Feeding of large pine weevil on Scots pine stem triggers localised bark and systemic shoot emission of volatile organic compounds

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ABSTRACT

Feeding by the large pine weevil (*Hylobius abietis* L.) causes severe damage to the bark of Scots pine (*Pinus sylvestris* L.) seedlings. We measured photosynthesis, the emission of volatile organic compounds from intact and weevil-damaged bark and systemic emissions from undamaged foliage. Feeding damage did not affect photosynthesis. Monoterpenes dominated the emissions from the feeding site, although some sesquiterpenes were also emitted. Weevil feeding increased bark emission of monoterpenes by nearly 4-fold and sesquiterpenes by 7-fold. The influence of weevil damage on systemic monoterpene emissions from shoots was more profound. Several compounds were substantially induced, including linalool, β-phellandrene, limonene and 1,8-cineole. Sesquiterpenes contributed only 1.2% of the total foliage emission, but comprised eight different compounds including (*E,E*)-α-farnesene, β-bourbonene and (*E*)-β-farnesene. The total emission of monoterpenes and sesquiterpenes from shoots was respectively 2.8-fold and 2.9-fold higher in the pine weevil damaged plants than the undamaged plants. As many of the induced compounds are highly reactive in the atmosphere and form organic aerosol particles, our results suggest that conifers damaged by insects could become a more important source of secondary organic aerosols than healthy trees.

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1. Introduction

Scots pine (*Pinus sylvestris*) is one of the most widely distributed conifers in the world with a geographical range from Scotland to the coastal area of eastern Siberia in Russia (Hämet-Ahti et al., 1992). It is a dominant tree species in European boreal conifer forests and has major economic importance in timber, pulp and paper industries. However, monocultured forest plantations of Scots pine have generated significant pest problems. Large pine weevil (*Hylobius abietis* L., Coleoptera: Curculionidae) adults emerge from conifer tree stumps and feed on the stem phloem and bark of young seedlings causing severe problems in cultivated areas (Örlander and Nilsson, 1999). Heavy infestations of this insect can result in the widespread loss of conifer seedlings for several years after plantation establishment in Europe (Danielsson et al., 2008; Dillon et al., 2008). Both large pine weevil sexes are attracted by volatile compounds, such as α-pinene and β-pinene emitted by fresh conifer stumps left during felling (Zagatti et al., 1997).

Conifer resistance to biotic damage is associated with constitutive and inducible defence compounds, i.e. a mixture of volatile monoterpenes (MTs), sesquiterpenes (SQTs) and non-volatile diterpenes (resin acids) in the oleoresin (Trapp and Croteau, 2001), and with structural defences e.g., resin canals. Concentrations of MTs in current-year needle extracts of Scots pine seedlings are only 10–30% of those in current year stem bark extracts and (*E*)-β-caryophyllene was the only SQT detectable in extracted samples (Sallas et al., 2003). When plants are damaged by herbivores they emit volatile organic compounds (VOCs) (e.g., Arimura et al., 2004; Rasmann and Turlings, 2007). Oleoresin stored in the bark of conifer seedlings could be a significant source of VOCs (Ghirardo et al., 2010). Damage by bark phloem feeding insects causes massive resin flow that can passively release VOCs that may function as airborne signals within-plant. The defence compounds in conifers have been found to deter insect pests and fungal pathogens (Gershenzon and Croteau, 1991) and act in direct and indirect ecological interactions (Mumm and Hilker, 2006; Gershenzon, 2007). It has also been shown in conifers that volatiles are induced by herbivory and egg deposition in needles (Mumm and Hilker, 2006). These volatiles often function in the attraction of natural enemies of insect herbivores (Keeling and Bohlmann, 2006). Many of these volatiles are emitted by foliage but also from resin canals in stem (Sallas et al., 1999; Heijari et al., 2005) and roots (Janson, 1993), especially in monoterpene-storing conifers.

The induction of resistance in non-affected parts of a plant when a distant part of the same plant is challenged by a pathogen or herbivore is called induced systemic resistance (ISR) and it has

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been observed in conifers (Bonello et al., 2001, 2006). Previous research on systemically induced volatile organic compounds has revealed the involvement of three major signal-transduction pathways and their key intermediates; octadecanoid, shikimate and ethylene pathways producing: jasmonic acid (JA) salicylic acid (SA) and ethylene (ET), respectively. Evidence exists that SA, JA and ET are involved in localised and systemic induced defence responses in conifers (Fäldt et al., 2003; Heijari et al., 2005; Bonello et al., 2006; Zeneli et al., 2006). Local and systemically induced VOC emissions from needles of Scots pine have been found after egg deposition by pine sawfly (Hilker et al., 2002).

In this study we focus on emissions of volatile organic compounds (VOC) from Scots pine seedlings after stem bark wounding below the foliage by the large pine weevil, *H. abietis.* Our aim was to clarify the quality and quantity of both local emissions from the feeding site at the stem base and the systemic emission from intact shoots. Furthermore, this study will give insight as to how outbreaks of bark-feeding insects affect the quality and quantity of reactive carbon compounds emitted from conifers to the atmosphere and thus the possible consequences on atmosphere-biosphere interactions e.g., the formation of secondary organic aerosols from plant volatiles (e.g., Bonn and Moortgat, 2003; VanReken et al., 2006; Pinto et al., 2007; Boy et al., 2008; Spracklen et al., 2008; Hao et al., 2009; Jimenez et al., 2009; Virtanen et al., 2010).

