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Impact of heat stress on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species

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Abstract

Changes in the biogenic volatile organic compound (BVOC) emissions from European beech, Palestine oak, Scots pine, and Norway spruce exposed to heat stress were measured in a laboratory setup. In general, heat stress decreased the de novo emissions of monoterpenes, sesquiterpenes and phenolic BVOC. Decreasing emission strength with heat stress was independent of the tree species and whether the de novo emissions being constitutive or induced by biotic stress.

In contrast, heat stress induced emissions of green leaf volatiles. It also amplified the release of monoterpenes stored in resin ducts of conifers probably due to heat-induced damage of these resin ducts. The increased release of monoterpenes could be strong and long lasting. But, despite of such strong monoterpene emission pulses, the net effect of heat stress on BVOC emissions from conifers can be an overall decrease. In particular during insect attack on conifers the plants showed de novo emissions of sesquiterpenes and phenolic BVOC which exceeded constitutive monoterpene emissions from pools. The heat stress induced decrease of these de novo emissions was larger than the increased release caused by damage of resin ducts.

We project that global change induced heat waves may cause increased BVOC emissions only in cases where the respective areas are predominantly covered with conifers that do not emit high amounts of sesquiterpenes and phenolic BVOC. Otherwise the overall effect of heat stress will be a decrease in BVOC emissions.

1 Introduction

Terrestrial vegetation is a key player in the biogeochemical cycles of carbon and water and thus a key player for Earth's climate (e.g. Carslaw et al., 2010). Therefore, possible changes in the photosynthetic capacity or transpiration by plants caused by future climatic conditions are of high importance for predicting future climate. Vegetation emits reactive biogenic volatile organic compounds (BVOC). The source strength of these

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BVOC exceeds that of organic volatile emissions due to human activities by an order of magnitude (Guenther et al., 1995). BVOC play important roles in atmospheric chemistry as they are involved in photochemical ozone formation and impact the oxidative capacity of the atmosphere. Indirectly BVOCs also affect Earths' climate as they are precursors of secondary organic aerosols (Hallquist et al., 2009 and references cited therein).

In light of these important roles, it is crucial to understand and quantify possible changes in BVOC emissions under climatic changes. Vegetation models predict that under future global climatic changes, forests in Temperate and Boreal regions will flourish and spread, suggesting that this expansion will increase the annual global production of BVOC (Lathiere et al., 2005). The prediction of future BVOC emissions as proposed by Lathiere et al. assumes equilibrium between vegetation and climate. However, considerable climatic changes will be apparent already within the next decades whereas vegetation propagates by only a few times 10 km in 100 yr (Chen et al., 2011). Although vegetation can adapt to climate warming (Wolkovich et al., 2012), the time scale of climate change is short compared to typical adaptation times of ecosystems.

The mean temperature increase is often used to estimate future trends in BVOC emissions. However, future climatic scenarios predict frequent heat waves and modification of other environmental conditions, such as amount of rain and soil water content, probably inducing stress to vegetation (e.g. Sitch et al., 2007; Trenberth et al., 2007). It is thus expected that vegetation in many regions will be forced out of the optimal living conditions, exposing it to more intense and elongated heat waves, dryness, pollution or various diseases. It is projected that such extreme conditions will impose stress and disturb the functioning of plants. Therefore, it may not be sufficient to consider only constitutive isoprene- and monoterpene emissions in future climate scenarios. Other BVOC classes such as sesquiterpenes and oxygenated BVOCs may play an important and hitherto not considered role.

The most obvious abiotic stressors to vegetation that are expected with ongoing climate change are more intense and elongated heat waves and drought. Here we

investigated impacts of heat stress on the emissions of BVOC. As stress impacts we termed only those that were irreversible on a time scale of hours to days, meaning that BVOC emissions did not recover to the emission pattern and strength observed before heat application. Reversible impacts of enhanced temperatures were not considered as stress impacts in this study.

Scots pine and European beech were used as they are widely spread species of Boreal and Temperate forests. For comparison to beech we also studied Palestine oak as a typical Mediterranean species. To check data obtained with Scots pine we used Norway spruce. Monoterpenes, sesquiterpenes and phenolic BVOC were considered. It was found that the response of different BVOC classes to heat stress depended on the basic emission mechanism. Hence an overall clear mechanistic concept on the impacts of heat stress could be obtained.

2 Material and methods

Experiments were conducted in the Jülich Plant – Atmosphere Chamber facility (JPAC). A detailed description of the chamber set up and its performance is given in e.g. Schimang et al. (2006) and in Mentel et al. (2009). In short, the facility consisted of three borosilicate glass chambers (164 l, 1150 l, and 1450 l) with Teflon floors. Either one of the chambers was used as plant chamber. All chambers were operated as continuously stirred tank reactors and each chamber was mounted in separate climate controlled housing.

The range of photosynthetic photon flux density (PPFD) at mid canopy height was between 0 and 800 µmol m^{-2} s^{-1} in the 164 l chamber, between 0 and 480 µmol m^{-2} s^{-1} in the 1150 l chamber, and between 0 and 360 µmol m^{-2} s^{-1} in the 1450 l chamber. Concentrations of water vapor, CO_2, O_3, and NO_x were measured as described in Schuh et al. (1997) and Heiden et al. (2003). All measurements were performed under low NO_x and O_3 conditions ($[NO_x] < 300$ ppt, $[O_3] < 1$ ppb).

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BVOC concentrations were mostly determined using gas chromatography – mass spectrometry systems with similar configuration (Agilent GC-MSD-systems: GC HP5890 Series II + MSD HP5972A, GC HP6890 + MSD HP5973, GC HP7890 + MSD HP5975C). All GC-MS systems were equipped with on-line thermodesorption units (Gerstel, TDSG). For more details on the systems see e.g. Heiden et al. (2003). In one long term experiment we used a GC-FID system (Airmotec HC 1010) optimized for measuring monoterpenes. For more details on this system see Schuh et al. (1997).

BVOC calibration was performed using a permeation source containing pure chemicals in individual vials in combination with a dynamic dilution system. Concentrations of the compounds released from the calibration source were determined from the mass loss rates of the individual compounds and the dilution fluxes. The BVOC mixing ratios were in the lower ppb range. The reproducibility of BVOC concentration measurements was in the range of 10 % and the detection limits of our analytic equipment were between 1 ppt and 5 ppt for individual BVOC.

Identification of BVOC was based on mass spectra and retention times of pure standards (Fluka/Aldrich, purity > 93 %). Some compounds were only tentatively identified by using reference mass spectra from the NBS library. For these compounds, no individual calibration was conducted. In these cases we used the method described in Heiden et al. (2003) to quantify the concentrations.

Three to four years old tree seedlings were used in all measurements. As typical species of Boreal or Temperate forest we used Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), and European beech (*Fagus sylvatica* L.). For comparison to European beech Palestine oak (*Quercus calliprinos* L.) was used as typical species of Mediterranean areas. The conifers were used as a proxy for plant species with storage pools for monoterpenes, the broad leaf species were used as proxy for species without storage pools. Experiments were performed with individual plants with one exception. In this one experiment monoterpene emissions from four Scots pine seedlings together were studied.

Before the experiment the Palestine oak was stored in a growth room. The other tree seedlings were stored outside near to a forest. Thus they were exposed to realistic conditions with all the impacts that plants experience in their environment. This also included insect or pathogen infestation.

