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Temporal and species-dependent variation in foliar monoterpene compositions of conifers under spruce budworm defoliation

Sirine Boubeker^{1*}, Mathieu Cusson¹, Emma Despland², Éric Bauce³, Lionel Ripoll¹, Andre Pichette¹, John MacKay⁴ and Annie Deslauriers¹

Abstract

Key message No induced herbivory effect under defoliation by Eastern spruce budworm (*Choristoneura fumiferana* Clemens) was detected in young conifer saplings, but a marked difference among species—balsam fir (*Abies balsamea* (L.) Mill.), white spruce (*Picea glauca* [Moench] Voss), and black spruce (*Picea mariana* [Mill.])—in monoterpene compositions.

Context Terpenoids are among the most common defensive compounds in conifers, found in foliage, wood, and bark. Induction of terpenoids by chewing folivorous insects is less understood than stem-feeding xylophagous insects.

Aims This study examined whether three conifers —balsam fir (*Abies balsamea* (L.) Mill.), white spruce (*Picea glauca* [Moench] Voss), and black spruce (*Picea mariana* [Mill.])— induce terpenoid variation in response to Eastern spruce budworm (*Choristoneura fumiferana* Clemens) defoliation.

Methods A total of 360 4-years-old saplings from three coniferous species were grown in a greenhouse for a 2-years-long experiment, with half exposed to defoliation and half as controls. Buds and larvae phenology were monitored, and foliage was collected throughout the growing season. Terpenoid compounds from needles were analysed using static headspace gas chromatography (HS-GC). Statistical analyses were performed on the 14 most common compounds that were found.

Results A total of 43 terpenoids were identified, of which 67% were monoterpenes. Defoliation did not significantly alter monoterpene composition, except in *Picea glauca* in 2015, where differences were mainly driven by eucalyptol and β -myrcene. Species identity thus accounted for major variation (26%) in monoterpene profiles. Monoterpene composition also varied with phenological stages, reflecting developmental changes in foliar chemistry during shoot growth.

Conclusion Although defoliation by the eastern spruce budworm impacted bud phenology and shoot growth, monoterpene pool composition was not significantly affected by defoliation. Species identity accounted for the largest portion of the variation in monoterpene composition, with specific compounds distinguishing each conifer species.

Keywords *Choristoneura fumiferana*, Terpenoids, Monoterpenoid profiles, Induced defence

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*Correspondence:

Sirine Boubeker

sboubeker@etu.uqac.ca

Full list of author information is available at the end of the article



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1 Introduction

In plants, secondary metabolites, which are also called plant specialised metabolites, are crucial in maintaining organismal homeostasis, particularly with respect to protection against herbivores (Singh and Singh 2021). Secondary metabolites include terpenoids and many other molecular classes. They affect herbivorous insects in different ways, by acting as signalling molecules, feeding deterrents, and toxins (Koul 2008; Erb et al. 2012; Fürstenberg-Hägg et al. 2013). The production and profiles of secondary metabolites are influenced by multiple environmental and genetic factors, resulting in a vast array of chemical compounds within different molecular classes (Sampedro et al. 2010; Ramawat and Goyal 2020; Zhan et al. 2022).

In terms of quantity and diversity, most secondary metabolites in conifers are composed of terpenoid compounds (Kopaczky et al. 2020). Terpenoids, which are also known as terpenes or isoprenoids, are organic molecules that are composed of isoprene units. Structural subcategories are distinguished according to the number of isoprene units that they contain: monoterpenes (two units), sesquiterpenes (three units), diterpenes (four units), and so on. In plants, terpenoid production takes place mainly in microsomes and the cytoplasm through the mevalonic acid (MVA) pathway, and in chloroplasts by the methylerythritol phosphate (MEP) pathway (Kasahara et al. 2002; Tarkowská and Strnad 2018). These biochemical pathways are directly involved in conifer host-insect herbivore relationships, where some intermediates or products are toxic to herbivores. Indeed, this is the case for α -pinene and δ -3-carene, which incur mortality in late-instar Eastern spruce budworm (*Choristoneura fumiferana* Clemens) at concentrations that are naturally present in *Abies balsamea* (L.) Mill. (Kumbaşlı and Bauce 2013).

Some conifer species within *Picea* and *Abies* produce, and then following their biosynthesis, store terpenoids within resin ducts and resin blisters in the cambial zone and the bark of the trees (Abbott et al. 2010; Kopaczky et al. 2020). These compounds increase in response to attacks by xylophagous stem-feeders (e.g., Cerambycidae, Scolytinae). Conifers under stress can also increase emissions of volatile monoterpenes and isoprene from their foliage (Blanch et al. 2009; Kopaczky et al. 2020). Yet, stress responses and emission profiles differ according to the nature of the stress and its intensity. Both the geographical location and provenance of trees have also been demonstrated to influence the terpenoid diversity that is observed in trees, both under stress and in its absence (Manninen et al. 2002; Martz et al. 2009; Clark et al. 2010; López-Goldar et al. 2019).

Defoliation that is caused by spruce budworm outbreaks represents a major perturbation in eastern North

American forests (MacLean 2016). It elicits multiple adverse effects in host conifers, including reduced carbon allocation and radial growth (Deslauriers et al. 2015; Fierravanti et al. 2019; Subedi et al. 2023) that in turn reduces wood quality (Paixao et al. 2019), potentially leading to tree mortality (Colford-Gilks et al. 2012; MacLean 2016). Defoliation thus requires physiological adaptations for host maintenance and survival (Deslauriers et al. 2019; Beaulieu et al. 2020), but evidence for induced defences in response to budworm defoliation is equivocal. One study showed that mono- and sesquiterpene compounds decreased in *A. balsamea* needles with increasing levels of budworm defoliation (Deslauriers et al. 2015). In contrast, LeClair et al. (2015) observed that spruce budworm defoliation triggered the release of terpenes as volatile organic compounds (VOC), indicating an active defence response. Likewise, Litvak and Monson (1998) noted an increase in terpenoid production activity as a result of herbivory by tiger moth larvae (*Halisdota ingens*) in the western North American pines, spruce, and firs (*Pinus ponderosa* Douglas ex C. Lawson, *Pinus contorta* Douglas subsp. *latifolia* Engelm., *Picea engelmannii* [Parry] Engelm., *Abies concolor* [Gordon & Glend.] Lindl. ex Hildebrand) that were studied. The extent to which conifers adjust their chemical defences in response to defoliation thus remains unclear. The variability in chemical responses could be influenced by several factors, including species, foliage age, and needle developmental stage.

Fir and spruce needles contain a variety of chemicals that vary depending upon many factors, including needle age. The composition of foliar terpenoids changes during shoot elongation and needle growth (von Rudloff 1972, 1975; Despland et al. 2016; Lirette and Despland 2021). Needle growth affects foliar chemical composition, which in turn affects budworm growth (Carisey and Bauce 1997a; Clancy et al. 2004; Nealis and Nault 2005). Spruce budworm also exhibits feeding preferences, showing a clear preference for *A. balsamea*, with higher defoliation rates when trees are more mature (70 vs. 30 years) (Bauce et al. 1994). It feeds mainly on new annual shoots, but may also consume older foliage. All of this is closely linked with the foliar composition, including the content of monoterpenes, tannins (Bauce et al. 1994; Ennis et al. 2017), and nitrogen (Albert and Bauce 1994). The composition of certain monoterpenes in foliage under defoliation by spruce budworm has been identified (Bauce et al. 1994; Carisey and Bauce 1997b), but a comprehensive analysis of all terpenoids, particularly those found in young seedlings, remains lacking.

Some monoterpenes, including α -pinene, δ -3-carene and bornyl acetate, are recognized for their detrimental

impacts on eastern spruce budworm larvae growth and budworm survival, which is contingent upon their concentrations (Kumbaşlı and Bauce 2013). In mixtures, monoterpenes have been shown to possess properties that are toxic to eastern spruce budworm larvae, resulting in their reduced growth and mortality at concentrations that are naturally present in host conifers (Kumbaşlı et al. 2011; Ullah et al. 2024). Understanding the nuanced chemical interactions between the eastern spruce budworm and its host trees sheds light on the intricate dynamics governing their relationship.

This paper characterises the early seasonal pattern of terpenoids in white spruce (*Picea glauca* [Moench] Voss), black spruce (*Picea mariana* [Mill.]), and balsam fir (*Abies balsamea* (L.) Mill.), which is the main host of spruce budworm. The objectives of this study were to verify whether there is an induced defence in conifers due to defoliation by spruce budworm, and whether terpenoid compounds varied among these species and how they are influenced by the age of the foliage (newly growing versus mature), defoliation, and the timing of sampling. To achieve these goals, we tested the hypothesis that the composition of terpenoids in developing buds and expanding new foliage differs among species and, in turn, is influenced by defoliation and shoot phenology (stage of growth), with potential evidence of induced responses.

2 Material and methods

The experiment ran from spring to autumn, 2015 and 2016 in a greenhouse that was located at the University of Quebec at Chicoutimi, Québec, Canada (Deslauriers et al. 2019). Three boreal conifer species that were considered to

be hosts of spruce budworm were used: *Picea glauca*, *Picea mariana*, and *Abies balsamea*. A split-split plot experimental design of 360 saplings (120×3 species) was set up in 2015 (for a full description of the design, see Deslauriers et al. 2019) using 4-years-old saplings. Three adjacent greenhouses, each containing two tables, were used for the experimental design. Therefore, saplings were placed on six tables containing a total of 60 plants (20 plants×3 species). The control and defoliated saplings were placed on two separate tables (7.94 m×1.83 m) and enclosed by a white netting (Proteknet 60 g, mesh size of 1.9 mm×0.95 mm), supported by a wooden frame (7.30 m×1.83 m) to prevent movement of larvae and moths between treatments.

About sixty larvae of diapausing spruce budworm (Standard code Glfc:IPQL:Cfum01 to Cfum16; Roe et al. 2018) were placed on saplings during spring (25–28 May 2015). Larvae were placed in groups of fifteen individuals upon apical and lateral branches once more than 50% of buds had reached stage 3 (refer to the bud phenology description, Fig. 1) for each species. In July 2015, the females laid eggs, which subsequently hatched into first-instar larvae by the end of the summer. The control treatment remained undefoliated (for details, see Deslauriers et al. 2019). All saplings were transferred to an open field in mid-September and exposed to natural winter conditions until the following spring (i.e., between 19 September 2015 and 4 May 2016). Larvae on defoliated saplings overwintered within hibernacula on the trees, and second-instar larvae (L2) emerged naturally in the spring of 2016. Consequently, no additional L2 larvae were intentionally introduced in 2016. Thus, defoliated saplings had experienced two seasons of defoliation, in both 2015 and 2016.