2. Materials and methods

2.1. Experimental plants and herbivores

Scots pine seedlings (2-years-old) were obtained from a commercial nursery (Taimityllilä Oy, Mäntyharju, Finland). Seedlings were individually planted in 7.5 l plastic pots in 2:1 (v/v) quartz sand (Ø 0.5–1.2 mm; SP Minerals Partek, Finland) and fertilized sphagnum peat (Kekkilä PP6, Finland). After planting, the seedlings were fertilized with a slow-release fertilizer (Taimiston kestolannos 9% nitrogen, 5% phosphorous, 5% potassium and 5% magnesium, 4% sulphur and micronutrients, Kemira Oyj, Finland), 4 g of which was added to the surface layer of the growth medium in each pot. The seedlings were grown outside with natural rainfall and supplementary watering as required.

Large pine weevils (*H. abietis* L., Coleoptera: Curculionidae) were collected from a saw dust storage heap of a sawmill (Iisveden Saha, Suonenjoki, Finland). Prior to experiments weevils were kept in an 8 °C controlled temperature room, they were stored in plastic containers filled with wood shavings and fed with small pine twigs.

2.2. Study site and design

The study site was the Kuopio campus research garden of the University of Eastern Finland (62°53′N, 27°37′E, and 80 m above sea level). The first experiment tested the effects of weevil feeding on systemic emissions from the shoots of Scots pine seedlings. Scots pine seedlings were grown on twelve separate wooden platforms. On six platforms, three Scots pine seedlings per platform were infested with large pine weevils (*H. abietis* L., Coleoptera: Curculionidae), while on the remaining six platforms the seedlings were non-infested controls. Seedlings to be infested were selected randomly and infested with two large pine weevil adults enclosed in mesh bags attached to basal stem bark of each seedling. Weevils were selected at random, irrespective of sex, based on earlier experiments where no differences were observed in their feeding activity (Heijari et al., 2005). Three days (72 h) after the initiation of feeding (June 4–7, 2007), the experimental insects were removed and volatile organic compounds (VOC) were collected from the whole foliage of 17 damaged and 16 non-damaged plants.

The second experiment tested the effects of weevil feeding on emissions from the site of damage. Five Scots pine seedlings were infested with weevils, as described above, and placed one per platform on five randomly separate platforms. A further five seedlings were selected as non-infested controls and placed on five different platforms. After 4 days (96 h) of feeding, seedlings were transported to the laboratory for collection of VOCs.

2.3. Photosynthesis measurements

Net photosynthesis (Pn) was measured from the top shoots of a subset of the sampled seedlings (8 damaged and 7 non-damaged seedlings). After measurements, the seedlings were harvested for determination of shoot dry weight (dried in an oven for 72 h at 60 °C) and needle area for use in Pn calculations. Pn measurements were conducted in direct sunlight, but with some of the light blocked with a light mesh fabric (Photosynthetically active radiation was on average ± SD, 758 ± 145 $\mu mol\, m^{-2}\, s^{-1}$). Measurements were made between 10:00 AM and 3:00 PM with a CI-510 Ultralight Portable Photosynthesis System (CID, Inc., Vancouver, WA, USA). The needle lengths of ten two-needle fascicles per annual shoot and the number of needles were measured. The total area (A_t) of needles was calculated using the model, $A_t = 4.2235 \times$ (Mean needle length) $- 15.6835$ (Flower-Ellis and Olsson, 1993).

Pn: net photosynthesis rate ($\mu mol/m^2/s$) for the open system was calculated using the formula:

$$Pn = -W \times (C_o - C_i) = -2005.39 \times \frac{V \times P}{T_a \times A} \times (C_o - C_i)$$

where C_o (C_i) is the outlet (inlet) CO_2 concentration (ppm or $\mu mol/mol$), V is the volume flow rate (l/min), P is the atmospheric pressure (bar), T_a: air temperature (K), A: leaf area (cm^2), W: mass flow rate per leaf area ($mol\, m^{-2}\, s^{-1}$).

2.4. Collection of volatile organic compounds

We collected VOCs (mono-, sesquiterpenes and green leaf volatiles (GLV)) from the foliage of 16 non-damaged and 17 damaged Scots pine seedlings. The mesh bags on each tree were opened immediately before sampling and the feeding weevils were removed. Polyethylene terephthalate (PET) bags (size 45 cm × 55 cm, LOOK, Terinex Ltd., Bedford, England) were heated at +120 °C for 1 h before collections to remove any contaminants from the bag, and subsequently cooled. The main shoot and side branches were enclosed inside the PET bag and fastened securely to the stem bark taking care not to damage any foliage. One of the two outermost bag corners was cut and an air inlet and data recording device were inserted and supported by a tripod. Clean charcoal-filtered and MnO_2 scrubbed air was pumped through Teflon tubing and into the bag at 600 ml min^{-1} to flush the system, and then reduced to 250 ml min^{-1} during collections. The remaining bag corner was cut and a stainless steel tube containing approximately 150 mg of Tenax TA-adsorbent (Supelco, mesh 60/80) was inserted and fastened into position. Air was pulled through the Tenax tube by battery-operated sampling pumps (Rietschle Thomas, Puchheim, Germany). The air flow through the Tenax tube was set to 200 ml min^{-1} with an M-5 bubble flowmeter (A.P. Buck, Orlando, FL, USA). The VOC collection system including pumps, air filters, Teflon tubing, data logging device and batteries was installed into a portable plastic toolbox. During the sampling period (30 min), the temperature, photosynthetically active radiation (PAR) and air humidity inside the plastic bags were monitored with a HOBO Micro Station Data Logger (MicroDAQ.com, Ltd., Contoocook, NH, USA).

