Biogeosciences Discuss., doi:10.5194/bg-2016-464, 2016
Manuscript under review for journal Biogeosciences
Published: 22 November 2016

Passive adsorption of neighbouring plant volatiles linked to associational susceptibility in a subarctic ecosystem.

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Abstract

Neighbouring plants may affect volatile compound emissions of a focal plant and confer associational resistance or susceptibility. Associational resistance has been reported as a result of adsorption of neighbouring plant volatile and semivolatile compounds on focal plant foliage in field experiments. However, these associational effects in a natural ecosystem remain largely unknown.

The effects of the presence and density of *Rhododendron tomentosum (Rt)* understorey on the volatile profile and herbivore density of mountain birch, *Betula pubescens ssp. czerepanovii* (MB) was investigated in a subarctic forest site.The monoterpene β-myrcene, sesquiterpene aromadendrene and sesquiterpene alcohols, palustrol and ledol were recovered from the foliage of MB trees that had *Rt* growing in the understorey. The number of *Rt* shoots growing directly under the MB trees correlated positively with the rate of recovery of adhered compounds and negatively with total MB emissions. Palustrol and β-myrcene recovery from MB leaves showed the highest positive correlation with *Rt* density. Recovery of adhered compounds was higher at lower sampling temperatures. Herbivory was at very low levels both in control and *Rt* plots. The proportion of foliage infected by a gall mites (*Acalitus spp.)* was positively correlated with the recovery of the adhered ledol and palustrol from MB foliage. These results indicate that understorey plant volatiles, both sesquiterpene and highly volatile monoterpenes, may adhere onto and be subsequently re-released from MB foliage at low temperatures during the subarctic growing season. The *Rt* density also plays an important role in the adherence and re-release rates of neighboring plant volatiles and may induce a response in MB volatile emission. Presence of *Rt* volatiles on MB foliage may make them more susceptible to gall mite infestation suggesting that high *Rt* density in the subarctic ecosystem may confer associational susceptibility to herbivores on MB.

1. Introduction

Plants growing in the subarctic are adapted to fast growth owing to the relatively short growing season in the region. The vegetation composition of subarctic ecosystems typically consists of sedges, bryophytes and shrubs; the majority of the tree species in these ecosystems are dwarf or miniature versions of the same species found in warmer climates – a coping mechanism for low nutrient availability as well as harsh environmental conditions (Rinnan et al. 2014). Apart from physical adaptation, plants also use chemical means to cope in the subarctic. For example, individual birch trees change the proportion of their primary and secondary metabolites pools continuously in a growing season (Riipi et al. 2004). Emission of volatile organic compounds (VOCs) is another chemical means by which plants adapted to the subarctic climate cope. Historically, the emission of VOCs from arctic and Antarctic regions were regarded to be minimal (Guenther et al. 1995). More recent evidence however suggests that volatile emissions from these regions may be much higher than earlier thought due to the sensitivity of vegetation to temperature regimes (Holst et al. 2010; Faubert et al. 2010a; Michelsen, Rinnan & Jonasson 2012; Schollert et al. 2014), especially large discrepancies between atmospheric and leaf and soil level temperatures (Svoboda 2009; Rinnan et al. 2014) and 24 hours daylight during growing season (Lindwall, Faubert & Rinnan 2015).

Plant-emitted VOCs mediate various ecological interactions within plants, between plants and between plants and other organisms (Dicke & Baldwin 2010). They can serve as info-chemical cues used for host location and host recognition by insects (Visser 1986; Bruce, Wadhams & Woodcock 2005), and attraction of natural enemies of arthropod herbivores (Karban 2011). In plant to plant interactions, volatile compounds released by a plant can convey improved fitness such as enhanced levels of herbivore resistance on neighbouring plants through passive or active means. In active interactions, volatile signal reception by the neighbouring plant results in a physiological change (Karban et al. 2006; Kost & Heil 2006) while the passive interaction involves adsorption of volatiles to the surface of neighbouring plants and chemical change only at the leaf surface level (Himanen et

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al. 2010; Blande, Holopainen & Li 2010; Li & Blande 2015). This beneficial effect in reduced herbivory on a plant due to coexistence with a neighbouring plant is known as associational resistance (AR) (Karban 2007) while the negative effects marked by increased herbivory and oviposition is described as associational susceptibility (AS) (Li & Blande 2015). Volatile mediated AR/AS have been shown in a number of plant associations both in field and laboratory conditions (Himanen et al. 2010; Himanen et al. 2015; Li & Blande 2015). Some plant species chemically hide other plant species or deter feeding herbivores by chemical means (Visser 1986, Bernays & Chapman 1994, Karban 2007). In agroecosystems, specific aromatic plant volatiles have been successfully tested for the purpose of repelling or masking host cues towards herbivores (Song et al. 2010; Mauchline et al. 2013).

The likelihood of herbivory on a plant is dependent on the plant’s inherent qualities as well as other qualities such as the guild, chemistry, morphology and distribution of neighbouring plants (Atsatt & O'dowd 1976; Karban 2007). The concentration or intensity of specific neighbouring plant traits that affect herbivory on a focal plant is the most important determinant of herbivory reduction (Barbosa et al. 2009). As such, AR or AS mediated by volatiles can be determined by the concentration or intensity of the specific neighbouring VOCs available.

In subarctic ecosystems, the sparse of plant distribution is characterized by relatively short vertical distances between tree canopies and shrubs growing in the understorey. Two co-existing species in the sub-arctic are the mountain birch tree, *Betula pubescens* ssp. *czerepanovii* (N. I. Orlova) Hämet-Ahti (henceforth referred to as MB) and the shrub *Rhododendron tomentosum* Harmaja (henceforth referred to as *Rt*) growing in the understorey. In northern Finland, MB trees are common and coexist with *Rt.* MB trees are introgressive hybrids between the diploid (n = 28) dwarf (*Betula nana* L.) and the tetraploid (n = 56) downy (*Betula pubescens* Ehrh.) birch (Vaarama & Valanne 1973; Kallio et al. 1983; Haukioja 2003; Klemola et al. 2010). MB leaves are located on either long or short shoots (Macdonald & Mothersill 1983; Macdonald, Mothersill & Caesar 1984; Ruohomäki et al. 1997;

Klemola et al. 2010). Long shoots account for the growth of branches and thus canopy expansion, whereas short shoots carry most of the photosynthesizing biomass of a tree individual (Maillette 1982; Macdonald & Mothersill 1983; Macdonald, Mothersill & Caesar 1984).

