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Published in:
Science of the Total Environment

DOI:
10.1016/j.scitotenv.2021.148516

Publication date:
2021

Document version
Publisher's PDF, also known as Version of record

Document license:
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Citation for published version (APA):
Contrasting responses of major and minor volatile compounds to warming and gall-infestation in the Arctic willow Salix myrsinites

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HIGHLIGHTS

• Isoprene accounts for 91% of the total volatile emissions from Salix myrsinites.
• Warming increases isoprene emissions, but gall-infestation does not.
• Gall-infestation increases emissions of DMNT and green leaf volatiles.
• Warming compromises the effects of gall-infestation.

GRAPHICAL ABSTRACT

ABSTRACT

Climate change is altering high-latitude ecosystems in multiple facets, including increased insect herbivory pressure and enhanced emissions of volatile organic compounds (VOC) from vegetation. Yet, joint impacts of climatic drivers and insect herbivory on VOC emissions from the Arctic remain largely unknown. We examined how one-month warming by open-top plastic tents, yielding a 3–4 °C air temperature increase, and the natural presence of gall-forming eriophyoid mites, Aculus tetanothrix, individually and in combination, affect VOC emissions from whortle leaved willow, Salix myrsinites, at two elevations in an Arctic heath tundra of Abisko, Northern Sweden. We measured VOC emissions three times in the peak growing season (July) from intact and gall-infested branches using an enclosure technique and gas chromatography–mass spectrometry, and leaf chemical composition using near-infrared reflectance spectroscopy (NIRS). Isoprene accounted for 91% of the VOCs emitted by S. myrsinites. Isoprene emission rates tended to be higher at the high than low elevation during the measurement periods (42 μg g−1 DW h−1 vs. 23 μg g−1 DW h−1) even when temperature differences were accounted for. Experimental warming increased isoprene emissions by approximately 54%, but decreased emissions of some minor compound groups, such as green leaf volatiles (GLV) and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT). In contrast, gall-infestation did not affect isoprene emissions but stimulated emissions of DMNT, sesquiterpenes and GLVs, particularly under ambient conditions at the low elevation. The NIRS-based chemical composition of the leaves varied between the two elevations and was affected by warming and gall-infestation. Our study suggests that under elevated temperatures, S. myrsinites increases emissions of isoprene, a highly effective compound for protection against oxidative stress, while an infestation by A. tetanothrix mites induces emissions of herbivore enemy attractants like DMNT, sesquiterpenes and GLVs. Under both conditions, warming effects on isoprene remain but mite effects on DMNT, sesquiterpenes and GLVs diminish.

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1. Introduction

Climate change is expected to have severe impacts on the Arctic climate even under the moderate carbon mitigation strategies put forward by the IPCC (Post et al., 2019). Annual air temperatures in the Arctic have already increased by 2.7 °C in the period 1971–2017 and are projected to rise by another 5–13 °C by 2100 (Box et al., 2019). This considerable warming has already caused drastic changes in the Arctic carbon cycle, including emissions of volatile organic compounds (VOCs) from vegetation (Kramshøj et al., 2016; Rinnan et al., 2020). Biogenic VOCs, especially isoprene with global annual emissions of ca. 500 TgC (Guenther et al., 2012), are known to affect climate via the formation of secondary organic aerosols (SOA) and ozone, and the reduction of the atmospheric oxidative capacity (Monson and Baldocchi, 2014; Pacifico et al., 2009). Such biogenic VOC-mediated climate feedback is thought to be especially significant in remote regions with low anthropogenic emissions, such as the Arctic (Rinnan et al., 2014). However, the impacts of climatic and biotic drivers on biogenic VOC emissions are still understudied in the Arctic.

Apart from influencing atmospheric chemistry and climate feedback, biogenic VOCs have multiple ecological functions, for example attracting and/or repelling herbivorous insects, recruiting natural enemies of the attacking herbivores, and mediating between-and-within-plant communication, among others (De Moraes et al., 1998; Karban et al., 2014; Li and Blande, 2017; Tooker and Hanks, 2006). Moreover, some plant VOCs, such as isoprene and monoterpenes, have been shown to confer plants with thermal protection against high temperature-induced oxidative stress (Loreto et al., 2014; Loreto and Fineschi, 2015). Isoprene is also one of the main compounds emitted from Arctic vegetation, in which typical isoprene emitters, such as mosses, graminoids, and willows (Salix spp.) are common (Rinnan et al., 2014). Some studies even suggest that ongoing Arctic warming may further favor VOC-emitting species (e.g., Angot et al., 2020). Thus, estimating the potential effects of biogenic VOCs on Arctic ecosystem processes and climate requires a better understanding of the underlying processes driving biogenic VOC emissions in the Arctic.

Early modeling studies suggested minimal VOC emissions from Arctic ecosystems due to sparse vegetation and cold temperatures (e.g., Guenther et al., 2012). However, recent field measurements at the scales of branches, communities and ecosystems have repeatedly shown that the Arctic is indeed a significant source of biogenic VOCs into the atmosphere and that ongoing Arctic warming will further elevate biogenic VOC emissions either through direct stimulation of VOC production and release by higher air temperature, or through indirect effects via long-term changes in vegetation composition and biomass induced by warming (Angot et al., 2020; Kramshøj et al., 2016; Rinnan et al., 2020; Seco et al., 2020). These studies have also revealed that the temperature dependency of VOC emissions seems to be particularly pronounced in tundra ecosystems compared to tropical and subtropical ecosystems (Faubert et al., 2011; Rinnan et al., 2014; Seco et al., 2020). For instance, experimental warming by 3–4 °C has led to a 240–280% increase in VOC emissions from Greenlandic tundra ecosystems (Kramshøj et al., 2016; Lindwall et al., 2016a).

