Eesti Maaülikool
Estonian University of Life Sciences

THE EFFECTS OF HEAT STRESS SEVERITY ON PHOTOSYNTHESIS AND VOLATILE ORGANIC COMPOUND EMISSIONS IN BLACK MUSTARD AND TOBACCO

KUUMASTRESSI MÕJU MUSTA KAPSASROHU (*BRASSICA NIGRA* L.) JA VÄÄRISTUBAKA (*NICOTIANA TABACUM* L.) FOTOSÜNTEESILE JA LENDUVÜHENDITE EMISSIOONIDELE

KAIA KASK

A thesis
for applying for the degree of Doctor of Philosophy
in Applied Biology

Väitekiri
filosoofiadoktori kraadi taotlemiseks rakendusbioloogia erialal

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Kõik, mida vajad, tuleb su juurde
ühel või teisel varjatud kujul.
Kui tunned ta ära,
saab ta su omaks.

Kõik, mida tahad, tuleb su juurde,
tunneb su ära ja saab sinu osaks.

Hinga, loe kümneni.

Hind selgub hiljem.

Doris Kareva

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The current thesis is based on the following research papers, which are referred to by their Roman numerals:

I **Kask K.**, Kännaste A. & Niinemets Ü. (2013) Emission of volatile organic compounds as a signal of plant stress. *Scientific Bulletin of ESCORENA 8*, 79–93.

II **Kask K.**, Kännaste A., Talts E., Copolovici L., Niinemets Ü. (2016) How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*. *Plant, Cell and Environment 39*, 2027–2042.

III Turan S., **Kask K.**, Kanagendran A., Li S., Anni R., Talts E., Rasulov B., Kännaste A., Niinemets Ü. (2019) Lethal heat stress-dependent volatile emissions from tobacco leaves: what happens beyond the thermal edge? *Journal of Experimental Botany, 70*, Issue 18, 5017–5030.

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ABBREVIATIONS

A	Net assimilation rate
BVOC	Biogenic volatile organic compound
g_s	Stomatal conductance to water vapour
GC-MS	Gas chromatography-mass spectrometry
GGDP	Geranylgeranyl diphosphate pathway
GDP	Geranyl diphosphate pathway
GLS	Glucosinolate
GLV	Green leaf volatile
LOX	Lipoxygenase pathway
MEP/DOXP	2C-methyl-D-erythritol-4-phosphate pathway
PSII	Photosystem II
PTR-QMS	Proton transfer reaction-mass spectrometer with a quadrupole detector
ROS	Reactive oxygen species
$T_{control}$	Temperature of control treatments
T_{leaf}	Leaf temperature
T_{stress}	Applied stress temperature

1. INTRODUCTION

Lichtenthaler (1996) defined plant stress as "any unfavourable condition or substance that affects or blocks a plant's metabolism, growth or development". The unfavourable conditions that trigger plant stress responses at the molecular and cellular level are divided among abiotic and biotic stress factors (Pereira, 2016; Rejeb et al., 2014; Suzuki et al., 2014). Abiotic stress factors are for example low or high temperature, extreme levels of light, drought, flooding, ozone, etc. (Guy et al., 2003; Hu et al., 2013; Karlsson et al., 1997). At the same time viruses, pathogens and herbivorous insects are considered biotic stress factors (Mumm et al., 2008; Shen et al., 2016; Toome et al., 2010).

Climate change and its impact on nature are widely discussed topics. It is predicted that in the near future global temperature may rise up to 2°C (IPCC, 2014; Kinley, 2017). Such a change in temperature will have a negative impact on the production of several primary crops such as maize, rice and wheat (Fahad et al., 2017; Lesk et al., 2016). Due to heat stress, plant morphology, physiology and biochemistry changes, and this leads to reduced photosynthesis, although photosynthesis is able to recover when the threshold for permanent damage is not exceeded (Hüve et al., 2011; Song et al., 2014).

During plant lifetime, exposure to different abiotic and biotic stresses is a routine event; however, not always stress leads to damage. Injury symptoms may not be detected if stress period and/or intensity are not too long and high (Lichtenthaler, 1996, 1998; Niinemets, 2010).

Model plants such as thale cress (*Arabidopsis thaliana* L.), tobacco (*Nicotiana tabacum* L.), European aspen (*Populus tremula* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) have been used in numerous studies; most of them are agriculturally valuable plants due to their biological characteristics (Chang et al., 2016; Jansson & Douglas, 2007; Vos et al., 2010). Knowledge about model plant biochemistry, development and physiology can be applied to other plants (Chutteang et al., 2016; Street et al., 2011). Nevertheless, a growth environment with its abiotic and biotic stress factors that is suitable for one plant species may be stressful to another plant species. For example, accessions of *A. thaliana* winter annuals tolerate aphids, thrips and drought, but summer annuals survive

the *Pieris rapae* (L.) and *Plutella xylostella* (L.) caterpillar attacks (Olivas et al., 2017). In addition, Angadi et al., (2000) found that *Brassica napus* (L.) and *B. juncea* (L.) are more resistant to heat stress than *B. rapa* (L.).

Given the high genetic diversity within species, plant responses to stressors are also varying (Evans et al., 2017; Nankishore & Farrell, 2016). For example, in key model plant species such as *A. thaliana*, bean, and poplar both ozone-sensitive and -tolerant genotypes are available (Beauchamp et al., 2005; Brosché et al., 2010; Guidi et al., 2009; Street et al., 2011). Yet, given the species richness of the plants, studies involving abiotic and biotic factors constitute a great challenge and questions of how different plant species sense various stressors, which biosynthetic pathways and enzymes are evolved for biosynthesizing the stress signals, how the signals are transmitted within the plant leaves and between different organs, are still waiting for detailed answers (Mengiste et al., 2003; Wu et al., 2009; Yoshida et al, 2014).

1.1. Volatile organic compound emissions by plants

Plant roots, flowers, fruits, and leaves release complex biogenic volatile organic compounds (BVOCs), which serve as info-chemicals between plants or plants and herbivores (Delory et al., 2016; Maron, 1998). It has been demonstrated, that neighbouring plants affect the volatile blend of a target plant; for example, *Trifolium pratense* (L.) monoterpene and green leaf volatile emissions increased when growing together with *Dactylis glomerata* (L.)(Broz et al., 2010; Kigathi et al., 2013, 2019). BVOCs also take part in atmospheric chemistry and composition processes, react with ozone and other air oxidants and form secondary aerosols (Calfapietra et al., 2009; Fuentes et al., 2000; Loreto et al., 2014).

Plants typically release BVOC blends with one or a few main volatile compounds that either repel pests or attract pollinators and/or parasitoids (Arimura et al., 2005; Junker et al., 2017; Piesik et al., 2013). Monoterpenes α-pinene and limonene and sesquiterpenes β-caryophyllene and α-copaene, were the most common compounds emitted by the 55 tropical tree species (Courtois et al., 2009). Methanol was the main volatile in the young leaves of some Mediterranean species, and the emissions decreased when leaves became mature (Bracho-Nunez et al., 2011). It has been found that α-farnesene acted as a repellent to coffee berry borer, *Hypothenemus hampei* (Ferrari) (Vega et al., 2017), and

N. tabacum odour was a repellent to coffee berry borer in laboratory and field experiments (Castro et al., 2017). The general attractiveness to parasitoids can be reduced by some volatiles. For instance, indole makes *Spodoptera littoralis* (Boisduval) caterpillars undesirable to the parasitoid *Microplitis rufiventris* (Kokujev) (Ye et al., 2018). Appearance of green leaf volatiles (GLVs) (various C6 aldehydes and alcohols) like (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol in plant emissions shows a stress response (**Paper I**) and the total amount of GLVs released indicates the severity of stress (Arimura et al., 2009; Farag & Pare, 2002; Niinemets, 2010).

Stress-induced BVOCs are emitted in response to abiotic or biotic stresses (**Paper I**) of different severity, reflecting changes in the activity of biochemical pathways or mechanical damage that can release stored volatiles. Such stress-induced emissions have been observed in plants subjected to heat and drought stress or after insect biting/wounding (Allmann & Baldwin, 2010; Gutbrodt et al., 2012; Joó et al., 2011).

Plant-emitted BVOCs are biosynthesized via several biochemical pathways: i) the lipoxygenase pathway (LOX) for green leaf volatiles in cytosol (GLVs); ii) methylerythritol 4-phosphate (MEP) pathway for monoterpenes, isoprene and GGDP-pathway (geranylgeranyl diphosphate) compounds in plastids; iii) mevalonic acid (MVA) pathway for volatile sesquiterpenoids in cytosol and iv) shikimic acid pathway for aromatic volatiles in plastids (Mejia-Garibay et al., 2015; Niinemets et al., 2013). The rate of synthesis of GGDP-pathway compounds like geranyl acetone and 6-methyl-5-hepten-2-one (also called apocarotenoids) increases, as fruits ripen (Simkin et al., 2004). Their biosynthesis takes place in the plastids, where monoterpene synthases are situated (Tholl, 2006). The bouquet of emitted BVOCs is plant species-specific, however, environmental conditions, including season, temperature, light, humidity, the damage of herbivores, also leaf and plant age strongly affect BVOC emission (Arimura et al., 2005; Bracho-Nunez et al., 2011; Gols et al., 2008; Rosa & Rodrigues, 1998; Staudt et al., 1997; Usano-Alemany et al., 2014; Zhang et al., 2009).

