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Volatile organic compound emission from holm oak infested by gypsy moth larvae: evidence for distinct responses in damaged and undamaged leaves

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Summary Foliage of *Quercus ilex* L. (holm oak), a widespread Mediterranean species, constitutively emits large quantities of a complex genotype-dependent mixture of volatile organic compounds (VOCs). During a mass outbreak of gypsy moth (*Lymantria dispar* L.) in southern France, we examined the effects of gypsy moth feeding on VOC production from whole apices and single leaves of *Q. ilex*. Feeding induced the emission of new VOCs at rates up to 240 ng m^{-2} s^{-1} (16% of the total VOC release), which mainly consisted of sesquiterpenes, a homoterpene and a monoterpene alcohol. The new compounds were emitted after a delay of several hours following infestation and their production declined rapidly when caterpillars were removed. Undamaged leaves of infested trees emitted new VOCs, but with a different composition to those of damaged leaves and at lower rates. Neither caterpillars nor caterpillar excrement released VOCs. Emission of constitutive VOCs by undamaged leaves of infested trees temporary increased by up to 30%, whereas, in damaged leaves, they remained stable and decreased after some days when necrotic spots occurred around the feeding sites. In continuous light and at constant temperature, emissions of new VOCs showed a marked diurnal cycle, whereas those of constitutive VOCs did not. The results suggest that induced VOCs make a significant contribution to the atmospheric VOC load and may mediate trophic interactions. The observed differential local and systemic responses in composition, quantity and time courses of emissions mirror the existence of several regulation processes triggered by different signaling compounds and elicitors.

Keywords: biogenic volatile organic compounds, biosphere atmosphere interactions, herbivores, homoterpene, Lymantria dispar, monoterpene, plant defense, Quercus ilex, sesquiterpene, wounding.

Introduction

Gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), is one of the most economically important pests in the temperate forests of the Northern Hemisphere (Liebhold et al. 1992). The larvae hatch from eggs during bud break in spring and typically pass through five or six instars before entering the pupal stage in summer. The caterpillars secrete a fine silk thread on which they are suspended and are rapidly spread by the wind within the canopy. Gypsy moth is native to Europe and Asia where episodic mass outbreaks lead to the defoliation of large forested areas. Introduced to North America in the Nineteenth century, the gypsy moth annually defoliates over 4000 km^2 of hardwood forests in the United States (McManus et al. 1989). Oaks are the gypsy moth's preferred host, although they can feed on many other broad-leaved tree and shrub species. In 2004 and 2005, mass attacks of gypsy moth caterpillars in southern France caused the partial defoliation of thousands of hectares of holm oak (*Quercus ilex* L.) forests. For instance, in the department Hérault, 385 km^2 of the 830 km^2 of holm oak forests were defoliated during the 2004 outbreak, of which 317 km^2 was defoliated by more than 50% (Joffre et al. 2005).

Holm oak is one of the most widespread tree species of the Mediterranean basin, and is a major source of volatile organic compounds (VOCs) in that region (Staudt et al. 2001). Although leaves of holm oak lack specific VOC producing organs, they emit huge amounts of monoterpenes (Staudt and Seufert 1995, Pasqua et al. 2001). The synthesis and emission of these unsaturated compounds are continuously modulated by environmental factors, whereas the compositional fingerprint of the emissions is mainly genetically controlled (Staudt et al. 2003, 2004). The monoterpenes released by holm oak forests are of interest to environmental and atmospheric scientists because their chemical breakdown in the troposphere has a role in the formation of oxidants and secondary aerosols (Kesselmeier and Staudt 1999), with consequences for air quality and visibility, cloud properties, radiative forcing, and nitrogen transport and deposition on a regional scale, which in turn can affect the primary and secondary metabolism of the emitting vegetation (Fuentes et al. 2001, Lerdau and Slobodkin 2002, Heiden et al. 2003, Niyogi et al. 2004).

These VOC driven atmosphere–biosphere interactions may change substantially during mass outbreaks of gypsy moth in

southern France. Given that the feeding activity of the caterpillars cause great loss of foliar biomass, VOC fluxes from holm oak forests are expected to decrease in infested regions unless caterpillar feeding triggers some compensatory increase in VOC production by retained foliage or additional unknown VOC sources emerge during periods of infestation. There is increasing evidence that plants release an array of new VOCs in response to biotic as well as abiotic stresses (Holopainen 2004). These stress-induced emissions may represent an important contribution to the atmospheric VOC load (Loreto et al. 2006), but may also be involved in plant defensive reactions to stress. Several case studies have demonstrated that herbivore-induced de-novo synthesized VOCs can act as signaling compounds for predators or parasitoids of the herbivore insects to track their prey, or to induce defence reactions in neighboring non-attacked con-specific individuals (Kessler and Baldwin 2001, Thaler et al. 2001).

