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Needle Removal by Pine Sawfly Larvae Increases Branch-Level VOC Emissions and Reduces Below-Ground Emissions of Scots Pine

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Supporting Information

ABSTRACT: Climate warming is expected to increase the frequency of insect outbreaks in Boreal conifer forests. We evaluated how needle removal by the larvae of two diprionid sawfly species affects the composition and quantity of VOC emissions from *Pinus sylvestris* L. saplings. Feeding damage significantly increased the rate of localized VOC emissions from the damaged branch. The emissions of total monoterpenes (MTs) were dominating (96−98% of total VOCs) and increased by14-fold in *Neodiprion sertifer*-damaged branches and by 16-fold in *Diprion pini*-damaged branches compared to intact branches. Emissions of δ-3-carene, α-pinene, sabinene, and β-phellandrene were most responsive. Feeding damage by *N. sertifer* larvae increased the emission rates of total sesquiterpenes by 7-fold (4% of total VOCs) and total green leaf volatiles by 13-fold (<1% of total VOCs). The VOC emissions from *N. sertifer* larvae constituted nearly 25% of the total branch emissions. *N. sertifer* feeding in the lower branches induced 4-fold increase in MT emissions in the top crown. Defoliation of Scots pine by *D. pini* significantly reduced the below-ground emissions of total MTs by approximately 80%. We conclude that defoliators could significantly increase total VOC emissions from the Scots pine canopy including MT emissions from resin storing sawfly larvae.

INTRODUCTION

The total land surface area of Earth is approximately 148×10^8 ha,[1] one-third of which represents forest vegetation.[2] Boreal Conifer Forest constitutes the largest terrestrial biome (Taiga) of the Earth and it covers about 15×10^8 ha (33%) of the total forest area[3] and has the potential to affect regional climatic conditions. The boreal forests are dominated by needle-leaved conifers of the family Pinaceae, including spruce (*Picea*), fir (*Abies*), pine (*Pinus*), and larch/tamarack (*Larix*). In Finland, forest land covers about 26.3 million hectares, the total growing stock is 2206 million m^3 and Scots pine (*Pinus sylvetris* L.) covers 50%.[4]

Biogenic volatile organic compounds (BVOCs) refer to organic atmospheric trace gases and nonmethane hydrocarbons, which are emitted by plants or other organisms.[5] Based on structure and atmospheric lifetime, BVOCs are grouped into four main categories: isoprene, monoterpenes, other reactive VOCs and other less reactive VOCs.[6] Biogenic VOCs constitute more than 50% of all atmospheric VOCs.[7] Globally, the annual natural VOC flux from biogenic sources is estimated to be 1150 Tg C, composed of 44% isoprene, 11% monoterpenes, 22.5% other reactive VOCs, and 22.5% other VOCs.[7]

Abiotic and biotic stresses such as high temperature, high light level and herbivore or pathogen attack together or separately increase the emission of VOCs from vegetation and affect communication with other plants and other organisms.[8−11] VOCs produced by plants are also involved in plant growth, development, reproduction, and defense.[8,11]

In the troposphere, VOCs react with hydroxyl (OH) and nitrate (NO_3) radicals and ozone (O_3), and play an important role in the chemistry of the lower atmosphere.[12] Oxidation of some VOCs by O_3, results in a series of new compounds that can very rapidly form secondary organic aerosols (SOAs).[13] On the other hand, phytotoxic tropospheric O_3 is formed by photochemical reactions involving primary pollutants such as nitrogen oxides (NO_x) and VOCs.[14] At least 50% of primary organic aerosol mass converts to SOA on the global scale.[15] Atmospheric aerosols can scatter or absorb solar radiation, which therefore changes the radiative balance of the atmosphere,[16] which can also affect light diffusion into the canopy.[17] Ozonolysis of monoterpene-dominated VOCs

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appears to be an important contributor to the aerosol particle size growth process following nucleation.[18] Regionally, insect outbreaks in conifer forests may have a significant impact on monoterpene emissions and atmospheric SOA concentrations.[19]

Scots pine (*Pinus sylvestris* L.) (Pinaceae) is one of the most widely distributed conifer tree species in the world.[20] The VOC emissions from Scots pine forests are dominated by monoterpenes (MTs)[21] such as α-pinene, β-pinene, δ-3-carene, limonene, myrcene, and β-phellandrene, which comprise more than 95% of total VOC emissions.[18] Foliage and branches of Scots pine are known to be an important source of reactive terpenoid compounds.[21−23] However, the concentration of MTs (α- pinene, β-pinene, δ-3-carene and myrcene) is also high in the soil under a Scots pine canopy.[24]

Defoliation of Scots pine by the European pine sawfly (*Neodiprion sertifer* Geoffroy) and the Large pine sawfly (*Diprion pini* L.) decreases tree growth and timber yield, increases tree mortality and causes economic losses.[25−28] In northern Europe, *N. sertifer* larvae consume mature needles of Scots pine, usually feeding between June and mid-July, whereas *D. pini* feeds on all age class needles between late July and August. The impact of needle feeding on VOC emissions from young and mature conifers is poorly known, although herbivore damage to conifer bark is known to increase local bark emissions[29−31] and systemic needle emissions.[29,30]

The main objective of the present study was to evaluate the composition and quantity of VOC emissions triggered by feeding damage by two species of diprionid sawfly larvae on Scots pine saplings in laboratory and field conditions. VOC emission from vegetation is mostly related to photosynthetic leaf area or leaf dry mass.[6,7] In conifers, substantial monoterpene emission can be detected from intact or insect damaged bark.[30,31] Therefore we focused on branch level VOC emissions. We wanted to know if defoliating insects also induce systemic VOC emissions and if the herbivore is a significant source of VOCs. Our testable hypotheses were (1) feeding damage by diprionid sawfly larvae increases VOC emissions from the damaged and fully defoliated branches of Scots pine, (2) VOC emissions from larval resin storage per se could be substantial, and (3) feeding damage by the larvae induces systemic shoot and below-ground VOC emissions from the pine saplings.