In all cases the plants were left for at least 1 day in the plant chamber for adaptation before starting the experiments. After adaptation, we checked for the basic emission mechanisms by inspecting the diurnal modulation of the emissions. In particular it was tested whether or not there was a distinct dependence of the emissions on photosynthetic photon flux density (PPFD) and furthermore if there were significant emissions during darkness.

For heat stress studies we applied a basic procedure. The plants were held at moderate chamber temperatures (between 12 and 31 °C) for several days to control the stability of BVOC emissions. Then the plants were exposed to elevated temperatures for time periods between 1 h and 5 days. Thereafter the plants were investigated at moderate temperatures again, i.e. at conditions comparable to those before the heat. BVOC emissions as measured before and after the heat application at the same temperature and light intensity were compared. We considered differences of less than 10 % to be indicative of negligible stress impact of the elevated temperature. When the emissions before and after heat application differed significantly the effect was ascribed to heat stress. In all cases the plants were sufficiently watered, thus decoupling the impacts of heat from drought impacts.

The temperatures given here are chamber temperatures. Measurements with temperature sensors (Newport, type K, Ni-CrNi) showed that leaf temperatures were up to 4 °C higher than chamber temperatures. However leaf temperatures depended on the distance of the respective leaf to the chamber lamps and it was impossible to exactly determine dependence of needle temperature on the distance from the lamps. We suggest that mean leaf and needle temperatures were approximately 2 to 3 °C higher than chamber temperatures for the well watered plants used here. Table 1 gives an overview of the experiments conducted with respect to heat application.

To assess the impact of heat stress on BVOC emissions it is required to know the basic emission mechanisms. In particular it had to be known whether the emitted BVOC were recently biosynthesized BVOC (de novo emissions) or stored in plant organs prior to emission (pool emissions). These mechanisms were tested for by exposing plants to $^{13}CO_2$ (99 % ^{13}C, ~ 350 ppm, exposure durations ~ 8 h). For further interpretation we used the ratio of ^{13}C-labeled over unlabeled BVOC:

$$R_{iso} = \frac{\text{labeled}}{\text{unlabeled}} = \frac{C(m+1) + C(m+2) + \ldots + C(m+n)}{C(m)} \quad (1)$$

In Eq. (1), C(m) is the count rate obtained from the mass spectrum for the molecular ion. C($m+1$) to C($m+n$) are the count rates obtained at the masses $m+1$ to $m+n$ with n being the number of C atoms of the respective BVOC. Equation (1) gives the ratio R_{iso} of all molecules containing excess ^{13}C to the unlabeled molecules. We assume that all molecules containing excess ^{13}C were synthesized during the exposure to $^{13}CO_2$. Under this assumption, R_{iso} gives the fraction of molecules biosynthesized during the time of $^{13}CO_2$ exposure. As there may be other carbon sources for BVOC biosynthesis than the carbon taken up as CO_2 (Schnitzler et al., 2004), Eq. (1) gives a lower limit for the fraction of BVOC synthesized shortly prior to their emissions. The natural ^{13}C abundance of 1 % per C atom in the BVOC was always considered in these calculations.

3 Results

3.1 BVOC emissions from unstressed plants

All investigated plants emitted only low amounts of isoprene and therefore isoprene is not included in the further analysis and discussion. Unstressed plants emitted mainly monoterpenes (MT) (> 85 %). In some cases also emissions of phenolic BVOC originating downstream of the shikimate pathway (e.g. methyl salicylate, MeSa) or

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sesquiterpenes were found for European beech and Scots pine, although the plants showed no obvious symptoms of infestation or injury. We interpret these emissions as indications of slight and unintended stress. The plants were nevertheless classified as "unstressed plants" due to the small contribution of these BVOCs to the total emissions (< 10 %).

European beech and Palestine oak do not possess storage organs for monoterpenes. Thus, MT emissions from these species are de novo emissions. For another Mediterranean oak, Holm oak, this was shown by Loreto et al. (1996) in $^{13}CO_2$ exposure experiments. In consistence MT emissions from these species are nearly zero during darkness and exhibit a strong dependence on PPFD (e.g. Peñuelas and Llusiá, 1999). A PPFD dependence in parallel with low emissions during darkness was shown for the MT emissions from beech (Schuh et al., 1997) indicating negligible contributions of pool emissions from European beech (see also Fig. 1).

MT emissions from conifers originate mainly from MT diffusion from the resin ducts wherein they are stored (e.g. Janson, 1993). Accordingly we found only a low degree of ^{13}C labeling in the MT emitted from Scots pine. Even after 8 h of $^{13}CO_2$ exposure α-pinene, camphene, and Δ^3-carene exhibited labeling ratios between 0.1 and 0.3 (R_{iso}, Eq. 1) indicating that the majority of these emissions (70–90 %) originate from storage pools, in line with other studies (Shao et al., 2001; Ghiarado et al., 2010).

For the oxygenated MT 1,8-cineole emitted from pine, a strong labeling was found, indicating that more than 90 % of the emitted 1,8-cineole was synthesized from the CO_2 taken up within the last 3 to 4 h before emission. Consistently, 1,8-cineole emissions from Scots pine were negligibly low in darkness and exhibited a profound PPFD dependence (see also Tarvainen et al., 2005). This de novo emission of 1,8-cineole from Scots pine enabled studying impacts of heat stress on de novo and on diffusive pool emissions together using a single plant (pine).

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3.2 Impact of heat stress on constitutive de novo monoterpene emissions

Heat was applied to European beech and to Palestine oak, both species without storage pools. In three experiments with beech the temperature was increased up to 40 °C for 1 to 4 h. Consistent to the results of Dindorf et al. (2006) we found no impacts of these short time exposures on the BVOC emissions except of the normal temperature dependence. We therefore conducted an experiment with longer application of heat stress. A three years old beech seedling was held at a temperature of 31 °C for two days (PPFD = 800/0 µmol m^{-2} s^{-1} day/night). At the third day the temperature was increased to 40 °C for 5 days to apply a long period of heat stress. Thereafter, the temperature was decreased to 24 °C for two days and the initial temperature of 31 °C was restored again. The diurnal variation of MT emissions during the whole time period is shown in Fig. 1 at the example of sabinene emissions.

Figure 1 shows higher emission rates at higher temperatures. Comparing the data obtained at 31 °C before and after applying the elevated temperature we found a reduction of sabinene emissions by about 30 % although rates of net photosynthesis ($1.9 \pm 0.5/2.2 \pm 0.4$ µmol m^{-2} s^{-1} before/after heat application) and transpiration ($2.5 \pm 0.1/2.5 \pm 0.15$ mmol m^{-2} s^{-1} before/after heat application) were not significantly affected. Also the sum of all MT emissions dropped by 30 % from 0.19 ± 0.01 to 0.13 ± 0.02 nmol m^{-2} s^{-1}. Considering the extreme high temperature and the long application time, the decrease in MT emissions was moderate and it seemed that beech was quite resistant to pure heat stress as long as the water supply was sufficient. However, the reduction in MT emissions was significant.