To date, the effects of gypsy moth attack or other insect pests on VOC release from oaks that constitutively produce monoterpenes, such as *Q. ilex*, are unknown. In the present study, we examined whether gypsy moth feeding induces quantitative and qualitative changes in VOC emissions from holm oak leaves. We followed the emissions from damaged and undamaged leaves of infested plants and those of control plants under physiologically realistic conditions. We also monitored VOC emissions from both caterpillars and caterpillar excrement, which covers the forest floor in considerable quantities during mass attack.

Material and methods

Plant material and experimental protocol

Six-year-old potted holm oak saplings of a single origin were used in two experiments. For each experiment, test trees were randomly chosen and subdivided into two groups, one to be infested with gypsy moth caterpillars (treatment) and one to serve as the control.

In Experiment 1, the upper parts of two saplings were permanently enclosed in two cylindrical, 1.5-l volume Teflon chambers (see Staudt et al. (2000) for a detailed description of the enclosure system) and flushed with charcoal filtered ambient air (relative humidity of about 30%) at a constant rate of 500 ml min^{-1}. Air flow was regulated with a mass flow controller (MKS, Alborn). The system was installed in a small air-conditioned laboratory close to a greenhouse where the plants were grown before the experiment. After acclimation for 24 h, one of the chambers was infested with five to six caterpillars for three days. The other chamber, without infestation, served as the control. During the experiment, the plants were kept in a 14-h photoperiod and illuminated at a photosynthetic photon flux (PPF) of about 1000 μmol m^{-2} s^{-1} at the upper plant level with a white light source (OSRAM 1000 W, filtered by a 5-cm water bath). On the last day, caterpillars and caterpillar excrement (plus particles of leaf frass) were removed from the infested chamber and transferred to glass U-tubes for separate emission measurements. The tube ends were each secured with a small plug of deactivated glass wool (Varian, Palo Alto, CA) and flushed with air at 100 ml min^{-1} using the same air source as for the chambers. The VOCs were collected from the outlet air stream as described below.

Experiment 1 was repeated four times, each time with a different plant and with different caterpillars. During each experiment, foliar VOC emissions and photosynthetic rates were measured in both chambers twice on the first day (once before infestation and once after infestation), three to four times on the second and third days of infestation, and once on the last day after removal of the caterpillars, caterpillar excrement and leaf frass from the infested chamber. Measurements were made between 1000 and 1700 h at a temperature of 30 ± 1 °C. During VOC emission measurements, the photosynthetic rate of the shoot apex was recorded three times with a gas-exchange system (CI-301 CO2 Gas Analyzer, CID, Vancouver, WA). Before each emission measurement in the infested chamber, leaf damage caused by caterpillar feeding was estimated as the percent loss of lamina area of each enclosed leaf. Feeding activity of the caterpillars was highly variable, occurring mainly at night and exclusively on current-year leaves. Losses of total enclosed leaf area ranged between 6 and 24%. Enclosed foliage was hence composed of damaged and intact current-year leaves and intact 1-year-old leaves.

In Experiment 2, VOC emissions of intact and damaged leaves of infested trees were studied independently in order to differentiate between local and systemic responses to caterpillar feeding. Three trees were chosen for caterpillar infestation and four terminal test shoots with two to four mature current-year leaves were selected on each tree. In parallel, one terminal shoot of each of three control trees was also selected. We determined rates of VOC emission and gas exchange of these 15 test shoots once during the 48 h preceding caterpillar infestation. In the evening before infestation, all test shoots were enveloped by a fine-mesh net bag taking care that leaves were untouched by the net. Three to four caterpillars were introduced in three of the four shoot bags of the three infested trees and 8–10 caterpillars were randomly distributed in their canopy to increase the feeding impact at the whole-canopy level to mimic a mass outbreak. The fourth test shoot of each of the infested trees as well as those of the control trees were kept un-infested. Gypsy moth caterpillars were allowed to feed on the trees overnight. The following morning the net bags and caterpillars were removed from the infested trees and the shoots that had been enclosed with caterpillars were examined for leaf tissue loss. One test shoot showing 25 to 50% loss of leaf tissue was selected on each infested tree for further measurements. Rates of VOC emissions and photosynthesis were measured on 6 days (nine measurements per day) over a period of 10 days on the attacked and non-attacked test shoots of infested trees and on the test shoots of control trees. Throughout the experiment, infested and control plants were kept separately in adjacent greenhouse compartments to prevent possible tree-to-tree transmission of airborne signals. We determined rates of VOC emissions and photosynthesis at 30 ± 0.1 °C leaf temperatures and 1000 μmol m^{-2} s^{-1} PPF by means of a custom-made, temperature-controlled gas exchange

