvality potravy pro herbivory. Tyto změny byly napříč experimenty relativně konzistentní, avšak jejich přenos do odezvy herbivorů byl slabý a bez jednotného směru. Změny sekundárního metabolismu a metabolomických profilů byly výrazně závislé na konkrétním druhu a experimentálním uspořádání.

V experimentu zaměřeném na přímou reakci borovice na poškození kmene klikorohem se chemická odezva projevila především změnami primárního metabolismu v kořenech, zatímco chemické složení jehlic zůstávalo krátkodobě stabilní. Typ herbivorie a zasažený orgán se tak ukázaly jako významné faktory určující charakter chemické odezvy rostliny. Provenience hostitelských dřevin ovlivňovala některé chemické charakteristiky pletiv, avšak její vliv se neprojevil v odezvě herbivorů ani v intenzitě poškození sazenic.

Terénní studie ukázaly, že výskyt klikoroha je významně ovlivněn stanovištními podmínkami, zejména vlhkostí stanoviště, stářím paseky a způsobem managementu pasek. Intenzita poškození sazenic přitom nebyla přímo úměrná početnosti herbivora,

což ukazuje, že vztah mezi abundancí a skutečným poškozením je komplexní a kontextově podmíněný.

Disertační práce ukazuje, že změny chemického složení lesních dřevin vyvolané klimatickými faktory samy o sobě nepředstavují spolehlivý prediktor odezvy hmyzích herbivorů. Naopak se ukázalo, že intenzita herbivorního tlaku je výrazně ovlivňována konkrétními stanovištními podmínkami a způsobem managementu. Výsledky tak naznačují, že budoucí výzkum i praktická opatření by měla zohledňovat kombinaci klimatických vlivů s kontextem prostředí a zaměřit se nejen na změny kvality hostitelské rostliny, ale i na faktory, které mohou herbivorní tlak přímo ovlivňovat.

Klíčová slova: CO₂, herbivor, metabolity, provenience, teplota

Abstract

This dissertation examined the indirect effects of climate change on the interactions between forest trees and insect herbivores, specifically examining how changes in the chemical composition of host plants influence these interactions. It evaluates the relationship between these chemical changes and herbivore responses across various experimental settings. The first objective was to identify the connections among climate, plant physiological changes, and the responses of herbivores feeding on both deciduous and coniferous tree species. The second objective was to assess the impact of site conditions, tree species, and provenance on the presence of the large pine weevil, as well as how seedlings respond to its feeding.

The study examined the relationships between host plant chemistry and herbivore responses in laboratory and semi-field conditions. It also assessed how site and management factors influence herbivore occurrence and seedling damage in field conditions.

In laboratory experiments, the combined effects of elevated atmospheric CO₂ concentrations and increased temperatures on the chemical composition of pedunculate oak (*Quercus robur*) and Norway spruce (*Picea abies*) were assessed. The experiment evaluated the performance of defoliating herbivores, specifically *Lymantria dispar* and *Lymantria monacha*. Under semi-field conditions, the study examined how elevated CO₂ levels interacted with factors such as water availability, nitrogen supply, and spruce provenance, focusing on needle chemistry and the growth of three sawfly species: *Pristiphora abietina*, *P. leucopodia*, and *Euura scutellata*. Another experiment investigated the chemical responses of two Scots pine (*Pinus sylvestris*) provenances and the damage caused by the large pine weevil (*Hylobius abietis*). Additionally, field studies examined the effects of site conditions, clear-cut age, and management practices on weevil occurrence and seedling damage intensity.

The results showed that different combinations of climatic factors altered the chemical composition of plants, particularly the basic stoichiometric characteristics, such as the C:N ratio. This ratio is an important indicator of the nutritional quality of herbivore diets. While these changes were generally consistent across experiments, their direct impact on herbivore responses was weak and inconsistent. Additionally, alterations in secondary metabolism and metabolomic profiles were strongly influenced by plant species identity and experimental design.

In the experiment examining how pine trees respond directly to stem damage caused by weevils, the chemical response primarily involved changes in primary metabolism in the roots. Meanwhile, the needles' chemistry remained stable in the short term. The type of herbivory and the specific plant organ affected were significant factors influencing the plant's chemical responses. Although the provenance of the pine trees

affected certain chemical characteristics of their tissues, this influence did not affect herbivore performance or the severity of damage to seedlings.

Field studies have shown that pine weevil presence is significantly influenced by site conditions, particularly moisture levels, clear-cut age, and management practices. Furthermore, the level of seedling damage does not directly correlate with the number of herbivores present. This suggests that the relationship between herbivore abundance and the actual damage they cause is complex and context-dependent.

The dissertation shows that changes in the chemical composition of forest trees due to climate change are unreliable predictors of how insect herbivores will respond. Instead, herbivore pressure is largely influenced by specific site conditions and management practices. The findings suggest that future research and practical measures should account for interactions between climatic factors and the environmental context. It is essential to focus not only on changes in host plant quality but also on factors that directly influence herbivore pressure.

Keywords: CO₂, herbivore, metabolites, provenance, temperature

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1. Úvod

Lesní ekosystémy jsou v současnosti vystaveny řadě environmentálních stresorů, které na ně nepůsobí izolovaně, ale současně v různých kombinacích (Dudney et al., 2025). Jejich intenzita a frekvence se v důsledku probíhající klimatické změny výrazně zvyšují (IPCC, 2022). Mezi hlavní faktory patří zejména rostoucí koncentrace oxidu uhličitého (CO₂) v ovzduší a nárůst průměrné teploty, které vedou ke změnám ve srážkovém režimu, zvýšené evapotranspiraci a častějším periodám sucha (IPCC, 2022; Wu & Cao, 2024). Tyto změny mají velký dopad na lesní dřeviny, jejich růst, alokaci zdrojů a schopnost reagovat na biotické stresory, mezi které patří i hmyzí herbivoři (Hamann et al., 2021).

Hmyzí herbivoři představují v lesních porostech významnou skupinu škodlivých činitelů, jelikož mohou napadat nejen oslabené, ale i zdravé stromy (Bracalini et al., 2024). V podmínkách klimatické změny může docházet ke zvýšení jejich populačních hustot, zrychlení vývoje, změnám ve fenologii a rozšiřování areálů výskytu, což může vést k zesílení jejich negativních dopadů na lesní ekosystémy (Johnson & Haynes, 2023). Klimatické faktory zároveň ovlivňují kvalitu hostitelských rostlin, zejména chemické složení jejich pletiv, které je považováno za jeden z klíčových mechanismů určujících interakce mezi rostlinami a herbivory (Jamieson et al., 2017).

Zvýšená koncentrace CO₂ v atmosféře patří mezi nejčastěji studované faktory globální změny v kontextu rostlino-hmyzích interakcí (Robinson et al., 2012). Řada studií ukazuje, že zvýšená koncentrace CO₂ vede ke změnám v chemickém složení rostlinných pletiv, zejména ke zvýšení poměru uhlíku a dusíku (C:N), poklesu obsahu dusíkatých látek a změnám v produkci sekundárních metabolitů (AbuEIEla et al., 2025). Tyto změny mohou snižovat nutriční kvalitu potravy pro herbivory a prodloužit jejich vývoj nebo vést ke kompenzačnímu zvýšení žíru (Stiling & Cornelissen, 2007). Přímý efekt zvýšené koncentrace CO₂ na herbivory je přitom považován za slabý, zatímco nepřímé efekty zprostředkované změnami hostitelské rostliny mohou být významnější (Bede & Blande, 2025).

Kromě koncentrace CO₂ hrají pro rostliny důležitou roli také teplota a dostupnost vody. Zvýšená teplota ovlivňuje fotosyntézu, respiraci i produkci obranných látek a může modifikovat kompromis mezi investicemi do růstu a do obrany (Qaderi et al., 2023). Stres suchem představuje další významný faktor, jehož účinky se liší v závislosti na intenzitě stresu a druhu hostitele (Descamps et al., 2021). Zatímco mírný vodní stres může vést ke zvýšení koncentrace některých obranných látek, dlouhodobé nebo intenzivní sucho obvykle snižuje vitalitu rostlin (Carvajal Acosta et al., 2022).

Klimatické faktory však nepůsobí pouze prostřednictvím hostitelské rostliny, ale mohou mít i přímé účinky na herbivory. Zvýšená teplota může urychlovat metabolismus

herbivorů, zkracovat jejich vývojový cyklus a zvyšovat intenzitu žíru (Hamann et al., 2021).

Většina uvedených studií se však zaměřuje na působení jednotlivých klimatických faktorů izolovaně, přestože v přirozených podmínkách na rostliny obvykle působí více stresorů současně (Hamann et al., 2021). Kombinované působení klimatických a environmentálních faktorů může vést k odezvám, které se liší od účinků pozorovaných při jejich samostatném působení, přičemž se vlivy jednotlivých stresorů mohou vzájemně zesilovat, oslabovat nebo měnit směr očekávaných reakcí rostlin i herbivorů (Gely et al., 2020; Zvereva & Kozlov, 2006).

Přestože je změna chemického složení hostitelských rostlin často považována za klíčový mechanismus vysvětlující odezvu herbivorů na klimatickou změnu, empirické studie poskytují nejednoznačné výsledky (Robinson et al., 2012). Zatímco některé práce dokumentují snížení prospívání herbivorů v důsledku zhoršené kvality potravy (Stiling & Cornelissen, 2007), jiné ukazují slabou odezvu (Zvereva & Kozlov, 2006), případně výraznou variabilitu mezi druhy herbivorů a experimentálními podmínkami (Robinson et al., 2012). Jedním z možných vysvětlení této variability je schopnost herbivorů regulovat příjem a využití živin prostřednictvím kompenzačního žíru a stechiometrické homeostázy, která může oslabovat přímou vazbu mezi chemií rostlin a prospíváním herbivorů (Behmer, 2009; Johnson et al., 2014).

Vedle klimatických a stanovištních faktorů se v literatuře opakovaně objevuje také otázka role genetické variability hostitelských dřevin, včetně rozdílů mezi populacemi či genotypy, v jejich obranyschopnosti vůči hmyzím herbivorům (Moreira et al., 2013; Zas et al., 2011). Existence genetické variability v náchylnosti dřevin k poškození herbivory i ve fyziologických reakcích na stres byla prokázána u řady druhů (Barbour et al., 2015; López-Goldar et al., 2018). Provenience jsou proto zvažovány jako jeden z možných nástrojů ke zvýšení odolnosti sadebního materiálu, zejména prostřednictvím selekce či šlechtění (Sinclair et al., 2015; Alfaro et al., 2014). Míra, do jaké se genetický původ skutečně uplatňuje ve srovnání s aktuálními klimatickými a stanovištními podmínkami, však zůstává nejasná a dostupné studie ukazují, že její význam je výrazně podmíněn konkrétními podmínkami prostředí a charakterem sledovaných interakcí (Park & Rogers, 2023). Dosavadní poznatky tak naznačují, že odezvy rostlin i herbivorů nelze spolehlivě vysvětlit na základě izolovaných účinků jednotlivých faktorů, ale je nutné je hodnotit jako výsledek jejich vzájemného působení (Erkelenz et al., 2025).

Přesto zůstává otázkou, do jaké míry lze změny chemického složení hostitelských rostlin považovat za spolehlivý prediktor odezvy hmyzích herbivorů. Empirické studie často dokumentují posuny ve stechiometrických charakteristikách či ve složení primárních a sekundárních metabolitů, avšak vztah těchto změn k růstu, vývoji a intenzitě žíru herbivorů není jednoznačný a může být výrazně závislý na konkrétních podmínkách

prostředí (Divekar et al., 2022). Současně není zcela jasné, zda geneticky podmíněné rozdíly mezi proveniencemi hostitelských dřevin mohou modifikovat jejich fyziologickou a chemickou odezvu na stres a zda se tyto rozdíly skutečně promítají do míry herbivorního poškození (Moreira et al., 2013; Zas et al., 2011).

Disertační práce na uvedenou problematiku navazuje prostřednictvím souboru tří publikovaných studií a dvou rukopisů připravených k publikaci. Zaměřuje se na interakce mezi lesními dřevinami a hmyzími herbivory v kontextu probíhající klimatické změny a sleduje, jak kombinace vybraných klimatických faktorů ovlivňuje chemické složení hostitelských rostlin a odezvu herbivorů. Současně zohledňuje vliv stanovištních podmínek na intenzitu herbivorního tlaku i roli genetické variability dřevin v těchto interakcích. Práce tak vytváří rámec pro komplexní posouzení toho, do jaké míry mohou změny chemického složení hostitelských rostlin skutečně vysvětlovat dynamiku herbivorního poškození v podmínkách měnícího se klimatu.

2. Cíle práce

Prvním cílem této disertační práce bylo nalezení vztahů mezi klimatem, fyziologickými změnami rostlin a herbivory vázaných na tyto rostliny. Tyto vztahy ověřit u herbivorů živících se jak na listnatých, tak na jehličnatých dřevinách.

Druhým cílem bylo stanovit, zda environmentální podmínky prostředí, druh dřeviny a jejich provenience ovlivňují výskyt klikoroha borového a fyziologické reakce sazenic na jeho žír.

3. Rozbor literární problematiky

3.1. Hlavní faktory klimatické změny

Současná klimatická změna je především důsledkem zvyšování koncentrace skleníkových plynů v atmosféře v důsledku lidské činnosti, zejména oxidu uhličitého (CO₂) (IPCC, 2021). Zvýšená koncentrace CO₂ zesiluje skleníkový efekt a vede ke zvyšování průměrné teploty na Zemi (IPCC, 2021). V kontextu suchozemských ekosystémů však CO₂ nepředstavuje pouze klimatický činitel, ale současně i klíčový zdroj uhlíku pro fotosyntézu (Chen et al., 2022). Jeho zvýšená koncentrace tak může ovlivňovat fyziologii a chemické složení rostlin (Roy et al., 2024). Nárůst teploty působí jako integrující klimatický faktor, který ovlivňuje rychlost metabolických procesů, růst a vodní režim rostlin a modifikuje jejich odezvu na další environmentální stresory (Samat et al., 2025). Zvýšení koncentrace CO₂ a nárůst teploty jsou zároveň úzce propojeny se změnami vodního režimu, včetně změn srážkového režimu, zvýšené evapotranspirace a častějšího výskytu epizod sucha (Guan et al., 2024). Tyto faktory často působí současně a jejich kombinované účinky mohou zásadně ovlivňovat růst, alokaci zdrojů a stresovou fyziologii lesních dřevin (Stinziano & Way, 2014). Z tohoto důvodu jsou změny koncentrace CO₂, teploty a dostupnosti vody považovány za hlavní klimatické faktory určující odezvu lesních ekosystémů na probíhající klimatickou změnu (IPCC, 2021).

3.1.1. Zvýšená koncentrace CO₂

Množství oxidu uhličitého (CO₂) v atmosféře patří mezi klíčové faktory globální klimatické změny, které v posledních desetiletích výrazně ovlivňují fungování suchozemských ekosystémů (IPCC, 2023). Zatímco v předindustriálním období se koncentrace CO₂ pohybovala přibližně kolem 280 ppm, v roce 2019 již dosáhla 410 ppm a v roce 2022 překročila 417 ppm. Podle emisních scénářů IPCC lze očekávat její další nárůst v průběhu 21. století (IPCC, 2021; IPCC, 2023). Tento trend má zásadní dopad na rostliny, zejména na proces fotosyntézy, růst a hospodaření s uhlíkem (Dusenge et al., 2019).

U většiny C₃ rostlin vede zvýšená koncentrace CO₂ ke zvýšení rychlosti fotosyntézy a krátkodobému nárůstu produkce biomasy (Poorter et al., 2022). Tento počáteční nárůst je často spojen s akumulací uhlíkatých sloučenin v pletivech rostlin, avšak v delším časovém horizontu se nemusí udržet, protože růst začíná být limitován omezenou dostupností živin, typicky dusíku (Fleischer & Terrer, 2022). Odezva rostlin na zvýšenou koncentraci CO₂ je tak výsledkem interakce mezi zvýšenou asimilací uhlíku a dostupností dalších limitujících zdrojů (Terrer et al., 2019).

Jedním z nejčastěji popisovaných důsledků zvýšené koncentrace CO₂ je změna poměru uhlíku a dusíku (C:N) v rostlinných pletivech (Du et al., 2019). Zvýšená asimilace uhlíku při současném omezení příjmu nebo dostupnosti dusíku vede ke snížení koncentrace dusíkatých látek, včetně proteinů a aminokyselin, v listech a jehlicích (Ekele et al., 2025).

Tento proces je v literatuře označován jako efekt zředění dusíku (nitrogen dilution effect), tedy relativní pokles koncentrace dusíku při zvýšené akumulaci uhlíku v rostlinných pletivech (Welti & Kaspari, 2024). Zvýšená dostupnost uhlíku může zároveň ovlivňovat alokaci zdrojů v rámci rostliny a modifikovat syntézu primárních i sekundárních metabolitů jako součást komplexní fyziologické odezvy rostlin na zvýšenou koncentraci CO₂ (Sun & Fernie, 2024).

3.1.2. Změna teploty

Změny teplotního režimu představují jeden z hlavních faktorů probíhající klimatické změny s významnými dopady na rostliny (IPCC, 2021). Podle klimatických modelů a scénářů budoucího vývoje emisí skleníkových plynů lze v průběhu 21. století očekávat zvýšení průměrné globální teploty o více než 2 °C ve srovnání s předindustriálním obdobím, s možností překročení i o 3 °C při pokračujících vysokých emisích (IPCC, 2023). Tyto změny ovlivňují celou řadu fyziologických procesů, včetně fotosyntézy, respirace, růstu a celkového metabolického fungování rostlin (Yamori et al., 2014).

Teplota má přímý vliv na biochemické reakce a metabolické toky v rostlinách (Dang et al., 2025). Obecně platí, že fotosyntetická aktivita a další metabolické procesy se s rostoucí teplotou zrychlují, dokud se teplota nepřiblíží optimu specifickému pro daný druh; nad touto hranicí se efektivita fotosyntézy postupně snižuje (Liu, 2020). Při překročení teplotního optima dochází ke snížení fotosyntetické aktivity a omezení růstu (Teskey et al., 2015). Zvýšená teplota rovněž ovlivňuje chování stomat a vodní režim rostlin (Urban et al., 2017). Vyšší teploty zpravidla zvyšují evapotranspiraci a atmosférické napětí par (VPD), což může vést k uzavírání průduchů a omezení vstupu CO₂ do listů a tím ke snížení čistého zisku uhlíku pro růst (Grossiord et al., 2020). Dlouhodobé teplotní zatížení tak může představovat významné omezení pro uhlíkovou bilanci a metabolickou stabilitu rostlin (Dang et al., 2025).

Vliv teploty se navíc často uplatňuje v kombinaci s dalšími environmentálními faktory, zejména dostupností vody (IPCC, 2022). Zvýšená teplota může zesilovat účinky vodního stresu a tím dále modifikovat fyziologickou odezvu rostlin (Teskey et al., 2015).

3.2. Sucho a vodní stres

Dalším významným stresovým faktorem ovlivňujícím fyziologii lesních dřevin v podmínkách probíhající klimatické změny je dostupnost vody (Choat et al., 2018). Zvýšená frekvence suchých period a vyšší evapotranspirace spojená s oteplováním vedou ke snížení dostupnosti půdní vody a vzniku vodního stresu u řady druhů dřevin (IPCC, 2022).

Vodní stres se u rostlin projevuje omezením příjmu vody kořenovým systémem a poklesem vodního potenciálu pletiv (Torres-Ruiz et al., 2024). Jednou z klíčových fyziologických reakcí je uzavírání průduchů, které omezuje transpiraci, ale současně

snižuje vstup CO₂ do listů (Buckley, 2019). To vede ke snížení fotosyntetické aktivity a omezení dostupnosti uhlíku pro růst (Drake et al., 2018). Při dlouhodobém působení se tyto procesy projevují zpomalením růstu, omezením tvorby biomasy a poklesem vitality rostlin (McDowell et al., 2020).

Omezení fotosyntézy a růstu se promítá také do chemického složení pletiv (Ballhorn et al., 2014). Zpomalení růstu může vést k relativní koncentraci některých metabolitů, zatímco při silném nebo dlouhodobém suchu dochází ke změnám v primárním i sekundárním metabolismu (He et al., 2022; Selmar & Kleinwächter, 2013). Směr a rozsah těchto změn závisí na intenzitě a délce trvání stresu (Haghpanah et al., 2024).

3.3. Klimatická změna a interakce mezi rostlinami a hmyzími herbivory

Poškození rostlin hmyzími herbivory nepředstavuje pouze ztrátu biomasy, ale aktivní stresový podnět, který spouští obranné reakce rostlin (Kloth & Dicke, 2022). Obrana vůči herbivorům může být konstitutivní, tedy založená na trvale přítomných mechanických a chemických vlastnostech pletiv, nebo indukovaná, aktivovaná v reakci na poškození (Blanchard et al., 2024). Indukovaná obrana zahrnuje změny v primárním i sekundárním metabolismu, přesměrování alokace zdrojů a aktivaci signálních drah spojených s obranou (Kessler & Mueller, 2024). Tyto reakce jsou energeticky nákladné a jejich intenzita i účinnost jsou podmíněny fyziologickým stavem rostliny (Züst & Agrawal, 2017). V podmínkách klimatické změny mohou environmentální faktory, jako je zvýšená koncentrace CO₂, vyšší teplota či menší dostupnost vody, modifikovat schopnost rostlin reagovat na žír herbivorů a ovlivňovat rovnováhu mezi růstem a obranou (Hamann et al., 2021).

3.3.1. Nepřímé efekty klimatické změny zprostředkované hostitelskou rostlinou

Klimatická změna ovlivňuje interakce mezi rostlinami a hmyzími herbivory především nepřímo, prostřednictvím změn fyziologického stavu a chemického složení hostitelských rostlin (Kuczyk et al., 2021). Faktory, jako je zvýšená koncentrace CO₂, nárůst teploty a změny dostupnosti vody, modifikují růst, metabolismus a hospodaření s živinami u rostlin, což se následně promítá do kvality pletiv využívaných herbivory (Hamann et al., 2021).

Jak bylo uvedeno výše (kap. 3.1.1), zvýšená koncentrace CO₂ může vést ke změnám stechiometrických charakteristik pletiv, zejména ke zvýšení poměru uhlíku a dusíku (C:N) a poklesu obsahu dusíkatých látek (Loladze, 2014). Dusík představuje klíčovou živinu pro hmyzí herbivory, neboť je nezbytný pro syntézu proteinů, růst a vývoj (Lee et al., 2021) a jeho relativní nedostatek tak může ovlivňovat nutriční hodnotu potravy (Li et al., 2024). Změny v C:N poměru tak představují jeden z hlavních mechanismů,

jimiž klimatické faktory mohou nepřímo ovlivňovat prospívání herbivorů (Kaspari & Welts, 2024).

Vedle stechiometrických charakteristik mohou klimatické faktory ovlivňovat také složení primárních metabolitů v rostlinných pletivech, zejména obsah sacharidů, aminokyselin a proteinů (Neto et al., 2021). Změny v obsahu rozpustných cukrů a dusíkatých látek mohou modifikovat energetickou hodnotu potravy a její nutriční vyváženost, což může ovlivňovat růst, vývoj i schopnost herbivorů kompenzovat sníženou kvalitu potravy zvýšeným příjmem (Deans et al., 2022).

Klimatická změna může dále ovlivňovat syntézu sekundárních metabolitů, které tvoří klíčovou součást obranných strategií rostlin (Kivimäenpää et al., 2022). Fenolické sloučeniny, třísloviny či terpeny mohou snižovat stravitelnost pletiv, působit toxicky nebo ovlivňovat chování herbivorů při výběru hostitelské rostliny (Holopainen et al., 2018). Změny v produkci a složení těchto látek tak mohou modifikovat nejen atraktivitu hostitelské rostliny, ale i prospívání a intenzitu žíru herbivorů (War et al., 2012).

Nepřímé efekty klimatické změny zprostředkované hostitelskou rostlinou jsou proto považovány za významný mechanismus utvářející interakce mezi rostlinami a hmyzími herbivory (Cornelissen, 2011). Zatímco některé klimatické faktory, zejména teplota, mohou působit na herbivory i přímo, zvýšená koncentrace CO₂ se projevuje převážně prostřednictvím změn kvality hostitelské rostliny (DeLucia et al., 2012). Hostitelská rostlina tak funguje jako klíčový filtr, který zprostředkovává vliv klimatické změny na vyšší trofické úrovni (Kuczyk et al., 2021).

3.3.2. Reakce hmyzích herbivorů na změny chemického složení rostliny

Změny v kvalitě hostitelské rostliny vyvolané klimatickými faktory se u hmyzích herbivorů mohou projevit celou řadou fyziologických, vývojových i behaviorálních reakcí (Bede & Blande, 2025). Tyto odezvy jsou určovány zejména nutriční hodnotou pletiv, složením primárních metabolitů a přítomností obranných látek, které společně formují podmínky pro růst, vývoj a reprodukci herbivorů (Behmer, 2009).

V souvislosti se zvýšeným poměrem C:N a poklesem obsahu dusíku může docházet ke zpomalení růstu a prodloužení vývojového cyklu herbivorů (Li et al., 2024). Nedostatek dusíku omezuje syntézu proteinů a může vést ke snížení tělesné hmotnosti či zvýšené mortalitě, zejména v raných vývojových fázích (Ren et al., 2025).

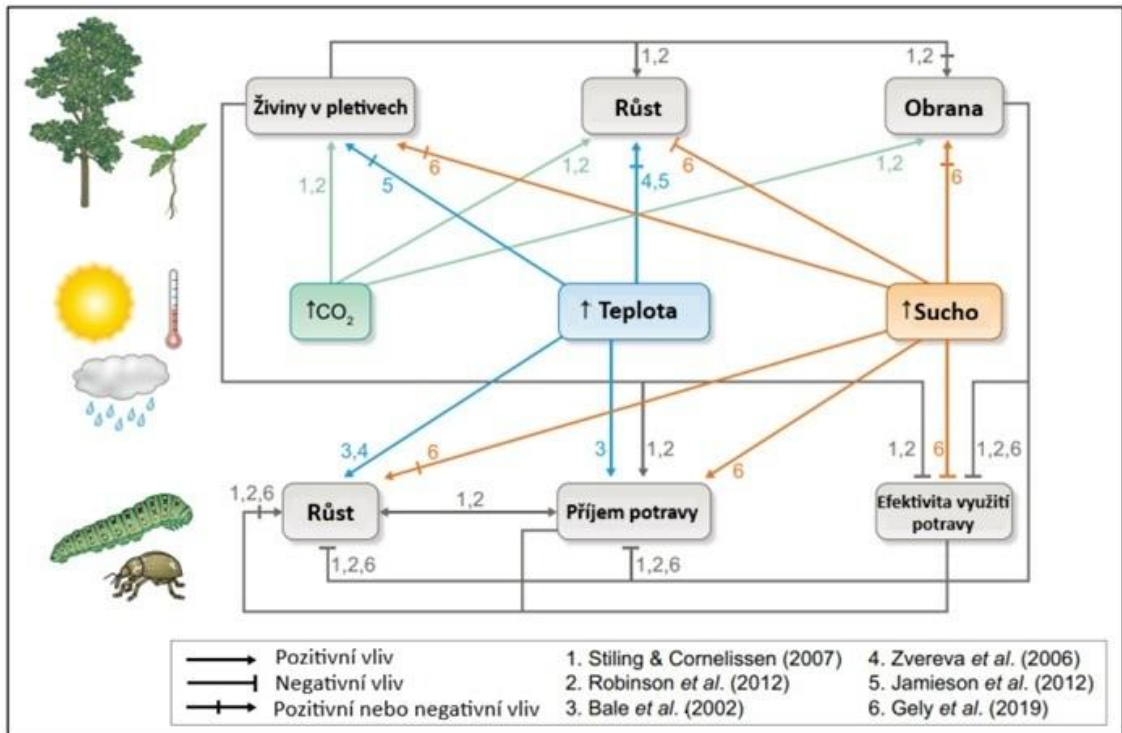
Změny v obsahu sacharidů, aminokyselin a proteinů tak mohou vést k nerovnováze mezi energetickými a stavebními složkami potravy (Le Gall & Behmer, 2014). Pokud je potrava bohatší na uhlík, ale chudší na dusík, mohou herbivoři reagovat kompenzačním žírem, tedy zvýšením příjmu potravy s cílem pokrýt své nutriční

požadavky (Stiling & Cornelissen, 2007). Úspěšnost této strategie je však závislá na schopnosti regulace příjmu a využití živin, tedy na míře stechiometrické homeostázy (Meunier et al., 2023).

Sekundární metabolity rostlin mohou odezvu herbivorů dále modifikovat (War et al., 2012). Fenolické sloučeniny, třísloviny či terpeny mohou snižovat stravitelnost potravy, působit toxicky nebo ovlivňovat preference při výběru hostitele (Khan et al., 2025). Změny v jejich produkci tak mohou ovlivňovat intenzitu žíru i energetické náklady spojené s detoxikací (Jeckel et al., 2022).

Reakce herbivorů na změny kvality hostitelské rostliny se může výrazně lišit v závislosti na druhu (Forister et al., 2015), vývojovém stádiu (Barton & Koricheva, 2010) i na tom, zda jde o specialistu nebo generalistu (Ali & Agrawal, 2012). Zatímco někteří herbivoři jsou schopni změny v kvalitě potravy kompenzovat, jiní reagují výrazným poklesem prospívání (Behmer, 2009). Tato závislost přispívá k nejednoznačným výsledkům v literatuře (Zvereva & Kozlov, 2006).

Vztahy mezi hlavními faktory klimatické změny, fyziologickou odezvou rostlin a reakcemi herbivorů jsou schematicky znázorněny na obrázku 1. Schéma shrnuje přímé i nepřímé vazby popisované v literatuře a zdůrazňuje roli hostitelské rostliny jako klíčového zprostředkujícího článku mezi klimatickými faktory a vyššími trofickými úrovněmi (Hamann et al., 2021).



Obrázek 1. Schématické znázornění přímých a nepřímých účinků klimatických faktorů na fyziologii rostlin a trofické odezvy hmyzích herbivorů. Převzato a upraveno podle přehledové studie Hamann et al. (2021).

3.4. Genetická variabilita a provenience lesních dřevin

Výše popsané mechanismy, jimiž klimatické faktory ovlivňují fyziologii rostlin a jejich interakce s hmyzími herbivory, představují obecně popisovaný rámec napříč různými rostlinnými druhy (Zvereva & Kozlov, 2006). Intenzita těchto procesů a výsledná odezva však nemusí být u všech jedinců stejná. Zdrojem variability může být genetický původ hostitelských rostlin, který ovlivňuje jejich růstové vlastnosti, fyziologickou odezvu na stresory i obranné strategie (Zas et al., 2011).

Genetická variabilita lesních dřevin a rozdíly mezi proveniencemi jsou formovány dlouhodobým působením klimatických a stanovištních podmínek v místě jejich původu (Whittet et al., 2019). Tyto rozdíly se mohou projevovat ve zvýšené či snížené toleranci k abiotickým stresům, jako jsou sucho nebo teplotní extrém (Alberto et al., 2013), a zároveň se promítat do chemického složení pletiv (Lieutier et al., 2003), alokace zdrojů a charakteru obranných mechanismů (Moreira et al., 2014).

Řada studií prokázala existenci geneticky podmíněné variability v náchylnosti lesních dřevin k poškození hmyzími herbivory (Lämke & Unsicker, 2018; Hochwender & Fritz, 2004). Rozdíly mezi proveniencemi se mohou projevovat jak v míře poškození, tak v následném prospívání rostlin a souviset s variabilitou v konstitutivní i indukované obraně (Moreira et al., 2014). Odlišnosti v chemickém složení pletiv, produkci

sekundárních metabolitů či emisích těkavých látek mohou ovlivňovat atraktivitu hostitelské rostliny i prospívání herbivorů (Hamann et al., 2021).

Význam genetické variability se dále zvyšuje v kontextu klimatické změny, která může zvýrazňovat rozdíly mezi rostlinami různého geografického původu v jejich schopnosti čelit kombinaci abiotických a biotických stresů (Schwinning et al., 2022). Provenience tak představuje faktor, který může modifikovat odezvu lesních ekosystémů na klimatickou změnu (Aitken & Whitlock, 2013).

Studium vztahů mezi klimatickými faktory, fyziologickou odezvou rostlin a reakcemi herbivorů poskytuje základ pro pochopení těchto interakcí. V reálných podmínkách lesních ekosystémů však klimatické, stanovištní a biotické faktory nepůsobí samostatně, ale v kombinaci, přičemž kombinované účinky abiotických a biotických stresorů mohou výrazně modifikovat výslednou odezvu systému (Teshome et al., 2020).

4. Metodika a výsledky jednotlivých experimentů

Disertační práce je založena na souboru tří publikovaných studií a dvou rukopisů připravených k publikaci, které se liší experimentálním přístupem a mírou kontroly podmínek. Jednotlivé experimenty jsou řazeny od plně kontrolovaných laboratorních podmínek přes semi-field uspořádání až po terénní studie, aby bylo možné sledovat, jak se vztahy mezi chemií hostitelských rostlin a odezvou herbivorů mění s rostoucí komplexitou prostředí.

Podrobné popisy experimentálního uspořádání, použitých metod a statistických analýz jsou uvedeny v jednotlivých publikovaných pracích a rukopisech. Následující podkapitoly shrnují metodiku podle typu experimentálního přístupu.

4.1. Přehled experimentů disertační práce

Laboratorní experiment – plně faktoriální uspořádání ($\text{CO}_2 \times$ teplota), herbivoři krmeni standardizovanou dietou z experimentálních rostlin (Příloha 1).

Semi-field experiment I – ekosystémové sféry; manipulace CO_2 , vody, dusíku a provenience smrku; larvy pilatek krmeny přímo na stromech (Příloha 2).

Semi-field experiment II – replikované venkovní boxy; hodnocení chemické odezvy borovice na přímé poškození klikorohem (Příloha 3).

Terénní studie I – 20 pasek; faktory pasek a poškození sazenic různých druhů (Příloha 4).

Terénní studie II – 60 lokalit; vztah mezi výskytem klikorohů, stářím paseky a post-těžebním managementem (Příloha 5).

4.2. Laboratorní experiment: CO₂, teplota a interakce rostlina-herbivor

Tento experiment je detailně zpracován v publikované studii uvedené jako Příloha 1 této disertační práce.

Tato kapitola popisuje experiment realizovaný v plně kontrolovaných podmínkách, jehož cílem bylo otestovat, zda kombinace různých koncentrací atmosférického CO₂ a teplot vedou ke změnám chemického složení hostitelských rostlin a zda se tyto změny promítají do prospívání listožravých herbivorů. Experiment byl navržen jako plně faktoriální uspořádání umožňující testovat hlavní efekty obou faktorů i jejich interakce.

4.2.1. Studované organismy

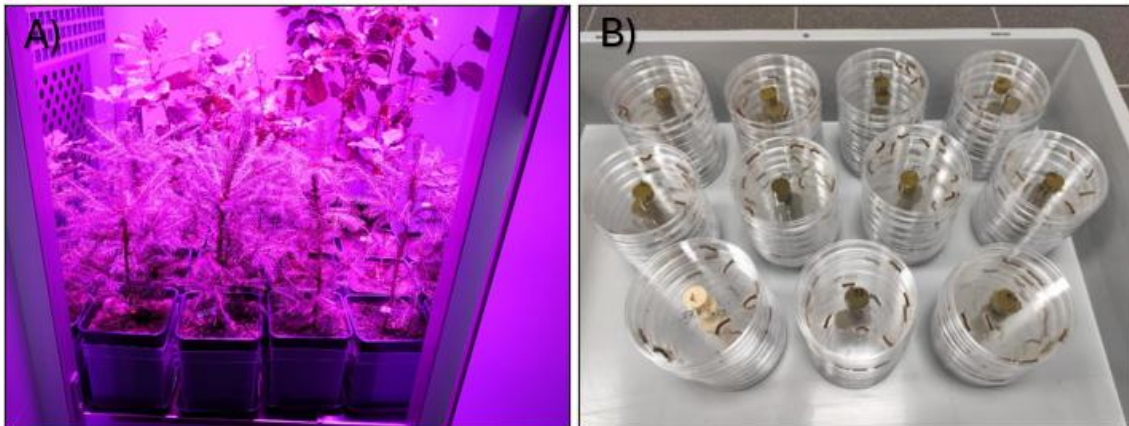
Experiment zahrnoval dva druhy hostitelských dřevin reprezentující kontrastní typy potravy: dub letní (*Quercus robur* L.) a smrk ztepilý (*Picea abies* (L.) Karst.). Tyto druhy se liší chemickým složením pletiv, zejména obsahem dusíku, sacharidů a sekundárních metabolitů a představují vhodný model pro testování rozdílů v kvalitě potravy pro herbivory (Xie et al., 2024).

Jako modeloví herbivoři byly použity dva druhy motýlů (Lepidoptera: Erebidae): bekyně velkohlavá (*Lymantria dispar* (Linnaeus, 1758)) a bekyně mniška (*Lymantria monacha* (Linnaeus, 1758)). *L. dispar* je polyfágní druh preferující listnaté dřeviny, zatímco *L. monacha* je vázána především na jehličnany (Nakládal & Brinkeová, 2015; Milanović, 2014). Škodlivým stádiem jsou larvy, které při vyšší populační hustotě mohou způsobovat úplnou defoliaci porostů (Nakládal & Brinkeová, 2015).

4.2.2. Design experimentu

Sazenice obou hostitelských dřevin byly po dobu jednoho roku pěstovány v růstových komorách ve čtyřech kombinacích klimatických ošetření vzniklých kombinací dvou úrovní koncentrace atmosférického CO₂ (ambientní a zvýšená) a dvou teplotních režimů (nižší a vyšší teplota) (Obr. 2). Experiment měl plně faktoriální uspořádání umožňující testovat hlavní efekty jednotlivých faktorů i jejich interakce.

Po ukončení kultivace byly z listů dubu a jehlic smrku připraveny agarové diety obsahující jemně mletý rostlinný materiál. Tyto diety sloužily jako standardizovaný zdroj potravy pro larvy a umožnily přesnou kontrolu množství zkonsumované potravy (Obr. 2B). Larvy byly experimentálním dietám vystaveny ve čtvrtém instaru, kdy bylo hodnoceno jejich prospívání.



Obrázek 2. Experimentální sazenice smrku ztepilého (*Picea abies*) a dubu letního (*Quercus robur*) pěstované ve fotokomoře (A) a Petriho misky s agarovou dietou z experimentálního materiálu s larvami *Lymantria dispar* a *Lymantria monacha* (B) (Foto: David Musiolek, Barbora Dvořáková).