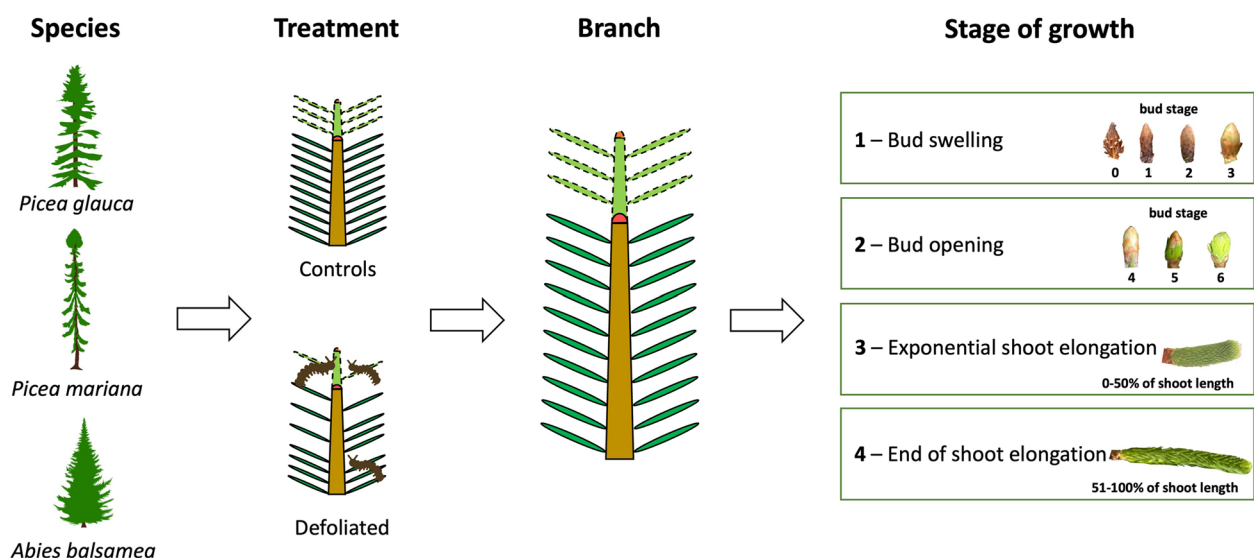


Fig. 1 Overview of experimental conditions and sampling design. General setup of the 2-years-long experiment under greenhouse conditions, including defoliation treatments and plant species. Definition of the stage of growth according to the bud stages

2.1 Branch sampling, phenological, and growth measurements

Foliage was sampled on four dates during the growing season. In 2015, these corresponded to Day-of-Year (DOY) 140, 154, 168, and 176; in 2016, DOY 127, 141, 155, and 169. For each sampling date that follows, branches were collected on 36 saplings for terpenoid measurements (6 of each from the two defoliation treatments among the three greenhouses). In 2015, only expanding foliage was sampled, while mature foliage (from 1- to 3-years-old needles) was also sampled in 2016, from the same branches as the expanding foliage (Fig. 1). Stage of growth (SG), corresponding to both phenological stage and growing foliage, was defined to account for growth and phenological differences between the two years (Fig. 1).

The stages of growth were defined according to bud opening phases (Dhont et al. 2010) and shoot elongation (Fig. 1): (1) bud swelling, corresponding to phase 0–3 (open bud with a lightly colored tip, elongated bud, with scales stretching and swollen bud with smooth scales); (2) bud opening, corresponding to phase 4–6 (translucent bud, bud broken, exposed shoot and spread needles); (3) exponential shoot elongation, corresponding to <50% of total shoot length; (4) end of shoot elongation, corresponding to >50% of total shoot length. Those stages were recorded every 3–4 days during the experiment on 72 plants (12 plants $\times$ 3 species $\times$ 2 treatments). Shoot elongation was measured by the length of two lateral shoots using an electronic caliper (resolution: 0.1 mm). Simultaneously, the larval instar (phenological stage of development from 2 to 6) of spruce budworm was determined (Bouzidi et al. 2019) on three randomly chosen larvae per sapling belonging to the defoliated treatment.

2.2 Terpenoid analyses

Once collected, the needles were separated from the twigs and flash-frozen in liquid nitrogen (-196°C) to stop all enzymatic activities and then stored at -20°C . The needles were then cut into small pieces to obtain a mass of 50 mg in vials, which were sealed and stored in the freezer. The analysis of terpenoids was conducted using static headspace gas chromatography (HS-GC) (Caron et al. 2013), within 6 months of the harvesting of the needles. To maintain repeatability, each sample was prepared in a 10 ml HS-vial and analysed twice. Samples were analysed with an Agilent 7890C GC system coupled to an Agilent PAL autosampler (headspace module), using helium as the carrier gas (flow 1 ml/min). The column that was used was a DB-5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μm ; Agilent Technologies), and a MS detector 5975C model XL-EI/CI triple AXIS was used consistent with analyses conducted by Caron et al. (2013). Samples were incubated at 80°C for 15 min. The temperature of the injector and needle were maintained respectively at 250°C and 90°C ; the injection volume was 500 μl

in split-less mode (for further details, see Caron et al. 2013). The temperature was set at 40°C for 2 min, then ramped up by $2^\circ\text{C}/\text{min}$ and held at 135°C for 15 min. These incubation conditions, adapted from Caron et al. (2013), were selected to promote reproducible partitioning of terpenoids between the needle matrix and the headspace. All samples were subjected to identical equilibration time, temperature, and injection parameters. Each sample was analysed in duplicate to ensure analytical repeatability. The use of GC–MS allowed reliable identification of compounds based on retention times and mass spectra, using both the internal LASEVE laboratory of the University of Quebec at Chicoutimi (UQAC) database and a commercial database. Separation was performed on a nonchiral column, and therefore individual enantiomers of chiral monoterpenes were not resolved. As a result, reported compounds represent combined enantiomeric forms rather than enantiomer-specific profiles. Given the number of terpenoids that were identified (Table 1), a semi-quantitative index (the percentage of the mass spectra, %) was used for statistical comparisons.

2.3 Statistical analyses for terpenoids

Since many of the terpenoid compounds were only observed in some samples or they were near to the detection level, fourteen out of 43 identified compounds were retained for further comparison (see below). The compounds were α -pinene, β -myrcene, β -phellandrene, β -pinene, borneol, bornyl acetate, camphene, camphor, δ -3-carene, eucalyptol, fenchyl acetate, limonene, sabinene, and tricyclene. They were selected based on their occurrence in more than 10% of the samples. Therefore, only the most common compounds were retained across factors. We included fenchyl acetate because it was present only in *P. mariana* and considered important for that species. Few samples ($n=5$) were excluded since they showed extreme values. Averaged values (percentage of the mass spectra, %) from two trials per sample were used in the analysis.

Change in the terpenoid compounds (the percentage of the mass spectra, %) was analysed across species (fixed factor, 3 levels: *P. glauca*, *P. mariana*, and *A. balsamea*), defoliation treatments (fixed factor, 2 levels: defoliation with spruce budworm and control), years (fixed factor, 2 levels: 2015 and 2016), and stages of growth (fixed factor nested in year, 4 levels; see above categories and Fig. 1) using permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2008, with 999 permutations). Euclidean distance matrices were calculated on normalized data. A similar analysis was also performed for 2016 data only, which includes species, stage of growth, and foliage age (fixed, 2 levels: expanding and mature foliage). The age factor was nested within an additional random factor that considered the effects of each tree. When significant differences were found,

Table 1 Names of the main terpenoids (43 listed) that were found in foliage of spruces and fir. The fourteen compounds that were selected for PERMANOVA are shown in bold

Type of compounds	Compounds	Abbreviation
Monoterpene (67.4%)	α -Pinene oxide	α -pin-ox
	α -Phellandrene	α -phell
	α-Pinene	α-pin
	α -Terpineol	α -terpin
	β-Myrcene	β-myrc
	β-Phellandrene	β-phell
	β-Pinene	β-pin
	Borneol	borneol
	Bornyl acetate	bor-acet
	Camphene	cam
	Camphene hydrate	cam-hyd
	Camphor	camphor
	Citronellol	citron
	δ -2-Carene	δ -2-car
	δ-3-Carene	δ-3-car
	δ -3-Carene-4-ol	δ -3-car-ol
	Eucalyptol	eucal
	Fenchone	fenchone
	Fenchyl acetate	fench-acet
	γ -Terpinene	γ -terpin
	Limonene	lim
	Linalool	linalool
	Nerol	nerol
	Piperitol	piperitol
	Piperitol acetate	acet-pip
	Sabinene	sab
	Sabinol	sabinol
	Terpinolene	terpin
	Tricyclene	tricyc
Diterpene (2.3%)	Neophytadiene	neophyta
Sesquiterpene (30.2%)	α -Bisabolene	α -bis
	α -Caryophyllene	α -caryo
	α -Himachalene	α -hima
	α -Longipinene	α -longi
	β -Bisabolene	α -bisa
	β -Cadinene	β -cadi
	β -Caryophyllene	β -caryo
	β -Cubebene	β -cub
	β -Elemene	β -elem
	δ -Cadinene	δ -cadi
	Hexahydrofarnesol	hexa
	Longifolene	longifolene
	Zonarene	zonarene

pairwise post hoc permutational *t*-tests (999 runs) were used to identify differences among levels.

We visualised the effects of the treatments using principal component analysis (PCA) ordinations. We evaluated the contribution of each variable to the observed distance between treatments using a similarity percentage analysis (SIMPER). Analyses were run using PRIMER+PERMANOVA v.7.0.23 (PRIMER-E Ltd., Auckland, New Zealand). We used a significance level of $\alpha=0.05$ for all

statistical analyses, but *P* values that were marginally higher than 0.05 were carefully considered. No corrections to the significance level in our multiple comparison tests were applied.