EXPERIMENTAL SECTION

Study Design and VOC Sampling. *Chamber Experiment.* Five Scots pine saplings of their sixth growing season (details in the Supporting Information (SI)) were randomly selected for control and feeding damage treatments. The saplings of each treatment were placed separately in two identical 2.6 m^3 growth chambers with the same controlled environmental conditions.[33] The saplings inside the chambers and the treatments between chambers were rotated weekly in order to prevent any effects of chamber-specific growth conditions.

A group of 25−30 *N. sertifer* larvae of second or third instars was used to defoliate a lower branch (a branch of the third whorl from the top) of each sapling for the damage treatment. Larvae-damaged branches and similar branches of intact control plants were covered with mesh sleeves so that two needle age classes of each branch were inside. Both ends of the sleeves were then closed with shutters to prevent larvae escaping. On the eighth day, most of the larvae were transferred to another adjacent branch of the same whorl while a few were left on the same branch to finish the rest of the needles and simulate the natural feeding and movement behavior of a pine sawfly larval brood under conditions of food shortage. The few larvae on the first defoliated branch were transferred to the adjacent branch before the next sampling on the 17th day. The new branch was also covered with a mesh sleeve in the way described above, with controls treated the same. This transferring process of larvae to the adjacent branches was continued until larvae finished consuming all needles of the third whorl branches.

The VOC sampling was conducted in a laboratory at 23 °C and c.a. 300 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) with lighting provided by portable lamps (OSRAM Dulux F 24W/41-827 Fluorescent tube, Osram-Melco Ltd., Japan). Two types of *P. sylvestris* VOC emissions (localized emissions and systemic shoot emissions) were studied in this experiment. Additionally, direct VOC emissions by a group of *N. sertifer* larvae were also assessed.

For localized emissions, VOC samples were always collected from the first larvae-damaged branch (stated as damaged branch hereafter) together with the larvae until eighth day and without larvae afterward. Prior to insect defoliation, a background sampling (0 h sampling) was done and then 1.5, 3, 22, 27, and 46 h and 3rd, 6th, 8th, 17th, and 32nd day sampling from the start of insect feeding. The mesh sleeves on each sapling were opened, and the part of the branch inside the sleeve was enclosed by a precleaned (120 °C for 1 h) polyethylene terephthalate (PET) bag (25 × 55 cm, LOOK, Terinex Ltd., England). Teflon lines channelling charcoal filtered and MnO_2 scrubbed air were inserted through openings cut at the corners of the PET bags and fastened with shutters to make the connection airtight. The bags were flushed for 10 min to let the bag fill-up and air was replaced at flow rate of 0.3 L/min. The headspace was pulled through to steel tubes filled with 150 mg of Tenax TA-adsorbent (Supelco, mesh 60/80, Perkin-Elmer) at rate of 0.2 L/min through an opening cut at the corner of the bag. The collection period was 30 min. The flow calibration and sample storing was done as described by Heijari et al.[30]

For systemic shoot emissions, VOC samples were collected from the top of the crown consisting of the two uppermost whorls of the saplings. The VOC samples were collected on the 3rd, 6th, 8th, 17th, 22nd, and 32nd days of insect damage. The size of the PET bags was 45 × 55 cm, the flushing period was 15 min and collection period also15 min. In all other ways, the sampling procedure was the same as described above.

The samples of VOCs emitted by *N. sertifer* larvae were collected on the eighth day from the start of the experiment by enclosing the groups of all larvae feeding on an individual larvae-damaged plant separately inside 25 × 55 cm PET bags. First, each larval group was transferred to cleaned Petri dishes for their fresh biomass measurement and then the group was enclosed in cleaned PET bag and VOC samples were collected for 1.5 h following the same procedures as described above. In order to calculate the proportion of larval emissions, both the larval group emissions and the emissions from branch plus larvae together were expressed as μg h^{-1}. In addition, larval emissions were calculated per unit of their fresh biomass as described in the SI.

After sampling on each day, the length of all current-year growing shoots that were enclosed inside sampling bags during VOC collection was measured, while the length of previous-year shoots enclosed inside the collection bags was measured at

the start of the experiment only. Likewise, the proportion of needle loss (mature needles) in all larvae-damaged branches was estimated. Each day after sampling, the control and larvae-damaged saplings (with mesh sleeves enclosing the lower branches) were kept in the separate chambers. After the last sampling day, VOC-sampled lower branches and the top crowns were cut, oven-dried (+60 °C) and the dry biomass of stem and needles were determined.

Open-Field Experiment. Five Scots pine saplings were randomly selected for control and feeding damage treatments. A group of twenty *D. pini* larvae of approximately the same size and weight were used to defoliate a lower branch (a branch of the third whorl from the top) of damaged plants, designated the lower original branch, LOB. Each larva-damaged branch was enclosed in a mesh sleeve. One branch from each control plant was enclosed in the same way and used for VOC sampling. On the sixth day, all larvae of each larvae-damaged plant were transferred to another adjacent branch of the same plant, designated the lower adjacent branch, LAB. However, the larvae were kept on the LOB for the whole experiment on one of the saplings due to the larvae not consuming all the needles of that branch. After transferring larvae, the new branches were also covered with mesh sleeves.

Three types of VOC emissions, that is, localized emissions, systemic shoot emissions, and below-ground emissions were studied in this experiment. Just before larval feeding, a background sample was also made from the LOB of each sapling. For localized emissions, the first VOC samples were made from the LOB on the sixth day of feeding damage, whereas the second sampling was made from the LAB on the 5th day of feeding damage on the branch (10th day of feeding damage on plants). All the larvae were transferred to Petri dishes and VOCs were collected from the damaged branches without larvae. The VOC sampling was done with pump-operated VOC-collection system designed for the field work,[34] the collection period was 15 min but otherwise the same as in the chamber experiment.