To determine the impact of insect feeding on the emission of volatile organic compounds from stem bark we measured emis-

sions from 5 undamaged and 5 pine weevil damaged seedlings. We used a similar sampling system to that described above but used a transparent tube (heated pre-sampling at 60 °C for 1 h) made from a split 0.5 l PET plastic bottle, and conducted all VOC collections in controlled laboratory conditions (temp: 23 °C). Following the period of weevil feeding the sampling tube was fastened around the stem enclosing the weevil-damaged area. Stems of undamaged seedlings were enclosed in the same way. In all cases the PET tubes enclosed only a 60 mm length of stem bark and no foliage.

2.5. Analysis of volatile organic compounds

The VOC samples were analyzed with a gas chromatograph-mass spectrometer (Hewlett-Packard GC 6890, MSD 5973). Trapped compounds were desorbed with a thermal desorption unit (Perkin-Elmer ATD400 Automatic Thermal Desorption system) at 250 °C for 10 min, cryofocused at −30 °C, and injected onto a HP-5 capillary column (50 m × 0.2 mm i.d. × 0.5 μm film thickness, Hewlett-Packard) with helium as a carrier gas. The temperature program was as follows: 40 °C 1 min, 5 °C min^{-1} to 210 °C, 20 °C min^{-1} to 250 °C, 250 °C 8 min. All mass numbers between 30 and 350 m/z were recorded using SCAN technique (Manninen et al., 2002). The compounds (mono-, sesquiterpenes and green leaf volatiles, GLV) were identified by comparing their mass spectra with the Wiley library and with pure standards. For compounds whose standard was not available, α-pinene for monoterpenes and (E)-β-farnesene for sesquiterpenes were used to calculate the concentration of these compounds assuming that the responses would be the same as the responses of α-pinene and (E)-β-farnesene, respectively. This was the case for the monoterpenes α-terpinene, (E)-β-ocimene, β-phellandrene and the sesquiterpenes (E)-β-caryophyllene, (E,E)-α-farnesene and β-bourbonene. Emission rates are presented in ng g(DW)$^{-1}$ h^{-1}. Needle dry weights for shoot emissions and enclosed stem dry weights for bark emissions were used in calculations. As biogenic emissions depend strongly on light and temperature all terpene emissions collected from shoots under variable conditions in the field were made comparable by standardizing them to a temperature of 30 °C using the classic algorithm established by Guenther et al. (1993). We used the temperature coefficient (β (K^{-1})) of 0.09 recommended by Guenther et al. (1993) to standardise monoterpene emissions, and the (β (K^{-1})) of 0.18 as used by Helmig et al. (2006) to standardise sesquiterpene emissions.

2.6. Statistical analyses

A t-test was used to analyze differences between control and insect damaged Scots pine needle VOC emissions, stem emission and photosynthesis. If emissions data were not normally distributed they were analyzed with a Mann–Whitney test. The statistical analyses were conducted using SPSS 14.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

Large pine weevil damage on the lower half of stem bark (3 days prior to VOC measurements) of Scots pine did not significantly influence ($t = 0.417$, df = 13, $P = 0.684$) net photosynthesis (means ± SD) of Scots pine shoots in damaged seedlings (9.17 ± 7.53) when compared to control (10.84 ± 7.98), but the average net photosynthesis rate was 15.4% lower in the damaged plants.

Feeding by large pine weevils on stem bark caused increased emissions of several VOCs from the shoots (Fig. 1a). Pine weevil feeding had the most significant inductive effect on shoot emissions of the monoterpene alcohol linalool (12.1-fold) and the

MTs β-phellandrene (7.4-fold), limonene (6.8-fold) and 1,8-cineole (5.4-fold). A total of 28 compounds were identified in the volatile profiles with the majority of the emission made up by the MTs Δ^3-carene, α-pinene and myrcene with lesser amounts of limonene, sabinene, β-phellandrene and β-pinene. All of the 20 detected MTs were emitted by the foliage of both intact and damaged seedlings. The SQT emission was only 1.2% of the total emission but included eight different compounds (Figure 1b). Two of these, (E)-β-farnesene and α-humulene, were emitted in trace amounts by the foliage of intact seedlings.

The MT and SQT emissions from shoots were normalised using the algorithm (Guenther et al., 1993) described above. The normalised total needle MT emission was 2.8-fold higher ($t = -2.696$, df = 31, $P = 0.011$) and the total SQT emission 2.9-fold higher ($t = -3.367$, df = 31, $P = 0.003$) in pine weevil damaged plants than in the undamaged plants. The actual total needle MT emission was 2.6-fold higher ($t = -2.640$, df = 31, $P = 0.013$) and the total SQT emission also 2.6-fold higher ($t = -2.946$, df = 31, $P = 0.006$) in pine weevil damaged plants than in the undamaged plants (Fig. 2).