2.4 Statistical analyses for phenology

The average date ($\bar{x}$) and standard deviation ($s_{\bar{x}}$) at which the E_i stage occurred were calculated by using a frequency table:

$$\bar{x} = \frac{\sum_{i=1}^k f_{E_i} \times x_i}{n}$$

$$s_{\bar{x}} = \sqrt{\frac{\sum_{i=1}^k (x_i - \bar{x})^2}{n - 1}}$$

where x_i is the date expressed in day, f_{E_i} is the frequency of the E_i stage of bud ($i=1$ to 6) or instar ($i=2$ to 6) development, and k is the number of the sampling date (adapted from Scherrer 2007). Non-parametric logistic models were then used to infer differences in bud phenology between treatments (control versus defoliated) and species (*A. balsamea*, *P. glauca*, and *P. mariana*) using the *Logistic* procedure in SAS (version 9.4, SAS Institute, Inc., Cary, NC, USA). This procedure produces logit probability models, where the explanatory variable (phenological stage) was considered as a qualitative nominal value, and treatment and species were considered as fixed effects (Deslauriers et al. 2019). Differences between treatments and species were identified through least-squares means (*LSMEANS* option) using a Bonferroni multiple test comparison ($P < 0.05$, *Logistic* procedure, SAS).

For lateral growth (mm), mixed models (*Mixed* procedure, SAS) with repeated measurements (Day-of-Year) were used to characterise the effect of species and treatments, which were considered as fixed variables, while blocks and trees were considered as random variables. Normalized residuals were calculated with the *VCIRY* option to examine their distribution and detect extreme values. Differences between treatments and species were determined using the *LSMEANS* option of the *Mixed* procedure, together with Bonferroni-adjusted multiple comparison tests.

3 Results

3.1 Phenology and defoliation

Bud and shoot phenology progressed consistently across species, with significant inter-annual differences (Appendix Result; Tables 6, 7). In 2016, defoliated trees showed earlier bud break and shoot elongation than controls, while spruce budworm development was also advanced and more synchronised with host bud stages (Appendix Result; Tables 6, 7; Figs. 5, 6). All trees that were subjected to the defoliation treatment exhibited extensive needle loss on developing shoots (Fig. 2). Yet, higher levels were observed in 2015

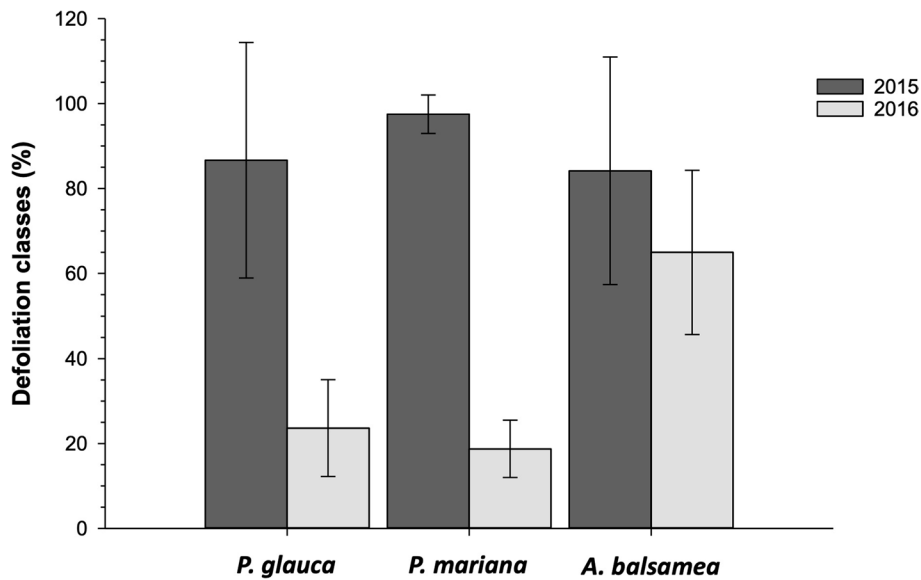


Fig. 2 Mean defoliation observed in 2015 and 2016 on the growing shoots of *Picea glauca*, *Picea mariana*, and *Abies balsamea*. The vertical bars show the standard error

compared with 2016. In 2015, the level varied between 80% and nearly 100% defoliation of the new shoot in all species. In 2016, a lower degree of defoliation was observed on spruce species (~15 to 20%), while *A. balsamea* showed a higher percentage, reaching up to 60% defoliation (Fig. 2).

3.2 Terpenoid compounds

A total of forty-three major chemical compounds were determined by static headspace gas chromatography (HS-GC), which are listed in Table 1. Most compounds that were found belong to the class monoterpene (29 of 43), while the others were classed as sesquiterpenes (13

of 43), with one diterpene (Table 1). Within the terpenoid classes, some are present in a linear form like β -myrcene, citronellol, and linalool, or in a cyclic form like α -pinene, borneol, and eucalyptol. Several compounds that were detected were derived terpenes with additional functional groups, such as bornyl acetate and fenchyl acetate.

3.3 Effect of species, stage of growth, and defoliation in the newly growing foliage

In the newly growing foliage, differences in the composition of terpenoids were found among species, years, and stage of growth (Table 2). The interaction between

Table 2 Summary of PERMANOVA showing the effects of species, year, defoliation treatment, and the stage of growth (nested in year) on the terpenoid profile of new expanding foliage in both 2015 and 2016. Significant values ($P < 0.05$) are shown in bold

Source of variation	df	Pseudo-F ratio	P	Variance explained (%)
Species	2	31.86	0.001	25.9
Year	1	8.77	0.001	10.8
Defoliation	1	1.61	0.116	3.3
Stage of growth (year)	6	2.87	0.001	8.3
Species × year	2	1.49	0.121	4.6
Species × defoliation	2	1.28	0.199	3.7
Species × stage of growth (year)	11	1.74	0.010	8.5
Year × defoliation	1	1.18	0.290	2.5
Defoliation × stage of growth (year)	4	1.00	0.485	0
Species × year × defoliation	2	1.20	0.294	4.4
Species × defoliation × stage of growth (year)	4	0.96	0.527	0
Residual	223			29.0
Corrected total	259			

species and the stage of growth was also significant, meaning that the composition of terpenoids changes between stages of growth according to species ($P=0.010$; Table 2). The species effect explained most of the variance in the model (25.9%; Table 2), followed by the sampling year (10.8%; Table 2). The variance that was explained by the stage of growth alone (8.3%; Table 2) or by its interaction with species (8.5%; Table 2) highlighted the changes occurring in terpenoid compounds during bud and shoot development. The effect of defoliation and its interaction with the other factors, however, was not significant with respect to the terpenoid profile (Table 2).

Several terpenoids explained the significant difference between species in the newly growing foliage (Table 3, Table 8 in Appendix; Fig. 3). As shown in Fig. 3, the first axis (PC1), which explained 26.6% of the total variance, divides the compounds characterising *A. balsamea* from the two spruce species. The second axis (PC2), explaining 14.6%, mainly divides *P. mariana* from *P. glauca* (Fig. 3). Therefore, the separation between the species was clear with few samples overlapping between the species. The compounds characterising *A. balsamea* included β -pinene, β -phellandrene, sabinene, and δ -3-carene. On one hand, compounds that most clearly defined *P. mariana* were camphene, bornyl acetate, tricyclene, and α -pinene. On the other hand, compounds such as camphor, β -myrcene, limonene, and eucalyptol characterised *P. glauca*.

Differences that were observed in compounds between pairs of species were identified by SIMPER as shown in Table 3 and Table 8 in Appendix. Five compounds helped

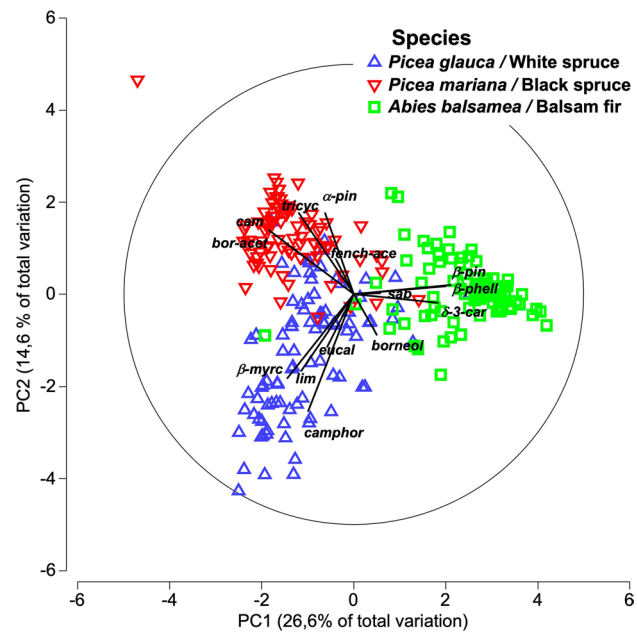


Fig. 3 Principal component analysis (PCA) illustrating the different terpenoid compounds, according to three conifer species. The first two axes were represented with their relative variance contribution explained (%). See Table 1 for compounds abbreviations

Table 3 Summary of SIMPER for terpenoid composition in different groups. Only compounds responsible for differences between pairs of species were reported (cut-off for low contributions limited to 50%)

Compounds	<i>P. glauca</i> vs. <i>P. mariana</i>	<i>P. glauca</i> vs. <i>A. balsamea</i>	<i>P. mariana</i> vs. <i>A. balsamea</i>
α -Pinene			
β -Myrcene	11.42		
β -Phellandrene		10.03	11.15
β -Pinene		10.76	11.52
Borneol			
Bornyl acetate			10.10
Camphene			10.52
Camphor	16.30	9.51	
δ -3-Carene		9.55	10.31
Eucalyptol	12.76		
Fenchyl acetate			
Limonene		8.34	
Sabinene		8.15	
Tricyclene	14.90		

to discriminate between *A. balsamea* and *P. mariana*, which together represent 10% and 12% (Table 3) of the selected terpenoids (14 of 43 identify compounds, see “2”): these included β -phellandrene, β -pinene, and δ -3-carene. These last compounds helped also to differentiate *A. balsamea* from *P. glauca*, together with three other compounds, i.e., camphor, limonene, and sabinene. Camphor is the compound that most clearly distinguishes *P. glauca* from *P. mariana* (16.30%; Table 3). Other compounds that were identified to distinguish *P. mariana* from *P. glauca* are tricyclene, eucalyptol, and β -myrcene (Table 3).

