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Herbivory by an outbreaking moth increases emissions of biogenic volatiles and leads to enhanced secondary organic aerosol formation capacity

Pasi Yli-Pirilä^{†,*,}, Lucian Copolovici^{‡,§}, Astrid Kännaste[‡], Steffen Noe[‡], James D. Blande[#], Santtu Mikkonen[†], Tero Klemola[§], Juha Pulkkinen[‡], Annele Virtanen[†], Ari Laaksonen^{†,⊥}, Jorma Joutsensaari[†], Ülo Niinemets^{‡,¶}, and Jarmo K. Holopainen[#]

[†]Department of Applied Physics, University of Eastern Finland, P.O. Box 1626, 70211 Kuopio, Finland

[‡]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia

[§]Institute of Technical and Natural Sciences Research-Development of Aurel Vlaicu University, 2 Elena Dragoi St., 310330 Arad, Romania

[#]Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

[§]Section of Ecology, Department of Biology, University of Turku, 20014 Turku, Finland

[⊥]School of Pharmacy, University of Eastern Finland, P.O. Box 1627, 70211 Kuopio, Finland

[⊥]Finnish Meteorological Institute, P.O. Box 503, 00101 Helsinki, Finland

[¶]Estonian Academy of Sciences, Kohtu 6, 10130 Tallinn, Estonia

Abstract

In addition to climate warming, greater herbivore pressure is anticipated to enhance the emissions of climate-relevant biogenic volatile organic compounds (VOCs) from boreal and subarctic forests and promote the formation of secondary aerosols (SOA) in the atmosphere. We evaluated the effects of *Epirrita autumnata*, an outbreaking geometrid moth, feeding and larval density on herbivore-induced VOC emissions from mountain birch in laboratory experiments, and assessed the impact of these emissions on SOA formation via ozonolysis in chamber experiments. The results show that herbivore-induced VOC emissions were strongly dependent on larval density. Compared to controls without larval feeding, clear new particle formation by nucleation in the reaction chamber was observed and the SOA mass loadings in the insect-infested samples were significantly higher (up to 150-fold). To our knowledge, this study provides the first controlled documentation of SOA formation from direct VOC emission of deciduous trees damaged by known defoliating herbivores, and suggests that chewing damage on mountain birch foliage could significantly increase reactive VOC emissions that can importantly contribute to SOA formation in subarctic forests. Additional feeding experiments on related silver birch confirmed the SOA results. Thus, herbivory-driven volatiles are likely to play a major role in future biosphere-vegetation feedbacks such as sun-screening under daily 24-hours sunshine in the subarctic.

*Corresponding Author: Pasi Yli-Pirilä, University of Eastern Finland, Department of Environmental and Biological Sciences, P.O.Box 1627, FI-70211 Kuopio, Finland, pasi.yli-pirila@uef.fi.

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Introduction

Plant-generated volatile organic compound (VOC) emissions have various protective functions against abiotic stresses at cellular and whole plant levels [1–3]. The rates of VOC emission are controlled by compound physicochemical characteristics, such as low volatility or diffusion, and the biochemical and physiological controls over compound synthesis [4, 5]. Previous work on VOCs has mainly focused on constitutive emissions under abiotic stress conditions, but different stresses alone and in combination can induce emission of many new compounds and alter constitutive emissions [5, 6]. When feeding on plant leaves, insect herbivores act as biotic stressors and induce a specific emission profile of VOCs which are also called herbivore-induced plant volatiles (HIPV) [7–10]. These volatiles have ecological effects in ecosystems ranging from plant to plant communication to plant herbivore and plant carnivore interactions [11–14]. However, specific herbivore-induced VOCs such as methyl salicylate (MeSa), numerous lipoxygenase (LOX) pathway products (e.g. (*E*)-2-hexenal, (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate), homoterpenes (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), monoterpene (MT) (*E*)- β -ocimene, and sesquiterpene (SQT) α -farnesene, can be induced in infections by fungal and bacterial plant pathogens as well [15–17]. Herbivore-feeding significantly increases the constitutive emission of VOCs stored, e.g. in resin canals [18, 19]. Biotic infections can also reduce emissions of constitutively emitted compounds such as isoprene [16] or the proportion of constitutively emitted compounds can decrease in the total emissions [20, 21].