4.2.3. Hodnocené proměnné

U hostitelských rostlin byly hodnoceny základní stechiometrické charakteristiky (obsah C, N a poměr C:N) a vybrané skupiny primárních a sekundárních metabolitů, zejména rozpustné sacharidy a fenolické sloučeniny.

Prospívání herbivorů bylo hodnoceno pomocí gravimetrických nutričních indexů: relativní růstové rychlosti (RGR), aproximativní stravitelnosti (AD), efektivity konverze přijaté potravy (ECI) a efektivity konverze strávené potravy (ECD) podle Waldbauera (1968).

4.2.4. Výsledky experimentu

Chemické složení hostitelských rostlin se výrazně lišilo mezi oběma dřevinami. Smrk vykazoval nižší obsah dusíku a vyšší poměr C:N než dub. Zvýšená koncentrace CO₂ vedla ke zvýšení poměru C:N u obou druhů, zatímco teplotní režim ovlivňoval zejména obsah dusíku, především u dubu.

Rozdíly byly zaznamenány i u sekundárních metabolitů. Dubové listy obsahovaly vyšší množství fenolických látek a kondenzovaných tříslovin, zatímco smrk vykazoval vyšší obsah flavonoidů. Zvýšená koncentrace CO₂ neovlivnila sledované sekundární metabolity konzistentním způsobem. Teplotní režim však jejich koncentrace ovlivňoval, přičemž směr změn byl závislý na druhu hostitele a kombinaci ošetření.

U primárních metabolitů byly mezi druhy patrné rozdíly zejména v koncentracích rozpustných sacharidů, které byly vyšší u dubu. Kombinace CO₂ a teploty však nepředstavovala dominantní zdroj variability v obsahu cukrů.

Prospívání herbivorů bylo primárně ovlivněno druhem hostitelské rostliny. U *L. dispar* byly nutriční indexy (s výjimkou ECD) vyšší na dubových dietách než na dietách ze smrku, přičemž larvy na smrku vykazovaly obecně nejnižší výkonnost. Vliv klimatických

kombinací byl ve srovnání s efektem hostitele slabý a projevil se pouze v několika dílčích interakcích bez jednotného směru. U *L. monacha* byla výkonnost larev obecně nižší na smrkových dietách ve srovnání s kontrolní dietou a klimatické efekty se projevíly jen omezeně a nekonzistentně napříč jednotlivými indexy. Celkově nebyl zjištěn konzistentní vzorec systematické změny prospívání larev v závislosti na testovaných klimatických kombinacích.

4.2.5. Interpretace v rámci experimentu

Výsledky ukazují, že změny chemického složení hostitelských rostlin vyvolané kombinací zvýšené koncentrace CO₂ a teploty se pouze omezeně promítly do prospívání listožravých herbivorů. Přestože byly zaznamenány změny ve stechiometrických charakteristikách i v některých metabolitech, tyto změny nebyly doprovázeny konzistentní odezvou larev.

Dominantním faktorem ovlivňujícím prospívání herbivorů byl druh hostitelské rostliny, zatímco kombinace klimatických faktorů měla ve srovnání s tímto efektem pouze omezený vliv. Výsledky ukazují, že při kombinovaném působení více environmentálních faktorů nemusí být vazba mezi chemickým složením rostlin a odezvou herbivorů lineární ani snadno předvídatelná (Scherber et al., 2013).

Zatímco laboratorní experiment umožnil testování vztahů při plně kontrolovaných podmínkách, následující kapitola rozšiřuje tuto problematiku do semi-field prostředí s nižší mírou kontroly a vyšší mírou přirozených podmínek.

4.3. Semi-field experiment I: CO₂, voda, dusík a provenience ve vztahu k chemii smrku a pilatkám

Tento experiment je detailně zpracován v rukopise připraveném k publikaci, uvedeném jako Příloha 2 této disertační práce.

Tento semi-field experiment zkoumal vztah mezi chemickým složením hostitelské rostliny a prospíváním specializovaných listožravých herbivorů v podmínkách částečné manipulace environmentálních faktorů. Experiment byl koncipován jako plně faktoriální uspořádání zahrnující koncentraci atmosférického CO₂, dostupnost vody, dostupnost dusíku a provenience *P. abies*.

Chemické složení jehlic bylo hodnoceno na úrovni základních stechiometrických charakteristik, vybraných primárních metabolitů a celkového metabolického profilu bez cílené kvantifikace jednotlivých skupin sekundárních metabolitů. Larvy pilatek byly umístěny přímo na experimentální stromy, což umožnilo hodnotit jejich prospívání při přirozeném způsobu příjmu potravy.

4.3.1. Studované organismy

Hostitelskou rostlinou byl s. ztepilý. Do experimentu byly zahrnuty dvě provenience reprezentující původ ze smrkobukového lesního vegetačního stupně (6. LVS) a smrkového lesního vegetačního stupně (8. LVS), což umožnilo zohlednit variabilitu spojenou s genetickým původem hostitelských rostlin.

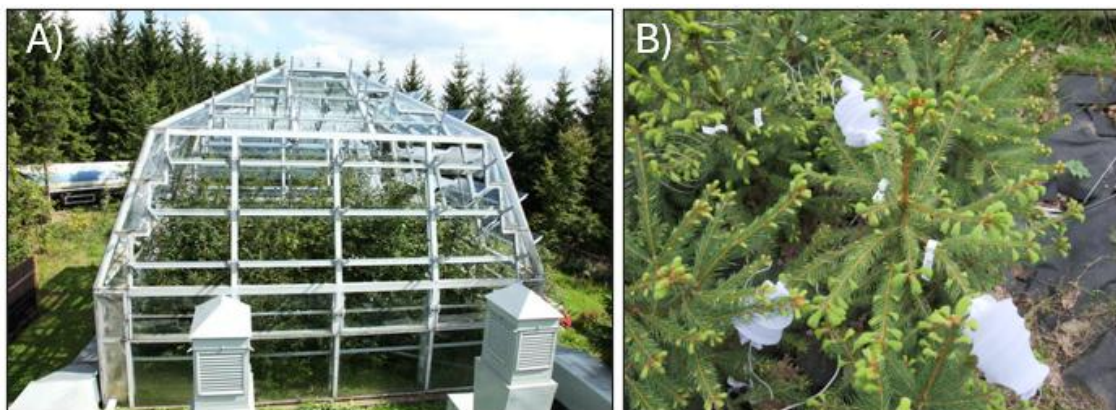
Jako modeloví herbivoři byly použity larvy tří druhů smrkových pilatek (Hymenoptera: Tenthredinidae; *Pristiphora abietina* (Christ, 1791), *Pristiphora leucopodia* (Hartig, 1837) a *Euura scutellata* (Hartig, 1837), specializovaných defoliátorů vázaných na smrk (Holuša & Lubojacký, 2007). Larvy se živí čerstvě rašícími jehlicemi a při vyšší populační hustotě mohou způsobovat lokální defoliace. Druhy mají zpravidla jednorocní vývojový cyklus a přezimují v půdě ve stádiu praepupy (Liston et al., 2017).

4.3.2. Design experimentu

Experiment byl realizován ve skleněných ekosystémových sférách umožňujících manipulaci vybraných environmentálních faktorů při zachování přirozenějších podmínek než v laboratorním experimentu (Obr. 3A). Design zahrnoval plně faktoriální kombinaci:

- koncentrace atmosférického CO₂ (ambientní vs. zvýšená),
- dostupnosti vody (plná vs. omezená zálivka),
- dostupnosti dusíku (hnojení vs. bez hnojení),
- provenience smrku (6. LVS vs 8. LVS).

Ošetření byla aplikována kontinuálně. Larvy pilatek byly na stromky nasazovány a ponechány k přirozenému žíru (Obr. 3B). Uspořádání umožnilo testovat hlavní efekty jednotlivých faktorů i jejich interakce ve vztahu k chemickému složení jehlic a prospívání larev.



Obrázek 3. Skleněné ekosystémové sféry pro semi-field experiment (A) a detail stromku smrku ztepilého (*Picea abies*) se síťovým vakem pro umístění larev pilatek (B) (Foto: převzato z webu Abicko – AV ČR, David Musiolek).

4.3.3. Hodnocené proměnné

Prospívání pilatek bylo hodnoceno pomocí relativní růstové rychlosti (RGR). Chemická analýza trusu byla využita k posouzení stechiometrických vztahů mezi poměrem C:N jehlic, růstem larev a poměrem C:N trusu.

Chemické vlastnosti jehlic zahrnovaly obsah uhlíku, dusíku a poměr C:N, vybrané primární metabolity a celkový metabolomický profil.

4.3.4. Výsledky experimentu

Všechny experimentální faktory – koncentrace atmosférického CO₂, dostupnost vody, dusíkaté hnojení i provenience významně ovlivnily poměr C:N jehlic. Zvýšená koncentrace CO₂ vedla ke zvýšení poměru C:N, zatímco dusíkaté hnojení a plná zálaha tento poměr snižovaly. Poměr C:N se lišil také mezi proveniencemi a byla zaznamenána významná interakce mezi CO₂ režimem a proveniencí.

Navzdory těmto změnám chemického složení nebyl u pilatek zaznamenán žádný vzorec odezvy. Relativní růstová rychlost larev se mezi jednotlivými ošetřeními nelišila a nereagovala ani na změny dostupnosti vody, dusíku či provenience. Stechiometrie trusu larev rovněž nevykazovala systematické rozdíly mezi experimentálními variantami.

Metabolomické analýzy ukázaly, že zvýšená koncentrace CO₂ představovala hlavní zdroj variability v celkovém chemickém profilu jehlic. PLS-DA analýza zároveň jasně oddělila vzorky z ambientního a zvýšeného CO₂ režimu, přičemž mezi nejvýznamnější metabolity přispívající k této separaci patřily především aminokyseliny. Vliv dusíkatého hnojení

a závlahy byl slabší a méně konzistentní, zatímco provenience se v metabolomických profilech významně neprojevila.

Celkově tedy experiment prokázal výrazné změny chemie hostitelské rostliny, které se však nepromítly do měřitelných rozdílů v růstu ani stechiometrii herbivorů.

4.3.5. Interpretace v rámci experimentu

Výsledky ukazují, že kombinované působení zvýšené koncentrace CO₂, dostupnosti vody, dusíku a provenience vedlo ke změnám chemie hostitelské rostliny, zejména ve stechiometrických charakteristikách. Metabolomická odezva byla primárně spojena se zvýšenou koncentrací CO₂.

Navzdory těmto chemickým změnám nebyla zaznamenána odpovídající konzistentní odezva v prospívání pilatek. Výsledky opět ukazují, že změny chemického složení jehlic, včetně posunů v poměru C:N a metabolomického profilu, nemusí být samy o sobě dostatečné k predikci reakce specializovaných herbivorů v podmínkách kombinovaného působení více environmentálních a genetických faktorů (Ogwu et al., 2025).

4.4. Semi-field experiment II: chemická odpověď borovice na poškození klikorohem

Tento experiment je detailně zpracován v rukopise připraveném k publikaci, uvedeném jako Příloha 3 této disertační práce.

Tento experiment se zaměřil na chemickou odezvu hostitelské rostliny na přímé poškození herbivorem. Na rozdíl od předchozích experimentů nebyly manipulovány klimatické faktory; cílem bylo porovnat chemické složení napadených a nenapadených sazenic borovice lesní (*Pinus sylvestris* L.) po krátkodobé expozici žíru klikorooha borového (*Hylobius abietis* (Linnaeus, 1758)).

Pozornost byla věnována rozdílům mezi jednotlivými rostlinnými orgány, zejména kořeny a jehlicemi, aby bylo možné posoudit, zda se poškození kmene promítá do změn chemického složení nadzemních a podzemních částí rostliny.

4.4.1. Studované organismy

Hostitelskou rostlinou byla b. lesní (*P. sylvestris*). Do experimentu byly zahrnuty sazenice dvou proveniencí (děčínské a třeboňské) reprezentujících původ z nižší a vyšší nadmořské výšky.

Studovaným herbivorem byl k. borový, polyfágní druh napadající široké spektrum jehličnatých dřevin. U tohoto druhu dochází k poškození kmínku sazenic žírem na kůře a lýku, zejména v oblasti kořenového krčku.

4.4.2. Design experimentu

Experiment probíhal v semi-field podmínkách na zelené střeše bez manipulace klimatických faktorů. Sazenice obou proveniencí byly umístěny do boxů, do nichž byli umístěni dospělci klikorooha (Obr. 4B). Síťová konstrukce na plastových boxech zamezovala úniku hmyzu a umožňovala přirozený průběh herbivorie (Obr. 4A).

Napadené sazenice byly vystaveny žíru po dobu sedmi dnů, zatímco kontrolní jedinci borovice byli po stejnou dobu ponecháni bez přítomnosti herbivora.

Po ukončení expozice byly odebrány vzorky kořenů a jehlic pro následné chemické analýzy.



Obrázek 4. Rozmístění experimentálních boxů na zelené střeše (A) a detail sazenic umístěných v jednom boxu (B) (Foto: Barbora Dvořáková).

4.4.3. Hodnocené proměnné

U kořenů i jehlic byly stanoveny základní stechiometrické charakteristiky (obsah C, N a poměr C:N), koncentrace rozpustných sacharidů a fenolických sloučenin. Hodnocení chemického složení bylo doplněno metabolomickou analýzou.

Průměr kmene byl zahrnut jako doplňková proměnná ve statistickém vyhodnocení.

4.4.4. Výsledky experimentu

Poškození herbivorem vedlo k orgánově specifickým změnám chemického složení rostliny. Nejvýraznější odezva byla zaznamenána v kořenech, zatímco chemické změny v jehlicích nebyly zjištěny.

V kořenech napadených sazenic došlo k významnému poklesu koncentrace rozpustných sacharidů ve srovnání s kontrolními jedinci, a to konzistentně napříč oběma proveniencemi. Naopak jejich koncentrace v jehlicích nebyla poškozením významně ovlivněna. Pokles koncentrace vybraných rozpustných sacharidů v kořenech navíc souvisel s intenzitou žíru. Koncentrace fenolických látek v jehlicích se mezi napadenými a kontrolními rostlinami významně nelišila.

Metabolomická analýza neprokázala zřetelnou separaci vzorků podle napadení ani provenience.

Rozsah poškození sazenic byl ovlivněn průměrem kmene, zatímco mezi proveniencemi nebyly zjištěny rozdíly v intenzitě žíru.

Výsledky tedy ukazují, že krátkodobé poškození kmene klikorohem bylo spojeno se změnami primárního metabolismu v kořenech, zatímco chemické složení jehlic zůstalo stabilní.

4.4.5. Interpretace v rámci experimentu

Krátkodobé poškození kmene borovice se chemicky projevilo především v podzemních částech rostliny. Pokles koncentrace rozpustných cukrů v kořenech pravděpodobně souvisí s narušením transportu asimilátů v důsledku poškození kůry a lýka (Rademacher et al., 2019).

Absence změn v jehlicích naznačuje, že poškození kmene nevedlo v daném časovém horizontu k systémové aktivaci obranného metabolismu v nadzemních orgánech. Reakce rostliny tak byla prostorově omezená a soustředěná především na změny primárního metabolismu (Schultz et al., 2013).

Tyto výsledky doplňují předchozí experimenty zaměřené na defoliátory a ukazují, že charakter chemické odezvy hostitelské rostliny závisí na typu herbivorie a zasaženém orgánu (Xiao et al., 2019).

4.5. Terénní studie: prostředí, management a herbivorní tlak

Zatímco předchozí experimenty byly zaměřeny na chemickou odezvu hostitelských rostlin a prospívání herbivorů, následující terénní studie hodnotí herbivorní tlak v reálných podmínkách obnovy lesa. Vedle vlastností hostitelské rostliny zde hrají klíčovou roli stanovištní podmínky a způsob hospodaření, které určují intenzitu herbivorního tlaku na sazenice.

Pro terénní část byl jako modelový druh zvolen k. borový a *Hylobius pinastri* (Gyllenhal, 1813), významní škůdci mladých jehličnatých porostů v Evropě (Lalík et al., 2021). Jejich význam je spojen zejména s obnovou lesa po těžbě, kdy dospělci nalétávají na paseky a poškozují vysazené sazenice (Wallertz et al., 2016).

Terénní experimenty se zaměřují na vztah mezi charakteristikami pasek, způsobem jejich post-těžebního managementu a výskytem k. borového a dále na míru poškození sazenic různých druhů dřevin. Tato část práce tak poskytuje pohled na herbivorní tlak v podmínkách, kde se kombinují biologické, stanovištní a managementové faktory.

4.6. Terénní studie I: faktory pasek a poškození sazenic

Tato studie je detailně zpracována v publikované studii uvedené jako Příloha 4 této disertační práce.

4.6.1. Design studie

Studie byla provedena na 20 lesních pasekách ve středních Čechách vzniklých po těžbě v důsledku kůrovcových kalamit. Paseky se nacházely převážně v jehličnatých porostech s dominancí s. ztepilého.

Na každé pasece byly současně hodnoceny stanovištní charakteristiky, výskyt k. borového a poškození sazenic. Paseky byly osázeny třemi běžně používanými druhy dřevin: b. lesní, modřínem opadavým (*Larix decidua* Mill.) a s. ztepilým.

4.6.2. Hodnocené proměnné

Na úrovni pasek byly sledovány vybrané stanovištní charakteristiky, konkrétně:

- půdní vlhkost,
- průměrná vzdálenost mezi pařezy,
- průměr pařezů,
- podíl ostatních jehličnatých dřevin,
- mulčování pařezů.

Příklady pasek s různými stanovištními charakteristikami zobrazuje Obrázek 5.

Výskyt klikorohů byl hodnocen počtem jedinců zachycených do zemních pastí s návnadou. Poškození sazenic bylo hodnoceno na základě intenzity žíru na kmínku, výšky nejvýše položeného poškození a mortality.



Obrázek 5. Příklady sledovaných typů pasek: suché stanoviště (A), vlhké stanoviště (B) a mulčované stanoviště (C) (Foto: Barbora Dvořáková).

4.6.3. Výsledky studie

Výskyt k. borového se mezi jednotlivými pasekami lišil v závislosti na stanovištních podmínkách. Z hodnocených proměnných byla s početností klikoroha spojena především půdní vlhkost. Ostatní charakteristiky pasek nevykazovaly jednoznačný vztah k celkovému výskytu.

Poškození sazenic se lišilo mezi druhy dřevin. Intenzita žíru byla vyšší u borovice lesní a modřínu opadavého než u smrku ztepilého. Kromě typu sazenice ovlivňoval intenzitu žíru také průměr pařezů na pasece.

4.6.4. Interpretace v rámci studie

Výsledky ukazují, že výskyt k. borového na pasekách souvisí především se stanovištními podmínkami, zejména s půdní vlhkostí. Naopak vlastnosti pařezů a způsob jejich ošetření nebyly hlavním faktorem určujícím jejich početnost.

Míra poškození sazenic se lišila mezi druhy dřevin, což ukazuje rozdílnou náchylnost hostitelských druhů k napadení. Kromě druhu sazenice ovlivňoval intenzitu žíru také průměr pařezů na pasece, což ukazuje na význam strukturálních charakteristik prostředí pro rozsah poškození.

4.7. Terénní studie II: vliv mulčování pařezů na výskyt klikorohů

Tato studie je detailně zpracována v publikované studii uvedené jako Příloha 5 této disertační práce.

4.7.1. Design studie

Studie byla provedena na 60 lesních pasekách ve středních Čechách lišících se způsobem post-těžebního managementu. Sledovány byly paseky s mulčováním pařezů (Obr. 6A, B) a paseky s ponechanými pařezy (Obr. 6C, D), přičemž obě varianty zahrnovaly nově vzniklé (Obr. 6A, C) i roční paseky (Obr. 6B, D). Současně byl zohledněn typ původního porostu (jehličnatý vs. smíšený).

Výskyt k. borového byl hodnocen pomocí zemních pastí s návnadou, rozmístěných na jednotlivých lokalitách. Odchyty probíhaly opakovaně od května do srpna.

4.7.2. Hodnocené proměnné

Na úrovni pasek byl sledován:

- způsob ošetření pařezů (mulčování vs. ponechání),
- stáří paseky (nová vs. roční),
- typ původního porostu (jehličnatý vs. smíšený).

Hlavní sledovanou proměnnou byla početnost k. borového zachycených do pastí.



Obrázek 6. Příklady sledovaných typů pasek podle stáří a typu post-těžebního managementu: nová mulčovaná paseka (A), roční mulčovaná paseka (B), nová paseka s ponechanými pařezy (C) a roční paseka s pařezy (D) (Foto: Barbora Dvořáková).

4.7.3. Výsledky studie

Početnost k. borového se lišila mezi pasekami v závislosti na způsobu managementu pařezů a stáří paseky. Na nově vzniklých pasekách byl zaznamenán vyšší výskyt jedinců na mulčovaných plochách než na pasekách s ponechanými pařezy. Na ročních pasekách byl naopak výskyt nižší na mulčovaných plochách. Nejvyšší početnost byla zaznamenána na začátku sledovaného období, zatímco v dalších měsících zůstával jejich výskyt nižší a relativně stabilní.

Na nově mulčovaných pasekách byl výskyt k. borového srovnatelný v jehličnatých i listnatých porostech, přestože za běžných podmínek bývají jehličnaté porosty výrazně atraktivnější.

4.7.4. Interpretace v rámci studie

Výsledky ukazují, že vliv mulčování pařezů na výskyt k. borového je časově podmíněný. Zatímco bezprostředně po vzniku paseky bylo mulčování spojeno s vyšší početností klikorohů, na starších pasekách byl zaznamenán opačný trend.

Tyto rozdíly dokazují, že význam post-těžebního zásahu se mění v čase a také to, že krátkodobý efekt mulčování může dočasně překrýt vliv typu původního porostu na atraktivitu pasek. Sezónní dynamika výskytu dále ukazuje, že tlak k. borového je nejvyšší v období jarního rojení a následně klesá.

Celkově výsledky naznačují, že hodnocení účinnosti post-těžebních opatření musí zohledňovat časový vývoj stanoviště i strukturu původního porostu.

5. Syntéza výsledků

5.1. Vztah mezi chemickými změnami rostlin a odezvou herbivorů

Napříč laboratorními a semi-field experimenty se opakovaně ukázalo, že změny chemického složení hostitelských rostlin vyvolané klimatickými faktory nebo herbivorií byly často výrazné, ale jejich přenos do odezvy a prospívání hmyzích herbivorů zůstával slabý. Tento vzorec byl velmi podobný napříč různými typy experimentálních podmínek i skupinami herbivorů.

V laboratorních experimentech vedly klimatické faktory k výrazným změnám chemického složení hostitelských rostlin, ale tyto změny se však pouze omezeně promítaly do růstu, vývoje nebo do způsobu využívání potravy listožravými herbivory. Podobný vzorec byl pozorován i v semi-field experimentech, kde kombinované působení zvýšené koncentrace CO₂, dostupnosti vody a dusíku významně ovlivnilo chemické vlastnosti pletiv, aniž by se tyto změny promítly do odezvy listožravých herbivorů.

Celkově tak napříč experimentálními úrovněmi nebyl potvrzen předpoklad, že změny chemického složení hostitelských rostlin představují spolehlivý prediktor odezvy herbivorů.

V experimentech zaměřených na k. borového byla v semi-field podmínkách hodnocena především intenzita poškození sazenic, přičemž chemické změny hostitelské rostliny byly zaznamenány v kořenových pletivech oproti nadzemním částem.

5.2. Typ herbivorie a zasažený orgán jako faktor chemické odezvy

Charakter chemické odezvy rostlin se napříč experimenty lišil v závislosti na typu herbivorie a lokalizaci poškození. Rozdíly mezi jednotlivými experimenty souvisely s tím, zda byla chemie rostlin hodnocena primárně v kontextu působení environmentálních faktorů nebo v přímé souvislosti s herbivorním poškozením.

U defoliátorů byly chemické vlastnosti asimilačních pletiv hostitelských rostlin hodnoceny zejména jako odezva na klimatické a environmentální faktory. V laboratorním experimentu byla přítomnost herbivora oddělena od samotné rostliny prostřednictvím standardizované potravy, zatímco v semi-field experimentu s pilatkami působily klimatické faktory a žír současně. Změny chemického složení jehlic tak odrážely především fyziologickou reakci rostlin na kombinované působení environmentálních podmínek, přičemž u pilatek nelze zcela vyloučit i podíl indukované reakce na žír.

Naproti tomu u k. borového, jako herbivora poškozující kmen sazenic, byla chemická odezva hostitelské rostliny hodnocena přímo v souvislosti s jejím poškozením. Změny

chemických vlastností se projevovaly především v podzemních částech rostliny, zatímco chemické vlastnosti nadzemních orgánů zůstávaly méně ovlivněny.

Tyto výsledky ukazují, že chemická odezva hostitelských rostlin není univerzální reakcí na herbivorii, ale je výrazně podmíněna typem poškození a zasaženým orgánem, což dále komplikuje přímé propojování změn chemického složení rostlin s odezvou herbivorů.

5.3. Kombinované působení environmentálních faktorů

V experimentech založených na kombinovaném působení environmentálních faktorů vykazovaly hostitelské rostliny výrazné chemické odezvy, jejichž charakter se však lišil mezi jednotlivými skupinami sledovaných ukazatelů.

Zatímco základní stechiometrické charakteristiky, zejména poměr C:N, reagovaly na kombinace environmentálních ošetření směrově konzistentním způsobem, odezvy sekundárního metabolismu a metabolomických profilů byly výrazně závislé na konkrétní kombinaci faktorů a experimentálním uspořádání.

Stechiometrické změny tak představovaly nejstabilnější chemickou odezvu hostitelských rostlin, avšak ani tyto konzistentní změny nebyly doprovázeny systematickou odezvou herbivorů.

Růstové a nutriční parametry bekyní i pilatek zůstávaly stabilní napříč sledovanými kombinacemi environmentálních podmínek; u pilatek se navíc neměnila ani stechiometrie trusu.

5.4. Role provenience hostitelských rostlin

Provenience hostitelských rostlin ovlivňovala některé chemické vlastnosti pletiv, zejména poměr C:N a jeho interakci s režimem CO₂. Tento efekt potvrzuje existenci geneticky podmíněné variability ve fyziologické odezvě stromů na změněné environmentální podmínky.

Navzdory této variabilitě se vliv provenience konzistentně nepromítl do odezvy k. borového a pilatek ani do intenzity poškození sazenic. V experimentu se sazenicemi borovice a k. borového byly navíc reakce hostitelské rostliny na poškození mezi proveniencemi velmi podobné, což dokazuje závěr, že v rámci sledovaných systémů nepředstavovala provenience rozhodující faktor určující riziko herbivorního poškození.

5.5. Role prostředí při utváření herbivorního tlaku

Terénní experimenty prokázaly výraznou variabilitu početnosti k. borového mezi sledovanými lokalitami. Početnost byla významně ovlivněna vlhkostí stanoviště a interakcí mezi stářím paseky a způsobem post-těžebního managementu.

Na nově vzniklých pasekách byl výskyt k. borového vyšší na mulčovaných plochách, zatímco na ročních pasekách byl zaznamenán opačný trend. Na nově mulčovaných

pasekách byl výskyt srovnatelný v jehličnatých i listnatých porostech, přestože za běžných podmínek bývají jehličnaté porosty výrazně atraktivnější. Tento vzorec ukazuje, že krátkodobé změny prostředí po zásahu mohou dočasně překrýt běžné rozdíly v atraktivitě jednotlivých typů porostu. Současně rozdíly mezi jednotlivými terénními experimenty ukazují, že efekt post-těžebního managementu se může výrazně lišit v závislosti na konkrétním experimentálním uspořádání a souboru zahrnutých faktorů, což zdůrazňuje citlivost výsledků na kontext studie.

Dále se faktory ovlivňující početnost klikorohů lišily od faktorů určujících intenzitu poškození sazenic, což naznačuje, že vztah mezi abundancí herbivora a skutečným herbivorním tlakem nemusí být přímočarý. Vedle druhové náchylnosti sazenic se na intenzitě žíru podílel i průměr pařezů, což poukazuje na význam podmínek vzniklých po těžbě pro rozsah následného poškození.

Zatímco experimentální studie prokázaly změny chemického složení hostitelských rostlin bez odpovídající odezvy herbivorů, terénní experimenty ukázaly výraznou citlivost výskytu klikorohů na stanovištní a managementové podmínky.

Výsledky napříč experimentálními úrovněmi ukazují, že v reálných terénních podmínkách mohou stanovištní a managementové faktory hrát významnější roli při utváření herbivorního tlaku než samotné chemické vlastnosti hostitelských pletiv. Prostředí tedy nepůsobí pouze jako modifikátor účinků klimatické změny, ale jako klíčový determinant toho, zda a s jakou intenzitou se herbivorní tlak skutečně projeví.

6. Diskuse

Hlavním zjištěním této disertační práce je, že klimatické faktory vedly k měřitelným změnám chemického složení hostitelských rostlin, avšak tyto změny se pouze omezeně promítly do odezvy hmyzích herbivorů. Zatímco základní stechiometrické charakteristiky reagovaly relativně konzistentně, změny sekundárního metabolismu a metabolomických profilů byly výrazně kontextově podmíněné. Navzdory těmto chemickým posunům byla odezva herbivorů slabá a bez jednotného směru. Naopak terénní experimenty ukázaly, že intenzita herbivorního tlaku byla výrazně formována stanovištními a managementovými podmínkami. Práce tak ukazuje, že změny chemického složení hostitelské rostliny samy o sobě nevysvětlují odezvu herbivorů tak přímočaře, jak by mohl naznačovat zjednodušený model „klima → chemie → herbivor“. Vztah mezi těmito úrovněmi je pravděpodobně tlumen schopností herbivorů vyrovnávat se změnám kvality potravy a zároveň je silně ovlivněn konkrétním prostředím, v němž interakce probíhá (Lee, 2017; Zeng, 2024). Tyto výsledky potvrzují nutnost hodnotit vztahy mezi klimatickými faktory, fyziologickou odezvou rostlin a reakcemi herbivorů v kontextu kombinovaného působení více faktorů (Jamieson et al., 2017).

6.1. Chemické změny hostitelských rostlin

Chemické složení hostitelských rostlin reagovalo na působení klimatických a environmentálních faktorů relativně citlivě, avšak charakter této odezvy se lišil mezi jednotlivými složkami rostlinné chemie. Nejkonzistentnější změny byly zaznamenány u základních stechiometrických charakteristik, zejména u poměru C:N, který se při zvýšené koncentraci atmosférického CO₂ opakovaně zvyšoval. Tento vzorec odpovídá obecně popisovanému efektu relativního snížení dusíkatých látek v pletivech při zvýšené dostupnosti uhlíku (Robinson et al., 2012).

Zvýšení poměru C:N bývá v literatuře často interpretováno jako klíčový mechanismus, jímž zvýšená koncentrace CO₂ snižuje kvalitu potravy pro herbivory (AbuEIEla et al., 2025; Chen & Markham, 2021). V tomto kontextu se předpokládá, že pokles relativního obsahu dusíku povede ke zpomalení růstu nebo zvýšení kompenzačního žíru (Stiling & Cornelissen, 2007). Naše výsledky však ukazují, že samotná změna stechiometrie nemusí být dostatečná k vyvolání biologicky významné odezvy herbivorů, zejména pokud nedochází k extrémním hodnotám nebo pokud jsou současně aktivní kompenzační mechanismy.

Odezvu sekundárního metabolismu nelze mechanicky zobecňovat napříč experimenty, neboť změny sekundárních metabolitů jsou silně závislé na typu stresoru, druhu rostliny i experimentálních podmínkách (Zandalinas et al., 2021).

Variabilita sekundárního metabolismu napříč experimenty zároveň naznačuje, že obranné reakce rostlin nejsou univerzální odpovědí na stres, ale výsledkem jemné

regulace mezi růstem, alokací zdrojů a typem poškození (Züst & Agrawal, 2017). To podporuje koncept, že změny sekundárních metabolitů nelze interpretovat izolovaně, ale vždy v kontextu fyziologického stavu rostliny a konkrétní kombinace působících faktorů (Jangpangi et al., 2025).

6.2. Odezva herbivorů na změněnou kvalitu hostitelské rostliny

Přestože se chemické složení hostitelských rostlin v reakci na klimatické faktory měnilo, odezva hmyzích herbivorů zůstala celkově slabá a bez jasného směru. Růstové a nutriční parametry defoliátorů se mezi jednotlivými variantami výrazně nelišily.

Tento výsledek je významný zejména proto, že zpochybňuje implicitní předpoklad silného bottom-up řízení herbivorů prostřednictvím kvality potravy. Ačkoli chemické změny byly statisticky průkazné, jejich skutečný dopad na herbivory se ukázal jako omezený. To naznačuje, že mezi změnou kvality potravy a výkonem herbivora existuje filtrační mechanismus, který tlumí přímý přenos těchto změn (Lee, 2017).

Slabá odezva může souviset se schopností herbivorů regulovat příjem a využití živin prostřednictvím stechiometrické homeostázy a kompenzačního příjmu potravy (Hillebrand et al., 2009; Simpson & Simpson, 1990). Herbivoři tak mohou aktivně vyrovnávat mírné posuny v kvalitě potravy, což vede k relativní stabilitě jejich růstových parametrů napříč environmentálními variantami (Behmer, 2009). Tento mechanismus může vysvětlovat, proč řada studií nachází pouze slabé nebo nekonzistentní efekty zvýšené koncentrace CO₂ na prospívání herbivorů (Kuczyk et al., 2021; Zvereva & Kozlov, 2006; Robinson et al., 2012).

Současně je třeba zdůraznit, že klimatické faktory mohou působit na herbivory i přímo, například prostřednictvím změn vývojové rychlosti či fenologie (Lehmann et al., 2020; Bale et al., 2002). Absence testování přímých teplotních efektů v této práci zároveň ukazuje na limit přístupu zaměřeného výhradně na nepřímé, hostitelem zprostředkované efekty. Pokud jsou přímé fyziologické účinky teploty na herbivory silnější než změny kvality potravy, může být význam nepřímých efektů v reálných podmínkách relativně malý (Lehmann et al., 2020).

6.3. Význam typu herbivorie a zasaženého pletiva

Výsledky ukazují, že chemická odezva hostitelských rostlin je úzce spjata s typem herbivorie a s orgánem, který je poškozením přímo zasažen (Touw & van Dam, 2025). Reakci rostlin nelze chápat jako jednotný proces, ale jako soubor orgánově a funkčně specifických odezev, jejichž charakter je podmíněn povahou poškození (Erb & Reymond, 2019).

U defoliátorů byly chemické vlastnosti asimilačních pletiv hodnoceny především v souvislosti s působením klimatických faktorů. Změny chemického složení listů a jehlic tak odrážely zejména fyziologickou reakci rostlin na kombinované působení faktorů

prostředí, nikoli bezprostřední lokální odezvu na samotnou herbivorii. Tento přístup odpovídá běžnému experimentálnímu uspořádání studií zaměřených na nepřímé klimatické efekty zprostředkované hostitelem (DeLucia et al., 2012; Robinson et al., 2012). U pilatek navíc docházelo k souběhu klimatických manipulací a žíru, což ztěžuje jednoznačné oddělení fyziologické a indukované odezvy rostlin.

Naproti tomu u klikorohů byla chemická odezva sledována přímo jako reakce na poškození transportních pletiv. Tento typ herbivorie zasahuje kůru a lýko, tedy klíčová pletiva zajišťující transport asimilátů v rámci celé rostliny (De Schepper et al., 2013). Změny se proto projevily především v primárním metabolismu kořenů, což pravděpodobně souvisí s narušením toku asimilátů a energetické bilance rostliny. Tento vzorec odpovídá poznatkům o prostorové specifitě obranných reakcí, kdy se lokální a systémová odezva mohou výrazně lišit v závislosti na typu poškození (Kloth & Dicke, 2022).

Rozdíly mezi defoliátory a herbivory poškozujícími kmen tak ukazují, že lokalizace poškození zásadně ovlivňuje nejen charakter indukovaných metabolických změn, ale i jejich ekologický význam (Mostafa et al., 2022). Zatímco poškození asimilačních orgánů může vést k indukci obranných látek a změnám kvality potravy, poškození transportních pletiv může primárně ovlivňovat alokaci zdrojů a metabolickou stabilitu celé rostliny (Harner et al., 2022). Typ herbivorie a zasažený orgán tak představují klíčové faktory, které podmiňují přenos chemických změn do vyšších trofických úrovní.

6.4. Role provenience hostitelských rostlin

V této práci byla provenience hostitelských rostlin zahrnuta jako faktor, který by mohl modifikovat chemickou odezvu stromů na působení klimatických faktorů nebo na poškození herbivorem. Napříč jednotlivými experimentálními přístupy se však její význam ve vztahu k odezvě herbivorů ukázal jako omezený.

V experimentech zaměřených na klimatické manipulace se provenience promítala do některých chemických charakteristik pletiv, zejména do poměru C:N a jeho interakce s režimem CO₂. Tyto rozdíly potvrzují existenci geneticky podmíněné variability ve fyziologické odezvě stromů na změněné podmínky prostředí a ukazují přítomnost interakcí typu provenience × prostředí (Alberto et al., 2013). Přesto se tyto chemické odlišnosti neprojevily v růstu ani ve vývoji pilatek, což ukazuje, že genetická variabilita hostitele sama o sobě nemusí být dostatečná k ovlivnění výkonu specializovaných herbivorů, pokud nejsou rozdíly v kvalitě potravy výrazné (Moreira et al., 2014; Leimu et al., 2005).

Podobný vzorec byl pozorován i v experimentu zaměřeném na reakci sazenic borovice na poškození k. borovým. Chemická odezva rostlin na žír byla mezi sledovanými proveniencemi srovnatelná, což ukazuje, že při krátkodobém a intenzivním poškození

transportních pletiv nebyl genetický původ hostitelské rostliny rozhodujícím faktorem určujícím charakter metabolické reakce.

Celkově lze konstatovat, že ačkoli provenience ovlivňovala některé chemické vlastnosti pletiv, její vliv se konzistentně nepřenesl do odezvy herbivorů ani do intenzity poškození sazenic. Tyto výsledky nenaznačují, že by volba provenience představovala samostatný a spolehlivý nástroj ke snížení rizika herbivorního poškození v podmínkách sledovaných experimentů. Spíše podporují předpoklad, že význam genetického původu je silně kontextově podmíněn a může být překryt účinky prostředí nebo typem herbivorie (Zas et al., 2011; Alberto et al., 2013).