When compound compositions in the newly growing foliage were analysed within each species, the effect of year was significant in the three species and explained between 32 and 39% of all variances (Table 4a, b, c). In *P. mariana*, β -myrcene, tricyclene, bornyl acetate, and fenchyl acetate collectively explained up to 64% of the difference between the two years; all these compounds are more abundant in the year 2015 than in 2016 (Table 9a in Appendix). For *P. glauca*, as shown in Fig. 4, β -myrcene, limonene, and camphor were the most important, explaining up to 64% of the difference between years (Table 10c in Appendix; Fig. 4C), also with an abundance higher in 2015 for every compound. For *A. balsamea*, yearly difference was explained, in contrast, by up to 53% contributed by α -pinene, δ -3-carene, and borneol (Table 11 in Appendix). The α -pinene was more abundant in year 2016, δ -3-carene was

Table 4 Summary of PERMANOVA showing the effects for each species, of year, defoliation treatment, and the stage of growth (nested in year) on the terpenoid profile. In this analysis, only expanding foliage was considered in both 2015 and 2016. Significant values ($P < 0.05$) are shown in bold

Source of variation	df	Pseudo-F ratio	P	Variance explained (%)
a. <i>P. mariana</i>				
Year	1	15.66	0.001	32.3
Defoliation	1	0.58	0.764	0
Stage of growth (year)	5	2.43	0.002	16.2
Year × defoliation	1	1.83	0.097	10.7
Defoliation × stage of growth (year)	2	1.55	0.150	11.8
Error	30			29.0
Corrected total	40			
b. <i>P. glauca</i>				
Year	1	10.97	0.001	34.1
Defoliation	1	1.53	0.135	6.3
Stage of growth (year)	5	1.47	0.108	10.8
Year × defoliation	1	2.49	0.009	15.1
Defoliation × stage of growth (year)	1	0.31	0.959	0
Error	38			33.7
Corrected total	46			
c. <i>A. balsamea</i>				
Year	1	8.11	0.001	38.9
Defoliation	1	0.62	0.700	0
Stage of growth (year)	5	1.31	0.204	13.9
Year × defoliation	1	0.91	0.475	0
Defoliation × stage of growth (year)	1	0.64	0.619	0
Error	27			47.2
Corrected total	36			

approximately in the same abundance, and borneol was more important in year 2015.

In the case of *P. mariana*, the effect of stage of growth was significant (Table 4a). In the first growth stage (SG 1, from closed to swelling buds), many compounds had higher concentrations compared with the later stage of growth, especially bornyl acetate, tricyclene, fenchyl acetate, and β -myrcene (Table 9b in [Appendix](#)). During the second stage of growth (SG 2, from bud swelling to bud opening), the compounds camphene, bornyl acetate, β -myrcene, α -pinene, and tricyclene were more abundant. Yet, camphene, α -pinene, tricyclene, and bornyl acetate were less abundant in SG 2 than in the other stages of growth. In contrast, β -myrcene was more abundant in SG 2 than in SG 3 (exponential shoot growth) (Table 9b in [Appendix](#)). During exponential shoot growth (SG 3), α -pinene was more abundant compared to other stages of growth. Indeed, a decrease in the abundance of fenchyl acetate was found between SG 1 and SG 3. Camphene and bornyl acetate increased in abundance in SG 3 compared to SG 2. However, while camphene was slightly less

abundant in SG 4, bornyl acetate showed manifestly lower levels in both SG 1 and SG 4 (Table 9b in [Appendix](#)).

No analyses indicated a significant main effect of spruce budworm defoliation on the terpenoid profile. A significant interaction was observed only in *P. glauca* between defoliation and year, explaining 15% of the variation (Table 4b; Fig. 4C), further suggesting that defoliation only affected terpenoid composition in 2015. For *P. glauca* (Fig. 4C), the first axis (PC1), explaining 53.6% of the total variance, indicates the year effect. In 2015, the second axis (PC2), explaining 11.6%, divides the effect of defoliation from control. Spruce budworm defoliation in *P. glauca* increased the percentage of β -myrcene and reduced the percentage of eucalyptol. Together these two compounds explained up to 50% of the differences between both defoliation treatments (Table 10a in [Appendix](#)).

3.4 Differences between newly growing foliage and mature needles for each species

In all species, the composition of the terpenoid profile was affected by the age of foliage, i.e., newly developing foliage

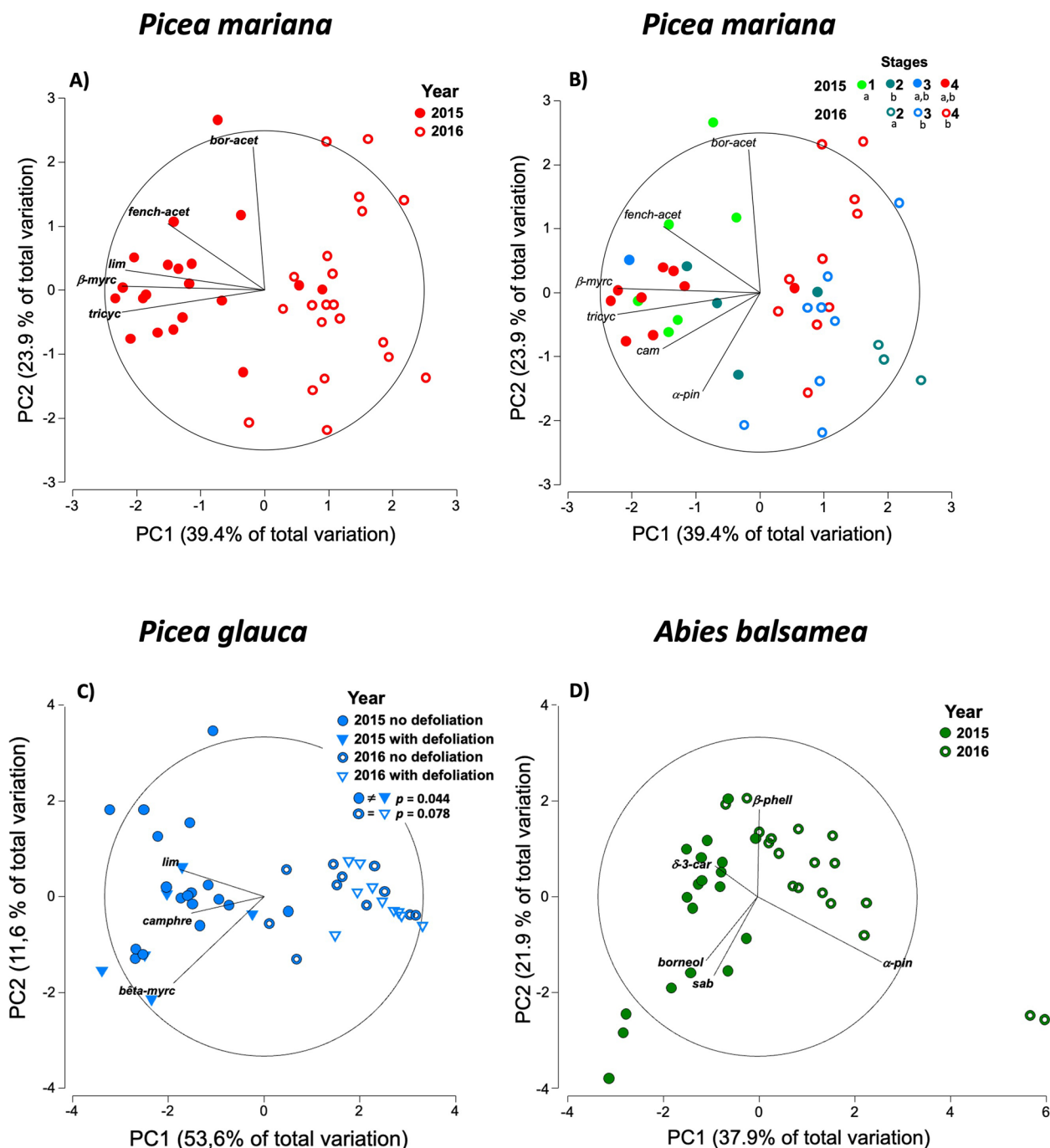


Fig. 4 Principal component analysis (PCA) illustrating the different terpenoid compounds, according to *Picea mariana* **A** between years and **B** stage of growth; *Picea glauca* **C** between years and defoliation; and *Abies balsamea* **D** between years. According to the factors illustrated here, only compounds with high contributions (%) that were detected using SIMPER were presented. The first two axes were represented with their relative variance contributions explained (%). See Table 1 for compounds abbreviations

versus 1- to 3-years-old needles (Table 5). Such effects vary according to species and explained up to 26% of variability in compounds across species (Table 5b, c, d). The compounds explaining this age effect in foliage were different in each species (Table 12 in Appendix). In *P. mariana*,

γ -terpinene, camphene, and tricyclene were more abundant in 1- to 3-years-old needles, cumulatively explaining 58% of differences. In *P. glauca*, β -myrcene, eucalyptol, and limonene each explained about 16% of differences, with a cumulative value of 48%. In this case, β -myrcene

Table 5 Summary of PERMANOVA showing the effects of factors of a) species, age of the foliage (expanding foliage versus 1- to 2-years-old needles) within individuals (as a random block) on the terpenoid profile. In this analysis, only the year 2016 was considered, as both expanding and 1- to 3-years-old foliage were analysed. Only data from controls (no defoliation treatment) were used here. Results considering the factors ages and individuals are also shown for each species in b) *P. mariana*, c) *P. glauca*, and d) *A. balsamea*. Significant values ($P < 0.05$) are shown in bold

Source of variation	df	Pseudo-F ratio	P	Variance explained (%)
a) All species				
Species	2	15.35	0.001	29.0
Foliage age	1	7.39	0.001	11.2
Individual (species)	37	2.15	0.001	19.8
Species $\times$ age	2	4.60	0.001	14.2
Error	35			25.8
Corrected total	77			
b) <i>P. mariana</i>				
Foliage age	1	3.74	0.009	26.2
Individual	9	1.46	0.053	23.8
Error	9			50.0
Corrected total	19			
c) <i>P. glauca</i>				
Foliage age	1	10.64	0.001	25.7
Individual	18	3.26	0.001	38.3
Error	18			36.0
Corrected total	37			
d) <i>A. balsamea</i>				
Foliage age	1	3.76	0.007	26.4
Individual	10	1.53	0.109	26.0
Error	8			47.6
Corrected total	19			

and limonene had higher abundance in the new expanding foliage compared to the older needles. Finally, in *A. balsamea*, borneol and δ -3-carene accounted for up to 52% of observed variation, with borneol exhibiting a remarkably higher value compared to δ -3-carene, which showed a slightly elevated abundance in the old needles. The variance that is explained for the individual foliage interaction with species is 19.8% (Table 5a).