Total MT (means ± SE: 12657.4 ± 6389.36 ng g(DW)$^{-1}$ h^{-1} control; 43319.6 ± 7186.7 ng g(DW)$^{-1}$ h^{-1} damaged) and total SQT (29.4 ± 19.6 ng g(DW)$^{-1}$ h^{-1} control; 208.0 ± 71.9 ng g(DW)$^{-1}$ h^{-1} damaged) emissions from the lower stem bark including the feeding site were significantly increased by weevil feeding damage ($t = -3.189$, df = 8, $P = 0.013$) and ($t = -2.396$, df = 8, $P = 0.043$), respectively.

The number of detected MT compounds in bark emissions was 18 in both intact and damaged seedlings (Fig. 3a). The only significantly increased MT component was sabinene ($t = -2.59$, df = 8, $P = 0.045$) (5.8-fold increase), although β-phellandrene (7.1-fold), γ-terpinene (8.0-fold), limonene (7.5-fold), α-phellandrene (7.1-fold), terpinolene (6.2-fold) and bornyl acetate (5.7-fold)) had high average emission increases. The typical foliage MT 1,8-cineole was not found in stem emissions. Stem bark emissions only included five SQT compounds (Fig. 3b). Emissions of longifolene ($t = -2.52$, df = 8, $P = 0.049$) and δ-cadinene ($t = -5.191$, df = 8, $P = 0.005$) were significantly increased by herbivore feeding while α-copaene and α-humulene were only detected in emissions from damaged seedlings.

The number of terpenes emitted was much lower from bark than from shoots and there were qualitative differences between bark and shoot emissions. E.g., linalool was found in the foliage emission of both intact and weevil-damaged seedlings, while in bark emissions only a minor amount of linalool was detected after weevil-damage. The relative impact of weevil damage on total MT and SQT emission pools at injured bark tissue sites was slightly higher than that of distal shoots. Total VOC emission was increased 3.4-fold in bark and 2.8-fold in shoots of damaged seedlings and it was the same for total MTs. Total SQT emissions were increased 7.1-fold in bark and 2.9-fold in shoots.

4. Discussion

4.1. Net photosynthesis in damaged seedlings

Photosynthetic capacity of plants is known to be reduced after herbivore attack (Mäntylä et al., 2008; Mitra and Baldwin, 2008). An impact of pine saw fly oviposition on photosynthesis of P. sylvestris needles was reported by Schröder et al. (2005) showing that the net photosynthetic rate of oviposition-induced pine twigs was lower than that of untreated control twigs. We observed that net photosynthesis in the foliage of damaged pine seedlings was not significantly affected by herbivory, although there was a slight trend indicating a reduction rather than an increase. Similarly, Staudt and Lhoutellier (2007) did not find insect feeding to affect
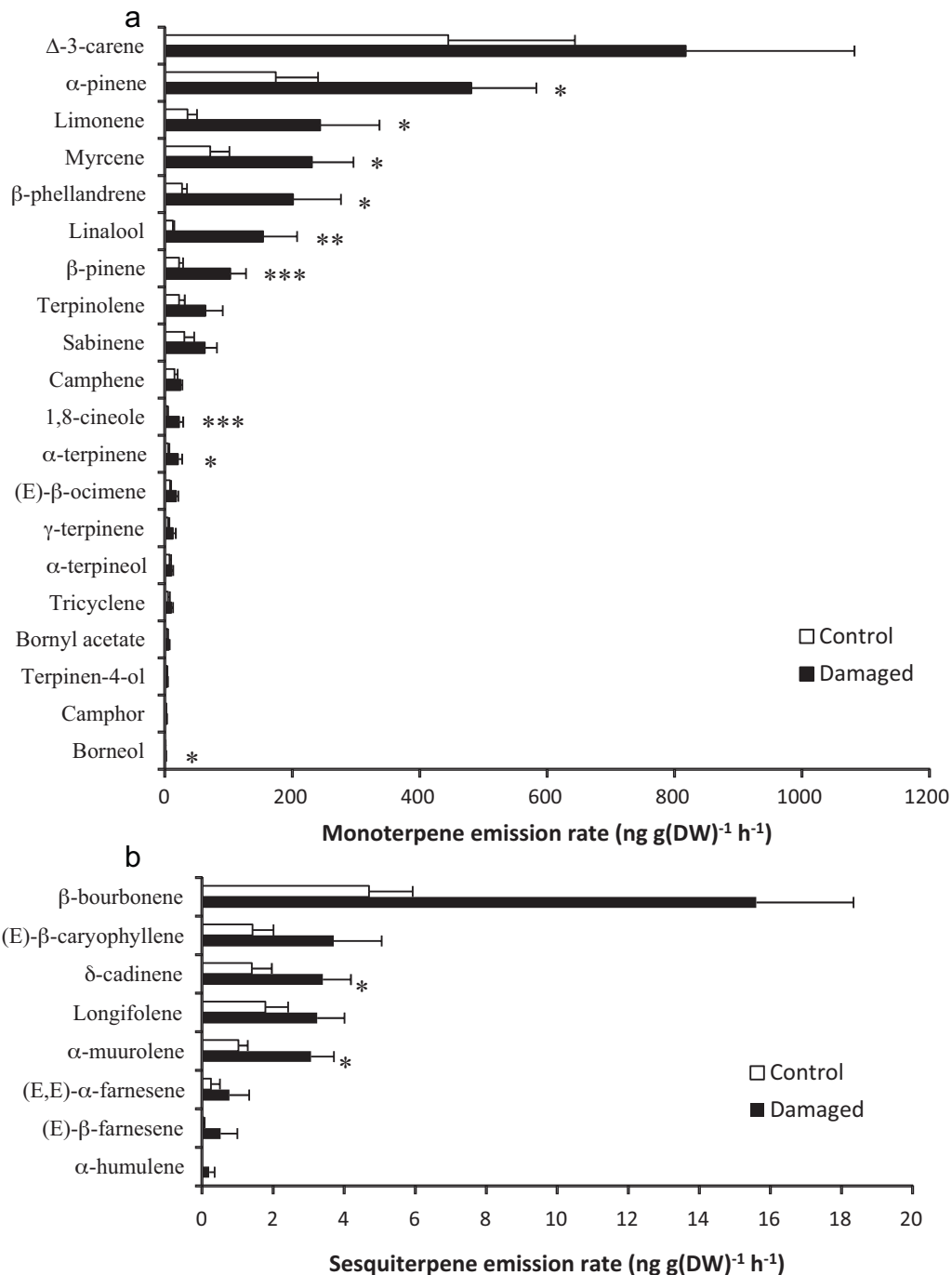
Fig. 1. Profiles of (a) monoterpene and (b) sesquiterpene emissions (ng g(DW)$^{-1}$ h^{-1} ± SE) from Scots pine foliage of undamaged (n = 16) and large pine weevil damaged (n = 17) seedlings. Compounds tested with Mann-Whitney test include α-muurolene, β-bourbone, β-phellandrene, α-terpinene, Linalool 1,8-cineole, Limonene and β-pinene (DW, dry weight). All values are normalised using the algorithm developed by Guenther et al. (1993) with a temperature coefficient (β (K^{-1})) of 0.09 for MTs and 0.18 for SQTs.