While the last decade has witnessed great strides toward understanding the impact of climate drivers, particularly warming, on Arctic VOC emissions (Rinnan et al., 2020), little attention has been paid to assessing the impact of biotic drivers such as insect herbivory. Warming of the Arctic leads to changes in the occurrence of insect herbivory because higher temperatures remove physiological constraints on insect expansion in these ecosystems (Li et al., 2019). These changes lead to increased risk of herbivore attacks causing severe foliar damage (Barrio et al., 2017). Of insect herbivores, the impact of gall-inducing insects on plants is particularly profound (Coelho Kuster et al., 2020; Tooker et al., 2008).

Galling insects induce a tumour-like growth on their host leaves and the insects live within plant tissues, consuming plant resources, which results in reduced growth, development, and reproduction of host plants (Mosbacher et al., 2013; Petanović and Kielkiewicz, 2010; Tooker and Hanks, 2006; Ye et al., 2019). However, research examining VOC emissions in plants under herbivory has largely focused on chewing and sucking insects, and the effects of gall-inducing insects on VOC emissions are still largely unknown (Borges, 2018). It has been suggested that infestation from different galling species leads to various defense responses in host plants, with induced emissions of benzenoids, monoterpenes, sesquiterpenes, and green leaf volatiles (GLVs) in some studies (Jiang et al., 2018; Ye et al., 2019) and no change in VOC emissions in others (Tooker and De Moraes, 2007). A reduction in isoprene emissions under exposure to galling is expected due to galling-induced inhibition of photosynthesis (Noe and Niinemets, 2020). However, results from recent studies show both induced and reduced isoprene emissions from gall-infested foliage (Jiang et al., 2018; Ye et al., 2019). Even though herbivory by gall insects is widespread in the High Latitudes (Mosbacher et al., 2013; Patankar et al., 2013a; Patankar et al., 2013b), we lack understanding on the effects of galling on VOC emissions from Arctic species.

The direct and indirect effects of climate change on biogenic VOC emissions are occurring rapidly and simultaneously. Most studies have focused on the individual effects of abiotic or biotic factors on VOC emissions, yet interactive effects of multiple stressors have been underappreciated (Holopainen et al., 2018; Li et al., 2019). It is important to investigate the joint effects of multiple stressors to better represent the natural environment and conditions. Furthermore, plant responses to multiple stressors are complex and not just the sum of individual effects (Holopainen et al., 2018). Therefore, field research examining the interactive effects of rising temperatures and herbivory on VOC emissions is required.

In this study, we aimed to examine how short-term temperature increase and foliar damage caused by the gall-forming eriophyoid mite, Aculus tetanothrix, individually and in combination, affect VOC emissions from the whorled leaved willow, Salix myrsinifolia, in an Arctic tundra ecosystem. We measured in situ VOC emissions at two elevations during the peak growing season. Warmer and longer growing seasons increase the abundance of deciduous woody shrubs in the High Latitudes (Callaghan et al., 2013; Rundqvist et al., 2011; Tape et al., 2006). Of these, Salix spp., is an effective isoprene emitter, one of the most abundant shrubs present in the Arctic tundra, and is highly susceptible to leaf galls (Patankar et al., 2013b; Rinnan et al., 2011; Vedel-Petersen et al., 2015). A. tetanothrix is a widely distributed mite species in Salix species in the Arctic, which causes often irregularly rounded galls on upper leaf surface, and appears to occur less frequently at high elevations than at low elevations (Kuczynski and Skoracka, 2005). We set out to assess how experimental warming and leaf infestation by A. tetanothrix, alone and in combination, affect VOC emissions and leaf chemical composition in S. myrsinifolia, and whether these effects differ between elevations. We hypothesized that warming augments VOC emissions as VOC biosynthesis and volatility are temperature-dependent (Guenther et al., 2012; Peñuelas and Staudt, 2009). The effects of leaf-infestation by A. tetanothrix mites are compound-specific, but we were not able to predict the direction of these effects as the very limited number of studies on plant VOC responses to gall-infestation have led to inconsistent results (Borges, 2018; Jiang et al., 2018; Tooker and De Moraes, 2007). Under both conditions, warming either amplifies or attenuates the effects of gall-infestation, depending on the identity of compounds. We finally hypothesized that the effects of warming and gall-infestation are stronger at the low than high elevation as air temperatures and herbivory pressure generally decrease with elevation (e.g., Moreira et al., 2018).