4 Discussion

4.1 No clear induction of terpenoid compositions under defoliation

Terpenoid composition did not significantly change in any species under defoliation, indicating no induced herbivory effect. We can thus partially reject our hypothesis, given that defoliation only affected the terpenoid composition of *P. glauca* in 2015, i.e., the year corresponding with the

higher shoot defoliation level (>80%). However, the lack of a clear defoliation effect on terpenoid composition does not necessarily imply an absence of a response on their production and synthesis activity. Indeed, Litvak and Monson (1998) observed that defoliation by tiger moth larvae led to increased enzymatic activity in conifers, despite no measurable change in the total terpenoid pool. This apparent discrepancy may be attributed to the highly volatile nature of these compounds, given that their low boiling points facilitate rapid evaporation, potentially masking subtle sub-effects and complicating their accurate assessment. For instance, activity of monoterpene cyclase (responsible for monoterpene synthesis) in *Abies concolor* was elevated under defoliation, yet monoterpene volatilisation compensated for this increased production, thereby maintaining a stable overall pool size (Litvak and Monson 1998).

Numerous sub-effects, which are not directly linked to defoliation, can also affect the emission profiles of volatile organic compounds (VOCs), including monoterpenes. Temporal factors, such as time of day, diurnal variation (Komenda and Koppmann 2002; Zulak and Bohlmann 2010; Kopaczky et al. 2020), and seasonal variation (Pokorska et al. 2012), can indeed influence VOC profile emissions. It is further noteworthy that other abiotic factors and stresses, such as the temperature, have been demonstrated to influence terpenoid emissions (Kopaczky et al. 2020). Abiotic factors interact with herbivores and influence VOC emissions. For instance, in a study of Scots pine (*Pinus sylvestris* L.), the interaction was observed between the web-spinning pine sawfly (*Acantholyda posticalis*) and other factors such as warming, high ozone levels, and greater nitrogen availability, in addition to the herbivory (Ghimire et al. 2017). The combined effect of these factors on VOC emissions was found to be significant, with sesquiterpene and monoterpene emissions being particularly influenced (Ghimire et al. 2017).

In addition to these sources of variability, methodological constraints may also influence the interpretation of terpene profiles. Monoterpenes were analysed using a nonchiral column, and therefore enantiomers were not resolved. This distinction may be biologically relevant, as different enantiomers can be produced by different pathways and terpene synthases (Lücker et al. 2002; Pateraki et al. 2015; Tholl 2015), and may vary in their ecological functions including their roles in plant–insect interactions (Wibe et al. 1998; Asztemborska and Ochocka 2002). As a result, potential enantiomer-specific responses to herbivory could not be detected in our analyses. It is therefore possible that subtle changes in terpene composition occurred at the enantiomeric level without being captured in the present study (Yassaa and Williams 2007). Future work using chiral analytical approaches would help refine our understanding of inducible defences in conifers.

Most of the terpenoids that were found in needles are also present in oleoresin, suggesting a common biosynthetic origin (Chang and Hanover 1991; Francezon and Stevanovic 2017). These compounds are primarily synthesised by terpene synthases in resin ducts (Zulak and Bohlmann 2010), which are distributed throughout different conifer organs, including stems and needles (Celedon and Bohlmann 2019). While the role of terpenoids in conifer defence against phloem-feeding insects, including bark beetles and weevils, is well documented (Miller et al. 2005; Keeling and Bohlmann 2006; Hall et al. 2011), their function in responses to needle herbivory remains not well understood, particularly in developing foliage. The variability that is observed in terpenoid responses under defoliation suggests that these compounds may not always be induced in the same manner as in stem tissues, or that their role in foliage defence could extend beyond direct herbivore deterrence. In this context, our results are consistent with the optimal defence theory, predicting that plants allocate defenses preferentially to tissues that are most valuable for survival and least replaceable (Meldau et al. 2012). Indeed, eastern spruce budworm mainly affects the photosynthetic apparatus by decreasing the surface leaf area (Deslauriers et al. 2025), thus directly affecting carbon assimilation, growth, and survival (Krause et al. 2003; Deslauriers et al. 2015; Fierravanti et al. 2019). By contrast, bark beetles frequently result in the death of coniferous trees, primarily due to the disruption of water and nutrient transport in the phloem (Franceschi et al. 2005; Krokene 2015). In addition, bark beetles introduce symbiotic fungi that further compromise host survival (Lieutier et al. 2009; Krokene 2015). Therefore, cambial and phloem tissues, being essential for tree survival, are more likely to exhibit strong induced defenses, such as increased terpenoid accumulation during bark beetle attacks. In contrast, during herbivory, conifers can more easily replace the damaged needles, and therefore attacks to these organs could potentially result in a weaker or absent inducible chemical response. These observations suggest that defence allocation patterns under defoliation may be influenced by tissue importance and physiological constraints, and do not necessarily result in measurable changes in monoterpene composition.

A similar lack of induced volatile terpenes in response to foliar herbivory has also been reported in other conifer–herbivore systems. Moreira et al. (2013) showed that herbivory by pine processionary caterpillar *Thaumetopoea pityocampa* (folivore) on two species of pine did not result in a significant induction of volatile terpenes (monoterpenes and sesquiterpenes) in the needles. However, it did lead to a significant increase in the concentration of polyphenols in the phloem (Moreira et al. 2013). These findings support the hypothesis that the absence of

significant monoterpene induction in our study following spruce budworm feeding does not necessarily reflect an absence of defensive response. Rather, the choice of tissue and the class of compounds analysed strongly influence the ability to detect induced effects (Carrillo-Gavilán et al. 2012; Moreira et al. 2012a, 2013; López-Goldar et al. 2020). Collectively, these observations support the hypothesis that terpenoids may play a broader role in conifer defense, contributing not only to resistance against spruce budworm attack, but also to protection from abiotic stress, fungal pathogens, and bacteria. Given their multiple biological activities (Pichette et al. 2006; Zamponi et al. 2007; Kusumoto et al. 2014), these compounds likely serve functions beyond a direct inducible response to needle herbivory. Their presence in both woody tissues and foliage suggests they are part of a more complex and multi-faceted defense strategy rather than being exclusively associated with localized responses to defoliation.

Furthermore, insect herbivore–plant interactions involve complex mechanisms, including the ability of some lepidopteran herbivores to manipulate plant defenses through oral secretions (OS) (Musser et al. 2002; Weech et al. 2008; Consales et al. 2012; Savchenko et al. 2013). These effects can be triggered not only by enzymes such as glucose oxidase, but also by small RNAs (sRNAs) and other effectors present in OS (Louis et al. 2013; Han et al. 2025; Mobarak et al. 2025; Cedden and Güney 2026). These effectors may alter plant defense pathways and volatile organic compound (VOC) emissions (Bede et al. 2006; Savchenko et al. 2013; Gao et al. 2024). Although glucose oxidase has not been detected in the oral secretions of spruce budworm (LeClair et al. 2015), the involvement of other effectors remain a plausible hypothesis. Such mechanisms could contribute to the absence of induced changes in monoterpene composition observed in our study.

The induction of monoterpenes by herbivory appears to vary among conifer species, having been more commonly reported in pines (Litvak and Monson 1998; Loreto et al. 2000), especially in the case of stem attack by bark beetles (Miller et al. 2005; Keeling and Bohlmann 2006). The impact of defoliation on terpenoid dynamics likely depends on multiple factors, including the conifer host–herbivore pairing (Priemé et al. 2000; Faiola and Taipale 2020), as well as climate conditions (Trowbridge et al. 2014). In other conifer systems, Howe et al. (2020) reported that the relationship between constitutive and induced monoterpene defences can shift from negative (high constitutive levels associated with low inducibility) at the genotypic level to positive (high constitutive levels associated with high inducibility) among populations and species. These responses illustrate how

physiological trade-offs may be modulated by environmental heterogeneity (Moreira et al. 2014). In addition to ecological and environmental influences, plant genetics also play a significant role in modulating the response to biotic stress (Sampedro et al. 2011), contributing to the observed variability in terpenoid induction across species and conditions (Ralph et al. 2006; Ott et al. 2011; Niinemets et al. 2013).

4.2 The exception of *Picea glauca*

In 2015, *P. glauca* exhibited a distinct terpenoid response to defoliation. Two monoterpenoids, i.e., β -myrcene and eucalyptol, which are both characteristic of this species, primarily accounted for observed differences between treatments (50.48% cumulative contribution, Table 10 in Appendix). While concentrations of β -myrcene increased in defoliated saplings, eucalyptol was more abundant in the control group (Table 10 in Appendix). Such contrasting patterns are not uncommon; for instance, Deslauriers et al. (2015) have reported higher levels of certain terpenoids, such as 3-carene, in nondefoliated *A. balsamea* needles compared to defoliated trees.

Regarding β -myrcene, it consistently emerges as a prevalent monoterpene in numerous studies involving conifers (von Rudloff 1972; Grant et al. 2007; Blanch et al. 2009). Recent studies have demonstrated that β -myrcene exerts a disruptive effect on the organisation of bacterial and fungal membranes, thereby enabling the effective incorporation of other active substances (Połec et al. 2020, 2021). Like many other monoterpenes, β -myrcene exhibits significant antibacterial and antifungal properties (Rasoul et al. 2012; Wang et al. 2019; Albayrak et al. 2023), including activity against plant pathogens. Most notably, β -myrcene has also been implicated in stimulating oviposition behavior in spruce budworm, similar to the effects that have been observed with other host monoterpene blends (Grant et al. 2007; Ennis et al. 2017). This response highlights the complex roles that these compounds play in acting as both host identification stimuli and defensive molecules.