For comparison to European beech we used the Mediterranean species Palestine oak. As in the case of beech the impact of elevated temperature on MT emissions from a Palestine oak was measured by comparing the emission rates under the same conditions before and after a heat application. A temperature of 24 °C and a PPFD of 800 µmol m^{-2} s^{-1} were used as reference before and after heat application. Between the measurements at 24 °C the temperature of the plant chamber was increased to

40 °C (leaf temperature ∼ 43 °C). The plant was kept at 40 °C chamber temperature for 16 h (2 h at PPFD = 800 µmol m^{-2} s^{-1}, 1 h at twilight, 10 h at darkness, 1 h twilight and 2 h at PPFD = 800 µmol m^{-2} s^{-1}). Thereafter the temperature was reduced back to 24 °C. Compared to the emissions before the heat stress, the emissions were reduced by about 50 %. Table 2 lists the emissions measured at 24 °C/800 µmol m^{-2} s^{-1} before and after the heat stress for individual MT.

As shown in Table 2, absolute and relative decreases varied for the different MT, indicating that the heat stress had different effects on the emissions of individual MT thus not only altering emission strength but also the emission pattern.

In summary, when elevated temperatures acted as stress to broad leaf species constitutive MT emissions were decreased thereafter. Increased MT emissions due to the heat stress were not observed for these non-storing species.

3.3 Impact of heat stress on constitutive monoterpene emissions from pools

Table 3 gives an overview of the experiments conducted with respect to impacts of elevated temperatures on monoterpene emissions from conifers. Three to four years old seedlings were used to study the impacts of heat on MT-storing conifers. Four experiments were made with non-infested Scots pine, another four were made with insect infested Scots pine and Norway spruce. Experiments with non-infested plants are described in this section; experiments with infested plants are described in Sect. 3.4.

Before applying elevated temperatures, the plants were kept at moderate temperatures (12 °C–30 °C) for some days. Then the plants were exposed to temperatures between 31 °C and 51 °C for 1 to 48 h. In all cases, the MT emissions increased with increasing temperature. In one case (experiment 1 in Table 3) the increases could be described by the usual exponential temperature dependence with temperature coefficients between β = 0.1 K^{-1} to 0.12 K^{-1}. The temperature reduction after heat application also caused a reduction of the emission rates with the same temperature coefficients. Thus, after applying the enhanced temperatures, MT emissions returned to the initial values upon return to the initial conditions. This behaviour implies that the high

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temperatures caused a higher diffusion of MT out of the resin ducts but did not induce excessive stress. In this case no emission pulses of green leaf volatiles (GLV) were found.

Contrary, in other cases (experiments 2, and 3, in Table 3) weak but distinct pulses of GLV emissions appeared during heat stress application. In parallel strong MT emissions were found and, after reducing the temperature to the initial value, MT emissions remained much higher than before the heat stress. As indicated by GLV emissions, in these cases the high temperatures caused membrane damage. We hypothesize that also membranes surrounding the resign ducts were damaged by the heat. This may have caused an increased release of MT during and after the heat stress.

To test this hypothesis, a Scots pine was exposed to a severe heat stress (46–51 °C for 3 h, experiment 2 in Table 3) during an exposure to $^{13}CO_2$ (99 % ^{13}C, 350 ppm, 8 h). In the beginning of the experiment (starting from time = 0, see Figs. 2 and 3) the pine was exposed to $^{13}CO_2$ at 28 °C i.e. without heat application. This moderate temperature of 28 °C was kept for 3 h. Then the temperature was raised to about 50 °C for three hours and thereafter set back to 28 °C. The $^{13}CO_2$ exposure was stopped another two hours later, as indicated by the brown bars in Figs. 2 and 3.

Before applying the heat, most of the emitted MT were labeled to a low degree (R_{iso} = 0.1 – 0.3) indicating that 70 to 90 % of these emissions were pool emissions. Nevertheless, the labeling with ^{13}C was distinct. During the third hour at elevated temperature the strong pulse of MT emissions appeared together with a pulse of GLV emissions (not shown). Together with the onset of the MT emission pulse the labeling of the MT decreased to non-measurable amounts. Reaching 28 °C again after the heat pulse, the release of the stored MT was still 8-fold higher than before the heat stress. Although exposure to $^{13}CO_2$ continued, the degree of labeling remained zero (Fig. 3).

As mentioned above, the emission of 1,8-cineole from Scots pine is a pure de novo emission. Thus, under stress free conditions i.e. within the first three hours of the $^{13}CO_2$ exposure, we found a fast and strong labeling of 1,8-cineole ($R_{iso} \approx 11$, see Fig. 3). As expected from the results obtained with European beech and Palestine oak the

behaviour of de novo emission was different from that of the α-pinene emission representing pool emissions. Upon start of the heat application, 1,8-cineole emissions increased two fold. But, together with the onset of emissions of GLV and enhanced emissions of MT stored in the resin ducts, 1,8-cineole emissions dropped to non-measurable amounts (Fig. 3). After the heat stress the plant was investigated for 5 more days. The pool emissions decayed exponentially but the 1,8-cineole emissions did not recover and remained below detection limit.

For one experiment where Scots pine seedlings were exposed to severe heat stress (experiment 4 in Table 3) we continued the measurements for another 6 weeks. In this experiment four Scots pine seedlings were investigated together (diurnal rhythm: 12 h illumination, 10 h darkness, and 1 h twilight in the morning and in the evening, respectively). PPFD during illumination varied from day to day ranging between 30 and 360 $\mu mol\,m^{-2}\,s^{-1}$ and plant chamber temperatures were between 12 and 30 °C.

During a time period of about ten weeks (mid of April to end of June, Fig. 4) the plants were held without stress application. MT emissions were fairly stable during that time (e.g. emission rate of α-pinene = 4 $nmol\,m^{-2}\,s^{-1}$, 1σ standard deviation of $\pm$ 0.7 $nmol\,m^{-2}\,s^{-1}$, see Fig. 4). Then the plant chamber temperature increased to 45 °C for 48 h. As observed for the other pines this heat stress induced MT emissions. To check for possible long-term effects, the plants were investigated further on at moderate temperature conditions.

It was found that photosynthesis was strongly reduced following the heat stress and it took several days until the plants began to recover. However the plants recovered and 4 weeks after the heat stress, rates of net photosynthesis and transpiration were similar to those measured before the heat (difference $< 20\,\%$). In addition, MT emissions dropped back to the values similar to those before the heat application. Six weeks after the heat stress the plants behaved as before and showed no visible symptoms of injury. Thus, the heat stress application had no long term impacts on the plants.

As obvious from Fig. 4, the additional release of MT could be quite large. Estimating the total amount of released MT (compare integral emissions = $\Phi \cdot t$ for emissions

without and emissions with heat stress in Fig. 4) the additional MT amount released could be as large as the emissions without heat stress over a time period of several months.

3.4 Impact of heat on biotic stress induced BVOC emissions

To investigate impacts of heat stress on BVOC emissions induced by biotic stress we used selected Scots pine seedlings that were stored outside and were obviously infested by insects. The visible insects were aphids. We neither made an exact identification of the insects nor looked for other infestations than the visible ones. For our purpose it was sufficient to first check the basic mechanisms of stress induced emissions and thereafter check the impact of heat on these emissions.

Most prominent emissions from the insect infested Scots pine were sesquiterpenes (SQT) and phenolic BVOC. The emissions of α-farnesene and β-farnesene contributed to more than 80 % of the SQT emissions. Methyl salicylate (MeSa) contributed more than 80 % of the phenolic BVOC emissions. Emission rates of SQT and phenolic BVOC depended on PPFD (Fig. 5).