chamber (85-ml volume). The chamber was flushed with humidified air (about 40% relative humidity) at a constant rate of 350 ml min^{-1}. The air and the light source were the same as in Experiment 1. Before each emission measurement, a shoot was carefully clamped in the chamber and acclimated to measurement conditions for 30 to 45 minutes.

After the experiments, the leaves of all test shoots and apices were harvested to determine their projected area (Delta-T area meter MK2, Delta-T Devices, U.K.). Leaf areas of the infested shoots before the caterpillar attack were deduced from the length and width measured on each leaf by applying the equation: $(\chi lw)/4$, where l and w are maximal length and width of the leaf lamina, and χ is an empirical coefficient, which was determined by regression analysis from leaf samples of the same population covering a large range of leaf sizes ($n = 44$, $r^2 = 0.99$).

VOC sampling and analysis by capillary gas chromatography

The VOC emissions were determined by drawing 1–3 liters of chamber air at a constant flow rate of 100 ml min^{-1} through commercial VOC traps containing either 120 mg Tenax TA plus 80 mg Carbotrap B (VOC traps, Varian) or 290 mg Tenax TA (Perkin Elmer). The traps were analyzed immediately after sampling or the next day (storage at 4 °C) either by gas chromatography with a flame ionization detector (GC/FID) or by gas chromatography coupled with mass spectrometry (GC/MS). In both systems, VOC traps were thermically desorbed and injected into the GCs after pre-concentration on a cold trap (2-stage thermo-desorption).

Additional VOC sampling was undertaken for solvent extraction allowing repeated GC/MS analysis of the same sample. For this purpose, 30–60 l of air was sampled from traps filled with Porapaq Q (VTC, ARS, Florida). These traps were eluted with 150 µl of dichloromethane, spiked with an internal standard and stored at –20 °C until analyzed. For analysis, an aliquot of 1 µl was injected using a split ratio of 1:10.

The GC/FID system consisted of a Chrompack CP9003 gas chromatograph equipped with a Chrompack TCT 4002 thermo-desorber (Varian). The GC/MS analyses were performed with a Varian CP3800 gas chromatograph combined with a Varian Saturn 2000 ion trap mass spectrometer. This system was equipped with a Perkin Elmer Turbomatrix thermo-desorption unit and a CP-4810 autoinjector coupled to a temperature programmed split/splitless injector (Varian) for automatic liquid injections. Temperature programs of the 2-stage desorption were: GC-FID: desorption 10 min at 220 °C, pre-concentration at –120 °C on a piece of deactivated capillary column (0.53 mm ID), injection 1 min at 220 °C, transfer-line 220 °C; GC-MS: desorption 15 min at 230 °C, pre-concentration at –30 °C on a Tenax TA cold trap, injection 5 min at 230 °C, transfer-line 200 °C.

In both GCs, VOCs were separated on a Chrompack Sil 8 CB low bleed capillary column (30 m × 0.25 mm × 0.25 µm) using the following temperature program: 3 min at 40 °C, 3 °C min^{-1} to 100 °C, 2.7 °C min^{-1} to 140 °C, 2.4 °C min^{-1} to 180 °C, 6 °C min^{-1} to 250 °C. Carrier gas was helium (1.2 and 1.0 ml min^{-1}) in all cases.

Peaks were identified by comparing their mass spectra (fragmentation pattern) and retention times with those from authentic standards analyzed under the same conditions. In the absence of standards, we used mass spectra from MS libraries and Covats-indices for peak identification. All GC systems were calibrated with authentic standards (Fluka Chemie AG, Buchs, Switzerland; Roth, Karlsruhe, Germany) diluted in methanol or dichloromethane: 0.5 to 3 µl of standard solution was injected on the head of the VOC trap through a T-fitting equipped with a septum and purged with 300 ml of pure nitrogen at a flow rate of 50 ml min^{-1}. Precision as determined by repeated measurements of standards at realistic concentrations was within 2–5% for monoterpenes and most sesquiterpenes, and 5–10% for C6 aldehydes, alcohols and esters, and other oxygenated compounds such as methyl-jasmonate and methyl-salicylate. Oxygenated compounds had lower FID responses than non-oxygenated compounds. For unknown peaks and compounds for which authentic standards are commercially unavailable, we used the mean response factors of the same compound classes.