For systemic shoot emissions, VOC samples were collected from the uppermost whorl on the 30th day of larval damage on plants. The size of the PET bags was 45 × 55 cm and the collection period was 15 min. The VOC samples for below-ground emissions were also collected on the 30th day of larval damage. The root system with tight soil around it was lifted from the pot, without breaking any root, placed in a 45 × 55 cm PET bag and VOCs were collected for 30 min as described above. Plants were not cut, but laid down horizontally during VOC collection.

Temperature, air humidity and PAR level outside the plastic bags were monitored with a HOBO Micro Station Data Logger.[30] Temperature inside the bags was monitored by wireless temperature/humidity loggers (Hygrochron DS1923-F5 i Button, Maxim Integrated Products, Inc., CA). The details of temperatures during VOC collections and the temperature standardization (at 30 °C) procedure of VOC emission data are given in the SI.

After each sampling, the shoot lengths of VOC-collected branches were measured. We estimated the proportion of mature needles damaged by larvae and performed dry biomass analysis in the same way as described in the chamber experiments.

VOC Analyses. The VOC samples were analyzed by gas chromatography−mass spectrometry (GC-MS). The details of the VOC analyses and the emission calculations are given in the SI.

Statistical Analyses. Normality of the VOC emissions data was tested. In cases of non-normality, logarithm transformation (log 10(x+1)) was done to make the data follow a normal distribution, and treatments were compared using Independent Sample T-Tests. In cases where the transformation did not normalize the data, Mann−Whitney U Tests were used. The VOC emission rates for each time point were studied separately. The statistical analyses were conducted using SPSS 14.0.1 for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Foliar VOC Emissions. *Insect-Damaged Lower Branch (Localized Emissions).* The most dominant individual MTs induced by *N. sertifer* feeding on Scots pine saplings were α-pinene and δ-3-carene, which accounted for an average of 26% and 35% of the total MT emissions, respectively (SI Table 1a and 1b). Feeding damage significantly increased the emission rate of α-pinene and δ-3-carene, on average by13- and 50-fold, respectively. Increase in δ-3-carene can be partly attributed to a higher proportion of seedlings of the δ-3-carene dominating genotype[35,36] in the insect-damaged group. Larval feeding significantly increased the emission rate of camphene, β-phellandrene and sabinene by up to 11-fold, 25-fold, and 28-fold, respectively. The MT emissions increased shortly after larval feeding, were detected highest on the sixth day of feeding and declined after 8 days of larval feeding (SI Table 1a and 1b). The MT emissions of the damaged branches were nearly 96% of the total VOCs, and on average 14-fold greater than the emissions of the control (Figure 1a). The same compounds dominated total MT emissions of mechanically damaged seedlings[18] and the forest canopy atmosphere.[21] β-phellandrene has the largest scale-up factor in bark beetle-damaged pine forests.[19] However, the emissions of typical herbivore-inducible MTs such as β-ocimene or linalool in conifer seedlings[29,30] were not detected in our experiments.

N. sertifer feeding significantly increased the emission rates of sesquiterpenes (SQTs) such as trans-caryophyllene, germacrene-D and δ-cadinene at four of the observation points and α-muurolene at seven observation points. The average emission rates of trans-caryophyllene, germacrene-D, δ-cadinene, and α-muurolene from the damaged branches were 32%, 19%, 24%, and 12% of the total SQTs emissions, respectively. The emission rates of germacrene-D and δ-cadinene from the damaged branches were the highest of all SQTs, where this rate was increased by15- and 11-fold, respectively compared to the controls. All dominant SQTs had highest emission rates from the damaged branches on the sixth day of larval damage (SI Table 1a and 1b). SQT emissions comprised nearly 4% of the total VOC emissions, being on average 7-fold greater in damaged branches than the controls (Figure 1b).

The emission rate of GLVs from *N. sertifer*-damaged branches was significantly increased. The emission rate of trans-2-hexenal was increased at seven, and cis-3-hexen-1-ol and cis-3-hexenyl-acetate at four of the observation points. The average emission rates of trans-2-hexenal, cis-3-hexen-1-ol and cis-3-hexenyl-acetate were 27%, 22%, and 37% of the total GLV emissions, respectively. Larval feeding increased the emission rates of trans-2-hexenal and cis-3-hexenyl-acetate by 5- and 6-fold, respectively. The maximum emission rates of GLVs were detected on the eighth day of larval damage (SI Table 1a and 1b). The GLV emissions of the damaged branches were nearly