Global warming is expected to most strongly affect the northern latitudes resulting in significantly increased VOC emissions from vegetation [2, 22, 23]. Global warming is further predicted to induce large-scale insect outbreaks in the northern forests [24–27] and recent observations from subarctic mountain birch forest indicate outbreak range expansion of the geometrid moths [26, 28–30]. Mountain birch (*Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti) is a subspecies of downy birch and a dominating tree species in subarctic areas of Greenland, Iceland, Norway, Sweden, Finland and Russia [31]. Mountain birch has been found to be a significant source of VOCs dominated by sesquiterpenes [32] and insect feeding on foliage has induced typical emissions of herbivore-induced VOCs in forest sites of northern Lapland [33]. Mountain birch ramets (multiple stems) infested by foliage-feeding autumnal moth (*Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae)) larvae induced emissions of monoterpenes (*E*)- β -ocimene and linalool, homoterpene (*E*)-DMNT, and several sesquiterpenes [33]. Among the herbivore-induced plant VOCs, there are several highly reactive compounds which have shorter lifetimes than most of the compounds in the constitutive plant emissions in reactions with atmospheric oxidants [34–36].

A large-scale data analysis of long-term atmospheric observations showed that VOCs are emitted from the vegetation at higher rates under a warming climate [37]. This will substantially stimulate oxidation of the VOCs to secondary organic aerosols (SOA); increase the number concentration of particles large enough to act as cloud condensation nuclei (CCN) and result in an increase of cloud albedo. This biogenic VOC-based SOA growth mechanism produces roughly 50 % of aerosol particles at the size of CCN (30–100 nm [38])

across Europe [37]. There is evidence of the existence of a negative aerosol climate feedback mechanism in the continental biosphere, which is directly dependent on biogenic VOC emissions [37, 39]. A link between herbivore-induced VOCs and formation of SOA in the atmosphere was suggested by Holopainen [40] and demonstrated with elicitor-stimulated herbivore-induced VOC emissions of cabbage plants by Joutsensaari et al. [41]. Recently it has been suggested that climate warming may substantially promote the emission of reactive HIPVs from boreal and subarctic forests through insect outbreak effects and promote SOA and cloud formation by herbivore-induced VOC reactions with atmospheric oxidants [14, 36, 42]. Hence, a modeling analysis of bark beetle outbreak areas in Northern America demonstrated that the substantial increase of reactive VOC emissions from bark beetle-attacked conifer forests may significantly increase the atmospheric SOA concentrations in the affected areas [43]. Aerosol optical density analyses of satellite data from Western Canada [44] supported the modeling results of Berg et al. [43]. However, the composition of VOC emissions may have an effect on the overall SOA particle formation potential and the SOA formation may be even suppressed by highly reactive VOCs with small individual SOA mass yields [42, 45, 46].

Our aim was to assess the role of herbivore-induced emissions on SOA formation under controlled laboratory conditions to address the following questions: 1) how does *E. autumnata* feeding at different densities on mountain birch affect herbivore-induced VOC emission rates from the foliage? 2) How does the composition of VOC emissions differ between intact and herbivore-damaged plants? and 3) does herbivore-induced VOC emissions stimulate formation and growth of SOA particles in reactions with ozone? The results provide conclusive evidence of a major increase in SOA formation in herbivore-attacked plants.

Experimental Section

Study design

We performed two sets of experiments to elucidate the effects of herbivory on plant emissions and concurrent SOA formation. The first series of experiments assessed the influence of varying degrees of *Epirrita autumnata* herbivory (0-16 larvae per small seedling) on the plant physiological responses and induction of volatile emissions from *Betula pubescens* ssp. *czerepanovii* leaves. VOC emissions were collected and analyzed by GC-MS. The second series of experiments linked the herbivore-induced VOC emissions to SOA formation, using either 0 (control) or 6 larvae per mountain birch seedling, and measuring the volatiles emitted from the plants at the inlet of the reaction chamber, representing the ingoing VOCs available for SOA production. The number of larvae (6) used in the second set of experiments was optimized on the basis of the results of the first experiments.

Plant and insect material

Mountain birch, *Betula pubescens* ssp. *czerepanovii* (N. I. Orlova) Hämet-Ahti, seedlings were grown from seed material originating from Swedish Lapland (Kiruna, 67°51' N 20°13' E). Seeds were sown in a 1:1 mixture of sand and commercial fertilized peat (VAPO

B2), and grown in 0.5 L pots in greenhouses. They were left overwinter under snow for one winter before they were used in experiments. Autumnal moth (*Epirrita autumnata* (Lepidoptera: Geometridae)) larvae were reared on silver birch (*Betula pendula*) seedlings from eggs collected from *E. autumnata* reared in Finnish Lapland (the Kevo Subarctic Research Station, 69°45'N, 27°01'E). For feeding experiments on mountain birch, larvae representing the 3rd and 4th instars were used.