Rt is a small woody perennial evergreen shrub distributed throughout boreal ecosystems (Urcelay et al. 2003; Himanen et al. 2010). The plant species has a high volatile terpenoid content that gives it a characteristic smell (Butkienė et al. 2008), some of these terpenoid compounds are the species-specific arthropod-repelling (Egigu et al. 2011) C_{15} semivolatile compounds ledene ($C_{15}H_{24}$), ledol ($C_{15}H_{26}O$), and palustrol ($C_{15}H_{26}O$) (Dampc & Luczkiewicz 2013). In addition, this plant emits β-myrcene as a major monoterpene compound (Jaenson, Pålsson & Borg-Karlson 2006; Butkienė et al. 2008; Himanen et al. 2010). This acyclic monoterpene with three double bonds is very reactive with ozone and OH radicals and capable of forming reaction products such as terpenylic acid in the atmosphere (Böge et al. 2013). Oxygenated sesquiterpenoids such as ledol and palustrol are resistant to degradation by ozone in the atmosphere (Pinto et al. 2010), have semivolatile characteristics, and could therefore persist on leaf surfaces (Helmig et al. 2004). The emission of these specific sticky volatiles makes *Rt* an excellent species for studying how passive adsorption of volatiles might contribute to AR or AS and neighbouring plant interactions (Himanen et al. 2010). *Rt* mediated AR/AS may be dependent on the amount of *Rt* plants available within the vicinity of the focal plant. Other factors that affect volatile emissions such as herbivory, mechanical damage and abiotic stress (Mithofer, Wanner & Boland 2005; Kigathi et al. 2009) may also play important roles.

The arctic and subarctic regions are the most at risk of increasing global temperature (Mitchell et al. 1990), with climate models predicting of up to 4°C increases in temperature for arctic summers (IPCC. 2014). Volatile emissions, especially terpenoid compounds are strongly dependent on temperature (Kesselmeier & Staudt 1999) and an increase as low as 2°C has been shown to double terpenoid emissions in Swedish subarctic heath vegetation (Faubert et al. 2010b; Valolahti et al. 2015). Temperature increase is likely to increase the volatile emissions of vegetation growing in the

Finnish subarctic, including emissions from *Rt* and MB. The adsorption and evaporation of *Rt* semivolatiles on neighbouring plant surfaces is also likely to be affected by temperature changes.

In this study, we investigated the effects of abundance and coverage of *Rt* ramets growing in the understorey of MB trees on the branch scale recovery of *Rt* volatiles from MB trees and also the effect of neighbouring *Rt* branches and their volatile compounds on arthropod herbivore on MB trees. We hypothesise that (a) The amount of *Rt* volatiles recovered from neighbouring MB foliage is dependent on the density of *Rt* shrub in the understorey. (b) Recovery of adhered *Rt* compounds is temperature dependent, higher day-time temperatures will lead to loss of adhered compounds and reduced recovery. (c) The abundance of arthropod herbivores and their damage to MB foliage will be affected by the amount of *Rt* volatiles adsorbed on MB foliage.

2. Materials and Methods

2.1. VEGETATION AND HERBIVORE ASSESSMENTS

The research site, the Kevo Subarctic Research Institute of the University of Turku is located in upper Finnish Lapland (69° 45´ N, 27° 01´ E) within the Kevo strict nature reserve area. All assessment and VOC sampling was between 30th June and 2nd July 2015. We selected and grouped the mountain birch, *Betula pubescens* ssp. *czerepanovii* (N. I. Orlova) Hämet-Ahti trees based on the density of *Rhododendron tomentosum (Rt)* shoots growing in their understorey (Appendix A1). Apart from *Rt* shrubs, we found a number of other species growing in the understorey (Appendix A1).

Under 24 mountain birch trees, a 1m^2 quadrant was marked and a MB branch growing directly above each quadrant was selected for VOC sample collection. The density of the *Rt* shoots growing in the quadrant was used to place the trees into three different categories; low, medium and high density. The low *Rt* density group had *Rt* coverage under the tree of 2-17% in the 1m^2 quadrant (n=8). The medium density group was considered to have an average *Rt* density, which was 20 – 25% of the quadrant (n = 4). The high *Rt* density group had a coverage of 40-80% (n = 6). We marked 6 trees with no *Rt* in the understorey as the control group. Due to the small sample size of the medium *Rt* density sites, we merged low and medium density into a new 12 tree group – Moderate density. The branch used for VOC sampling as well as three other branches at the same heights were used for arthropod analysis. The damage levels on leaves and number of arthropods on leaves were observed visually. We recorded and counted the species of arthropods on the leaves and the number of leaves with gall mite colonies per branch. We also counted the number of leaves with holes and other injuries per branch and visually estimated the size of the damage area.

2.2. VOC SAMPLING

The dynamic headspace sampling technique was used to collect VOCs for determination of emission rate (Mäntylä et al. 2008; Blande, Turunen & Holopainen 2009). One branch from each of the 24 MB trees and 10 *Rt* branches were selected for volatile sampling: one *Rt* branches from 6 high *Rt* density quadrants and a branch each from 4 moderate *Rt* density quadrants (control had no *Rt* branches). VOC sampling was done by enclosing branch in a pre-heated multi-purpose cooking bag [polyethylene terephthalate, 25 × 55 cm]. The disposable bags had been pre-heated at 120ºC for an hour before use. Bags were tied at the base of the stem and a hole made at the top corner of the bag, through which airflow of about 300ml min^{-1} was passed. A Tenax TA adsorbent tube was attached to a hole made in the second top corner of the bag and connected to a suction tube that sucked air at a rate of about 250ml min^{-1}. All openings in the bag were made as air-tight as possible by tightening with a flexible clip. The VOC samples from the MB branches were collected for 30 minutes and sample tubes were tightly sealed with brass caps and placed stored in a cold box before analysis. The same sampling system was used for volatile collection of vegetative *Rt* shoots but with collection time reduced to 10 minutes. After sampling, photos of the birch leaves were taken for leaf area measurements. *Rt* branches with narrow leaves were cut and dried at 60°C for 3days after which dry weight was measured. The emission rates of the MB branch were expressed as a function of the leaf area while those of the *Rt* branches were expressed as a function of the plant's dry weight as also the stem bark is covered with glandular trichomes, an important storage organs of volatiles.