Besides previously mentioned pathways, black mustard contains different sulphur- and nitrogen-containing compounds called glucosinolates (GLSs) that are derived from amino acids and sugars (Ishida et al., 2014; Sønderby et al., 2010). The aforementioned metabolites give the brassicaceous species-specific taste and smell and have allelopathic

effects (Klopsch et al., 2018). Glucosinolate content varies quantitatively and qualitatively across the plant species and cultivars (Cartea et al., 2012; Kirkegaard & Sarwar, 1998; Sotelo et al., 2016). Besides, the content of glucosinolates is related to the plant growth phase and plant physiological status (Dicke, 2016; Pashalidou et al., 2015).

Usually, there are three to four dominant glucosinolates in the blend, but up to 15 various metabolites can be found in the same plant (Cartea et al., 2012; Klopsch et al., 2018). Nowadays the documented number of glucosinolates is up to 130 (Sønderby et al., 2010) and more than 30 are found in the *Brassica* species (Sarwar & Kirkegaard, 1998). For the adjustment to biotic and abiotic stresses, *Brassica* species have huge genetic and morphological variety. Many studies have explored the great diversity of glucosinolates and their breakdown products in *B. rapa*, *B. oleracea*, *B. napus*, *B. nigra* and also in *Eruca sativa* (Mill.) and *Arabidopsis* (Bell et al., 2015; Gielen et al., 2006; Kliebenstein et al., 2001; Klopsch et al., 2018; Newton et al., 2009; van Dam et al., 2004).

Plant cells that contain glucosinolates are relatively stable, but when they get damaged (for example, during feeding of a herbivore or mechanical damage), the enzyme β-thioglucosidase also called myrosinase is released and volatile glucosinolate breakdown products like isothiocyanate, nitriles, thiocyanates are formed (Bones & Rossiter, 2006; Halkier & Du, 1997; Rask et al., 2000; Sønderby et al., 2010). Depending on the plant species, typically, the myrosinase is stored in idioblasts (myrosin cells), while glucosinolates are stored in intracellular compartments in vacuoles (Augustine & Bisht, 2017; Kelly et al., 1998; Redovniković et al., 2008). Glucosinolate breakdown products act as repellents for insects, microorganisms and generalist herbivores or attractants for insect specialists (Agrawal & Kurashige, 2003; Mumm et al., 2008; Pashalidou et al., 2015). Glucosinolate breakdown products have also fungicidal and bactericidal effects (Barba et al., 2016).

2. REVIEW OF THE LITERATURE

2.1. Black mustard

Black mustard (*Brassica nigra* L.) as a crop plant is common in Asia and Europe. It grows and spreads widely to old agricultural areas and has thus become a troublesome weed (Gomaa et al., 2012). Nevertheless, due to the rapid growth, high stress tolerance, and complex genome, it is providing novel information to the brassicaceous model system (Bischoff & Hurault, 2013; Duke, 1983; Westman & Kresovich, 1999). Furthermore, certain *B. nigra* ecotypes are used for phytoremediation (Cevher-Keskin et al., 2019). In a recent study of Farré-Armengol et al., (2016) ozone dose determined the behaviour of *B. nigra* pollinators towards ozone-treated plants. In another study, depending on ozone stress severity, the parasitoid *Cotesia glomerata* (L.) discriminated the *Pieris brassicae* (L.) attacked plants from the non-attacked ones (Khaling et al., 2016). There are limited data about *B. nigra* stress tolerances, especially about tolerance to heat stress (Waters & Schaal, 1996), although there are studies looking at plant-insect interactions (Blatt et al., 2008; Lucas-Barbosa et al., 2017; Ponzio et al., 2017).

2.2. Tobacco

Besides being a model plant, tobacco (*Nicotiana tabacum* L.) has the key tool, the BY-2 plant cell line, which is widely used in plant molecular investigations (Nagata et al., 1992). Tobacco gives valuable input to plant disease susceptibility as it shares some diseases with tomato, potato and pepper that all belong to the *Solanaceae* family (Sierro et al., 2014; Srba et al., 2016). Tobacco stands out in all crop plants that are generally cultivated around the world in open fields and nowadays a special market for organic tobacco is a novel direction (Bilalis et al., 2009, 2010; Chantal et al., 2013). Its traditional agricultural value is in its biomass and alkaloid production, but its importance is rising in the production of beneficial recombinant pharmaceutical proteins (Colgan et al., 2010; Fischer & Emans, 2000; Schillberg et al., 2013). Earlier, drought and temperature stress and ozone tolerance of tobacco have been studied (Gerardin et al., 2018; Jud et al., 2016; Pollastri et al., 2019; Yang et al., 2018). Additionally, a gene (BcICE1) from cold-resistant *Brassica*

campestris 'Longyou 6' has been transferred to tobacco to investigate its cold tolerance (Zhang et al., 2018). Extracts made from tobacco can be used as insecticides in agriculture (Sarker & Lim, 2018).

2.3. Elevated temperature stress and its effect on photosynthesis

In natural conditions, temperature fluctuations within a 24 h period are a common phenomenon. Additionally, during heatwaves, leaf temperatures can exceed 50°C (Singsaas et al., 1999; Singsaas & Sharkey, 2000). Hüve et al., (2019) have shown that for any given leaf surface minimum and maximum temperatures can differ even up to 10 ºC during sunflecks. Heat episodes differ in their longevity and temperature levels (Sharkey, 2005; Talukder et al., 2014; Zhang et al., 2009). Temperature increases from low values at night-time to high values in direct sunlight, and strongly influences the rates of all plant physiological processes (Sung et al., 2003). In fact, even a mild temperature change is adequate to trigger multiple cellular responses such as diminished photosynthesis, reduced chlorophyll content or metabolite transport and cell expansion and division (Allakhverdiev et al., 2008; Hüve et al., 2011; Niinemets, 2010).

Temperatures between 35 to 40°C (42°C) are typically considered moderate stress (Mainali et al., 2014; Sharkey, 2005; Sinsawat et al., 2004). Temperatures above 43-45°C are considered as severe heat stress, but the tolerance to high temperatures is species-specific and even depends on the genotypes within species (Camejo et al., 2005; Mittler et al., 2012; Ortiz & Cardemil, 2001).

Severe heat stress can cause irreversible damage to plant photosynthetic apparatus, resulting in reduced plant growth and development, ultimately leading to yield losses (Hüve et al., 2011; Siebert et al., 2014). For example, CO_2 assimilation depends on the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity (Du et al., 1999; Haldimann & Feller, 2004; Kim & Portis, 2005; Perdomo et al., 2017; Yan et al., 2013). Moderate temperatures slow down photosystem II (PSII) activity, but severe temperatures cause impairment of PSII, stomatal closure, membrane leakiness, production of different reactive oxygen species (ROS), ultimately leading to cellular death (Niinemets, 2010; Sharkey, 2005; Song et al., 2014; Zhang et al., 2009). For example, net photosynthesis decreased over 50% when *Quercus ilex* (L.) leaf

temperature exceeded 37 ºC and in *Solanum lycopersicum* (L.) it reached close to zero when treated with 49 ºC, indicating great damage in foliar photosynthesis (Copolovici et al., 2012; Gratani et al., 2000).

2.4. Heat stress effects on constitutive and induced volatile emissions

Heat stress causes the release of various constitutive and induced volatiles (**Paper I**) (Usano-Alemany et al., 2014; Velikova et al., 2009). Constitutive volatiles are emitted from special storage structures, for example from glandular trichomes present on the leaf surface of *N. tabacum* (**Paper III**) (Lange & Turner, 2013; Nayidu et al., 2014). *B. nigra*, on the other hand, has non-glandular trichomes (**Paper II**) that probably provide a mechanical barrier against herbivory as shown previously in a study done with *Phaseolus vulgaris* (L.) (Nayidu et al., 2014; Xing et al., 2017). Constitutive emissions are released both from plants growing under favourable conditions and under stress, but stress modifies the rate of emissions (Niinemets, 2010). Under stress conditions, the release of BVOCs can become either amplified (stress-induced volatiles) or reduced (constitutively emitted volatiles) (Klaiber et al., 2013; Lehrman et al., 2013; Niinemets et al., 2013). For example, heat stress increases the emission of BVOCs stored inside the leaf or in glandular trichomes, as demonstrated for 2-carene and limonene emissions from *S. lycopersicum* (Copolovici et al., 2012; Loreto et al., 1998, 2004). In addition, induced emissions can contain several novel compounds that are emitted immediately or with some delay from the plants after the stress factor has activated the volatile biosynthetic pathways (Beauchamp et al., 2005; Kleist et al., 2012). For example, the main stress volatiles that indicate cellular damage and are emitted upon heat stress are GLVs like (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate and 1-hexanol, all formed via lipoxygenase (LOX) pathway (Copolovici et al., 2012; Kask et al., 2016).

Methanol is emitted by most plants during the growth of leaves at the time of cell wall formation as a result of pectin demethylation (Fall & Benson, 1996). Yet, methanol emissions also tend to increase as leaf temperature increases (Folkers et al., 2008; Macdonald & Fall, 1993).

Plant emissions contain several MEP/GDP (methylerythritol/ geranyl diphosphate) pathway compounds like monoterpenes. Their biosynthesis takes place in the plastids, where the final enzymes and monoterpene

synthases are situated (Chen et al., 2011; Tholl, 2006). The release of monoterpenes may be either constitutive or induced. For example, in control treatments, α-pinene, β-pinene, 3-carene have been found in *B. nigra* emissions (Khaling et al., 2016). In *Q. ilex*, temperature rise up to 35 °C increased α-pinene and β-pinene emission, but emissions declined when leaf temperature further increased (Loreto et al., 1998). Additionally, increased emission of GGDP-pathway compound geranyl acetone is related to the oxidative cleavage of carotenoids in heat stressed plants (García-Plazaola et al., 2017; Goff & Klee, 2006).