Plant physiological measurements

Whole plant was placed in a dynamic headspace sampling cuvette system with a setup described in detail earlier [47, 48]. Light intensity was kept at 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature at 28 °C, and ambient CO₂ concentration was 380-400 $\mu\text{mol mol}^{-1}$. The air flow through chamber was 1.4 L min⁻¹. After hermetic installation of the plants they were stabilized under chamber conditions for 1 h. CO₂ and H₂O concentrations at the chamber in- and outlets were measured with an infra-red dual-channel gas analyzer operated in differential mode (CIRAS II, PP-systems, Amesbury, MA, USA). The rates of net assimilation, transpiration, and stomatal conductance to water vapor were calculated from these measurements according to von Caemmerer and Farquhar [49]. By the end of the stabilization period (time 0), the treatment was started by placing 5 larvae of *E. autumnata* on the plants. Control plants were left untreated and measured in the same way. After introduction of the larvae, the plants were stabilized in the system in the dynamic air flow for 20 minutes before VOC sampling. The larvae were allowed to feed from the leaves for 24 hours.

Herbivore-induced VOC emission measurements

The impact of *E. autumnata* larval density on the emission rates was assessed using one-year-old non-branching seedlings by enclosing the above-ground part of each seedling together with various numbers (0, 4, 8, or 16) of *E. autumnata* 3rd to 4th instar larvae inside 3 L glass chambers for GC-MS measurements of emitted volatiles as described by Copolovici et al. [8]. In the 24-hour experiment, larvae were allowed to feed for 7 hours after which they were removed. In the long-term experiment (0-3 days), larvae were removed after 2.2 days and the light cycle was 14 h light followed by 8 h darkness. VOC sampling was performed via the outlets of each cuvette every hour with a flow rate of 200 mL min⁻¹ for 20 min by using a constant flow air sample pump (1003-SKC, SKC Inc., Houston, TX, USA). In addition, a sample was taken from the air inlet prior to the cuvettes to measure the background VOC concentrations. Adsorbent cartridges were analyzed for LOX pathway products, mono-, homo- and sesquiterpene emissions with a combined Shimadzu TD20 automated cartridge desorber and Shimadzu 2010 plus GC-MS instrument (Shimadzu Corporation, Kyoto, Japan) using a method as detailed in [16, 50]. The background (blank) VOC concentrations were subtracted from emission samples of the seedlings. Separate samples were collected from larvae without plants. The emissions of volatile compounds from insects were below the detection limits of our system (see Copolovici et al. [50]). At the end of the experiment, all the leaves were harvested and scanned at 300 dpi. Projected leaf area was determined from the scanned images with custom made software (see Copolovici et al. [8]).

SOA formation measurements

Six 3rd to 4th instar *E. autumnata* larvae were transferred to shoots of mountain birch seedlings and enclosed within mesh bags and kept in growth chambers (Bioklim 2600T, Kryo-Service Oy, Helsinki) at 23 °C (day)/18 °C (night), 60-80% relative humidity, and 22 h photoperiod (350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active quantum flux density during the light period). Larvae were allowed to feed for 48 h before the start of the SOA experiments to maximize the production of herbivore-induced VOCs [51]. After the mesh bags were removed, eight control seedlings or eight herbivore-damaged seedlings (each seedling still having 6 feeding larvae) were transferred into each of two 22.3 L glass vessels (Schott Duran, Schott AG, Mainz, Germany). The plant pots were covered with aluminum foil to minimize root system emissions. Two lamps (Lival Shuttle Plus, Lival Oy, Sipoo, Finland, with OSRAM Dulux F 24W/41-827 Fluorescent tubes, Osram-Melco Ltd., Japan) were positioned above each vessel to ensure sufficient lighting level (350 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for photosynthesis activity and VOC emissions at laboratory conditions (24 °C).