2.3. VOC ANALYSIS

The VOCs collected from the plants were stored in adsorbent tubes at +4ºC during transportation and analysed by gas chromatography-mass spectrometry GC–MS (Hewlett Packard GC 6890, MSD 5973) as reported by (Girón-Calva et al. 2014). The compounds adsorbed in the tube were desorbed in a thermal desorption unit at 250ºC for 10 minutes, cryofocused in a cold trap at -30ºC and injected into an HP-5MS capillary column (50 m × 0.2 mm i.d. × 0.5 µm; Agilent Technologies, USA), helium was the carrier gas. Oven temperature was at 40°C for one minute, then raised to 210°C at 5°C min^{-1} and further to 250°C at 20°C min^{-1}. The compounds (Terpenes and GLVs) were identified by comparing their mass spectra, retention time and peak with those in the Wiley library and pure standards. A palustrol standard was unavailable, so a ledol standard was used to calculate its emission. Other unavailable compounds were quantified by comparing peak areas to corresponding peak area and amount (ng) of α-pinene in pure standard. Emission rates were expressed as ng g^{-1} LA m^{-2} h^{-1} for birch leaves and ng g^{-1} leaf DW h^{-1} for *R. tomentosum* shoots.

Due to the temperature dependence of plant volatile emissions, we calculated the MB emissions with temperature standardization as reported by (Guenther et al. 2012). The standardized measurements were calculated using algorithms relative to temperatures of 30°C, which is a common reference temperature. These algorithms were inapplicable for emissions of adhering compounds (compounds emitted by *Rt* shoots and recovered from MB branches) on MB foliage which were not related MB photosynthesis activity, so unstandardized values were used. To compare emission rates of *Rt* with earlier work by (Himanen et al. 2010), and as a result of high proportion of terpene glands on bark surface that are not related to photosynthetic emissions, *Rt* emission rates were also unstandardized. The temperature and humidity in the PET bag was measured during collection using the wireless

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Manuscript under review for journal Biogeosciences
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temperature/humidity loggers (Hygrochron DS1923- F5 i Button, Maxim Integrated Products, Inc., CA). These temperatures were used in the standardized emission rate calculations as well as temperature correlation analyses.

2.4. STATISTICAL ANALYSIS

All statistical analyses were performed using the SPSS statistics 21.0 package (SPSS, Inc, Chicago, IL, USA). The variables were tested for normality and equality of variance and the Student's t-test was used to compare the difference between volatile emissions of moderate and high density *Rt* shoots. Due to emissions of many compounds below detection limit in some of the MB samples, most of the variables were not normally distributed. We used the non-parametric Kruskal-Wallis test for comparison between the three groups and Mann-Whitney test was used for pairwise comparisons, because the assumptions of ANOVA were not met. Due to the small sample size and non-normality of data, the non-parametric Kendall’s tau-b rank order correlation co-efficient was used to test the relationship between the recovery of *Rt* compounds from MB foliage and *Rt* shoot numbers and herbivore abundance and damage on MB foliage.

3. Results

3.1.*RHODODENDRON. TOMENTOSUM* (*RT*) SHOOT EMISSIONS

The volatile compounds emitted by *Rt* branches included the monoterpene, β-myrcene (58% of total VOC emissions), the sesquiterpene, aromadendrene (8%) and the sesquiterpene alcohols palustrol and ledol (15 and 3% respectively) (Table 1). There was no difference in the means of *Rt* compounds emitted from sampled branches from high and moderate *Rt* quadrants. *Rt* branches from high density quadrants had higher emission rates per emitting unit (ng g^{-1} h^{-1}) of these four compounds than those from moderate *Rt* density quadrants – 60 vs 38% for β-myrcene, 8.9 vs 4.4% for aromadendrene, 16 vs 10% and 3.9 vs 2.4% for palustrol and ledol respectively.

3.2. MOUNTAIN BIRCH (MB) SHOOT EMISSIONS

The total monoterpenoid emissions from MB trees was highest in the control group and then decreased with increasing *Rt* density. The control (Mann–Whitney $U = 4.0$; $P = 0.026$) and moderate *Rt* (Mann–Whitney $U = 14$, $P = 0.04$) groups differed significantly from the high *Rt* group (Table 2). There was a marginally significant decrease in the emission of α-pinene from MB branches across the three *Rt* groups. In between groups, trees growing above high *Rt* density had significantly lower α-pinene emission compared to the control group (Mann–Whitney U = 3.5, P = 0.037) (Table 2). There was also a non-significant decreasing trend in sesquiterpene emissions from MB branches with increasing *Rt* density, the lowest total sesquiterpene emission was from trees growing above high *Rt* density (Table 2).

There was a marginally significant reduction in total MB VOC emission across the three groups (Table 2), control group (Mann–Whitney $U = 3$, $P = 0.015$) had significantly higher total volatile emissions compared to high *Rt* group (Table 2).

3.3. ADHERED COMPOUNDS ON MOUNTAIN BIRCH (MB) SHOOTS

The emission rates of β-myrcene, the major monoterpene released by *Rt* increased across the MB tree groups from control to high *Rt* quadrants (Table 2). There was higher emission of β-myrcene from MB trees in high and moderate *Rt* quadrants compared to control (Mann – Whitney $U = 3.5$, $P = 0.015$; and Mann–Whitney $U = 18.5$, $P = 0.05$ respectively) (Table 2).

The other terpenoid compounds recovered from sampling the MB branches were sesquiterpenoid compounds emitted characteristically by *R. tomentosum* (palustrol, ledol and aromadendrene). There was increase in the palustrol recovery from MB trees across the three treatment groups (Table 2). Palustrol emissions from MB branches sampled from both moderate (Mann–Whitney $U = 9$, $P = 0.01$) and high density (Mann–Whitney $U = 3$, $P = 0.015$) *Rt* quadrants were significantly higher than emissions from control trees (Table 2).

The emission of ledol was only found in six trees both in moderate and high density quadrants and recovered at temperatures below 12.5°C. Aromadendrene, the other volatile not emitted by the MB trees was recovered from foliage in both moderate and high density quadrants at rates below 5ng/m^2/h. (Table 2).

There was also an increase in total adhered emissions (TAE) (i.e. β-myrcene, aromadendrene, ledol and palustrol) across the three groups. The adhered emissions in MB branches in moderate *Rt* (Mann–Whitney *U* = 11, *P* = 0.018) and high *Rt* (Mann–Whitney *U* = 3.5, *P* = 0.05) quadrants were higher than those from the control group (Table 2).

3.4.*R. TOMENTOSUM* ABUNDANCE AND RECOVERY RATE OF ADHERED COMPOUNDS

The total MB VOC emission was negatively correlated (n = 24, τ_b = -0.399, P = 0.007) with the number of *Rt* shoots growing in the understorey (Fig. 1a). The recovery of β-myrcene (n = 24, τ_b = 0.550, P < 0.001), palustrol (n = 24, τ_b = 0.452, P = 0.004) and total adhered emissions (n = 24, τ_b = 0.476, P = 0.02) from MB branches were all positively correlated with the number of shoots growing in the understorey (Figs. 1b,c,d respectively).