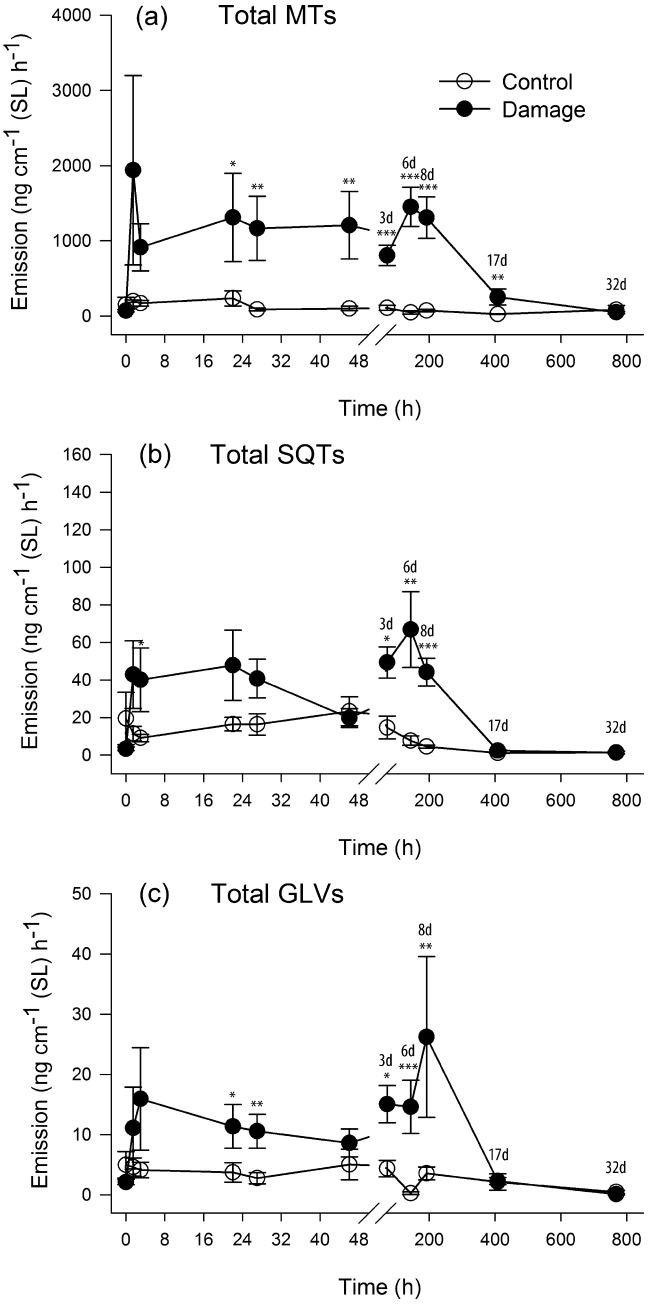

Figure 1. Mean (±SE, n = 5) emissions of (a) total MTs, (b) total SQTs, and (c) total GLVs measured from the control and *N. sertifer*-damaged original branch of Scots pine saplings at different time points in the chamber experiment. The statistical significance is represented by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ (independent sample t-test). SL refers to shoot length.

1% of the total VOCs, and on average 13-fold greater than those of the controls (Figure 1c). The emissions trend of the total VOC was the same as the total MTs. The proportion of mature needles damaged by the larvae in each sampling point was 2% (1.5 h), 4% (3 h), 7% (22 h), 9% (27 h), 12% (46 h), 15% (3 d), 40% (6 d), 75% (8 d), and 97% (17 and 32 d).

D. pini feeding in the field site induced significantly higher emission rates of the dominant MTs (α-pinene, camphene, β-pinene, myrcene, and limonene) in the damaged LOB compared to the branch of control saplings (data not shown). Larval feeding also significantly increased the emission rates of α-pinene, β-pinene, myrcene, and terpinolene from the damaged LAB compared to the control. Larval feeding increased total MT emissions by 17- and 15-fold in LOB and LAB, respectively, which comprised 98% of the total VOC emissions (Figure 2a).

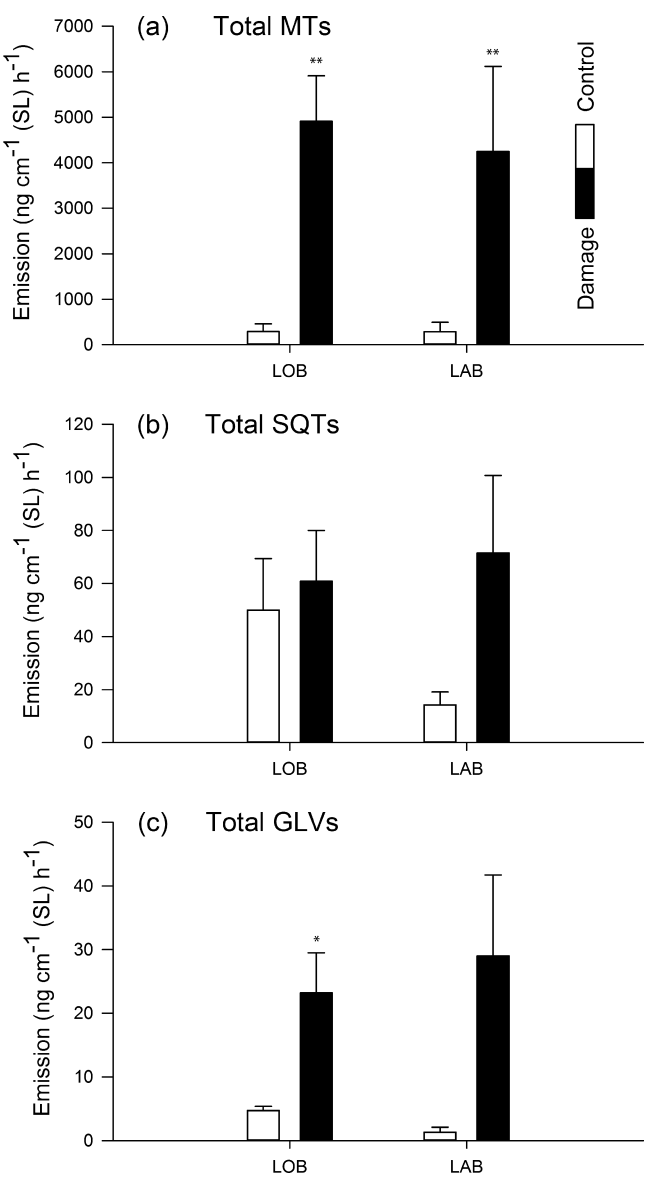

Figure 2. Mean (±SE) emissions of (a) total MTs, (b) total SQTs, and (c) total GLVs measured from the control (n = 4) and *D. pini*-damaged lower original branch (LOB) (n = 5) of Scots pine saplings on the 6th day of larval feeding, and from the control (n = 4) and *D. pini*-damaged lower adjacent branch (LAB) (n = 4) on the 5th day of larval feeding in the field experiment. The statistical significance is represented by $*p < 0.05$ and $**p < 0.01$ (independent sample t-test). VOC emissions data was temperature standardized at 30 °C. SL refers to shoot length.