2. Materials and methods

2.1. Location

Our field experiments were located on two Arctic tundra heaths in Abisko, Swedish Lapland. The growing season lasts from late May until...
the end of August, with mean annual temperature and precipitation of −0.5 °C and 304 mm, respectively (30-year average from 1961 to 1990) (Alexandersson and Eggertsson Karlström, 2001). Experiments were conducted at two elevations during the period July 12–30, 2019. The low elevation (68° 20′ N, 18° 49′ 34″ E; 385 m a.s.l.) was located in a wet mesic heath at the edge of a birch forest, below the tree line (Faubert et al., 2011). The high elevation (68° 19′ 21″ N, 18° 50′ 40″ E; 560 m a.s.l.) was located right above the tree line in a tundra heath (Rousk et al., 2016). The soil pH at both elevations is neutral and the vegetation is similar, dominated by dwarf shrubs, mosses, and graminoids (Rinnan et al., 2007; Rousk and Michelsen, 2017). Common dwarf shrubs included Vaccinium uliginosum L., Betula nana L., Andromeda polifolia L., Empetrum nigrum spp. hermaphroditum (Lange ex Hagerup) Böcher and Rhododendron laponicum (L.), and common graminoids included Carex vaginata Tausch and Carex paralella (Laest.) Sommerf.

2.2. Experimental design

The field treatments consisted of a warming treatment and a control treatment. All treatments were established as 1 × 1 m experimental plots and replicated six times in a randomized block design. The warming treatment was created using dome-shaped open-top polyethylene plastic tents (0.05 mm thick, approximately 80 cm high, 1.2 × 1.2 m at the base, 40 cm diameter at the top) with an expected temperature increase of up to 4 °C and 1–2 °C for air and soil, respectively (Rinnan et al., 2011). The open-top tents were set up at each elevation on June 12–13, 2019, when the galls from A. tenuothrix had not yet emerged. On July 12, approximately one month after the warming setup, branches of S. myrsinites (approximately 5–8 cm long) infested with and without A. tenuothrix galls were marked in each plot for VOC measurements. We initially intended to target one pair of branches (i.e., one branch with galls and one branch without galls) in each plot. However, due to the low abundance of gall-infested branches, we used branches from outside the marked plots in some control plots, while in some warming plots we selected two pairs of infected and uninfected branches from separate plants. At the low elevation, 34 branches of S. myrsinites (n = 10 for control and warming, n = 7 for gall-infestation and warming plus gall-infestation) were sampled, of which 12 branches (n = 3 in each case) were likely hybrids with S. phylicifolia. At the high elevation, 30 branches of S. myrsinites (n = 9 for control and warming, n = 6 for gall-infestation and warming plus gall-infestation) were sampled. The selected branches within each plot were from different plants distanced 20–70 cm apart.

2.3. VOC sampling

At each elevation, three VOC sampling campaigns were made in situ on the same branches (July 13, 19, and 27 at the high elevation and July 14, 20, and 28 at the low elevation). The selected branches were enclosed in transparent polyethylene terephthalate (PET) containers with a volume of 500 mL. Each container was closed with a lid at the base of the branch, and tubing for in- and outflow air was inserted through holes in the bottom. A new container was used for every sample to avoid cross-contamination and memory effects. Prior to sampling and immediately after enclosing the branch, the container was flushed for approximately 3 min at a flow rate of 1000 mL min⁻¹. During sampling, the inlet flow was lowered to 300 mL min⁻¹. The incoming air was purified by an air filter and an activated carbon filter to remove particles and VOCs, and by a copper tube coated with potassium iodide to scrub ozone (Kramshøj et al., 2016; Ortega et al., 2008). Headspace air was pulled out of the container through a stainless steel adsorbent cartridge filled with 150 mg Tenax TA and 200 mg of Carbograph 1TD (Markes International Limited, Llantrisant, UK) at a flow rate of 200 mL min⁻¹ for 10 min. After sampling, the cartridges were sealed with Teflon coated brass caps and stored in the refrigerator until analysis. During each VOC sampling campaign, two blank measurements from empty containers were conducted in the middle and end of the branch VOC sampling, respectively, to correct for any contaminants released from the container and the sampling system. PET containers have been used in several studies for VOC collection (e.g., Li et al., 2019; Stewart-Jones and Poppy, 2006). Compared to some semi-neutral materials such as polytetrafluoroethylene (PTFE/Teflon) and glass, PET containers may adsorb greater amounts on surfaces (Stewart-Jones and Poppy, 2006), leading to underestimation of the emission rates of certain compounds. Since we used small PET containers and short sampling time, such underestimation is likely to be relatively small and would not hamper detection of treatment effects, which was the main aim of the study.

During the VOC sampling, temperature and relative humidity (RH) were measured inside the enclosed container using data loggers (DS1923, iButton Hygrochron, Maxim Integrated, San Jose, USA). Moreover, for the duration of a measuring day, photosynthetic photon flux density (PPFD) (Onset Computer Corporation, Bourne, USA) and temperature (DS1923, iButton Hygrochron, Maxim Integrated, San Jose, USA) were measured at canopy level inside and outside the warming tents. Soil temperature was measured at 5 cm depth at three different places within each plot using a soil thermometer (Delta-T Devices, Cambridge, England). Seasonal climate data on PPFD, temperature, and precipitation were retrieved from the climate station at the Abisko Scientific Research Station for the 2019 growing season.

At the end of the last campaign, the branches were harvested and all leaves were scanned. Leaf area was measured using scanned images and Imagej software (National Institutes of Health, Bethesda, Maryland, USA), and leaf dry weights were determined after 72-h of oven drying at 60 °C. To estimate the gall-infestation degree, we measured the leaf area covered by a representative gall using Imagej, and counted the total number of representative galls present on each target branch as well as the total number of leaves with galls. The gall-infestation degree, which was expressed as the percentage of leaf area covered by galls, was estimated by first multiplying the total number of representative galls and the leaf area infested by the representative gall, and then dividing it by the total leaf area.