3.5. TEMPERATURE AND RECOVERY RATE OF ADHERED COMPOUNDS

The relationship between the recovery of adhered compounds and temperature was tested only among treatment groups – moderate and high *Rt*. The recovery of all adhered compounds except β-myrcene showed a negative correlation with temperature. The association of temperature with the recovery of adhered compounds from MB leaves was strongest in ledol (n = 18, τ_b = -0.598, P = 0.002) (Fig. 2a). Aromadendrene and palustrol showed negative correlation with temperature (n =18, τ_b = -0.425, P = 0.024; τ_b = -0.433, P = 0.013) respectively (Figs 2b,c). The total recovered adhered emissions including β-myrcene (TAE) did not show a statistically significant correlation with temperature (n = 18, τ_b = -0.304, P = 0.081) (Fig. 2d).

3.6. EFFECTS ON HERBIVORY

Herbivore density was very low during our sampling. We found a small number of insects (aphids, sawfly larvae and homopteran nymphs) on a number of assessed branches. There were also leaves with colonies of white gall mites, *Acalitus rudis* (Canestrini), and red gall mites, *A. longiseotus* (Nalepa) (Acari, Eriophyiidae). The proportion of leaves infested with white gall mites correlated positively with the recovery of palustrol ($n = 24$, $\tau_b = 0.424$, $P = 0.006$), ledol ($n = 24$, $\tau_b = 0.59$, $P < 0.001$) and total adhered compounds ($n = 24$, $\tau_b = 0.34$, $P = 0.02$) from MB branches. The proportion of leaves with arthropods (aphids, sawfly larva, homopteran nymphs and gall mites) correlated with the recovery of palustrol and ledol, while the proportion of leaves with holes was positively correlated with β-myrcene recovery. (Table 3).

4. Discussion

4.1. *R. TOMENTOSUM* VOLATILES ADHERE TO MOUNTAIN BIRCH BRANCHES

Our results gave a clear indication that *Rt* vegetation in the understorey of MB resulted in the adherence and re-release of *Rt* volatiles on MB foliage. This agrees with earlier observations by Himanen et al, (2010) where the *Rt* volatile sesquiterpene, ledene as well as the sesquiterpene alcohols, ledol and palustrol were recovered from silver birch (*Betula pendula* Roth) growing in the vicinity.

The dominating *Rt* monoterpene, β-myrcene was also recovered in significantly higher amounts in MB trees growing above high density *Rt* shoots compared to the control group in our study. Although, β-myrcene is among the monoterpenes synthesized and emitted in minor quantities by *Betula* spp. (Mäntylä et al. 2008; Maja et al. 2015), only one out of the six control MB branches sampled in our study emitted the compound. Our results suggest that monoterpenes as well as sesquiterpenes can be adsorbed and re-released by non-source emitters in subarctic ecosystems. There was no significant adherence and rerelease of β-myrcene in previous field (Himanen et al. 2010) and laboratory (Himanen et al. 2015) studies using *Rt* in plant associations. The emission rate of β-myrcene from the *Rt* shoots in our study was similar to the rates reported by (Himanen et al. 2010). However, the β-myrcene/palustrol ratio in *Rt* was only 1.7 in a natural habitat and 2.8 in a field experiment (Himanen et al. 2010), while in our study the β-myrcene/palustrol ratio in *Rt* shoots was 3.6 in moderate *Rt* coverage and 3.7 in high *Rt* coverage. Higher β-myrcene concentration in *Rt* shoot emissions in the subarctic site suggests that although similar amounts of β-myrcene were available in *Rt* emissions, a higher proportion of β-myrcene in the adhered pool is expected. Furthermore, the comparatively higher tropospheric ozone levels in boreal zones compared to the subarctic (Cooper et al. 2014) could reduce the amount of β-myrcene available for adherence due to its high reactivity with ozone and hydroxyl radicals (Böge et al. 2013; Li, Blande & Holopainen 2016).

Generally, the deposition and rerelease of compounds on leaf surfaces is expected to be governed largely by the physicochemical properties of the compound, leaf surface characteristics as well as air and leaf temperature (Niinemets et al. 2014). Due to the lipophilic nature of cuticular waxes of leaf surfaces, lipophilic uncharged volatiles can be adsorbed and retained on a plant surface (Müller & Riederer 2005). The hydrophobic nature of non-oxygenated monoterpenes and sesquiterpenes including β-myrcene and aromadendrene means they can be directly adsorbed onto leaf cuticle upon gas deposition and can be taken up through the cuticle into the leaf through diffusion (Bakker 2001; Keyte et al. 2009; Burkhardt & Pariyar 2014). Monoterpene uptake and rerelease by non-emitting plant foliage has been observed in a number of plants in experiments exposing plants to pure compounds. (Noe et al. 2008) showed that limonene can be adsorbed and rereleased by plant foliage of 13 different species. The uptake of this compound scaled positively with individual leaf lipid content (Noe et al. 2008). The hydrophobic nature of deoxygenated monoterpenes like β-myrcene mean that they tend to partition in the leaf lipid phase, rather than the aqueous phase (Niinemets & Reichstein 2002; Noe et al. 2006) which means that leaf lipid content can positively affect the amount of β-myrcene or other monoterpenes it adsorbs. The leaf surface structures can also play a role in the leaf's adsorptive capacity; the MB leaves (used in our experiment) differed from silver birch (Himanen et al. 2010) by having a higher number of short and long non-glandular trichomes (Valkama et al. 2003), which are secretors of lipids and waxes (Valkama et al. 2004). These could be possible explanations for high β-myrcene recovery in MB branches growing above *Rt* shoots in our study.

4.2. SIGNIFICANCE OF THE *R. TOMENTOSUM* SHOOT DENSITY

According to our categorization at the natural growing site, there was an *Rt* density effect on the amount of palustrol and β-myrcene recovered from the MB branch growing directly above. The other sesquiterpenoid compounds, ledol and aromadendrene emitted by *Rt* were also recovered only in trees with *Rt* shoots in the understorey. The total MB emissions correlated negatively with *Rt* abundance, which raises the question of whether the presence of *Rt* or its volatile emissions induces a response in neighbouring MB trees. Induced responses as a result of neighbouring plant volatiles have been observed both in field (Karban 2001; Karban et al. 2006) and agricultural (Khan et al. 1997) settings.