In contrast to β -myrcene, the role of eucalyptol (1,8-cineole) in the *P. glauca*–spruce budworm interaction remains less clear. Although no studies to date have evaluated its isolated effects on spruce budworm performance or behavior, eucalyptol is known to possess a range of biological defensive activities, including antibacterial (Magiatis et al. 2002; Aldoghaim et al. 2018; Mączka et al. 2021), antifungal (Morcia et al. 2011; Yu et al. 2015; Kim et al. 2018), and insecticidal properties (Yang et al. 2004; Toloza et al. 2006; Batish et al. 2008). It occurs at high concentrations in the phloem of camphor trees (*Camphora officinarum* Nees = *Cinnamomum*

camphora (L.) J.Presl.) and has been identified as one of the compounds that is most toxic against phloem-feeding insects such as *Pagiophloeus tsushimanus* (Coleoptera: Curculionidae) (Li et al. 2022). Yet, some specialised herbivores have developed microbial symbioses that enable them to detoxify such compounds, as demonstrated by the degradation of eucalyptol by terpenoid-metabolizing gut bacteria in *Pagiophloeus tsushimanus*, thereby allowing continuous feeding on terpenoid-rich hosts (Qiao et al. 2023). While these findings stem from systems outside the spruce budworm context, they highlight the dual nature of monoterpenes such as eucalyptol, which may act defensively under some circumstances, yet may be tolerated or circumvented in others. The reduced abundance of eucalyptol under defoliation in *P. glauca* may reflect such complex and context-dependent roles.

4.3 Species represents the most important factor explaining compound variability

The terpenoid composition in newly expanding foliage exhibited variation, depending principally upon species, and explaining 25% of the variation in our results. Each conifer species had a different terpenoid profile, with compounds mainly distinguishing the two spruce species from fir. The compounds characterising spruce included β -pinene, β -phellandrene, δ -3-carene, borneol, and sabinene. Between the two spruce species, *P. mariana* was characterised by α -pinene, bornyl acetate, camphene, fenchyl acetate, and tricyclene; in the case of *P. glauca*, the compounds were β -myrcene, borneol, camphor, eucalyptol, and limonene. Garneau et al. (2012) identified the chemical composition of the extracted hydrosol (herbal distillate) and oil on the same tree species. Despite a different extraction method and tissue that was being used, the terpenoid compositions were quite similar: camphor for *P. glauca*; camphene, α -pinene, and bornyl acetate for *P. mariana*; β -pinene, β -phellandrene, and δ -3-carene for *A. balsamea* (Garneau et al. 2012, 2014). This is also consistent with the fact that terpenoid profiles are species-dependent (Kopaczyn et al. 2020), even under individual variability (Komenda and Koppmann 2002; Deslauriers et al. 2015). Such variation could stem from genetic differences, leading to diverse enzyme synthesis pathways (Komenda and Koppmann 2002). Furthermore, spruce species, in contrast to *A. balsamea*, possess resin ducts that contain terpenoids within the tissues of their needles. In contrast, the genus *Abies* does not possess these ducts; rather, it has resin blisters in the stem (Zulak and Bohlmann 2010; Lange 2015).

Fenchyl acetate was exclusively detected in black spruce (*Picea mariana*) in our study and has similarly been identified in previous studies involving this species (Tang et al. 2023). Beyond *P. mariana*, fenchyl acetate is rarely

reported in conifers, but does occur in substantial quantities in the essential oils of several angiosperms, such as *Strobilanthes sessilis* Nees (Acanthaceae; Murali Sharma et al. 2025), certain species of the genus *Croton* (Euphorbiaceae; Compagnone et al. 2010), and the genus *Alpinia* (Zingiberaceae; Van et al. 2021). Although fenchol was not detected in our samples, it shares a molecular structure similar to fenchyl acetate (Noma and Asakawa 2010; Blagojević et al. 2017) and has been reported in several conifer extracts (Garneau et al. 2012, 2014; Garcia et al. 2017; Ankney et al. 2022). Notably, fenchyl acetate has been endowed with bioactive properties, such as diuretic effects (Bashir et al. 2023) and larvicidal activity against the Asian tiger mosquito, *Aedes albopictus* (Brandão et al. 2020).

4.4 Inter- and intra-annual influences on the terpenoid variability

The phenology of bud development follows a succession of metabolically active stages, involving many biochemical processes that lead to bud break and the emergence of expanding shoots and needles. These phenological stages are shaped by both plant genetics and environmental conditions (Rossi and Bousquet 2014). Bud break period is known to be species-specific (Hennigar et al. 2008; Pureswaran et al. 2015; Podadera et al. 2024), but climatic variability also contributes to variation in growing season dynamics (Bouzidi et al. 2019), potentially influencing the composition of defensive compounds such as terpenoids. Beyond phenology, herbivory pressure exerted by spruce budworm defoliation, for example, is also known to affect shoot growth (Piene and MacLean 1999). This is a pattern that we also observed in our study, with significant differences between controls and defoliated plants 1 year after the introduction of larvae. Consistent with this response, the 2 years of experimentation exhibited distinct growing seasons with variable timing of bud break and shoot elongation, which reflects species-specific phenological patterns and temporal factors. Although differences in bud break were notably observed between species and defoliation treatments in 2016, these phenological variations did not appear to influence overall terpene composition between treatments. Yet, the stage of growth itself, which ranges from early bud opening to active shoot expansion, did influence terpenoid profiles. Six monoterpenes were identified as discriminating growth stages in *P. mariana*, i.e., α -pinene, β -myrcene, bornyl acetate, camphene, fenchyl acetate, and tricyclene.

Inter-annual variability in terpenoid profiles revealed key differences between the two experimental years, suggesting that year-specific factors such as defoliation intensity and shoot development may have influenced terpene expression. Most of the compounds that

discriminated between years were more abundant in 2015, a year that was characterised by greater shoot elongation and higher average defoliation levels. In *P. glauca*, β -myrcene, limonene, and camphor accounted for 60% of the cumulative contribution to inter-annual variation and were all more concentrated in 2015, which was the year with the highest defoliation. In *P. mariana*, five key compounds explained 74% of the variation and were likewise more abundant in the first year of the experiment. In *A. balsamea*, inter-annual patterns were more variable: bornol was more abundant in 2015, δ -3-carene remained relatively stable over time, and α -pinene was more abundant in 2016. It is important to note that greenhouse conditions differed between years, which may have contributed to the observed variation in terms of both shoot development and terpenoid composition. While defoliation pressure likely played a role, environmental factors may explain some of the inter-annual differences.

4.5 Mature foliage versus newly growth foliage

Across all species, the terpenoid composition was also influenced by the age of the foliage (1- to 3-years-old needles versus newly growing shoots), highlighting the importance of ontogeny in shaping chemical defence profiles. Conifer needles are long-lived organs, and their chemical composition changes substantially during shoot elongation and needle maturation (von Rudloff 1972, 1975; Despland et al. 2016; Lirette and Despland 2021). Such developmental shifts are known to affect eastern spruce budworm performance, as needle growth alters concentrations of defensive compounds and nutrients, including monoterpenes, tannins, and nitrogen (Albert and Bauce 1994; Carisey and Bauce 1997a; Clancy et al. 2004; Nealis and Nault 2005).

In our study, it was observed that some compounds were more abundant in old foliage than in expanding foliage, as was the case of maltol, which was found to be more abundant in 1-year-old foliage than in current-year foliage (Williams et al. 2019). The progression of bud phenology and shoot growth during the two study years illustrates the insect-host relationship with insect phenology closely linked with bud and shoot development. Because spruce budworm larvae primarily target newly expanding shoots but may also consume older needles, they are exposed to foliage that differs markedly in chemical composition over the course of development.

Evidence from other conifer–herbivore systems further supports the idea that needle age can shape defense expression and herbivore performance. In *Pinus sylvestris*, needle age influences both insect preference and defense traits as it is the case with the sawfly *Diprion pini* preferentially feeding on older needles, which is associated with an increased larval weight (Rahman-Soad et al.

2025). At the same time, age-dependent differences were observed in phytohormonal responses, with young needles showing stronger induction of salicylic acid and abscisic acid following herbivory, whereas proteinase inhibitor activity was constitutively lower in older needles (Rahman-Soad et al. 2025). These findings illustrate that defense traits in conifer foliage are developmentally plastic (Rahman-Soad et al. 2025) and that herbivores may exploit predictable ontogenetic variation in host chemistry.

5 Conclusion

Our main findings reveal distinct species-specific patterns in terpenoid composition and suggest that developmental and intrinsic factors outweigh defoliation in shaping these chemical traits, thereby highlighting their relevance in understanding conifer responses to biotic stress. The limited effect of defoliation on monoterpenes highlights the need to expand the scope of investigation when assessing conifer defences against defoliators. In particular, other classes of volatile organic compounds such as nitrogenous compounds and fatty acid derivatives, as well as nonvolatile metabolites, may contribute to defence responses and remain insufficiently explored. Future studies could also benefit from integrating complementary approaches, such as the analysis of terpene biosynthetic activity or enzymatic pathways as performed by Litvak and Monson (1998), to better capture dynamic changes in defence regulation. Expanding analyses across tissues, including phloem-associated compounds such as polyphenols, may further improve our understanding of how conifers allocate defences under herbivore pressure as Moreira et al. (2012b, 2013) observed in their results. Overall, this study contributes to a growing body of work suggesting that defence responses to defoliators differ fundamentally from those induced by more lethal attackers such as bark beetles, and emphasises the importance of considering both tissue type and ecological context when interpreting plant chemical defences.

Appendix

Results—shoot and spruce budworm phenology

Over the observed period, all tree species showed a consistent and gradual progression in bud break over time as shown in Fig. 5, corresponding to stage of growth 1 (SG 1) and stage of growth 2 (SG 2). The effect of Day-of-Year (DOY) was significant for both 2015 and 2016 (Table 6). During the first year of defoliation, no substantial difference was observed between control and defoliated plants (Table 6; Fig. 5). However, in the subsequent year (2016), significant differences emerged, with earlier bud break under defoliation (Fig. 5; $P < 0.001$; Table 6). For both years, species exhibited differences in bud phenology ($P < 0.001$; Table 6). In 2016, the species $\times$ defoliation effect was significant ($P < 0.001$; Table 6), with defoliated plants exhibiting both an earlier onset and end to bud break compared to control plants.

The phenology of shoot elongation, which corresponded to SG 3 and SG 4, differed between years as shown in Fig. 6. DOY was significant for both years ($P < 0.001$; Table 7). Generally, the transition between SG 3 and SG 4 was observed earlier in the defoliation treatment compared to control (Fig. 6). A significant difference in shoot length was found between the treatments in 2015 ($P < 0.001$), but not in 2016 (Table 7). Shoot elongation did not exceed 40 mm in defoliated plants for any species; it ranged between 20 and 40 mm, whereas in control plants, elongation varied between 30 and 90 mm (Fig. 6). Maximum shoot length was greater in the control treatment, especially in 2015. No species $\times$ treatment or species effect was found in either year ($P > 0.05$; Table 7) indicating similar shoot growth between tree species under control or defoliation treatments.