photosynthesis in undamaged foliage of moth-infested *Quercus ilex*. Reduction of photosynthesis in foliage after herbivore attack, is explained by increased assimilate transport to roots instead of to young leaves (Schwachtje et al., 2006). This could be part of a herbivory tolerance strategy, as when plants allocate more carbon to roots and less to new leaves the foliar quality for herbivores is reduced (Mitra and Baldwin, 2008). In our case, the herbivore attack was to the stem phloem, which could directly harm carbon transport to roots, but the carbon allocation pattern was probably not altered as no significant effect on photosynthesis was detected.

4.2. Effects of feeding damage on bark and systemic VOC emissions from shoots

We observed that feeding by pine weevils on the stem of Scots pine seedlings induced emission of a VOC blend from the foliage, which differed from that emitted by the foliage of intact seedlings, and that emitted from the feeding site on the bark. Furthermore, we also detected MT and SQT emissions from the intact stem bark of healthy control seedlings, suggesting that branch bark could be a significant VOC emitter in terpene-storing gymnosperms. This also means that part of the foliar VOC emission we measured could
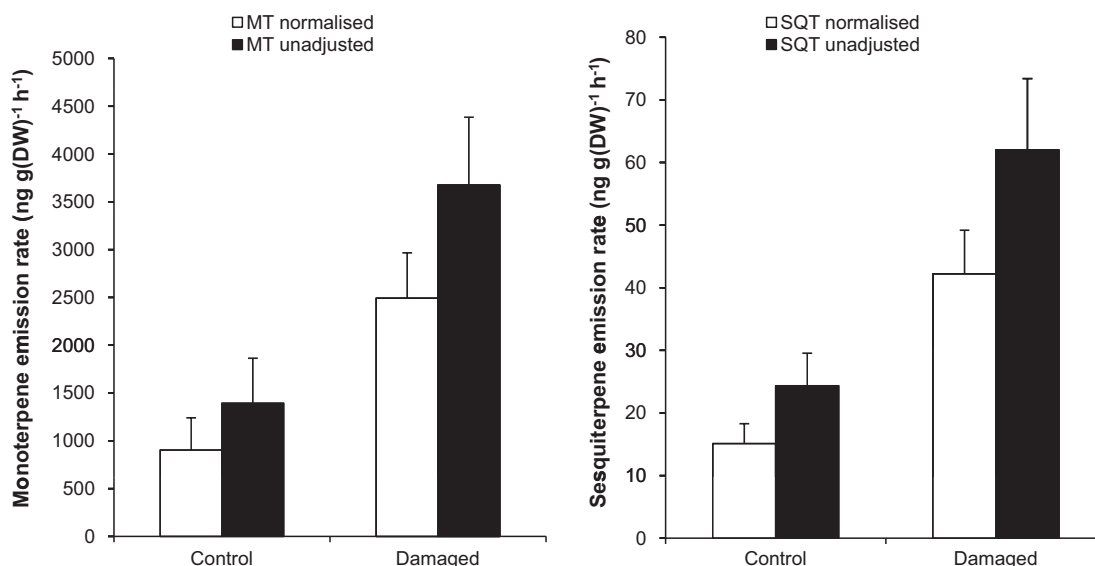
Fig. 2. Comparison of total monoterpene and sesquiterpene emissions from control and damaged plants, before and after normalisation of the data using the Guenther algorithm (1993).

be emitted from the resin canals in the branch bark of needle-covered shoots and from the thinner cortex of the current-year elongating shoots. As the amounts of MT and SQT in emissions from stem bark were larger than the amounts of these compounds in foliar emissions, the importance of bark emission in shoot emission analysis using branch enclosures needs further elucidation. Nicole et al. (2006) found that weevil damage on the leader shoot bark of Norway spruce seedlings induced expression of genes encoding monoterpene or sesquiterpene synthases in bark tissue, but not in needles.