The recovery of other *Rt* compounds (aromadendrene and ledol) from a small number of MB branches with *Rt* in the understorey, suggests that *Rt* presence and density alone may not be enough for adherence of sticky volatile compounds on neighbouring plant foliage. Other factors like temperature (Niinemets et al. 2014) and distance (Heil & Adame-Alvarez 2010) may play important roles. The vertical distances between the base of *Rt* shoots and the sample MB branches in our study ranged between 100 – 106cm. For comparison, small amounts of *Rt* volatiles have been recovered on birch emissions at distances of up to 5m (Himanen et al. 2010). The distances in our study is higher than usually reported for volatile mediated intraspecific and interspecific interactions between plants. For example, in the case of interspecific volatile mediated plant interactions between sagebrush and tobacco plants, effective distances were restricted to about 15cm (Karban 2001). Lima bean volatiles have been shown to affect neighbouring plants at a distance of no more than 50cm in field conditions or 70 cm in controlled chamber environments (Blande, Holopainen & Li 2010) and clipped sagebrush induced a response in conspecifics at a distance of 60cm (Karban et al. 2006).

4.3. EFFECTS ON HERBIVORY

Due to low levels of MB herbivory in the study site, the effect of the adsorption and rerelease of *Rt* volatiles on AR/AS in mountain birch could not be properly elucidated. However, there was a positive correlation between the proportion of leaves infested with white gall mites on sampled branches and recovery of adhered compounds as well as the number of *Rt* branches in the understorey. This raises the question of whether *Rt* presence or adhered volatiles enhance MB trees susceptibility to gall mite infestation. However, *Rt* presence, might also indicate more benign environment for all organisms and support insect and mite preference towards *Rt* rich habitats.

AS mediated by the adsorption of neighbouring conspecific plant volatiles has been shown by (Li & Blande 2015), who found increased oviposition by *Plutella xylostella* on plants and artificial surfaces exposed to herbivore induced sesquiterpenes from neighbouring *Brassica oleracea* plants. Experiments in a silver birch/*Rt* association (Himanen et al. 2010) and *Brassica*/*Rt* system (Himanen et al. 2015) showed that adhered *Rt* compounds can protect focal plants against some herbivores in an AR relationship. In field observations by (Himanen et al. 2010), only one (*Euceraphis sp.)* of three aphid genera found on birch seedlings, was found in higher amounts on control birch leaves compared to *Rt*-exposed birch leaves. Similarly, only one birch feeding herbivore, *Phaedinus flavipes* preferred non-exposed birch leaves to *Rt*-exposed leaves when two different herbivores were tested (Himanen et al. 2010). These observations suggest that volatile mediated AR/AS can be very species-specific in action and many herbivores can be unaffected or even attracted to the host plant as a result of the same adhering compounds. Furthermore, the different ratio of monoterpene β-myrcene to sesquiterpene alcohols in the adhered compound pool might be a crucial factor to explain the AS effects of *Rt* in this study when compared to AR in earlier studies (Himanen et al. 2010, 2015).

Herbivore responses in plant associations have also been shown to vary among host species. In a comparison of three forest tree species growing in mixed and monoculture forests, (Vehviläinen et al. 2006; Vehviläinen, Koricheva & Ruohomäki 2007) reported that lower insect herbivory (AR) on silver birch growing in mixed cultured trees compared to monocultures, while sessile oak and black alder had higher insect herbivory (AS) in mixed cultures compared to monocultures. These discrepancies in response of herbivore hosts can also be affected by sampling time, tree age and location of trees (Vehviläinen, Koricheva & Ruohomäki 2007). AR in terms of lower density of insect defoliators reported for mixed cultured silver birch compared to monocultures in Finland was only observed during the early part of the growing season (early June), by the end of the season, the effect was lost (Vehviläinen et al. 2006). Analysis based on more extensive datasets by (Vehviläinen, Koricheva & Ruohomäki 2007) showed that effects of forest diversity on herbivores can change direction from AR to AS over the course of a season.

Associational effects are therefore species-specific in action both in terms of herbivores and also host plant species. A plant – neighbour association mediated by a known mechanism can possibly confer AR and AS or have no effect at all based on the specific herbivore involved, the type of plants and time of season.

4.4. SIGNIFICANCE OF TEMPERATURE

BVOC emissions from plants growing in subarctic ecosystems are very susceptible to temperature changes (Rinnan et al. 2014). Monoterpene emissions doubled and sesquiterpene emissions increased five-fold in subarctic heath vegetation in northern Sweden as a response to 2°C increase in temperature (Valolahti et al. 2015). This means that temperature increase, which is predicted to be double in arctic regions compared to other regions of the world (Pachauri et al. 2014), is likely to increase plant emission of volatile and semi-volatile compounds in these ecosystems.

The semi-volatile nature of the sesquiterpenes emitted by the *Rt* shoots make them subject to temperature controlled condensation and evaporation. There was a strong association between temperature and recovery rate of the *Rt* volatiles from MB branches. Ledol, for example was only recovered at sampling bag temperatures less than 12.5°C indicating rapid release of these compounds at higher temperatures before sampling. The results support our hypothesis that increasing temperature may result in re-release of the adhered semi-volatile compounds from MB foliage. This is in line with the results of (Himanen et al. 2015) who found stronger associational resistance in *Brassica oleracea* due to *Rt* volatiles at 12°C compared to 22°C. The temperature dependence of the adherence and rerelease of sesquiterpenes from surfaces have also been reported by Schaub et al., (2010).

The mean atmospheric temperature during the sampling period ranged between 5.2 – 9.8°C night time and between 8.3 -13.3°C daytime (FMI). In subarctic climates there can be significant discrepancy between atmospheric and leaf temperatures (Svoboda 2009), high solar radiation can lead to temperature increases of up to 15°C in microclimates by heating up soil and consequently the low-stature plants (Rinnan et al. 2014). These differences in temperature make the adsorption and evaporation behaviour of semi-volatile compounds difficult to study and predict in a natural ecosystem. The increasing warming trend may also alter the vegetation composition and subsequently affect the quantity and quality of volatile emissions in these regions (Valolahti et al. 2015). In the Swedish subarctic, a 19% increase in the MB density was observed over a period of 13 years (Hedenås et al. 2011) while the coverage of shrubs like *Rt* are increasing in warm arctic locations (Elmendorf et al. 2012).