2.4. VOC analysis

VOC samples were analysed by gas chromatography–mass spectrometry (GC–MS, Agilent 7890A GC and 5975 C Vl MSD). Compounds were subjected to thermal desorption at 250 °C for 10 min using a TD100-xr (Markes International Limited, Llantrisant, UK) and were then separated through an HP-5 capillary column (50 m × 0.2 mm × 0.33 μm, Agilent, Santa Clara, USA) with helium as the carrier gas at a flow rate of 1.2 mL min⁻¹. The GC oven was heated to 40 °C for 3 min, and then raised at 5 °C min⁻¹ to 210 °C and finally raised at a rate of 20 °C min⁻¹ to 250 °C and held for 8 min, resulting in a total runtime of 47 min. The chromatograms were analysed using PARADiSe software (Johnsen et al., 2017). Compounds were tentatively identified by comparing mass spectra to those in the NIST2014 Mass Spectral Library (National Institute of Standards and Technology, USA), verified using pure standards (Supplementary data, Table S1) when available, and quantified using external standards. Compounds without pure standards were matched with structurally related standards for quantification, namely, α-pinene for monoterpenes and (E)-4,8-dimethylno-1,3,7-triene (DMNT), (E)-4-caryophyllene for sesquiterpenes (SQT), hexanal for green leaf volatiles (GLV), and toluene for benzenoids and other compounds. If compounds were detected in at least 50% of the samples of one treatment, they were included in the analysis. VOC concentrations in blanks were subtracted from the samples prior to calculation of emission rates. Compounds were divided into eight compound groups: isoprene,
GLVs, monoterpenes, benzenoids, sesquiterpenes, DMNT, and other VOCs, which included nitrogen-containing compounds, furans, alcohols, aldehydes, alkanes, alkenes, and ketones. In order to tease apart the immediate effect of temperature fluctuations during the measurement periods from the warming treatment effects, we also reported the isoprene emission rates standardised to a fixed temperature of 30 °C and PPFD-level of 1000 µmol m⁻² s⁻¹, according to Guenther et al. (1993). The emission rates of other compounds were non-standardised as there is no established methodology for the large majority of these compounds and as isoprene dominates Salix VOC emissions (Angot et al., 2020; Guenther et al., 2012; Svendsen et al., 2016).

2.5. Chemical composition of leaf samples

Oven-dried leaf material was crushed to powder and analysed using Near-infrared reflectance spectroscopy (NIRS) (Antaris II FT-NIR Analyzers, Thermo Fisher Scientific, Waltham, USA). Each NIR spectrum of the reflected light as a whole describes the specific chemical composition and serves as a chemical fingerprint for the analysed sample (Foley et al., 1998). NIRS measurements were conducted with three technical replicates per sample over a spectral range of 4000–10,000 cm⁻¹, where each spectrum was an average of 32 scans.

2.6. Statistical analysis

All statistical analyses were run with R studio software (R Studio, Boston, USA). We analysed for the effects of warming, gall-infestation, elevation, and campaign, as well as their interactions on VOC emission rates using linear mixed-effect models (lmer function from lme4 package). Warming, elevation, gall-infestation, and campaign were set as predictor variables, while block, plot, and plant were treated as random factors with plant nested within plot and plot nested within block. Light and temperature are well known environmental factors driving emissions of biogenic VOCs, in particular isoprene. When conducting experimental work in nature under varying environmental conditions, we introduce unwanted variation in the emission rates of all light- and temperature-dependent VOCs. To account for this variation, we included light and enclosure temperature as covariates in our statistical models. Significant linear mixed-effect models were followed by Tukey’s post-hoc multiple comparisons to test for the effects of warming and gall-infestation during each campaign at each elevation (emmeans function in the emmeans package). Prior to analysis, Shapiro-Wilk and Levene’s tests were performed to evaluate normality and homogeneity of error variances (shapiro_test and levene_test function from rstatix package). Log (x) or log (x + 1) transformations were applied to VOC data to account for violation of the assumptions of normal distribution and homogeneity of variance. To test if the effect of the warming treatment on soil and air temperature and gall-infestation varied between the two elevations, two-sample t-tests were conducted (t.test function from the stats package).

VOC emission profiles and NIRS spectra were analysed using Principal Component Analysis (PCA) in SIMCA software 13.0.3 (Umetrics, Umeå, Sweden). For the VOC data, PCAs were used to assess how the VOC blends – regardless of the emission rates – varied between experimental treatments (i.e., warming and gall-infestation) and elevations during each measurement campaign. We were not interested in the temporal variation within the relatively short experimental period, but wanted to assess differences between elevations and experimental treatments. Therefore, PCAs were run separately for each campaign. For the NIRS data, the PCA was used to assess how the spectral signatures of the leaf material varied between experimental treatments and elevations. The VOC data were unit-variance scaled and the NIRS data were pre-processed using multiplicative scatter correction (MSC) and centred prior to PCA. Scores of each principal component were tested for significant main effects and interactions between elevations, warming, and gall-infestation, as described above.