Data analysis

Emission rates were calculated as the difference between the air concentration in the chamber enclosing leaves and the concentration measured in the empty chamber multiplied by airflow and divided by the projected leaf area or by the leaf dry mass. Because of the high variability of VOC emission rates among individual trees, the effects of caterpillar feeding on rates of VOC emission and photosynthesis were evaluated in two ways. First, by comparing data of infested and control plants on a given day of measurement (*t*-test), and second, by comparing data measured at a given day after infestation with data measured before infestation (paired *t*-test).

Results

At standard light and temperature conditions, uninfested holm oak foliage emitted large quantities of monoterpenes. The main compounds of these constitutive emissions were α-pinene and β-pinene, sabinene, myrcene and limonene. Minor and trace compounds in the emissions were tricyclene, α-thujene, camphene, α-terpinene, *p*-cymene, β-phellandrene, eucalyptol (both closely eluting with limonene), (Z)- and (E)-β-ocimene, γ-terpinene, (Z)-linalool oxide, terpinolene, linalool, alloocimene, terpinen-4-ol and terpineol. The sesquiterpenes germacrene-D and β-caryophyllene were also regularly emitted in trace quantities. After infestation, the emissions of germacrene-D, β-caryophyllene, linalool and (Z)-linalool oxide strongly increased (Figure 1b) and an array of new compounds appeared among the emissions. These new caterpillar-induced VOCs were mainly the sesquiterpenes β-bourbolene, α-humulene, δ–cadinene and the homoterpene (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT, Figure 1b). α- and β-Cubebene, α-copaene, (E)-β-farnesene, bicyclogermacrene, γ-cadinene, (E)-nerolidol, methyl-jasmonate and two unidentified compounds eluting late on our column were