5. Conclusion

We demonstrate the phenomenon of adherence and rerelease of *Rt* volatiles from nearby mountain birch foliage in a natural sub-arctic ecosystem. Furthermore, we were able to show a *Rt* density and temperature effect on the adsorption and rerelease rate of these volatiles from neighbouring plants. We also observed that the presence of *Rt* or it's volatiles may make MB trees subject to gall mite infestation and thus have an associational susceptibility relationship. Our results provide more evidence on the complexities and possible variation of associational relationships between plants.

Acknowledgement

This study was funded by the Academy of Finland (project no. 278424). We thank the staff in Kevo Subarctic Research Station and Mr. Rana Siddiq for their support during our sampling. KM was supported by FY 2014 Researcher Exchange Program between Japan Society for the Promotion of Science and the Academy of Finland. We are also thankful to Dr Tero Klemola, Dr Minna Kivimäenpää, Dr James Blande and Dr Tao Li for their comments on the manuscript.

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TABLES AND FIGURES

Table 1. Mean volatile compounds emitted by *Rhododendron tomentosum* branches in moderate and high density coverage in order of retention time. Emission rates expressed as ng g^{-1} DW h^{-1} ± SE $n = 6$ in high *R. tomentosum* density and $n = 4$ in moderate *R. tomentosum* density.

Compound	**Moderate *Rt* Coverage**	**High *Rt* Coverage**
α-Pinene	82.85 ± 39.64	67.34 ± 15
Camphene	54.76 ± 37.04	23.58 ± 5.12
Sabinene	107.59 ± 37.77	173.41 ± 57.75
β-pinene	44.31 ± 21.22	46.88 ± 14.75
6-methyl-5-hept-2-one	37.10 ± 15.00	38.78 ± 17.51
Myrcene	31429.51 ± 13267.39	43092.38 ± 12703.5
δ-3-carene	10.84 ± 6.43	20.37 ± 6.93
α-Terpinene	265.25 ± 98.34	313.84 ± 127.02
Cymene	116.65 ± 46.09	116.99 ± 26.66
Limonene	133.75 ± 66.25	127.29 ± 38.21
Cis ocimene	271.69 ± 143.84	897.37 ± 225.49
β-ocimene	238.4 ± 69.02	481.49 ± 83.47
γ-Terpinene	76.33 ± 25.91	90.86 ± 34.02
Terpinolene	18.52 ± 12.7	36.31 ± 16.24
Nonanal	0.00 ± 0.00	71.22 ± 47.83
Allo-ocimene	0.00 ± 0.00	5.13 ± 5.13
Borneol	22.2 ± 22.2	0.65 ± 0.65
Terpinen-4-ol	0.59 ± 0.59	0.00 ± 0.00
α-Terpineol	1.62 ± 1.62	0.00 ± 0.00
Bornyl acetate	89.75 ± 76.19	57.52 ± 18.83
Citronellyl acetate	1000.12 ± 604.57	244.20 ± 31.99
Geranyl acetate	4351.16 ± 3251.37	1261.49 ± 291.19
β-Elemene	33.46 ± 19.79	57.34 ± 18.80
Azulene	1967.64 ± 1075.28	2659.76 ± 1016.09
(E)-β-caryophyllene	2299.14 ± 799.48	174.17 ± 77.28
(E)-β-farnesene	147.29 ± 130.64	0.00 ± 0.00
Calarene	101.19 ± 59.82	207.72 ± 47.13
Aromadandrene	3635.26 ± 1122.34	6343.90 ± 2309.45
α-Humulene	92.49 ± 27.11	185.08 ± 100.00
Ledene	117.42 ± 46.57	119.09 ± 28.84
Palustrol	8654.52 ± 2707.18	11442.50 ± 1964.00
Ledol	1929.68 ± 596.12	2767.60 ± 371.38
Total	**57328.9 ± 26462.27**	**71124.26 ± 19700.93**

Table 2. Median values (IQR) for volatile emissions (ng m^{-2} LA h^{-1}) from mountain birch (MB) trees in three different categories of *Rhododendron tomentosum* (*Rt)* growing in understorey, median (IQR), P-values for difference across 3 groups – (Kruskal-Wallis test) are shown, statistically significant values emboldened. Different letters ^{ab} represents significant (p<0.05) difference between groups (Mann-Whitney U test)

Compounds	Control	Moderate *Rt*	High *Rt*	P-value
Monoterpenoids (MT)				
α-pinene	59.00 (75.15 – 28.30)^{a}	37.40 (66.39 -19.99)^{ab}	9.72 (31.48 – 1.27)^{b}	0.098
Camphene	0.00 (2.58 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.195
Sabinene	8.60 (56.78 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.140
β-pinene	5.33 (16.27 – 0.00)	0.00 (12.22 – 0.00)	0.00 (0.00 – 0.00)	0.343
δ-3-Carene	5.02 (9.05 – 1.19)	0.00 (10.80 – 0.00)	5.48 (11.16 – 0.00)	0.809
α-terpinene	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.607
Limonene	96.45 (119.65 – 52.23)	81.77 (128.29 – 31.35)	34.51 (56.72 – 4.37)	0.112
1,8 Cineole	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.571
β-Ocimene	0.00 (0.00 – 0.00)	0.00 (8.07 – 0.00)	0.00 (4.71 – 0.00)	0.830
γ-terpinene	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.612
Linalool	74.72 (93.59 – 24.61)	17.03 (41.63 – 0.00)	4.94 (19.81 – 0.00)	0.163
Total Monoterpenoids	277.10 (631.88 – 162.60)^{a}	210.09 (393.15 – 113.65)^{a}	71.05 (129.77 – 8.11)^{b}	**0.044**
Sesquiterpenoids (ST)				
α-copaene	45.80 (97.17 – 0.00)	0.00 (26.8 – 0.00)	0.00 (43.79 – 0.00)	0.516
(E)-β-caryophyllene	308.36 (209.46 – 170.24)	170.24 (413.27 – 117.39)	63.23 (200.63 – 51.65)	0.094
α-humulene	0.00 (0.00 – 0.00)	0.00 (35.38 – 0.00)	0.00 (31.04 – 0.00)	0.740
Germacrene	57.85 (96.44 – 6.29)	8.69 (83.87 – 0.00)	0.00 (18.38 – 0.00)	0.354
Caryophlyllene oxide	265.02 (375.56 – 160.00)	144.11 (255.75 – 0.00)	81.09 (193.92 – 51.6)	0.518
Total Sesquiterpenoids	809.85 (1814.65 – 593.56)	475.78 (657.94 – 249.09)	212.13 (414.94 – 112.17)	0.098
Adhered Compounds				
β-myrcene (MT)	0.00 (0.00 – 0.00)^{a}	14.10 (26.13 – 0.00)^{b}	26.32 (33.87 – 21.6)^{b}	**0.039**
Aromadendrene (ST)	0.00 (0.00 – 0.00)	0.00 (4.39 – 0.00)	1.49 (3.32 – 0.00)	0.206
Palustrol (ST)	0.00 (0.00 – 0.00)^{a}	3.50 (15.19 – 1.24)^{b}	8.18 (10.98 – 7)^{b}	**0.014**
Ledol (ST)	0.00 (0.00 – 0.00)	0.00 (3.49 – 0.00)	0.00 (1.69 – 0.00)	0.269
Total Adhered Emissions (TAE)	0.00 (0.00 – 0.00)^{a}	23.87 (50.42 – 1.76)^{b}	38.21 (46.27 – 31.8)^{b}	**0.026**
GLVs				
(E)-2-Hexenal	0.00 (0.00 – 0.00)	0.00 (1.19 – 0.00)	0.00 (4.59 – 0.00)	0.872