Emission of volatile glucosinolate breakdown products upon heat stress indicates that glucosinolates and myrosinases are engaged (Burow et al., 2007; Kelly et al., 1998; Zhao et al., 2008). The blend of released volatiles is affected by the combination of various glucosinolates, protein cofactors and pH in plant cells (Ahuja et al., 2010; Bones & Rossiter, 2006; Yan & Chen, 2007). For example, Fe^{+} and acidic pH are required for the epithiospecifier (ESP) protein, that converts glucosinolates with aliphatic side chain via hydrolysis to nitriles, but under neutral pH, isothiocyanates like allyl isothiocyanate and methyl isothiocyanate are formed (Backenköhler et al., 2018; Lambrix et al., 2001).

3. AIMS OF THE STUDY

The general aim of this thesis was to quantify the responses of *Brassica nigra* L. and *Nicotiana tabacum* L. to heat stress in order to understand whether the plant response is related to the stress severity, to what extent the foliage photosynthetic characteristics and volatile emissions change and finally, whether the stress response is plant species-specific.

The specific aims of the thesis were:

1. to gain an insight into the relationships among ubiquitous stress responses and brassicaceous-specific stress responses through the different heat stress treatments in *Brassica nigra;*
2. to characterize the effects of heat shock varying from moderate (46 °C) to extremely severe (55 °C) on *Nicotiana tabacum;*
3. to evaluate species differences in responding to heat stress of varying severity;
4. to study the relationships between the critical heat stress thresholds for impairment of foliage photosynthetic characteristics and stress volatile emissions.

Hypotheses:

Paper I is a review paper, due to that no hypotheses were created. However, presented topics give an overview of plant volatiles and stress and their interactions with the environment.

Paper II hypothesized that severe heat stress leads to the elicitation of glucosinolate volatiles and that the emissions are quantitatively more significant upon long-term stress due to the elicitation of induction responses.

Paper III hypothesized that once the critical heat stress threshold is reached, foliage photosynthetic characteristics are impaired, while key stress volatile emissions increase even above the temperatures that lead to the collapse of the photosynthetic machinery.

4. MATERIALS AND METHODS

4.1. Plant material

Seeds of black mustard (*Brassica nigra* L.)(**Paper II**) were received from the Department of Entomology, University of Wageningen, the Netherlands. Seeds were sown in a commercial garden soil (Biolan Oy, Finland) mixed with quartz sand and kept at day/night temperature of 24/20 °C and 60% of air humidity. Light intensity at the level of plant leaves was 400 μmol m^{-2} s^{-1} (metal halide lamps, HPI-T Plus 400 W, Philips, Eindhoven, Netherlands) provided for 12 h light period. Plants were watered regularly and 5 to 6 weeks old plants were used in the experiments.

Seeds of tobacco (*Nicotiana tabacum* cv. Wisconsin 38) (**Paper III**) were germinated in Kekkilä garden soil (Kekkilä Group, Vantaa, Finland). Upon germination, each seedling was replanted to 4 L plastic pots, and cultivated in the similar substrate in the plant growth room at day/night temperatures of 27/23 °C and 60% of relative humidity. The day length was 14 h and the light irradiance at plant level was 400-500 μmol m^{-2} s^{-1} (metal halide lamps, HPI-T Plus 400 W, Philips, Eindhoven, Netherlands). Plants were watered daily and fertilized with 0.5% fertilizer solution (Baltic Agro, Lithuania; NPK content ratio: 5:5:6; and micronutrients B (0.01%), Cu (0.03%), Fe (0.06%), Mn (0.028%), and Zn (0.007%)) on a weekly basis.

Fully mature non-senescent leaves of nine- to ten-week-old and 40 to 60 cm tall tobacco plants were used. All measurements were done with attached leaves. Two to three days before the stress treatments, part of the axial leaf tissue was removed, so that the remaining portion of the leaf of 25-40 cm^2 could be efficiently analysed (see Chapter 4.5 for experimental setup). The integrity of major veins was retained such that the photosynthetic activity of the preserved portion of the leaf was not significantly different from the intact leaf. Leaf wounding elicited a major release of short-lived LOX pathway volatiles (Brilli et al., 2012; Brilli et al., 2011; Portillo-Estrada et al., 2015). Yet, on a day of heat stress treatments, no LOXs or other stress volatiles were observed (Kanagendran et al., 2018; Li et al., 2018). There were also no quantitative

or qualitative variances in the bouquets of base-level volatile emissions among mechanically wounded and intact leaves (data not shown).

4.2. Gas-exchange measurements and collection of BVOCs

Foliage photosynthetic rates for *B. nigra* (**Paper II**) and *N. tabacum* (**Paper III**) were measured in a closed gas-exchange chamber, made of double-walled glass placed above stainless steel bottom (Copolovici & Niinemets, 2010). Leaf temperature (T_{leaf}) in the chamber was controlled by the temperature of water in a water bath that circulated water between chamber glass layers. Other conditions for gas-exchange measurements and BVOC collection, including light intensity, chamber CO_2 concentration, humidity and gas flow through the chamber are briefly explained in Table 1 and explained in detail in **Papers II** and **III**.

Table 1. Gas-exchange measurement system conditions for *Brassica nigra* and *Nicotiana tabacum* for measuring foliage photosynthetic rates.

	Brassica nigra	***Nicotiana tabacum***
Foliage photosynthesis recording device	Ciras II	Li-7000
Light at plant level (μmol m^{-2} s^{-1})	800	500
CO_2 concentration (μmol m^{-2} s^{-1})	380-400	380-400
Humidity, %	~60	~60
Flow (L/min)	1.2	1.26
Ambient air to the system	drawn from outside	drawn from outside
Ozone removal	HCl-activated copper tubing	ozone scrubber
Stabilization for plant, min	on average 20	10-20

4.3. Temperature response curve measurement

No less than three top leaves of *B. nigra* (**Paper II**) were enclosed in the gas-exchange system and left to stabilize for 20-30 min at the temperature of 20 °C (control) (Fig. 1).

Gas-exchange measurement conditions are shown in Table 1. When net assimilation rate (A) and stomatal conductance to water vapour (g_s) were

stable (in 20-30 min), A and g_s values were recorded and BVOCs were collected. The gas-exchange chamber temperature ($T_{chamber}$) was then set to the next higher temperature. Again, the photosynthetic characteristics were recorded and BVOCs collected after A and g_s values became stable. Altogether, foliage photosynthetic characteristics were measured at $T_{chamber}$ of 20 °C (control plants), 25 °C, 30 °C, 35 °C and 40 °C and BVOCs were collected at 20 °C, 25 °C, 30 °C and 40 °C.

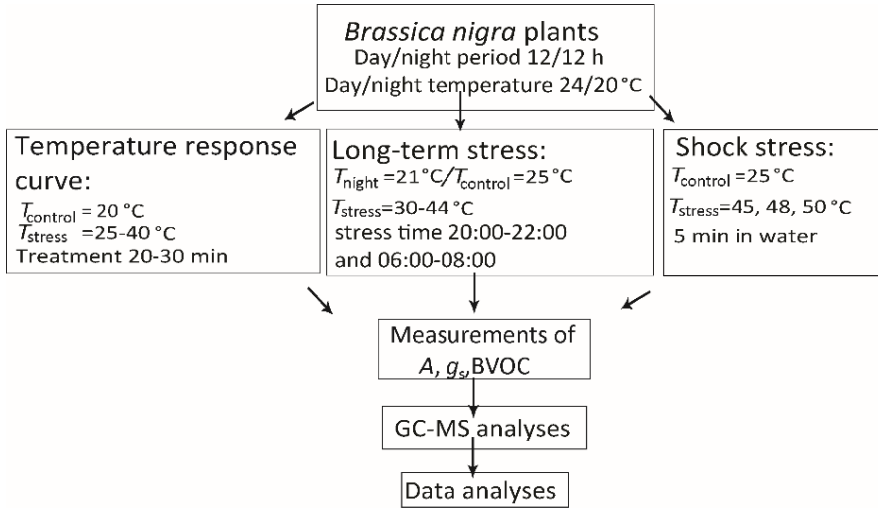

Figure 1. A schematic representation of the experimental design for *Brassica nigra* plants subjected to three diverse heat treatments: temperature response curve measurement, long-term heat stress, and shock stress. Plants were placed in a gas-exchange system to measure foliage net assimilation (A) rate and stomatal conductance to water vapour (g_s) and to collect BVOCs (analysed with a GC-MS system). In the case of temperature response curve measurements, the stress treatments and physiological measurements occurred simultaneously at the treatment temperature. In the long-term and shock stress, the physiological measurements were taken after the heat stress treatment at 25 °C (modified from **Paper II**).

4.4. Long-term heat stress treatment

For long-term heat (Fig. 1) stress study (**Paper II**), potted *B. nigra* plants were transferred from the growth room to a Percival growth chamber (model E-36HO, Percival Scientific, Inc., Perry, IA, USA). Before stress, the plants acclimated for 24 h at controlled day/night temperature of 25/21 °C and a humidity level of 60%. Day length was 16 h and light intensity at plant level was set to 400 μmol m^{-2} s^{-1}. Heat stress was applied in two impulses, the first one in the evening between 20:00-

22:00 h, and the second one the next morning between 06:00-08:00 h. In total, stress treatment lasted for 4 h, but during night time between stress treatments, the temperature was 21 °C, which enabled a recovery of plants. Growth chamber temperatures were set to 25 °C (control), 30 °C, 35 °C, 40 °C and 44 °C. After the stress treatment, individual plants were placed in the gas-exchange system for foliage photosynthesis and BVOCs measurements, which were done promptly at 25 °C.