3. Results

3.1. Environmental conditions and gall-infestation severity

The month of July was the warmest of the year, with a mean temperature of 12.5 °C, which was higher than the 30-year average of 11.3 °C (Alexandersson and Eggertsson Karlström, 2001). Ambient air temperatures measured within the canopy during VOC sampling ranged from 14.9 to 29.4 °C (Table S2). The warming treatment had a stronger effect at the high elevation, where it gave a mean temperature increase of 8.6 °C relative to ambient temperatures, while the temperature increase at the low elevation was 5.7 °C on average (p < 0.001 in all measurement campaigns). Soil temperatures were increased by an average of 1.8 °C in the tents at the high elevation (p < 0.05). However, the 1.2 °C average increase in soil temperature observed in the warming plots at the low elevation was not statistically significant (p > 0.05). The tents resulted in an unintended shading effect; the incoming PPFD was 17% lower inside than outside the tents averaged over the measurement period (p = 0.04; Table S2).

The percent leaf area covered by A. tetanothrix galls was on average 2.5% and did not vary significantly between the two elevations (p = 0.73; Table 1). Gall-infestation levels on branches used for VOC measurements were similar between control and warming plots at the low elevation, but higher in control plots at the high elevation (p = 0.03; Table 1).

3.2. VOC emissions

A total of 134 VOCs were detected and divided into 7 compound groups (Fig. 1; Table S3). Of these, isoprene was the dominant VOC emitted, accounting for 91% of the total VOC emission amount throughout the three measurement campaigns. After isoprene, the largest contributions were from GLVs, monoterpenes, and other VOCs (Fig. 1a).

The PCA performed on the VOC emission profiles for each campaign showed that in the first and the last measurement campaigns, the first principal component (PC) accounted for differences between the two elevations while the second PC described mainly the effects of warming in the first campaign (Fig. 1b) and the effects of gall-infestation in the last campaign (Fig. S1). The corresponding loading plots indicate that the VOC blends from the high elevation were described by high loading values for most detected compounds, monoterpenes in particular (Figs. 1c, S1). The VOC blends at the low elevation were characterised by a higher relative contribution of a few other VOCs such as heptane, 6-methyl-5-heptene-2-one, furfural and geranyl acetone. The effect of warming was mainly demonstrated by higher relative contributions from sesquiterpenes and lower relative contributions from GLVs (Fig. 1b-c). During the second measurement campaign, the variation was generally larger and the second PC was significantly affected by elevations (Fig. S2). Gall-infestation had no significant effects on the VOC emission profiles.

Emissions of most VOC classes varied significantly with measurement campaigns, which was in line with the variations in enclosure temperatures and PPFD (Fig. 2). This was particularly the case for isoprene. Isoprene emission rates increased exponentially within a temperature range of 16–43 °C recorded inside the enclosure during measurements and increased linearly with increasing PPFD (Fig. S3). Emission rates peaked when the recorded temperatures were highest, on July 20 and 27 for the low and high elevation, respectively (Fig. 2c).

Table 1 Severity of gall-infestation (mean ± SE) by A. tetanothrix mites on S. myrsinites leaves selected for VOC measurements in control and warming treatment plots. Asterisks indicate significant differences according to two-sample t-test (*p < 0.05).

<table>
<thead>
<tr>
<th>Elevation</th>
<th>Leaf area infested by galls (%)</th>
<th>Leaves infested by galls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Warming</td>
<td>Control</td>
</tr>
<tr>
<td>Low elevation</td>
<td>2.7 ± 0.7</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>High elevation</td>
<td>3.7 ± 0.7</td>
<td>1.6 ± 0.4*</td>
</tr>
</tbody>
</table>
Overall, the one-month experimental warming significantly increased isoprene emission rates (Fig. 2c), which, when averaged across elevations, gall-infestation, and measurement campaigns, increased by 54%. The warming effects appeared to be most pronounced on June 28 at the low elevation, and on June 13 and 19 at the high elevation. Warming also increased isoprene emission rates standardised to fixed temperature and PPFD \( (p = 0.006, \text{Fig. S4}) \). Surprisingly, warming significantly reduced emissions of DMNT, GLVs, and other VOCs, across measurement campaigns and elevations (Fig. 2d, g, i). As revealed by the significant interactive effects between warming and campaign or elevation, the negative warming effects on DMNT emissions varied with measurement campaigns, being most obvious at the second campaign for both elevations (Fig. 2d), while the most notable effects on GLV emissions were observed at the low elevation (Fig. 2g).

The presence of *A. tetanothrix* galls did not significantly affect isoprene emissions (Fig. 2c), and there was no relationship between the isoprene emission rates and the gall-infestation levels (Fig. S5). However, gall-infestation significantly increased emissions of DMNT, sesquiterpenes and GLVs (Fig. 2d, f, g). There was a significant interaction effect on GLV emissions among gall-infestation, warming and elevation (Fig. 2g), showing that under ambient conditions at the low elevation, gall-infested plants emitted greater quantities of GLVs consistently over the three measurement campaigns. Of the individual GLVs, the most responsive compounds included (Z)-3-hexenal, (E)-2-hexenal, (Z)-3-hexenyl acetate and (E)-4-oxohex-2-enal \( (p < 0.05 \text{ for gall-infestation \times elevation}) \), all of which were released in greater amounts from gall-infested plants at the low elevation (Table S3). With respect to DMNT and sesquiterpene emissions, the most notable gall effects were observed in the second campaign (i.e. June 20) under ambient conditions at the low elevation (Fig. 2d, f). The individual sesquiterpenes that were most responsive to gall-infestation or its interactions with warming, elevation or campaign included \( \alpha \)-farnesene \( (\text{gall-infestation \times warming}: p = 0.038), (\text{gall-infestation \times warming}: p = 0.04) \), and isogermacrene D \( (\text{gall-infestation \times warming}: p = 0.03) \). Their emissions were substantially enhanced by gall-infestation in the second campaign, particularly under ambient conditions (Table S3). \( \alpha \)-farnesene had the largest contribution \( (15\%) \) to total sesquiterpene emissions.