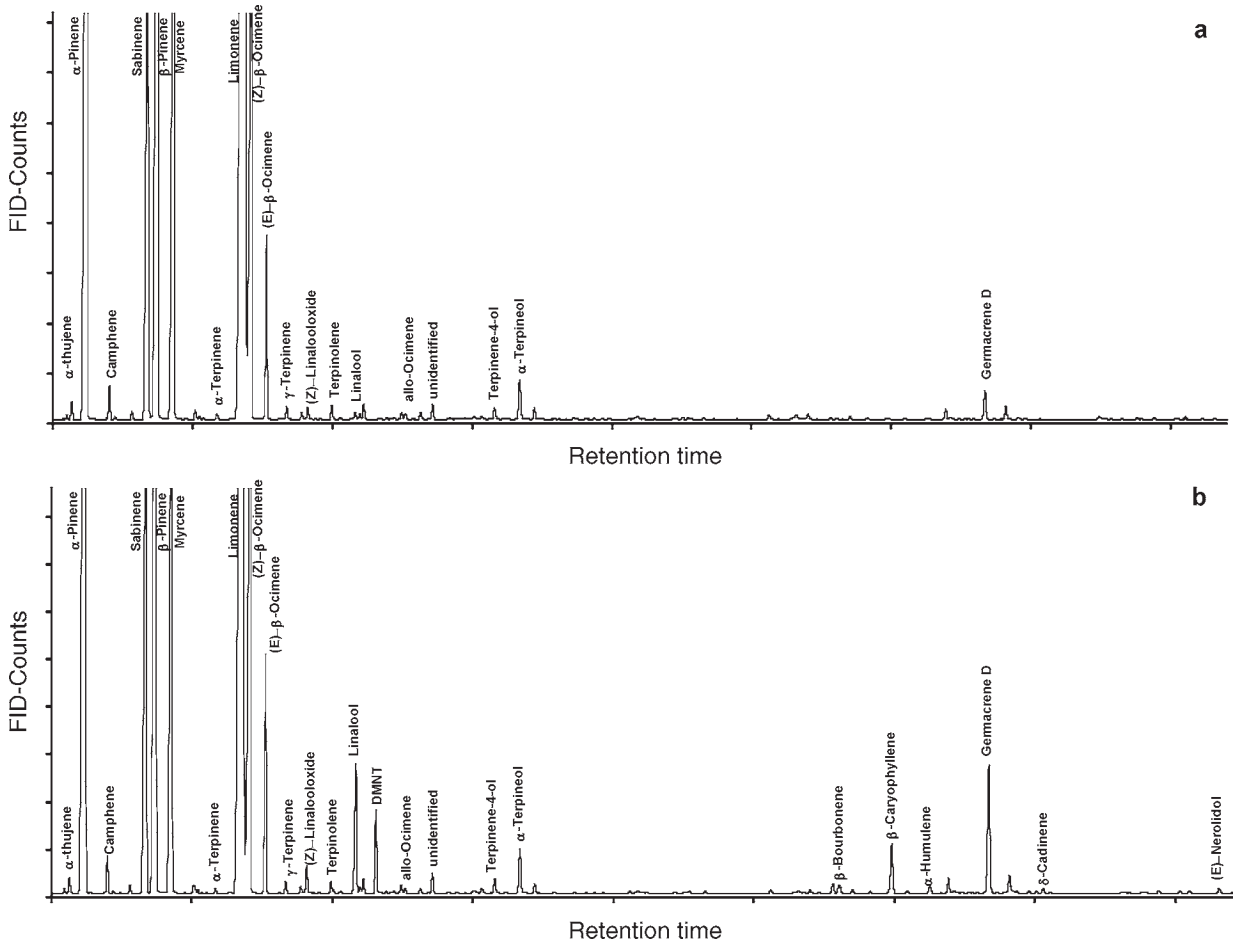

Figure 1. Induction of new VOC emissions in *Quercus ilex* foliage by gypsy moth caterpillars. The chromatograms display the VOC profiles of the same *Q. ilex* apex measured under the same climatic conditions before and 2 days after infestation with gypsy moth larvae. Before infestation (a), the leaves emitted almost exclusively monoterpenes, mainly α- and β-pinene, sabinene, myrcene, limonene and (Z)- and (E)-β-ocimene. After infestation (b), the emissions of germacrene-D, β-caryophyllene, linalool and (Z)-linalool oxide strongly increased and additional semi-volatiles such as DMNT emerged in the emission profile.

emitted from most but not all infested trees. We also observed occasional emissions of C6-volatiles, mainly (E)-2-hexenal, (Z)-3-hexenol, 3-hexanol and (Z)-3-hexenyl acetate. Methyl-salicylate was seen in only a few samples during the post-infestation period, when the emissions of other induced VOCs were declining.

In Experiment 1, in which whole tree shoot apices were permanently enclosed with caterpillars, induced VOCs were undetected in the first hours following infestation. Induced VOC usually emerged on the first day after caterpillar infestation and peaked on the second or third day. During the day, emission rates of induced VOC measured in continuous light and at constant temperature exhibited a marked diurnal profile, whereas emission rates of constitutive monoterpenes did not (Figure 2). When caterpillars were removed from the chamber, shoot apices continued to emit induced VOC, whereas no significant VOC emissions were detected from either caterpillars or caterpillar excrement (data not shown). Generally, the emission rates of induced VOCs per enclosed leaf area were low compared with constitutive monoterpene emission rates and highly variable among individual trees (Figure 3). Maximum emission rates of induced VOCs from shoot apices ranged between 20 and 100 ng m^{-2} s^{-1}, accounting for 5 to 10% of total VOCs. Constitutive monoterpene emission rates (Figure 3a) as well as photosynthetic rates (not shown) were not significantly affected by caterpillar feeding: in two experiments, monoterpene emission rates slightly decreased during caterpillar infestation, whereas in the other two they tended to increase.

In Experiment 2, in which damaged and undamaged leaves of attacked trees were monitored separately after 1 night of feeding, constitutive emission rates of monoterpenes from damaged leaves decreased slightly during the first 4 days and dropped significantly at the end of the experiment (Figure 4a). Constitutive monoterpene emission rates from undamaged leaves of infested plants increased over the first week and decreased to initial values at the end, whereas emission rates from control plants remained fairly constant over the measuring period. Mean emission rates from damaged leaves differed significantly from those from undamaged leaves of infested