Of the SQTs, *D. pini* feeding significantly decreased the emission rates of α-farnesene in the damaged LOB compared to the control. The emission rates of trans-β-caryophyllene were significantly higher in damaged LAB than control branches. However, larval feeding did not increase the emission rates of total SQTs, either from LOB or LAB (Figure 2b). Larval feeding significantly increased the emission rates of total

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Table 1. Mean (± SE) VOC Emissions from *N. sertifer* Larvae[a]

VOCs	branch and larval emissions together (μg h^{-1})	larval emissions per 28 larvae in group (μg h^{-1})	proportion (%) of larval group emissions from whole branch emissions	emissions per larva (μg h^{-1})	emissions per larval fresh mass (μg g^{-1} h^{-1})
total monoterpenes	43.9 ± 6.3	8.8 ± 1.0	21 ± 2	0.3 ± 0.0	0.4 ± 0.1
total sesquiterpenes	1.5 ± 0.2	1.3 ± 0.3	83 ± 10	0.0 ± 0.0	0.1 ± 0.0
total GLVs	0.7 ± 0.2	0.0 ± 0.0	1 ± 0	0.0 ± 0.0	0.0 ± 0.0
total VOCs	46.1 ± 6.5	10.1 ± 1.1	23 ± 2	0.4 ± 0.0	0.4 ± 0.1

[a]VOC emissions from the larvae-damaged branches together with a group of *N. sertifer* larvae (n = 5), emissions from the group of larvae (n = 5), proportion of larval group emissions from whole branch emissions, emissions per larva and per unit of larval fresh mass (mean wt. of 1 larvae =0.0323 g) measured on the 8th day of larval feeding on the Scots pine saplings. Approximately third-instar *N. sertifer* larvae were weighed in groups as described by Lyytikäinen.[45]

GLVs from the LOB (Figure 2c). Larvae damaged approximately 80% of needles on both LOB and LAB.

Our laboratory and open-field experiments showed that the most significant MTs emitted by sawfly damaged branches were the same as those emitted constitutively by control plants, but with higher emission rates. This is in agreement with earlier observations in *Pinus ponderosa*[37] where feeding by folivorous moth larvae increased MT cyclase activity in needles by 4.5-fold compared to intact plants. However, Litvak and Monson[37] reported ca. 6-fold increases in the emission of constitutively emitted MTs after 8 days of larval feeding, whereas our observations after 6 days of *D. pini* feeding indicated a 16-fold increase in total MT emission rate in the open-field experiment. Ghirardo et al.[32] reported that 58% of the MT emission would originate directly from de novo synthesis in Scots pine. The extremely high fraction (96%) of the total VOCs comprised of MTs in our experiment may originate from both terpene storage pools and de novo synthesis of damaged seedlings.

In the *N. sertifer* feeding experiment, the peak MT emission rate observed for the damaged branches on the 6th and 8th days of larval feeding coincide with active larval feeding on several needles and an observed flow of resin droplets from the base of totally consumed needles. This may be caused by the flow of resin from the bark to the base of needles freshly consumed by the larvae, but also by high de novo synthesis of MTs at the base of needles damaged from the tip.[37] As there is no increase in the pool size of total MTs in wounded needles, but an increase only in emission rates of MTs,[37] the volatile pool may function as immediate defense compounds against feeding larvae. The emission rates of all MTs from defoliated branches were rapidly decreased on day 17, after removal of larvae from the original branch on the eighth day, but still stayed above the total MT emission rate of control branches. Thirty days later, the emission rate was still as high as in intact controls. This indicates that needle stumps, and possibly the slight biting injury observed on bark, could be an important source of MT emissions from fully defoliated conifer branches.

A number of SQTs such as trans-caryophyllene, trans-β farnesene, germacrene-D, α-muurolene, and δ-cadinene were also induced as defense compounds in response to sawflies feeding in Scots pine. As a chemical defense of pine against herbivorous insects, a huge variety of pine terpenoid compounds mediate numerous specific food web interactions.[38] Induction of SQTs by larval feeding was delayed and highest on the 6th day of feeding damage. Our results of a high proportion of SQT in larval emissions suggest that SQT accumulation in larvae could be another explanation for the highest SQT emission rates. The emission rates of the total GLVs were highest on the eighth and last day of larval feeding. After that, on day 17, there were still some minor emissions of these compounds, although GLV emissions are fast indicators of mechanical cellular damage[39] and emissions cease rapidly when feeding episodes stop.[40]

The *D. pini* feeding experiment was conducted in the open-field later in the growing season, when this species also feed on current-year needles, and can defoliate whole branches. The most dominant induced VOCs in *D. pini* damaged LOB and LAB branches were the same constitutive MTs (α-pinene, camphene, β-pinene, myrcene, limonene, and terpinolene) emitted by control plants. Larval feeding caused strong induction of SQT (trans-β-caryophyllene) from the damaged LAB, but did not significantly affect the total SQT emission rate, which contrasts with the results of the laboratory experiment. Larvae were not included in the branch scale sampling of VOCs in the field experiment, which suggests that larval emission could be an important source of SQT emissions from diprionid damaged Scots pine branches.

Pine weevil (*Hylobius abietis* L.) feeding on Scots pine bark increased localized emission of MTs by nearly 4-fold and some SQTs by 7-fold.[30] Our results suggest that defoliating sawflies affect branch-based SQT and GLV emissions more efficiently than the herbivore-induced bark emissions.[30]

Undamaged Top Crown (Systemic Shoot Emissions). The dominant MTs and SQTs emitted from the intact top crown in both experiments were the same as in the lower branches. Early season *N. sertifer* feeding on the lower branches significantly increased the total MT emission rates (4-fold) of the top crown, but only on the 22nd day of larval damage, when MTs comprised 99% of the total VOCs (SI Figure 1a). At the compound level, this increase was significant to sabinene emission (3% of total MTs) which was 6-fold higher in the damaged saplings than controls. At this time point, the larvae had damaged approximately 65% of the needles on the lower branches. The emission rates of total SQTs and total GLVs from the top crown were not increased at any of the time points (SI Figure 1b and 1c). In contrast to *N. sertifer* feeding, *D. pini* feeding did not induce systemic shoot emissions of total MTs, SQTs and GLVs (data not shown).