(Z)-3-Hex-1-ol	353.98 (862.73 – 32.15)	146.30 (444.75 – 40.08)	102.92 (187.14 – 43.13)	0.698
(E)-2-hexen-1-ol	11.76 (22.75 – 0.65)	0.00 (8.28 – 0.00)	3.13 (6.68 – 0.00)	0.563
1-Hexanol	1.90 (25.77 – 0.00)	0.00 (8.44 – 0.00)	6.42 (7.97 – 1.5)	0.861
(Z)-3-hexenyl acetate	483.83 (882.53 – 130.46)	140.61 (922.53 – 61.13)	110.06 (191.79 – 35.96)	0.425
(E)-2-hexenyl acetate	18.58 (170.91 – 3.28)	9.84 (29.71 – 0.00)	15.69 (35.96 – 3.05)	0.841
Nonanal	53.90 (85.94 – 46.41)	46.81 (81.18 – 35.62)	24.59 (25.56 – 21.93)	0.181
(Z)-3-hexenyl butyrate	53.60 (83.97 – 28.92)	44.79 (89.79 – 7.37)	9.58 (32.71 – 5.62)	0.398
(Z)-3-hexenyl isovalerate	7.79 (17.3 – 16.84)	5.51 (10.65 – 2.55)	1.09 (3.09 – 0.00)	0.128
Total GLVs	1256.94 (2193.07 – 252.39)	345.51 (1741 – 172.44)	320.27 (511.85 – 165.88)	0.544
Other Compounds				
MeSA	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.567
DMNT	0.77 (6.91 – 0.00)	0.00 (0.32 – 0.00)	0.00 (0.00 – 0.00)	0.116
Phenol	0.00 (5.48 – 0.00)	5.08 (8.72 – 0.00)	0.00 (4.98 – 0.00)	0.488
6-Methyl-5-hept-2-one	5.35 (8.26 – 2.29)	3.83 (5.32 – 0.00)	0.00 (2.34 – 0.00)	0.502
Acetic acid, hexyl ester	10.71 (47.74 – 2.29)	7.08 (29.62 – 1.89)	3.18 (5.23 – 0.65)	0.404
Butanoic Acid 2-hexenyl ester	0.00 (10.27 – 0.00)	0.00 (17.45 – 0.00)	0.00 (3.8 – 0.00)	0.889
4-Methylene-2,8,8-trimethyl-2-vinyl-bicyclo(5.2.0) nonane	91.56 (146.23 – 20.31)	96.92 (118.02 – 35.7)	41.53 (70.01 – 32.47)	0.710
Total MB Emissions	4059.67 (5643.7 – 02085.58)^{a}	2034.22 (4473 – 100.47)^{ab}	996.16 (1937.55 – 746.72)^{b}	0.067

Table 3. Kendall tau's non parametric correlation between recovery of adhered volatile parameters from sampled mountain birch branches and arthropod parameters observed on same trees. n = 24 in all cases.

Volatile compounds	Arthropod parameters	Kendall Tau's B	P-value
Myrcene	Proportion of leaves with holes	0.309	0.046
Palustrol	Proportion of leaves with arthropods	0.432	0.005
	Proportion of leaves with white gall mites	0.424	0.006
Ledol	Proportion of leaves with white gall mites	0.592	< 0.001
	Proportion of leaves with arthropods	0.613	< 0.001
Total adhered emissions (TAE)	Proportion of leaves with white mites	0.336	0.02