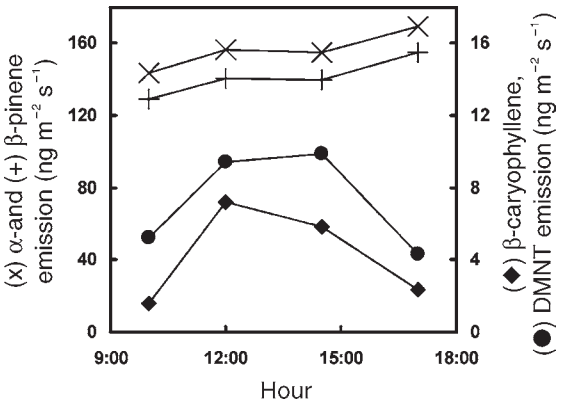

Figure 2. Examples of the diurnal variation of VOC emissions from a *Quercus ilex* apex infested with gypsy moth caterpillars for two days. At constant temperature and in continuous light, herbivore induced emissions of semi-volatiles (see DMNT, β-caryophyllene) displayed a marked diurnal cycle, whereas the constitutive emissions of monoterpenes (see α- and β-pinene) did not.

plants from the second day after infestation onward, but they were significantly different from the controls, which showed high variability, only on the last 2 days of measurements (*t*-tests, $P < 0.05$). However, compared with the pre-infestation emission rates, monoterpene emission rates of infested plants after caterpillar feeding were significantly higher in undamaged leaves on Days 2, 3 and 5, and significantly lower in undamaged leaves on the last 2 days of measurements (paired *t*-tests, $P < 0.05$).

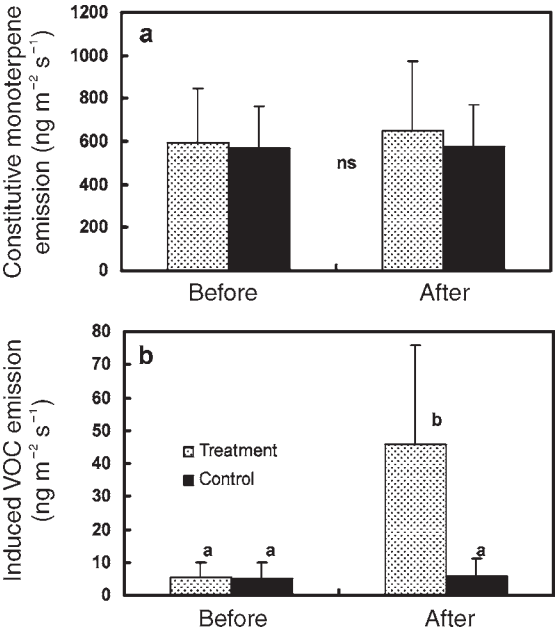

Figure 3. Effects of feeding gypsy moth larvae on VOC emissions from *Quercus ilex* apices: (a) sum of constitutively emitted monoterpenes and (b) sum of induced VOC emission immediately before infestation and on the second day after infestation. Small letters denote significant differences ($P < 0.05$; ns = not significant) between caterpillar infested (treatment) and control plants. Values are means of $n = 4 \pm$ SD from measurements made around midday under the same temperature and light conditions.

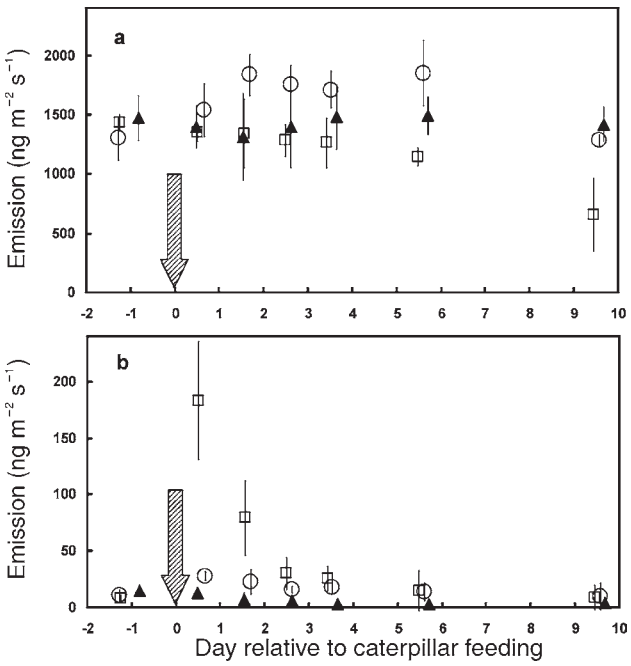

Figure 4. Local and systemic effects of gypsy moth caterpillar feeding on the constitutive emission of monoterpenes (a) and induced-emission of semi-volatiles (b) from *Quercus ilex* leaves. Caterpillars fed on trees over one night (Day 0, arrows) and were then removed. Emissions of VOCs were measured at standard light and temperature conditions on attacked leaves (□) and intact leaves (○) of infested plants and on intact leaves of non-infested control plants (▲). Values are means of $n = 3$ replicates ± SD.