The pronounced PPFD dependence indicated that these stress-induced emissions were de novo emissions. To corroborate this finding we exposed the pine to $^{13}CO_2$ (99 % ^{13}C, 350 ppm, exposure time 8 h, PPFD = 800 µmol m^{-2} s^{-1}, temperature = 28 °C). As observed also for non-infested Scots pine, the MT emitted from storage pools were only slightly labeled (α-pinene, $R_{iso} \sim 0.1$). In contrast, most SQT (e.g. α-farnesene, $R_{iso} \sim 28$) were significantly labeled, confirming that SQT emissions are de novo emissions. The labeling ratio of MeSa was lower ($R_{iso} \sim 0.6$) suggesting that parts of the MeSa was formed from stored carbon.

After the labeling experiment the plant was exposed to 46 °C for 1 h but no irreversible effect was observed (experiment 5 in Table 3). Thus the elevated temperature did not act as stress. The plant's emissions were measured for another week (diurnal rhythm: 12 h illumination, PPFD = 800 µmol m^{-2} s^{-1}, 1 h twilight in the morning and in the evening, respectively, 10 h darkness, PPFD = 0 µmol m^{-2} s^{-1}, T = 24/20 °C

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illumination/darkness). During this period, BVOC emissions including the emissions induced by biotic stress were quite stable allowing to test the impact of heat stress on these stress induced emissions in a second experiment with the same individual.

Exposing this plant to 46 °C for 4 h caused irreversible effects (experiment 6 in Table 3). A pulse of GLV emissions appeared and MT emissions increased by an order of magnitude. Thus the behavior of MT emissions was the same as in case of the plants that were not infested by insects. Emissions of phenolic BVOC increased 3-fold during the heat application while SQT emissions dropped. Two days after the heat stress the plant was investigated again under the same conditions as prior to the heat stress ($T = 24$ °C, PPFD = 800 µmol m^{-2} s^{-1}). MT emission rates were about 2-folds higher than before the heat stress; emissions of SQT and phenolic BVOC had dropped to about 1/3rd and to about 1/5th, respectively (Fig. 6).

When heat acted as stress it increased the pool MT emissions but decreased the de novo emissions induced by the biotic stress. As the de novo emissions were higher than the pool MT emissions before the heat stress, their suppression outbalanced the increase of pool emissions (compare Fig. 6: emissions 2 h before heat stress application to emissions 52 h thereafter). The total BVOC emissions rates from this conifer decreased due to the heat stress.

To check if the impacts of heat stress on top of the impacts of insect infestation were also observable for another coniferous species we applied elevated temperature (35 °C for 9 h) to a Norway spruce. For this experiment we also used a plant that was stored outside before the measurements and showed visible aphid infestation. The same general behavior was observed for the Norway spruce as for the Scots pine. Before the heat application the spruce emitted high amounts of SQT (sum of SQT ~ 60 nmol m^{-2} s^{-1}) and MT (sum of MT ~ 43 nmol m^{-2} s^{-1}) and remarkable amounts of phenolic BVOC (sum of phenolic BVOC ~ 4 nmol m^{-2} s^{-1}). In particular, emissions of the SQT β-farnesene (~ 35 nmol m^{-2} s^{-1}) and α-farnesene (~ 17 nmol m^{-2} s^{-1}) were quite high before the heat stress. After the heat stress, the biotic stress induced emissions of SQT and phenolic BVOC decreased to low amounts but pool MT emissions were higher than

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before the heat stress. Pool emissions decreased with time and de novo emissions did not recover within three days. Three days after the heat stress the total amount of emitted BVOC was lower than before the heat stress.

Including the data obtained for plants without obvious insect attack (Sect. 3.3) eight experiments were conducted on the impacts of elevated temperatures on BVOC emissions from Boreal conifers (see Table 3). In three cases no impacts on the emissions were observed except for the normal temperature dependence. In five cases there were irreversible impacts. No relationship was found between occurrence of stress impacts and the maximum applied temperature.

4 Discussion

Significant parts of Earth's vegetation, especially in middle and high latitudes, will experience more frequent heat waves and drought periods as a consequence of climate change. Both stresses will have combined effects as heat and drought periods are often coupled. The impact of heat may be enhanced by parallel drought because reduction of transpiration causes less cooling of leaves. During drought periods, leaf temperatures may exceed air temperatures (e.g. Hamerlynck and Knapp, 1994; Singsaas et al., 1999). Hence, the temperatures used here to apply heat stress were not unrealistically high and may occasionally be reached in future climate. As a consequence of these stresses, BVOC emissions will change. Considering the basic emission mechanisms this seemingly complex system can be unravelled at least qualitatively on a mechanistic basis.

We first separate the emissions of green leaf volatiles (GLV) from other BVOC emissions because GLV emissions behave differently from the other de novo emissions. Thereafter, we investigate terpenoid emissions and consider the two basic emission mechanisms: de novo emissions and pool emissions. As a last step we focus on the impacts of heat stress on the BVOC emissions induced by biotic stress.

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4.1 GLV emissions in response to heat

Green leaf volatiles are synthesized via a sequence of enzymes within the octadecanoid pathway. Due to their instantaneous release short after their biosynthesis (e.g. Fall et al., 1999), we consider GLV emissions as de novo emissions although we found neither ^{13}C labeling nor any distinct diurnal variation. The lack of labeling in GLV emissions is most probably due to insignificant labeling of the substrate. GLV are produced from membrane lipids that were synthesized long before the exposure to $^{13}CO_2$. Thus the ^{13}C abundance in these membrane lipids is similar to the natural ^{13}C abundance. Significant degree of labeling therefore cannot be expected for the GLV. Furthermore, GLV emissions appear as pulses exhibiting high temporal dynamics (see Heiden et al., 2003). This temporal dynamics certainly superimposes any diurnal variation. Therefore typical diurnal variations such as observed for constitutive monoterpene emissions cannot be expected.

GLV synthesis and thus GLV emissions involve membrane damage (e.g. Croft et al., 1993). Once membrane damage has occurred, GLV are released independent of the stressor that induced the damage (Heiden et al., 2003). It is therefore reasonable that heat induces GLV emissions if the stress is above a threshold that causes membrane damage. This was shown for common reed (*Phragmites australis*) by Loreto et al. (2006), for tomato (*Solanum lycopersicum*) by Copolovici et al. (2012) and also observed in this study for pine and spruce. In four cases of heat stress application to conifers the severity of the applied stress was above the membrane damage threshold (in one case no reliable data on GLV emissions were obtained due to use of GC-FID system). In the other three cases, we did not find GLV emissions as the applied stress was below the membrane damage threshold.

GLV emission strengths are related to the degree of damage (Fall et al., 1999; Beauchamp et al., 2005; Behnke et al., 2009). We therefore assume that heat stress on top of another stress that had induced membrane damage before, can either induce additional membrane damage or have no effect. It is assumed that heat stress does

not cause repair of damaged membranes and therefore heat stress should not cause lower GLV emissions. We therefore propose that future heat waves will increase GLV emissions, as long as the stress is above the threshold for membrane damage but below the threshold that affects the activity of enzymes involved in GLV synthesis.