6.5. Role prostředí při utváření herbivorního tlaku

Dosavadní části diskuse se soustředily na vztahy mezi klimatickými faktory, fyziologickou odezvou rostlin a reakcemi herbivorů. Terénní výsledky však ukazují, že samotná intenzita herbivorní interakce je do značné míry podmíněna podmínkami stanoviště a způsobem hospodaření.

V této práci byla početnost klikorohů významně ovlivněna vlhkostí stanoviště a interakcí mezi stářím paseky a způsobem post-těžebního managementu. Vyšší výskyt k. borového na nově mulčovaných pasekách, následovaný opačným trendem na ročních pasekách, svědčí o tom, že účinek managementového zásahu je časově proměnlivý. Krátkodobé změny prostředí po zásahu mohou dočasně zvýšit atraktivitu lokality pro kolonizaci, zatímco s odstupem času se význam těchto změn snižuje. Pozorovaná časová dynamika odpovídá obecným poznatkům o rychlé kolonizaci disturbovaných ploch, jejíž intenzita se s postupnou stabilizací prostředí snižuje (Prach & Walker, 2011).

Pozorované rozdíly mezi jednotlivými terénními experimenty zároveň ukazují, že účinek post-těžebního managementu není univerzální, ale závisí na konkrétním experimentálním uspořádání, věku paseky a kombinaci dalších stanovištních faktorů. To podtrhuje význam kontextu při interpretaci role managementu v utváření herbivorního tlaku.

Skutečnost, že početnost k. borového a rozsah poškození sazenic byly spojeny s odlišnými faktory prostředí, naznačuje složitější vztah mezi přítomností herbivora a jeho skutečným dopadem na sazenice. Stanoviště tak může ovlivňovat nejen celkovou početnost klikorohů, ale i podmínky, za nichž se jejich žír na jednotlivých sazenicích projeví. To může vést k výrazné variabilitě poškození mezi jednotlivými sazenicemi i v rámci stejného stanoviště (Nordlander et al., 2023; Nordlander et al., 2003).

V reálných podmínkách lesa je tak intenzita herbivorního tlaku výrazně formována mikroklimatickými, strukturálními a managementovými faktory (De Frenne et al., 2021; Castagnyrol et al., 2014; Raffa et al., 2008). Chemické vlastnosti hostitelských pletiv představují pouze jednu složku systému, jehož výsledná podoba je určována širším

ekologickým rámcem, v němž se interakce mezi rostlinou a herbivorem skutečně odehrává.

Výsledky této práce ukazují, že vztah mezi klimatickými změnami, chemií hostitelských rostlin a odezvou herbivorů není lineární ani snadno předvídatelný (Rosenblatt & Schmitz, 2016). Přestože klimatické a environmentální faktory opakovaně ovlivnily chemické složení pletiv, tyto změny se do výkonu herbivorů promítly jen omezeně a bez jednotného směru. Herbivorní tlak se naopak v terénu výrazně měnil v závislosti na stanovištních podmínkách a způsobu managementu. Chemické vlastnosti rostlin tak představují pouze jednu část systému, jehož výsledná podoba je formována souběhem fyziologických regulačních mechanismů herbivorů a konkrétními podmínkami prostředí (Deans et al., 2022; Gely et al., 2020).

7. Závěr a doporučení

Tato disertační práce přispívá k porozumění vztahům mezi klimatickými faktory, fyziologickou odezvou lesních dřevin a reakcemi hmyzích herbivorů napříč různými experimentálními úrovněmi. Kombinace laboratorních, semi-field a terénních přístupů umožnila sledovat tyto interakce v podmínkách od plně kontrolovaných až po reálné lesní prostředí, kde se uplatňuje širší soubor ekologických a managementových faktorů.

Výsledky prokázaly, že klimatické faktory mohou vyvolávat měřitelné změny chemického složení hostitelských rostlin, zejména ve stechiometrických charakteristikách a vybraných metabolických profilech. Přenos těchto změn do odezvy herbivorů se však ukázal jako omezený a výrazně kontextově podmíněný. V rámci sledovaných experimentálních systémů se samotná změna chemického složení hostitelských pletiv neprojevila jako spolehlivý ukazatel budoucí intenzity herbivorního tlaku.

Charakter odezvy rostlin se lišil v závislosti na typu herbivorie a lokalizaci poškození. Reakce na defoliaci a na poškození transportních pletiv vykazovaly rozdílné metabolické vzorce, což potvrzuje, že vztahy mezi chemií rostlin a prosperitou herbivorů nelze zobecňovat napříč funkčními skupinami hmyzu ani mezi různými orgány rostliny. Interpretace těchto vztahů proto vyžaduje zohlednění typu interakce, časového horizontu i experimentálního kontextu.

Vliv provenience hostitelských dřevin se projevil především ve fyziologických a chemických charakteristikách rostlin, avšak bez konzistentního přenosu do odezvy herbivorů. Genetický původ stromů tak v podmínkách této práce nepředstavoval samostatný faktor určující dynamiku herbivorního poškození, ale spíše jeden z prvků širší interakční sítě, jehož význam je silně podmíněn prostředím.

Naopak terénní část práce zdůraznila zásadní roli stanovištních a managementových podmínek při utváření skutečného herbivorního tlaku. Ukázalo se, že riziko poškození sazenic nelze vysvětlit pouze vlastnostmi hostitelské rostliny, ale že je výrazně ovlivněno charakterem a managementem prostředí. Tyto faktory modifikují mikroklimatické podmínky, dostupnost zdrojů i kolonizační možnosti herbivorů a tím zásadně ovlivňují výslednou podobu interakce.

Hlavním přínosem této práce je empirické propojení fyziologických experimentů s terénní realitou lesa a ukázání toho, že predikce herbivorního rizika nelze stavět na izolovaném hodnocení chemických změn hostitelských rostlin. Výsledky ukazují, že vztah mezi klimatem, rostlinou a herbivorem je nelineární, víceúrovňový a výrazně kontextově podmíněný.

Z hlediska dalšího výzkumu je žádoucí širší integrace přímých účinků klimatických faktorů na herbivory do studia jejich populační dynamiky. Zvláštní pozornost si zaslouží vliv teploty na rychlost vývoje, fenologii a potenciální změny počtu generací a jejich

kombinace se stanovištními a managementovými podmínkami obnovy lesa. Budoucí výzkum by měl více propojovat experimentální manipulace s dlouhodobým sledováním populačních procesů v terénu, aby bylo možné lépe kvantifikovat relativní význam nepřímých a přímých klimatických efektů.

Z pohledu lesnické praxe výsledky ukazují, že samotná manipulace s chemickými vlastnostmi sadebního materiálu či volba provenience nebude ve většině případů představovat dostatečný nástroj ke snížení herbivorního tlaku. Větší význam má práce s prostorovým a časovým uspořádáním obnovy lesa, zohlednění sezónní dynamiky výskytu herbivorů a promyšlené načasování managementových zásahů. Tyto závěry podporují přístup založený na integrované ochraně lesa (IPM), který kombinuje preventivní, ekologická a managementová opatření namísto spoléhání na jediný regulační mechanismus.

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9. Seznam příloh

Příloha 1:

Dvořáková, B., Holuša, J., Musiolek, D., Kalyniukova, A., Hradecký, J., Čepl, J., & Schopf, A. (2025). Host tree impact on *Lymantria* species under CO₂ and temperature changes. *Frontiers in Forests and Global Change*, 8, 1564011.

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Příloha 4:

Dvořáková, B., Holuša, J., Horák, J., Hradecký, J., Bledý, M., & Zelenka, M. (2024). Pine weevil (*Hylobius abietis*) preferences among species of conifer seedlings planted on clear-cuts in Central Europe. *Frontiers in Forests and Global Change*, 7, 1399405.

Příloha 5:

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Příloha 1.
Host tree impact on *Lymantria* species
under CO₂ and temperature changes



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Host tree impact on *Lymantria* species under CO₂ and temperature changes

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Introduction: Climate change has led to rising atmospheric CO₂ levels and temperatures, projected to double CO₂ concentrations and increase temperatures by 2–5°C by the end of the 21st century. These environmental changes influence plant primary and secondary metabolism, potentially altering plant-insect interactions. Herbivore performance depends on the nutritional quality of host plants, which may decline with elevated CO₂ due to an increased carbon-to-nitrogen (C:N) ratio. To explore these effects, the performance of spongy moth larvae (*Lymantria dispar*) was assessed on oak (*Quercus robur*) and spruce (*Picea abies*) seedlings grown under varying climatic conditions. This approach compares a preferred host with a non-preferred one in the case of *L. dispar*, providing insight into how host plant selection may be influenced under future climate scenarios. In addition, the nun moth (*Lymantria monacha*), a conifer-feeding species, was also studied on the experimental spruce seedlings to facilitate a comparison with a specialist herbivore.

Methods: Three-year-old oak and spruce seedlings were reared for 1 year under four climate scenarios combining two CO₂ levels (ambient: 410 ppm and elevated: 820 ppm) and two temperature regimes (20:15°C and 25:20°C). Seedlings were then processed into leaf powder diets for laboratory bioassays with larvae. Secondary metabolites in the seedlings were analyzed to assess climate-induced changes in tree composition and their effects on herbivores.

Results: Elevated CO₂ increased the C:N ratio in both tree species, with spruce showing a higher ratio than oak. Higher temperatures led to increased nitrogen content, particularly in oak seedlings. *L. dispar* performed better on oak despite higher secondary metabolite concentrations, while *L. monacha* exhibited minimal variation in performance on spruce across climate treatments.

Conclusion: The combined effects of elevated CO₂ levels and increased temperatures impacted plant quality; however, there were nearly no differences in the performance of *Lymantria* larvae. Despite the higher concentrations of secondary metabolites in the trees, the larvae were able to thrive effectively, demonstrating their resilience to environmental changes.

KEYWORDS

climate change, herbivory, plant chemistry, plant-insect interactions, secondary metabolites

1 Introduction

1.1 Human activity changes the atmospheric composition

Human activities have resulted in an increase in greenhouse gasses, particularly carbon dioxide (CO₂), which contributes significantly to global warming (IPCC, 2021; International Energy Agency [IEA], 2017; Xi-Liu and Gao, 2018; Yu et al., 2008). Despite established agreements and advancements in technology, there has been no meaningful reduction in CO₂ emissions and other harmful pollutants (Charfeddine, 2017). At the start of the Industrial Revolution, the concentration of atmospheric CO₂ was 280 ppm. Currently, it is approximately 400 ppm (Sodiq et al., 2023) and is projected to double by the end of the 21st century (IPCC, 2014). This increase in CO₂ levels is primarily due to the burning of fossil fuels and deforestation (IPCC, 2014; Begum et al., 2020; Uddin et al., 2021; Keeling, 1995). As a result, plants will need to adapt to the current conditions of CO₂ and temperature and future scenarios involving elevated CO₂ levels, higher temperatures, and potentially more frequent extreme weather events (Slot et al., 2021).

1.2 CO₂, temperature and plants

Increased levels of atmospheric CO₂ are resulting in higher global temperatures and more severe droughts (Diffenbaugh et al., 2017). Consequently, many trees have died (Hartmann et al., 2018). Forests are responsible for two-thirds of global photosynthesis (Giri, 2017) and cover over a third (43%) of the Earth's land surface (Bonan, 2008; Karnosky, 2003). While forests are directly exposed to the effects of climate change, they are also impacted by this phenomenon in other ways. Given forests crucial role in mitigating carbon emissions, it is important to understand how elevated CO₂ levels affect tree growth and ecosystem functions within forested areas (Psistaki et al., 2024).

Elevated levels of CO₂ and temperature can influence ecological processes in several ways: by directly affecting plant or insect herbivore species separately (Murray et al., 2013; Bale et al., 2002; Ellsworth et al., 2004), in combination (Zvereva and Kozlov, 2006), or indirectly through biotic interactions (Blois et al., 2013; Montoya and Raffaelli, 2010; Tylianakis et al., 2008). The complex relationships among abiotic stress factors, host trees, and insect herbivores present significant challenges in predicting the overall impacts of climate change on forest ecosystems (Jactel et al., 2019).

1.2.1 CO₂ concentration

Numerous tree-ring studies have indicated that radial growth increases with rising atmospheric CO₂ concentration (Guo et al., 2022; Marchand et al., 2020; Zhang et al., 2003), but it seems that only for a short time period (Körner et al., 2005). Elevated levels of CO₂ in the atmosphere can change the chemistry of plants. This includes enhancements in photosynthesis and increases in carbohydrates (Zheng et al., 2019; Hartmann et al., 2018; Boullis et al., 2015; Zhang and Dang, 2007; Lindroth et al., 1993; Williams et al., 1998), as well as non-structural carbohydrates and carbon-based allelochemicals (Bae et al., 2019; Rajashekar, 2018). Additionally, the C:N ratio is affected (Sheng et al., 2021; Roy and

Mathur, 2021; Watanabe et al., 2021; Lotfiomran et al., 2016; Ziska, 2002). The C:N ratio reflects the plant's chemical composition, with an increase in carbohydrate content and a decrease in nitrogen concentration (by about 1/10–1/3) (Robinson et al., 2012; Lindroth et al., 2002; Ehleringer et al., 2002), a phenomenon referred to as the nitrogen dilution effect (Strain and Cure, 1985). However, these changes can vary among different tree species (Foss et al., 2013).

1.2.2 Air temperature

It is expected that the rise in atmospheric CO₂ levels will result in a global average surface temperature increase of at least 1–3°C within this century (Rapp, 2024; Adak et al., 2023; Ciaia et al., 2013; Solomon, 2007). Temperature is the primary factor limiting photosynthesis and plant growth (see Kaiser et al., 2015; Yamori et al., 2014; Kellomäki and Väisänen, 1988). Higher air temperatures are expected to enhance photosynthetic rate, which in turn will boost leaf biomass production and accelerate plant growth, further amplifying the effects of elevated CO₂ (Way and Oren, 2010; Farrar and Williams, 1991; Long, 1991). Notably, higher temperatures tend to promote growth in deciduous species more than in coniferous trees (Way and Oren, 2010). Additionally, the nutritional quality of host trees for herbivores is influenced by temperature (Holopainen et al., 2018). The temperature-induced changes in phytochemistry can vary significantly among different traits and tree species (Jactel et al., 2019). While increasing temperatures have a weak effect on nitrogen content or the C:N ratio, they significantly impact other characteristics, such as carbohydrates, phenols, and terpenoid content (Zvereva and Kozlov, 2006).

1.3 Plant-herbivore interaction

Herbivorous insects are closely connected to specific host plant species, which serve as their food source. The presence and success of herbivorous insects depend significantly on the quality of these host plants. The quality, in turn, is influenced by both primary and secondary metabolite composition and content (Schmitt et al., 2020). Organic carbon, bound in a wide range of plant-building compounds is the primary source of energy for insects, while their bodies are predominantly composed of proteins, containing also significant amounts of nitrogen. As a result, insects must consume not only a carbon-rich diet but also must contain substantial amounts of nitrogen to completely meet their nutritional needs (Zhou et al., 2015).

1.3.1 Nitrogen and carbon as important elements of life

Nitrogen is a crucial element for insect growth (Ren et al., 2022), and it plays a key role in plant metabolic processes, cellular structure, and genetic coding (Bala et al., 2018; Mattson, 1980). When nitrogen levels in leaves are low, the development of leaf-chewing insects is prolonged because they need to consume more plant tissue to obtain enough nitrogen-based nutrients (Stiling and Cornelissen, 2007; Johnson and McNicol, 2010; Johnson et al., 2014; Yin et al., 2010; Xie et al., 2015). In high CO₂ treatments, insect larvae can consume up to 80% more leaves (Lincoln et al., 1984). Additionally, insects that feed on plants grown in elevated CO₂

levels exhibit reduced efficiency in utilizing ingested food (Zhang et al., 2018; Teawkul and Hwang, 2019; Marks and Lincoln, 1996), which correlates with a reduced nitrogen content in their food source (White and Warrington, 1984). An increased feeding rate also leads to a higher intake of defensive compounds from host trees (Richardson et al., 2015).

Carbon provides the energy needed for metabolic processes (Bringaud et al., 2006). When the diet is too high in carbon compared to nitrogen, larvae may have plenty of energy but not enough protein for optimal growth. On the other hand, a diet high in nitrogen relative to carbon can enhance growth but may lack sufficient immediate energy. Although it is known that temperature changes can affect the plant nutritional value for many insect species, there is less understanding of how these temperature variations impact insect development (Stamp and Yang, 1996).

1.3.2 Secondary metabolites

Changes in the chemical composition of plants, particularly the levels of plant carbon-based secondary metabolites such as phenolic glycosides and tannins, can significantly affect the quality of plant foliage as food for insect herbivores (Boeckler et al., 2011; Singh et al., 2021; Moreira et al., 2017; Barbehenn and Constabel, 2011; Lindroth et al., 2001). Although secondary metabolites are not essential for the growth and development of organisms, they often serve other important ecological functions (Salam et al., 2023). These compounds can help plants adapt to environmental stresses and act as chemical defenses against various threats, including insects (Reddy and Guerrero, 2004; Chen et al., 2005). For example, phenolic compounds like tannin, lignin, and flavonoids play a role in defending against herbivores and pathogens. Condensed tannins can hinder the growth and development of herbivores by precipitating proteins (Croteau et al., 2000).

1.4 Hypothesis and goals

Previous studies have demonstrated that the effects of elevated CO₂ levels and temperature on plants vary among different tree species (Jactel et al., 2019; Way and Oren, 2010; Foss et al., 2013; Lindroth et al., 1993; Roth and Lindroth, 1994). Numerous studies have focused on the impact of elevated CO₂ on plants, trees and forests (Klein et al., 2016; Ceulemans and Mousseau, 1994; Curtis et al., 1996; Curtis and Wang, 1998; Saxe et al., 1998; Norby et al., 1999; Karnosky et al., 2001; Karnosky, 2003). Reviews focused on the combination of these factors report that the anticipated negative impacts of elevated CO₂ on herbivores may be mitigated by rising temperatures (Zvereva and Kozlov, 2006; Robinson et al., 2012).

Most studies on insect performance in elevated CO₂ atmospheres or varying air temperatures have primarily focused on a single host plant species, with herbivores raised directly on those plants, or considered only one climatic factor at a time (Henn and Schopf, 2001; Milanović et al., 2015; Xiaowei et al., 2006). Our research aim was to test the interactions between two climatic factors, temperature and CO₂ levels, on both broadleaf and coniferous trees, using two polyphagous herbivores that are significant forestry pests; one herbivore prefers conifers while the other prefers broadleaves. Furthermore, most of the studies cited in this article didn't analyze changes in plant chemical composition when evaluating larval performance.

In our study, we planted seedlings in four different combinations of climatic conditions, using two host tree species [*Quercus robur* (L.) and *Picea abies* (L.) Karst. and two closely related herbivores] [*Lymantria dispar* (L., 1758) and *Lymantria monacha* (L., 1758)]. We also prepared leaf powder diets from these seedlings to ensure precise scoring of herbivore performance. To analyze changes in plant chemistry, we conducted a chemical analysis of the leaves and needles, focusing on alterations in selected secondary metabolites. We expected that seedlings grown under less favorable conditions (higher temperature and CO₂ levels) would produce more defensive compounds, potentially leading to slower growth rates in the larvae.

The objectives of this study were: (I) to compare the effects of different combinations of CO₂ levels and temperatures on seedlings and the performance of two closely related herbivorous insects (II) to test how the secondary metabolic compounds in these seedlings are affected by varying environmental conditions.

2 Materials and methods

2.1 Study species

Our study examines two species of lepidopterans, the spongy moth (*Lymantria dispar*) and the nun moth (*Lymantria monacha*), along with two types of trees: the coniferous Norway spruce (*Picea abies*) and the broadleaf pedunculate oak (*Quercus robur*) from the nursery of the Tree Breeding Station in Kostelec nad Černými lesy in the Czech Republic.

The larvae of *L. dispar* originate from a laboratory rearing (the New Jersey Standard Strain) of USDA-APHIS (Animal and Plant Health Inspection Service, OTIS Methods Development Center, MA 02542). Nun moth larvae were reared from eggs collected around the town of Zgierz in Poland in February 2021.

Both larvae species belong to the family *Erebidae*. The spongy moth (*L. dispar*) is a common pest found in temperate forests across Europe. *L. dispar* typically feeds on broadleaves, especially oaks, rarely feeding on pines in natural environments (Castedo-Dorado et al., 2016). This study compared the response to a preferred broadleaf host with a non-preferred coniferous host. Its larvae are folivores, feeding on the leaves of over 300 tree species, including *Betula* sp. (birch), *Crataegus* sp. (hawthorn), *Larix* sp. (larch), *Populus* sp. (aspen), *Quercus* sp. (oak), *Salix* sp. (willow), and *Tilia* sp. (basswood) (Elkinton and Liebhold, 1990; Liebhold et al., 1995). The nun moth (*L. monacha*) primarily feeds on the needles of conifers, especially spruces and pines, as well as larch and fir (Nakládal and Brinkeová, 2015). In spring, the larvae of both species hatch from overwintering eggs and progress through five instars for males or six instars for females (Doane and McManus, 1981; Schwenke, 1978). The larvae feed on leaves from April to June, at which point they pupate. Adult moths have a short lifespan of less than 7 days, and females are unable to fly (Keena et al., 2007). Both species experience regular and cyclical population outbreaks (Hlásny et al., 2016), leading to extensive defoliation that has been documented since the Middle Ages (Komárek, 1931; Schwenke, 1978).

Norway spruce and pedunculate oak are common and economically important tree species in Europe. English oak

typically grows well in lowland areas, while Norway spruce is more prevalent at higher elevations. The economic significance of Norway spruce is highlighted by its extensive use in the forestry and timber industries (Jansson et al., 2013). However, its growth and resilience are increasingly threatened by climate change, especially at the trailing edge of its distribution (Honkaniemi et al., 2020).

2.2 Plant growing conditions

The experiment was conducted in four growth chambers (Step-In Fytoscope FS-SI 3400), which were set either to ambient CO₂ levels (410 ppm) or elevated CO₂ levels (820 ppm) at day and night temperatures to 20:15°C and 25:20°C under a light-dark cycle of 16:8 h. Relative humidity remained consistent at 70% in all chambers.

Oak and spruce seedlings were planted in trays (20 cm × 20 cm × 30 cm) filled with AGRO substrate (Agro CS a.s., Czech Republic) in the year 2016 in the nursery. On April 1st, 2019, nine spruce and nine oak seedlings were placed in each growth chamber. From November 10th to December 6th, 2019, the air temperature was gradually lowered from 20 and 25°C by 5 and 3°C per week until the temperature reached 5°C. On December 7th, 2019, the seedlings were placed outside for dormancy until February 17th, 2020, when they were returned to the chambers, and both the photoperiod and temperature were gradually increased again by 5 and 3°C each week until they finally reached 20 and 25°C. The collection of leaves was conducted in May 2020. The collected leaves were weighed, placed in paper bags, and frozen in liquid nitrogen. A freeze dryer (Alpha 2-4 LSC basic, Christ) was used for lyophilization, and the samples were then ground and homogenized using a mixer mill (MM 400, Retsch) and stored in a deep freezer at -80°C until diet preparation.

2.3 Chemical analysis

2.3.1 Elementary N and C analysis

For the analysis, 10–30 mg of the pure sample of the leaf powder was weighed into tin capsules designed for the instrument used. The capsules were then introduced into the combustion chamber of the Thermo Scientific Flash 2000 Elemental analyzer (Thermo Scientific, United States). The samples were combusted in a stream of pure oxygen at a temperature of 1,000°C. The resulting carbon and nitrogen oxides were passed through a copper reduction column and directed to a separation column, where moisture was removed. Helium was used as the carrier gas. The content of the separated oxides was determined using a thermal conductivity detector, and signal processing was performed with Eager Xperience software (Thermo Scientific).

2.3.2 Metabolite analysis

2.3.2.1 Extraction of carbohydrates

A total of 40 mg of freeze-dried samples (72 spruce and 36 oak samples) was extracted by 500 µL of methanol:chloroform: water in the ratio 12:5:3 and kept in the water bath at 60°C for 30 min. After 1,000 µL of water was added to the test tube. The solution was vortexed and then centrifugated for 3 min at 13,000 rpm. The

supernatant was filtered using a 0.22 µm PVDF filter prior to LC-MS-qTOF analysis.

2.3.2.2 Determination of total phenolic content

TCT was performed according to Makkar et al. (1993). Briefly, 20 µL of the extract was placed in the test tube and made up the volume to 0.5 mL of water. Then, 250 µL of the Folin Ciocalteu reagent was added. After 3 min, 1 mL of 20% sodium carbonate was added. The test tube was well mixed and kept in a dark place for 40 min. The absorbance was recorded at 725 nm using a spectrophotometer. The concentration of TPC was expressed in tannin acid equivalent.

2.3.2.3 Determination of total flavonoid content

The determination of TFC was carried out according to Zengin et al. (2016). Briefly, 150 µL of sample extract was placed into the 2 mL test tube and filled up to 1 mL with 70% of acetone. Then, a 2% solution of aluminum trichloride prepared in methanol was added. After 15 min, the absorbance was recorded at 415 nm, against blank. The concentration of flavonoid content was expressed in rutin equivalent (mg g⁻¹).

2.3.2.4 Determination of total condensed tannin content

The total flavonoid content was determined following the method described by Porter et al. (1985). 150 µL of sample and 150 µL of 70% acetone were placed in 2-mL test tube. Then, 1.5 mL of butal-HCL reagent was added [butanol-HCL reagent was prepared by mixing 95 ml of butanol and 5 mL of concentrated HCL (37%)]. After, 50 µL of ferric reagent was added (ferric reagent was prepared as 2% ferric ammonium sulfate in 2N HCl). The solution was well mixed and placed in the thermoshaker for 1 h at 95°C and 1,000 rpm. When the solution was cooled, the absorbance was recorded at 550 nm. The concentration of TCT was expressed in cyanidin equivalent (mg g⁻¹).

2.3.2.5 LC-MS-qTOF analysis of carbohydrates

LC-qTOF-MS/MS analysis was performed using Agilent 1290 Infinity II coupled with Agilent 6546 LC/MS QTOF system (Agilent, United States). Chromatographic separation was performed on Asahipak NH2P40 3E column 3.0 × 250 mm, 4 µm (Shodex, Japan) operated at 30°C. The mobile phase was composed of acetonitrile (A) and water (B). The isocratic elution was achieved with the started condition of mobile phase 70:30 (A:B) and increased up to 75:25 (A:B) for 30 min with a flow rate of 0.3 mL min⁻¹. The injection volume was set to 1 µL. The system was operated in negative ionization mode. The QTOF parameters were as follows: scan range 100–760 m/z; drying gas temperature, 280°C; sheath gas flow rate, 12.0 L/min; sheath gas temperature, 275°C; capillary voltage, 3.0 kV; fragmentor, 160 V; collision energy at 10, 20 and 40 eV. MS/MS data were acquired at a scan range was 50–760 m/z. During the analysis, two reference masses: 112.9855 m/z and 966.0007 m/z were continuously measured for mass correction. The data collection was carried out using Agilent Mass Hunter Acquisition software. The data analysis was performed using Agilent Mass Hunter Qualitative Analysis 10.0 and Q-TOF Quantitative analysis. Identification of monosaccharides and oligosaccharides was determined according to precursor ions, the fragment ions, and retention time compared with the standards of sucrose, glucose, fructose, and raffinose.

2.4 Leaf powder diet preparation

The larvae were fed a wheat control diet after Bell (1981) from emergence until the end of the second instar (the diet recipe can be found in Supplementary Table S1). This diet (later called “wheat control diet”) was also provided to control group larvae, which were fed this diet for the entire experimental duration.

We prepared leaf powder diets from leaves of spruce and oak experimental seedlings to feed the fourth instar larvae. The preparation process is similar to that of the wheat diet and includes the following steps: (1) Boil water and dissolve agar in it. (2) Allow the mixture to cool down to 50°C. (3) Add leaf powder, Sorbic acid, and Methyl Parabenzenes to the mixture. (4) Mix all the ingredients thoroughly. (5) Store the prepared mixture in Petri dishes or syringes in the refrigerator. All the ingredients can be found in Supplementary Table S2.

We used plastic syringes to maintain the freshness of the leaf powder diets, which made it easy to measure the amount of food served to the larvae. When opening the syringes, we cut off the tops and then covered them with parafilm before storing them in the refrigerator.

2.5 Experimental design

The eggs of both species (*L. dispar* and *L. monacha*) were placed separately in 250 mL plastic box containing a wheat control diet. They were kept at 20°C under a light:dark cycle of 16:8 h. Once the larvae emerged, they were allowed to feed on the wheat control diet until they reached the end of the second instar. Beginning in the third instar, the larvae were reared in groups of 15 within Petri dishes (ϕ 6 cm) and provided with leaf powder diets. *L. dispar* larvae were fed both spruce and oak diets, while *L. monacha* larvae were fed only the spruce diet. We monitored the larvae hourly (besides night time (7:00 a.m.–10:00 p.m.)), to identify the premolt stage, which is indicated by the larvae being stuck with their heads moved forward. When in the premolt stage, the larvae were transferred to individual Petri dishes containing a small piece of leaf powder diet

(2 g). Immediately after molting into the fourth instar, we weighed both the larva and the piece of diet. During the fourth instar, the larvae were left to feed *ad libitum* for 5 days (the duration of the fourth instar at 20°C), with additional food provided if necessary. Once the larvae reached the premolt stage to the fifth instar, they were weighed, placed in small Eppendorf tubes, and frozen along with any remaining food and frass. The collected material was then dried in an incubator with blue desiccant and stored refrigerated at -20°C.

To ensure accurate measurements of evaporation-induced weight loss in artificial larval diets, we stored 10 samples of each diet type at 20°C for 5 days and determined the water loss thereafter. This information was then used to adjust our data analysis accordingly.

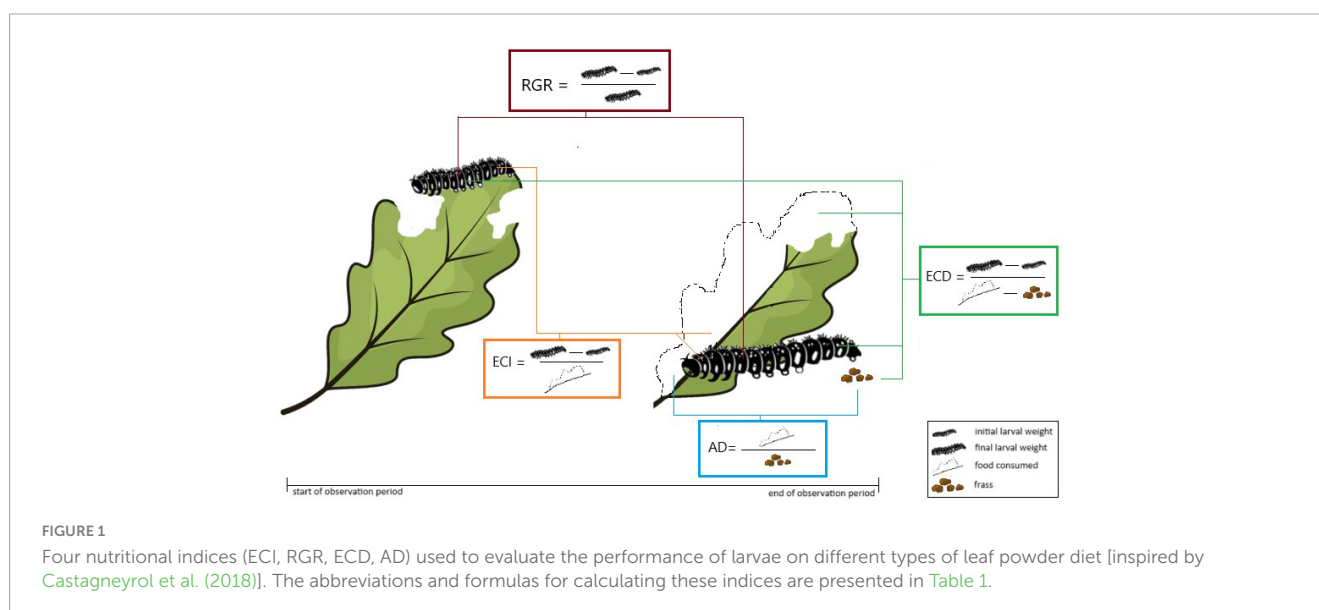
2.6 Evaluating larval performance

According to the gravimetric method of Waldbauer (1968), four nutritional indices were used to evaluate larval performance on the different diets (Table 1). These indices include AD (approximate digestibility), ECD (efficiency of conversion of digested food), ECI (conversion of ingested food), and RGR (relative growth rate) (Figure 1). All indices are calculated based on dry weights.

TABLE 1 Formulas of all nutritional indices used for evaluating larval performance, including their abbreviations.

Index	Description	Formula
RGR	Relative growth rate	$(\log W_2 - \log W_1) / t$
AD	Approximate digestibility (%)	$[(Q - F) / Q] \times 100$
ECI	Efficiency of conversion of ingested food (%)	$[(W_2 - W_1) / Q] \times 100$
ECD	Efficiency of conversion of digested food (%)	$[(W_2 - W_1) / (Q - F)] \times 100$

In the formulas, W represents the dry weight of larvae, Q the dry weight of consumed food, F the dry weight of frass, and t the time period.



Approximately 25 larvae were weighed immediately after molting from the third to the fourth instar (FW—Fresh weight). They were then frozen and dried (DW—Dry weight) to determine the FW/DW ratio. This ratio was used to estimate the dry weight of the larvae at the beginning of the fourth instar using the formula $WC = [(FW-DW) \times 100]/FW$. To determine the dry weight of the food at the start of the observed period, we applied this formula to 10 pieces (2 g) of each type of diet (as described in the previous chapter), and the average of these results was used as the initial diet weight.

The relative growth rate (RGR) explains the amount of larval gain within a specific unit of time. Approximate digestibility (AD) measures the efficiency of food conversion. The efficiency of conversion of ingested food (ECI) is an overall index of the insect's ability to utilize foliage for growth. The conversion efficiency of digested food (ECD) is an index of the insect's ability to assimilate and convert food into body substances (Waldbauer, 1968).

2.7 Statistical analysis

Statistical analyses were performed using R statistical software (R Core Team, 2021). Principal Component Analysis (PCA) was conducted using the *prcomp* function from base R.

To assess the effects of different diets on larval growth, estimated by four nutritional indices, an analysis of variance (ANOVA) was first performed using the *aov* function. This compared the means of the selected indices across diet groups for each moth species separately. Tukey's Honest Significant Difference (HSD) test was then applied to the ANOVA models to conduct pairwise comparisons between food groups, with the results summarized in plots using letter groupings (above each tasted treatment).

For further analysis of diet effects on nutritional indices, linear mixed-effects models were fitted for each growth index and moth species using the *lmer* function from the *lme4* package (Bates et al., 2015). The initial models included tree species, cultivation temperature, and CO₂ regimes as fixed effects, with individual food batches treated as random effects. Since only the tree species factor showed statistically significant differences, diets were categorized into three diet groups: control, oak-based and spruce-based. Subsequently, a simplified linear mixed-effects model was fitted with these diet categories as fixed effects and individual food batches as random effects. Then *emmeans* function from the *emmeans* package (Russell et al., 2021) was used to estimate marginal means and perform pairwise comparisons between diet categories. Results were visualized using letter groupings in summary plots (above each diet group).

To compare carbon (C), nitrogen (N), the carbon-to-nitrogen ratio (C:N), sugar compounds, and phenolic compounds among food batches, ANOVA models were employed. These included tree species, cultivation temperature, CO₂ regimes, and interactions of tree species with sampling date, cultivation temperature and CO₂ regimes as explanatory variables.

Effect sizes (η^2 values) were calculated using the etaSquared function from the *lsr* package (Navarro and Navarro, 2022) to evaluate the contribution of individual factors and interactions to variation in the response variables. The η^2 statistic quantifies

the proportion of the total variance in a response variable that is explained by each explanatory variable or interaction in the model. This provides a standardized measure of effect size, allowing for the comparison of the relative importance of different factors.

For all models, the normality of residuals was checked. Several outlying values, likely due to measurement errors, were omitted, and the data were reanalyzed to ensure robustness.

3 Results

3.1 Nitrogen, carbon, and C:N ratio in spruce and oak

The nitrogen concentration varied significantly among tree species, with lower levels observed in spruce. This difference was logically reflected in the C:N ratio, which was significantly higher in spruce. Seedlings growing in doubled CO₂ concentration had a higher C:N ratio. Seedlings growing at higher temperatures contained higher nitrogen content and didn't affect the C:N ratio (Table 2 and Figure 2).

3.2 Chemical analysis of oak leaves and spruce needles

The leaves of oak seedlings contained higher levels of phenolics and condensed tannins compared to spruce needles. Spruce had higher flavonoid content. However, there was no significant difference in non-tannins between oak and spruce seedlings (Tables 3, 4).

Higher temperatures affected phenolic and flavonoid concentrations. Phenolic concentrations increased at higher temperatures, except in oak under ambient CO₂ concentration. Flavonoid concentrations lowered at higher temperatures except

TABLE 2 Influence of tree species (spruce and oak), elevated temperature (20°C vs. 25°C), and doubled air CO₂ concentration (410 ppm vs. 820 ppm) on nitrogen, carbon, and the C:N ratio in leaves.

N	F-value	p-value	Eta ²	Eta ² part
Tree species	411.570	<0.001	0.954	0.991
Temperature	17.509	0.014	0.039	0.814
CO ₂	5.369	0.081	0.012	0.573
C	F-value	p-value	Eta ²	Eta ² part
Tree species	1.295	0.319	0.061	0.168
Temperature	1.791	0.252	0.135	0.309
CO ₂	3.040	0.156	0.228	0.432
C:N	F-value	p-value	Eta ²	Eta ² part
Tree species	224.088	<0.001	0.915	0.983
Temperature	7.460	0.052	0.030	0.651
CO ₂	11.233	0.029	0.044	0.737

Bold numbers represent significant impact.