The SOA formation experiments from the ozonolysis of mountain birch emitted VOCs were carried out in a rectangular continuous flow-through chamber made of fluorinated ethylene propylene (FEP) film. The experimental set-up and the chamber have been described elsewhere [44]. Briefly, the reaction chamber volume was 2 m³. Air flow (8 L min⁻¹) from the two glass vessels (later on referred to as plant chambers), each having either 8 control plants or 8 herbivore-damaged plants, was pooled to one line (Teflon tubing) and mixed with an ozone-enriched air flow (generated with a UV lamp generator, with a target concentration set at 200 nmol mol⁻¹) at the inlet of the reactor. The average ozone concentration inside the reaction chamber varied between 65-90 nmol mol⁻¹ during the experiments. The total air flow into the reaction chamber was 16 L min⁻¹ giving an average residence time of 2 h in the chamber. The chamber was kept at constant room temperature (23 $\pm$ 1 °C) during the experiment. Relative humidity in the chamber varied between 30-35 % depending on the water vapor coming from the seedlings. Before each experiment, the chamber was flushed continuously with purified dry air (dew point -40 °C, filtered by active carbon and Purafil Select Media[®] pellets and HEPA filter) at least for 24 h to ensure minimal contamination from previous experiments.

At the beginning of each experimental run, VOCs from seedlings were introduced to the reaction chamber for 90 minutes before starting the ozone addition. The duration of the chamber experiment was ca. 21 h. The plants' diurnal cycle was mimicked by turning off the lights over the plant chambers from 00:00 to 03:00. In all experiments, concentrations of ozone (measured with a DASIBI 1008-RS O3 analyzer, Dasibi Environmental Corp. Glendale, CA, USA), NO_x (with Environnement AC 30M NO_x analyzer, Environnement S.A., Poissy, France) and SO₂ (with Environnement AF21M SO₂ analyzer, Environnement S.A., Poissy, France) were monitored. NO_x and SO₂ concentrations were below the analyzers quantification limits (3 nmol mol⁻¹) during the experiments.

VOC concentrations in the ingoing (before the reaction chamber) and outgoing (after the reaction chamber) air were measured by GC-MS. The VOCs were trapped on a Tenax TA (Supelco, mesh 60/80) cartridge with an air flow of 200 mL min⁻¹ for 30 minutes. For the outgoing air, the sampled air passed first through a potassium iodine-coated copper tube to

scrub the ozone. The trapped compounds were desorbed from the collected VOC samples with a thermal desorption unit (Perkin-Elmer ATD400 Automatic Thermal Desorption system) and analyzed with a gas chromatograph-mass spectrometer (Hewlett-Packard GC 6890 and MSD 5973). A detailed description of the VOC analysis can be found in Pinto et al.[52].

The ozonolysis of BVOCs resulted in a clear particle nucleation and growth event which was monitored with a scanning mobility particle sizer (SMPS, consisting of DMA 3071 & CPC 3022A, TSI Inc., St. Paul, MN, USA) by measuring particle number size distribution in the diameter range of 15 to 700 nm. The total particle concentration (particle diameter > 4 nm) was monitored with a condensation particle counter (CPC 3775, TSI Inc.). The total mass concentration of the produced SOA was calculated from the measured number size distribution using a particle density of 1.3 g cm^{-3} [53] and corrected particle wall losses (see Supplement). The SOA mass yields were estimated by dividing the formed SOA mass (maximum SOA mass) by the sum of reacted VOC concentrations (the total VOC concentration at the reactor inlet minus the concentration at the outlet) [54]. The experiments were conducted without seed particles, which could lower SOA mass yields by increasing loss of low volatile organics to the chamber walls [55]. The effect of VOC losses on SOA mass yields has been discussed in more detail in the supplement.

These exactly same experimental settings were repeated in another set of experiments, where silver birch (*Betula pendula*) seedlings were used as plant material. Results of these experiments are shown in Supporting Information (S5-S7, Figure S3).

Statistical analysis

Plant physiological responses of control and herbivore-damaged plants were compared by t-tests and considered significant at $p < 0.05$ (Prism 5, GraphPad Software, Inc., La Jolla, CA, USA). Long-term (3 days) variation in emission rates was studied with Mixed Model ANOVA (MMA) which takes into account the repetitive structure of the measurements and thus gives valid interpretations for the between group comparisons. Nonlinear regression analysis was used to study the emission rates of different compounds in relation to leaf area consumed by *E. autumnata* larvae. This analysis was made in R-software (R Core Team, 2014). Concentrations of VOCs emitted by mountain birch at the reaction chamber inlet in the SOA formation experiments were compared with Mann-Whitney U-test. The Mann-Whitney U-test and MMA analyses were performed in SAS 9.4 (SAS Institute, Cary NC).