These results might indicate that systemic emissions are only induced by diprionid feeding during shoot elongation growth of *P. sylvestris* in the early growing season, when leaf based MT emissions are peaking in *P. sylvestris* stands.[21] Herbivore-induced VOCs are known to function as external signals for within-plant communication and play a role in the systemic response of a plant to local damage.[41] It is obvious that there is still limited information about the systemic response of VOCs

induced by insects feeding on conifers, but this type of response could be stronger earlier in the growing season.

Larval Emissions. The emissions by the groups of *N. sertifer* larvae showed a varying proportion of MTs. The average emission proportion of dominant MTs by the group of larvae was 27% of the total branch VOC emissions (branch and larval emissions together). The total MT and total SQT emissions by the larvae were approximately 20 and 80% respectively, of total branch VOC emissions (Table 1). Total GLVs were emitted in very low proportions (<1%) by larvae. Total VOC emissions by larvae contributed nearly 25% of total branch VOC emissions (Table 1).

The share of larval emissions in the total branch VOC emissions was surprisingly high. A source of high VOC emissions could partly be the stored resin in the larval body and partly the droplets regurgitated in defense by larvae under stressed conditions.[42] Therefore, our results probably overestimate the proportion of continuous larval emissions by nondisturbed feeding larvae, but may reflect conditions at forest sites where larvae are under predation by birds, small mammals and invertebrates.

VOC Emissions from Below-Ground Plant Part. The dominant MTs emitted from the below-ground part of pine saplings were α-pinene, camphene, myrcene, δ-3-carene, limonene, γ-terpinene, and terpinolene. Defoliation by *D. pini* significantly decreased the emission rates of total MTs in the below-ground part of the saplings with approximately 80% decrease compared to the controls (Figure 3).

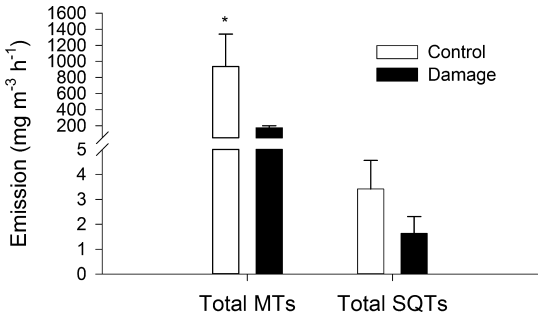

Figure 3. Mean (±SE, n = 5) emissions of total MTs and total SQTs measured from the below-ground parts of control and *D. pini*-damaged Scots pine saplings on the 30th day of feeding damage on plants in the field experiment. The *asterisk* (*) represents significant differences at $p < 0.05$ (Mann−Whitney U test). VOC emissions data was temperature standardized at 30 °C.

Major SQTs emitted from the below-ground parts of the pine saplings were α-longipinene, longifolene, and δ-cadinene. The emission rates of total SQTs from the below-ground parts did not differ significantly between undamaged and damaged saplings (Figure 3). GLV emissions were not detected from the below-ground parts of the saplings.

High concentrations of MTs are found in the soil of Scots pine forests[43] originating from roots and needle litter.[44] Our study suggests that needle damage by larvae on the Scots pine foliage may substantially reduce MT emission from the below-ground parts of the plants probably due to the reduction in carbon fixation by needles and a subsequent reduction in allocation to roots. Our results may exaggerate the change in emission rate from roots to the atmosphere as whole root system was sampled.

Defoliator-Induced VOCs and Biogenic SOA Formation. This study demonstrated that Scots pine saplings, both intact and herbivore-damaged, are important sources of reactive VOC emissions, and potential precursors of biogenic secondary aerosols. Insects feeding on the pine needles can increase localized VOC emissions by up to 16-fold, and total MT emissions from the undamaged top crown can be found up to 4-fold. The share of the emission coming from larvae can be substantial in the early season, although defoliation may significantly reduce carbon fixation and the below-ground emissions of MTs from the saplings.

MT emissions from *P. sylvestris* have previously been found to be significant natural precursors of rapid secondary aerosol particle formation in O_3 and $OH^{\bullet}$ reactions in the laboratory[13,18] and in the forest environment.[13] Pine sawfly larvae feed on the needles of both young and mature Scots pine in the nature. The results of this study convincingly indicate that local branch-level MT emissions will substantially increase during pine sawfly outbreaks. Therefore, more extensive VOC surveys, for example, see refs 19,31, and SOA measurement campaigns should be conducted at forest outbreak sites of both conifer bark damaging and needle defoliating insects to assess the impact of insect outbreaks on atmospheric SOA load from forests.

■ ASSOCIATED CONTENT

Supporting Information

Description of plant and insect material; VOC analyses; a figure indicating VOC emission rates from undamaged top crown; two data tables showing the emission rates of some significantly induced MTs, SQTs, and GLVs from the insect-damaged branches in the chamber experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) *Earth Science*. Coble, C. R., Murray, E. G., Rice, D. R., Eds.; Prentice-Hall: Englewood Cliffs, NJ, 1987.

(2) FAO. *Global Forest Resources Assessment 2010, Main Report*, FAO Forestry Paper; Food and Agriculture Organization of the United Nations: Rome, Italy, 2010; Vol. *163*.

(3) Taggart, R. E.; Cross, A. T. Global greenhouse to icehouse and back again: The origin and future of the boreal forest biome. *Global Planet. Change* **2009**, *65*, 115−121.