Systemic increase in tissue concentration and emission of VOCs from stem- and root-damaged conifer seedlings has been reported previously. Blande et al. (2009) found that *H. abies* feeding on branch bark induced monoterpene and sesquiterpene emissions from distal foliage of the same branch in *Picea abies* saplings. An increase in foliar terpene concentrations was found shortly after mechanical root cutting and transplantation of Scots pine seedling (Sallas et al., 1999), although later in the growing season root-stressed transplanted seedlings had lower terpenoid concentration than non-transplanted seedlings. A topical treatment with the elicitor compound methyl jasmonate (MeJA) to *Pinus pinaster* branches did not induce a systemic terpene response in distal branches (Moreira et al., 2009) suggesting the systemic response to biotic stresses is not universal in conifers.

The detected systemic emission of MTs from shoots, were mostly dominated by the same compounds as detected in stem bark emissions. This is an indication that MT composition of Scots pine are under strong genotypic control and that the composition of the MT pool in different plant parts is qualitatively very similar (Sallas et al., 2003). Our observation of induced emission of MTs and SQT in the pine shoot foliage after herbivore feeding on the stem could have at least four different explanations. Detected induced emissions from the foliage could be (1) simply a result of adsorption of higher quantities of VOC compounds emitted from the damage site and then re-emitted from needle surfaces during sampling. The possibility that they are synthesized in the bark or needle tissues in shoots requires a signal transduction, (2) which could be transmitted via vascular tissue or (3) via airborne signals from the damage site to the foliage. Wounding of oleoresin storing pine stems leads to resin outflow from the damaged site in the bark. This could induce (4) increased resin flow from foliage via resin ducts in the

branch bark causing concurrent leakage of volatile MTs and SQT through the bark. All of these proposed mechanisms of systemic foliage emission after stem bark damage need further experimental testing. Below we briefly summarise the current evidence from the literature.

The first explanation is based on the fact that tree foliage can uptake hydrophobic MTs in the leaf lipid phase from the atmosphere when the monoterpene concentration in the ambient air is high, and release them when the concentration is low (Noe et al., 2008). However, deposition and re-release of SQTs on plant surfaces is more pronounced than that of MTs (Schaub et al., 2010; Himanen et al., 2010). This evidence means that at least a minor proportion of the MTs and SQTs in the foliar emissions we detected could be the molecules released earlier from the stem bark and adsorbed by the needles above. However, the fact that compounds like the monoterpene alcohol linalool and SQTs β-bourbonene and α-muurolene were emitted by foliage in significant amounts, and that these compounds were practically undetectable in stem bark emissions, strongly imply that the induced MTs and SQTs detected in the foliage were synthesized there.