Results and Discussion

We have shown that the VOC emission rate from herbivore-damaged plants is related to the extent of the damaged leaf area and the number of damaging herbivores. Aerosol measurement in reaction chambers gave evidence that the quantitative and qualitative changes in VOC emissions of mountain birch after herbivory are sufficient to promote secondary aerosol formation in the atmosphere.

Impact of herbivore density on VOC emissions

In the 24 h feeding data (Fig. 1), emissions of different compounds were induced with different time kinetics. The first major emissions observed were LOX products, which were induced immediately after the onset of larval feeding (Fig. 1a) and stayed at higher levels for at least 7 hours. Instead, the emissions of monoterpenes did not show such a rapid response to larval feeding; a slight increase in emission rates was detected just after the start of feeding at $t = 0.5$ h, but the maximum emission rate was observed at $t = 5$ h (Fig. 1c). Sesquiterpenes, especially α -farnesene, were induced after 3 h from the start of the insect feeding (Fig. 1e). No comparable increases in the VOC emissions were observed in the control plants (Fig. 1b,d,f). When plant physiological responses were monitored, larval feeding on mountain birch leaves did not affect plant photosynthesis (see Supplement Fig S1).

Over the longer term (3 days), the larval density was positively correlated with the emission of LOX products, monoterpenes and (*E*)-DMNT (Fig. 2). Among the individual LOX products, emission rates of (*Z*)-3-hexenol, (*E*)-2-hexenal and (*Z*)-3-hexenyl acetate were all dependent on larval density (Fig. 2a,b,c). LOX products are excellent indicators of leaf mechanical damage, e.g., caused by chewing mouth parts of herbivores [56, 57]. These emissions are short-lived and emerge immediately when the feeding starts and disappear quickly when the feeding activity ends [57].

However, emission of monoterpenes and the homoterpene (*E*)-DMNT were more variable and were not quite as obviously dependent on larval density as the LOX products (Fig. 2d,e). For these compounds, eight larvae induced emissions nearly equal to the emission rates induced by sixteen larvae. However, all tested larval densities differed statistically significantly from the control (Mixed Model ANOVA analysis pairwise comparisons are shown in Supplement Table S1). In general, the emission rates could be expressed as a nonlinear function of leaf area eaten (see Supplement Fig S2).

Our results confirmed the fact that herbivore-damaged plants with clearly diminished photosynthetic leaf area are still capable of emitting VOCs at a higher rate than healthy plants with intact foliage [19, 58]. Emission rates of the induced LOX compounds, monoterpene (*E*)- β -ocimene and sesquiterpene (*E,E*)- α -farnesene were also positively correlated with the number of larvae (0 to 8) feeding on the foliage of *Alnus glutinosa* seedlings [8, 59]. In our experiments *B. pubescens* ssp. *czerepanovii* seedlings gave very similar results, but increasing the larvae number from 8 to 16 per seedling did not considerably increase emissions rates of VOCs. This might be related to a very rapid decline in the whole plant leaf area in these two treatments with high insect density. The presence of a quantitative relationship between VOC emissions and the degree of herbivore feeding is not only conceptually important, but also provides a basis for quantitative assessment of induced emissions in large-scale biosphere-atmosphere models [60, 61].

Epirrita autumnata feeding-induced VOC emissions in relation to SOA formation

Four chamber experiments were conducted to see SOA forming potential of mountain birch seedlings, two with control plants and two with herbivore-damaged plants (Table 1). Our

results show that the seedlings damaged by *E. autumnata* larvae had a significantly higher potential for SOA formation when compared with intact seedlings (Table 1, Fig. 3). The VOCs (monoterpenes, a homoterpene, sesquiterpenes, LOX products and methyl salicylate), acting as precursors to SOA formation, were emitted in higher rates in the herbivore-damaged mountain birch seedlings than in the control seedlings (Table 1). The same phenomenon was observed for a boreal forest species, silver birch (*Betula pendula*) seedlings in the same experimental settings (see Supporting Information Tables S5 and Figure S3).

In addition to the greater emission strength, the emission profile was more varied in the damaged seedlings vs. the controls, i.e., there were more individual terpenoid compounds identified in the emissions from damaged seedlings than in that of the intact seedlings (see Supplement Table S2). The greatest differences in the levels of VOCs emitted were seen in the LOX compounds: in the SOA formation experiments, LOXs were present only in the damaged samples. The main LOX compounds measured were the typical C6 aldehydes and alcohols, e.g. (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol. In our control plants, VOC emissions were similar to those earlier reported from mountain birch; e.g. limonene, sabinene and linalool of the MTs and β -caryophyllene and α -farnesene of the SQTs [32, 33], while herbivore damage resulted in enhanced emissions of these ubiquitous terpenoids and additional emissions of (*E*)- β -ocimene, (*E*)-DMNT and LOX compounds as also reported earlier by Mäntylä et al. [33]. In our small-sized herbivore-damaged seedlings, higher MT emissions than SQT emissions were detected, which is different from the observation in older mountain birch ramets in a forest site [33].