5 We observed GLV emission pulses up to temperatures of 51 °C when all other de novo emissions collapsed. From this observation we conclude that the enzyme system producing the GLV emissions is quite heat tolerant at least more than the enzyme systems synthesizing MT, SQT or phenolic BVOC. Denaturation of enzymes synthesizing the GLV is expected only at extreme temperatures, such that severely damage

10 the plant. As long as the heat stress remains below a threshold that induces denaturation of enzymes within the octadecanoid pathway, GLV emissions are the only de novo emissions increasing during heat stress.

With respect to global change induced impacts, we project that GLV emissions will increase with increasing heat stress. However, as observed for Scots pine and Norway

15 spruce, GLV emission pulses were at least 2 orders of magnitude lower than the MT emission pulses due to pool damage. GLV emission pulses after heat stresses were observed for several hours only whereas the increased MT emissions lasted for days to weeks. From this observation we conclude that heat stress induced GLV emissions are less important for total BVOC emissions from conifers than the changes in MT

20 emissions at least for the investigated tree species which were all strong MT emitters.

4.2 Impact of heat on constitutive de novo monoterpene emissions

Temperature is a key variable in controlling MT emissions from vegetation and it is widely accepted that MT emissions increase exponentially with temperature. However, if leaf temperature exceeds a certain optimum, MT emissions can drop (e.g. Bertin

25 et al., 1997; Staudt and Bertin, 1998), similar to isoprene emissions (e.g. Guenther et al., 1993). Reductions of isoprene emissions as a consequence of heat are attributed to an overall reduction of biosynthetic activity with the heat stress (Zhang et al., 2009; Zhang and Sharkey, 2009; Niinemets et al., 2010).

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Similar to isoprene, de novo MT emissions depend on the activity of the enzymes producing the respective BVOC. Such enzymes may denature at temperatures above 40 to 45 °C (Loreto and Schnitzler, 2010; Loreto et al., 2006). Hence, the observed decreases of de novo MT emissions during heat stress can be explained by denaturation of MT synthesizing enzymes as a consequence of heat. When constitutive de novo emissions were significantly reduced, they did not recover when returning to normal temperature. The heat-induced effects were not reversible on a time scale of hours to days, which is a typical effect for a denaturation.

We observed heat stress induced decreases of de novo MT emissions from Scots pine, Palestine oak and from European beech, with varying magnitude. In case of Scots pine, the 1,8-cineole emissions diminished and did not recover for days. This implies a long recovery time for the enzymes synthesizing 1,8-cineole in Scots pine and thus a long lasting impact of the heat. For Palestine oak whereof all emissions were of de novo nature, the degree of decrease varied for the different MT species. This observation indicates that the sensitivity of different MT synthesizing enzymes to heat stress is different. This was already shown by Staudt and Bertin (1998) who found decreasing emissions of cyclic MT above 40 °C but still increasing emissions for acyclic MT. For European beech, the heat stress induced changes in MT emissions were moderate despite the high and long lasting heat application. It is therefore obvious that the impacts of heat stress on different plant species may differ largely.

Beech as species of Temperate forests should be less heat tolerant than Mediterranean species. It was therefore expected that denaturing the MT producing enzymes may require higher temperatures to induce heat stress in Mediterranean species. We nevertheless found comparable decreases of de novo MT emissions after exposing European beech and Palestine oak to 40 °C. Moreover, Bertin et al. (1997) show that MT emissions from the Mediterranean species Holm oak can drop already when the temperature exceeds 35 °C implying that the plants used in our experiments were less sensitive to heat stress than Holm oak. But the plants investigated in our experiments were well watered and impacts of heat stress may strongly depend on the plants water

status. Differences in water availability may explain the seemingly higher heat tolerance of European beech compared to Holm oak.

To summarize, temperatures above which de novo MT emissions decrease vary broadly. Such threshold temperatures depend on the heat sensitivity of the enzymes producing individual MT species, on the individual plant as well as on the environmental conditions the plants experience. Therefore general statements on such threshold temperatures are not possible yet. However one general statement can be made: de novo MT emissions decrease when enhanced temperatures act as stress.

4.3 Impact of heat on monoterpene emissions from pools

The impacts of heat stress on pool MT emissions from conifers species differ substantially from those on de novo emissions. Pool MT emissions increased considerably more than expected from the usual exponential temperature dependence. We conclude that the excessively high increase was due to damage of membranes surrounding the resign ducts.

The MT emission pulses were accompanied by a simultaneous pulse of GLV emissions, also indicating membrane damage in the plant. The similar timing between the onset of GLV emissions and increased MT releases suggest that membranes surrounding the resin ducts were also damaged, causing a decrease of the diffusive resistance from the resign ducts to the air. The MT stored in the large pools of the pine were produced before the $^{13}CO_2$ exposure, therefore the MT released in excess contained only the natural ^{13}C abundance. As the MT synthesizing enzymes were denatured by the heat, no MT containing excess ^{13}C were synthesized and emitted after the heat stress. Thus, the labeling of the emitted MT decreased to immeasurable amounts.

All our observations regarding heat stress induced increases of MT emissions can be explained by these assumptions. On the contrary our observations cannot be explained by an increased de novo MT production. If the emissions were de novo emissions, the loss of labeling parallel to the increased emissions would require a carbon source for MT synthesis other than the $^{13}CO_2$ taken up. The respective enzymes should then have

been active although other enzymes producing the respective MT from CO_2-carbon were already denatured. Moreover, those enzymes should have produced the MT with the same pattern as the former pool emissions. As we did not observe such increase of MT emissions when exposing non-storing species to heat stress, this hypothetical de novo synthesis should be restricted to plants with pools. All these prerequisites together are highly improbable leading to the conclusion that the observed increases in MT emissions were due to a release of MT out of the heat-damaged pools.

Not much data exist regarding the impact of heat stress on pool MT emissions. Tingey et al. (1980) show a purely exponential increase of MT emissions from Slash pine up to a temperature of 46 °C indicating that this temperature had no stressing impact on Slash pine. This seems to be in contrast to our observations for Scots pine where we found MT emission pulses even at lower temperatures. Such differences may be explained by different heat stress tolerance of different species. But in some cases we also observed no impacts of high temperatures on MT emissions except of the usual exponential increase. Seven of the experiments with heat stress on conifers were conducted with plants of the same species and the results listed in Table 3 imply that thresholds above which temperature acts as stress can also differ between individuals. Reasons for the different temperatures above which MT pools are damaged may be differences in water supply, differences in the temperature history of the individual, or differences in the duration of the heat stress.

As described in the review by Niinemets et al. (2010) the impacts of heat pulses on isoprene emissions depend on the duration of the heat. Short heat pulses may have lower impacts on isoprene emissions than longer lasting heat intervals. Similar behavior was also observed in this study for pool MT emissions: no irreversible impacts were observed when exposing a plant to 46 °C for one hour, whereas irreversible effects were observed when exposing the same individual to 46 °C for four hours (see Table 3, experiments 5 and 6). This observation implies that the duration of the heat stress is of importance for a threshold when damage of membranes surrounding the resign ducts appears.

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Considering that besides degree and duration of heat stress other environmental factors may impact temperature thresholds, it is obvious that exact threshold temperatures or duration after which MT emission pulses appear cannot be provided at this stage. However, it is possible to state that enhanced temperatures either do not affect such pool emissions irreversibly or increase the emissions when the severity is above the threshold. As global change is assumed to induce longer lasting heat waves than in present climate we suspect that heat stress induced MT releases from MT-storing conifers will be more frequent in the future.