Induced systemic resistance (ISR) of conifers, where pathogenic infection or herbivore infestation induce resistance and related changes in plant biochemistry in previously non-infected parts of a plant, was found to be communicated via the vascular system (Wang et al., 2006). The proteins expressed in the intact area of *Pinus nigra* phloem were not pathogenenesis related PR proteins which are normally found localized to the infection site, but proteins similar to small heat shock proteins and other proteins related to abiotic stresses (Wang et al., 2006). It is possible that these proteins prime the intact tissues to increase their resistance to attack by biotic stressors. Studies with tobacco and chili pepper (Maldonado-Bonilla et al., 2008) demonstrated that a particular systemic gene expression for enzymes directly involved in sesquiterpene biosynthesis in leaves was localized to the immediate vascular tissues in *Pseudomonas syringae* infected leaves and in neighbouring leaves. Authors suggested that this expression pattern might be mediated by reactive oxygen species. Thus, it is highly likely that feeding by weevils on Scots pine stem bark induced MT and SQT synthesis in foliage and that the induction was mediated at least partly via the vascular system.
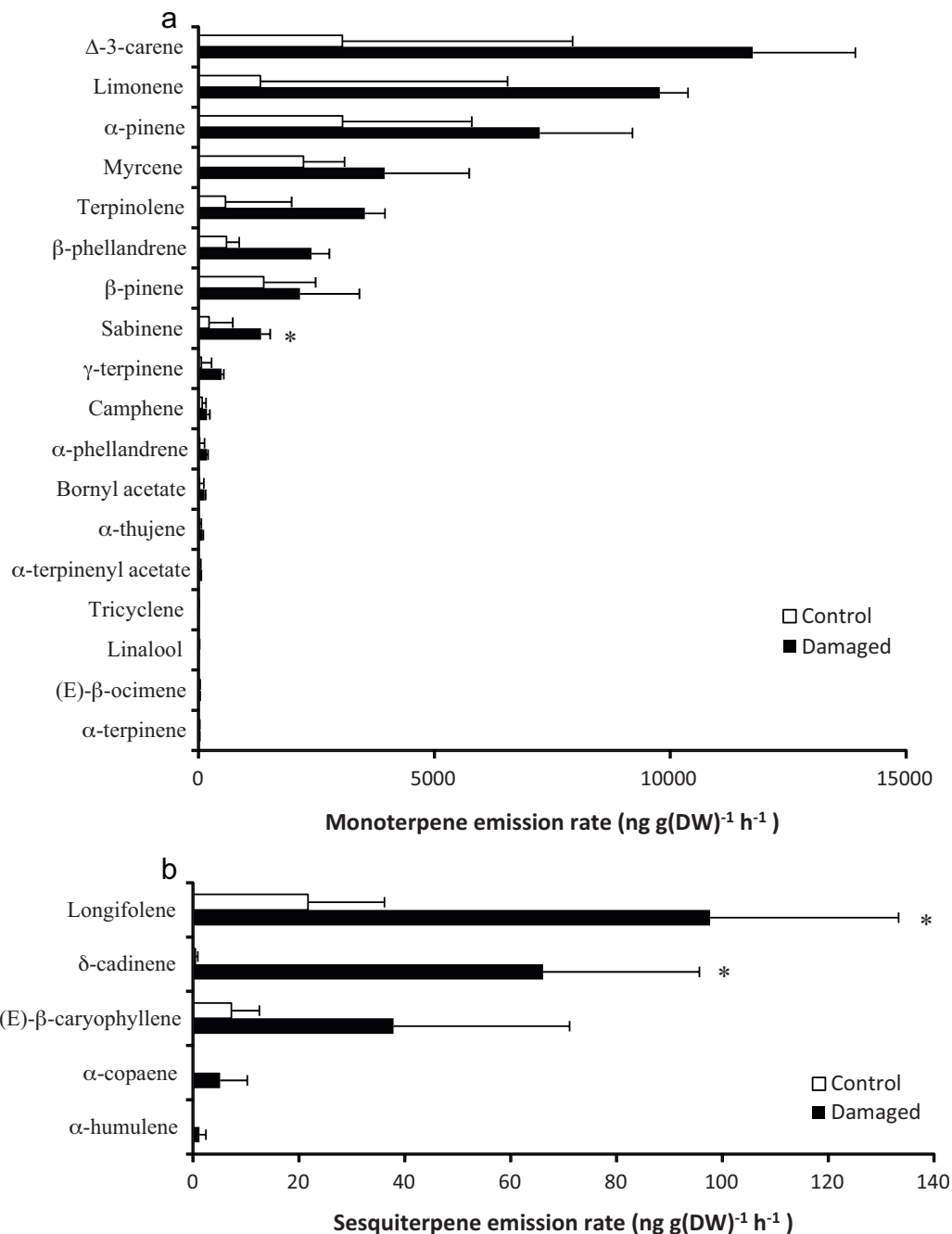
Fig. 3. Profiles of (a) monoterpene and (b) sesquiterpene emissions (ng g(DW)$^{-1}$ h^{-1} ±SE) from Scots pine stem of undamaged and large pine weevil damaged seedlings (n = 5) (DW, dry weight). All values are actual emission rates at a constant temperature of 23 °C.

The third option for foliar VOC emission induction, that the signal molecules are airborne, has some support from studies on deciduous trees. In vascularly unconnected plant parts within-plant signalling in poplar (*Populus deltoides × nigra*) (Frost et al., 2007, 2008) and *Vaccinium corymbosum* (Rodriguez-Saona et al., 2009) saplings can be mediated by VOCs released from damaged leaves. The induction of systemic VOC emissions in Scots pine may have been mediated by VOCs released directly from the damage site. It has previously been shown that monoterpene concentration in the phloem tissue (Wallin and Raffa, 1999) or VOC emissions (e.g., Mumm and Hilker, 2006; Blande et al., 2009) from *Pinaceae* species are induced in adjacent foliage when a distant part of a branch is damaged by insects. Volatile signal compounds, like MeJA, applied exogenously to Scots pine foliage enhance needle MT concentration

(Heijari et al., 2008) and MT emission (Holopainen et al., 2009) from foliage, but the emitted VOC blend is more dominated by myrcene and linalool than the *Hylobius* induced foliar emissions in the current study. MeJA treatment at the base of 30-years-old Norway spruce (*Picea abies*) stems (Franceschi et al., 2002) and on a basal branch of *P. pinastri* seedlings (Moreira et al., 2009) affected resin duct formation in the application zone, but not in distal branches above or on the opposite side to the damage site. This suggests that MT and SQT released from the damage site could affect distal induction of foliar VOC emission even more than better known volatile signal compounds such as MeJA or cis-3-hexenyl acetate (Frost et al., 2008).

Increased resin flow and formation of traumatic resin ducts locally and systemically are immediate responses to treatment with

the elicitor MeJA, wounding and damage by the weevil *Pissodes strobi* in *P. abies* (Nicole et al., 2006). In Scots pine stem bark, fungal pathogens (Luchi et al., 2005; Knebel et al., 2008) and MeJA (Heijari et al., 2005) induce a resin flow response, which can last for up to three months (Knebel et al., 2008) after damage, and extend inside the seedling for at least 30 cm. We found that healthy bark can release MTs and SQTs, which are probably leaked from resin ducts. Therefore, it is possible that *Hylobius* feeding on tree stem bark induced resin flow in the resin ducts of branches above as well as synthesis of terpenes including diterpenes (resin acids) in the needles, which together with VOC leakage through the branch bark and soft cortex of young shoots will lead to enhanced MT and SQT emissions from the whole of the foliage.