While the overall VOC emissions were greater in the damaged seedlings than in the control seedlings, there was a difference between the two controls. Especially the SQT and MeSa emissions were higher in Control 2 than in either of the insect-damaged samples (see Table 1 and Supplement Table S2). Higher SQT and MeSa emissions observed in Control 2 may indicate unnoticed fungal pathogen attack or damage by sucking herbivores such as aphids [62] in the seedlings or in their root system. Our supplemental material of *B. pendula* seedlings in similar conditions (Supplement Table S5-S7) suggests that intact *Betula* sp. seedlings do not emit MeSa. However, the differences in SQT emissions could also have been due to natural variability as significant fluctuation in VOC emissions from visibly intact mountain birch in a natural growing site has been reported by Haapanala et al. [32]. The emissions of SQT and MeSa in Control 2 experiments may have caused the higher SOA formation since these compounds have high SOA formation potential [42, 63], as observed in the SOA formation experiment (discussed later).

The clear differences in SOA production observed between the controls and herbivory experiments reflected the differences in the VOC emissions of the samples. While MT and SQT emissions in the herbivore-damaged seedlings were approximately twice those from intact seedlings, greater differences were seen in SOA formation (Table 1, Fig. 3). The SOA mass loading was as much as two orders of magnitude higher in herbivore-damaged experiments compared to Control 1 where the SOA formation was very low ($0.01 \mu\text{g m}^{-3}$). While some SOA production ($0.17 \mu\text{g m}^{-3}$) was also observed in the Control 2, the SOA mass loadings in the herbivory samples were 3 to 7-fold higher ($0.43 \mu\text{g m}^{-3}$ - $1.25 \mu\text{g m}^{-3}$).

The mass of produced SOA was moderate, less than $1.3 \mu\text{g m}^{-3}$ for both control and herbivory samples. Main reasons for these low SOA mass loadings are low initial VOC concentrations and low consumption of precursor VOCs. However, the VOC and organic particle mass concentrations used and observed in our study, as well as particle formation and growth rates (see Supplement Table S3), are coincident with atmospheric concentrations in forested remote areas (e.g. in Hyytiälä, Finland) [64–67]. We suggest that our results better represent natural conditions than the results from many other chamber studies, which have been conducted much higher VOC and organic particulate mass levels. Furthermore, our SOA yields are in line with reported α -pinene yields by Shilling et al. [54] at low mass loadings (below $2 \mu\text{g m}^{-3}$). In fact, there is a need to collect SOA formation data in atmospherically relevant organic aerosol mass loadings ([68] and references therein).

SOA formation petered out when the lights were off in the plant chamber (00:00 - 03:00 am), and commenced again when the lights were turned on (Fig. 3). Our diurnal experiment thus demonstrates that the formation of SOA from mountain birch VOC emissions is not only dependent on the severity of herbivore damage but it is also strongly light-dependent. This is perhaps not surprising as monoterpene emissions from broad-leaved species lacking storage structures are in general typically photosynthesis- and light-dependent (e.g. [1, 60]). Even in species having constitutive emissions from storage structures, the induced terpenoid emissions are characteristically dependent on light [6, 69–71]. On the other hand, in the case of compounds immediately released after herbivory such as LOX compounds, the emissions can be importantly driven by insect feeding habits. Thus, predicting SOA formation due to herbivory-driven VOC emissions requires consideration of both plant physiological responses to light and insect-specific feeding patterns.

Our chamber experiments showed that herbivore-damage induced by six *E. autumnata* larvae per seedling can increase the SOA formation potential of mountain birch due to increases in VOC emissions. Thus our study expands the earlier findings which show that stress, both biotic and abiotic, can increase the VOC emissions and concurrent SOA formation in plants [41, 42, 44, 52]. However, in the view of several previous studies, the effect of the VOC composition on SOA production is less clear [42, 45, 46].