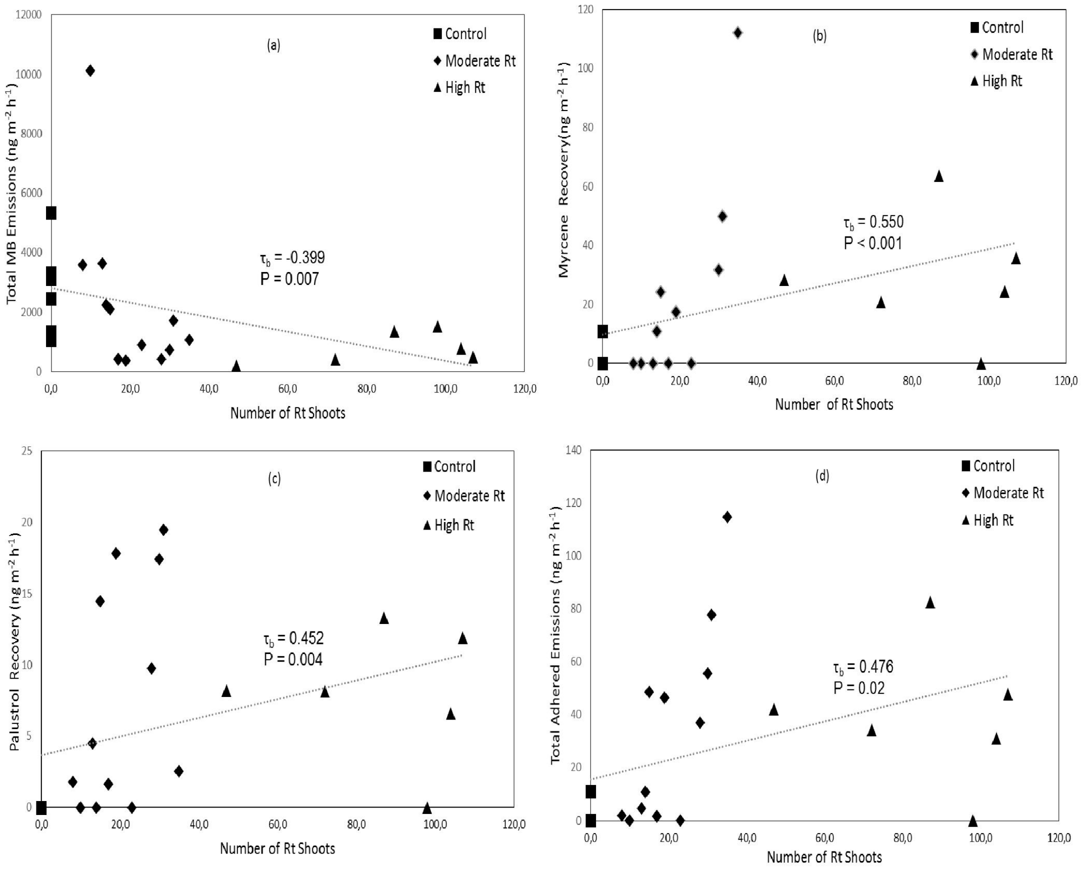

Fig 1. Relationship between Number of *R. tomentosum* shoots and (a). Total MB emissions (b). myrcene (c). palustrol (d). Total adhered compounds recovery from MB branches (n = 24) in all cases.

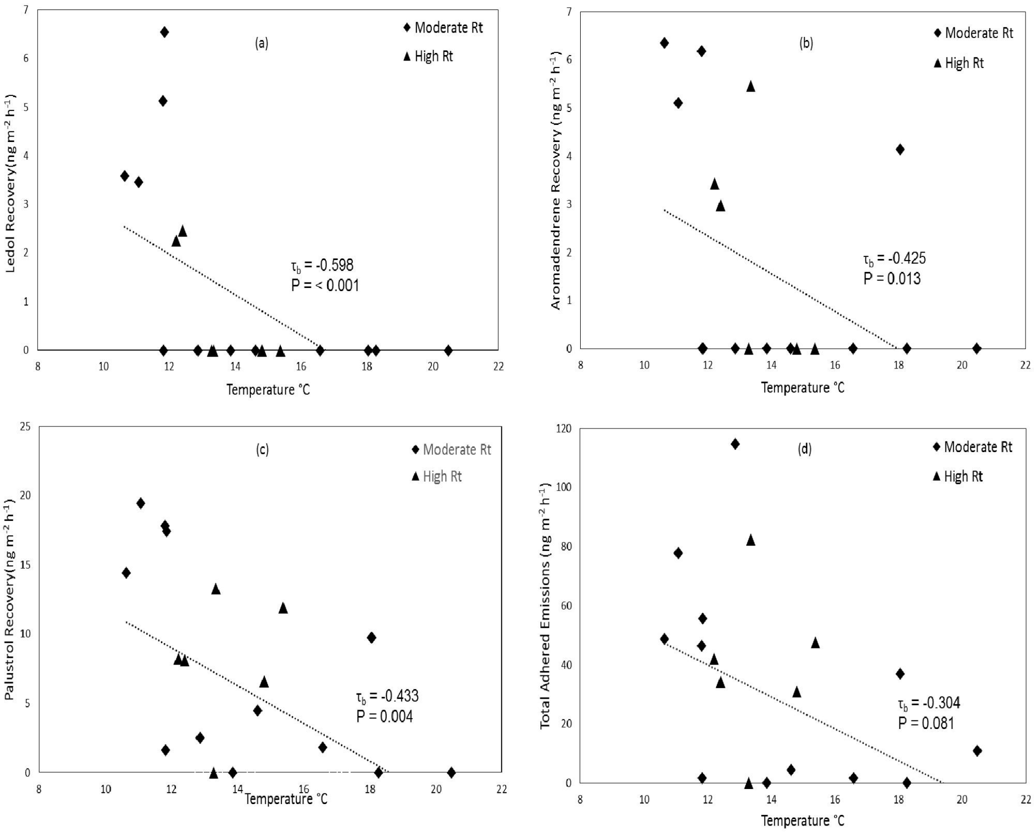

Fig. 2. Relationship between Temperature and recovery of (a) ledol (b) aromadendrene (c) palustrol and (d) total adhered compounds from mountain birch branches, (n = 18) in all cases.

APPENDIX

(A1) – Vegetation analysis of 1m^2 plots of understorey of sampled mountain birch (MB) trees including distance between sampled branch and *Rhododendron tomentosum (Rt)* shrubs. Average values ± SE represented (n=6 for control and high *Rt* plots and 12 for moderate *Rt* density plots).

Treatment	**Control**	**Moderate *Rt* Density**	**High *Rt* Density**
Tree Height (m)	3.5 ± 0.4	4.1 ± 0.3	5.3 ± 0.17
Plant Densities			
Rt Shoots	0 ± 0	20.3 ± 2.6	85.8 ± 9.4
Rt flowering shoots	0 ± 0	0.2 ± 0.2	2.0 ± 0.9
Distance from base of sampled MB branch (cm)[a*]	105.7 ± 7	105.9 ± 5.4	100.2 ± 9.2
Plant Coverage (%)[b*]			
VASCULAR PLANTS			
Angiosperms			
Rhododendron tomentosum	0 ± 0	14.1 ± 2	60 ± 5.3
Vaccinium vitis-idea	30 ± 8.7	17.1 ± 3.1	20 ± 7.4
Vaccinium myrtilus	6.7 ± 2	4.8 ± 1.6	1.2 ± 0.8
Empetrum nigrum	35 ± 8.8	45.4 ± 7.1	18 ± 5.3
Linnea borealis	5.2 ± 3.1	4.0 ± 1.1	6.5 ± 3.1
Vaccinium uliginosum	2.5 ± 1.7	11.2 ± 2.9	0.7 ± 0.4
Club mosses (Lycopodiopsida)			
Lycopodium annotinum	0 ± 0	0.1 ± 0.1	0 ± 0
BRYOPHYTES			
Mosses			
Pleurozium schreberi	65 ± 8.5	21.7 ± 4.9	22.5 ± 6.7
Hylocomium splendens	14.2 ± 9.3	31.4 ± 6.9	13.7 ± 3.1
Lichens			
Cladonia rangiferina	1.2 ± 0.6	1.8 ± 1.2	0 ± 0
Cladonia arbuscula	1 ± 0.5	0.6 ± 0.3	0 ± 0
Peltigera apthosa	2.2 ± 1.6	0.5 ± 0.4	0 ± 0
Cetraria nivalis	1.7 ± 1.7	0.9 ± 0.5	0 ± 0

[a*] In control plots, distance is measured from the base other shrubs growing in the understorey.

[b*]Total leaf area coverage can exceed 100% of the soil area as leaves of different plant species are in different layers