4.5. Heat shock stress

To apply the heat shock stress treatment, *B. nigra* plants (**Paper II**) were treated according to the protocol of Copolovici et al., (2012). In a temperature-controlled glass vessel equipped with a magnetic stirrer (Heidolph MR Hei-Standard with an EXT Hei-Con temperature sensor, Heidolph, Schwabach, Germany) distilled water was heated to 25 °C (control), 45 °C, 48 °C and 50 °C. The uppermost leaves (no less than three) were placed in the water for 5 min. Wet leaves were allowed to air dry for approximately 5 min. The same protocol was used for studying the stress response of *N. tabacum* (**Paper III**), except that the same tobacco leaf was used for both, the control measurement and the given stress treatment. After evaluating the response of a non-stressed leaf (25 °C), the same leaf was exposed to either 46, 47, 48, 49, 50, 51, 52, 53, 54 or 55 °C (Fig. 2). Hence, in total 34 leaves were studied. In addition, differently from the *B. nigra* experiment, the water bath MB-5 (Julabo GmbH, Germany) was used and stress application lasted for 10 min. Finally, after heat stress application, gas-exchange rates and BVOCs were collected from *B. nigra* and *N. tabacum*.

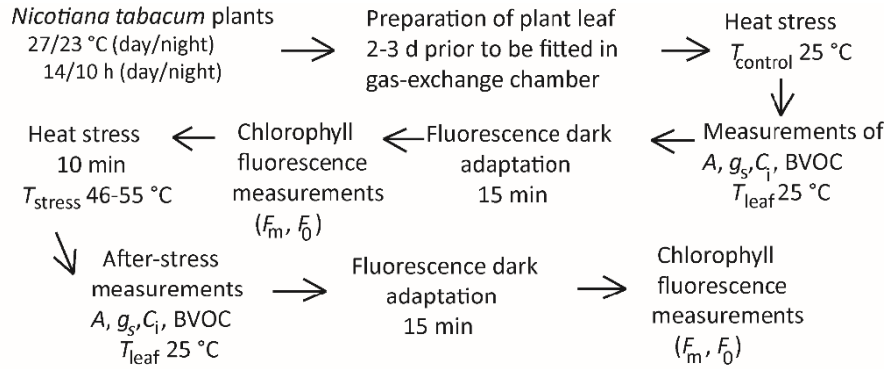

Figure 2. A schematic representation of the experimental design for tobacco (*Nicotiana tabacum* cv. Wisconsin 38) heat stress experiment. Two to three days prior to heat stress treatment, a part of the leaf tissue was removed so, that the leaf could be fitted in the gas-exchange system. Heat treatments were applied for 10 min. by immersion of leaves in water (25 °C for control leaves, 46-55 °C for heat stressed leaves). After this, the leaf was placed in the gas-exchange system for net assimilation rate (A), stomatal conductance to water vapour (g_s), intercellular CO_2 (C_i) and BVOCs measurements at leaf temperature (T_{leaf}) of 25 °C. After these measurements, the leaf was dark-adapted for 15 min. for chlorophyll fluorescence measurements. Each individual leaf was treated only once at one of the heat shock temperature intervals indicated above (reproduced and modified from **Paper III**).

4.6. Collection of BVOCs and their quantification with GC-MS and PTR-QMS

A brief explanation of BVOCs collection and detection in *B. nigra* and *N. tabacum* is provided in Table 2 and explained in details in the Materials and Methods part in **Papers II** and **III**, respectively.

Table 2. BVOCs collection and detection in *B. nigra* and *N. tabacum*.

	BVOCs collection and detection	
	Brassica nigra	***Nicotiana tabacum***
Pocket pump flow, ml/min	200	200
Collecting time of BVOCs, min	20	15
Cartridges	stainless steel multi-bed cartridges	stainless steel multi-bed cartridges
Device for the detection of volatiles	Shimadzu TD20 connected with Shimadzu 2010 Plus GC-MS system	Shimadzu TD20 connected with Shimadzu 2010 Plus GC-MS system; PTR-QMS (for methanol)

BVOC emissions were calculated as in Niinemets et al., (2011):

$$\Phi_i = \frac{peak\ area_i * F * 10^4 * 10^9}{calibration\ factor * M * 60 * S * V}$$

Φ_i – emission rate of a compound (nmol m^{-2} s^{-1})
Peak $area_i$ – peak area of an identified volatile
F – gas flow in the gas-exchange chamber (L/min)
M – molar mass of the volatile (g/mol)
S – leaf area (cm^2)
V – volume of the gas through the cartridge (L)

4.7. Data analysis

Net assimilation rate (A) and stomatal conductance to water vapour (g_s) for *B. nigra* (**Paper II**) and *N. tabacum* (**Paper III**) were calculated according to von Caemmerer and Farquhar (1981).

Foliage photosynthetic characteristics and emission rates of volatiles were expressed as average ± SE. Data were log-transformed and foliage gas-exchange and volatile emission rates of *B. nigra* (**Paper II**) and *N. tabacum* (**Paper III**) at different temperatures were compared with one-way ANOVA followed by a Tukey test. Linear and non-linear relationships were tested to estimate the co-variance between the gas-exchange characteristics and emission rates of BVOCs (Statistica, StatSoft Inc., Tulsa, OK, USA; SigmaPlot ver. 12.5, Systat Software, Inc., San Jose California USA). All statistical tests were considered statistically significant at $P<0.05$.

5. RESULTS

5.1. Photosynthesis affected by different heat stresses

Generally, the temperature rise inhibited the photosynthetic capacity of both plants, *Brassica nigra* (**Paper II**) and *Nicotiana tabacum* (**Paper III**). For the temperature response curve treatment (**Paper II**), a stepwise temperature increase from 20 °C (control) to 35 °C slightly reduced the net assimilation rate (A) of *B. nigra* from 8.5±1.7 to 5.5±1.7 μmol m^{-2} s^{-1}. Yet, after 40 °C treatment, A fell to 2.6±0.8 μmol m^{-2} s^{-1}, which was considerably lower than A of control plants (Fig. 3a). Together with A, the stomatal conductance to water vapour (g_s) also strongly decreased from 99±13 mmol m^{-2} s^{-1} in control plants to 13±4 mmol m^{-2} s^{-1} measured after 40 °C treatment (Fig. 3b).

Long-term heat stress (**Paper II**) caused a noticeable decrease in A from 10.4±0.9 μmol m^{-2} s^{-1} (25 °C) to 2.5±0.9 μmol m^{-2} s^{-1} (44 °C). The highest g_s – value (69±10 mmol m^{-2} s^{-1}) was characteristic to the control plants (25 °C). After 30 °C treatment g_s fell to 35±9 μmol m^{-2} s^{-1} and remained stable for 35 °C and 40 °C treatments. Yet, a further 4 °C temperature increase reduced g_s to 24±5 mmol m^{-2} s^{-1} (Fig. 3b).

Differently from temperature response curve and long-term heat stress experiments, the heat shock treatment (**Paper II**) had a major effect on A and g_s in *B. nigra*, because temperatures 48 °C and 50 °C lead to negative A values and g_s became almost zero (Fig. 3).

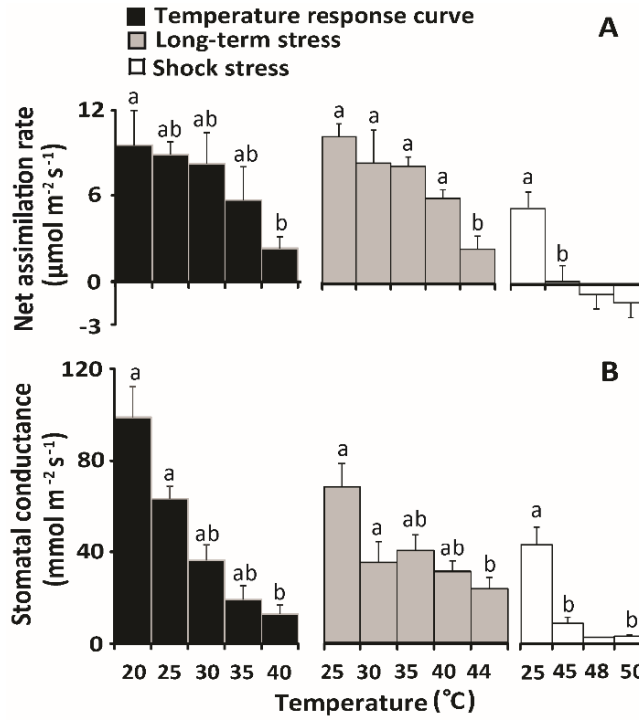

Figure 3. Effect of temperature through temperature response curve measurements (mild stress), long-term stress (chronic stress), and short-term heat shock stress (severe stress) on net assimilation rate (A) and stomatal conductance to water vapour (B) (mean ± SE) in leaves of *Brassica nigra (*experiment details are shown in Figure 1). Data were log-transformed and compared with one-way ANOVA followed by Tukey's *post-hoc* test. Different letters indicate significant differences at $P<0.05$ (reproduced from **Paper II**).