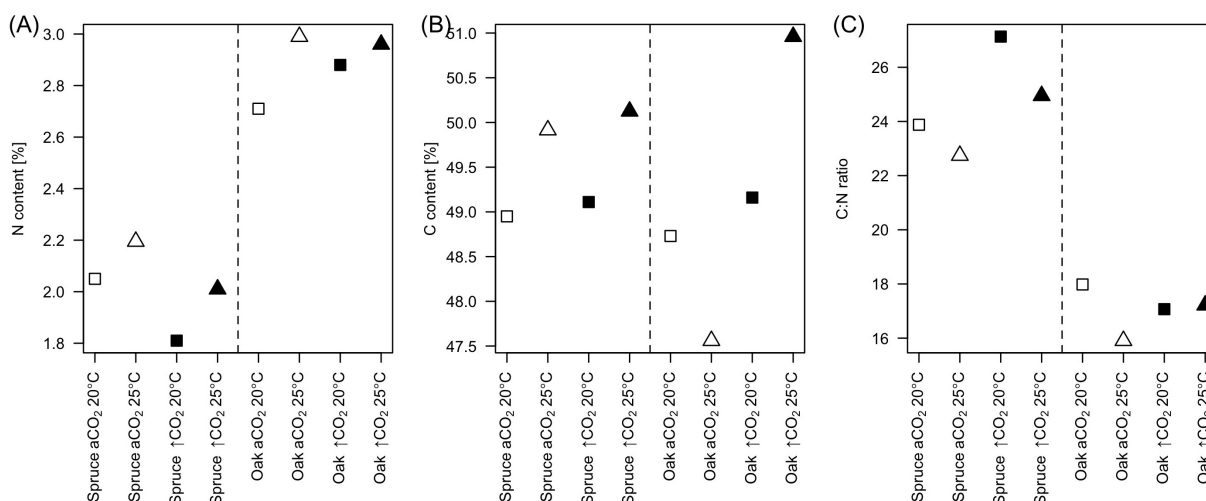


FIGURE 2 Influence of CO₂ concentrations and temperature on the nitrogen (A), carbon (B) content and the C:N ratio (C) in leaf powder diets prepared from spruce and oak seedlings. In all cases, 36 samples were analyzed.

in spruce under doubled CO₂ concentration (Tables 3, 4). The CO₂ treatments didn't affect any secondary metabolites we studied (Table 4).

Sucrose and raffinose concentrations were significantly higher in oak compared to spruce. The levels of all sugar types in diets from seedlings grown under both temperature regimes and in both ambient and doubled CO₂ concentrations were similar (Tables 5, 6).

3.3 The performance *Lymantria* species on different leaf powder diets prepared from oak (*L. dispar*) and spruce (*L. dispar* and *L. monacha*)

For *L. dispar*, the nutritional indices, except for the ECD, were significantly higher in larvae reared on oak diets compared to those reared on spruce diets. Except for the ECD index, the oak diet showed a significant difference when compared to the wheat control diet. Except for the ECD index, larvae reared on spruce diets performed worst.

Only a small influence of climatic conditions on *L. dispar* larval performance was found, but significant differences were found between different climatic conditions in three cases. AD index was higher for larvae reared on spruce needles grown at 20°C than at 25°C when growing in doubled CO₂ concentration. ECI index was higher for larvae reared on spruce needles grown at 20°C and doubled CO₂ concentration than other spruce variants. ECD index was lower for larvae reared on spruce needles grown at ambient CO₂ and 20°C compared to all other spruce variants (Figure 3).

For *Lymantria monacha*, the RGR, AD, and ECI indices were significantly higher in the wheat control diet than in the spruce needle powder diet.

Only a small influence of climatic conditions on *L. monacha* larval performance was found. Still, significant differences were found between different climatic conditions in three cases. RGR

index was higher for larvae reared on spruce grown at ambient CO₂ and 20°C than fed on spruce grown in doubled CO₂ concentrations and both temperatures. AD index was higher for larvae reared on spruce grown in doubled CO₂ when comparing both CO₂ treatments at 20°C. ECD index was higher for larvae reared on spruce grown at ambient CO₂ when comparing both CO₂ treatments at 25°C (Figure 4).

4 Discussion

In our experiment, seedlings of two tree species, spruce and oak, were reared under four different climatic conditions for 1 year. The study aimed to examine how these factors interact and their potential effects on the food quality for herbivorous insects. We compared a preferred host tree (oak) with a non-preferred host tree (spruce) and the response of Spongy moth (*L. dispar*) larval performance and conifer-preferring nun moth (*L. monacha*) on spruce, providing insight into how host plant selection may be influenced under future climate scenarios.

4.1 Effects of doubled CO₂ concentration and higher temperature on C:N ratio and water soluble carbohydrates in seedlings

Our findings support the hypothesis that the plant C:N ratio in plants increases with elevated air CO₂ concentrations, as observed in previous studies (Vencl et al., 2024; Zheng et al., 2019; Lotfiomran et al., 2016; Du et al., 2019; Hikosaka et al., 2006; Luo et al., 2006). The extent of this impact can vary based on plant species (Kubiske et al., 2015), growth conditions, and other environmental factors (Gifford et al., 2000).

Understanding how climate warming affects ecosystem function and stability is crucial for predicting the impacts of global climate change (Liu et al., 2021). Our results showed

TABLE 3 Contents of secondary metabolites in leaves of oak (n = 36) and spruce (n = 72) seedlings grown under lower (20°C) and higher (25°C) temperatures, as well as ambient (410 ppm) and doubled (↑, 820 ppm) CO₂ concentrations.

Diet	Total phenolic and tannins content (mean, mgTA/g)	Total phenolics (SD)	Total non-tannins (mean, mgTA/g)	Total non-tannins (SD)	Total flavonoids (mean, mgRU/g)	Total flavonoids (SD)	Total condensed tannins (mean, mgCD/g)	Total condensed tannins (SD)
aCO ₂ , 20°C spruce	49.87	3.69	0.42	0.01	34.30	0.40	6.59	0.01
aCO ₂ , 25°C spruce	57.53	0.83	0.41	0.05	32.44	0.61	1.29	0.03
↑CO ₂ , 20°C spruce	51.84	0.27	0.35	0.02	27.81	0.21	2.25	0.03
↑CO ₂ , 25°C spruce	52.29	4.37	0.38	0.01	30.42	0.76	3.56	0.05
aCO ₂ , 20°C oak	91.67	2.12	0.47	0.03	36.29	0.65	6.21	0.10
aCO ₂ , 25°C oak	52.74	1.75	0.30	<0.01	25.93	0.42	3.28	0.01
↑CO ₂ , 20°C oak	74.01	2.90	0.43	<0.01	32.52	0.33	5.66	0.01
↑CO ₂ , 25°C oak	77.35	0.86	0.46	0.02	26.54	1.39	6.21	0.03

TABLE 4 Comparison of the contents of individual groups of secondary metabolites in leaves of oak (n = 36) and spruce (n = 72) seedlings grown under lower (20°C) and higher (25°C) temperatures, as well as ambient (410 ppm) and doubled (820 ppm) CO₂ concentrations.

Phenolics	F-value	p-value	Eta ²	Eta ² part
Tree species	224.545	<0.001	0.720	0.894
Temperature	12.394	0.002	0.044	0.341
CO ₂	3.729	0.065	0.013	0.135
Non-tannins	F-value	p-value	Eta ²	Eta ² part
Tree species	0.455	0.507	0.015	0.017
Temperature	0.011	0.917	<0.001	0.001
CO ₂	0.002	0.961	<0.001	<0.001
Flavonoids	F-value	p-value	Eta ²	Eta ² part
Tree species	68.339	<0.001	0.474	0.705
Temperature	12.091	0.002	0.100	0.335
CO ₂	0.133	0.718	0.001	0.006
Condensed tannins	F-value	p-value	Eta ²	Eta ² part
Tree species	69.563	<0.001	0.740	0.747
Temperature	1.436	0.243	0.015	0.056
CO ₂	0.048	0.829	<0.001	0.002

that seedlings grown at higher temperatures had increased nitrogen concentrations in their leaves and needles. This finding aligns with research by Yuan et al. (2021), which reported an almost 8% decrease in the C:N ratio at elevated temperatures in *Quercus mongolica* Fisch. ex Turcz. and *Fraxinus mandshurica* Rupr. Similarly, Zhang et al. (2011) indicated that increased nitrogen concentrations in leaves at higher temperatures are linked to enhanced photosynthesis and a greater capacity for nitrogen uptake. This mechanism likely explains our findings, where doubled CO₂ levels did not result in a significant increase in carbon content in the seedlings.

The response of the C:N ratio varied between tree species. Spruce seedlings exhibited a higher C:N ratio compared to oak trees, which had correspondingly higher nitrogen concentrations in their leaves. The low C:N ratio in oak indicates a greater investment in proteins essential for photosynthesis (Zhang et al., 2020). Additionally, oak leaves contained a higher content of certain sugars, particularly sucrose and raffinose. These sugars are critical products of photosynthesis, providing carbon and energy necessary for synthesizing secondary metabolites (Rolland et al., 2006). Compounds such as phenols, flavonoids, and condensed tannins are synthesized via the shikimate pathway, which relies on intermediates derived from sugar metabolism, including phosphoenolpyruvate and erythrose-4-phosphate as primary substrates (Marchiosi et al., 2020). This relationship may explain why oak, with its higher sugar content, also exhibited elevated levels of these carbon-based secondary metabolites.

TABLE 5 Sugar contents in oak (n = 36) and spruce (n = 72) seedlings grown under lower and higher temperatures (20°C and 25°C), as well as ambient (410 ppm) and doubled (820 ppm) CO₂ concentrations.

Diet	Sucrose content (mean, mg/g)	Sucrose content (SD)	Fructose content (mean, mg/g)	Fructose content (SD)	Glucose content (mean, mg/g)	Glucose content (SD)	Raffinose content (mean, mg/g)	Raffinose content (SD)
aCO ₂ , 20°C spruce	23.35	0.68	2.61	0.15	1.93	0.05	0.09	0.01
aCO ₂ , 25°C spruce	20.76	0.70	2.67	0.15	1.85	0.22	0.13	0.03
↑CO ₂ , 20°C spruce	23.47	0.80	3.03	0.06	2.33	0.09	0.22	0.01
↑CO ₂ , 25°C spruce	22.46	0.94	2.55	0.09	1.88	0.19	0.11	0.03
aCO ₂ , 20°C oak	27.50	0.11	3.14	0.11	2.74	0.04	0.22	0.11
aCO ₂ , 25°C oak	26.87	0.13	2.75	0.31	2.56	0.12	0.24	0.03
↑CO ₂ , 20°C oak	25.68	0.84	2.82	0.07	2.29	0.27	0.25	0.01
↑CO ₂ , 25°C oak	23.89	0.36	3.78	0.07	3.90	0.35	0.29	0.01

TABLE 6 Comparison of the sugar contents in oak (n = 36) and spruce (n = 72) seedlings grown under lower and higher temperatures (20°C and 25°C), as well as ambient (410 ppm) and doubled (820 ppm) CO₂ concentrations.

Sucrose	F-value	p-value	Eta ²	Eta ² part
Tree species	20.676	0.010	0.477	0.806
Temperature	5.861	0.073	0.168	0.594
CO ₂	0.737	0.439	0.021	0.156
Glucose	F-value	p-value	Eta ²	Eta ² part
Tree species	5.164	0.086	0.360	0.546
Temperature	0.084	0.787	0.006	0.020
CO ₂	0.002	0.963	<0.001	<0.001
Fructose	F-value	p-value	Eta ²	Eta ² part
Tree species	2.017	0.229	0.209	0.309
Temperature	0.148	0.720	0.017	0.036
CO ₂	0.017	0.902	0.002	0.004
Raffinose	F-value	p-value	Eta ²	Eta ² part
Tree species	20.576	0.011	0.631	0.824
Temperature	0.843	0.411	0.029	0.174
CO ₂	0.496	0.520	0.017	0.110

Bold numbers represent significant impact.

4.2 Effects of doubled CO₂ concentration and higher temperature on plant secondary metabolites

4.2.1 Phenolics

Our research shows that higher temperatures in both tree species, along with doubled CO₂ in oak, lead to an increase in phenolic concentrations. Only one opposite finding was in oak at ambient CO₂ with rising temperature, which aligns with a meta-analysis by Zvereva and Kozlov (2006) and a review by Julkunen-Tiitto et al. (2015), which stated that phenolic concentrations tend to decrease as temperatures rise under ambient CO₂ conditions. In contrast, spruce needles exhibited a different response under ambient conditions, with no significant differences observed in seedlings exposed to elevated CO₂ levels, as reported in previous studies (Koricheva et al., 1998; Zvereva and Kozlov, 2006; Lindroth, 2012; Julkunen-Tiitto et al., 2015; Robinson et al., 2012). Moreover, our findings indicate that the interaction between CO₂ and temperature can interfere with each other's effects, suggesting that the impact of these factors varies depending on the specific context (Julkunen-Tiitto et al., 2015; Zvereva and Kozlov, 2006; Veteli et al., 2007).

4.2.2 Flavonoids

We observed that flavonoid levels significantly decreased at higher temperatures in oak leaves under both ambient and elevated CO₂ conditions. This finding is consistent with the research conducted by Julkunen-Tiitto et al. (2015). In contrast, we found

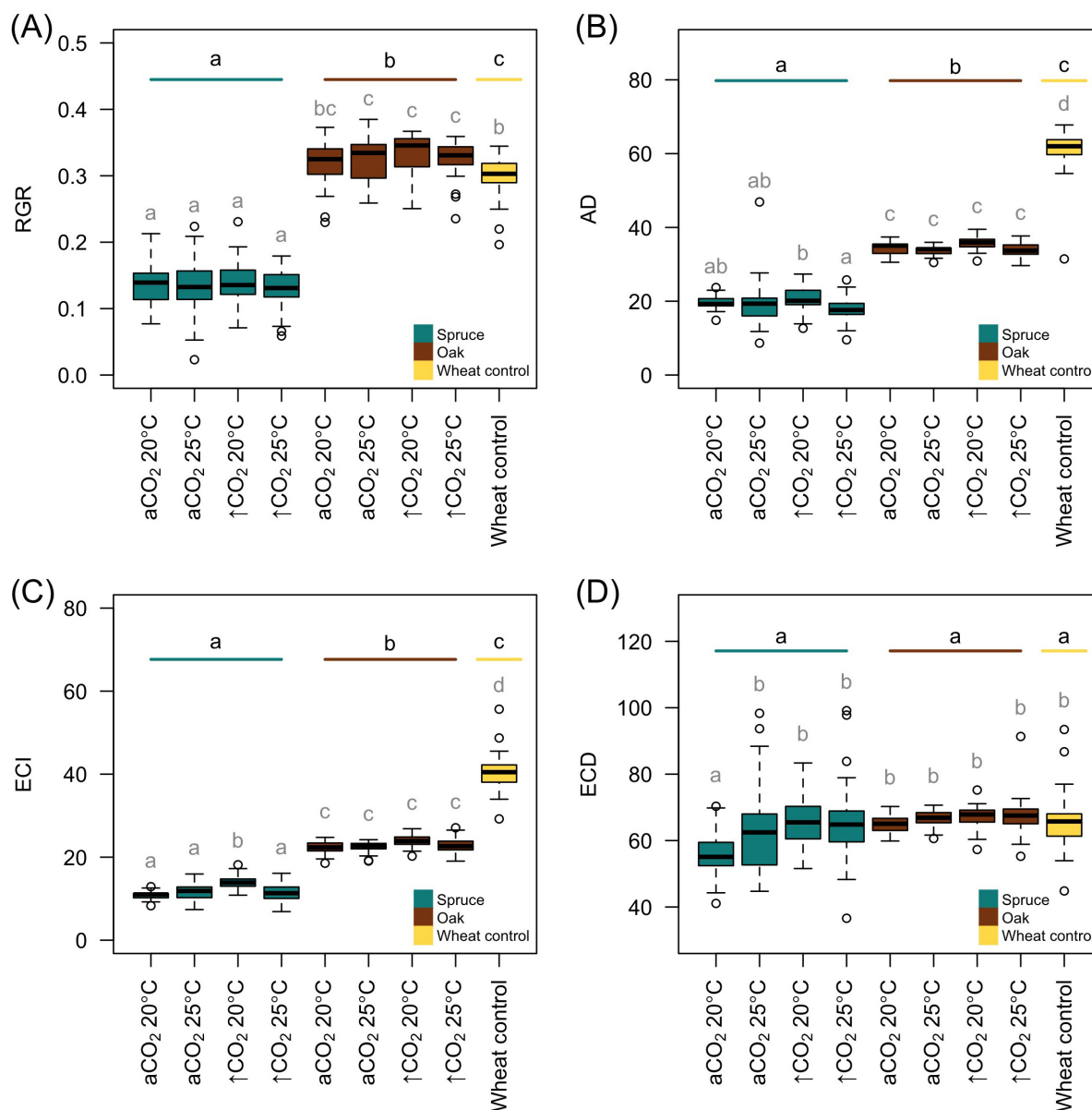


FIGURE 3
 The influence of spruce and oak leaf powder diet from trees grown under two temperatures (20°C and 25°C) and two CO₂ concentrations (410 ppm and 820 ppm) on the growth rate (RGR) (A), approximate digestibility (AD) (B), efficiency of conversion of ingested food to body substances (ECI) (C), and efficiency of conversion of digested food to body substances (ECD) (D) of *L. dispar* compared to the growth on wheat germ diet (controls) (after Bell, 1981). Colorful lines with different letters in the upper part of graphs summarize the significant differences between spruce, oak, and wheat control diet groups. Different gray letters above boxplots indicate significant differences between the tested treatments. For further details on the indices, refer to Table 1 and Figure 1.

an opposing result in spruce needles grown under doubled CO₂ concentration, where the flavonoid content was higher at 25°C compared to 20°C. This trend was also reported in studies by Li et al. (2011) and Shamloo et al. (2017). Additionally, Holopainen et al. (2018) mentioned that elevated CO₂ levels should increase flavonoid concentrations, although this effect is weaker than that of temperature changes. This discrepancy may help explain the differences we observed, but a similar trend was not found in oak trees. Some studies, including those by Holopainen et al. (2018) and Julkunen-Tiitto et al. (2015), indicated that the combination of temperature and CO₂ had no significant impact on flavonoid levels.

In summary, our findings indicate that the effects of climate change factors, such as elevated temperatures and increased CO₂ concentrations, on secondary metabolites are highly variable and depend on the specific context. The responses of phenolics and flavonoids differ, suggesting that the interaction between CO₂ levels and temperature can either enhance or reduce the impacts on plant metabolites. Further studies with a longer exposure period of the trees under the corresponding temperature and CO₂ conditions could possibly provide a clearer picture of the factors influencing the changes in leaf constituents and contribute to a better understanding of the

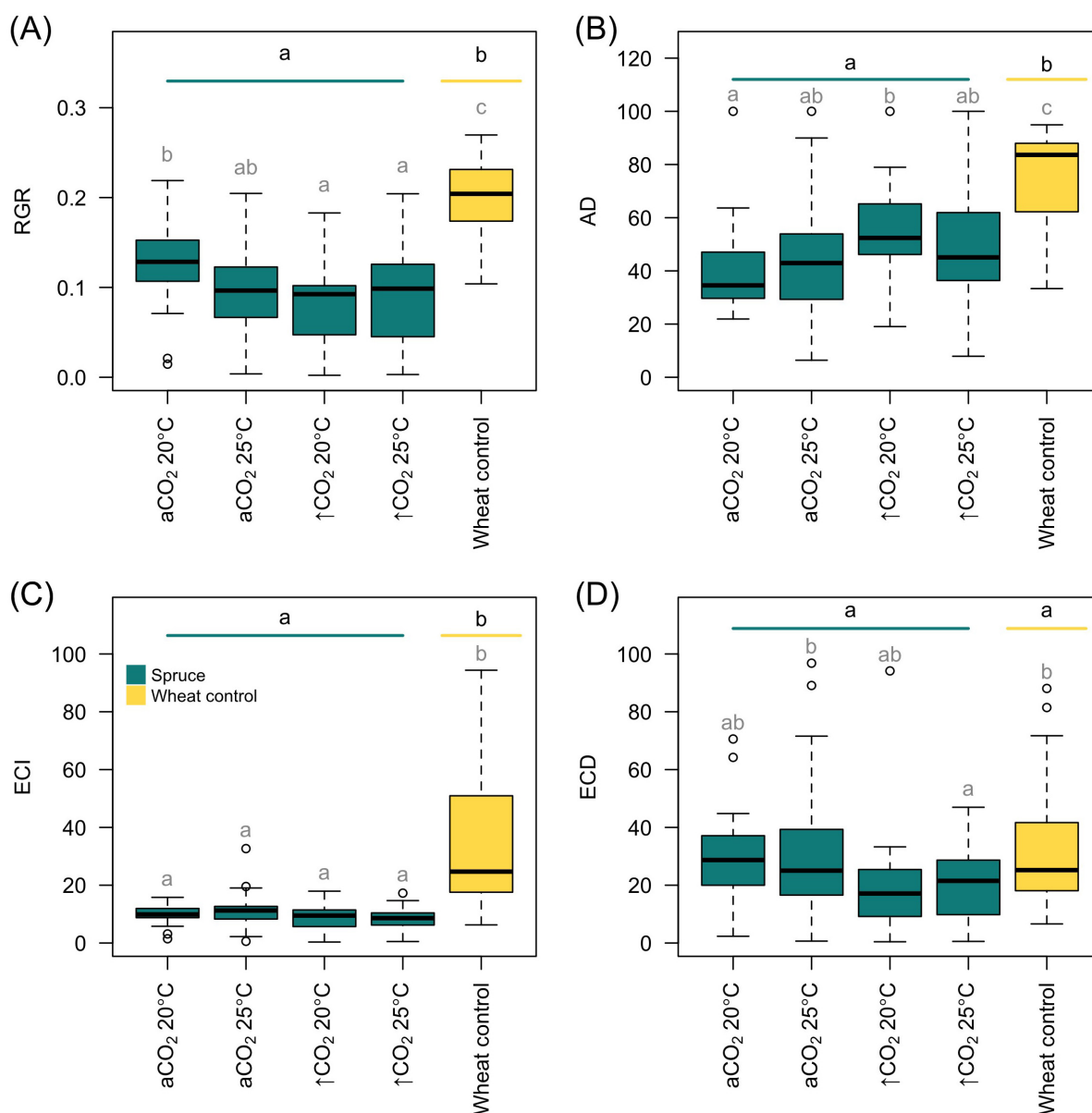


FIGURE 4
 Influence of spruce leaf powder diet from trees grown under two temperatures (20°C and 25°C) and two CO₂ concentrations (410 ppm and 820 ppm) on the growth rate (RGR) (A), approximate digestibility (AD) (B), efficiency of conversion of ingested food to body substances (ECI) (C), and efficiency of conversion of digested food to body substances (ECD) (D) of *L. monacha* compared to the growth on wheat germ diet (controls) (after Bell, 1981). Colorful lines with different letters in the upper part of graphs summarize the significant differences between spruce, oak, and wheat control diet groups. Different gray letters above boxplots indicate significant differences between the tested treatments. For further details on the indices, refer to Table 1 and Figure 1.

complex interactions between climate factors and their effects on plant chemistry.

4.3 Effects of doubled CO₂ concentration and higher temperature on larval performance

Both *Lymantria* species examined are polyphagous insects, with *L. dispar* preferring deciduous trees and *L. monacha* preferring coniferous species as their host trees. The larvae of *L. dispar*

have been reared in a laboratory for decades on the wheat germ diet, which was used as a control diet in our study, while the nun moth larvae were collected from the field. Therefore, it was not surprising that in the gravimetric analyses, the spongy moth larvae showed the highest AD and ECI values on the artificial wheat control diet, which lacked the secondary metabolites found in their natural food sources. Interestingly, development on the high wheat germ diet showed similar RGR values to those on the oak powder diet; however, both diets produced RGR values that were more than twice as high as those on the spruce powder diet. The effects of different temperatures and CO₂ exposure on

the trees were only evident with the less suitable spruce needle diet.

The reason why the effects were evident only in spruce can be explained by Stowe and Osborn (1980), as they reported that the toxicity of phenolic compounds is closely linked to nutrient concentrations. Specifically, phenolic acids have been shown to inhibit growth only at low nutrient levels significantly. This helps clarify why *L. dispar* performs better on oak, despite its higher levels of phenolics, and condensed tannins (see Barbehenn et al., 2013; Milanović et al., 2015). Additionally, in another study, *L. dispar* demonstrated significantly better performance on white alder (*Alnus rhombifolia* Nutt.) compared to Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Joseph et al., 1991). Interestingly, the alder had higher levels of phenolic compounds and greater nitrogen content, contributing to its overall nutritional value (Joseph et al., 1991; also see Hättenschwiler and Schafellner, 1999).

For *L. dispar*, the effects of increased CO₂ on ECI and ECD were more pronounced at the lower temperature (20°C) compared to the higher temperature (25°C). This finding is supported by higher flavonoid levels in spruce trees at higher temperatures with doubled CO₂ levels. Conversely, flavonoid concentrations decreased at lower temperatures, which allowed the larvae to perform better when exposed to doubled CO₂ concentration in spruce. Additionally, for *L. monacha*, the lower temperature (20°C) seems to positively impact larval performance at ambient CO₂ concentrations, resulting in higher RGR and ECD. This effect can be attributed to the higher phenol concentrations in the needles from trees at higher temperatures, thereby underscoring the role of secondary metabolites in influencing larval performance.

The diet of larvae from oak trees also contained higher levels of sugars, particularly sucrose and raffinose. Glucose seemed to be higher in oak in most cases, especially when temperatures were higher. Still, the result is not significant, probably because the numbers are similar to those of spruce, and the amount of oak is much higher in ambient CO₂ and lower temperature treatment. This likely explains why *L. dispar* larvae performed better on oak than on spruce. Sugars serve as a primary energy source for insects (see Skowronek et al., 2021). Simple sugars like glucose and fructose are quickly metabolized, providing immediate energy necessary for essential activities such as growth, movement, and reproduction (Sheng et al., 2019; Mayack et al., 2020).

The overall quality of a plant as a food source is influenced by several factors, including the balance of nutrients, the presence of secondary metabolites, and the plant's digestibility (Scogings et al., 2013; Wink, 2018). The artificial diet was prepared by powdering lyophilized leaves, a standardized method for the chemical analysis of leaves (Šulc et al., 2021). However, this method overlooks physiological factors that are also crucial in plant-herbivore interactions. For instance, leaf hardness, which can be increased by higher levels of structural carbohydrates resulting from elevated CO₂ concentrations (Prior et al., 2004; Gely et al., 2020; Münzbergová and Skuhrovec, 2020), cannot be taken into account when using dried leaves.

Escalating temperatures lead to the combined effects of drought and heat waves (Sato et al., 2024; Cook et al., 2018).

These factors have become significant direct consequences that severely impact ecosystems (Bailey-Serres et al., 2019). Including drought as an additional variable in studies like ours would provide more complex insights into the effects of climate change on forest ecosystems (Clark et al., 2016). Our study focused on elevated CO₂ concentrations in the atmosphere and their direct consequence: higher air temperatures. The results indicate that trees respond to changes in climate conditions, which in turn affects their chemical composition. However, herbivorous insects can still thrive on these trees despite variations in nutritional quality, suggesting that climate change does not significantly impact their performance via this indirect effect.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements because the partner who sent us the eggs of larvae breeds them especially for this kind of experiments. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BD: Conceptualization, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. JHo: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. DM: Investigation, Methodology, Writing – review & editing. AK: Formal Analysis, Validation, Writing – review & editing. JHr: Formal Analysis, Validation, Writing – review & editing. JČ: Data curation, Formal Analysis, Visualization, Writing – review & editing. AS: Conceptualization, Methodology, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2025.1564011/full#supplementary-material>

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Příloha 2.

Ecological realism weakens predicted plant–herbivore responses to climate change in a semi-field spruce forest

1 **Ecological realism weakens predicted plant–herbivore responses**
2 **to climate change in a semi-field spruce forest**

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10 **Abstract**

11 Global climate change significantly affects the chemical composition of plant
12 tissues, which is often a key factor influencing the responses of folivorous
13 herbivores. However, it remains unclear whether and under what conditions
14 changes in host plant quality affect herbivore performance in more complex
15 ecological environments. In this study, we investigated whether climate change-
16 induced alterations in host plant chemistry, along with nutrient availability, affect
17 the performance of specialized folivorous herbivores under semi-field conditions.
18 We conducted a fully factorial experiment that included variations in CO₂
19 concentration, water availability, nitrogen availability, and plant provenance.
20 Norway spruce (*Picea abies*) was cultivated in glass ecosystem spheres, and sawfly
21 larvae were fed directly on the freshly flushing shoots of these experimental trees.
22 Our findings revealed that elevated CO₂ levels, reduced water availability, and
23 contrasting nitrogen supply led to significant changes in needle chemistry, with CO₂
24 as the dominant factor affecting both the C:N ratio and overall metabolomic
25 profiles. However, despite these chemical changes, we observed no differences in
26 larval growth, development, or frass stoichiometry across treatments or tree
27 provenances. These results indicate that even substantial climate-induced changes
28 in host plant chemistry, potentially amplified by variations in nutrient and water
29 availability, do not necessarily affect the performance of specialized herbivores in
30 semi-field conditions. This suggests that predictions of climate change impacts on
31 herbivores, based solely on plant chemical traits, may overestimate their biological
32 consequences in some ecological systems.

33 **Keywords:** elevated CO₂, *Picea abies*, plant chemistry, sawfly, semi-field
34 experiment

35

36

37 **1. Introduction**

38 **1.1. Climate change as a factor influencing plant chemistry**

39 Forests play a crucial role in the global carbon cycle and provide a range of
40 ecosystem services, including carbon sequestration, timber production, and
41 biodiversity support. This makes them vital in the context of ongoing climate change
42 (Psistaki et al., 2024; Pan et al., 2011). Changes in the ecological interactions within
43 temperate forest ecosystems can lead to significant ecological and socio-
44 economic consequences (Kutnar, 2022). Thus, understanding how temperate forest
45 communities respond to climate change is essential for predicting the future
46 functioning and stability of these ecosystems. Research indicates that climate
47 change can significantly affect the growth and physiology of forest trees, leading to
48 changes in growth patterns (Blanco et al., 2021), phenology (Montgomery et al.,
49 2020), and the chemical composition of plant tissues (Stiling & Cornelissen, 2007;
50 Holopainen et al., 2018). These alterations at the plant level can have a cascading
51 effect on a wide range of trophic interactions, including plant-herbivore
52 relationships (Robinson et al., 2012), interactions between plants, herbivores, and
53 natural enemies (Ode, 2006), plant-pollinator interactions (Hegland et al., 2009),
54 and associations between plants and microorganisms (Sharma et al., 2022).
55 However, significant uncertainty remains, as many studies on temperate forests
56 have focused on individual climatic drivers or short-term experiments. This focus
57 limits our understanding of the combined and mechanistic effects of climate
58 change in ecologically realistic scenarios (Korell et al., 2020).

59 Climate change in natural ecosystems does not occur in isolation; rather, it results
60 from a combination of environmental pressures (Korell et al., 2020). These include
61 increased atmospheric CO₂ concentrations, changes in water and nutrient
62 availability, and rising year-to-year climate variability (Van Moorsel et al., 2023).
63 Plant responses to these factors can be nonlinear and are influenced by their
64 interactions, meaning that the combined effects often differ from those observed in
65 experiments examining individual factors (Korell et al., 2020). While nutrient
66 availability, especially nitrogen, is not a direct driver of climate change, it
67 significantly alters plant responses to climatic factors. Therefore, understanding
68 nutrient dynamics is crucial for evaluating the ecological significance of climate
69 change-induced effects (Terrer et al., 2019).

70 In long-lived woody species, responses to climate change can be significantly
71 influenced by ontogeny. Older trees typically have larger nutrient reserves, deeper
72 root systems, and different carbon allocation strategies compared to seedlings
73 (Niinemets, 2010). These variations may affect both the extent of chemical changes
74 in their tissues and the ecological significance of these changes for higher trophic
75 levels, including herbivores and their feeding relationships (Robinson et al., 2012;

76 Wetzel et al., 2023). However, research on mature trees and their long-term
77 exposure to climatic factors remains relatively limited compared to studies on
78 seedlings (Koricheva et al., 2025).

79 **1.2. Changes in plant quality and their effects on herbivores**

80 Climate change drivers, such as increased atmospheric CO₂ concentrations,
81 altered nutrient availability, and more frequent droughts, can significantly change
82 the chemical composition of plant tissues, ultimately affecting food quality for
83 herbivores (Jamieson et al., 2017). Elevated CO₂ levels are generally linked to a
84 reduction in tissue nitrogen content due to enhanced carbon assimilation, resulting
85 in a higher C:N ratio, a phenomenon known as "nitrogen dilution." This mechanism
86 is often viewed as a factor that diminishes the nutritional value of foliage for leaf-
87 feeding insects (Kaspari et al., 2024; Strain & Cure, 1985). Soil nitrogen availability
88 can modify these effects by partially offsetting the changes induced by CO₂.
89 However, limited water availability can amplify the situation by negatively impacting
90 nitrogen uptake and transport within plants (Dong et al., 2024; Gessler et al., 2017).

91 Changes induced by climate change are not limited to basic stoichiometric traits,
92 such as nitrogen content or the C:N ratio. They also involve shifts in both primary
93 and secondary plant metabolism (Dvořáková et al., 2025; Sun et al., 2024). These
94 chemical changes are often seen as a potential cause of negative impacts on
95 herbivores; however, empirical studies result in mixed results (Liu et al., 2024;
96 Lindroth et al., 2010). While some studies have reported reduced growth, prolonged
97 development, or altered herbivore stoichiometry in response to decreased food
98 quality (Zhang et al., 2018; Rao et al., 2012), others indicate weak or no responses
99 (Roberts et al., 2022; Van De Velde et al., 2019), especially when plants are
100 simultaneously exposed to multiple environmental factors (Hamann et al., 2021).
101 The relationship between changes in host plant chemistry and herbivore
102 performance is thus highly context-dependent, making it one of the key
103 uncertainties in predicting the impacts of climate change on plant–insect
104 interactions (Wetzel et al., 2023).

105 One possible explanation for this variability is that herbivores are not just passive
106 consumers of food quality; they have mechanisms that allow them to actively
107 regulate their nutrient intake and utilization (Behmer et al., 2009). Nutritional
108 plasticity, compensatory feeding, and post-ingestive regulatory processes may
109 enable herbivores to maintain relatively stable growth and internal nutrient balance
110 even when host plant chemistry changes significantly (Cavigliasso et al., 2020). In
111 such cases, the connection between changes in plant chemistry and herbivore
112 performance may be partially or entirely disrupted (Robinson et al., 2012).

113 Due to these experimental limitations, it remains largely unclear how changes in
114 host plant chemistry affect herbivores, especially when responses are evaluated in

115 young plants over short time scales. This underscores the necessity for
116 experimental methods that incorporate older trees, extended exposure periods, and
117 more natural seasonal dynamics (Niinemets et al., 2010).

118 These considerations highlight the need for experimental approaches that go
119 beyond short-term, single-factor studies to achieve greater ecological realism. It is
120 important to recognize that no experiment can fully capture the complexity of
121 natural systems (Korell et al., 2020; Zvereva & Kozlov, 2006). By incorporating longer-
122 term exposure, older trees, multiple climate change drivers, and direct assessment
123 of herbivore performance in semi-field conditions, our study represents a step
124 toward understanding plant-herbivore interactions in the context of climate change.

125 **1.3. Hypothesis and aims**

126 Building on previous research, we investigated whether changes in host plant
127 chemistry, induced by major climate change drivers, are sufficient to affect
128 herbivore performance under semi-field conditions. To address this question, we
129 designed a multifactorial experiment that combined elevated CO₂ levels, nitrogen
130 availability, water limitation, and tree provenance.

131 We specifically aimed to evaluate how elevated atmospheric CO₂, nitrogen
132 availability, and water limitation impact the nutritional quality of Norway spruce
133 needles (*Picea abies*) and whether these changes influence the performance of
134 specialized folivorous herbivores.

135 In this semi-field experiment, we tested the following questions:

- 136 (i) Does elevated CO₂ increase the C:N ratio of the needles and affect larval growth,
137 development, and frass stoichiometry?
- 138 (ii) Can increased nitrogen availability counteract the changes in needle quality
139 induced by elevated CO₂ and modify larval performance?
- 140 (iii) Does reduced water availability amplify the CO₂-related changes in needle
141 chemistry and impact larval performance?
- 142 (iv) Do spruce provenances from contrasting elevations differ in their chemical
143 responses to environmental drivers and in associated herbivore performance?

144 **2. Materials and methods**

145 **2.1. Study site and experimental setup**

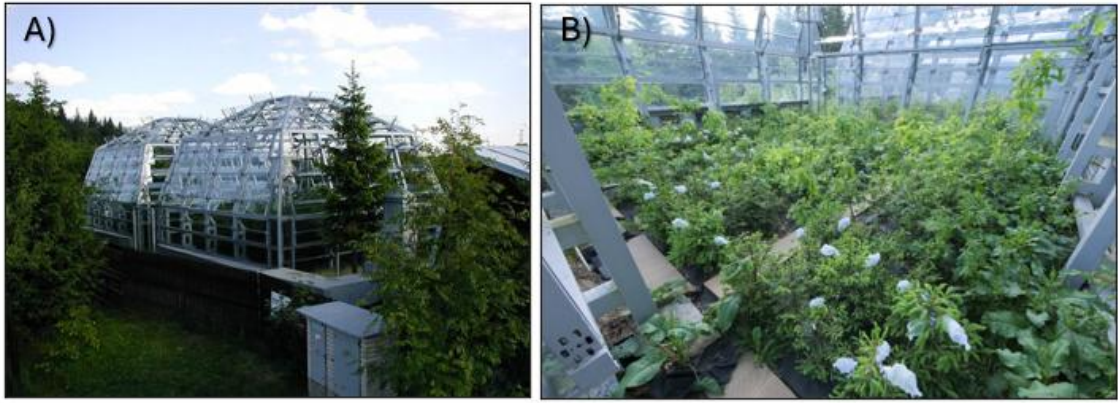
146 The experiment was conducted at the Bílý Kříž ecosystem research station in the
147 Moravian-Silesian Beskids (49.502449 N, 18.538938 E; 908 m above sea level) over
148 two growing seasons, from 2019 to 2020. The site is characterized by a mean annual
149 air temperature of 6.7 °C, a mean annual relative humidity of 80%, and a mean
150 annual precipitation of 1,374 mm (Ofori-Amanfo et al., 2023). The soil at the site is
151 classified as Haplic Podzol, which has developed on Mesozoic Godula sandstones
152 of flysch origin.

153 In 2017, two experimental Norway spruce (*Picea abies* (L.) Karst.) stands were
154 established at the site, each consisting of 384 seedlings planted at a spacing of 0.5
155 meters apart. The trees were sourced from two provenances representing different
156 forest altitudinal zones: the spruce–beech zone and the spruce zone. Each stand
157 was enclosed within a separate ecosystem sphere, also known as a "glass dome,"
158 with a ground area of 10 × 10 meters and a maximum height of 7 meters (Fig. 1A).