Regarding eastern spruce budworm larvae, based on the bud break chronology, larval instars began and ended earlier in 2016 compared to 2015 on all host species (Fig. 5). Their development appears to be better synchronised in 2016 than in 2015, given that the second larval instar co-occurs with SG 2, particularly bud stage 4 in 2015, whereas it aligns more closely with SG 1 in 2016 (Fig. 5). There were no significant differences between species (Table 6).

Table 6 Ordinal regressions resulting from the application of the effects: Day-of-Year (DOY), treatments (T) and tree species (Sp) on the bud stage, and spruce budworm in 2015 and 2016. For each effect, the results include the Wald chi-square statistic, degrees-of-freedom (df), and probability (*P*). For buds and instars, the ordinal regression models were significant in both 2015 and 2016 ($P < 0.001$). Probability (*P*) is reported as not significant (NS) when $P > 0.05$

Phenology	Year	2015			2016		
		Wald κ^2	ν	<i>P</i>	Wald κ^2	ν	<i>P</i>
Bud stages	DOY	414.07	1	< 0.001	513.75	1	< 0.001
	T	0.41	1	NS	247.12	1	< 0.001
	Sp	143.79	2	< 0.001	99.50	2	< 0.001
	T × Sp	4.40	2	NS	21.24	2	< 0.001
Larvae instar	DOY	106.23	1	< 0.001	132.31	1	< 0.001
	Sp	40.56	2	< 0.001	4.87	2	NS

Table 7 Repeated mixed models calculated for lateral shoot growth in 2015 and 2016. The effects that were considered in the model were treatment (T), tree species (Sp), and Day-of-Year (DOY). The latter was considered as the repeated measures variable. For each effect, the results include degrees-of-freedom (ν_1, ν_2), *F*-statistic, and probability (*P*). Probability (*P*) is reported as not significant (NS) when $P > 0.05$

Year		2015			2016		
Growth	Effect	ν_1, ν_2	<i>F</i>	$P > F$	ν_1, ν_2	<i>F</i>	$P > F$
Lateral (mm)	T	1, 66	79.43	< 0.001	1, 65	1.72	NS
	Sp	2, 66	0.94	NS	2, 65	0.92	NS
	T × Sp	2, 66	0.64	NS	2, 65	1.19	NS
	DOY	23, 1516	86.20	< 0.001	21, 1365	80.43	< 0.001
	DOY × T	23, 1516	18.48	< 0.001	21, 1365	8.74	< 0.001
	DOY × Sp	46, 1516	2.75	< 0.001	42, 1365	9.47	< 0.001
	DOY × T × Sp	46, 1516	1.01	NS	42, 1365	3.19	< 0.001

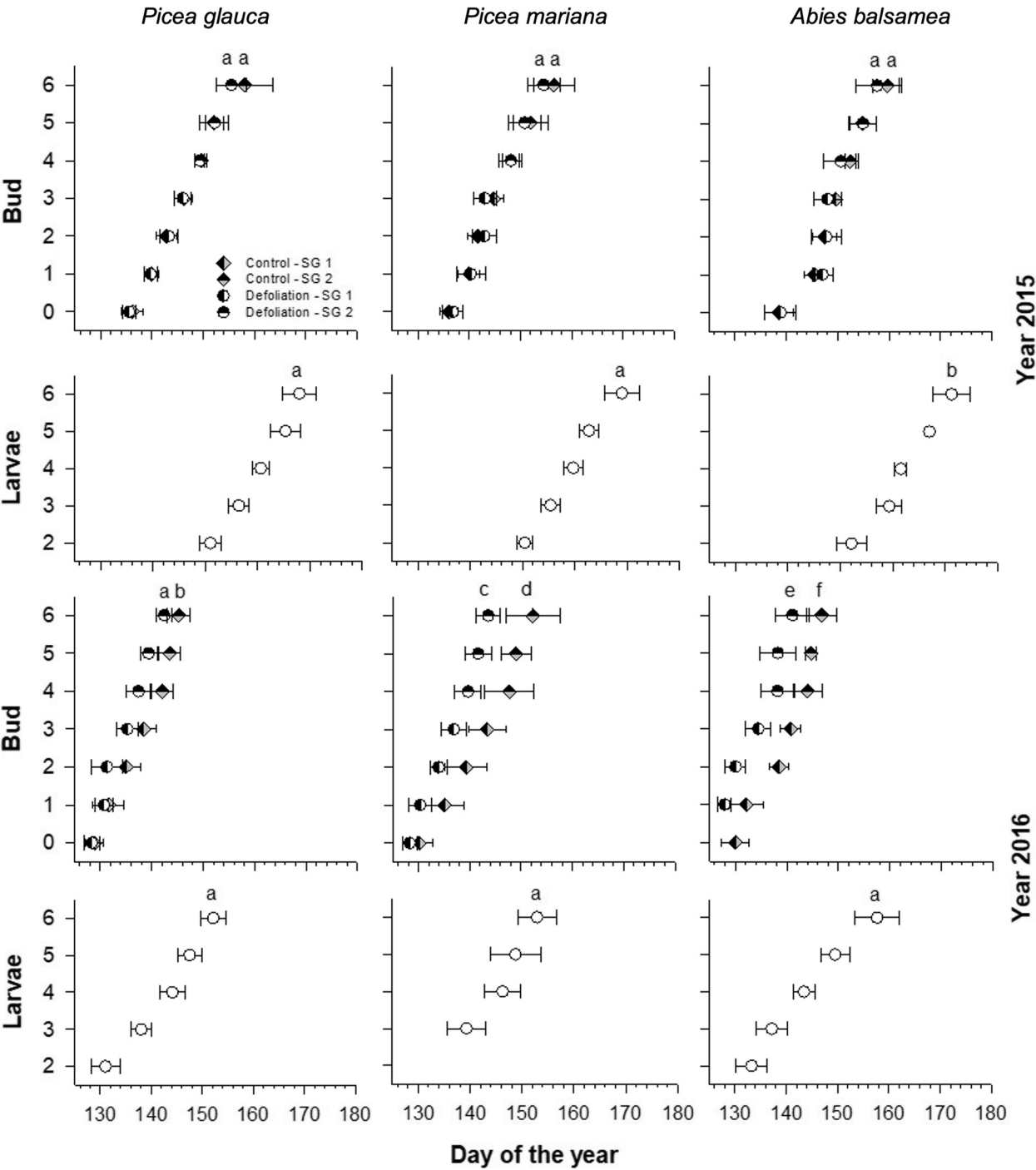


Fig. 5 Bud and larval instar development phases in 2015 and 2016 for *Picea glauca*, *Picea mariana*, and *Abies balsamea*. Means are represented with horizontal lines as standard deviations across Day-of-Year. Diamonds represent controls, while circles represent defoliated saplings. Each treatment was divided in two stages of growth (SG): SG 1 is expressed by a vertical half-half, while SG 2 is expressed as a horizontal half-half. Instar larval development was monitored on defoliated saplings only (open circles). Different letters (a–f) at the top of each series indicate significant differences between treatments and species ($P < 0.05$) according to the repeated mixed models

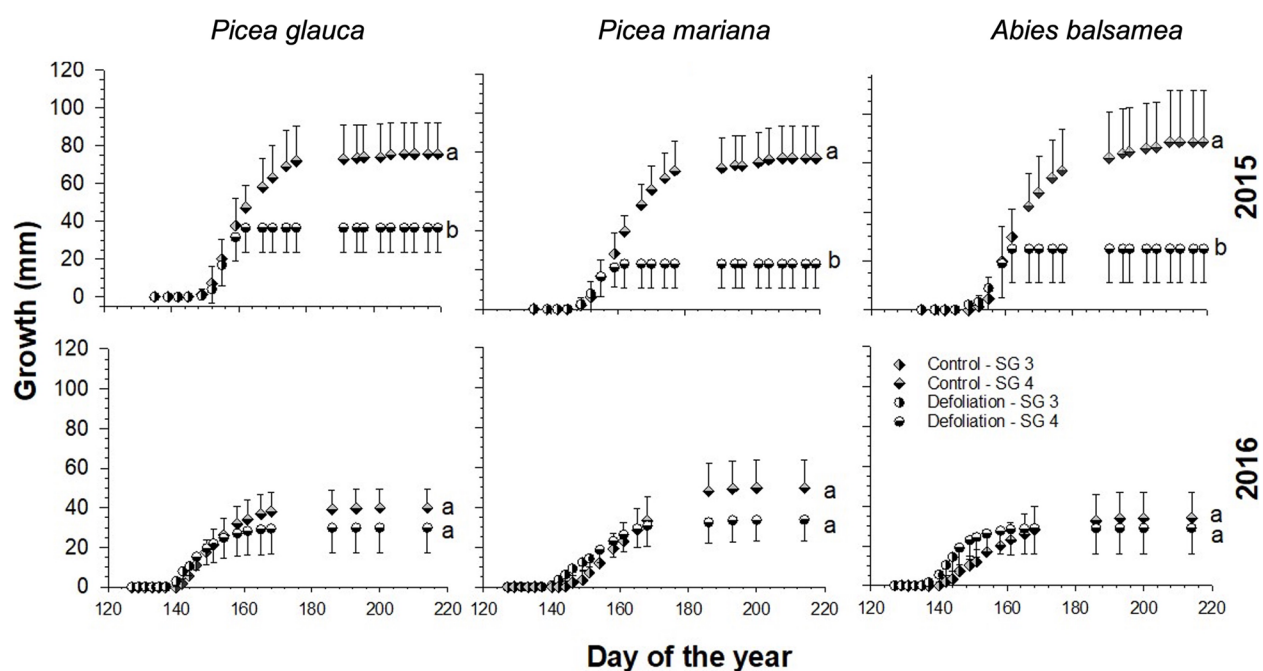


Fig. 6 Mean lateral shoot growth (mm) in 2015 and 2016 for *Picea glauca*, *Picea mariana*, and *Abies balsamea*. The vertical bars show the standard deviation, expressed only in a positive or negative way to avoid overlap. Diamonds represent controls, while circles represent defoliated saplings. Symbols were divided in two stages of growth (SG): SG 3 was expressed by a vertical half-half, while SG 4 is expressed as a horizontal half-half. Different letters (a, b) at the end of each series indicate significant differences between treatments and species ($P < 0.05$) according to the repeated mixed models