4.3. Composition and reactivity of VOC emission blends from bark and shoots

Our results indicate that insect feeding on stem bark activates resin flow and altered MT and SQT emission profiles from stem and induces systemic MT and SQT emissions from shoots of *P. sylvestris*. The chemical composition of shoot VOC emissions was more variable than VOC emissions from stems, with SQT showing higher diversity. MTs comprised the majority of the shoot-emitted VOCs with the dominant compounds, Δ-3-carene, α-pinene, limonene and myrcene, contributing almost 80% of the total VOC blend. The impact of large pine weevil feeding reduced the proportion of these four compounds to 70% of the total VOC blend. The relative proportions of the MTs β-phellandrene and linalool and the SQTs β-bourbonene and (E)-β-caryophyllene were increased in the shoot emissions.

This change of emission profile in herbivore-damaged plants is important if we are to consider the reactivity of MTs and SQT in the atmosphere. Of the dominating MTs of intact plants, Δ-3-carene and α-pinene, have atmospheric life times of hours while the induced compounds β-phellandrene, linalool and (E)-β-caryophyllene have life times of less than an hour under elevated OH and O₃ (Atkinson and Arey, 2003). Furthermore, limonene is more reactive than Δ-3-carene and α-pinene. Shorter atmospheric life times of induced emissions can affect atmospheric quality (Pinto et al., 2007) and have ecological effects (Yuan et al., 2009).

There is consensus that biogenic VOCs have an essential role in the formation and growth of secondary organic aerosol (SOA) particles (VanReken et al., 2006; Joutsensaari et al., 2005; Spracklen et al., 2008; Jimenez et al., 2009; Mentel et al., 2009; Virtanen et al., 2010). If the response of large conifer trees to stem bark damage, e.g., against bark beetle feeding, is similar to that observed in this study, local damage to trunk bark could lead to significantly enhanced MT and SQT emissions at the whole tree level. As insect outbreak areas could be hundreds of thousands of km^2 (Kurz et al., 2008) herbivore-induced conifer VOC emission from resin flow and foliage could be considered a significant source of reactive VOCs participating in SOA nucleation and even cloud formation (Spracklen et al., 2008). Induced emissions of reactive SOA-forming volatiles after herbivore damage may have partly evolved to compensate herbivore-induced reduction in photosynthesis. Recently it has been shown that aerosol particles increase diffuse radiation which promotes photosynthesis (Mercado et al., 2009).

4.4. Ecological effects of induced VOCs

Volatile terpenes released from damaged plants can activate transcriptional response in neighbouring plants of the same species or other species, but in conifers this has not yet been shown (Baldwin et al., 2006). If VOCs released from the feeding site in the stem induced a systemic response in the foliage of the same seedling, it is possible that neighbouring plants could have a sim-

ilar response. However, how the induced VOC emission increases the fitness of Scots pine remains unclear, along with the induced defence strategies of Scots pine (Mumm and Hilker, 2006). Direct defences against *Hylobius* adults in living conifer seedlings include oleoresin flow to the damage site. Resin flow can be vertical, e.g., from foliage to stem, but also horizontal from the stem wood to damaged bark, resulting in higher resin acid content of bark and parallel reduction in xylem resin acid content (Heijari et al., 2005). The MT limonene is known to be a repellent of *Hylobius* (e.g., Nordlander, 1990). We observed that the relative proportion of the total MT emission represented by limonene clearly increased in stem bark and shoot emissions, which could be an indication of plant direct defence against weevils. It is noteworthy that the emissions of stem bark VOCs were one order of magnitude higher than emissions from foliage, which suggest that bark emissions should have a stronger repellent effect. Kännaste (2008) observed that in herbivore-induced VOC emissions of *P. abies* (E)-β-farnesene was attractive to *H. abietis* while methyl salicylate and (−)-linalool acted as repellents. We found (E)-β-farnesene and linalool to be inducible in pine shoots after feeding damage, but not in injured bark emissions.

To understand induced response in conifers the ecological analysis should also include higher trophic levels. The implication of this work is that a relatively small amount of feeding by *H. abietis* can have a systemic effect on the plant including activation of volatile terpene production, which is commonly related to the indirect defensive mechanisms through VOC emission (Mumm and Hilker, 2006). Unfortunately, the evidence of multitrophic interactions mediated by VOCs from Scots pine foliage is still rather limited (Hilker et al., 2002; Mumm and Hilker, 2006). Release of VOCs from foliage may attract natural enemies of *Hylobius* adults, such as insectivorous birds (Petersson et al., 2006; Mäntylä et al., 2008), although major top-down control of this species takes place in tree stumps where the larvae develop and are exposed to predators and parasitoids (Dillon et al., 2008).

To conclude, we have shown that intact and herbivore-damaged bark could be a significant source of VOC emissions in conifer seedlings. Feeding damage on Scots pine stem bark did not significantly affect photosynthesis, but induced monoterpene and sesquiterpene emissions from damage sites and from the parts of the seedlings with intact foliage. This might be relevant to the direct and indirect defence mechanisms of these seedlings and improves our knowledge of the complex interactions between insects and trees in coniferous forests. We have outlined how relatively small amounts of insect feeding can have an impact on VOC emissions on the whole plant level. Induced emission blends have more compounds with a short atmospheric life time, which in turn can undergo reactions in the atmosphere, with a potential impact on secondary aerosol formation.

Acknowledgements

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