159 The interior of each sphere was divided into six blocks, each measuring 16.5 m² and
160 containing 12 individuals (Fig. 1B). To prevent lateral water movement within the soil
161 profile, the blocks were hydraulically separated using PVC panels that were inserted
162 to a depth of 0.5 m. Throughout the entire growing season, three randomly selected
163 blocks in each sphere were regularly irrigated (WW), while irrigation in the remaining
164 blocks was reduced from early July to mid-August (WL). The regularly irrigated
165 blocks received 20 mm of water per week through an automated irrigation system.

166 Soil moisture was continuously monitored using LM3 Theta Probe sensors (Delta-T
167 Devices, UK) at depths of 5–10 cm and 30–40 cm. One experimental sphere was kept
168 at the ambient CO₂ concentration of 385 ppm, while the other was set to 700 ppm.
169 An automated control system regulated the CO₂ concentrations based on prevailing
170 climatic conditions, maintaining the target levels for approximately 91% of the
171 growing season.

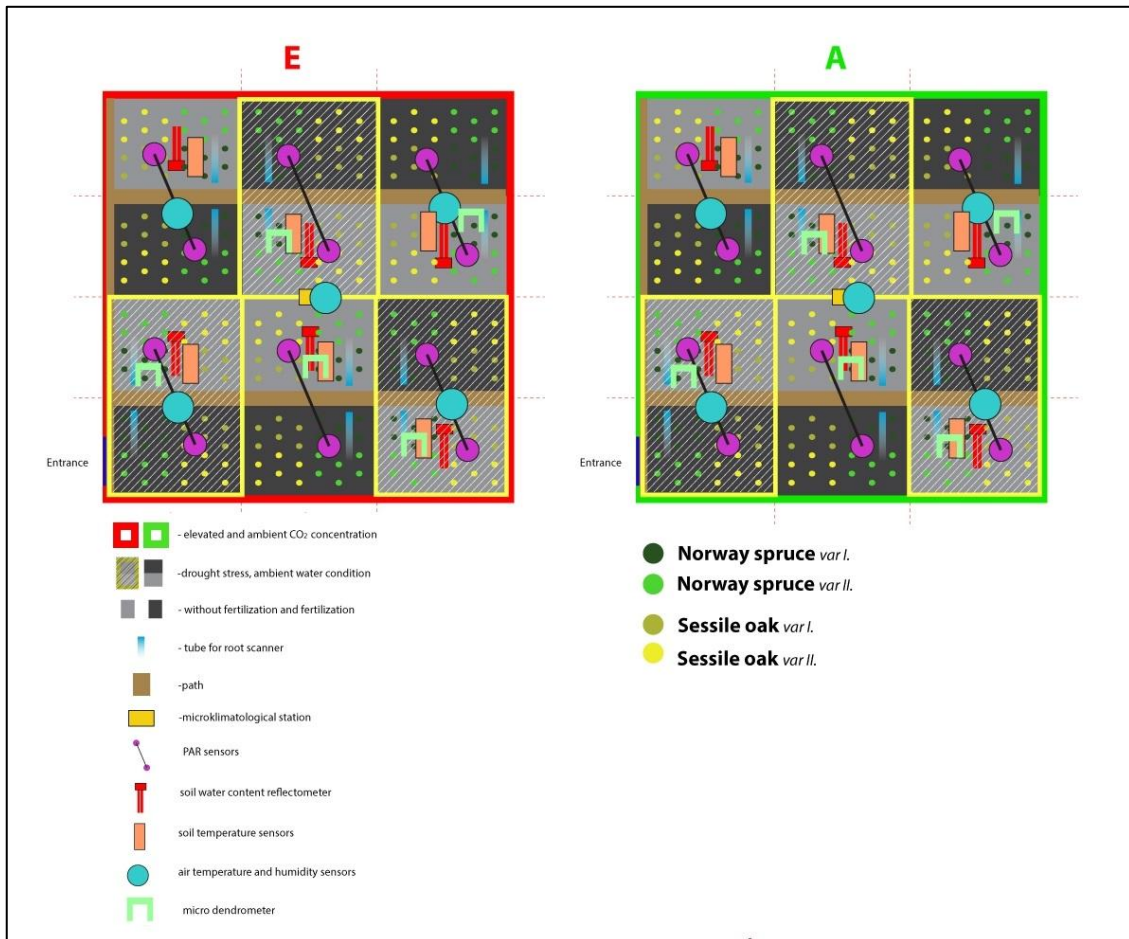
172 Fertilization was applied as an additional experimental factor to half of the trees
173 according to a predefined scheme at a rate of 60 kg N·ha⁻¹, while the remaining
174 individuals remained unfertilized.



175 **Figure 1. Glass domes (“ecosystem spheres”) at the Bílý Kříž research station**
176 **with Norway spruce seedlings exposed to combined environmental treatments**
177 **(CO₂, irrigation, fertilization, provenance). A) External view of the glass dome**
178 **structure. B) Interior arrangement of the dome with experimental spruce**
179 **seedlings.**

180 **2.2. Experimental Design and Replication Structure**

181 The experiment was designed as a fully factorial study with four factors: CO₂
 182 concentration (ambient vs. elevated), irrigation level (well-watered vs. reduced),
 183 fertilization (N⁺ vs. N⁻), and spruce provenance. In total, 16 treatment combinations
 184 were tested, representing all possible combinations of these factors (2 × 2 × 2 × 2)
 185 (Fig. 2).



186 **Figure 2. Layout of experimental seedlings and treatments within the two**
 187 **ecosystem spheres at the Bílý Kříž research station. Sphere E was supplied with**
 188 **elevated CO₂ (700 ppm), whereas sphere A was maintained at ambient CO₂**
 189 **concentration (385 ppm). Colored sectors indicate the respective**
 190 **environmental treatments (irrigation, fertilization) and the spatial distribution**
 191 **of spruce trees from the two provenances. Other tree species (sessile oak) and**
 192 **installed sensors formed part of the overall ecosystem sphere design but were**
 193 **not included in the feeding experiment.**

194 Each combination of experimental factors was represented by the same number of
 195 individuals in each year of the experiment. In 2019, 18 trees were used per treatment
 196 combination, for a total of 144 individuals. In 2020, the number of replicates was
 197 reduced to nine individuals per combination, resulting in a total of 72 individuals

198 due to lower tree availability. In both years, the trees were evenly distributed within
199 the two experimental spheres according to a predefined layout. This was done to
200 ensure spatial balance among treatment combinations and to minimize potential
201 positional effects within the spheres.

202 **2.2.1. Field Collection of Larvae**

203 In Central Europe, the larvae of the three most common and abundant spruce
204 sawfly species—*Pristiphora abietina* (Christ, 1791). Upon arriving at the Bílý Kříž
205 research station, the larvae were weighed to the nearest 0.1 mg, documented with
206 photographs, and individually placed on freshly flushing shoots of the experimental
207 trees., *P. leucopodia* (Hartig, 1837), and *Euura scutellata* (Hartig, 1837) were
208 collected during the spruce flushing period (May–June) in 2019 and 2020. The
209 collections took place at various sites on the Obořa massif (coordinates:
210 49.5870544° N, 18.4890597° E) at elevations ranging from 500 to 600 meters above
211 sea level. The larvae were collected in the early second instar and were found
212 exclusively on freshly flushing shoots. After collection, the individuals were
213 transported to the laboratory within two hours.

214 Spruce sawflies (Hymenoptera: Tenthredinidae) are common specialist herbivores
215 that have a close relationship with Norway spruce (*Picea abies*) (Holuša, 2002).
216 Their adult emergence is synchronized with bud flushing, making them an ideal
217 model for studying how the chemistry of host plants affects herbivore development
218 (Holuša & Lubojacký, 2007). The larvae feed solitarily. In *P. abietina*, feeding is
219 concentrated on the current year's flushing shoots and needles (Beier Petersen,
220 1956). In contrast, larvae of *P. leucopodia* and *E. scutellata* initially develop on
221 freshly flushed needles before later switching to older foliage (Nigitz, 1974; Křístek,
222 1957). Fully grown larvae are relatively small, typically weighing between 50 and 80
223 milligrams. As a result, they generally require only a limited amount of foliage from
224 a single twig to complete their development, which places minimal stress on the
225 host plant during experimental rearing conditions.

226 Upon transfer to the Bílý Kříž research station, the larvae were weighed to the
227 nearest 0.1 mg, photographically documented, and individually placed on freshly
228 flushing shoots of the experimental trees.

229 **2.2.2. Larval Feeding Experiment**

230 Larvae were individually placed on twigs of Norway spruce and enclosed in
231 protective sleeves made from spirally coiled wire, measuring 2 mm in diameter.
232 Each sleeve formed a tube approximately 5 cm in diameter and 10 cm in length. This
233 tube was covered with a fine mesh fabric composed of 97% polyamide and 3%
234 elastane, which prevented the larvae from escaping, protected them from
235 predation, and allowed sufficient air circulation and light penetration (Fig. 3A, B).

236 The nylon sleeves were securely fastened at both ends with elastic bands. In 2019,
237 two sleeves were installed per tree, whereas in 2020, up to three sleeves were used
238 (Fig. 3A, B). Control trees without sleeves were included in each treatment for
239 metabolomic analyses. The larvae were inspected approximately every 2 to 3 days,
240 during which their developmental stage and any mortality were recorded, and frass
241 was collected continuously. Each larva was monitored individually from placement
242 until it completed its development (either forming a cocoon or entering the
243 eonymph stage). The duration of monitoring varied among the individuals depending
244 on the timing of placement and their developmental rates. Larvae that died within
245 the first three days without any signs of feeding were replaced with new individuals;
246 however, later mortality was not replaced. After development was completed, each
247 larva was removed from the sleeve, weighed fresh, dried, and further analyzed.



248

249 **Figure 3. A) An experimental Norway spruce tree fitted with nylon sleeves, each**
250 **containing a single sawfly larva. B) A close-up of a sleeve surrounding a newly**
251 **flushing shoot, used to isolate individual larvae, prevent their escape, and**
252 **collect frass during the feeding experiment.**

253 **2.2.3. Collection and Analysis of Larval Frass**

254 All frass produced by each larva was collected from the sleeve during the
255 experiment, subsequently dried, weighed, and analyzed. The C:N ratio was
256 calculated as the molar ratio of carbon to nitrogen.

257 For the C and N analysis, 10–30 mg of the pure frass sample was weighed into tin
258 capsules designed for the instrument used. The capsules were then introduced into
259 the combustion chamber of the Thermo Scientific Flash 2000 Elemental analyzer
260 (Thermo Scientific, United States). The samples were combusted in a stream of pure
261 oxygen at 1,000°C. The resulting carbon and nitrogen oxides were passed through a

262 copper reduction column and directed to a separation column, where moisture was
263 removed. Helium was used as the carrier gas. The content of the separated oxides
264 was determined using a thermal conductivity detector, and signal processing was
265 performed with Eager Xperience software (Thermo Scientific).

266 **2.2.4. Needle Sampling and Chemical Analyses**

267 Control needles were sampled from separate control trees. The needles were dried
268 at 60 °C, homogenized, and analyzed for C and N content using the same
269 methodology as for frass samples. A subset of samples was additionally subjected
270 to metabolomic analysis (PLS-DA) to assess shifts in the profiles of primary and
271 secondary needle metabolites.

272 For metabolomic analysis, thirty milligrams of freeze-dried and homogenized
273 samples were accurately weighed into 2 mL test tubes, and 1 mL of 80% methanol
274 was added. The tubes were vortexed for a few seconds, then placed in an ultrasonic
275 bath for 10 minutes. Afterward, the samples were centrifuged for 5 minutes at
276 13,000 rpm. The supernatant was filtered using a 0.22 µm PTFE syringe filter prior to
277 analysis.

278 A metabolomic analysis was conducted using an Agilent 1290 Infinity II liquid
279 chromatography system coupled with an Agilent 6546 LC/MS quadrupole time-of-
280 flight (QTOF) mass spectrometer (Agilent Technologies, USA). Chromatographic
281 separation was achieved on an Agilent InfinityLab Poroshell 120 EC-C18 column (2
282 × 150 mm, 2.7 µm particle size). The mobile phase consisted of solvent A (0.1%
283 formic acid in water) and solvent B (methanol), with the following gradient elution
284 profile: 0–4 minutes at 85% A, 4–7 minutes at 75% A, 7–9 minutes at 68% A, 9–16
285 minutes at 60% A, 16–22 minutes at 45% A, and 22–28 minutes at 5% A, followed by
286 a 2-minute hold at 5% A. The flow rate was set to 0.5 mL/min, the column
287 temperature to 35 °C, and the injection volume to 1 µL. Measurements were
288 performed in negative ionization mode. QTOF parameters included a mass scan
289 range of 100–1000 m/z, drying gas temperature of 160 °C, sheath gas flow rate of
290 12.0 L/min, sheath gas temperature of 400 °C, capillary voltage of 5.0 kV, nozzle
291 voltage of 2.0 kV, fragmentor voltage of 140 V, and collision energies of 10, 20, and
292 40 eV. MS/MS analysis was performed with a scan range of 50–800 m/z, a retention
293 time window of 0.5 minutes, an isolation window of 1.3 amu, and an acquisition rate
294 of 3 spectra per second. Continuous mass calibration was maintained with two
295 reference masses: 112.9855 m/z and 966.0007 m/z.

296 Data preprocessing, including feature extraction and chromatographic alignment,
297 was performed using Mass Hunter Profinder 10.0 software. The alignment
298 parameters were set with a minimum intensity threshold of 5000 counts and a
299 maximum retention time difference of 0.5 minutes. Feature extraction was

300 conducted with an m/z range of 100–1000, a retention time error tolerance of 0.25
301 minutes, and a mass accuracy of 2 mDa.

302 The HPLC analysis was performed using a Thermo Fisher UltiMate 3000 UHPLC
303 system, equipped with a photodiode array detector for quantification of phenolic
304 compounds. Compound separation was achieved using Thermo Fisher Hypersil
305 GOLD C18 Column (150 mm × 2.1 mm i.d., 5 µm particle size). The mobile phase
306 consisted of water (solvent A) and acetonitrile (solvent B), each containing 3%
307 acetic acid. The column temperature was maintained at 35°C. Gradient elution was
308 applied for the separation as follows: 0–15 minutes, 93% A and 7% B; 15–17 minutes,
309 72% A and 28% B; 17–20 minutes, 73% A and 27% B; 20–23 minutes, 2% A and 98%
310 B; 23–25 minutes, 50% A and 50% B; 25–30 minutes, 93% A and 7% B, at a flow rate
311 of 0.5 mL/min. A 5 µL injection volume was used, and the total analysis time was 30
312 minutes. Data acquisition and processing were conducted using the Thermo
313 Scientific Chromeleon Chromatography Data System (Várfalvyová et al. 2023).

314 **2.2.5. Calculation of Larval Growth Parameters**

315 For each larva, the relative growth rate (RGR) was calculated according to
316 Waldbauer (1968) using the following equation:

$$317 \quad RGR = \frac{\log W2 - \log W1}{t}$$

318 *In the formula, „W“ represents the dry weight of larvae and „t“ the time period.*

319

320 **2.3. Statistical analysis**

321 All statistical analyses were conducted using R version 4.3.3. Data z let 2019 a 2020
322 byla analyzována společně a proměnná *year* byla ve všech modelech zahrnuta jako
323 pevný efekt, aby se zohlednily meziroční rozdíly v podmínkách prostředí. Mixed-
324 effects models and generalized mixed-effects models were fitted using the “lme4”
325 package (Bates et al. 2015). In each model, tree identity (treeID) nested within the
326 corresponding sphere was included as a random effect to account for hierarchical
327 structure in the data. Druh pilatky byl ve všech modelech zahrnut jako pevný efekt,
328 aby bylo možné zohlednit rozdíly v absolutní výkonnosti mezi druhy, přičemž hlavní
329 zájem byl zaměřen na účinky experimentálních ošetření a jejich interakce. For
330 continuous response variables, we considered three different model types: linear,
331 log-normal, and gamma. The model with the best fit was selected based on the
332 amount of explained variability. For discrete response variables, we assessed
333 overdispersion and, where necessary, used a negative binomial distribution from
334 the “MASS” package (Venables & Ripley 2002). Binary response variables were
335 modeled using a binomial distribution with a logit link function. We employed both

336 "bobyqa" ("Bound Optimization BY Quadratic Approximation") optimizer for all our
337 models, which is particularly suited for handling difficult optimization problems,
338 ensuring model convergence, especially in cases with complex random effects
339 structures.

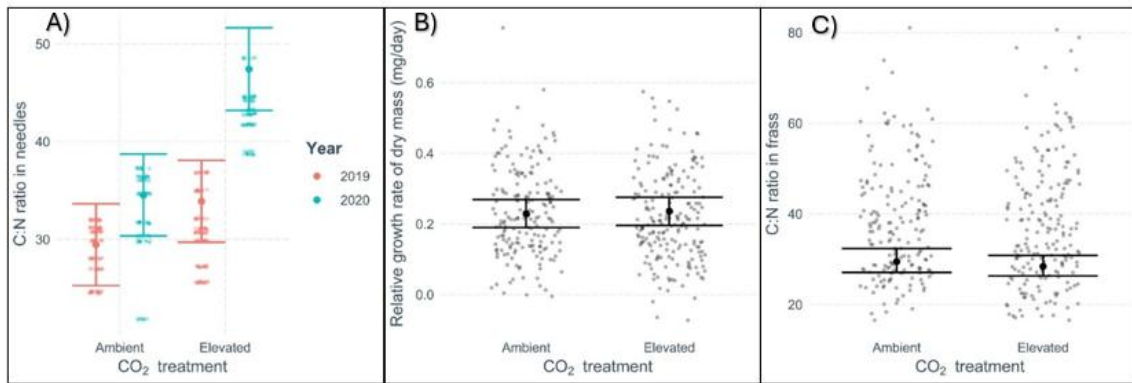
340 Model selection was performed using stepwise selection based on Akaike
341 Information Criterion (AIC), accounting for covariates and the main explanatory
342 variables as defined by the experimental design (see below). In the stepwise
343 process, we considered main effects, second-order polynomials for quantitative
344 variables, and all possible two-way interactions. In the final model, significant
345 covariates were retained, and we reintroduced the primary explanatory variables
346 dictated by the research design, even if they were non-significant, to examine their
347 effect. Thus, for the final model, we closely monitored multicollinearity using
348 variance inflation factor (VIF). Any VIF values greater than 2 were investigated, and
349 in justified cases, we allowed values up to 5 in the final model. For model evaluation,
350 we used the ANOVA from the "car" package (Fox & Weisberg, 2019). Graphical
351 representation of the final model estimates was produced using the "jtools",
352 "interactions", and "ggplot2" packages (Long 2022, Long 2019, Wickham 2016).

353 Metabolomic data were \log_2 -transformed, batch-corrected for sampling year (*sva*
354 package; Leek et al. 2012), and autoscaled (z-score). Then, principal component
355 analysis (PCA) was performed. Difference in multivariate metabolite profiles were
356 evaluated by two-factor permutational multivariate analysis of variance
357 (PERMANOVA, *vegan* package; Oksanen et al. 2013) and univariate linear modelling
358 with empirical Bayes moderation (*limma* package; Smyth, 2005). Then, partial least
359 squares–discriminant analysis (PLS-DA) was applied using the *mixOmics* package
360 for discriminant sample analysis.

361 **3. Results**

362 **3.1 Elevated CO₂**

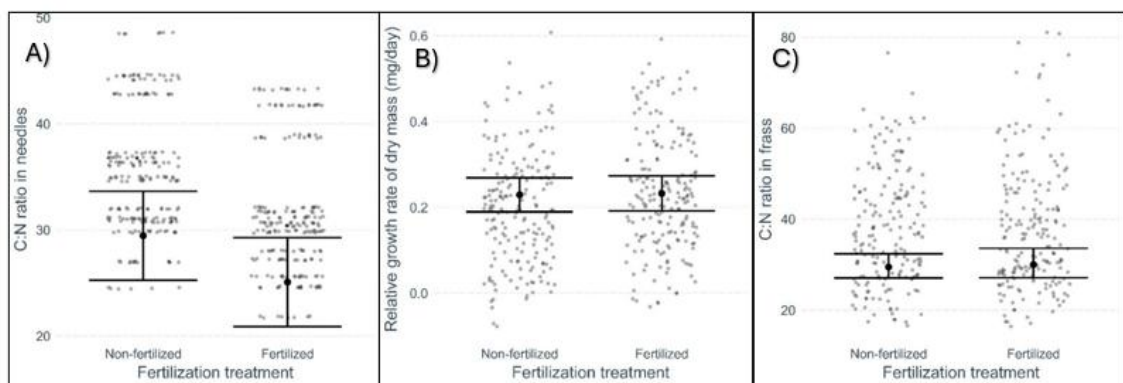
363 Elevated CO₂ affected needle quality, with the C:N ratio being higher under elevated
364 CO₂, and the effect was more pronounced in 2020 than in 2019 (Fig. 4A; $df = 417$, χ^2
365 $= 237.12$, $P < 0.001$). Relative larval growth rate did not differ between CO₂ regimes
366 (Fig. 4B); $df = 361$, $\chi^2 = 0.08$, $P = 0.773$). Likewise, no statistically significant
367 difference was detected in frass C:N ratio (Fig. 4C; $df = 355$, $\chi^2 = 0.73$, $P = 0.394$).



368 **Figure 4. Overview of the effects of elevated CO₂ on needle quality, larval**
 369 **growth, and sawfly frass stoichiometry. Left: C:N ratio in needles of *Picea abies***
 370 **under ambient (385 ppm) and elevated (700 ppm) CO₂ concentrations in 2019**
 371 **and 2020. Middle: Relative growth rate (RGR) of larvae fed on spruce trees**
 372 **exposed to the two CO₂ regimes. Right: C:N ratio of frass produced by larvae fed**
 373 **under ambient and elevated CO₂. Points represent individual measurements;**
 374 **lines with error bars indicate mean values ± 95% CI.**

375 3.2. Fertilization

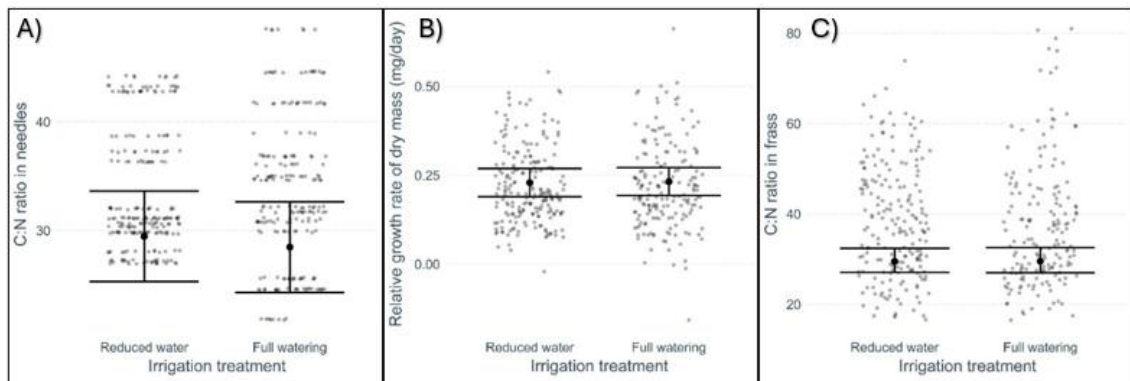
376 Fertilization had a statistically significant effect on needle C:N ratio, with fertilized
 377 trees exhibiting lower values than unfertilized trees (Fig. 5A; df = 417, $\chi^2 = 381.62$, P
 378 < 0.001). Relative larval growth rate did not differ between fertilization regimes (Fig.
 379 5B; df = 361, $\chi^2 = 0.26$, P = 0.611). Similarly, frass C:N ratio showed no statistically
 380 significant difference between treatments (Fig. 5C; df = 355, $\chi^2 = 0.06$, P = 0.802).



381 **Figure 5. Effects of nitrogen fertilization on needle quality, larval growth, and**
 382 **sawfly frass stoichiometry. A) C:N ratio of needles from fertilized and**
 383 **unfertilized trees. B) Relative growth rate (RGR) of larvae feeding on fertilized**
 384 **vs. unfertilized trees. C) C:N ratio of frass produced by larvae feeding on**
 385 **fertilized and unfertilized trees. Points represent individual measurements;**
 386 **error bars indicate mean ± 95% CI.**

387 3.3. Irrigation

388 Irrigation had a statistically significant effect on needle C:N ratio, with well-watered
389 trees exhibiting lower values than trees under reduced irrigation (Fig. 6A; $df = 417$, χ^2
390 $= 17.21$, $P < 0.001$). Relative larval growth rate did not differ between irrigation
391 regimes (Fig. 6B; $df = 361$, $\chi^2 = 0.17$, $P = 0.681$). Likewise, frass C:N ratio did not
392 respond to the irrigation treatment (Fig. 6C; $df = 355$, $\chi^2 = 0.02$, $P = 0.883$).



393 **Figure 6. Effects of reduced irrigation on needle quality, larval growth, and**
394 **sawfly frass stoichiometry. A) C:N ratio of needles under well-watered vs.**
395 **reduced irrigation conditions. B) Relative growth rate (RGR) of larvae feeding on**
396 **trees under full and reduced irrigation. C) C:N ratio of frass produced by larvae**
397 **under well-watered vs. reduced irrigation. Points represent individual**
398 **measurements; error bars indicate mean \pm 95% CI.**

399 3.4. Provenances

400 Needle C:N ratio differed between provenances, with trees originating from the
401 spruce altitudinal forest zone exhibiting higher values than those from the spruce-
402 beech zone (Fig. 7A; $df = 417$, $\chi^2 = 35.76$, $P < 0.001$). A significant interaction between
403 CO_2 regime and provenance was also detected ($df = 417$, $\chi^2 = 72.79$, $P < 0.001$).
404 Relative larval growth rate did not differ between provenances (Fig. 7B; $df = 361$, χ^2
405 $= 3.51$, $P = 0.061$). Similarly, frass C:N ratio did not show a statistically significant
406 difference between provenances (Fig. 7C; $df = 355$, $\chi^2 = 3.07$, $P = 0.080$).

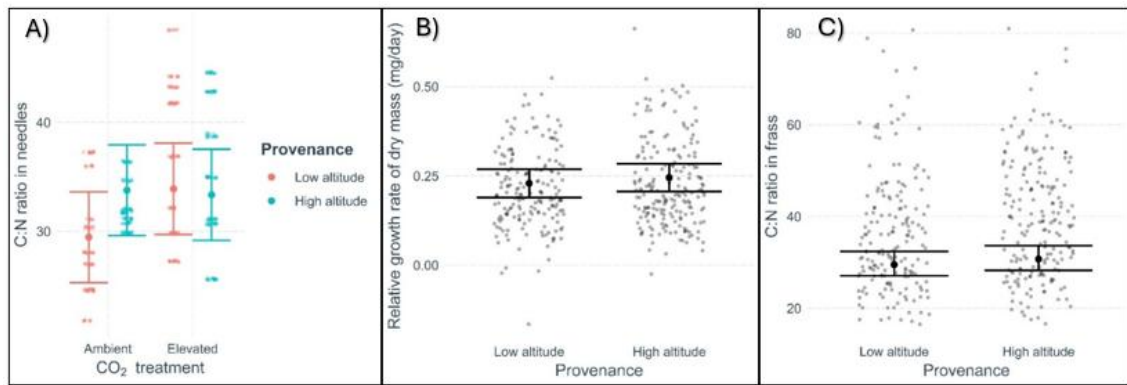
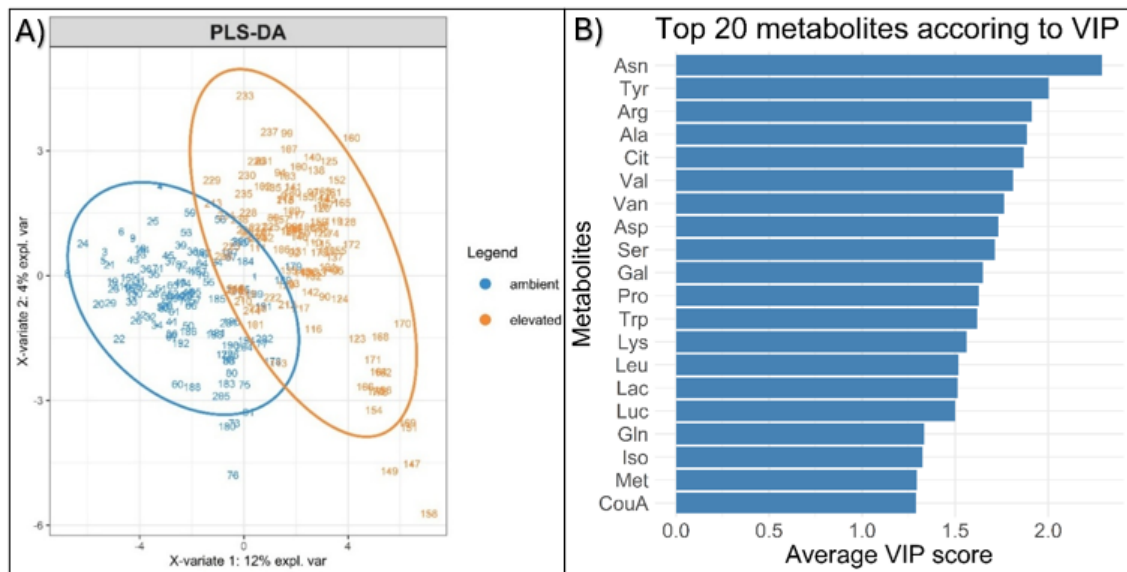


Figure 7. Differences between provenances (spruce–beech vs. spruce altitudinal forest zone) in needle quality, larval growth, and frass C:N ratio. A) C:N ratio of needles from the two provenances under ambient and elevated CO₂. B) Relative growth rate (RGR) of larvae feeding on spruce from the two altitudinal provenances. C) C:N ratio of frass produced by larvae feeding on spruce from the lower and higher elevation provenance. Points represent individual measurements; error bars indicate mean \pm 95% CI.

407 3.5. Metabolomic profiles

408 All experimental factors significantly influenced the C:N ratio, but their effects on
 409 the overall metabolomic structure varied considerably. Multivariate analysis
 410 (PERMANOVA) indicated that elevated CO₂ was the primary source of variability in
 411 the metabolomic data (Fig. 8A). In contrast, nitrogen fertilization (Supplementary
 412 Fig. 1) and summer irrigation (Supplementary Fig. 2) accounted for only a small
 413 portion of the variance, with their effects being significantly weaker. Additionally,
 414 tree provenance did not have a meaningful impact on metabolomic profiles
 415 (Supplementary Fig. 3).

416 Metabolomic analysis revealed a significant impact of elevated CO₂ levels on the
 417 chemical composition of spruce needles. Partial Least Squares Discriminant
 418 Analysis (PLS-DA) effectively separated between samples from ambient and
 419 elevated CO₂ environments (BER = 0.116, $p < 0.01$; Fig. 8A). The metabolites that
 420 contributed most strongly to this separation, as indicated by their high Variable
 421 Importance in Projection (VIP) scores, were mainly amino acids, particularly
 422 asparagine, tyrosine, arginine, and alanine (Fig. 8B).



423

424 **Figure 8. Metabolomic response of Norway spruce needles (*Picea abies*)**
 425 **exposed to elevated CO₂ in the presence of sawfly larvae. A) PLS-DA score plot**
 426 **of needle metabolomic profiles under ambient (385 ppm) and elevated (700**
 427 **ppm) CO₂ concentrations. Points represent individual samples, and ellipses**
 428 **indicate the multivariate space of the two groups. The first two latent variables**
 429 **explain 12% and 4% of the total variance, respectively, and show clear**
 430 **separation between CO₂ treatments. B) Twenty metabolites with the highest VIP**
 431 **(variable importance in projection) scores (>1) identified by the PLS-DA model,**
 432 **contributing most strongly to the discrimination between ambient and elevated**
 433 **CO₂. Bars represent mean VIP scores across the first two latent variables.**

434 **4. Discussion**

435 In our semi-field experiment, we found that elevated CO₂ levels, nitrogen
 436 availability, and water limitations significantly changed the chemistry of spruce
 437 needles. However, these notable chemical changes did not result in measurable
 438 differences in larval growth, development, or in the stoichiometry of frass (insect
 439 excrement). This consistent lack of response from herbivores across treatments
 440 suggests a disconnect between the chemistry of host plants and herbivore
 441 performance under more realistic ecological conditions. Therefore, for specialized
 442 herbivores in this system, simply changing leaf quality may not be enough to impact
 443 their performance.

444 Elevated CO₂ levels were identified as the strongest factor contributing to variability
 445 in needle chemistry. This increase led to a significant increase in the C:N ratio,
 446 consistent with the commonly observed effect of reduced nitrogen availability in
 447 plant tissues (Stiling & Cornelissen, 2007; Robinson et al., 2012). Additionally,
 448 higher CO₂ levels were linked to extensive changes in needle metabolomic profiles.
 449 Multivariate analysis confirmed that CO₂ was the primary factor influencing the

450 metabolome, while the effects of fertilization and irrigation were considerably less
451 significant. Despite these notable chemical changes, no measurable differences in
452 sawfly performance were observed. Specifically, there were no variations in relative
453 larval growth rate or frass C:N ratio between different CO₂ regimes, aligning with
454 studies that report weak or inconsistent herbivore responses to CO₂-induced
455 changes in host plant chemistry (Stiling & Cornelissen, 2007; Robinson et al., 2012;
456 Hall et al., 2020; Dvořáková et al., 2025).

457 A notable decoupling between the chemistry of host plants and herbivore
458 performance was observed relative to other environmental factors. Nitrogen
459 fertilization reduced the C:N ratio in the needles and partially mitigated the increase
460 in this ratio caused by elevated CO₂ levels. This is consistent with previous studies
461 showing that higher nitrogen availability can reduce the nitrogen dilution effect
462 (Stiling & Cornelissen, 2007; Robinson et al., 2012; Dong et al., 2024). In contrast,
463 reduced water availability increased the C:N ratio in needles, likely due to reduced
464 nitrogen uptake and transport under water-stress. Despite these clear changes in
465 host plant chemistry, neither fertilization nor irrigation had any effect on larval
466 growth, developmental rate, or frass stoichiometry. This decoupling between
467 alterations in the nutritional quality of host plants and herbivore performance has
468 been documented multiple times, especially among specialized species that can
469 maintain stable growth across a wide range of environmental conditions (Van
470 Hezewijk et al., 2008; Robinson et al., 2012; Yang et al., 2017).

471 The chemistry of needles varied among spruce provenances from different
472 elevations. Trees from higher elevations had a higher C:N ratio compared to those
473 from lower elevations. A significant interaction between provenance and CO₂
474 concentration was observed, indicating genetically determined differences in how
475 these trees respond to elevated CO₂ levels (Dillon et al., 2018; Provazník et al.,
476 2024). Provenance-specific responses to environmental factors have been
477 documented in forest trees and are often linked to adaptation to local climatic
478 conditions (Aranda et al., 2015; Kučerová et al., 2018; Provazník et al., 2024).
479 However, these differences in host plant chemistry had only a marginal impact on
480 sawfly performance and did not affect the C:N ratio of their frass (excrement). This
481 suggests that even genetically driven variations in food quality may not limit
482 specialized herbivores.

483 One key mechanism that likely explains this robustness is the combination of strong
484 stoichiometric homeostasis and post-ingestive nutrient regulation. The stable C:N
485 ratio of frass across all experimental treatments indicates that the larvae
486 maintained a relatively constant internal balance of carbon and nitrogen despite
487 variability in food chemistry. These post-ingestive mechanisms, which include
488 adjustments in assimilation efficiency and the internal regulation of nutrient fluxes,

489 are considered crucial traits that enable herbivores to stabilize their growth under
490 fluctuating food quality (Behmer, 2009; Joern et al., 2012; Cavigliasso et al., 2020).
491 Stoichiometric homeostasis has repeatedly been identified as a factor contributing
492 to the weak relationship between host plant nitrogen content and insect herbivore
493 performance (Stiling & Cornelissen, 2007; Robinson et al., 2012).

494 An important factor in our experiment may have been that the larvae fed on freshly
495 flushed needles. These tissues naturally have higher nitrogen concentrations than
496 older needles, making them a nutritionally rich resource for herbivores (Zhou et al.,
497 2015; Yuan et al., 2018). As a result, this may have reduced the biological
498 significance of shifts in the C:N ratio due to CO₂, drought, or fertilization, since the
499 overall nutritional quality of the food likely remained above the limit required for
500 larval growth. A similar trend of weak or absent herbivore responses to changes in
501 host plant chemistry was also observed in a related study in which moth larvae were
502 fed spruce needles (Dvořáková et al., 2025).

503 Although there were significant changes in needle metabolomic profiles due to
504 increased CO₂ levels, these changes did not result in measurable differences in
505 sawfly performance. This indicates that modifications in the primary and secondary
506 metabolism of host plants do not necessarily affect the functional quality of food for
507 herbivores (Riach et al., 2015). It may be that the extent of these changes did not
508 exceed a biologically relevant limit, or that the larvae have adaptations that allow
509 them to tolerate or compensate for fluctuations in host plant chemistry (Behmer et
510 al., 2009; Cavigliasso et al., 2020). This finding aligns with previous studies showing
511 that the impact of secondary metabolites on herbivores is highly context-dependent
512 and can vary with factors such as soil chemistry, nutrient availability, and herbivore
513 physiology (Hu et al., 2021).

514 Our results indicate that significant changes in the chemistry of host plants, driven
515 by global change factors, do not necessarily lead to altered performance in
516 specialized herbivores. This finding has important implications for predicting the
517 impacts of climate change on plant–insect interactions. Approaches based only on
518 shifts in leaf chemistry, such as changes in the C:N ratio, may overestimate the
519 biological consequences of global change in certain systems (Stiling & Cornelissen,
520 2007; Robinson et al., 2012). This study emphasizes the need to consider herbivore
521 traits in such predictions. Factors like nutritional regulation, stoichiometric
522 homeostasis, and the degree of specialization must be integrated to realistically
523 evaluate when and under what conditions changes in host plant quality will affect
524 higher trophic levels.

525 In our semi-field experiment, we observed that global change drivers significantly
526 altered the chemical composition of spruce needles. However, these changes did
527 not translate into noticeable effects on herbivore growth or development. This

528 suggests that variations in host plant chemistry alone do not automatically lead to
529 changes in herbivore performance, especially under more complex environmental
530 conditions. Our findings indicate that, in this particular system, food quality was not
531 the primary factor influencing sawfly responses under the specific conditions.
532 Nevertheless, the exact processes that contribute to this resilience cannot be
533 directly inferred from the data we have collected.