Next to *B. nigra* (**Paper II**), the photosynthetic characteristics of heat stressed *N. tabacum* were also studied (**Paper III**). Compared to the control plants, 46 °C heat shock led to remarkably declined *A* values (Fig. 4a). After 48 °C treatment, *A* was 0.35±0.07 μmol m^{-2} s^{-1} and then it became negative with further increases in temperature. Rising the temperature from 25 °C to 46 °C reduced g_s from 108±9 to 30±11 mmol m^{-2} s^{-1} ($P < 0.05$). At further temperatures up to 53 °C the g_s remained unchanged, yet after 54 °C and 55 °C treatments, g_s surprisingly increased to 66-77 mmol m^{-2} s^{-1}, which was similar to control plants ($P<0.05$) (Fig. 4b).

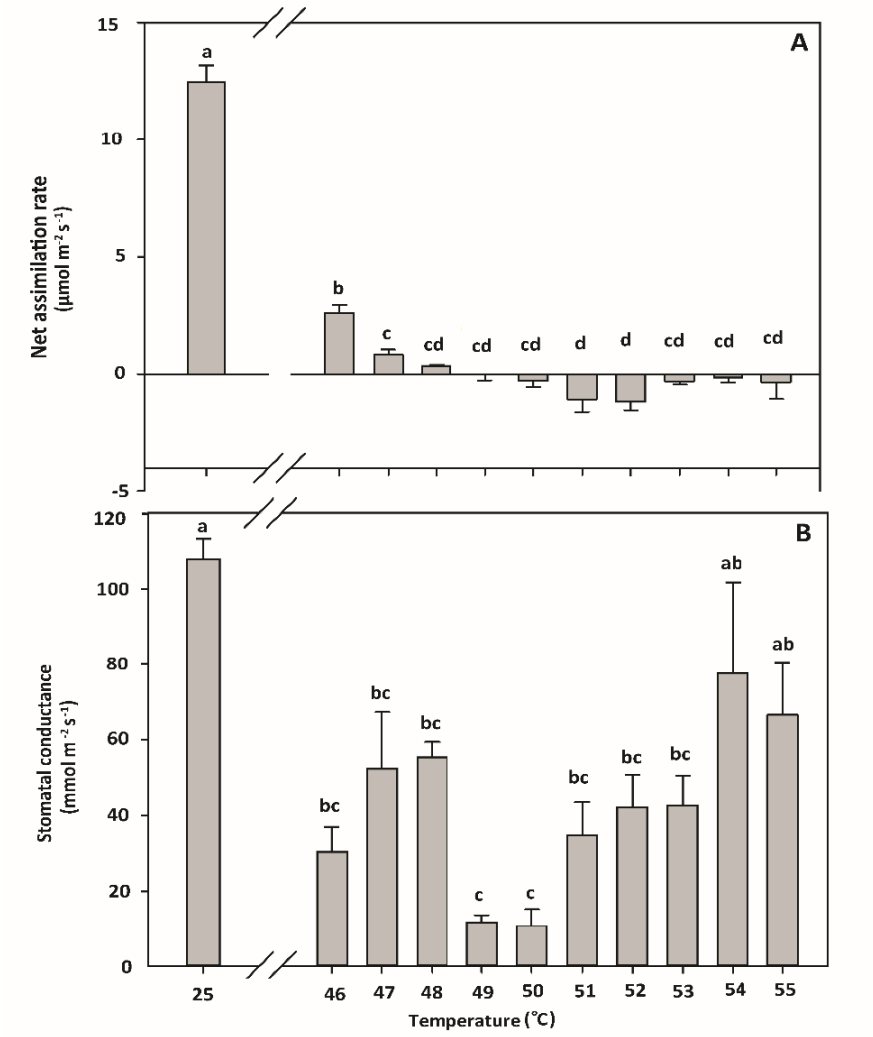

Figure 4. Impact of heat shock on net assimilation rate (A) and stomatal conductance to water vapour (B) (mean ± SE) in the leaves of *Nicotiana tabacum* cv. Wisconsin 38 (experiment details are shown in Fig. 2). Different letters indicate statistically significant differences (ANOVA followed by Tukey test, $P<0.05$) (reproduced from **Paper III**).

5.2. Emission of BVOCs in relation to heat stress severity

In general, the bouquets of heat stressed *B. nigra* and *N. tabacum* emissions consisted of various BVOCs such as green leaf volatiles (GLVs), monoterpenes, geranylgeranyl diphosphate (GGDP) pathway compounds, glucosinolate breakdown products and methanol,

2-ethylfuran, 2-methyl-2-cyclopenten-1-one, etc. The total emission rate of BVOCs strongly depended on heat stress duration and application (Fig. 5 and 6).

5.2.1. The response of *Brassica nigra* to heat stress

In the mild stress treatment, none of the volatile compounds dominated in the odour of *B. nigra* plants. The emission rates of fatty acid derived compounds (LOXs) and volatile glucosinolate breakdown products remained at the same level as in control plants (Fig. 5a, c). Yet, the release of GDP-compounds decreased considerably with increasing temperature from 20 °C to 40 °C (Fig. 5b). A similar trend from 0.0309±0.0027 nmol m^{-2} s^{-1} at 20-25 °C to 0.0043±0.0012 nmol m^{-2} s^{-1} at 30-40 °C was characteristic to GGDP-pathway compounds (Fig. 5d). In contrast, in the long-term stress treatment, the emissions of GDP- and GGDP-compounds did not change (Fig. 5b, d). Yet, as temperature increased from 35 °C to 40 °C, emissions of fatty acid derived compounds (including LOXs) showed an upward trend, increasing from 0.033±0.015 to 0.30±0.09 nmol m^{-2} s^{-1} (Fig. 5a). At 40 °C and 44 °C, the volatile bouquets were dominated by volatile glucosinolate breakdown products, which increased tremendously from 0.0087–0.0193 nmol m^{-2} s^{-1} at 25-35 °C to 1.10±0.43 nmol m^{-2} s^{-1} at 44 °C (Fig. 5c).

Finally, in the heat shock experiment, the total emissions of all aforementioned BVOCs except GGDP-compounds showed an increase with increasing temperature. The emission of GGDPs varied between 0.006 nmol m^{-2} s^{-1} and 0.012 nmol m^{-2} s^{-1} through treatments, but fatty acid derived compounds (including LOXs) increased from 0.036± 0.019 nmol m^{-2} s^{-1} (25 °C) to 1.4±1.0 nmol m^{-2} s^{-1} (50 °C). GDP-pathway compounds increased from 0.013±0.008 nmol m^{-2} s^{-1} (25 °C) to 0.048±0.016 nmol m^{-2} s^{-1} (50 °C), and finally glucosinolate breakdown products from 0.113±0.035 nmol m^{-2} s^{-1} (45 °C) to 0.37±0.09 nmol m^{-2} s^{-1} (50 °C) (Fig. 5a, b and c).

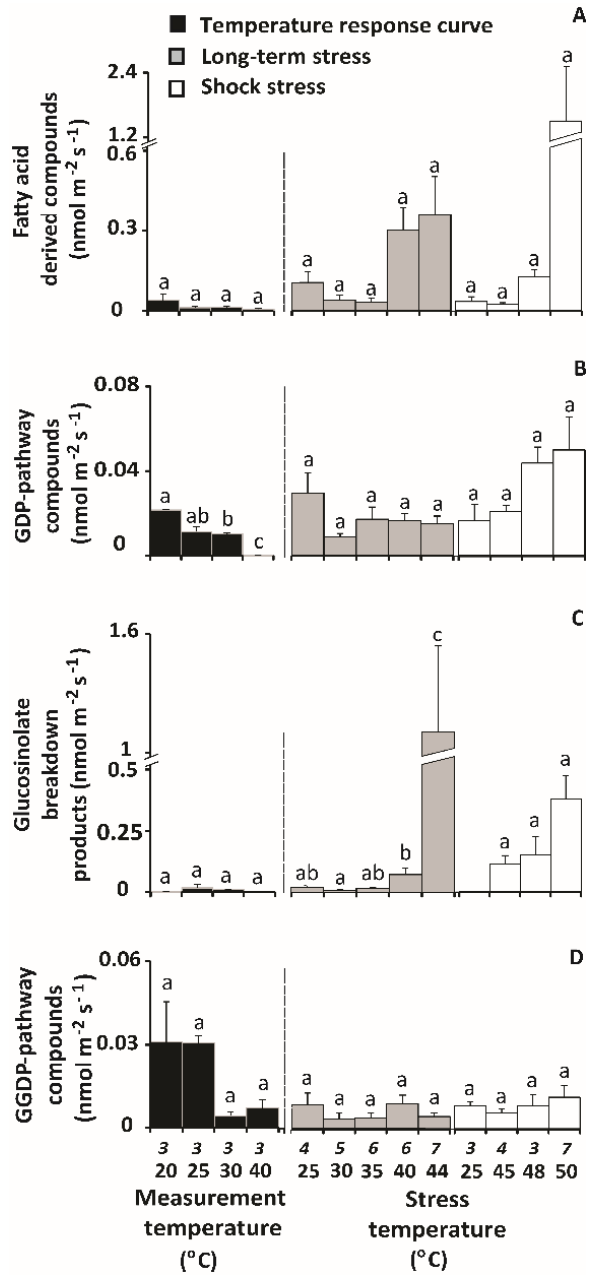

Figure 5. Emission rates (nmol m^{-2} s^{-1}, mean ±SE) of fatty acid derived compounds (A), GDP-pathway compounds (B), glucosinolate breakdown products (C) and GGDP-pathway compounds (D) from *Brassica nigra* in three diverse temperature stress treatments – temperature response curve (black bars), long-term (gray bars), and shock stress (white bars). The number of biological replicates is indicated above the temperature values. Emissions of individual BVOCs within each group are presented in Table 1 in **Paper II** (reproduced from **Paper II**).