No significant effects of elevation alone were observed for the emission rates of any VOC classes, except that plants at the high elevation tended to emit higher amounts of isoprene \( (p = 0.058) \) and DMNT \( (p = 0.076) \). There were significant interactions between elevation and campaign for sesquiterpenes and other VOCs, as the emissions of these VOC groups were higher at the high elevation than at the low elevation in both the first and second campaign (Fig. 2f, i). The emission rates of benzenoids and monoterpenes were not affected by warming, gall-infestation, or their interaction (Fig. 2e, h).

### 3.3. Leaf chemical composition

The chemical composition of the leaf samples analysed by NIRS was significantly altered by warming and gall-infestation, and it was also distinct for the two elevations (Fig. 3). The first PC, which explained 52% of the variation in the NIRS data analysed by PCA, separated the two elevations and showed a significant warming and gall effect \( (p < 0.001) \). Moreover, there was an interaction between elevation and warming \( (p = 0.03) \). The second PC explained 24% of the variation and likewise, separated the samples by warming treatment \( (p < 0.001) \). Furthermore, the effect of warming was different between the two elevations \( (p < 0.01 \text{ for the interaction}) \).

### 4. Discussion

#### 4.1. The effects of warming on VOC emissions

VOC emissions from *Salix* spp. are dominated by isoprene (Loreto et al., 2014; Rinne et al., 2009; Svendsen et al., 2016; Vedel-Petersen et al., 2015), as we also observed here for *S. myrsinites*. We found a
54% increase in isoprene emission rates under experimental warming of −5–8 °C on two Arctic heaths. An earlier study in the Arctic found that open-top chamber warming by 3 °C increased isoprene emission rates from *S. glauca* by 68% (Kramshøj et al., 2016). Rinnan et al. (2011) found no significant increase of isoprene emissions from *S. phylicifolia*, but the measurements were incidentally taken while temperature was similar in the control and warming treatments. Studies examining the effects of warming on tundra plant community emissions of isoprene have found larger, often several-fold increases in isoprene emissions compared to our results and other
and PPFD, using the G93 model (Guenther et al., 1993), were also
plant isoprene emissions in the Arctic follow a steeper temperature
ture up to 40 °C. Together, these studies clearly demonstrate that
2021), which all found an exponential increase with leaf tempera-
traits that may in
Physiological properties, maturity, and development of the plant,
regulation of genes and enzymes related to isoprene biosynthesis.
results from branch measurements (Kramshøj et al., 2016; Lindwall et al., 2016b; Valolaiht et al., 2015).

The strong temperature dependency of isoprene emissions is well established. We found an exponential temperature dependency of isoprene emission rates within a temperature range of 16–43 °C recorded inside the enclosure during measurements, and a linear correlation with PPFD. The isoprene-temperature response curve observed here did not fit well the G93 empirical leaf-level isoprene emission model (Guenther et al., 1993), which predicts an exponential increase with temperature up to 30–35 °C, then a rapid decay. However, our results are in agreement with several recent studies in the Arctic (Angot et al., 2020; Seco et al., 2020; Simin et al., 2021), which all found an exponential increase with leaf temperature up to 40 °C. Together, these studies clearly demonstrate that plant isoprene emissions in the Arctic follow a steeper temperature response than the G93 model assumes.

Isoprene emission rates standardised to a fixed temperature and PPFD, using the G93 model (Guenther et al., 1993), were also significantly increased by warming. This suggests that in addition to increasing isoprene volatilisation, warming likely leads to an up-regulation of genes and enzymes related to isoprene biosynthesis. Physiological properties, maturity, and development of the plant, all traits that may influence VOC emissions, may also have been affected by increased temperatures (Laathaworkitatkul et al., 2009).

In fact, our NIRS results clearly show that the chemical composition of the leaf material was altered by the warming treatment at both of our experimental elevations. Schollert et al. (2017) have reported physiological and morphological changes in tundra dwarf shrubs already in the first growing season after establishment of the warming treatment. They found that the epidemics of Betula nana had thickened and that the granular trichome density and photosynthes-
sis rates had increased in Empetrum hermaphroditum.

Compound groups other than isoprene made up less than 10% of the VOCs emitted by S. myrsinites and for these VOC groups, we found either no change or a decrease in emission rates in response to warming. We observed decreased emissions rates for GLVs, DMNT, and other VOCs. A decrease in emission rate is contrary to the expected temperature dependency of VOC biosynthesis and release (Peñuelas and Staudt, 2009). We suggest that the concurrent decrease in emission rates for GLVs, DMNT, and other VOCs, while isoprene emissions increased, may be related to a trade-off in biosynthesis of different compounds, especially those sharing the same biosynthesis pathways, due to competition for immediate substrates. Isoprene has been shown to play a major role against oxidative stress (Loreto et al., 2014; Loreto and Fineschi, 2015). It has been hypothesized that under mild and moderate stress, emissions of isoprene are favoured over other volatile compounds also synthesized through the methylerythritol phosphate (MEP) pathway (Fini et al., 2017). In contrast, isoprene emissions have been found to decrease under severe stress, in favor of other compounds more beneficial for specific stressors (Fini et al., 2017). Ghirardo et al. (2020) observed that warming tended to decrease emissions of sesquiterpenes from tundra heath microcosms dominated by Salix. They suggested that the warming-induced increase in isoprene emissions led to lower availability of biochemical pre-
cursors for the biosynthesis of sesquiterpenes (Ghirardo et al., 2020).