534 **5. Conclusion**

535 This study demonstrates that key global change factors, such as elevated
536 atmospheric CO₂ levels, changes in water availability, and variations in nitrogen
537 supply, significantly affect the chemical composition of Norway spruce needles.
538 However, these alterations did not lead to differences in larval growth,
539 developmental rates, or frass stoichiometry in the semi-field experiment. The
540 consistent lack of herbivore responses across all experimental treatments and
541 among different host tree provenances suggests that changes in host plant quality
542 alone may not be the primary factor influencing responses of specialized herbivores
543 in this system. Our findings indicate that predictions of the impacts of global change
544 on plant-insect interactions, based solely on changes in plant chemical traits, may,
545 in some cases, overestimate their biological effects.

546 **Authors' contributions**

547 Barbora Dvořáková: Investigation, Resources, Writing – original draft, Writing –
548 review & editing; David Musiolek: Conceptualization, data curation, Formal
549 analysis, Investigation, Methodology, Project administration, Resources, Validation,
550 Writing – review & editing; Alina Kalyniukova: Investigation, Formal analysis; Petr
551 Pyzsko: Data Curation, Formal analysis, Software, Writing – review & editing; Otmar
552 Urban: Formal analysis, Resources, Writing – review & editing; Karel Klem: Formal
553 analysis, Resources, Writing – review & editing; Jaroslav Holuša: Conceptualization,
554 Funding acquisition, Investigation, Project administration, Supervision, Resources,
555 Writing – review & editing.

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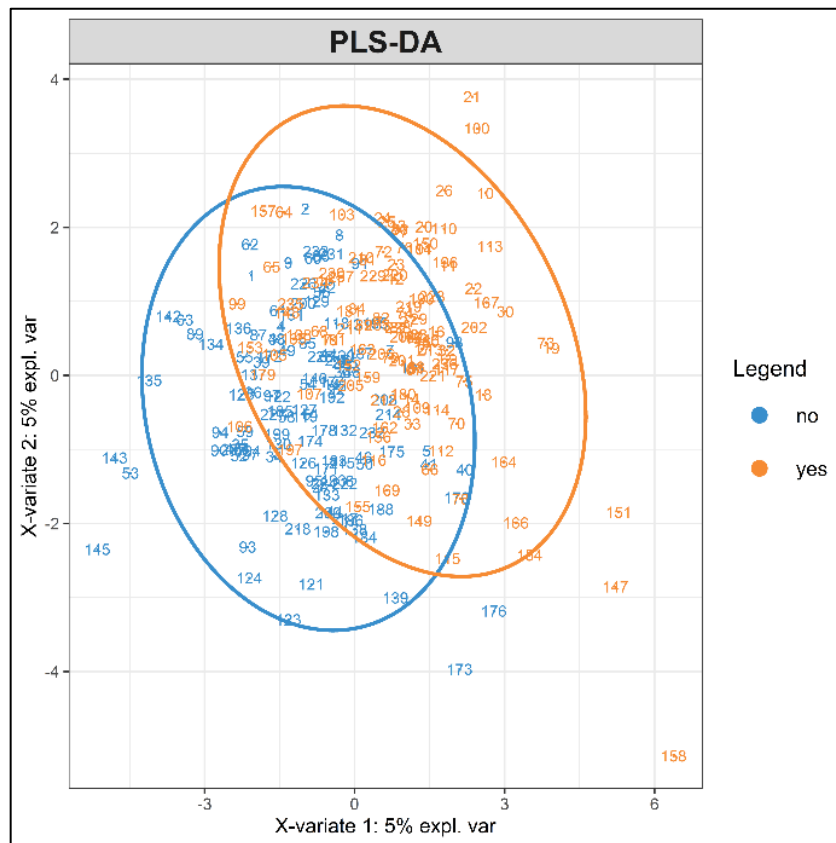
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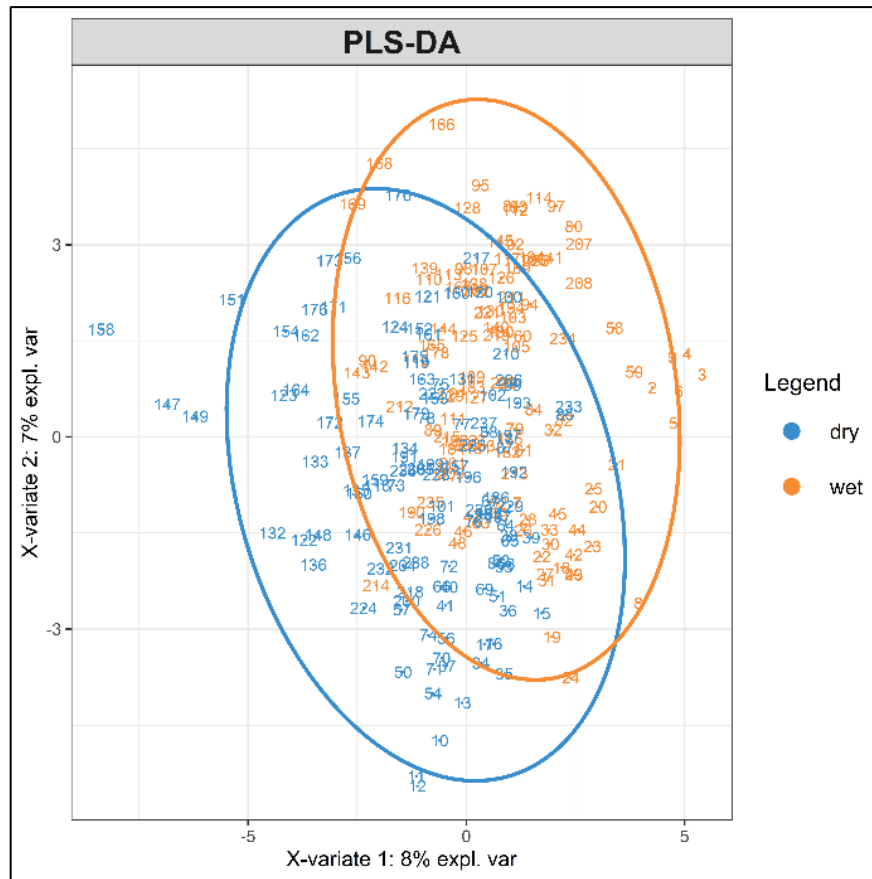
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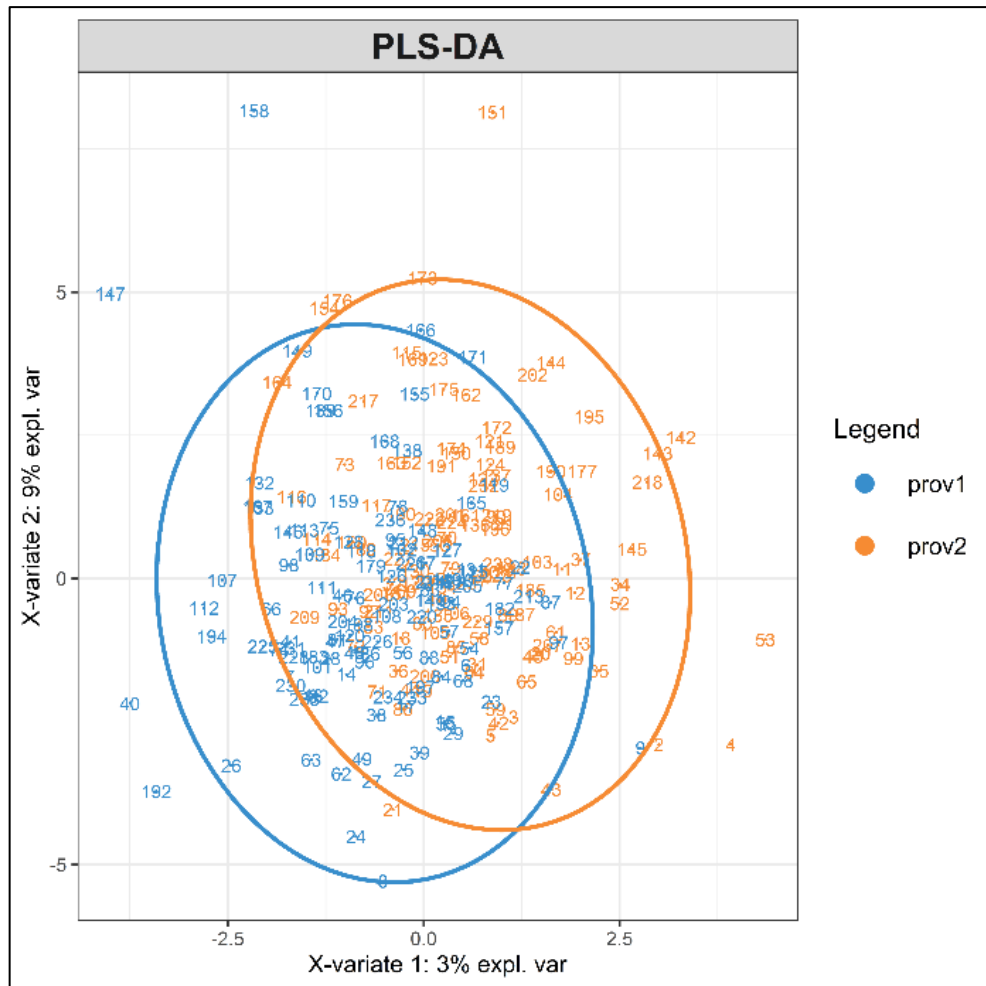
Supplementary material



786 **Figure 1S. Metabolomic response of Norway spruce needles (*Picea abies*) to**
787 **nitrogen fertilization. PLS-DA score plot showing metabolomic profiles of**
788 **needles from fertilized (yes) and unfertilized (no) trees. Points represent**
789 **individual samples, and ellipses indicate the multivariate space of the two**
790 **groups. The first two latent variables explain 5% and 5% of the total variance,**
791 **respectively.**



792 **Figure 2S. Metabolomic response of Norway spruce needles (*Picea abies*) to**
 793 **irrigation treatment. PLS-DA score plot showing metabolomic profiles of**
 794 **needles from trees under reduced water availability (dry) and well-watered**
 795 **conditions (wet). Points represent individual samples, and ellipses indicate the**
 796 **multivariate space of the two groups. The first two latent variables explain 8%**
 797 **and 7% of the total variance, respectively.**



798 **Metabolomic profiles of Norway spruce needles (*Picea abies*) from two**
 799 **altitudinal forest zones. PLS-DA score plot showing samples from the spruce–**
 800 **beech provenance (prov1) and the spruce provenance (prov2). Points represent**
 801 **individual samples, and ellipses indicate the multivariate space of the two**
 802 **groups. The first two latent variables explain 3% and 9% of the total variance,**
 803 **respectively.**

Příloha 3.

Pine weevil bark feeding depletes root carbohydrates in Scots pine seedlings

1 **Pine weevil bark feeding depletes root carbohydrates in Scots pine**
2 **seedlings.**

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9 **Key message**

10 Pine weevil bark feeding reduces root carbohydrates in Scots pine seedlings without
11 triggering systemic chemical defenses. The response was consistent across local
12 provenances and occurred primarily in belowground tissues.

13 **Abstract**

14 **Context:**

15 The large pine weevil (*Hylobius abietis* L.) is a major bark-feeding pest of conifer
16 seedlings in Europe. While defense responses in bark and phloem have been studied,
17 whole-plant chemical responses to feeding remain poorly understood.

18 **Aims:**

19 We investigated whether pine weevil feeding induces organ-specific changes in primary
20 and secondary metabolites in Scots pine (*Pinus sylvestris* L.) seedlings and whether these
21 responses differ between local provenances.

22 **Methods:**

23 Soluble sugars, phenolic compounds, and metabolomic profiles were analyzed in roots
24 and needles after seven days of pine weevil feeding. Stem damage was quantified, and
25 responses were evaluated using linear mixed models.

26 **Results:**

27 Pine weevil feeding significantly reduced soluble sugar concentrations in roots, whereas
28 needle sugars, phenolic concentrations, and metabolomic profiles remained unaffected.
29 Responses were consistent across two local provenances.

30 **Conclusion**

31 Short-term bark feeding reduces root carbohydrate concentrations without inducing
32 systemic chemical defenses, indicating an organ-specific physiological response that is
33 not provenance-dependent.

34 **Keywords:** carbon allocation, herbivory, *Hylobius abietis*, organ-specific response,
35 phenolic compounds, provenance

36 **1. Introduction**

37 The large pine weevil (*Hylobius abietis* L.) is one of the most damaging pests to conifer
38 plantations in Europe. It causes high mortality among newly planted seedlings and
39 results in significant economic losses (Skrzecz 2017; Lalík et al. 2021). With increasing
40 restrictions on the use of chemical insecticides in forestry, it has become crucial to better
41 understand how plants respond to pine weevil feeding. This understanding will help
42 develop alternative methods to reduce seedling damage while minimizing
43 environmental impact (Harvey et al. 2016; Moore et al. 2021).

44 Pine weevil feeding causes damage to the bark and phloem of young seedlings
45 (Berasategui et al. 2016). These tissues are essential for transporting assimilates and
46 signaling compounds throughout the stem (Liesche and Patrick 2017). As a result, stem
47 damage can affect not only the feeding place but also other parts of the plant, including
48 tissues not directly affected by the insect (Lei et al. 2021; Ryan and Asao 2014).

49 Plant responses to herbivory involve changes in both primary and secondary metabolism
50 (Kessler and Mueller, 2024). Secondary metabolites, such as phenolic compounds and
51 terpenoids, are often associated with defense mechanisms and have been the primary
52 focus of research on bark-feeding insects (Kumar et al., 2025). In contrast, changes in
53 primary metabolites may indicate more general physiological responses and can occur
54 in tissues that are far from the site of damage (Zhou et al., 2015). However, it remains
55 unclear how these two types of chemical responses are expressed in tissues outside the
56 damaged bark following pine weevil feeding.

57 Roots rely on a constant supply of resources from aboveground tissues and are crucial
58 for seedling survival and recovery after stress (Liesche and Patrick 2017; Kalra et al.
59 2024). Despite their importance, the chemical responses of roots to pine weevil feeding
60 have been overlooked, and information on organ-specific chemical changes at the
61 whole-plant level remains limited.

62 Variations in susceptibility to pine weevil feeding have been observed among different
63 pine species and across various geographic regions (Zas et al. 2006, 2011). However, at
64 local scales, genetic differences among provenances are often small, and within-
65 population variability can be substantial (Danusevičius et al. 2024; Whittet et al. 2019).
66 When genetic differentiation among provenances is low, and within-population
67 variability is high, the differences in plant responses to herbivory are likely to be small
68 (Alberto et al. 2013).

69 Previous research has shown that feeding by pine weevils triggers strong local defense
70 responses in bark and phloem tissues. This includes changes in gene expression and the

71 emission of volatile compounds (Kovalchuk et al., 2015; Heijari et al., 2011). While these
72 studies improve our understanding of local defense mechanisms, there is still limited
73 knowledge of how pine weevil feeding affects the plant's overall chemical status,
74 especially in organs far from the damaged area.

75 In this study, we investigated the chemical responses of Scots pine (*Pinus sylvestris* L.)
76 seedlings to pine weevil feeding. Our focus was on the differences between various plant
77 organs. We analyzed soluble sugars in both roots and needles, phenolic compounds, and
78 metabolomic profiles in needles after seedlings were exposed to pine weevil feeding.
79 We also assessed the extent of stem damage caused by the weevils. By comparing two
80 locally adapted Scots pine provenances, we aimed to determine whether the observed
81 chemical responses were consistent across ecotypes.

82 **2. Material and methods**

83 **2.1. Plant material and experimental design**

84 The experiment was conducted in the summer of 2023 at the Czech University of Life
85 Sciences campus in Prague (287 m above sea level; 50.1302328 N, 14.3689489 E). Two-
86 year-old Scots pine (*Pinus sylvestris* L.) seedlings from two local provenances with
87 significant ecological differences were used: a lowland ecotype from the Třeboň region
88 and an upland ecotype from the Děčín region. These provenances were selected
89 because both regions are areas where Scots pine naturally occurs within the Czech
90 Republic (Fig. 1).

91 These ecotypes represent contrasting growth strategies for Scots pine. The Třeboň
92 provenance corresponds to a lowland, pioneer ecotype that typically thrives on sandy,
93 nutrient-poor soils, often in monocultures. It regenerates in open habitats and exhibits
94 rapid early growth, but it has low tolerance to competition (Hejtmánek et al. 2023). In
95 contrast, the Děčín provenance represents a montane climax ecotype that generally
96 grows in mixed stands with other tree species. This ecotype can naturally regenerate
97 under canopy cover and is better adapted to competitive environments (Brichta et al.
98 2023). The seedlings were grown in a forest nursery before being transferred to outdoor
99 conditions at the experimental site, which is located on the green roof of the Faculty of
100 Forestry and Wood Sciences (Fig. 2).

101 The experiment included 16 plastic boxes arranged in four replicates (four boxes per
102 replicate). Half of the boxes were maintained as controls, which did not contain any
103 insects. Each box contained 12 seedlings, with 6 from each provenance. In the treated
104 groups, 12 adult pine weevils (one per seedling) were placed directly into each box and

105 allowed to feed freely for 7 days. The adult beetles were collected from the field during
106 the spring and summer of 2023. In total, 192 seedlings were evaluated.

107 **2.2. Seedling damage assessment**

108 After the experiment concluded, we assessed the extent of seedling damage. We
109 recorded the number of feeding scars on the stem and measured the total area of
110 damaged bark using transparent millimeter paper. Additionally, we measured the stem
111 diameter for each seedling.

112 **2.3. Chemical analysis**

113 **2.3.1. Determination of phenolic compounds and metabolomic analysis**

114 Thirty milligrams of freeze-dried and homogenized samples were accurately weighed
115 into 2 mL test tubes, and 1 mL of 80% methanol was added. The tubes were vortexed
116 for a few seconds, then placed in an ultrasonic bath for 10 minutes. Afterward, the
117 samples were centrifuged for 5 minutes at 13,000 rpm. The supernatant was filtered
118 using a 0.22 μm PTFE syringe filter prior to analysis.

119 A metabolomic analysis was conducted using an Agilent 1290 Infinity II liquid
120 chromatography system coupled with an Agilent 6546 LC/MS quadrupole time-of-flight
121 (QTOF) mass spectrometer (Agilent Technologies, USA). Chromatographic separation
122 was achieved on an Agilent InfinityLab Poroshell 120 EC-C18 column (2 \times 150 mm, 2.7
123 μm particle size). The mobile phase consisted of solvent A (0.1% formic acid in water)
124 and solvent B (methanol), with the following gradient elution profile: 0–4 minutes at
125 85% A, 4–7 minutes at 75% A, 7–9 minutes at 68% A, 9–16 minutes at 60% A, 16–22
126 minutes at 45% A, and 22–28 minutes at 5% A, followed by a 2-minute hold at 5% A. The
127 flow rate was set to 0.5 mL/min, the column temperature to 35 $^{\circ}\text{C}$, and the injection
128 volume to 1 μL . Measurements were performed in negative ionization mode. QTOF
129 parameters included a mass scan range of 100–1000 m/z, drying gas temperature of 160
130 $^{\circ}\text{C}$, sheath gas flow rate of 12.0 L/min, sheath gas temperature of 400 $^{\circ}\text{C}$, capillary
131 voltage of 5.0 kV, nozzle voltage of 2.0 kV, fragmentor voltage of 140 V, and collision
132 energies of 10, 20, and 40 eV. MS/MS analysis was performed with a scan range of 50–
133 800 m/z, a retention time window of 0.5 minutes, an isolation window of 1.3 amu, and
134 an acquisition rate of 3 spectra per second. Continuous mass calibration was maintained
135 with two reference masses: 112.9855 m/z and 966.0007 m/z.

136 Data preprocessing, including feature extraction and chromatographic alignment, was
137 performed using Mass Hunter Profinder 10.0 software. The alignment parameters were
138 set with a minimum intensity threshold of 5000 counts and a maximum retention time
139 difference of 0.5 minutes. Feature extraction was conducted with an m/z range of 100–
140 1000, a retention time error tolerance of 0.25 minutes, and a mass accuracy of 2 mDa.

141 The HPLC analysis was performed using a Thermo Fisher UltiMate 3000 UHPLC system,
142 equipped with a photodiode array detector for quantification of phenolic compounds.
143 Compound separation was achieved using Thermo Fisher Hypersil GOLD C18 Column
144 (150 mm × 2.1 mm i.d., 5 µm particle size). The mobile phase consisted of water (solvent
145 A) and acetonitrile (solvent B), each containing 3% acetic acid. The column temperature
146 was maintained at 35°C. Gradient elution was applied for the separation as follows: 0–
147 15 minutes, 93% A and 7% B; 15–17 minutes, 72% A and 28% B; 17–20 minutes, 73% A
148 and 27% B; 20–23 minutes, 2% A and 98% B; 23–25 minutes, 50% A and 50% B; 25–30
149 minutes, 93% A and 7% B, at a flow rate of 0.5 mL/min. A 5 µL injection volume was
150 used, and the total analysis time was 30 minutes. Data acquisition and processing were
151 conducted using the Thermo Scientific Chromeleon Chromatography Data System
152 (Várfalvyová et al. 2023).

153 **2.3.2. Determination of soluble sugars**

154 Thirty milligrams of freeze-dried and homogenized samples were weighed into 2 mL test
155 tubes, and 1,5 mL of 80% methanol was added. The tubes were vortexed for a few
156 seconds, then incubated in a thermoshaker at 1000 rpm for 60 minutes. Subsequently,
157 the samples were centrifuged for 5 minutes at 13,000 rpm. The supernatant was then
158 filtered through a 0.22 µm PTFE syringe filter before analysis (Šulc et al. 2021). LC-MS-
159 qTOF analysis for the determination of soluble sugars was performed using the same
160 system as metabolomic analysis. However, separation was achieved on Supelco apHera
161 NH₂ Polymer column (150 × 2 mm, 5 µm) maintained at a temperature of 30 °C. The
162 mobile phase consisted of acetonitrile (A) and water (B). A gradient elution method was
163 employed, starting with an 80:20 (A:B) ratio, followed by 0.5–13 minutes with a 55:45
164 (A:B) ratio, and 14–15 minutes at 80:20 (A:B), with a flow rate of 0.2 mL/min (Madsen
165 et al. 2015). The injection volume was set to 1 µL, and the analysis was carried out in
166 negative ionization mode.

167 QTOF parameters were optimized using a set of standards, including glucose, sucrose,
168 fructose, raffinose, and trehalose, with the following settings: mass range of 100–1000
169 m/z; drying gas temperature of 280 °C; sheath gas flow rate of 12.0 L/min; sheath gas
170 temperature of 400 °C; capillary voltage of 2.0 kV; fragmentor voltage of 120 V; and
171 collision energies of 10, 20, and 40 eV. MS/MS data were recorded in a 50–800 m/z
172 range. To ensure accurate mass calibration, the system continuously measured two
173 reference masses: 112.9855 m/z and 922.0098 m/z. Data collection was conducted
174 using Agilent Mass Hunter Acquisition software, while analysis was performed using
175 MassHunter Qualitative Analysis 10.0 and Q-TOF Quantitative Analysis. Metabolites
176 were identified and quantified through calibration curves for individual carbohydrates.

177 **2.3.3. Metabolomic profiling of volatile organic compounds (VOCs)**

178 Scots pine roots and needles were extracted with n-hexane at a ratio of 150 mg of
179 frozen, homogenized material to 1.5 mL of solvent. For metabolomic profiling of
180 extracted volatiles, two-dimensional comprehensive gas chromatography coupled with
181 time-of-flight mass spectrometry (GC×GC-TOF-MS) was used. The gas chromatograph
182 (Agilent Technologies 7890B, USA) was equipped with HP-5MS UI capillary column (30
183 m, 0.25 mm i.d., 0.25 µm film thickness) coupled via consumable-free modulator with
184 second dimension column VF-17 (1,5 m, 0.1 mm i.d., 0.1 µm film thickness, both columns
185 from Agilent USA). Helium at a flow of 1 ml/min was used as a carrier gas. The split
186 injection (1:5) was applied into a hot (275 °C) split/splitless injector. Oven temperature
187 increased from an initial 40 °C at 10 °C/min to 100 °C, then at 5 °C/min to 320 °C, and
188 held at this temperature for 3 min. The second-dimension GC oven and modulator
189 followed this temperature program, with 5 °C and 15 °C offsets, respectively. The
190 modulation period was 5 s. Separated compounds were ionized in the ion source of a
191 time-of-flight mass spectrometric detector (Pegasus 4D, LECO, USA) at 70 eV in electron
192 impact mode, and full spectral (35-500 Da) information was recorded with a frequency
193 of 100 Hz.

194 Signals with unique quantification mass signal-to-noise ratios greater than 50 were
195 aligned in deconvoluted records, creating a source data matrix (samples x variables).
196 Data were then normalized (constant row sums) and log-ratio centered. After Pareto
197 scaling, data were processed via principal component analysis (PCA) in Simca 18
198 (Sartorius Stedim, SWE) software.

199 **2.4. Statistical analysis**

200 All statistical analyses were performed in R (R Core Team 2024). Linear mixed models
201 were fitted using the ASReml-R package (Butler et al. 2017). Prior to analysis,
202 concentrations of each sugar were log-transformed to improve normality and
203 homoscedasticity. Fixed effects included beetle treatment (infested or control), origin
204 (Třeboň or Děčín), spatial replication (1–4), and their interactions. Random effects
205 consisted of box and box × origin.

206 The significance of fixed effects was assessed using Wald tests. Treatment effects were
207 derived from model-based marginal means, and contrasts were presented as estimated
208 differences on the log₁₀-transformed scale with associated p-values. Model
209 assumptions were evaluated by visual inspection of residual diagnostics.

210 The relationship between root sugar concentrations and damage intensity was
211 evaluated using linear mixed models fitted separately for each sugar, including total bite
212 area as a fixed effect. These analyses were conducted using data from infested seedlings
213 only.

214 The effect of seedling stem diameter on damage intensity (number of feeding scars and
215 total bark area consumed) was assessed using linear mixed models fitted to infested

216 seedlings. Stem diameter, origin, spatial replication, and their interaction were included
217 as fixed effects, while box and box × origin were fitted as random effects.

218 **3. Results**

219 **3.1. Seedling damage**

220 The number of feeding scars was strongly positively correlated with the total bark area
221 consumed ($r = 0.780$). There were no significant differences between the two
222 provenances of Scots pine regarding either the number of bites or the total bite area
223 (Fig. 3). The intensity of the damage was significantly influenced by the diameter of the
224 seedling stems ($p = 0.020$), while the influence of spatial replication was not statistically
225 significant ($p = 0.073$).

226 **3.2. Soluble sugars**

227 Pine weevil damage significantly decreased the total soluble sugar concentrations in the
228 roots ($p = 0.025$; Fig. 4). In contrast, there was no significant effect of damage on the
229 needle concentrations ($p = 0.370$; Fig. 4).

230 When examining individual carbohydrates, infested seedlings showed significantly
231 lower root concentrations of fructose, glucose, trehalose, raffinose, and sucrose than
232 the control group ($p < 0.05$ for all; Fig. 5). However, no significant differences were
233 observed in needle concentrations.

234 Root sugar concentrations decreased significantly with increasing total bite area in
235 damaged seedlings, with significant effects detected for fructose ($p = 0.008$), glucose (p
236 $= 0.026$), and sucrose ($p = 0.042$).

237 **3.3. Phenolic compounds**

238 Pine weevil damage did not significantly affect the overall phenolic concentrations in
239 the needles ($p = 0.11$; Fig. 6). Additionally, no significant differences were detected
240 between the provenances ($p=0.583$).

241 **3.4. Metabolomic profiles**

242 Multivariate analyses were conducted on both non-volatile compounds in the needles
243 and volatile metabolomic profiles in the needles and roots. The results showed no
244 distinct separation of samples based on infestation treatment or seedling origin.
245 Principal component analysis showed extensive overlap among the different treatments
246 and provenances. Specifically, the first two principal components accounted for 28% of
247 the variance in non-volatile needle metabolites, and 21% and 26% of the variance in the
248 volatile profiles of needles and roots, respectively.

249 **4. Discussion**

250 Pine weevil feeding was associated with distinct chemical changes in Scots pine
251 seedlings, specific to different plant organs, with the most notable effects observed in
252 the roots. These changes occurred outside the areas of damaged bark and were not
253 followed by similar changes in needle chemistry or overall metabolomic profiles. This
254 indicates that herbivory affecting the bark can trigger chemical responses beyond the
255 immediate area of damage, while still being highly specific to organs.

256 Pine weevil feeding disrupts bark and phloem tissues, thereby impairing the transport
257 of assimilates and other compounds along the stem (Berasategui et al. 2016; Kovalchuk
258 et al. 2015). One possible explanation for the observed reduction in root sugar
259 concentrations is that this disruption limits the supply of photo assimilates to
260 belowground sink tissues, which rely heavily on continuous input from aboveground
261 organs (Geiger, 2020). Additionally, the negative relationship between root sugar
262 concentrations and damage intensity suggests that the magnitude of root responses
263 increases with the level of stem damage, rather than reflecting an induced chemical
264 defense (Zhou et al. 2015; Aubrey et al. 2018).

265 In contrast to roots, the concentration of soluble sugars in needles was not affected by
266 pine weevil feeding. This disconnect between the responses of the aboveground and
267 belowground parts of the plant suggests that chemical changes following bark damage
268 do not occur uniformly across different plant organs. The stable sugar levels in needles
269 may support local metabolic activity despite impaired transport to sink tissues. This may
270 contribute to short-term stability and recovery after herbivory (Hartmann and Trumbore
271 2016). Similar organ-specific responses, primarily affecting sink tissues, have been
272 observed under other stress conditions that disrupt transportation or lead to resource
273 limitations. Examples include drought-induced phloem dysfunction (Hartmann et al.
274 2013), carbohydrate starvation (Li et al. 2003), and experimental disturbances in
275 belowground carbon supply (Aubrey et al. 2018).

276 Pine weevil feeding did not result in consistent changes in phenolic compound
277 concentrations in needles or in overall metabolomic profiles. The lack of significant shifts
278 in secondary chemistry indicates that the short-term responses to bark feeding are more
279 likely driven by physiological adjustments rather than a strong activation of chemical
280 defenses in undamaged tissues (Mason et al. 2017; Eyles et al. 2010). These physiological
281 adjustments may involve rapid changes in the dynamics of nonstructural carbohydrates,
282 which support metabolism and osmoregulation during stress (Hartmann and Trumbore
283 2016). Given the relatively short exposure period, this pattern likely reflects a
284 prioritization of maintaining essential metabolic functions over investing in energetically
285 expensive secondary metabolites, rather than an indication of a lack of defensive

286 capacity (Herms and Mattson 1992; Huot et al. 2014). Importantly, the absence of
287 detectable changes in phenolic compounds and metabolomic profiles is significant,
288 suggesting that the chemical responses to pine weevil feeding are selective rather than
289 generalized.

290 Despite the clear differences in organ-specific responses, no variations were found
291 between the two local ecotypes of Scots pine in terms of damage intensity, soluble sugar
292 concentrations, phenolic profiles, or metabolomic patterns. This consistency suggests
293 that the responses to pine weevil feeding were not influenced by ecotype-specific traits
294 but rather reflect a common early reaction shared among local provenances. Research
295 has documented significant genetic and phenotypic variation in susceptibility to pine
296 weevil feeding across different pine species and over broad geographic provenance
297 gradients (Zas et al. 2005, 2008, 2011). However, at local spatial scales, genetic
298 differentiation among provenances tends to be weak, and variability within populations
299 can exceed differences among populations (Boshier et al. 2015; Whittet et al. 2019;
300 Danusevičius et al. 2024). In such cases, short-term responses to herbivory are likely to
301 be driven primarily by common physiological processes rather than by strong,
302 provenance-specific resistance traits (Huot et al. 2014). It may take longer time frames
303 or repeated stress exposure for differences among provenances to become apparent.

304 In addition to chemical responses, the extent of stem damage was influenced by
305 seedling size. Larger stem diameters were associated with a greater total bark area
306 consumed. This relationship likely reflects an increased availability of feeding surface
307 rather than an increased susceptibility to damage. Generally, larger seedlings are more
308 tolerant of damage and tend to have a higher survival rate following a pine weevil attack
309 (Örlander and Nilsson 1999; Thorsen et al. 2001; Nordlander et al. 2011). Therefore,
310 seedling size should be considered a modifying factor that affects the extent of damage
311 rather than a determinant of resistance.

312 Our findings indicate that feeding by pine weevils leads to specific chemical changes in
313 Scots pine seedlings, particularly in their roots, which were notably responsive. These
314 changes were consistent across the two local provenances studied, suggesting that the
315 observed patterns were not influenced by provenance differences at this scale. By
316 examining chemical changes outside the damaged bark tissue, this study extends
317 previous research on local defense responses and provides new insights into the overall
318 chemical effects of bark-feeding herbivory on the plant.

319 **5. Conclusion**

320 Pine weevil bark feeding triggered distinct chemical responses in different parts of Scots
321 pine seedlings. Short-term feeding significantly decreased carbohydrate concentrations
322 in the roots, whereas sugar levels, phenolic compounds, and needle metabolomic
323 profiles remained mostly unchanged. These responses were consistent across two local
324 populations, indicating that the early chemical adjustments to bark feeding were not
325 dependent on provenance within the geographic range studied. Our findings suggest
326 that short-term bark herbivory primarily affects carbon allocation to belowground
327 tissues rather than triggering systemic chemical defenses. Understanding these organ-
328 specific physiological responses could enhance our knowledge of seedling performance
329 under bark-feeding stress in forest regeneration systems.

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332 **Authors' contributions**

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471 **Captions of figures**

472 **Fig.1** Natural distribution of Scots pine (*Pinus sylvestris* L.) in the Czech Republic, showing
473 the regions from which the two local provenances used in this study were selected.
474 Reproduced from Brichta et al. (2023) with permission.

475 **Fig.2** Experimental arrangement of Scots pine (*Pinus sylvestris* L.) seedlings. Left: a view
476 of the seedling layout within a plastic box. Right: a replicated arrangement of plastic
477 boxes at the outdoor experimental site located on the green roof of the Faculty of
478 Forestry and Wood Sciences at the Czech University of Life Sciences in Prague.

479 **Fig.3** Damage intensity caused by pine weevil feeding on Scots pine seedlings of two
480 provenances. Left panel: number of feeding scars per seedling (N_bites). Right panel:
481 total bark area consumed per seedling (area_bites, mm²). Boxplots represent the
482 median and interquartile range; whiskers show the range of observed values. Points
483 represent individual seedlings (circles = Děčín, triangles = Třeboň). Red symbols indicate
484 model-based means \pm 95% confidence intervals derived from linear mixed models. Panel
485 headers show the estimated difference between provenances (Třeboň – Děčín) and
486 associated p-values.

487 **Fig.4** Total soluble sugar concentrations in roots and needles of Scots pine seedlings
488 after pine weevil feeding. Left panel: roots. Right panel: needles. Values are log₁₀-
489 transformed total soluble sugars (mg g⁻¹ DW). Boxplots show the median and
490 interquartile range, which indicate the range of observed values. Points represent
491 individual seedlings (circles = Děčín, triangles = Třeboň). Red symbols indicate model-
492 based means \pm 95% confidence intervals derived from linear mixed models. Panel
493 headers show the estimated difference on the log₁₀ scale (Beetle – Control) and
494 associated p-values.

495 **Fig.5** Root concentrations of individual soluble sugars in Scots pine seedlings after pine
496 weevil feeding. Concentrations of fructose, glucose, trehalose, raffinose, and sucrose
497 are shown as log₁₀-transformed values (mg g⁻¹ DW). Boxplots represent the median and
498 interquartile range; whiskers show the range of observed values. Points represent
499 individual seedlings (circles = Děčín, triangles = Třeboň). Red symbols indicate model-
500 based means \pm 95% confidence intervals derived from linear mixed models. Panel
501 headers show the estimated difference on the log₁₀ scale (Beetle – Control) and
502 associated p-values.

503 **Fig. 6** Total phenolic concentrations in needles of Scots pine seedlings after pine weevil
504 feeding. Values represent log₁₀-transformed total phenolics (sum of quantified
505 compounds; mg g⁻¹ DW). Boxplots show the median and interquartile range, and

506 whiskers indicate the range of observed values. Points represent individual seedlings
507 (circles = Děčín, triangles = Třeboň). Red symbols indicate model-based means \pm 95%
508 confidence intervals derived from linear mixed models. Panel headers show the
509 estimated difference on the log₁₀ scale (Beetle – Control) and associated p-values.