5.2.2. The response of *Nicotiana tabacum* to heat stress

After heat shock treatments from 46 °C to 52 °C, the release rate of LOXs from *N. tabacum* (**Paper III**) remained low, ranging between 1-3 nmol m^{-2} s^{-1}. The highest emission of LOXs 14±5 nmol m^{-2} s^{-1} was observed after 54 °C treatment, yet by raising the temperature by 1 °C, the LOXs surprisingly declined to 2.8±1.6 nmol m^{-2} s^{-1} (Fig. 6). Finally, next to LOXs the total emission rate of monoterpenes increased with increasing temperature from 46 °C to 55 °C (r =0.41, P<0.05).

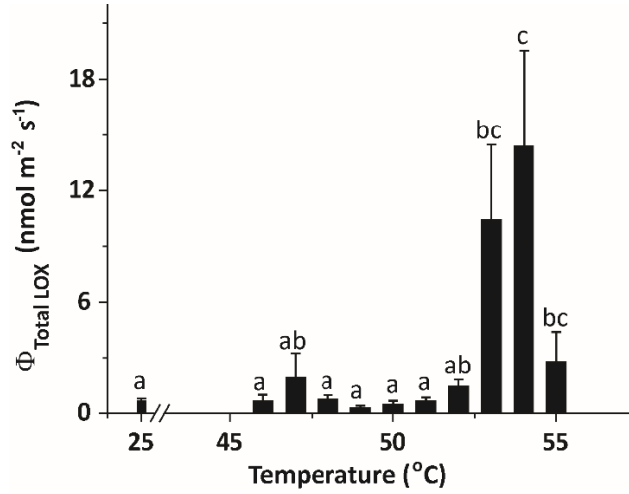

Figure 6. Influence of heat stress on the emissions of lipoxygenase pathway compounds (LOXs) in leaves of *N. tabacum* cv. Wisconsin 38. The emission rates (mean±SE) were quantified 15 min. after heat stress. Different letters indicate statistically significant differences (ANOVA followed by Tukey test, P<0.05) (reproduced from **Paper III**).

The emission rate of methanol was 2.9 ± 0.6 nmol m^{-2} s^{-1} in control plants, but the emission increased at 48-51 °C (Fig. 7). High methanol was characteristic to 52-55 °C treatments with the highest peak at 54 °C (270±50 nmol m^{-2} s^{-1}). At 55 °C, methanol emission was still high, especially when compared with strongly reduced emissions of LOXs (Figs 6 and 7).

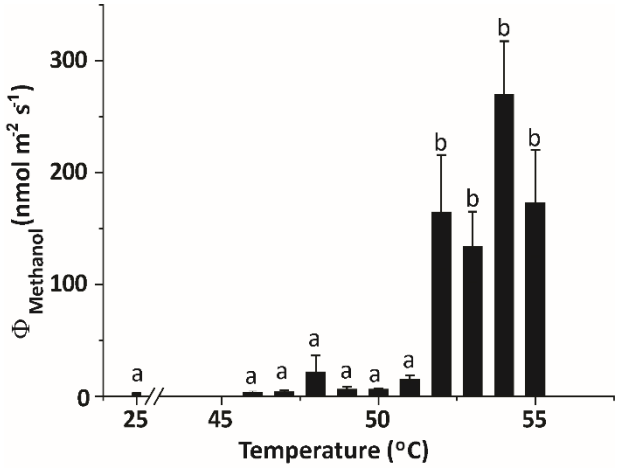

Figure 7. Methanol emission from control (25 °C) and heat stressed (46-55 °C) leaves of *N. tabacum* cv. Wisconsin 38. The emission rates (mean ± SE) were quantified 15 min after heat stress with PTR-QMS. Different letters indicate statistical differences (ANOVA followed by Tukey test, $P<0.05$) (reproduced from **Paper III**).

2-Ethylfuran and 2-methyl-2-cyclopenten-1-one were emitted in a similar manner from *N. tabacum* as methanol, i.e. between 25 °C and 49 °C the emission rates fluctuated around 4.65-18.1 pmol m^{-2} s^{-1}, but starting from 50 °C, they increased and reached the highest values at 53-54 °C (Table 1 in **Paper III**). The highest emission of 2-ethylfuran 2620±1260 pmol m^{-2} s^{-1} was measured at 53 °C and the highest emission of 2-methyl-2-cyclopenten-1-one 4430±1450 pmol m^{-2} s^{-1} at 54 °C (Table 1 in **Paper III**). Tobacco emission contained also methacrolein, with emission varying from 24 to 65 pmol m^{-2} s^{-1} over the temperature range of 25 °C to 52 °C. Yet, upon the 53 °C treatment, the tobacco released 240±100 pmol m^{-2} s^{-1} of methacrolein (Table 1 in **Paper III**).

5.2.3. Impact of heat stress on lipoxygenase pathway volatiles

Detailed identification of BVOCs showed that non-stressed *B. nigra* released a few C6-volatiles such as (*Z*)-3-hexen-1-ol, 1-hexanol and hexanal (Table 1 in **Paper II**). The latter compound was the only one, which was not quantitatively related to temperature in mild and heat shock treatments. Yet, hexanal was found in the bouquets of all the heated plants. Surprisingly, hexanal increased only in the long-term stress treatments by raising from 3.5-6.0 pmol m^{-2} s^{-1} at 30-35 °C to 106

pmol m^{-2} s^{-1} at 40 °C. The rest of the C5- and C6-volatiles (*E,E*)-2,4-hexadienal, 1-pentanol, 1-penten-3-ol and 1-penten-3-one were detected after 40 °C and/or 44 °C treatment of long-term treatments and 50 °C of heat shock treatments, respectively. In addition, (*E*)-3-hexen-1-ol and (*Z*)-3-hexenyl formate, were released by plants in the 50 °C treatment (Table 1 in **Paper II**).

In the case of *N. tabacum*, heat shock did not affect the emission rates of pentane and hexane and their emissions ranged from 33-165 pmol m^{-2} s^{-1} and 110-1600 pmol m^{-2} s^{-1}, respectively (Table 1 in **Paper III**). Meanwhile, (*E,E*)-2,4-hexadienal, hexanal, (*E*)-2-hexenal+(*Z*)-3-hexenol, 1-hexanol, 1-penten-3-one and propanal constituted the major burst of LOX emissions after 53-54 °C treatments (Table 1 in **Paper III)**. For example, 1-penten-3-one emission rose from 9.0±1.8 pmol m^{-2} s^{-1} (25 °C) to 180 pmol m^{-2} s^{-1} (52 °C) and propanal from 72±7 pmol m^{-2} s^{-1} (25 °C) to 3110±1450 pmol m^{-2} s^{-1} (54 °C).

5.2.4. Relationship between heat stress severity and the emission of GDP- and GGDP-pathway compounds

Next to LOX compounds, the variations among GDP- and GGDP-pathways derived compounds and their possible relationship to temperature were also evaluated (Fig 5; Table 1 in **Paper II**). Briefly, in the case of *B. nigra*, 3-carene, camphene, limonene, α-pinene, and β-pinene formed the emission of GDP-pathway volatiles and 6-methyl-5-hepten-2-one and geranyl acetone of GGDP-pathway volatiles (**Paper II**). In all treatments, α-pinene and 3-carene were the main monoterpenes. Surprisingly, the emission blends of plants exposed to mild stress lacked camphene and β-pinene (Table 1 in **Paper II**).

MEP pathway volatiles such as isoprene, (*Z*)-β-ocimene, limonene, α-pinene, and β-pinene were found in the bouquets of severely stressed *N. tabacum* plants. The emissions rates of monoterpenes were not related to the temperature (Table 1 in **Paper III**).

5.2.5. Impact of heat stress on the emission of volatile glucosinolate breakdown products

In general, various volatile glucosinolate breakdown products such as tetramethylthiourea, 2-propenenitrile, allyl isothiocyanate,

cyclohexyl isocyanate, cyclohexyl isothiocyanate, methanethiol, methyl isothiocyanate, and tetramethylurea were detected and quantified in the heat stressed *B. nigra* emissions (Table 1 in **Paper II**). Yet, the majority of the aforementioned volatiles, except cyclohexyl isothiocyanate, 2-propenenitrile and allyl isothiocyanate, were absent in the emissions of plants exposed to mild stress. Furthermore, after 40 °C treatment, the plants exposed to mild stress did not emit volatile glucosinolate breakdown products at all (Table 1 in **Paper II**).

At the same time, the emission rates of glucosinolate breakdown products changed considerably in the long-term study. For example, 25, 30 and 35 °C treatments did not affect the release rates of tetramethylthiourea, 2-propenenitrile, cyclohexyl isocyanate, cyclohexyl isothiocyanate, methanethiol, and tetramethylurea, but after 40 °C treatment, the emissions of BVOCs rose tremendously and a ´new´ compound methyl isothiocyanate appeared in the emissions. Increasing the temperature by another 4 °C further increased the emission of BVOCs. The largest changes for the temperature range of 40 to 44 °C were found for allyl isothiocyanate (from 12.3±4.8 pmol m^{-2} s^{-1} to 1300±700 pmol m^{-2} s^{-1}), tetramethylthiourea (from 14±7 pmol m^{-2} s^{-1} to 110±80 pmol m^{-2} s^{-1}), cyclohexyl isothiocyanate (from 28 pmol m^{-2} s^{-1} to 110±80 pmol m^{-2} s^{-1}) and finally 2-propenenitrile (from 30±8 pmol m^{-2} s^{-1} to 81±25 pmol m^{-2} s^{-1}) (Table 1 in **Paper II**).