4.2. Effect of gall-infestation on VOC emissions

While an increasing number of studies have investigated plant VOC responses to insect herbivory, few have focused on the impacts of gall-infestation. Previous studies documented that galls can reduce plant photosynthesis (Mosbacher et al., 2013; Patankar et al., 2013b), which in turn, decreases emissions of photosynthesis-dependent VOCs, such as isoprene (Noe and Niinemets, 2020; Tooker et al., 2008). We observed that infestation by A. tetanothrix mites did not influence isoprene emissions from S. myrsinites, which disagrees with the only two previous studies that investigated isoprene responses to gall-infestation (Jiang et al., 2018; Ye et al., 2019). Jiang et al. (2018) found decreased isoprene emissions in Quercus robur infested by gall wasp species, while Ye et al. (2019) showed increased isoprene emissions in Populus × petrovskiana infested by gall aphids. VOC emissions, including isoprene, are known to scale with the intensity of insect herbivory (Jiang et al., 2018; Niinemets et al., 2013; Ye et al., 2019). However, we observed no correlation between isoprene emissions and gall-infestation levels. The lack of a clear effect of gall-infestation on isoprene emis-
sions observed in our study might be due to VOC-mediated within- and between-plant signalling. VOCs emitted from herbivore-
damaged plants have been well documented to induce and/or prime VOC emissions from undamaged tissues of the same damaged plants as well as from neighbouring undamaged plants (Karban et al., 2014; Li and Blande, 2017).

In hybrid aspen, for instance, herbivore-induced VOCs were observed to enhance isoprene emissions from undamaged branches of the damaged saplings (Li and Blande, 2017). The galled and unga lled branches in our experimental plots were spaced 20–70 cm apart, which falls within the distance over which VOC- mediated within- and between-plant signalling can occur in nature (Karban et al., 2014), and further, the branches might be connected to each other below ground. Therefore, it is possible that gall-infested branches might have stimulated isoprene emissions from neighbouring uninfested branches via both vascular and airborne signals, which in turn may have overshadowed the direct effects of gall infestation on isoprene emissions. Finally, we could not rule out the possibility that S. myrsinites gets resistant to A. tetanothrix infestation by activating the production of other, more effective defense compounds while not altering isoprene production.

In contrast to isoprene, DMNT emissions increased significantly in response to A. tetanothrix infestation. DMNT is a typical herbivore-
induced volatile and known to act as a cue to attract predators or parasitoids to plants under herbivore attack (Copolovici et al., 2014; Tholl et al., 2011). However, to the best of our knowledge, our study is the first to show increased emissions of DMNT in response to gall- infestation. We also observed gall-induced emissions of GLVs, in agreement with previous studies (Borges, 2018; Ye et al., 2019). Many GLV compounds, such as (Z)-3-hexenyl (E)-2-hexenal, (Z)-3-hexenyl and (Z)-3-hexenyl acetate that were released in large quantities from gall-infested plants in the present study, have been demonstrated to be highly attractive to herbivore natural enemies (Borges, 2018; Kessler and Baldwin, 2011). We may speculate at this point that gall-induced GLV emissions from S. myrsinites may be implicated in the attraction...
of the natural enemies of *A. tetanothrix*. Lastly, gall-infestation significantly increased sesquiterpene emission rates by 60% compared to the intact branches on July 20 at the low elevation, while having no significant effects otherwise. Klimm et al. (2020) found that oak leaves galled by gall wasps emitted higher quantities of farnesene compared to controls. We also found that the emission rates of α-farnesene tended to increase under gall-infestation. Increased emission rates of farnesene due to herbivory have been recorded in previous studies (Kigathi et al., 2009; Pierre et al., 2011) and this compound has been suggested to both repel herbivores and attract their natural enemies (De Moraes et al., 1998; Wei and Kang, 2006).

The untargeted NIRS analysis indicates that the chemical composition of the sampled leaves was affected by *A. tetanothrix* infestation, as shown by our PCA. Our analysis preparation did not separate the galls from the leaves and as such, we cannot tease apart the relative contributions of galls themselves and gall-triggered leaf responses to the observed gall effects on leaf chemical composition. Earlier studies have shown that chemical compositions of both galls and un-galled leaf tissues in the galled leaves differ from that of un-galled leaves (Mosbacher et al., 2013; Nyman and Julkunen-Tiitto, 2000; Patankar et al., 2013b). For instance, Patankar et al. (2013b) found that in two Salix species (*S. pulchra* Cham. and *S. glauca* L.) growing in Alaskan Arctic tundra, galls caused by eriophyoid mites had higher levels of glucose and fructose, as well as a higher C:N ratio, but a lower level of starch, compared to the remaining leaf tissue of gall-infested plants and un-infested leaves of gall-free plants. Similarly, Mosbacher et al. (2013) found that in *S. arctica* growing in Northeast Greenland, eriophyoid mite-infested leaves had lower C and N contents than un-infested leaves. In combination with our results, these studies suggest that infestation by eriophyoid mites can alter leaf chemical composition.