In an earlier study by Mentel et al. [72], SOA particle formation rate and particle growth rate were found to be more efficient when the VOC emissions came from silver birch (*B. pendula*) than when the VOC emissions originated from conifer trees. Thus, they suggested that the oxidation of birch emitted SOA precursors can favor new particle formation. In their experiments, an incremental mass yield of $11 \pm 1 \%$ considering only the MT+SQT emissions from birch was observed. Our results presented in this study show yields of approximately the same level in the herbivore-damaged seedling experiments, whereas the levels in our controls (especially Control 1) were somewhat lower.

It has also been speculated that the mass yield can be significantly influenced by fractions of oxygenated VOCs (LOX compounds and MeSa) and SQT in the emissions. In the later study, Mentel et al. [42] studied the effect of VOC profiles on SOA formation further and they came to somewhat differing conclusion on the effect of LOX on SOA: LOX compounds inhibit SOA formation. They suspected that the suppressive effect of LOX compounds on

SOA formation is related to LOX capacity to scavenge OH radicals. When comparing our herbivore-damaged replicates 1 and 2, we found that replicate 1 had higher LOX emission rate but lower SOA mass loading. This finding could support the later hypothesis by Mentel et al.[42]. However, some other studies have found that LOX compounds reacted with ozone can be a substantial source of SOA globally [73–75]. They have speculated that ozonolysis of (*E*)-3-hexenol leads to significant formation of oligomers [73, 75] and this oligomerization mechanism has been shown to contribute significantly to SOA formation and it could also have an effect on the chemical and physical properties of SOA particles ([68] and references therein). In our experiments, LOX emissions dominated the VOC composition in the herbivore-damaged samples whereas they were almost absent in the control experiments. If LOX compounds completely inhibited the SOA formation as Mentel et al.[42] suggested, we probably would not have been able to observe such a clear SOA formation in these samples. However, LOX compounds could have some suppressive effect on SOA formation as the differences between our herbivory experiments 1 and 2 could be explained by the higher amount of produced and reacted LOX compounds. In conclusion, SOA formation yields from ozonolysis of LOX compounds, e.g. (*E*)-3-hexenol and (*E*)-3-hexenyl acetate, are lower than yields reported for MT or SQT only [73, 74, 76, 77], which indicates that LOX compounds can partly inhibit SOA formation and growth in mixtures of different VOCs.

Note that there were a variety of both MT and LOX compounds present in our experiment whereas Mentel et al.[42] performed their experiments only with single compounds (α -pinene as a monoterpene and (*Z*)-3-hexenol as a LOX compound). It is thus likely that the reactant composition has an effect on the inhibitory potential and therefore the phenomenon is not quite as straight-forward as suggested. On average, 80 % of the MT and 90-100 % of the SQT were consumed in the reaction chamber at the steady state conditions in our experiments. In contrast, there were substantial differences in the consumption of the individual LOX compounds; the consumption ranging from only 10 % up to 90 % in the chamber (see Supplement Table S4). In average, the consumption of the LOX products was lower than the consumption of other VOC groups measured (i.e. MT, SQT, HT, MeSa). One reason for this could be the preferred oxidant of the LOX compounds; LOX undergo oxidation more readily with OH radical than with ozone. For example, (*E*)-2-hexenal reaction with ozone is negligible but it reacts quickly with OH-radicals ([78] and references therein). Ozone was used as a main oxidant in the current study, and it could be anticipated that not all LOX products would have been oxidized. However, OH radicals are formed in reactions between terpenes and ozone in yields between 0.3 to 0.9 in relation to the reacted alkene [79]. Since we did not use any OH scavengers in our experiments, OH radicals were therefore also available for oxidation reactions in our experimental setting. Oxidation of LOX products could also have a role in the SOA formation, as both (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate have been shown to be consumed by reactions either with ozone or with OH-radicals formed in ozonolysis experiments. These LOX products are capable of forming oxidized compounds with vapor pressure low enough to take part of SOA formation and growth, although the yields are rather low when compared to MT or SQT SOA forming potential [73, 75].

To summarize, herbivore damage and the severity of the damage intensity has strong influence on the quantity and composition of mountain birch VOC emissions and consequent SOA formation. Hence, in extensive *E. autumnata* or other birch-feeding geometrid outbreak areas in the subarctic, we may expect to find increased SOA formation rates in the atmosphere. Although there is evidence that LOX compounds emitted after herbivore attack on plant foliage may induce SOA formation [73–75], our results also gave support to the results of Mentel et al. [42] that LOX emissions may suppress SOA formation in mixtures with terpenes. Future studies are needed to elucidate the role of LOX compounds in SOA formation in field conditions. However, chewing herbivores have such a strong impact on mono- sesqui- and homoterpene emissions and methyl salicylate emissions of mountain birch that higher a SOA formation rate is expected with outbreaks of defoliating moths. We argue that additional SOA formation due to insect-induced VOC emissions can affect the important sun-screening biosphere-atmosphere feedback system of aerosols under subarctic 24-hour sunshine periods.