(4) *Finnish Statistical Yearbook of Forestry. Official Statistics of Finland, Agriculture, Forestry and Fishery*; Peltola, A., Ed.; Finnish Forest Research Institute (METLA), 2009.

(5) Kesselmeier, J.; Staudt, M. Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. *J. Atmos. Chem.* **1999**, *33*, 23−88.

(6) Laothawornkitkul, J.; Taylor, J. E.; Paul, N. D.; Hewitt, C. N. Biogenic volatile organic compounds in the earth system. *New Phytol.* **2009**, *183*, 27−51.
(7) Guenther, A.; Hewitt, C. N.; Erickson, D.; Fall, R.; Geron, C.; Graedel, T.; Harley, P.; Klinger, L.; Lerdau, M. A global model of natural volatile organic compound emissions. *J. Geophys. Res., [Atmos.]* **1995**, *100*, 8873−8892.
(8) Peñuelas, J.; Llusia, J. BVOCs: Plant defence against climate warming? *Trends Plant Sci.* **2003**, *8*, 105−109.
(9) Heil, M.; Karban, R. Explaining evolution of plant communication by airborne signals. *Trends Ecol. Evol.* **2010**, *25*, 137−144.
(10) Holopainen, J. K.; Gershenzon, J. Multiple stress factors and the emission of plant VOCs. *Trends Plant Sci.* **2010**, *15*, 176−184.
(11) Loreto, F.; Schnitzler, J.-P. Abiotic stresses and induced BVOCs. *Trends Plant Sci.* **2010**, *15*, 154−166.
(12) Atkinson, R.; Arey, J. Gas-phase tropospheric chemistry of biogenic volatile organic compounds: A review. *Atmos. Environ.* **2003**, *37*, 197−219.
(13) Virtanen, A.; Joutsensaari, J.; Koop, T.; Kannosto, J.; Yli-Pirilä, P.; Leskinen, J.; Mäkelä, J. M.; Holopainen, J. K.; Poschl, U.; Kulmala, M.; Worsnop, D. R.; Laaksonen, A. An amorphous solid state of biogenic secondary organic aerosol particles. *Nature* **2010**, *467*, 824−827.
(14) Pinto, D. M.; Blande, J. D.; Souza, S. R.; Nerg, A. M.; Holopainen, J. K. Plant volatile organic compounds (VOCs) in ozone (O_3) polluted atmospheres: The ecological effects. (special issue: Human impact.). *J. Chem. Ecol.* **2010**, *36*, 22−34.
(15) Kanakidou, M.; Seinfeld, J. H.; Pandis, S. N.; Barnes, I.; Dentener, F. J.; Facchini, M. C.; Van Dingenen, R.; Ervens, B.; Nenes, A.; Nielsen, C. J.; Swietlicki, E.; Putaud, J. P.; Balkanski, Y.; Fuzzi, S.; Horth, J.; Moortgat, G. K.; Winterhalter, R.; Myhre, C. E. L.; Tsigaridis, K.; Vignati, E.; Stephanou, E. G.; Wilson, J. Organic aerosol and global climate modelling: A review. *Atmos. Chem. Phys.* **2005**, *5*, 1053−1123.
(16) IPCC. *Intergovernmental Panel on Climate Change, Climate Change 2001*, The Scientific Basis; Cambridge University Press: UK, 2001.
(17) Holopainen, J. K. Can forest trees compensate for stress-generated growth losses by induced production of volatile compounds? *Tree Physiol.* **2011**, *31*, 1356−1377.
(18) Hao, L. Q.; Yli-Pirilä, P.; Tiitta, P.; Romakkaniemi, S.; Vaattovaara, P.; Kajos, M. K.; Rinne, J.; Heijari, J.; Kortelainen, A.; Miettinen, P.; Kroll, J. H.; Holopainen, J. K.; Smith, J. N.; Joutsensaari, J.; Kulmala, M.; Worsnop, D. R.; Laaksonen, A. New particle formation from the oxidation of direct emissions of pine seedlings. *Atmos. Chem. Phys.* **2009**, *9*, 8121−8137.
(19) Berg, A. R.; Heald, C. L.; Huff Hartz, K. E.; Hallar, A. G.; Meddens, A. J. H.; Hicke, J. A.; Lamarque, J.-F.; Tilmes, S. The impact of bark beetle infestation on monoterpene emissions and secondary organic aerosol formation in Western North America. *Atmos. Chem. Phys.* **2013**, *13*, 3149−3161.
(20) Hämet-Ahti, L.; Palmén, A.; Alanko, P.; Tigerstedt, P. M. A. *Woody Flora of Finland*; University Press: Helsinki, 1992.
(21) Räisänen, T.; Ryyppo, A.; Kellomäki, S. Monoterpene emission of a boreal Scots pine (*Pinus sylvestris* L.) forest. *Agri. Fores. Meteorol.* **2009**, *149*, 808−819.
(22) Mumm, R.; Schrank, K.; Wegener, R.; Schulz, S.; Hilker, M. Chemical analysis of volatiles emitted by *Pinus sylvestris* after induction by insect oviposition. *J. Chem. Ecol.* **2003**, *29*, 1235−1252.
(23) Bäck, J.; Hari, P.; Hakola, H.; Juurola, E.; Kulmala, M. Dynamics of monoterpene emissions in *Pinus sylvestris* during early spring. *Boreal. Environ. Res.* **2005**, *10*, 409−424.
(24) Smolander, A.; Ketola, R. A.; Kotiaho, T.; Kanerva, S.; Suominen, K.; Kitunen, V. Volatile monoterpenes in soil atmosphere under birch and conifers: Effects on soil N transformations. *Soil Biol. Biochem.* **2006**, *38*, 3436−3442.
(25) Lyytikäinen-Saarenmaa, P. Growth responses of Scots pine (Pinaceae) to artificial and sawfly (Hymenoptera: Diprionidae) defoliation. *Can. Entomol.* **1999**, *131*, 455−463.