Whether or not future heat waves will cause increases or decreases of constitutive MT emissions from plants growing in a given area depends on the fraction of pool emitters and de novo emitters. It is expected that MT emissions will increase in areas predominantly covered with conifers (pool emitters) and will decrease in areas with predominantly de novo emitters.

However, such predictions are only valid for time scales of days to weeks. In all cases when the heat acted as stress it decreased actual MT biosynthesis, e.g. the MT production in conifers was suppressed. This counteracts the increased release of stored MT and it is not clear at this point which effect prevails on longer time scales. If the collapse of MT biosynthesis and de novo emissions last much longer than the emission pulse, the net effect of heat might also be a decrease of emissions.

The experiment with the four Scots pine seedlings showed that conifers can survive such periods of heat stress and that pool MT emissions will recover within few weeks. It is therefore likely that also the de novo MT emissions will recover after some time. However, so far we have no data on such a recovery.

4.4 Impact of heat on BVOC emissions induced by biotic stresses

High emissions of SQT and phenolic BVOC were observed only for Scots pine and Norway spruce showing visible insect infestation and we conclude that these emissions were induced by the insect attack. The same conclusion was drawn by Joó et al. (2011) who measured SQT and MeSa emissions from infested by aphids Douglas fir. We

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found this effect for Scots pine and Norway spruce which are wide spread species in Boreal and Temperate European forests. Furthermore, we found the same dominant SQT emissions, namely α- and β-farnesene from insect infested Norway spruce and Scots pine. This indicates that emissions of α- and β-farnesene as well as MeSa are
5 a quite typical reaction of Boreal conifers on aphid infestation.

Impacts of heat or drought on top of biotic stress can only be determined when stress induced emissions are either constant in time or when the stress impact is so unambiguous that fluctuations of the biotic stress induced emissions are negligible. We checked for stability before applying heat stress and found that the fluctuations from
10 day to day were by far less than the strong and abrupt changes during and after heat. We therefore conclude that the observed suppression of stress induced emissions was indeed caused by the heat stress.

As indicated by both, the low SQT emissions during darkness (Fig. 5) and by the high degree of labeling, SQT emissions were de novo emissions. The low labeling of MeSa
15 indicates that a considerable fraction was produced from a low labeled carbon source. We believe that a considerable carbon pool with an exchange time of several hours existed in the respective plant. Thereof the aromatic ring of MeSA was synthesized causing the low labeling. But we suggest that at least one of the steps in the cascade of enzymes synthesizing MeSa depends on PPFD causing the pronounced PPFD de-
20 pendence. Whichever step accounts for this PPFD dependence, we also consider the MeSa emissions as de novo emissions.

A characteristic of stress induced emissions is their absence in case of stress free conditions as well as their quick appearance when the plants experience stress. Considering this, it is comprehensible that such emissions cannot originate from diffusion of
25 these compounds out of large storage pools. We therefore believe that all BVOC emissions we observed during biotic stress were de novo emissions. Note that only BVOC emissions originating from plant internal signals are included in this assumption. Possible pulses of MT emissions due to mechanical damage of pools e.g. by herbivore attack are excluded here.

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The general response of such stress induced de novo emissions on heat stress was similar to that of constitutive de novo emissions. In all cases when the heat acted as stress these emissions decreased. An increase was not observed. The reason for this decrease is likely the same as that causing decreases of constitutive MT emissions; a general decrease of the plants performance as a consequence of the heat. The decrease in performance may be due to the denaturation of enzymes which synthesize the respective VOC, the breakdown of plant internal signaling cascades, or reduction of carbon supply caused by decreased CO_2 uptake.

Independent of the exact mechanism, the heat stress decreased the BVOC emissions induced by biotic stress. In cases when biotic stress induced emissions were stronger than the constitutive MT emissions from conifers the overall emissions were decreased by the heat stress (e.g. Fig. 6). The net impact of heat stress on BVOC emissions from Scots pine and Norway spruce depends on the impacts of biotic stress the plants experience. It is thus not a priori obvious whether heat stress on conifers increases or decreases total BVOC emissions. The net effect depends on the fraction of pool emissions on the one hand and on the fraction of constitutive and stress induced de novo emissions on the other hand.

Not much data are reported so far on the impact of heat on biotic stress induced emissions. Joó et al. (2011) show exponentially increasing emissions of SQT and MeSA with increasing temperature for Douglas fir. This situation is comparable to the cases where we found no GLV emissions and no pool-MT emission pulses. As listed in Table 3 (experiment 7) emissions of MT, SQT and MeSa were not affected irreversibly and GLV emissions were not induced when exposing a Scots pine to 35 °C for 22 h. Hence there is no difference between the findings of Joó et al. and the findings reported here, there is just a difference in terming stress. We propose to term only those effects as *heat stress induced* that appear on top of the usual exponential temperature dependence and are irreversible on time scales of hours to days.

With respect to impacts of future heat waves on stress induced de novo BVOC emissions the situation is somewhat more complex than in case of constitutive de

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novo emissions. Induction of biotic stress induced emissions requires activation of the respective biosynthetic pathways. This activation will certainly depend on the plant species, on the kind of infestation and probably also on the degree of infestation. The degree of infestation will also depend on changes of the plants environment with ongoing climate change. The development of interactions between plants and their biotic environment is uncertain (Arneth and Niinemets, 2010) and therefore no predictions are possible so far.

5 Summary and conclusions

Heat stress affected emissions of different BVOC classes in different ways. Emissions of GLV as well as MT emissions from pools increased due to heat induced damage of membranes including partial destruction of resin ducts. In contrast, de novo emissions of MT, SQT, and phenolic BVOC decreased. This general trend of decreasing emissions was independent of the de novo emissions being constitutive or induced by biotic stress prior to the heat. We believe that this behaviour was caused by a general decrease of the plants performance including denaturation of BVOC synthesizing enzymes.

This mechanistic model seems simple as it just requires discrimination between BVOC classes and their basic emission mechanisms to predict trends in emissions following future heat waves. Nevertheless, upscaling is impossible from our studies alone: In all cases the impacts of heat were only observable above thresholds. Quantitative information on such thresholds as well as on the dependence of these thresholds on other environmental variables is required before future trends in BVOC emissions can be assessed. With such quantitative information models containing parameters for vegetation types, their future spread and future heat stress episodes might yield trends for the impacts of heat waves on BVOC emissions from vegetation.

But still major information is missing before clear answers with respect to climate change induced modifications of BVOC emissions can be given. In particular the

uncertainty regarding development of biotic interactions prevents from reliable predictions (Arneth and Niinemets, 2010). The interactions between plants and their biotic environment may vary as the living conditions of aphids or pathogens may be favoured or worsened due to climate change. It is unclear how the whole system will develop under climate change and therefore our findings provide trends that have to be integrated in a larger context. However, one point is incontrovertible. Future trends of BVOC emissions cannot be estimated using constitutive emissions alone. BVOC emissions induced by biotic stress and their response to climate change have to be considered.

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Table 1. Overview of the measurements regarding the impacts of heat stress.