### 4.3. The effects of elevation on VOC emissions

When accounting for the variation in temperature and PPFD during the VOC measurement periods, we found no significant effects of elevation per se on VOC emission rates, although emissions of isoprene and DMNT tended to be higher at the high elevation than at the low elevation. However, significant interactions were seen between elevation and campaign for sesquiterpenes and other VOCs, which were released in larger quantities at the high than low elevation during the first and second campaigns. Moreover, both the VOC blends and foliar spectra were clearly separated between low and high elevations, and the effects of warming on VOC blends were stronger at the high than low elevation. Our results indicate that elevation can have variable effects on the emission rate and chemical composition of VOCs as well as the foliar chemical composition, as shown previously (Moreira et al., 2018; Ryde et al., 2021). The variable effects of elevation could be in part due to the fact that VOC emissions are highly affected by instantaneous weather that confounds other effects. Besides temperature, other environmental factors such as UV irradiation and soil moisture may partially drive the higher VOC emissions observed here at the high elevation.

In addition, we found higher responses of DMNT, sesquiterpene and GLV emissions to gall-infestation at the low elevation than at the high elevation. While we observed similar gall-infestation levels on the VOC-sampling branches at both elevations, this observation should be interpreted with caution as our study was not intended to investigate the elevation effects on gall infestation and thus we did not sample sufficient numbers of plants from a reasonable number of localities at each elevation. Indeed, an earlier study on five Arctic willow species showed consistently lower *A. tetanothrix* infestation levels at high than low elevation (Kuczyński and Skoracka, 2005). It is also known that insect herbivory pressure generally decreases with elevation due to physiological and metabolic constraints on insect habitat expansion imposed by the harsh and variable climate at high elevations (Moreira et al., 2018). It is therefore possible that in our experimental sites as well, the gall infestation levels might be actually higher at the low than high elevation, leading to stronger responses of DMNT, sesquiterpene and GLV emissions to gall-infestation at the low elevation.

### 4.4. Interacting effects of warming and gall-infestation

Although the individual effects of insect herbivores and elevated temperatures on plant phytochemicals have been increasingly documented, limited information exists on their combined effects (Li et al., 2019; Paudel et al., 2020), let alone the interactions associated with gall-forming herbivores. Emissions of isoprene, the dominant component in the VOC blend, increased under warming, independent of mite infestation, but did not change in response to galls. The relatively low levels of gall-infestation could partially explain the lack of interactive effects of warming and galls on isoprene emissions. Recently, Noe and Niinemets (2020) modelled the impacts of gall-forming insects on global VOC emissions and estimated a reduction in global isoprene emissions. Indeed, our results suggest that other climate change factors, such as warming, may be more important in controlling isoprene emission, at least compared to the effects of low levels of gall-infestation. Clearly, more studies using different plant-gall systems, especially under field conditions, are needed before a firm conclusion on the gall impacts on isoprene emissions can be drawn and used to parameterise VOC emission models.

We found an interacting effect of warming and gall-infestation on emissions of DMNT, sesquiterpenes and GLVs, particularly at the low elevation. In these cases, gall-infestation increased emissions of DMNT, sesquiterpenes and GLVs under ambient conditions, but not under warming. This suggests that in some conditions warming may cancel out the stimulatory effects of gall-infestation on GLV and DMNT emissions. In contrast, a recent study in an Arctic tundra on *B. nana* and jasmonate-mimicked herbivory treatments demonstrated a synergistic effect of warming and mimicked herbivory on DMNT and monoterpene emissions (Li et al., 2019). Collectively, these studies indicate that the interactive effects of warming and insect herbivory on plant defensive chemicals, including VOC emissions, appear to depend on the plant-insect systems and chemical identities. Nonetheless, our study suggests that under climatic warming, *S. myrsinites* may enhance emissions of isoprene, which is effective against warming-triggered oxidative stress. An inflection by *A. tetanothrix* mites stimulates emissions of DMNT, sesquiterpenes and GLVs, which are highly attractive to herbivore natural enemies. Under both conditions, the positive effects of warming on isoprene emissions are maintained but the positive effects of *A. tetanothrix* gall-infestation on emissions of DMNT, sesquiterpenes and GLVs are compromised. These results further suggest that when faced simultaneously by warming and *A. tetanothrix* gall-infestation, *S. myrsinites* may prioritize isoprene emissions against warming-induced oxidative stress over emissions of DMNT, sesquiterpenes and GLVs implicated in plant protection against gall-infestation.

**Funding**

This study was financially supported by the Marie Skłodowska-Curie Actions (grant agreement No. 751684), the European Research Council (grant agreement No 777102) under the European Union’s Horizon 2020 research and innovation programme, and the Danish National Research Foundation (DNRF100 CENPERM).

**CRediT authorship contribution statement**

**Laura Swanson:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Tao Li:** Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **Riikka Rinnan:** Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing.
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.

Acknowledgements

We would like to thank Anders Michelsen for setting up the warming treatments, Tommi Nyman for identifying the gall species, Roger Seco for helping with the C93 modeling, Zhengchao Ren for field assistance, and Cleo Davie-Martin for GC–MS analysis of the samples and language revision. The Abisko Scientific Research Station provided an excellent logistics base for the work.

Data availability

All VOC data that support the findings of this study can be found in Figshare at http://doi.org/10.6084/m9..figshare.13365392.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148516.

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