Rates of emission of induced VOCs from damaged leaves peaked on the first day after feeding and subsequently decreased exponentially (Figure 4b), and were significantly different from controls on Days 1–4 ($P < 0.05$). Maximum emission rates ranged between 140 and 240 ng m^{-2} s^{-1}, accounting for 9 to 16% of the total VOC released from damaged leaves. Several of these induced VOCs were also observed in the emissions of undamaged leaves of the same plants but in much lower quantities (significantly different from controls on Days 1, 3 and 4): their peak emission rates amounted to only 22–33 ng m^{-2} s^{-1} or 1–2% of the total VOC emitted from these leaves. Photosynthetic rates of undamaged leaves of infested plants as well as of control plants remained almost constant over the measuring period, whereas in damaged leaves, photosynthetic rates decreased by about 30% by the end of the experiment (data not shown).

Discussion

The major finding of our study is that caterpillar feeding on *Quercus ilex* foliage induces the temporary emission of new VOCs. After a delay of several hours following the introduction of caterpillars, *Q. ilex* leaves started to emit linalool,

DMNT, germacrene D, β-caryophyllene and several other sesquiterpenes that were not emitted or emitted only in trace amounts from non-infested leaves. Conversely, caterpillars and caterpillar excrement, which during mass attacks cover the forest floor in large quantities, were not a significant source of VOCs. Apparently, gypsy moth larvae are odorless, as has been reported for other caterpillar species (Turlings et al. 1990, Maes and Debergh 2003, Arimura et al. 2004).

The induced compounds we found in the emissions from *Q. ilex* are among the most commonly observed plant volatiles produced in response to herbivore attack, and the delay in the induction of these VOCs is consistent with previous studies showing that production of induced VOCs depends on de novo protein synthesis requiring gene activation (Kessler and Baldwin 2001, Holopainen 2004, Arimura et al. 2004, Keeling and Bohlmann 2006, Mumm and Hilker 2006).

Herbivore-induced emissions have been well investigated for several herbaceous species and some conifers, but they have rarely been described for oaks or other tree species, which produce large amounts of isoprenoids without storing them (Arimura et al. 2004) and are considered the major source of biogenic VOCs on a global scale (Guenther et al. 1995). Because induced VOCs are generally emitted in low quantities, in most past studies of herbivore-induced emissions, sampling and enclosure techniques were applied allowing maximum VOC enrichment, which makes reliable quantification of emission rates and their extrapolation to natural conditions difficult (cf. Tholl et al. 2006). Our study suggests that the emission rates of induced VOCs reach 100–200 ng m^{-2} leaf area s^{-1} or 2–4 μg g^{-1} leaf dry mass h^{-1} under physiologically realistic conditions. These emission rates are low compared with the constitutive emission rates of *Q. ilex*, which is an exceptionally strong monoterpene emitter, but they are in the same range as emission rates reported for other constitutive monoterpene emitters such as pines (Staudt et al. 2000, Nunez et al. 2002, Hakola et al. 2006), beech (Kahl et al. 1999), birch (Vuorinen et al. 2005), citrus (Ciccioli et al. 1999), cabbage (Vuorinen et al. 2004) and aromatic shrubs (Owen et al. 2001). Because oaks, like other forest trees, are permanently confronted by a multitude of phytophage insects and other biotic plagues, stress-induced VOC emissions are likely to be omnipresent and hence should be taken into account when estimating the atmospheric load of biogenic VOCs. Stress-induced VOC emissions may have particular relevance to the chemistry of the lower troposphere, because herbivore-induced VOCs consist mainly of sesquiterpenes and homoterpenes, which are large and highly reactive molecules (Ciccioli et al. 1999, Helmig et al. 2003). The degradation products of these semi-volatiles enhance the formation and growth of secondary aerosols (Bonn and Moortgart 2003, Joutsensaari et al. 2005) that affect the radiative properties of the atmosphere. Given that aerosols have a strong potential cooling effect on the atmosphere (Mitchell et al. 2001, Anderson et al. 2003), biogenic high-molecular-mass VOCs, including herbivore-induced volatiles, constitute a possible negative feedback loop on global warming because climate change and elevated CO_2 concentrations can have profound consequences for population dynamics and geographical ranges of herbivorous insect and host trees (Williams and Liebhold 1995) and therefore may dramatically change emissions of herbivore-induced VOCs on regional scales. For instance, Hättenschwiler and Schaffelner (2004) observed that an increase in atmospheric CO_2 concentrations affects host tree preference, feeding performance and growth of gypsy moth larvae in European temperate forests.