Species	No. of experiments	Range of max. temperatures [˚C]	Duration of heat stress
European beech	4	37–41	1 h–5 days
Palestine oak	1	44	16 h
Scots pine	7	31–51	1 h–2 days
Norway spruce	1	35	9 h

Table 2. Monoterpene emissions from *Q. calliprinos* measured at a temperature of 24 °C and a PPFD of 800 µmol m^{-2} s^{-1} before and after a heat stress application. Emission rates are the mean of at least 6 measurements and the error limits represent the 1σ standard deviations.

MT	Φ_{before} [pmol m^{-2} s^{-1}]	Φ_{after} [pmol m^{-2} s^{-1}]	Ratio: after/before heat stress
α-thujene	8.3 ± 0.4	4.2 ± 0.8	0.5 ± 0.1
α-pinene	260.0 ± 6.5	120.0 ± 15.2	0.46 ± 0.1
sabinene	22.0 ± 0.8	14.0 ± 2.4	0.64 ± 0.1
β-pinene	93.0 ± 2.7	50.0 ± 6.9	0.54 ± 0.1
myrcene	11.0 ± 0.9	2.6 ± 0.4	0.24 ± 0.04
α-phellandrene	5.4 ± 0.3	2.1 ± 0.4	0.39 ± 0.1
α-terpinene	16.0 ± 1.0	2.3 ± 0.5	0.14 ± 0.03
β-phellandrene	13.0 ± 0.6	6.4 ± 1.0	0.49 ± 0.1
γ-terpinene	39.0 ± 1.9	1.6 ± 0.3	0.04 ± 0.01

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Table 3. List of experiments investigating impacts of heat stress application on BVOC emissions from conifers and observed effects. rev: no irreversible effect observed. irr: irreversible effect observed with increases of pool MT emissions and decreases of SQT and MeSa emissions; b.l. = below detection limit; +: induced GLV emissions during the heat stress; –: GLV emissions not induced by the heat.

Experiment	Species	Max. T	Duration	MT	SQT	MeSa	GLV
1	Pine	33 °C	22 h	rev.	rev.	rev.	–
2	Pine	51 °C	3 h	irr.	b.l.	b.l.	+
3	Pine	31 °C	29 h	irr.	b.l.	b.l.	+
4	Pine[1]	45 °C	48 h	irr.	[1]	[1]	[1]
5[3]	Pine[2]	46 °C	1 h	rev.	rev.	rev.	–
6[3]	Pine[2]	46 °C	4 h	irr.	irr.	irr.	+
7[3]	Pine	35 °C	22 h	rev.	rev.	rev.	–
8[3]	Spruce	35 °C	9 h	irr.	irr.	irr.	+

[1] Experiment with 4 plants together, data obtained using a GC-FID system, no reliable information on GLV, MeSa, and SQT emissions.
[2] Two experiments with the same individual.
[3] Experiment with infested plants.

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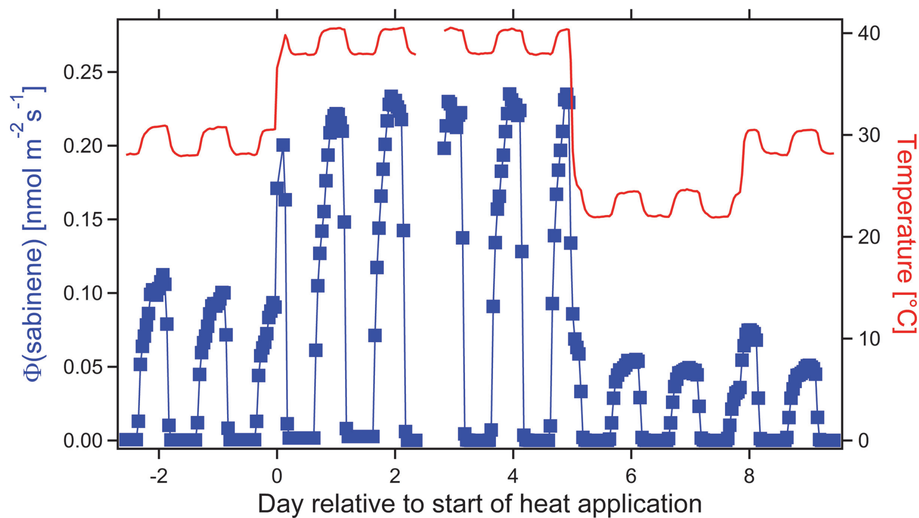

Fig. 1. Sabinene emissions (blue squares, left y-axis) from a beech under heat stress. The red line indicates temperature (right y-axis). PPFD = 800/0 µmol m^{-2} s^{-1}, daytime/darkness.

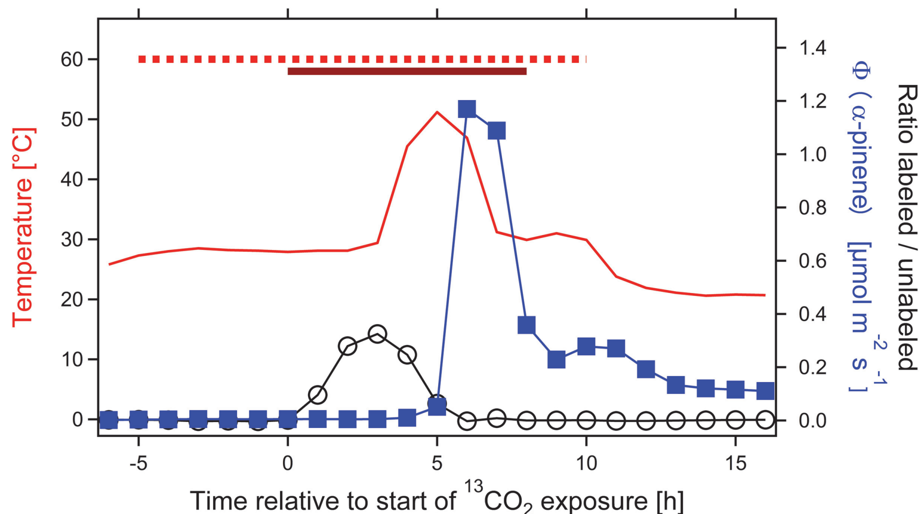

Fig. 2. α-pinene emissions from Scots pine under heat stress (blue squares, right hand scale). Open circles show the labeling ratio (R_{iso}) of α-pinene molecules calculated according to Eq. (1) (black circles, right hand scale). The red line shows the chamber temperature (left hand scale). The dashed red bar indicates the period of illumination (PPFD = 800 µmol m^{-2} s^{-1}) and the brown bar the period of $^{13}CO_2$ exposure.