Supporting Information

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Emission rate time series, 0-24 h, for different VOCs from herbivore-attacked and control plants. Figures represent LOX products from herbivore-attacked (a) and control (b) *B. pubescens* ssp. *czerepanovii* plants, different monoterpenoids and homoterpene (*E*)-DMNT from herbivore-attacked (c) and control (d) plants and different sesquiterpenes from herbivore-attacked (e) and control (f) plants. Each data point is the mean ($\pm$ SE) of 4 independent replicate experiments. At hour 0, the plants without the insects were measured. The insects were added at time $t = 0.5$ h and allowed to feed from the leaves for 7 hours. After that larvae were removed and plants were measured without them.

Figure 2.

Long-term, days 0-3, variation in emission rates of VOCs at varying herbivore damage severity. Figures represent LOX compounds (*E*)-2-hexenal (a), (*Z*)-3-hexenol (b), (*Z*)-3-hexenyl acetate (c), homoterpene (*E*)-DMNT (d) and monoterpenes (e) from the foliage of *B. pubescens* ssp. *czerepanovii* at different feeding densities of *E. autumnata* larvae. Sample size and measurement conditions as in Fig. 1. Larvae were removed after 2.2 days. The light cycle was 14 h light followed by 8 h darkness.

Figure 3.

Formation of secondary organic aerosols (SOA) from the ozonolysis of VOCs emitted from mountain birch control (a-d) and herbivore-attacked (e-h) seedlings. SOA formation is shown as particle size distribution (a-b, e-f); total particle number (c, g) and total particle mass concentration (d, h) as a function of time. Total particle mass was calculated from the measured size distributions using particle density of 1.3 g cm^{-3} . VOCs were fed into the reaction chamber for 1.5 h before the onset of the experiment (approx. 15:00). Herbivore-attacked experiment 2 was started 1 h earlier than others (approx. 14:00). The plant chamber illumination was off from 00:00 to 03:00 (indicated by red vertical lines).

Table 1

Concentrations of volatiles in the reaction chamber inlet emitted by *B. pubescens* ssp. *czerepanovii* plants and summary of the resulting secondary organic aerosol (SOA) concentrations and mass yields.

Exp.	MT ^a ($\mu\text{g m}^{-3}$)	SQT ^a ($\mu\text{g m}^{-3}$)	LOX ^a ($\mu\text{g m}^{-3}$)	MeSa ^a ($\mu\text{g m}^{-3}$)	(MT+SQT) ^a ($\mu\text{g m}^{-3}$)	LOX ^a ($\mu\text{g m}^{-3}$)	MeSa ^a ($\mu\text{g m}^{-3}$)	SOA ^a ($\mu\text{g m}^{-3}$)	SOA yield ^a (%)
Control 1	1.1±0.3	0.3±0.02	0	0	1.2±0.2	0	0	0.008±0.004	0.6±0.4
Control 2	1.6±0.3	2.2±0.4	0	0.7±0.3	3.5±0.8	0	0.3±0.1	0.168±0.025	4.4±1.2
Herbivore 1	2.7±0.4	0.5±0.1	27±17	0.1±0.1	2.7±0.4	24±16	0.1±0.1	0.431±0.030	1.6±1.0
Herbivore 2	2.9±0.2	1.1±0.2	6.4±2.4	0.3±0.1	3.2±0.5	5.9±2.5	0.3±0.1	1.248±0.060	13.3±4.4

^aThe data for monoterpenes (MT), sesquiterpenes (SQT), lipoxygenase pathway products (LOX) and methyl salicylate (MeSa) are averages ($\pm$ SE) for the duration of the experiment (3–5 samples in ca. 21 h). Homoterpene, (*E*)-4,8-dimethyl-1,3,7-nonatriene, was added to MTs for simplicity. The (MT+SQT), LOX and MeSa shows the concentration of the reacted MT, SQT, LOX and MeSa compounds. The produced SOA was corrected with particle wall losses (see Supplement) and calculated using the maximum total particle volume and particle density of 1.3 g cm^{-3} . The SOA yields are expressed as SOA divided by the sum of the (MT+SQT), LOX and MeSa.