Figures

Fig.1

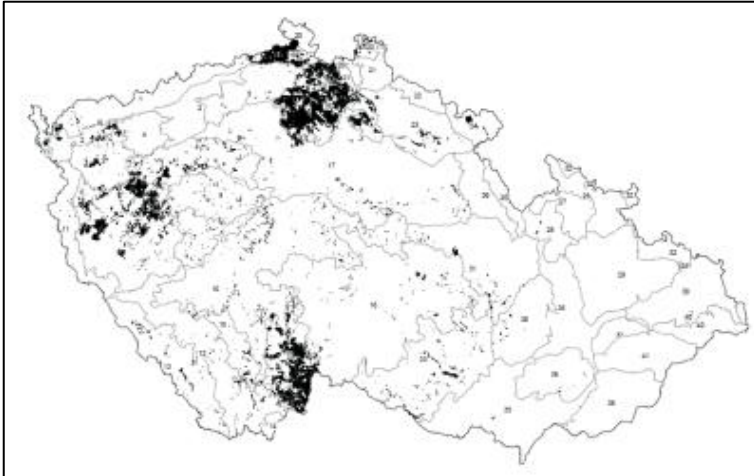


Fig.2



Fig.3

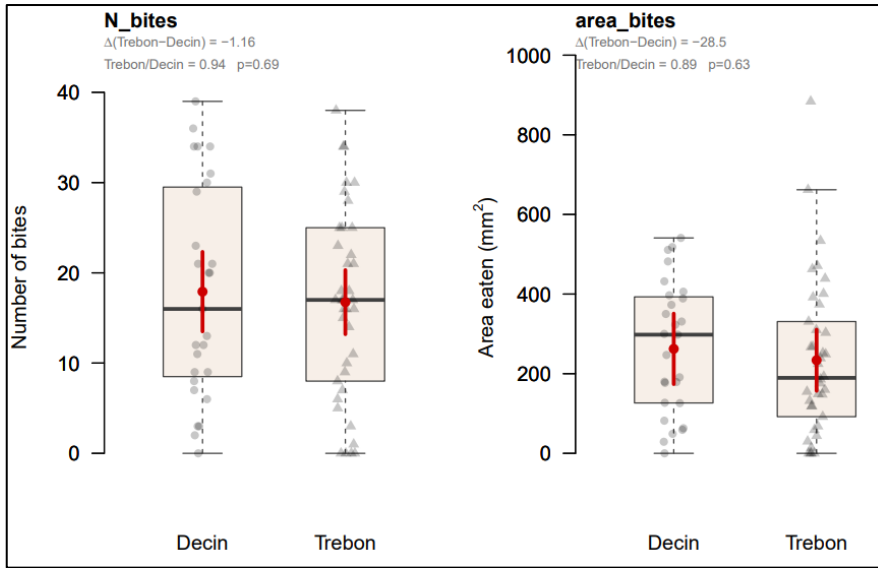


Fig.4

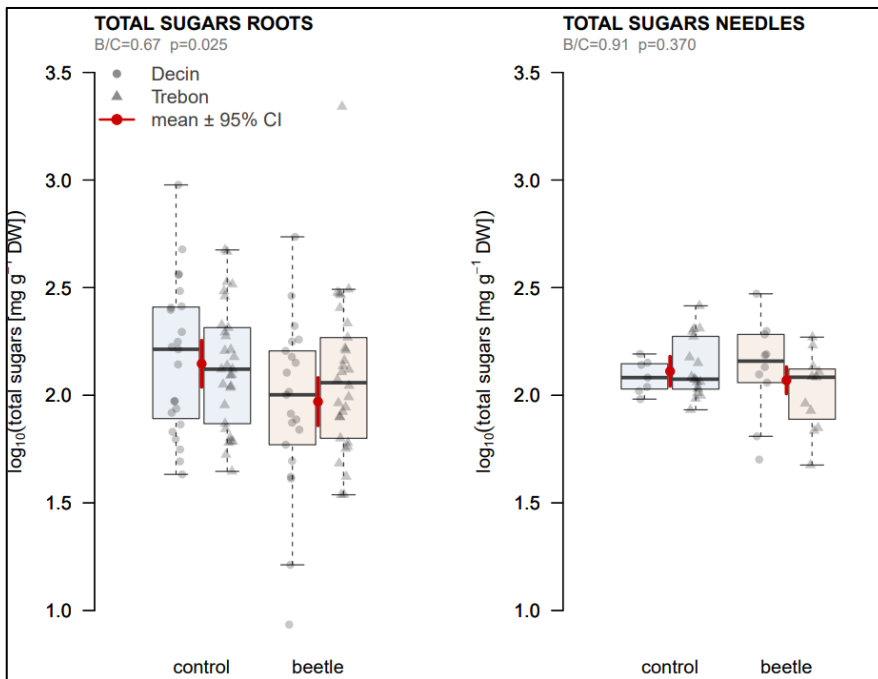
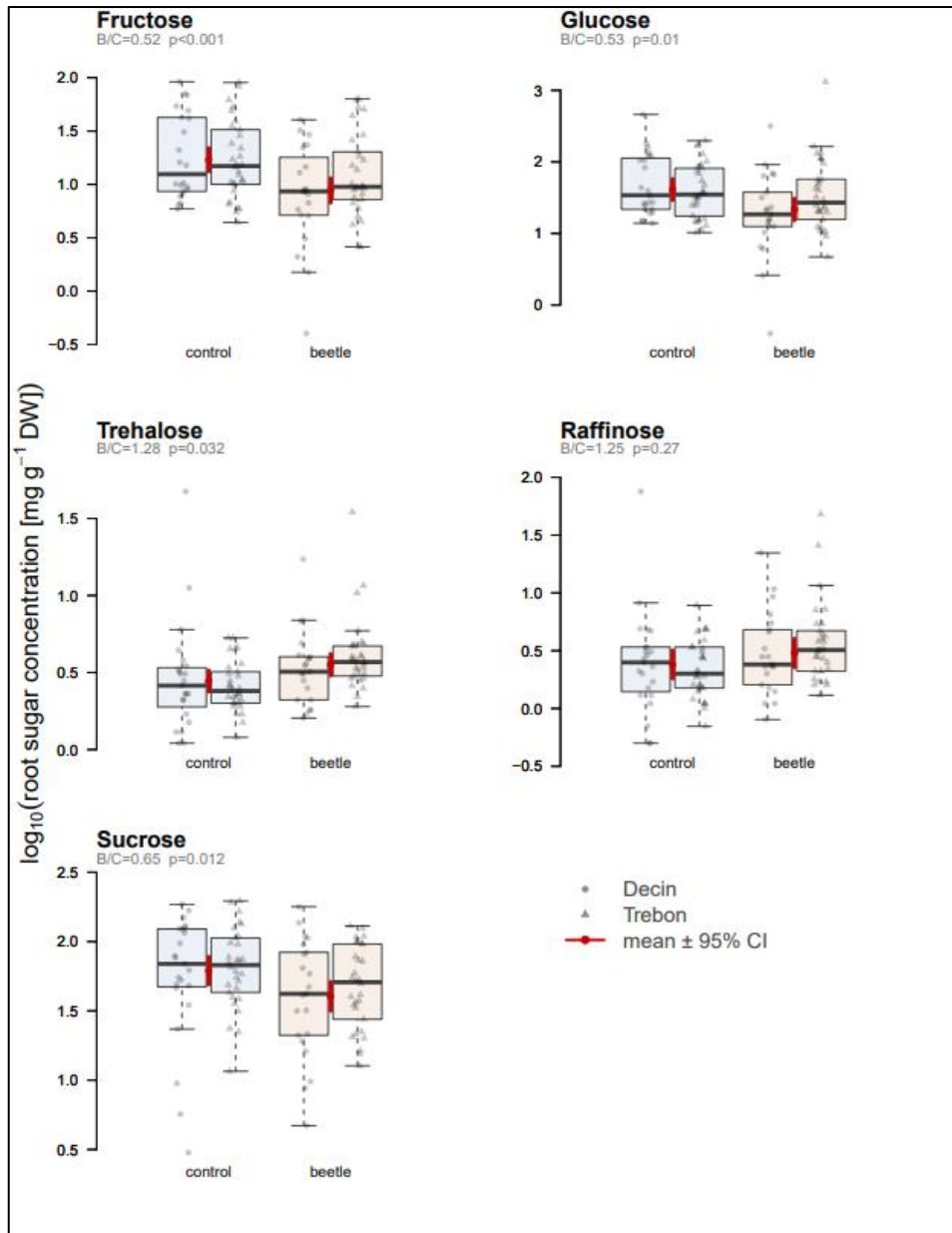
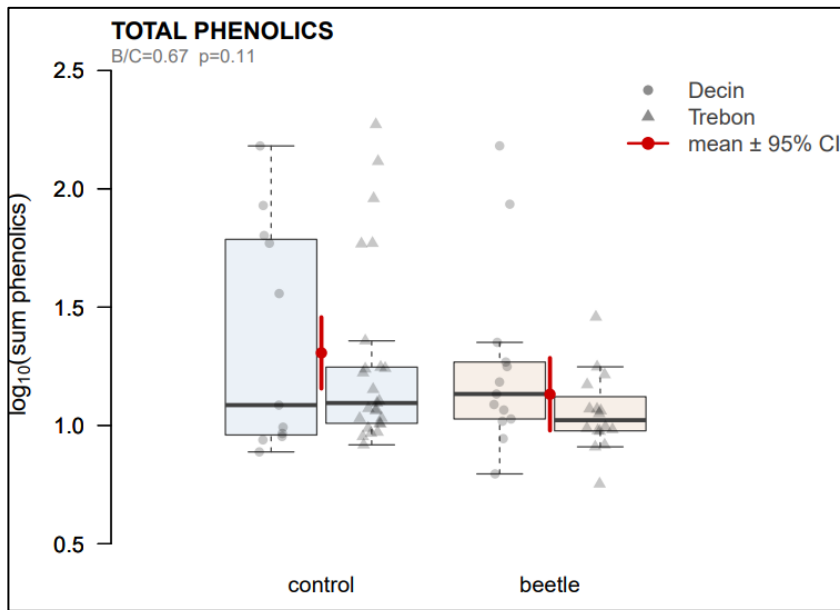


Fig.5



510 Fig.6



Příloha 4.

**Pine weevil (*Hylobius abietis*) preferences among species
of conifer seedlings planted on clear-cuts in central Europe**



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Pine weevil (*Hylobius abietis*) preferences among species of conifer seedlings planted on clear-cuts in central Europe

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Introduction: As a result of climate change and bark beetle outbreaks in forests, extensive salvage, and sanitary cutting have been undertaken in Europe, resulting in many clear-cuts with coarse stumps. The populations of pine weevils (*Hylobius abietis* and *Hylobius pinastri*) are steadily increasing. A high abundance of pine weevils feeding on seedlings typically results in significant economic losses and prolongs the forest establishment period. However, information on these species spatial distribution and habitat selection patterns, necessary for assessing their harmfulness and subsequently accurately estimating their threat to reforested areas, is lacking. To determine which factors influence spatial selection patterns and seedling type preference, this study investigated which clear-cut factors increase pine weevil abundance and which seedling types (species and age) are the most preferred.

Methods: The experiment was carried out on 20 clear-cuts in the central Czech Republic. We evaluated soil moisture level, average stump distance and diameter, proportion of other conifers, and whether the stumps were mulched. We detected the abundance of pine weevils using pitfall traps. We determined the feeding scar intensity on the first 10 cm of seedling stems on commonly used seedlings for reforestation in central Europe: 1-year-old Scots pine (*Pinus sylvestris*), European larch (*Larix decidua*), and 3-year-old Norway spruce (*Picea abies*), with 1,200 seedlings in total. The individual seedling types alternated in rows. The results were evaluated using generalized linear mixed-effect models (GLMMs). As dependent variables we used total numbers of *H. abietis* and *H. pinastri*. The soil moisture level, mulching, proportion of other conifers, average stump diameter, and distance were considered independent variables.

Results: We found that Norway spruce was the least attractive to pine weevils. We found a higher abundance of *H. abietis* females in moist clear-cuts, confirming that dry clear-cuts are less suitable for oviposition.

Conclusion: According to our findings, if foresters want to plant coniferous seedlings, it seems that planting older spruce is a better option than planting larch and pine. These findings provide valuable insights for forest management and reforestation strategies, equipping foresters with the knowledge to mitigate the threat of pine weevils and ensure successful forest establishment.

KEYWORDS

bark beetle, clear-cuts, *Hylobius pinastri*, pest, seedlings

1 Introduction

1.1 Pine weevil abundance increases as a result of bark beetle outbreaks

Ongoing climate change has increased water deficits, stressing trees and making them more vulnerable to insect pests (Holuša et al., 2018). Widespread bark beetle outbreaks have recently occurred across Europe. The most effective defense against the spread of bark beetles is to cut the infested trees and remove them from the forest before the beetle offspring leave the trunks (Mezei et al., 2017). The high number of newly emerging clear-cuts with stumps from felled trees provides suitable conditions for the spread and successful reproduction of the pine weevil (*Hylobius abietis*) (Linnaeus, 1758) (Coleoptera: Curculionidae) (Långström and Day, 2004). The weevils are attracted to places where fresh stumps emit volatiles (monoterpenes and ethanol) (Nordlander, 1987). Freshly made clear-cuts are suitable habitats for the pine weevil to breed (Solbreck, 1980). Therefore, the population of this species is expected to increase due to extensive clear-cut reforestation after bark beetle outbreaks (Doležal et al., 2021). The pine weevil is a widespread and severe pest associated with regenerated coniferous forests in northern and Central Europe (CAB International, 2003; Skrzecz, 2017). Forest management practices strongly influence the extent and severity of pine weevil damage (Jactel et al., 2009).

1.2 Pine weevil spread

Adult weevils, flying up to 10 km, use both smell and vision to greatly enhance their orientation when seeking a suitable breeding site (Örlander et al., 2000; Björklund, 2004). *H. abietis* may respond differently to primary attractants emitted by stumps depending on the season and the state of its reproductive development (Nordenhem and Eidmann, 1991). For example, the orientation of adult weevils involves responses to light and humidity, and their responses vary at different stages of the life cycle; e.g., older reproductive weevils are strongly photonegative (Havukkala and Selander, 1976; Havukkala, 1979). Nordlander et al. (2023) found that harvested stump species (coniferous vs. deciduous) and the age of clear-cuts affect pine weevil abundance and damage (Nordlander et al., 2023).

1.3 Pine weevil life cycle

The life cycle of *H. abietis* usually lasts 1 to 3 years. Adult pine weevils disperse to new clear-cuts during late spring and early summer (Nordenhem and Nordlander, 1994), where they lay eggs in stumps or soil on newly created clear-cuts (Nordlander et al., 1997; Tan et al., 2011). The larvae develop in the bark on the underground roots of dying stumps, where they eat the phloem and pass through four larval molts before pupation. Microclimatic

conditions influence the length of the life cycle. Pupation and emergence can happen the same year (at the end of summer), but usually 1 year after egg laying or in bad microclimatic conditions, it can be postponed until the late summer of the following year, or adults may emerge more than 2 years after occupying a clear-cut (Inward et al., 2012; Wainhouse et al., 2014).

The host tree species is essential for larval development, although larvae develop on all conifers (Véle, 2022). However, pine (*Pinus sylvestris* L.) is the preferred tree species for larval development over Norway spruce, *Picea abies* (L.) Karst. (Munro, 1928; Leather et al., 1994). Larvae develop faster and have lower mortality in pine stumps (Bejer-Petersen et al., 1962; Doom and Frenken, 1980; Thorpe and Day, 2002). Most individuals (ca. 75%) in Europe develop a little longer than 1 year (ca 14 months) (Bejer-Petersen et al., 1962).

Microclimate conditions, therefore, influence the length of the life cycle and determine how long reforested clear-cuts are attractive for repeated infestations (Leather et al., 1999; Inward et al., 2012). Therefore, fallow periods are the best option for integrated pest management (IPM), which varies in Europe according to geographical location. In southern and central Europe, postponing reforestation for 2 years is sufficient, whereas 4 years or more are necessary in Nordic countries (Bejer-Petersen et al., 1962; Bakke and Lekander, 1965; Bejer-Petersen, 1975; Moore et al., 2004).

Adults can live for up to 4 years (Eidmann, 1979). While larvae feed on stumps, adults typically feed on the root collar of seedlings that are planted in clear-cuts and other sources of thin conifer bark, such as twigs in crowns of mature trees and roots of larger trees (Örlander et al., 2000; Wallertz et al., 2006; Fedderwitz et al., 2018). Adults feeding begins when adults emerge from hibernation, i.e., in spring when temperatures are 8–9°C (Munro, 1928; Nordenhem, 1989), continues throughout the entire vegetative season, when oviposition occurs, and into autumn, when the first generation of beetles may appear (Moore et al., 2004). After this damage, the seedlings lose growth potential or die (Thorpe and Day, 2002; Day et al., 2004).

1.4 Food sources for pine weevils

H. abietis is a polyphagous species (Munro, 1928; Toivonen and Viiri, 2006); adults feed on many species of conifers (e.g., *P. sylvestris*, *Larix decidua* Mill., *P. abies*, *Pseudotsuga menziesii* (Mirb.) Franco) (Leather et al., 1999; Månsson and Schlyter, 2004). Researchers who studied the feeding preferences of pine weevils for specific tree species have reported no difference between Norway spruce and Scots pine (Toivonen and Viiri, 2006) but higher damages on Douglas fir (*Pseudotsuga menziesii*) and Sitka spruce [*Picea sitchensis* (Bong.) Carr.] compared to Norway spruce (Wallertz et al., 2014). A higher preference for Douglas fir over Norway spruce was also confirmed by Doležal et al. (2021). However, pine weevil feeding scars have also been recorded on deciduous trees, such as *Fraxinus excelsior* L., *Alnus glutinosa* (L.) Gaertn., *Fagus sylvatica* (L.) Gaertn., *Quercus robur* L., *Salix* L. spp., *Betula* L. spp. and others (Toivonen and Viiri, 2006; Manlove et al., 2013).

Abbreviations: IPM, Integrated pest management; Mts, Mountains; GLM, General linear model; GLLM, Generative large language model; VIF, Variance inflation factor.

1.5 Damage effects

The pine weevil severely damages all conifer species planted in large amounts in various parts of Europe, e.g., Norway spruce and Scots pine in Scandinavia (e.g., Johansson et al., 2015), Sitka spruce and Corsican pine in the UK (e.g., Wainhouse et al., 2009), and maritime pine and radiata pine in Spain (e.g., López-Villamor et al., 2019). In Europe, *H. abietis* causes annual damage of almost 120 million EUR (Lalík et al., 2021). Foresters have noted that damage intensity to the European larch (*L. decidua*) has increased recently. There are concerns that this increase may be greater than damage to Central Europe's other main economic seedling types.

1.6 Study aims

Based on these findings, we investigated the following questions: (I) Can different factors at clear-cuts increase the attractiveness of these sites for occupation by more pine weevil individuals, and do different management practices after logging also influence the pine weevil population? We included factors that may affect the quality (soil moisture level; see Carpenter et al., 1988) and the amount of breeding substrate (average distance between stumps and average diameter of stumps,) as well as a factor representing an increase in the emission of volatiles (presence of mulch). Recently, the amount of coarse logging residue harvested from clear-cuts, including stumps, has increased (Saarinen, 2006; Rahman et al., 2018), and we speculate that the odor of clear-cut mulch may attract more adult pine weevils (Nordlander, 1987; Brattli et al., 1998). Furthermore, we wanted to determine (II) if the attractiveness of European larch, Scots pine, and Norway spruce varies in relation to *H. abietis*.

2 Materials and methods

2.1 Study localities and design

The first part of the experiment investigated whether clear-cut factors affect the abundance of pine weevils in the chosen clear-cut samples. The second part of the experiment considered the feeding scar intensity on the three main economic seedling species of Europe: Norway spruce (*P. abies*), Scots pine (*P. sylvestris*), and European larch (*L. decidua*). The experiments were conducted at the same sites and time periods. The study localities were in the Czech Republic in the upper lands of central Bohemia in the Brdy Mts. In this region, rainfall varies between 550 and 600 mm, with average annual temperature ranging from 7 to 8°C, which is higher than the average temperatures observed in northern regions, making it a relatively dry area. The average monthly precipitation during the study period (May to August) was 70 mm. The tree species composition is dominated by Norway spruce (74%). It includes a mixture of Scots pine (15%), European larch (5%), European silver fir (*Abies alba* Mill.) (0.6%), oak (*Quercus* sp.) (2.5%), European beech (*Fagus sylvatica* L.) (2.1%) and others (0.8%).

The soils in the clear-cuts were dry Ranker Cambisol (vegetation coverage up to 10%), moderately moist mesobasic

Cambisol (vegetation coverage 10–50%) with a cover of low grasses <20 cm tall, and moist gley or pseudogley Cambisol (vegetation coverage above 50%) with wetland grasses taller than 1 m. The grasses represented a part of the moisture levels. The soil moisture level was divided into three levels: dry, moderately moist, and wet, how soil types are affected by water (Viewegh et al., 2003). There was no slash on the clear-cuts.

In total, we selected 20 clear-cuts (localities) from large-scale experiments, covering a total of 35 × 15 km in large spruce-dominated conifer stands with a maximum of 5% pine, which were harvested in October–December 2022 and reforested in March–spring 2023. The altitudes of the areas ranged from 420 to 700 m above sea level. The study clear-cuts were planted with Scots pine, Norway spruce, and European larch at a rate of 3,200 seedlings/ha. The individual tree species alternated in rows. The spruce seedlings were 1-year-old, the larch and pine seedlings were 1-year-old, the average height of the pine seedlings was 35 (±6) cm, that of the larch seedlings was 44 (±9) cm, and that of the spruce seedlings was 36 (±8) cm.

2.2 Calculated clear-cut factors

Five clear-cut factors that could affect the abundance of pine weevils were considered: the soil moisture level, the average distance between stumps, the average diameter of stumps, the proportion of other conifers, and whether the stumps were mulched (harvested). For the average diameter of the stumps of each tree species and the distances between them, ten stumps were measured in a line passing through each locality. Mulching was performed in March before the seedlings were planted by harvesting the stumps via a tractor with a cutter and rotavator. Woodchips of ca. 3 × 5 cm were evenly dispersed over the clear-cut study area, forming a layer of ca. 5 cm. To evaluate the impact of mulching on pine weevil abundance, only the presence or absence of mulch was considered.

2.3 Trapping *H. abietis*

The number of weevils caught in the pitfall traps determined the total abundance of adult weevils. Five pitfall traps were placed diagonally at a distance of 10 m from each other across each clear-cut area to catch pine weevil adults; thus, the traps were as representative as possible of the total abundance in the locality. Each pitfall trap was made from a 1-L plastic bucket containing bait in the form of a small 50-ml bottle filled with 70% ethanol with an ~5 cm-long stick of pine attached. At 2 cm below the edge, 8-mm-diameter holes were made so that pine weevil beetles, not larger beetles or small vertebrates, could be caught (see Lalík et al., 2019; Figure 1). The stick was regularly replaced every seven days to ensure that the concentration of the emitted volatiles did not decrease. Two inches of 4% formaldehyde were poured on the bottom of the pitfall traps to maintain the freshness of the trapped insects. Pine weevil collection was performed every 14 days from mid-May to early September 2023.



FIGURE 1
A ground trap with formaldehyde from a 1-L plastic bucket with 50 ml of ethanol bait and a pine twig 5 inches thick and 5 cm long was used for catching pine weevils in the Czech Republic.

Stereomicroscopy in the laboratory was used to determine the sex ratio of *H. abietis* weevils by the morphological depression (dimple) in the last body segment and to determine the number of *H. pinastri* (Gyllenhal, 1813) weevils based on the red coloration of the legs and the white coloration of the hairs (Brosset, 2017).

2.4 Characterization of feeding scars on seedlings

On each clear-cut we selected 60 seedlings (20 for each species) which we observed. We selected a transect in the middle of the clear-cuts, which contained seedlings of varying heights and overall vitality. These seedlings were marked and fenced to prevent them from being sprayed with insecticides, which are used to protect them from pine weevil damage (Figure 2). The study plots of the seedlings were always located in the middle of the clear-cut plots. The evaluation of feeding scar intensity was performed at the beginning of September. We evaluated the feeding scar intensity on the collar of the seedlings up to 10 cm from the ground, i.e., how many 1 cm sections were debarked; values ranged from 0 to 10 cm. We also recorded the height of the highest feeding scar (hereafter referred to as the “top feeding scar”) and the total height of the

seedlings. The mortality of the seedlings due to debarking was also recorded.

2.5 Statistical analysis

We split the study into two objectives (a determination of clear-cut factors and weevil abundance and a comparison of the damage to three seedling types (tree species and age of seedlings) because the relationship between the number of pine weevils and damaged seedlings is very weak, as evidenced by several studies (Wilson and Day, 1994; Örlander et al., 1997; Von Sydow, 1997; López-Villamor et al., 2019; Nordlander et al., 2023). The level of damage to seedlings cannot be related to the number of weevils caught in traps, as seedlings planted at the locality provide only part of the available food source for pine weevils (Nordlander et al., 2023). Adults naturally feed on branches in the crowns of mature trees and their roots or logging residues (Nordlander et al., 2003; Hansen et al., 2005; Fedderwitz et al., 2018). The following analyses were conducted in R Statistical Software (v. 4.2.1.).

The numbers of *H. abietis* females, males, and all adults of *H. pinastri* were used as dependent variables to evaluate the influence of the considered clear-cut factors. Adults were caught and summed from 5 pitfall traps on each of 20 clear-cuts (e.g., $N = 20$). The soil moisture level, mulching of stumps, proportion of other conifers, the average diameter of stumps, and the average distance between stumps were considered independent variables. In total, five models were analyzed. First, we tested for multicollinearity among the independent variables using the HH package (Heiberger, 2019) with the following criterion for excluding variables: $VIF > 2$. The exclusion of multicollinearity from statistical models enhances reliability and interpretation (Graham, 2003). The DHARMA package (Hartig, 2021) was used to assess the suitability of the GLM by testing the distribution of residuals; Gaussian, Poisson, and negative binomial distributions were considered for the dependent variables. If a negative binomial distribution was fit, the resulting Theta value was used in the final models. If none of these distributions were suitable, a quasi-Poisson distribution was used. We used generalized linear mixed-effect models (GLMMs) constructed using the R package MASS (Venables and Ripley, 2002) and generalized linear models (GLM). The numbers of *H. abietis* females, males, and all adults of *H. pinastri* were used as dependent variables. Clear-cut was used as a random factor. The results were visualized using the package visreg (Breheny and Burchett, 2017).

The same procedure was used to evaluate the effects of clear-cut factors on pine weevil (*H. abietis* and *H. pinastri*) feeding on seedlings. The feeding scar intensity was used as the dependent variable, and soil moisture level, mulching, proportion of other conifers, average stump diameter, and average distance of stumps were considered independent variables.

GLMM was used to evaluate the effects of seedling type on the intensity of *Hylobius abietis* and *Hylobius pinastri* feeding scars. The feeding scar intensity was used as a dependent variable, and seedling types (Species) and their heights were used as independent variables.



FIGURE 2 Experimental clear-cuts with stumps left and colored pins fencing the study plots with untreated pine, larch, and spruce seedlings; dry (a), moist (b), and mulched (c).

TABLE 1 The influence of clear-cut environmental variables (factors) on the total abundance of *Hylobius abietis*, its males, females, and *H. pinastri* beetles and the intensity of *Hylobius abietis* feeding scars in the Czech Republic.

Variable	<i>H. abietis</i>		<i>H. abietis</i> male		<i>H. abietis</i> female		<i>H. pinastri</i>		Feeding scars	
	z-value	P-value	z-value	P-value	z-value	P-value	z-value	P-value	t-value	P-value
Intercept	3.49	0.004	3.08	0.009	2.67	0.019	1.55	0.14	3.362	0.005
Soil moisture level	2.12	0.05	1.93	0.08	2.27	0.041	1.58	0.14	0.450	0.66
Mulching	1.57	0.14	1.63	0.13	1.49	0.16	0.62	0.55	0.096	0.92
Proportion of other conifers	0.43	0.67	0.41	0.69	0.42	0.68	-1.31	0.21	0.935	0.36
Stump diameter	0.85	0.41	0.79	0.45	0.92	0.38	0.70	0.49	2.131	0.05
Distance of stumps	-0.50	0.62	-0.49	0.64	-0.52	0.61	-0.88	0.40	1.458	0.17

The number of replicates used is $N = 20$. Data were analyzed as a generalized linear mixed model (GLMM).

To test the correlation between *H. pinastri* and *H. abietis* abundance, we used the Shapiro–Wilk test combined with a histogram to test the normality of the data distribution. Due to the non-Gaussian distribution, the Spearman correlation test was used.

The influence of seedling types on the height of the top feeding scar GLMM was used. The height of the top feeding scar was used as a dependent variable, and seedling types and their height were used as independent variables.

GLM was also used to evaluate the probability of survival of all seedling types. Survival was the dependent variable, and seedling type, plant height, and the feeding scar intensity were the independent variables.

3 Results

3.1 Clear-cut factors and presence of weevils

A total of 6,172 weevils were caught in all 20 clear-cuts. Of these, 5,918 were *H. abietis* and 254 were *H. pinastri*. There were 2,849 males and 3,069 females of the *H. abietis* species. Only the soil moisture level influenced the total number of *H. abietis* ($p = 0.05$) and female abundance ($p = 0.041$) on clear-cuts (Table 1). None of the other factors considered had a statistically significant effect on the total number of males or females of *H. abietis* caught in the

pitfall traps ($p > 0.05$). *H. pinastri* weevils accounted for 4.2% of all the caught weevils.

We found that the number of *H. pinastri* caught was strongly and significantly correlated with the number of *H. abietis* caught for both males and females [R_S (%) = 93.52].

3.2 Feeding on seedlings

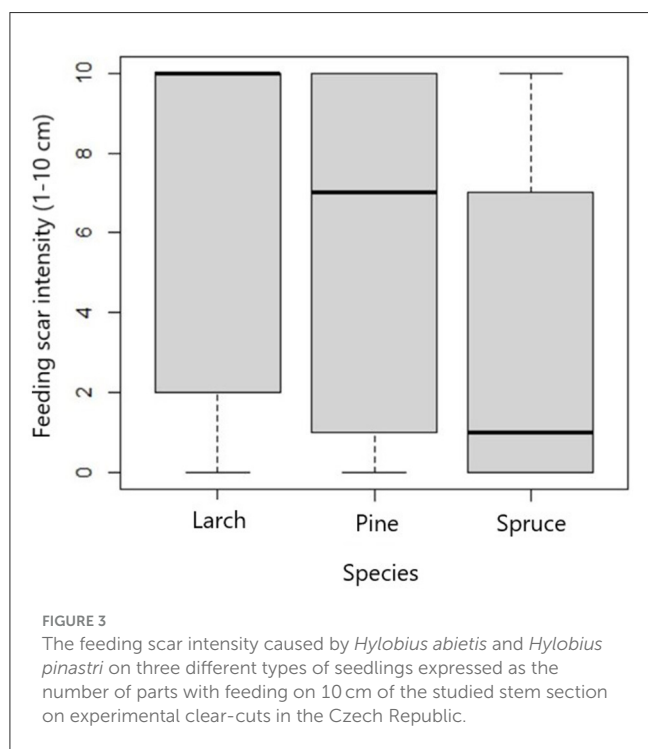
A comparison of pine weevil feeding intensity on the collar of individual trees showed that the tree seedling type had a statistically significant effect on the feeding scar intensity. Plant height didn't have any effect (Table 2). Pines and larches were significantly more debarked than spruces (Supplementary Figure 1). Besides the seedling parameters, we also evaluated how clear-cut factors affected feeding scar intensity. We found that only the average diameter of stumps influenced the feeding scar intensity ($P = 0.05$; Table 1).

We also evaluated the differences in the feeding scar intensity among seedling types (tree species and age of seedlings) with no influence of clear-cut factors (Figure 3). Larch and pine were significantly more damaged than spruce, but no difference was found between them (Table 2). On dead seedlings, the feeding scar intensity was greater. The average (\pm se) damage intensity in dead

TABLE 2 The influence of seedling types on the intensity of *Hylobius abietis* feeding scars, the height of the top feeding scar, and the function of seedling survival probability in clear-cuts in the Czech Republic.

Variable	Feeding scar intensity		Height of the top feeding		Seedling survival probability	
	t-value	P-value	t-value	P-value	t-value	P-value
Intercept	14.44	<0.001	13.21	<0.001	1.25	0.210
Pine vs. Spruce	-7.07	<0.001	-6.04	<0.001	2.87	0.004
Spruce vs. Larch	8.42	<0.001	14.22	<0.001	-0.39	0.69
Larch vs. Pine	-1.31	0.18	-4.87	<0.001	-2.48	0.01
Plant height	0.55	0.58	5.23	<0.001	-7.95	<0.001
Feeding scar intensity	-	-	2.87	0.004	8.63	<0.001

Data were analyzed as a generalized linear mixed model (GLMM).



seedlings was 7, and those that survived were 4.5 ($Z = -8.49$, $p \leq 0.001$) (Figure 4).

In addition to the feeding scar intensity, the height of the top feeding scar was evaluated. The effects of seedling type and height had a statistically significant effect on the top feeding scar (Table 2). The top feeding scar on larch was the highest, while that on spruce was the lowest (Supplementary Figure 2). The number of dead seedlings was greater than the number of surviving ones.

The average mortality rate was 22.7% (SE = 2.3) for larch, 22.4% (SE = 2.6%) for pine, and 23.2% (SE = 2.4%) for spruce. Pine is more likely to survive the damage than spruce and larch (Table 2).

4 Discussion

Our study focused on five factors that may affect the abundance of pine weevils and which seedling types they prefer as food sources.

Only the soil moisture level affected the pine weevil abundance. None of the other factors we examined, e.g., average stump diameter, the proportion of other conifers, the average distance between stumps, and the mulching of stumps, affected the overall number of pine weevils caught. We found that the weevils preferred 1-year-old Scots pine (*P. sylvestris*) and 1-year-old European larch (*L. decidua*) compared to 3-year-old Norway spruce (*P. abies*).

Because it is impossible to relate the number of beetles caught in traps and feeding damage on seedlings (Nordlander et al., 2023), we evaluated the influence of clear-cut factors on the feeding scar intensity caused by pine weevil adults. We didn't find any of the factors influencing feeding scar intensity. Only one factor, the average diameter of stumps tends to be significant but shows a higher feeding intensity with decreasing diameter. We would expect the opposite effect. We assumed that bigger stumps would emit more volatiles and allow more beetle breeding space. This finding needs to be studied more in the future.

4.1 *Hylobius* species

Among the *Hylobius* weevils caught, *H. abietis* was the most abundant, with a male-to-female ratio of 1:1.08, which is consistent with the findings of other studies (Nordenhem and Eidmann, 1991; Bylund et al., 2004; Voolma and Sibul, 2006; Heber et al., 2024). The proportion of *H. pinastri* in the total population was less than 5%. The abundance of *H. pinastri* is generally much lower and thus poses less of a threat of economic losses (Långström, 1982; Von Sydow and Örlander, 1994; Örlander et al., 1997; Voolma, 2001). The proportion of *H. pinastri* caught was similar in some studies (e.g., Nordlander, 1990) but significantly greater in others. In Estonia, *H. pinastri* accounted for a quarter of the caught weevils, but *H. abietis* was the dominant species (Luik and Voolma, 1989).

We found a high correlation between the number of *H. abietis* and *H. pinastri* weevils captured in each clear-cut area, suggesting that similar environmental factors suit these two species. The difference between these two species is that *H. pinastri* is less attracted to α -pinene than *H. abietis* (Von Sydow and Örlander, 1994). Unlike *H. abietis*, *H. pinastri* prefers to feed on spruce rather than pine (Viiri and Miettinen, 2013). Another difference in ecological niches may be that *H. pinastri* prefers damper sites more than *H. abietis* (Örlander et al., 2000).

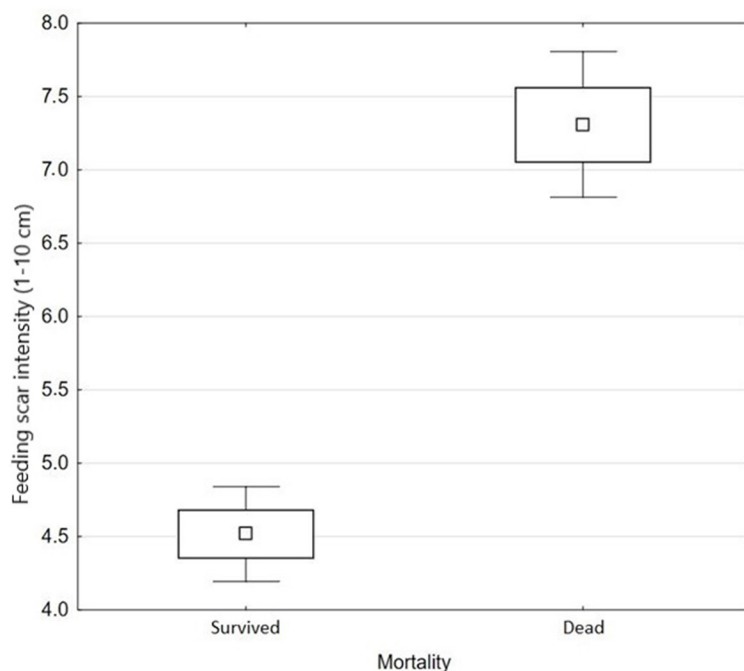


FIGURE 4
The average (\pm SE) damage intensity (on a scale of 1–10) in seedlings that died and survived.

4.2 Clear-cut factors

Our first objective was to identify the effect of clear-cut factors on the abundance of pine weevils. We did not include the size of the clear-cuts among our factors because the weevils fly in and then spread out, so the density is even throughout the clear-cut (Hansen et al., 2005). According to Örlander et al. (2000), weevils migrate by flight and land in the crowns of mature trees near clear-cut edges, where they feed for a short period before they spread over the clear-cut for reproduction (Örlander et al., 2000; Skrzeczek et al., 2021). The pitfall traps were equally dispersed throughout the clear-cut, so the number of caught weevils represented population density.

Neither stump diameters nor stump distances per unit area affected our experiment's abundance of pine weevils. Also, López-Villamor et al. (2019) reported that only two factors influenced the level of seedling damage: the age of the clear-cuts and the amount of logging residues left in the clear-cuts. The average stump diameter and basal area of the cut did not affect the damage (López-Villamor et al., 2019; Véle, 2022). Nordlander et al. (2023) reported that 2-year-old clear-cuts were more damaged than 1-year-old. We studied only 1-year-old clear-cuts because the pine weevil development in Central Europe is shorter compared to Scandinavia (Bejer-Petersen et al., 1962).

Deciduous tree stumps in clear-cuts could reduce beetle abundance, as pine weevils use conifer stumps for reproduction (Björkman et al., 2015). Our clear-cuts did not include any stumps of deciduous trees; we tested only the species composition with domination of spruce stumps and only a small proportion of pine and larch, which did not exceed 10%. Therefore, pine and larch stumps did not affect the number of pine weevils. We did not

find sufficient data supporting a significant correlation between the amount of substrate suitable for egg laying, e.g., the number of stumps and the number of pine weevils.

We hypothesized that stumps in damper habitats would emit more volatiles because they could be infested with fungi (Carpenter et al., 1988) and thus attract more pine weevils. This hypothesis was supported by the result that higher *H. abietis* female abundance was found on clear-cuts with higher soil moisture levels.

Adult pine weevils are attracted to volatile compounds emitted by stumps and woody residues in logged areas (Tan et al., 2011); we hypothesized that weevil abundance would be greater in mulched clear-cuts due to high concentrations of emitted volatiles, as was the case in a study with slash (López-Villamor et al., 2019). Mulched clear-cuts did not attract significantly more beetles; however, only three of the studied clear-cuts were mulched. This hypothesis requires further study.

4.3 Feeding on seedlings

For the experiment, we chose 1-year-old clear-cuts because these are the areas where seedlings are most threatened in central Europe. Over time, their vulnerability decreases (Örlander et al., 1997; Örlander and Nilsson, 1999; Nordlander et al., 2011; Paraschiv, 2020; Galko et al., 2022). The clear-cuts contained seedlings of European forests' three main economic tree species. We observed damage to the 1-year-old seedlings of *P. sylvestris*, 1-year-old *L. decidua*, and 3-year-old *P. abies*, which are commonly used in such seedling stages for reforestation in central Europe. The

seedlings were planted evenly (alternating rows) in clear-cuts. We predicted that pine seedlings would be the most damaged as they are the primary tree species for pine weevil development and have more larvae than spruce (Von Sydow and Birgersson, 1997). Vélé (2022) found that adult beetles hatching from spruce stumps are larger, but the abundance is smaller. This leads us to think that pine is more suitable for them (Vélé, 2022).