Besides their possible role in air chemistry and associated climate-driving processes, induced VOCs may play an important role in plant defence against environmental stresses (Holopainen 2004). In recent years, an increasing number of studies have shown that herbivore-induced VOCs can exert a protective role either directly by deterring herbivores or indirectly by attracting herbivore parasites or predators (e.g., Kessler and Baldwin 2001, Thaler et al. 2001). To our knowledge, such trophic interactions mediated by VOCs have never been reported for *Q. ilex* infested by gypsy moth or other phytophage insects. Past studies investigating the effects of abiotic stresses on *Q. ilex* emissions such as drought (Bertin and Staudt 1996), oxidants (Llusia et al. 2002, Loreto et al. 2004), heat (Staudt and Bertin 1998, Loreto et al. 1998) and wounding (Staudt et al. 2003) did not report the occurrence of new semi volatiles as we observed. Thus, it seems that the temporary synthesis of new VOCs in response to caterpillar feeding is not part of a general syndrome in response to stresses, but is a specific response to herbivory, which is consistent with its possible defensive role in trophic interactions.

Among the induced VOCs, the homoterpene DMNT was never found in emissions from intact leaves of caterpillar-infested *Q. ilex* plants, although it was one of the predominant compounds in the emissions from damaged leaves. This suggests that DMNT is formed as a local response, probably induced by caterpillar–specific elicitors such as acyl-glutamines found in the oral secretions of lepidopteran larvae (e.g., Halitschke et al. 2001). The emissions of other new VOCs, mainly sesquiterpenes, were also induced in intact leaves of infested plants but in much lower concentrations. Moreover, in these same intact leaves of infested plants, the bulk of the constitutively emitted monoterpenes increased by as much as 25% compared with control values but with completely different kinetics. These increases are a manifestation of a systemic response to caterpillar feeding, presumably activated by plant internal signals. Jasmonic acid and salicylic acid are key hormones involved in plant signaling cascades in response to biotic stresses, and may interact antagonistically (Rojo et al. 2003, Arimura et al. 2005, Leitner et al. 2005). Salicylic acid is more involved in local defence reactions against pathogens, such as the oxidative burst and subsequent activation of cell death during the so-called hypersensitivity response (Overmyer et al. 2003), whereas jasmonic acid seems to be particularly implicated in the mediation of local and systemic responses to herbivore insects (Li et al. 2002). In a recent study, Filella et al. (2006) sprayed *Q. ilex* leaves with jasmonic acid and observed a subsequent 30% increase in monoterpene emissions similar to what we observed in the systemic response to gypsy moth feeding.

However, in two earlier studies on velvet bean and poplar, systemic and local reductions in the constitutive emission of isoprene were found when leaves were mechanically damaged or wounded (Loreto and Sharkey 1993, Funk et al. 1999). In our study, constitutive monoterpene emissions from caterpillar-damaged leaves did not temporarily increase following feeding as in non-damaged leaves, but slightly decreased during the first days when induced emission rates were high. Subsequently, constitutive and induced emission rates significantly declined in parallel with photosynthesis, and necrotic spots emerged on the lamina close to the feeding sites. At the same time, significant amounts of methyl-salicylate were seen in the emissions of some samples. Hence, the final drop in constitutive emission rates from damaged leaves could be mainly attributed to the local decline in the quantity of leaf tissue and is probably associated with the accumulation of salicylic acid and reactive oxygen species (Leitner et al. 2005). During the initial phase, however, differential regulation processes should have occurred in the local response leading, on the one hand, to a strongly enhanced formation of sesquiterpenes and its derivative DMNT, and on the other hand, to unchanged or lowered formation of monoterpenes, two classes of isoprenoids that are synthesized by different pathways located in different cellular compartments. Differential up- and down-regulation of genes encoding enzymes in these pathways in response to caterpillar feeding have recently been found in alfalfa (Bede et al. 2006). In that study, down-regulation was likely linked to the presence of an enzyme in the caterpillar's saliva generating hydrogen peroxide, a reactive oxygen species involved in the cellular transmission of stress signals and often combined with the accumulation of salicylic acid and the occurrence of oxidative bursts and local cell death. Whether similar processes take place in *Q. ilex* leaves attacked by gypsy moth remains to be established. However, the differences we observed between the local and systemic responses in composition, quantity and time courses of VOC emissions clearly infer the existence of several regulatory processes mediated by different signaling compounds and elicitors.

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