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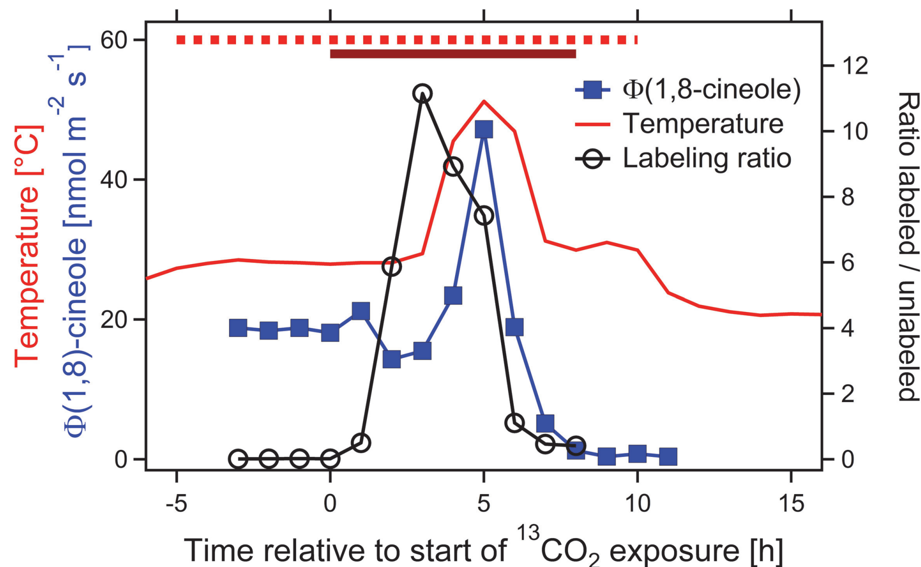

Fig. 3. 1,8-cineole emissions from Scots pine under heat stress (blue squares, left hand scale). The red line shows the chamber temperature (left hand scale). Open circles show the labeling ratio of 1,8-cineole molecules calculated according to Eq. (1), (right hand scale). The red dashed bar shows the period of illumination (PPFD = 800 µmol m^{-2} s^{-1}) and the brown bar shows the period of $^{13}CO_2$ exposure.

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Impact of heat stress on BVOC emissions

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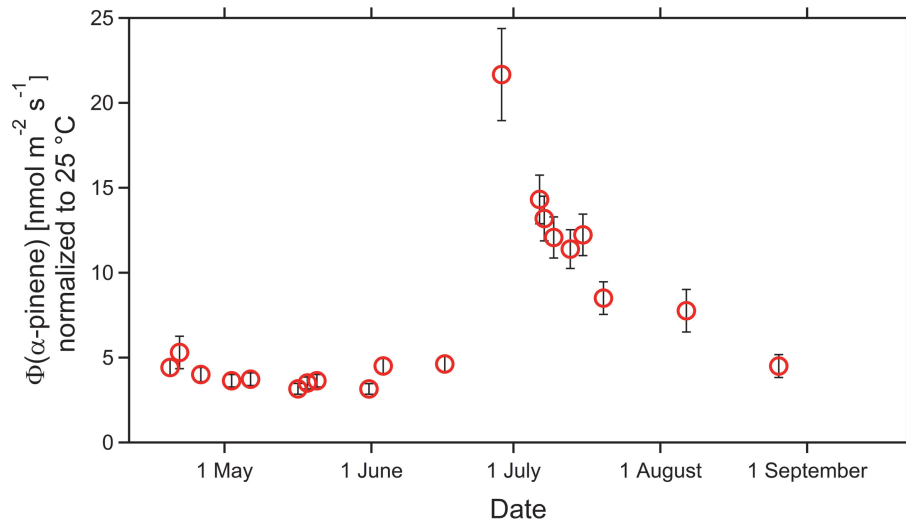

Fig. 4. Emission rates (Φ) of *α*-pinene from four Scots pine seedlings. Shown are only those data obtained at PPFD = 360 µmol m^{-2} s^{-1}. Temperatures were between 12 and 30 °C and the emission rates were normalized to T = 25 °C using the temperature dependence given in Shao et al. (2001).

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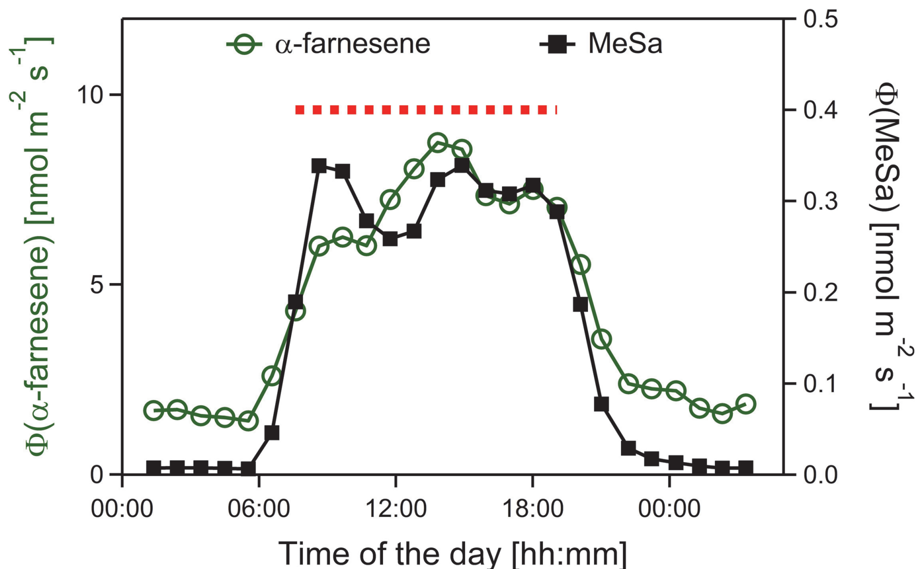

Fig. 5. Diurnal variation of the emission rates of α-farnesene (open circles, left hand scale) and MeSa (closed squares, right hand scale) measured at a chamber temperature of 20 °C during darkness (0:00 to 5:00 and 19:00 to 0:00 LT) and at 24 °C during illumination, respectively. The time period of full illumination (PPFD = 800 µmol m^{-2} s^{-1}) is indicated by the red dashed bar.

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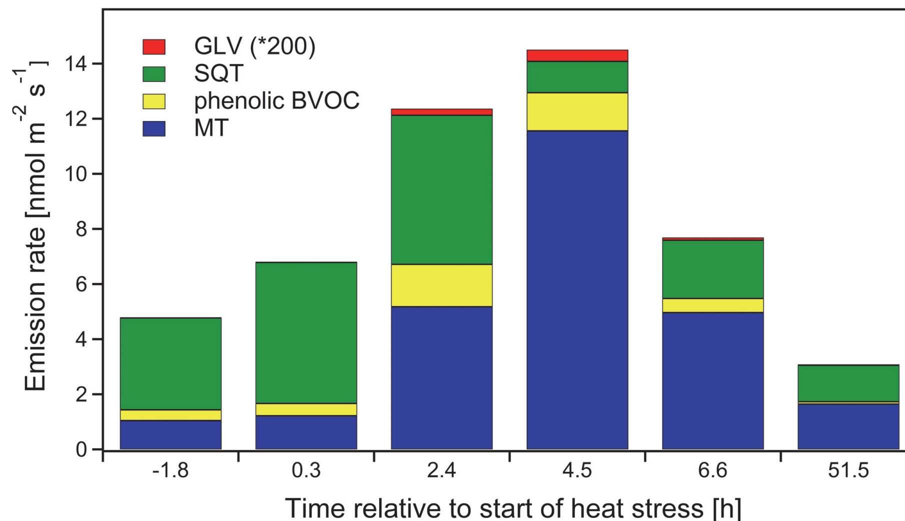

Fig. 6. Emission rates for different BVOC classes before, during and after a heat stress application to an insect infested pine. Blue bars = sum of MT emissions, yellow bars = sum of phenolic BVOC, green bars = sum of SQT emissions, red bars = sum of GLV emissions multiplied by 200 for visibility. Numbers at the x-axis indicate the time of measurement relative to starting heat stress application (46 °C, 4 h).

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