In our study, the feeding scar intensity on pine seedlings did not differ from feeding scars on larch. Larch was also found to be attractive by Doležal et al. (2021), but in their laboratory experiment, larch was preferred for almost the entire experimental period, while pine weevils started to prefer pine only during the last week of his experiment. However, it is interesting why the pine weevil prefers to eat larch, as female pine weevils feeding on larch lay far fewer eggs than females feeding on spruce or pine (Doležal et al., 2021).

However, pine was reported as the most preferred tree species in most other studies. Notably, pine was compared only with spruce (Örlander et al., 2000; Vélé, 2022) or deciduous seedlings or spruce, pine, and ash (Leather et al., 1994). *H. abietis* likely prefers Scots pine than Norway spruce because spruce in central Europe is usually planted as a bare-root seedling, which is thicker than pine and, therefore, probably less palatable. One reason may be that older spruce seedlings have a higher concentration of (-)-limonene than pine (Wibe et al., 1998), which may outweigh the smell of attractive compounds (Nordlander, 1990, 1991; Lindgren et al., 1996). Wallertz et al. (2014) found no differences between spruce, pine, or hybrid larch. A possible reason why the pine weevil might prefer larch to spruce could be the smaller size of larch seedlings than spruce seedlings. Larger seedlings, e.g., those with larger stem diameters, are known to survive the infestation of pine weevils better (Thorsen et al., 2001).

Certain seedlings may be more palatable to the pine weevil, so they return to the seedling and debark the entire stem. If pine weevils bite a seedling, resin, and volatiles are emitted and attract other weevils, which may be why the whole stem becomes debarked. We compared the height of damage to the seedlings because several studies have shown that larger seedlings are more resistant than thinner seedlings (Wainhouse et al., 2008). Because seedling size also influences weevil feeding, older seedlings are less preferred by pine weevils than younger ones (Thorsen et al., 2001); for larger seedlings with thicker bark, we believe that weevils are more likely to climb to taller heights and seek more palatable bark, which is why taller seedlings have more top feeding scars.

4.4 Implications for forest management

The protection of reforested areas from the pine weevil (*H. abietis*) is one of the largest problems in forest management in many European countries (Nordlander et al., 2011; Willoughby et al., 2017; Tudoran et al., 2021; Galko et al., 2022). Our experiment showed that in the coniferous forests of central Europe, all clear-cuts with fresh conifer stumps could be impacted by many pine weevils. If a clear-cut is reforested with conifer seedlings in the first year after logging, a high level of damage caused by pine weevils must be considered. Pine weevils preferred 1-year-old larch and pine over 3-year-old spruce. We would recommend foresters to plant deciduous trees.

However, when growing all three main coniferous species, e.g., pine, larch, and spruce, foresters must be prepared for pine weevil damage and the necessary use of IPM against them. Foresters may plant already treated seedlings and cause weevil mortality (with insecticide) or protect the seedlings (treating them with wax, sand, or collars); alternatively, foresters may have to monitor the seedlings and spray them when the economic damage threshold is exceeded. We advise foresters to use the fallow period, which in central Europe consists of postponing reforestation for 2 years while the amount of volatile compounds emitted from fresh stumps decreases, to decrease the attractiveness of clear-cuts to pine weevils (Rahman et al., 2018).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

BD: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. JHol: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. JHor: Writing – review & editing, Data curation. JHra: Writing – review & editing. MB: Writing – review & editing, Methodology. MZ: Writing – review & editing, Methodology.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/ffgc.2024.1399405/full#supplementary-material>

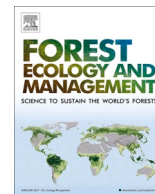
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Příloha 5.

Stump grinding management enhances pine weevil (*Hylobius abietis*) abundance on clear-cuts



Stump grinding management enhances pine weevil (*Hylobius abietis*) abundance on clear-cuts

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ABSTRACT

The pine weevil (*Hylobius abietis*) is a major pest that affects conifer reforestation, breeding in stumps left in clear-cuts after harvesting. Fresh stumps attract pine weevils by emitting volatile compounds during their decomposition. With recent bark beetle outbreaks leading to more clear-cuts, the economic losses from pine weevil feeding on young seedlings have risen. Stump grinding has become a popular post-harvest management strategy. This technique helps to remove stumps, prepare sites for reforestation, and reduce pine weevil populations. However, we observed a higher abundance of pine weevils in mulched clear-cuts, leading us to hypothesize that fresh stump grinding may increase volatile compound production, making these areas more attractive to the beetles. To test this, we studied 60 clear-cuts that varied by post-harvest management (mulched vs. retained stumps), clear-cut age (newly created vs. one-year-old), and stand type (coniferous vs. deciduous). We trapped pine weevils using five pitfall traps at each site throughout the summer season (June–August). Our results showed that pine weevils were significantly more abundant in newly mulched clear-cuts compared to those with retained stumps. In contrast, one-year-old mulched clear-cuts showed a lower abundance of pine weevils. Additionally, freshly mulched clear-cuts were highly attractive to pine weevils even in deciduous stands. These findings highlight that stump grinding strongly increases the short-term attractiveness of clear-cuts to pine weevils. Moreover, the timing of mulching is critical: applying it just before or during the swarming period greatly increases the risk of high pine weevil abundance on regeneration sites.

1. Introduction

In recent years, large-scale outbreaks of bark beetles have affected nearly the entire European continent (Das et al., 2025; Hlásny et al., 2021). Because the most effective way to slow the spread of the spruce bark beetle [*Ips typographus* (Linnaeus, 1758)] (Coleoptera: Curculionidae) is to remove infested trees before new beetles emerge, these outbreaks have led to a substantial increase in the extent of newly created clear-cuts (Véle and Frouz, 2023; Mezei et al., 2017). This increase has also led to a rise in another significant pest affecting conifer plantations: the large pine weevil [*Hylobius abietis* (Linnaeus, 1758)] (Coleoptera: Curculionidae). This pest is attracted to volatile compounds released from freshly cut stumps (Dvořáková et al., 2024; Tudoran et al., 2019). The large pine weevil causes considerable damage to conifer plantations by feeding on the bark of seedlings, often resulting in their death (Tudoran et al., 2021; López-Villamor et al., 2019; Nordlander et al., 2017; Wallertz et al., 2016). Annual economic losses in Europe due to this pest are estimated at around EUR 120 million (Lalík et al., 2021),

and no effective method has yet been found to prevent its spread.

1.1. Pine weevil life cycle

Understanding the biology of the pine weevil, particularly its life cycle, is key to explaining both its damaging potential and possible control measures. The pine weevil has a lifespan that ranges from one to four years (Lalík et al., 2021; Inward et al., 2012). The development of pine weevils relies on the stumps of coniferous trees, particularly their underground root systems (Conord et al., 2006). Adult weevils typically begin colonizing new clear-cuts in late spring and early summer, with dispersal generally occurring from May to August (Skrzecz, 2021; Nordenhem and Nordlander, 1994; Inward et al., 2012). During host finding, adults rely mainly on olfactory and visual orientation (López-Villamor et al., 2019; Örländer et al., 2000; Björklund, 2004).

On newly created clear-cuts, females lay their eggs either under the bark of stumps close to the soil surface or directly into the soil near the stumps (Tan et al., 2011; Scott and King, 1973; Nordenhem and

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Nordlander, 1994). The larvae feed on roots under the soil surface, undergoing four to five instars before pupation (Wainhouse and Brough, 2007; Leather et al., 1999). Overwintering typically occurs during the larval stage (third to fourth instar) and in adults (Nordenhem, 1989). Adults usually emerge in the spring of the following year, although the timing is significantly influenced by climatic conditions, especially temperature (Inward et al., 2012). Under favorable conditions, adults may emerge in the autumn of the same year after colonization, while in colder climates, development can take two to five years, although approximately 75 % of pine weevils in Europe emerge after one year (Wainhouse et al., 2014; Moore et al., 2004; Bejer-Petersen et al., 1962; Leather et al., 1999). Adults become active when temperatures reach 8–9 °C (Sibul et al., 2006; Nordenhem, 1989).

Before laying eggs, adults perform maturation feeding, consuming the bark of conifer seedlings and small branches of mature trees, while occasionally feeding on other woody species (Wallertz et al., 2006; Fedderwitz et al., 2018; Day et al., 2004). After this feeding period, they disperse to new clear-cuts (Nordlander et al., 2003).

1.2. Volatile organic compounds and pine weevil attraction

Understanding how adult pine weevils locate suitable host locations and colonize new clear-cuts is important, as these steps are crucial in their life cycle. Volatile organic compounds (VOCs) play a significant role as chemical cues in this process (López-Villamor et al., 2019). During the swarming period, adult pine weevils actively search for appropriate clear-cuts for feeding and reproduction, primarily guided by the volatile signals emitted from freshly cut stumps and woody residues (Nordlander et al., 2017; López-Villamor et al., 2019).

Monoterpenes are volatile organic compounds (VOCs) that readily evaporate into the gas phase (Malik et al., 2023). A synergistic mixture of ethanol and monoterpenes has been identified as the primary attractant for certain pests. Ethanol signals weakened or freshly damaged tissue, while monoterpenes indicate the species of the host tree (Heber et al., 2024; Sullivan et al., 2024; Kelsey, 2015; Althoff et al., 2023; Taft et al., 2015; Moreira et al., 2008). The combination of ethanol and α -pinene is particularly attractive and is commonly used in artificial lures for monitoring pine weevils (Nordlander, 1987; Lalík et al., 2019). Overall, the specific blend of ethanol and monoterpenes influences the attractiveness of clear-cut areas and provides information about host identity (Schlyter et al., 2004) and habitat suitability (Azeem, 2013).

The intensity of VOC emissions is highly influenced by the amount of evaporative surface (Yuan et al., 2025). Mechanical disturbances, such as cutting or chipping, increase the evaporation surface, which in turn enhances VOC emissions compared to intact wood (Zagatti et al., 1997; Fedele et al., 2007; Granström, 2007; Yuan et al., 2025). Coniferous trees release higher concentrations and a wider variety of monoterpenes than deciduous trees (Bao et al., 2023; Borsdorf et al., 2023; Granström, 2007). Additionally, the abundance of pine weevils is generally greater in coniferous stands than in deciduous stands (Nordlander et al., 2023). Furthermore, Nordlander et al. (2023) demonstrated that the species composition of harvested stumps and the age of clear-cuts can significantly affect pine weevil abundance.

1.3. Mechanical site preparation by stump grinding

Forestry practices have a significant impact on the amount and structure of woody residues, which can, in turn, affect VOC emissions and the attraction of pine weevils. One effective method for controlling pine weevil populations is stump removal (Piri et al., 2020; Rahman et al., 2015, 2018). Removing stumps and logging residues improves conditions for forestry operations and forest plantations. This practice reduces the effort needed for regeneration operations and enhances both the quality and efficiency of soil preparation and planting (Lazdinš and Matilla, 2012; Hjelm et al., 2019; Saarinen, 2006). Furthermore, stump removal can increase the survival rate of planted seedlings and promote

natural regeneration (Karlsson and Tamminen, 2013; Aosaar et al., 2020; Hyvönen et al., 2016).

As the available forestry workforce declines and the need for efficient post-harvest management increases, modern mechanization is becoming more prevalent (Ramantswana et al., 2020; He et al., 2021). One such advancement is the use of stump mulchers (Spinelli et al., 2016; Němečková, 2019). According to Willoughby et al. (2017), this method is effective in reducing populations of the pine weevil. Unlike stump harvesting, which removes the entire stump and root system from the site, stump grinding (or mulching) mechanically breaks the stump into wood chips that are left on the surface of the clear-cut area (Spinelli et al., 2016).

Stump grinding is an efficient method that provides various environmental and operational benefits (Angnes et al., 2021). This technique involves mechanically crushing tree stumps and leaving the fragmented material in place. The residues are small enough not to disrupt future operations and instead create favorable conditions for the establishment of new plant growth (Picchio et al., 2012; Berg, 2014). Moreover, the mulch left on the soil reduces the natural regeneration of unwanted tree species and debris. It also loosens the soil and mixes it with the crushed biomass, thereby increasing the organic matter content (Fontana et al., 2023; Novotný et al., 2012; Sanchez et al., 2003). One of the key advantages of mulching is the creation of a nutrient-rich layer from the residual biomass, which enhances soil biological activity (Stefani et al., 2023). The fragmented biomass increases the availability of essential nutrients such as nitrogen, phosphorus, and potassium (Remeš et al., 2016), while also improving the soil's physical properties, including its water-holding capacity (Tang et al., 2022; Wang et al., 2021). A 5–15 cm layer of crushed debris positively impacts soil temperature and moisture levels and supports natural nutrient cycling (Novotný et al., 2012). Additionally, mulching offers sanitary benefits by reducing coarse woody residues, which decreases the risk of infestations from pathogenic fungi and bark beetles (Modi et al., 2020; Piri et al., 2020; Aosaar et al., 2020; Jančařík, 1999). Unlike traditional methods where debris is either burned or left to decompose slowly, stump grinding followed by incorporating the biomass into the soil represents a more effective and sustainable management approach (Joslin et al., 2019).

The strong attraction of pine weevils to sawmills, where adults are often collected during their spring migration, highlights the role of VOC emissions from freshly processed wood in helping these insects find their hosts (Axelsson et al., 2017; Chen, 2021; Tudoran et al., 2021). This observation raises the question of whether similar effects occur in clear-cuts, where mechanically ground stumps may increase VOC emissions, thereby attracting migrating pine weevils.

1.4. Hypothesis and goals

Based on our previous field observations of higher pine weevil abundances in mulched clear-cuts, we hypothesized that grinding stump and slash increases the emission of volatile compounds that attract migrating pine weevils. Therefore, we expected to find a higher abundance of beetles in mulched clear-cuts compared to clear-cuts with retained stumps.

The goals of the experiment were: (i) to compare pine weevil abundance between mulched clear-cuts and clear-cuts with retained stumps; (ii) to determine whether this pattern is consistent across clear-cuts of different age categories (newly created and one-year-old sites); (iii) to examine seasonal changes in beetle abundance within a single season; and (iv) to investigate whether pine weevil abundance on mulched clear-cuts differs between coniferous and deciduous stands.

2. Materials and methods

2.1. Study sites and experimental design

The aim of the experiment was to compare the attractiveness of two

types of clear-cuts that employed different post-harvest management practices, with a focus on the occurrence of *H. abietis*. The first type involved mulched clear-cuts, in which the aboveground parts of stumps and slash were ground using a BFSP Stump and Depth Forest Mulcher, manufactured by Bugnot (Bugnot, 2018), later referred to as “grinding” in the figures (Fig. 1A, B). The second type consisted of clear-cuts with retained stumps, where the stumps were left intact after harvesting and the slash was removed manually, later referred to as “retained stumps or stumps” in the figures (Fig. 1C, D).

Besides the main hypothesis, we also aimed to test whether this pattern was consistent across clear-cuts of different age categories. Therefore, the experiment included both newly created clear-cuts [later referred to as “new clear-cuts” (harvested in the winter of 2024/2025 and mulched in February 2025)] and one-year-old clear-cuts [later referred to as “old clear-cuts” (harvested in the winter of 2023/2024 and mulched in February 2024)]. The same age categories were used for clear-cuts with retained stumps: older sites were harvested in the winter of 2023/2024, while younger sites were harvested in the winter of 2024/2025. In total, four experimental groups were established: (1) Grinding – new clear-cuts (Figs. 1A), (2) Grinding – old clear-cuts (Figs. 1B), (3) Retained Stumps – new clear-cuts (Figs. 1C), and (4) Retained Stumps – old clear-cuts (Fig. 1D). Each group consisted of 15 experimental plots, resulting in a total of 60 clear-cuts being monitored.

2.2. Study sites and their localization

The experiment was conducted in Central Bohemia, within the Brdy Highlands, the Czech Republic. The mean annual precipitation in this region ranges between 550–600 mm, and the mean annual temperature is 7–8 °C. The altitude of the plots ranged from 400 to 550 m a.s.l. (Culek et al., 2013). The vegetation is dominated by Norway spruce [*Picea abies* (L.) Karst.] (74 %), followed by Scots pine (*Pinus sylvestris* L.) (15 %), European larch (*Larix decidua* Mill.) (5 %), silver fir (*Abies alba* Mill.) (0.6 %), oaks (*Quercus* spp. L.) (2.5 %), European beech [*Fagus sylvatica* L.] (2.1 %), and other tree species (0.8 %) (Colloredo-Mannsfeld Ltd, 2023). The plots were distributed across the area between 49.7635469 N, 14.1417742E and 49.9391794 N, 13.7851617E.

To examine whether pine weevil numbers on mulched clear-cuts differ between coniferous vs. deciduous stands, both stand types were included in the experiment. A stand was classified as coniferous if conifers made up more than 90 % of its tree composition, and as deciduous if deciduous species accounted for more than 70 %. In total, the experiment included 38 coniferous stands and 22 deciduous stands.

The plot areas were relatively small, ranging from 0.13 to 0.61 ha.

The average stump density (based on 20 randomly chosen stumps on clear-cuts with stumps) was approximately 400 ± 168 stumps ha^{-1} , with a mean stump diameter of 32 ± 5.3 cm and a height of 12.3 ± 3.1 cm.

2.3. Pine weevil trapping

To monitor the number of pine weevils, pitfall traps were installed at each clear-cut site, following the designs outlined by Dvořáková et al. (2024) and Lalík et al. (2021). The traps were constructed from 1-liter plastic buckets (CIZOP, Dobříš, Czech Republic), which had holes (1 cm in diameter) drilled around the circumference just below the rim, allowing the beetles to enter. Each bucket was buried in the soil so that the holes were positioned just above the litter layer. Lids were placed on the buckets to prevent rainwater from getting inside. For attractants, artificial dispensers (Fytofarm, Bratislava, Slovakia) containing a mixture of $\pm\alpha$ -pinene (98 %) and ethanol/water (70:30; w/w) in a 1:3 ratio, with a total volume of 5 ml, were used. The dispenser tubes were attached to the underside of the trap lid with adhesive tape. To preserve the captured pine weevils, approximately 250 ml of a 4 % buffered formaldehyde solution (pH 7; Kulich, Hradec Králové, Czech Republic) was added to the bottom of each trap.

The pitfall traps were put in the clear-cuts at the end of April 2025. Five traps were installed at each site, arranged diagonally across the area with approximately 20-meter intervals to capture the spatial variability of pine weevil occurrence. The traps were inspected, and insects were collected once a month from June to August 2025, resulting in a total of three collections. This time frame was selected because pine weevils in the Czech Republic are most active from the end of April through August. The captured beetles were stored in plastic bags at -20 °C and were later counted manually.

2.4. Statistical analysis

All analyses were conducted using R (version 4.5.0; R Core Team 2025). The abundance of pine weevils was analyzed using generalized linear models (GLMs; MASS package, Ripley et al., 2013) or generalized linear mixed models (GLMMs; glmmTMB, Magnusson et al., 2017) employing a negative binomial distribution and a log link function, as preliminary Poisson models indicated strong overdispersion. The response variable was the number of beetles trapped per clear-cut. Explanatory variables varied based on the research question and included management type (Grinding and Retaining stumps), clear-cut age (New vs. Old), month of capture (June, July, and August), and



Fig. 1. Experimental clear-cuts with two types of post-harvest management (grinding vs. retained stumps) and two age categories. Displayed are: A) Grinding – new clear-cut, B) Grinding – old clear-cut, C) Retained stumps – new clear-cut, D) Retained stumps – old clear-cut.

forest type (Coniferous and Deciduous). Reference levels were set for the following: Grinding for post-harvest clear-cut management type, New clear-cuts for age, and Coniferous for forest type. The significance of model coefficients was evaluated using Wald tests (z-values) for GLMs and likelihood ratio tests for GLMMs.

To evaluate the impact of management practices and clear-cut age, as well as their interaction, we modeled total captures using a negative-binomial GLM that included management, age, and their interaction. We calculated estimated marginal means (EMMs) on the response scale using the emmeans function and performed pairwise comparisons between management types for each clear-cut age, adjusting the results with the Holm method. Since this represents the main outcome of the experiment, we also included a summary table with exact model estimates.

Seasonal changes in beetle numbers from June to August were analyzed separately for the mulched and stump-retained sites. We used negative-binomial GLMMs that included fixed effects for clear-cut age, month, and their interaction, along with a random intercept for site to account for repeated sampling. Differences among months within each clear-cut age were summarized using compact letter displays (CLD), which were derived from estimated marginal means with Holm adjustment.

The impact of forest type was analyzed only for mulched clear-cuts, comparing clear-cuts of coniferous and deciduous stands using negative-binomial GLMs. Pairwise comparisons were represented using CLD with Holm adjustment.

Data preparation was conducted using the dplyr package (Yarberry, 2021) and tidyr (Wickham, 2017). For statistical inference, we used the MASS package (Ripley et al., 2013), glmmTMB (Magnusson et al., 2017), emmeans (Lenth, 2018) for estimating marginal means and contrasts, and multcomp (Hothorn et al., 2016) for creating CLDs. Figures were generated with ggplot2 (Wickham, 2016). The graphs show either model predictions with 95 % confidence intervals or raw data represented as boxplots, with CLDs indicating significant groupings at $\alpha = 0.05$.

3. Results

3.1. Influence of post-harvest management and clear-cut age

The number of pine weevils was significantly affected by the type of post-harvest management in both new and old clear-cuts. In new clear-cuts, mulched sites (using grinding) had a significantly higher number of expected pine weevils compared to clear-cuts with retained stumps (Table 1, Fig. 2A). Conversely, in old clear-cuts, the result was reversed, with higher numbers of expected pine weevils in the clear-cuts that retained stumps compared to those that were mulched (Table 1, Fig. 2B).

3.2. Seasonal dynamics of pine weevil abundance

The influence of the month on pine weevil numbers was statistically significant for both management types and clear-cut ages (new clear-cuts: $\chi^2 = 31.41$ – 23.93 , $p < 0.001$; old clear-cuts: $\chi^2 = 18.20$ – 12.74 , $p \leq 0.01$; Fig. 3A, B). Pairwise comparisons revealed that the number of pine weevils was consistently higher in June than in July and August, with no significant differences between July and August.

Table 1

Predicted numbers of pine weevils (*H. abietis*) on clear-cuts of different ages: A) new clear-cuts and B) old clear-cuts, under two types of post-harvest management (grinding and retaining stumps). Within-age comparisons are expressed as incidence rate ratios (IRR = Grinding / Stumps) with 95 % confidence intervals. Significant differences are highlighted in bold (Wald z-tests, $p \leq 0.05$).

Clear-cut age	Comparison of managements	Estimate	95 % CI (lower)	95 % CI (upper)	z-value (z)	p-value (p)	Significance
New clear-cut	Grinding vs. Stumps	3.941	2.010	7.720	4.001	$p \leq 0.001$	***
Old clear-cut	Grinding vs. Stumps	0.313	0.155	0.630	-3.255	$p \leq 0.01$	**

3.3. Effect of stump grinding on pine weevil numbers in coniferous and deciduous stands

In mulched clear-cuts, there was no statistically significant difference in the number of pine weevils observed between coniferous and deciduous forest types in new clear-cuts ($\chi^2 = 1.09$; $p = 0.296$; Fig. 4A). However, in old clear-cuts, the forest type had a significant effect on the pine weevil numbers, with higher numbers recorded in coniferous stands ($\chi^2 = 5.21$; $p = 0.023$; Fig. 4B).

4. Discussion

Our study confirmed the main hypothesis that mulching increases the attractiveness of newly created clear-cuts to pine weevils. On these sites, we observed a significantly higher abundance of pine weevils in mulched clear-cuts compared to those with retained stumps. In contrast, on one-year-old clear-cuts, the number of pine weevils was lower in mulched areas. This finding suggests that stump grinding greatly enhances the short-term attractiveness of fresh clear-cuts, while this effect is not apparent in one-year-old sites. Although mulching is sometimes recommended as a method to reduce pine weevil damage by removing stumps, our results indicate that freshly mulched clear-cuts are highly attractive to these beetles. Therefore, this management strategy cannot be considered universally effective.

The significantly higher numbers of pine weevils on newly mulched clear-cuts can be attributed to the presence of wood chips and a significant amount of freshly damaged wood. Grinding the stumps greatly expands the evaporation surface, which in turn enhances the release of VOCs that attract these pests (Yuan et al., 2025; Duduman et al., 2009).

The contrasting pattern observed in older clear-cuts can be explained by the presence of stumps, which serve as a long-lasting breeding resource that keeps the populations of pine weevils high. Stumps support continuous reproduction, allowing new individuals to emerge and compensate for natural mortality (Nordlander et al., 2023). In contrast, mulching eliminates this breeding substrate, potentially resulting in lower populations on older mulched sites. Previous studies have also demonstrated that the removal of entire stumps can reduce the abundance of pine weevils in clear-cuts (Rahman et al., 2015, 2018; Piri et al., 2020). However, it is important to note that our experiment compared sites of two different age categories within a single season, and therefore, these results should not be interpreted as temporal changes within the same plots.

When examining seasonal population dynamics, the number of pine weevils was found to be highest in June, followed by a significant decline in July and August, with no differences between July and August. This trend was consistent across both management types and age categories, indicating that the main swarming event likely occurred earlier in the spring, probably in April or May (Skrzecz et al., 2021; Nordenhem, 1989). The observed decline in population was present in both mulched and stump clear-cuts, suggesting that the decrease cannot be attributed to changes in volatile signals from fresh wood. Ethanol, a primary attractant for pine weevils, is known to evaporate quickly (Fedele et al., 2007). Additionally, microbial decomposition can later produce more ethanol (Thakeow et al., 2007; Mäkinen et al., 2018). While similar processes occur in stumps, where decomposition can release ethanol and other VOCs, it is likely that these emissions are lower compared to those from freshly mulched materials. The biology of pine

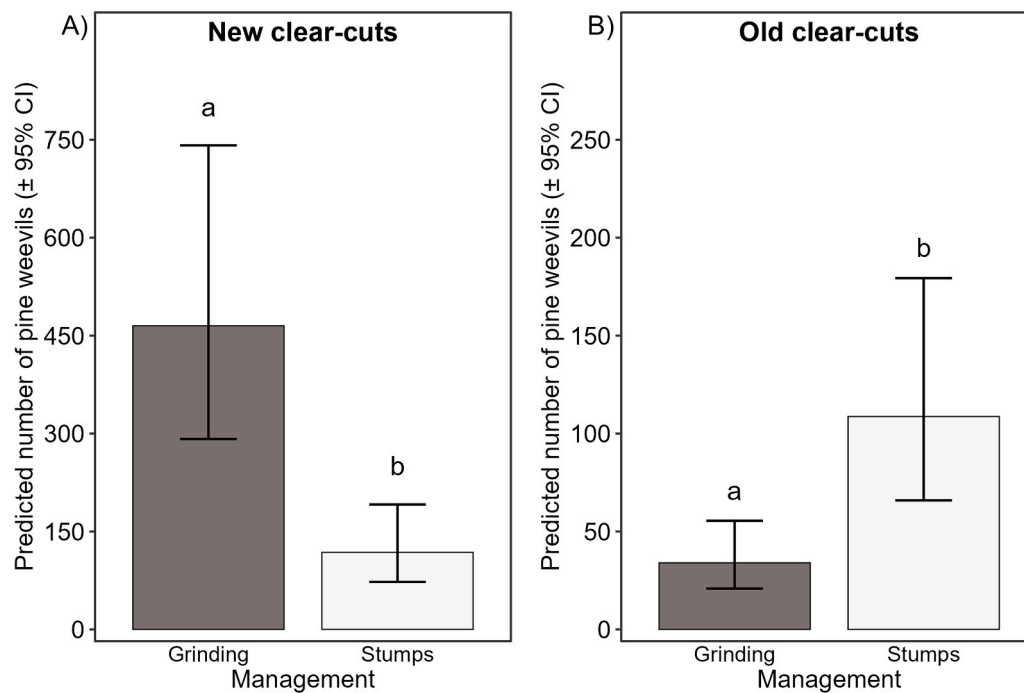


Fig. 2. Predicted number of pine weevils (*H. abietis*) on clear-cuts of different ages: A) new clear-cuts and B) old clear-cuts under two types of post-harvest management: grinding and retaining stumps (Stumps). Bars show estimated marginal means from a negative binomial GLM (log link), and vertical error bars represent 95 % confidence intervals on the response scale. Different letters above the boxes indicate statistically significant differences between management types within clear-cut ages ($p \leq 0.05$).

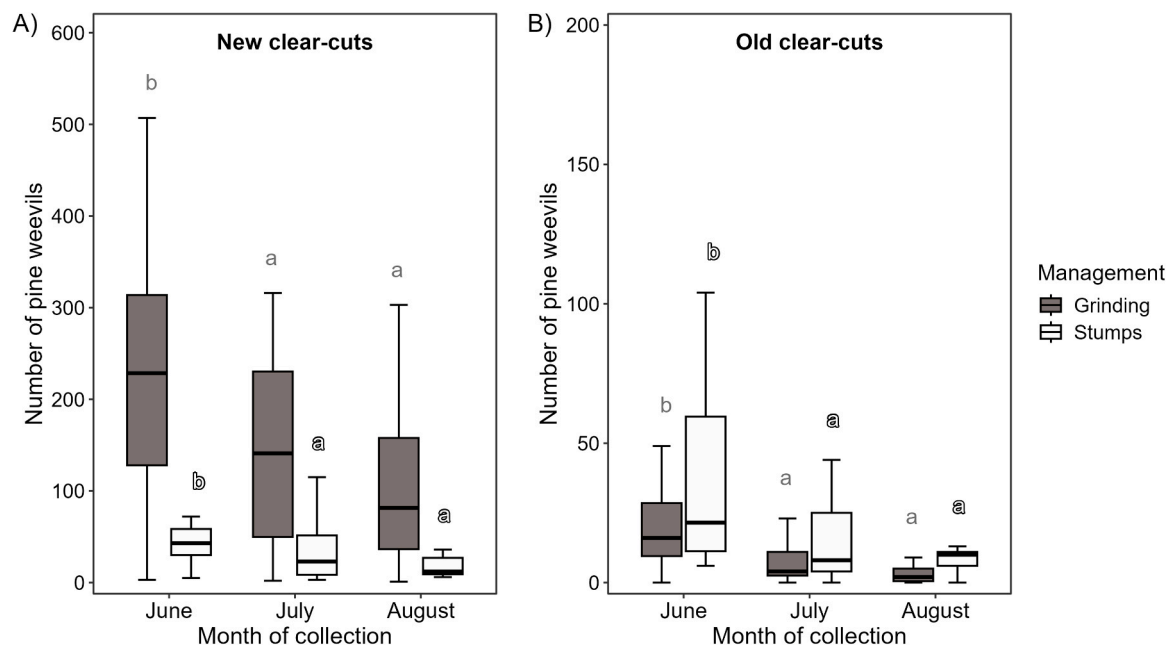


Fig. 3. Number of trapped pine weevils (*H. abietis*) on clear-cuts of different ages: A) new clear-cuts and B) old clear-cuts under two types of post-harvest management: grinding and retaining stumps (stumps), across three sampling dates (June, July, and August). Boxplots show the median (horizontal line), interquartile range (box), and whiskers extending to the most extreme values within $1.5 \times$ IQR; dots indicate outliers. Different letters above the boxes indicate statistically significant differences between months within years ($p \leq 0.05$).

weevils likely explains this observed pattern. Most individuals colonize clear-cuts early in the season, and as the season progresses, only limited immigration occurs, coupled with natural mortality that gradually reduces the population (Williams et al., 2013; Inward et al., 2012; Nordlander et al., 2017). These findings indicate that the attractiveness of clear-cuts to pine weevils is time-limited and tends to decline as the

season advances, regardless of the management type employed.

In forestry, stump grinding is mainly used as a method of site preparation before reforestation, as it helps facilitate subsequent planting (Löf et al., 2012; Klimek et al., 2020). Incorporating wood chips into the soil not only enhances short-term fertility but also reduces the growth of competing vegetation (Fontana et al., 2023; Gebretsadikan et al., 2023;

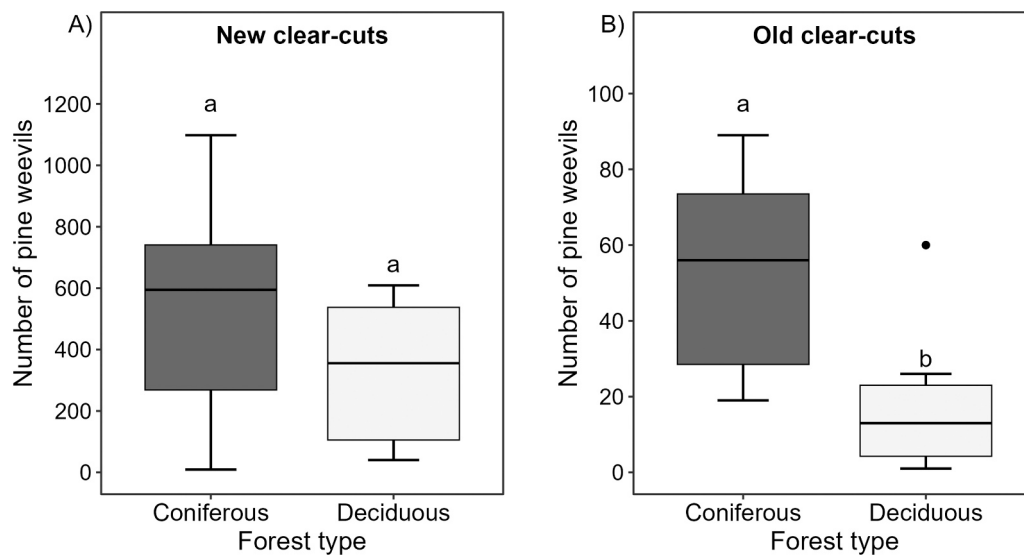


Fig. 4. Number of trapped pine weevils (*H. abietis*) on mulched clear-cuts, displayed for A) new clear-cuts and B) old clear-cuts, depending on the type of original stand (coniferous vs. deciduous). Boxplots show the median (horizontal line) and interquartile range (box); whiskers extend to the most extreme values within $1.5 \times \text{IQR}$, and dots indicate outliers. Different letters above the boxes indicate statistically significant differences between stand types ($p \leq 0.05$).

Pérez-Llorca et al., 2025). However, if reforestation is delayed by a year, the main benefits of mulching gradually decrease. By the following year, areas that have been clear-cut can quickly become overgrown with competing plants, which leads to intense competition for seedlings. This situation may need additional weeding or other vegetation control measures (Lesko et al., 2024; Yin et al., 2023; McCormick and Bowersox, 1981). Therefore, it is important to carefully consider these factors when making decisions about the timing of forest regeneration.

The timing of stump grinding plays a crucial role in determining how attractive clear-cut areas will be to pine weevils. If mulching is done in the spring, it leads to a significant release of ethanol and monoterpenes during the peak swarming period, which can increase colonization of these sites (Galko et al., 2022). Conversely, if mulching is postponed until after the swarming period, such as in the summer, the peak attractiveness occurs when most beetles are no longer searching for new sites (Leather et al., 2007). Therefore, from a management perspective, mulching should be scheduled to avoid the intense swarming period in order to minimize the risk of damage to seedlings.

In our study, we were unable to evaluate the overall impact of post-harvest management on seedling damage because the monitored clear-cuts were reforested with different tree species. It is well known that the intensity of damage caused by the pine weevil varies significantly among different seedling species (Leather et al., 2013; Doležal et al., 2021; Dvořáková et al., 2024). However, the higher abundance of beetles observed on mulched clear-cuts suggests a potentially higher risk of damage to seedlings compared to clear-cuts where stumps were retained, though this relationship was not directly examined in our study.

Although deciduous tree species generally contain fewer terpenes than conifers (Bao et al., 2023; Borsdorf et al., 2023; Granström et al., 2007) and are therefore expected to be less attractive to pine weevils, our study revealed that pine weevils were equally common in both coniferous and deciduous stands in newly mulched clear-cuts. The mechanical shredding of wood biomass generates a strong volatile signal that attracts beetles, even to clear-cuts in deciduous stands where they are typically not expected in large numbers (Fedele et al., 2007; Yuan et al., 2025; Wallertz et al., 2014). This suggests that stump grinding can temporarily increase the risk of pine weevil damage in deciduous stands, which is important because this pest has been shown to feed on deciduous seedlings, causing economically significant damage (Toivonen and Viiri, 2006; Manlove et al., 2013; Leather et al., 2013; Löf et al., 2004).

In contrast, in one-year-old mulched clear-cuts, we observed a significantly higher number of pine weevils in coniferous stands than in deciduous ones. This pattern is consistent with the species' preference for conifers and may indicate either dispersal away from deciduous areas or higher mortality in places where suitable hosts are less available. Together, these findings highlight the short-term, but strong attractiveness of mulched clear-cuts, even in deciduous stands, and underscore the importance of considering this risk in forest regeneration planning.

5. Conclusions

Our results show that stump grinding significantly increases the attractiveness of newly created clear-cuts to pine weevils. This increased attractiveness raises the risk of seedling damage, especially if stump grinding is conducted just before or during the main swarming period. In contrast, we observed that one-year-old, mulched clear-cuts had a lower population of pine weevils compared to sites with retained stumps. Furthermore, we found that freshly mulched clear-cuts were highly attractive even within mixed forest stands with a high proportion of deciduous trees. This suggests that the risk of pine weevil damage should not be overlooked in either coniferous or mixed forest regeneration efforts. From a management perspective, the timing of stump grinding is critical. Ideally, mulching should be done after the swarming period, with reforestation planned for at least the autumn of the same year or the following spring. Forest managers must carefully consider the purpose of mulching against the short-term risks associated with the increased attraction of freshly mulched clear-cuts to pine weevils.

CRedit authorship contribution statement

B. Dvořáková: Conceptualization, Investigation, Methodology, Data curation, Software, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration. **J. Hradecký:** Investigation, Data curation, Writing – review & editing. **M. Bledý:** Methodology, Writing – review & editing. **J. Holuša:** Supervision, Methodology, Conceptualization, Writing – original draft, Writing – review & editing, Validation, Funding acquisition.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to help with correcting English grammar and providing advice on R scripts. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of Competing Interest

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

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Data availability

Data will be made available on request.

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