Results – Monoterpene composition analyses

Table 8 Difference in compounds among three conifers species (*P. glauca*, *P. mariana*, *A. balsamea*). Summary of SIMPER (percentage of similarity) for compounds that cumulatively contribute up to 50% to the distance between factors considered. Av., average number (log values); Av. diss., average dissimilarity; Diss/SD, dissimilarity divided by standard deviation; Contrib.%, percentage of contribution; Cum.%, cumulated percentage of contribution

Difference between <i>P. glauca</i> and <i>P. mariana</i> (average squared distance = 22.97)						
Compounds	Av. <i>P. glauca</i>	Av. <i>P. mariana</i>	Av. diss	Diss/SD	Contrib.%	Cum.%
Camphor	1.02	−0.546	3.74	0.67	16.30	16.30
Tricyclene	−0.503	0.852	3.42	0.75	14.90	31.20
Eucalyptol	0.367	−0.114	2.93	0.25	12.76	43.96
β-Myrcene	0.335	0.0282	2.62	0.59	11.42	55.39
Difference between <i>P. glauca</i> and <i>A. balsamea</i> (average squared distance = 38.65)						
Compounds	Av. <i>P. glauca</i>	Av. <i>A. balsamea</i>	Av. diss	Diss/SD	Contrib.%	Cum.%
β-Pinene	−0.622	1.24	4.16	1.32	10.76	10.76
β-Phellandrene	−0.525	1.1	3.88	1.03	10.03	20.79
δ-3-Carene	−0.481	0.953	3.69	0.76	9.55	30.34
Camphor	1.02	−0.512	3.68	0.67	9.51	39.86
Limonene	0.569	−0.504	3.23	0.71	8.34	48.20
Sabinene	−0.187	0.389	3.15	0.29	8.15	56.35

Difference between *P. mariana* and *A. balsamea* (average squared distance = 34.92)

Compounds	Av. <i>P. mariana</i>	Av. <i>A. balsamea</i>	Av. diss	Diss/SD	Contrib.%	Cum.%
β-Pinene	−0.587	1.24	4.02	1.31	11.52	11.52
β-Phellandrene	−0.539	1.1	3.89	1.04	11.15	22.67
Camphene	0.812	−0.831	3.67	1.01	10.52	33.18
δ-3-Carene	−0.444	0.953	3.6	0.76	10.31	43.49
Bornyl acetate	0.76	−0.773	3.53	0.81	10.10	53.59

Table 9 Difference in compounds for *Picea mariana* between significant factors of A) year and B) stage of growth. Summary of SIMPER (percentage of similarity) for compounds that cumulatively contribute up to 50% to the distance between factors considered

A) *P. mariana* difference between year 2015 and 2016 (average squared distance = 12.83)

Compounds	Av. 2015	Av. 2016	Av. diss	Diss/SD	Contrib.%	Cum.%
β-Myrcene	0.956	−0.587	2.71	1.54	21.11	21.11
Tricyclene	1.13	0.0083	1.99	0.96	15.47	36.58
Bornyl acetate	0.611	0.582	1.96	0.75	15.28	51.86
Fenchyl acetate	0.732	−0.190	1.57	0.88	12.26	64.12
Limonene	0.351	−0.715	1.29	1.54	10.05	74.17

B) *P. mariana* difference between stage of growth

Difference between stage of growth 1 and 2 (average squared distance = 13.78)

Compounds	Av. 1	Av. 2	Av. diss	Diss/SD	Contrib.%	Cum.%
Bornyl acetate	1.3	−0.448	3.86	1.14	27.97	27.97
Tricyclene	0.919	−0.0398	2.26	0.82	16.40	44.37
Fenchyl acetate	0.771	0.0696	2.02	0.79	14.66	59.04

Difference between stage of growth 1 and 3 (average squared distance = 13.18)

Compounds	Av. 1	Av. 3	Av. diss	Diss/SD	Contrib.%	Cum.%
β-Myrcene	0.914	−0.455	2.43	1.83	18.43	18.43
Fenchyl acetate	0.771	−0.0825	2.18	0.80	16.51	34.94
Bornyl acetate	1.3	0.293	2.05	0.73	15.58	50.52

Difference between stage of growth 2 and 3 (average squared distance = 10.30)

Compounds	Av. 2	Av. 3	Av. diss	Diss/SD	Contrib.%	Cum.%
Camphene	−0.268	0.512	1.97	0.86	19.15	19.15
Bornyl acetate	−0.448	0.293	1.61	0.95	15.60	34.75
β-Myrcene	0.191	−0.455	1.54	0.94	14.99	49.74
α-Pinene	−0.103	0.361	1.41	0.81	13.72	63.46

Difference between stage of growth 1 and 4 (average squared distance = 9.11)

Compounds	Av. 1	Av. 4	Av. diss	Diss/SD	Contrib.%	Cum.%
Fenchyl acetate	0.771	0.331	1.84	0.82	20.24	20.24
Tricyclene	0.919	0.694	1.46	0.90	15.98	36.22
Bornyl acetate	1.3	0.904	1.42	0.83	15.54	51.75

Difference between stage of growth 2 and 4 (average squared distance = 10.78)

Compounds	Av. 2	Av. 4	Av. diss	Diss/SD	Contrib.%	Cum.%
Bornyl acetate	−0.448	0.904	3.1	0.77	28.73	28.73
Tricyclene	−0.0398	0.694	1.92	0.73	17.79	46.51
β-Myrcene	0.191	0.214	1.46	0.85	13.50	60.01

Difference between stage of growth 3 and 4 (average squared distance = 10.07)

Compounds	Av. 3	Av. 4	Av. diss	Diss/SD	Contrib.%	Cum.%
Bornyl acetate	0.293	0.904	1.88	0.62	18.62	18.62
β-Myrcene	−0.455	0.214	1.67	0.80	16.60	35.23
Camphene	0.512	0.574	1.32	0.55	13.09	48.31
α-Pinene	0.361	0.16	1.19	0.62	11.85	60.17

Table 10 Difference in compounds for *Picea glauca* between significant interactive factors of year (2015 and 2016) and defoliation (Def: defoliation with spruce budworm and Ctr: control). Summary of SIMPER (percentage of similarity) for compounds that cumulatively contribute up to 50% to the distance between factors considered

A) <i>P. glauca</i> difference between defoliation for year 2015 (average squared distance = 10.95)						
Compounds	Av. Ctr	Av. Def	Av. diss	Diss/SD	Contrib.%	Cum.%
β-Myrcene	1.48	2.12	3.49	0.99	31.84	31.84
Eucalyptol	0.585	−0.0817	2.04	0.58	18.64	50.48
B) <i>P. glauca</i> difference between defoliation for year 2016 (average squared distance = 8.70)						
Compounds	Av. Ctr	Av. Def	Av. diss	Diss/SD	Contrib.%	Cum.%
Bornyl acetate	0.353	0.438	2.83	0.71	32.55	32.55
Limonene	0.127	−0.538	1.7	0.67	19.52	52.08
C) <i>P. glauca</i> difference between year 2015 and 2016 (average squared distance = 8.70)						
Compounds	Av. 2015	Av. 2016	Av. diss	Diss/SD	Contrib.%	Cum.%
β-Myrcene	1.64	−0.405	5.83	0.97	24.63	24.63
Limonene	1.74	−0.19	5	1.28	21.13	45.76
Camphor	1.64	−0.274	4.35	1.50	18.40	64.16

Table 11 Difference in compounds for *Abies balsamea* between significant the factors of year (2015 and 2016). Summary of SIMPER (percentage of similarity) for compounds that cumulatively contribute up to 50% to the distance between factors considered

A. <i>balsamea</i> difference between year 2015 and 2016 (average squared distance = 24.20)						
Compounds	Av. 2015	Av. 2016	Av. diss	Diss/SD	Contrib.%	Cum.%
α-Pinene	−0.525	1.19	7.1	0.48	29.32	29.32
δ-3-Carene	0.887	0.869	2.99	0.80	12.37	41.68
Borneol	0.944	−0.388	2.85	0.79	11.78	53.47

Table 12 Difference in compounds between the significant factors of age needles (young: Y vs. old: O) for each studied species in the 2016 control samplings. Summary of SIMPER (percentage of similarity) for compounds that cumulatively contribute up to 50% to the distance between factors considered

Compounds	Av. Y	Av. O	Av. diss	Diss/SD	Contrib.%	Cum.%
A) <i>P. mariana</i> difference between age (average squared distance = 18.60)						
γ-Terpinene	−0.189	0.873	7.13	0.37	38.33	38.33
Camphene	0.596	1.63	1.91	0.94	10.25	48.58
Tricyclene	0.828	1.85	1.81	0.72	9.74	58.32
B) <i>P. glauca</i> difference between age (average squared distance = 19.90)						
β-Myrcene	0.316	−0.107	3.3	0.43	16.58	16.58
Eucalyptol	−0.0354	0.377	3.29	0.46	16.55	33.13
Limonene	0.655	0.254	3.12	0.70	15.69	48.82
Bornyl acetate	0.631	−0.266	2.65	0.68	13.30	62.12
C) <i>A. balsamea</i> difference between age (average squared distance = 16.14)						
Borneol	−0.404	1.04	4.37	0.70	27.09	27.09
δ-3-Carene	0.925	0.965	4.04	0.91	25.01	52.09

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Authors' contributions

All authors have contributed directly to the content of this work (study design, interpretation of the data, writing and critical revision) and approved the submitted version. JM, MC, ED and AD designed the study and interpreted the results; AP and LR performed the analyses; SB, AD and MC analyzed the data; SB, MC, ED and AD wrote the paper; ED, JM and EB reviewed and made substantial contributions.

Data availability

Terpenoids and phenological datasets are available at (<https://doi.org/10.5683/SP3/8VP1WN>) (Deslauriers 2026).

Declarations

Competing interests

The authors declare no competing interests.

Author details

¹Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Canada. ²Department of Biology, Concordia University, Montréal, Canada. ³Département des Sciences du Bois et de la Forêt, Université Laval, Québec, Canada. ⁴Department of Biology, University of Oxford, Oxford, United Kingdom.

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