In the heat shock treatment, the profile of glucosinolate breakdown products was less diverse. Only tetramethylthiourea, cyclohexyl isocyanate, and cyclohexyl isothiocyanate were detected in the emission bouquets in 45, 48 and 50 °C treatment. Similar to long-term stress, the highest emissions were quantified for tetramethylthiourea and allyl isothiocyanate (400±90 pmol m^{-2} s^{-1} and 250±15 pmol m^{-2} s^{-1}, respectively) (Table 1 in **Paper II**).

5.3. Relationships between photosynthetic characteristics and BVOCs

Relationships between the photosynthetic characteristics and the emission rates of the BVOCs of *B. nigra* (**Paper II**) and *N. tabacum* (**Paper III**) were examined. For example, in the long-term stress treatment of *B. nigra* (**Paper II**), there were non-linear relationships between net assimilation rate (*A*) and stomatal conductance to water

vapour (g_s) and emission rates of fatty acid derived (LOX) compounds and glucosinolate breakdown products (Fig. 7 in **Paper II**). However, the emissions of LOXs varied strongly among different leaves (Fig. 7 in **Paper II**), and only the negative correlations of the total emission rate of glucosinolate breakdown products with A and g_s through the stress treatments were significant (Fig. 8a, b). These relationships were driven by the high emission rate of glucosinolate breakdown products at 44 °C treatment. In addition, a significant relationship existed also between A and LOX emission rates in heat-stressed *N. tabacum* plants (Fig. 8c) (**Paper III**).

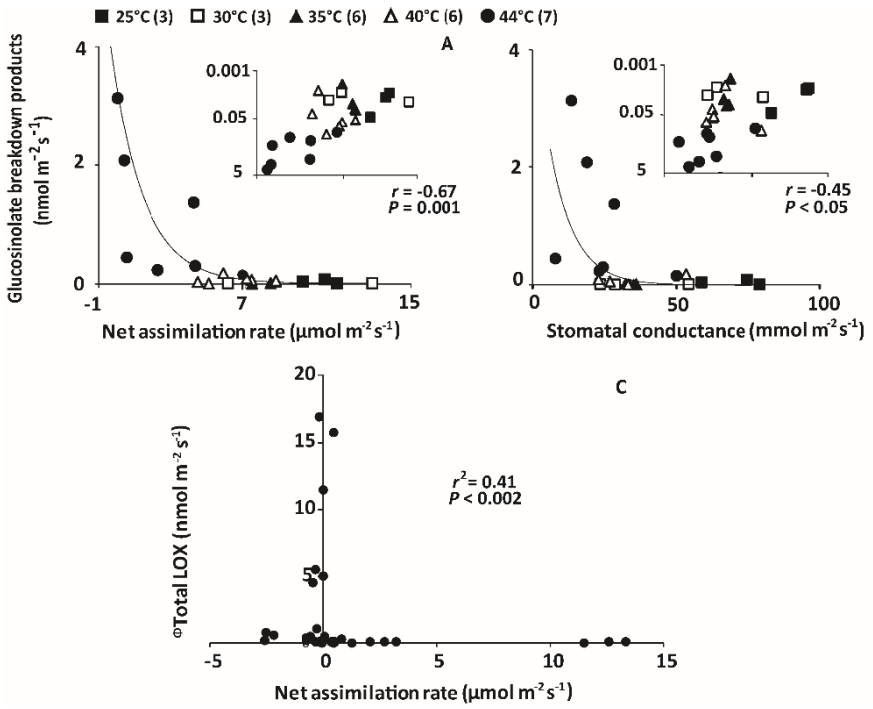

Figure 8. Non-linear relationships between glucosinolate breakdown products and net assimilation rate (A) and stomatal conductance to water vapour (B) in *Brassica nigra* in the long-term stress treatment (experiment details are shown in Figure 1) and between LOX compounds and net assimilation rate (C) through the severe heat stress treatments in *Nicotiana tabacum*. In A and B panels, the insets demonstrate the emissions with the y-scale reversed and log-transformed. Individual symbols stand for replicate experiments and different temperatures are shown by different symbols (A, B) and on (C) data corresponds to individual heat-stressed leaves (reproduced from **Papers II and III)**.

5.4. Heat stress driven relationships between the BVOCs

Finally, biochemical processes driven by the heat response of the plants were assessed by testing the relationships between the BVOCs of different pathways. In the long-term stress study of *B. nigra* (**Paper II**), emission rates of fatty acid derived compounds (including LOXs) correlated positively with the glucosinolate breakdown products at 25-40 °C treatments (Fig. 9). Yet, higher temperatures from 40 to 44 °C inhibited the further release of fatty acid derived compounds (including LOXs) and thus, the statistical dependence vanished (Fig. 9).

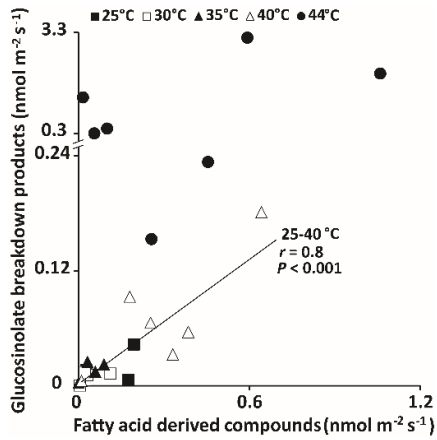

Figure 9. Relationship between the emission of glucosinolate breakdown products and fatty acid derived compounds in the long-term stress treatment in *Brassica nigra* (experiment details are shown in Figure 1). Each symbol corresponds to an individual plant (Table 1 in **Paper II** for stress effects on average emissions). Data over 25–40 °C were fitted by a linear regression (reproduced from **Paper II**).

In the long-term stress study and in the heat shock study, the release rates of GDP-pathway compounds and fatty acid derived compounds (including LOXs) were positively correlated (Fig. 10). Moreover, in both studies, the relationships depended on the temperature (r=0.91, P<0.001 for 25-35 °C and r=0.65, P<0.05 40-44 °C in the long-term study; and r=0.64, P<0.05 for 25-48 °C in the heat shock study) (Fig. 10).

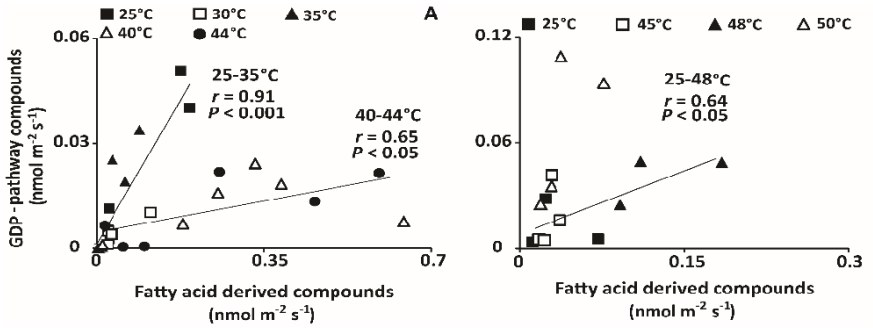

Figure 10. Linear relationships between the emission rate of GDP-pathway compounds (various monoterpenes, Table 1 in **Paper II**) and fatty acid derived compounds in *Brassica nigra* in long-term (A) and shock stress (B) treatments. After the heat treatments, the emission rates of BVOCs were measured at 25 °C (heat stress treatments as in Fig. 1). Each symbol corresponds to an individual replicate in the experiment (reproduced from **Paper II**).

In the *N. tabacum* study (**Paper III**), the emission rates of several volatiles were related to each other and similar to the long-term stress study of *B. nigra* (**Paper II**), the relationships were affected by temperature. For example, in the bouquets of control or heat stressed *N. tabacum* plants, the release rate of isoprene was correlated to the methacrolein emission rate (r = 0.67, P < 0.001 for 25 °C; r = 0.59, P < 0.01 at 50-55 °C). The temperature-driven difference was caused by the higher emission of methacrolein at higher temperatures (Fig. 11). On the other hand, methacrolein in turn correlated positively with 2-ethylfuran (r=0.48, P<0.01 for control plants; r=0.69, P<0.001 for heat-treated plants) (Fig. 12). Finally, different from the methacrolein – isoprene relationship (Fig. 11), the temperature increased the emission of both volatiles, methacrolein and 2-ethylfuran (Fig. 12).

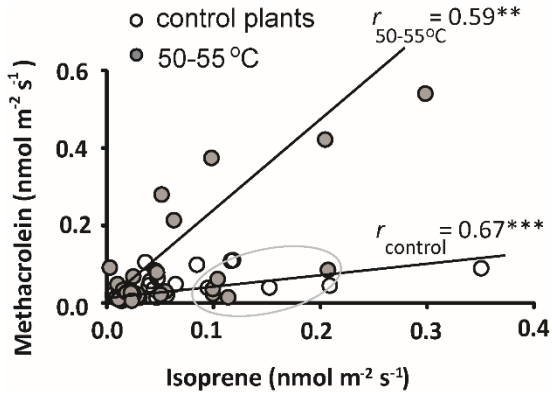

Figure 11. Linear regressions between the emissions of isoprene and methacrolein from control (white circles), and 50-55 °C treated (gray circles) leaves of *Nicotiana tabacum* cv. Wisconsin 38. Some stressed plants (within the ellipse), lacked the high emission of methacrolein and were excluded from the regression test of heat-stressed plants (50-55 °C). The relationships of control and stressed plants were significant at $P<0.001$ (**) and $P<0.01$ (***), respectively (reproduced from **Paper III**).