(26) Lyytikäinen-Saarenmaa, P.; Tomppo, E. Impact of sawfly defoliation on growth of scots pine *Pinus sylvestris* (Pinaceae) and associated economic losses. *Bull. Entomol. Res.* **2002**, *92*, 137−140.
(27) Kurkela, T.; Aalto, T.; Varama, M.; Jalkanen, R. Defoliation by the common pine sawfly (*Diprion pini*) and subsequent growth reduction in Scots pine: A retrospective approach. *Silva Fenn.* **2005**, *39*, 467−480.
(28) Kantola, T.; Vastaranta, M.; Yu, X.; Lyytikainen-Saarenmaa, P.; Holopainen, M.; Talvitie, M.; Kaasalainen, S.; Solberg, S.; Hyyppa, J. Classification of defoliated trees using tree-level airborne laser scanning data combined with aerial images. *Remote Sens.* **2010**, *2*, 2665−2679.
(29) Blande, J. D.; Turunen, K.; Holopainen, J. K. Pine weevil feeding on Norway spruce bark has a stronger impact on needle VOC emissions than enhanced ultraviolet-B radiation. *Environ. Pollut.* **2009**, *157*, 174−180.
(30) Heijari, J.; Blande, J. D.; Holopainen, J. K. Feeding of large pine weevil on Scots pine stem triggers localised bark and systemic shoot emission of volatile organic compounds. *Environ. Exp. Bot.* **2011**, *71*, 390−398.
(31) Amin, H. S.; Atkins, P. T.; Russo, R. S.; Brown, A. W.; Sive, B.; Hallar, A. G.; Huff Hartz, K. E. Effect of bark beetle infestation on secondary organic aerosol precursor emissions. *Environ. Sci. Technol.* **2012**, *46*, 5696−5703.
(32) Ghirardo, A.; Koch, K.; Taipale, R.; Zimmer, I.; Schnitzler, J.-P.; Rinne, J. Determination of *de novo* and pool emissions of terpenes from four common boreal/alpine trees by $^{13}CO_2$ labelling and PTR-MS analysis. *Plant Cell Environ.* **2010**, *33*, 781−792.
(33) Himanen, S. J.; Nerg, A.-M.; Poppy, G. M.; Stewart, C. N.; Holopainen, J. K. Abiotic stress and transgenics: Implications for reproductive success and crop-to-wild gene flow in *Brassicas*. *Basic App. Ecol.* **2010**, *11*, 513−521.
(34) Hartikainen, K.; Nerg, A.-M.; Kivimäenpää, M.; Kontunen-Soppela, S.; Mäenpää, M.; Oksanen, E.; Rousi, M.; Holopainen, T. Emissions of volatile organic compounds and leaf structural characteristics of European aspen (*Populus tremula*) grown under elevated ozone and temperature. *Tree Physiol.* **2009**, *29*, 1163−1173.
(35) Bäck, J.; Aalto, J.; Henriksson, M.; Hakola, H.; He, Q.; Boy, M. Chemodiversity of a Scots pine stand and implications for terpene air concentrations. *Biogeosci.* **2012**, *9*, 689−702.
(36) Hao, L. Q.; Romakkaniemi, S.; Yli-Pirilä, P.; Joutsensaari, J.; Kortelainen, A.; Kroll, J. H.; Miettinen, P.; Vaattovaara, P.; Tiitta, P.; Jaatinen, A.; Kajos, M. K.; Holopainen, J. K.; Heijari, J.; Rinne, J.; Kulmala, M.; Worsnop, D. R.; Smith, J. N.; Laaksonen, A. Mass yields of secondary organic aerosols from the oxidation of α-pinene and real plant emissions. *Atmos. Chem. Phys.* **2011**, *11*, 1367−1378.
(37) Litvak, M.; Monson, R. Patterns of induced and constitutive monoterpene production in conifer needles in relation to insect herbivory. *Oecologia* **1998**, *114*, 531−540.
(38) Mumm, R. Direct and indirect chemical defence of pine against folivorous insects. *Trends Plant Sci.* **2006**, *11*, 351−358.
(39) Brilli, F.; Ruuskanen, T. M.; Schnitzhofer, R.; Mueller, M.; Breitenlechner, M.; Bittner, V.; Wohlfahrt, G.; Loreto, F.; Hansel, A. Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction "time-of-flight" mass spectrometry (PTR-TOF). *Plos One* **2011**, *6*, e20419.
(40) Schaub, A.; Blande, J. D.; Graus, M.; Oksanen, E.; Holopainen, J. K.; Hansel, A. Real-time monitoring of herbivore induced volatile emissions in the field. *Physiol. Plantarum.* **2010**, *138*, 123−133.
(41) Heil, M.; Bueno, J. C. S. Within-plant signalling by volatiles leads to induction and priming of an indirect plant defence in nature. *Proc. Nat. Acad. Sci. U.S.A.* **2007**, *104*, 5467−5472.
(42) Björkman, C.; Larsson, S. Pine sawfly defence and variation in host plant resin acids: A trade-off with growth. *Ecol. Entomol.* **1991**, *16*, 283−289.
(43) Smolander, A.; Ketola, R. A.; Kotiaho, T.; Kanerva, S.; Suominen, K.; Kitunen, V. Volatile monoterpenes in soil atmosphere under birch and conifers: Effects on soil N transformations. *Soil Biol. Biochem.* **2006**, *38*, 3436−3442.

(44) Hayward, S.; Muncey, R. J.; James, A. E.; Halsall, C. J.; Hewitt, C. N. Monoterpene emissions from soil in a Sitka spruce forest. *Atmos. Environ.* **2001**, *35*, 4081−4087.
(45) Lyytikäinen, P. The success of pinesawflies in relation to 3-carene and nutrient-content of Scots pine foliage. *For. Ecol. Manag.* **1994**, *67*, 1−10.

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