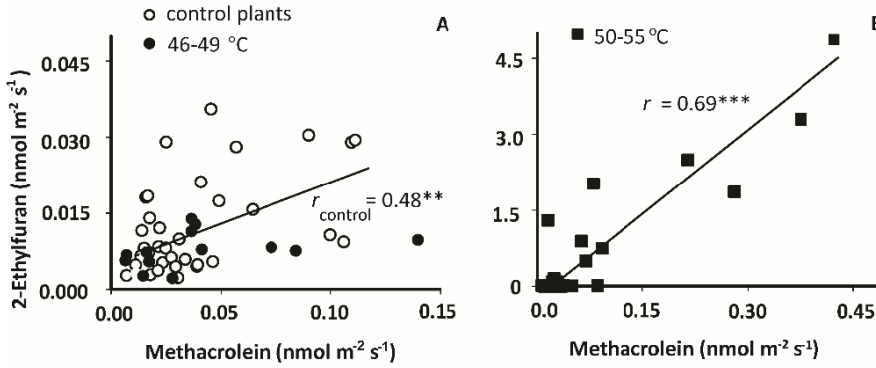

Figure 12. Linear regressions between the emissions of methacrolein and 2-ethylfuran in *Nicotiana tabacum* cv. Wisconsin 38 quantified in the control plants, and heat stressed plants of 46 to 49 °C (A) and the plants treated with 50-55 °C (B). The relationships for control and stressed plants were statistically significant at $P<0.01$ (**) and $P<0.001$ (***), respectively (reproduced from **Paper III**).

6. DISCUSSION

6.1. Impact of different heat stress treatments on leaf photosynthetic characteristics

Temperature as one of the key globally changing abiotic factors alters plant secondary metabolite emissions and thereby leads to changes in the interactions between plants and insects (**Paper I**). Furthermore, higher temperature promotes earlier flowering, modifies life cycle, changes plant physiology and productivity, and the capacity to cope with interspecific competition (Bidart-Bouzat & Imeh-Nathaniel, 2008; Dicke & Loreto, 2010; Svenning et al., 2014; Visser, 2008).

Impacts of severe heat shock stress on *B. nigra* (**Paper II**) and severe stress on *N. tabacum* (**Paper III**) were similar as net assimilation (*A)* values were strongly reduced and even became negative (Fig. 3 and 4). Moderate temperatures 35, 40 and 44 °C also caused a decrease in *A* in *B. nigra* (**Paper II***)*, but the response depended on the heat stress type and species. In tobacco, the response to 46-48 °C was actually a reaction to moderate stress (Fig. 4) (**Paper III**) (Niinemets, 2010; Salvucci & Crafts-Brandner, 2004; Scafaro et al., 2010). In studies by Chen et al., (2012) and Song et al., (2014), poplar treated with 42 °C and *Citrus medica* var. *sarcodactylis* (Swingle) leaves treated with 45 °C showed decreased *A*, while in *Eucalyptus camaldulensis* (Dehnh.), *A* became negative when the plants were treated with 50 °C (Guidolotti et al., 2019). Stomatal closure also occurred at high temperatures, and in the case of *B. nigra* exposed to the highest temperatures, more than half of the initial conductance was lost (Fig. 3b). In the case of *N. tabacum,* g_s was fluctuating or even increased between temperatures but did not reach to the pre-stress level (Fig. 4b). The same g_s increase tendency was observed in *Gossypium hirsutum* (L.) and *Oryza sativa* after 40 °C heat stress (von Caemmerer & Evans, 2015), but not in *N. tabacum* (Evans & von Caemmerer, 2013). Severe temperature stress in *B. nigra* (44-50 °C) (**Paper II**) and *N. tabacum* (49-55 °C) (**Paper III**) probably exceeded the temperature threshold for damage and resulted in damaged cells and metabolic processes, and photosynthetic activity did not recover to previous level (Hüve et al., 2011; Loreto et al., 1998). It has been shown, that for *N. tabacum* long-term growth temperature of 28 °C inhibits growth and photosynthesis leading to accelerated flowering and senescence (Yang et al., 2018).

Brassica oleracea and *B. acephala* seedlings growing under high temperature (32 °C) experienced decreased fresh weight on the aerial part that could lead to the possible decrease in photosynthetic activity (Rodriguez et al., 2015).

6.2. BVOC emissions related to photosynthetic characteristics

After exposure to high temperatures, stress-induced compound (LOXs) emissions increased simultaneously with decreasing A and g_s. In the case of *B. nigra* after long-term stress treatment (**Paper II**) photosynthetic characteristics decreased, but fatty acid derived compound emissions increased after 40 °C and 44 °C (Fig. 3 and 5a). At negative A, *B. nigra* (**Paper II**) and *N. tabacum* (**Paper III**) showed also enhanced LOX emission after exposure to damaging temperatures (Fig. 5a, 6). A heat shock study with *S. lycopersicum* showed also that A reached close to zero at temperatures 46, 49, 51 °C, whereas LOX compound emission rates increased at these temperatures (Copolovici et al., 2012). In these studies, the membrane breaking temperature that caused the increased LOX emissions in heat shock stress treatment was 53 °C in *N. tabacum* and 50 °C in *B. nigra*.

A similar response was observed for glucosinolate breakdown products in *B. nigra*, but the emissions of glucosinolate breakdown products increased mostly at 44 °C (Fig. 5c). It is suggested that the emissions of glucosinolate breakdown products reflect physiological modifications (Fig. 8b) (Hossain et al., 2013). It is further proposed, that the glucosinolate breakdown products may be connected to guard cell ABA responses (Zhao et al., 2008). A study done by Khokon et al., (2011) suggests that plant produced isothiocyanates may induce stomatal closure. This may explain allyl isothiocyanate occurrence in the emission of *B. nigra* together with decreased g_s after 40 °C as an opportunity to prevent water loss.

6.3. Characteristic LOX compounds upon different heat stresses

Characteristic LOX compounds are released also at low levels during flowering and fruit ripening (Ceuppens et al., 2015; Schiller et al., 2015), but herbivore attack, mechanical damage or high temperature may as well induce or increase the emission of LOX compounds (Aartsma et al., 2017; Hu et al., 2008; Kleist et al., 2012). Thus, the release of LOX

compounds (*E,E*)-2,4-hexadienal, 1-penten-3-one, 1-hexanol, (*Z*)-3-hexen-1-ol and hexanal from *B. nigra* and *N. tabacum* in response to heat stress is in agreement with the earlier findings (Copolovici et al., 2012; Filella et al., 2007). As the emission of LOX compounds is connected to the severity of membrane damage, we can assume that both plants experienced dose-dependent damage (Matsui, 2006; Matsui et al., 2012). It has been shown that (*E*)-2-hexenal reaches its maximum emission level about 45 min after treatment of *Phragmites australis* (Cav.) leaves with 45 °C, although the start of the rise of the emissions can be already seen a few minutes after the treatment (Loreto et al., 2006). In the severe stress treatment, *N. tabacum* emitted (*E*)-2-hexanal and (*Z*)-3-hexen-1-ol (Table 1 in **Paper III**) and these volatiles are considered to be the earliest compounds indicating damage (Allmann & Baldwin, 2010; Scala et al., 2013; Turlings et al., 1995). In the case of *B. nigra,* (*Z*)-3-hexen-1-ol increased in most cases with increasing of the treatment temperature in long-term and shock stress, except in the temperature response curve treatment (Table 1 in **Paper II)**.

Novel compounds emerging in the emission of *B. nigra* after the shock stress temperature at 50 °C were (*E*)-3-hexen-1-ol and (*Z*)-3-hexenyl formate, previously found in the emission of mechanically damaged *Camellia sinensis* (L.) or *Festuca rubra* (L.)(de Gouw et al., 1999; Han & Chen, 2002; Tietel et al., 2011). C5 volatiles 1-penten-3-ol and 1-penten-3-one emerged after 44 °C and 50 °C treatment (Table 1 in **Paper II**). 1-Penten-3-ol has been detected in the headspace of uninfested and *Pieris brassicae* caterpillar feeding on *B. nigra* plants and was induced by ozone in lima bean plants (Ponzio et al., 2014; Vuorinen et al., 2004). It is believed that C5 compounds are also formed via LOX pathway in dependence of hydroperoxide lyase (HPL) enzyme activity (Salas et al., 2006; Shen et al., 2014).

The highest LOX emission and negative *A* show that 53 °C is the temperature limit that causes damage at the membrane level in *N. tabacum* (**Paper III**). In the case of *B. nigra* (**Paper II**), *A* was close to zero at 45 °C, but only low levels of (*Z*)-3-hexen-1-ol and 1-hexanol were observed. Based on that, these results show species-specific responses, pathways involved and plant ability to cope with heat stress (Hasanuzzaman et al., 2013).

In general, all previously mentioned LOX compounds are present also as info-chemicals in tritrophic interactions and in plant-plant

communication (**Paper I**) (Girón-Calva et al., 2014, 2016; Scala et al., 2013; Shiojiri et al., 2006).

Other compounds from fatty acid derived pathway including hexanal, octanal, nonanal, and decanal could be constitutive volatiles emitted by *B. nigra* (**Paper II**). However, the emissions of these volatiles are also elevated after ozone, pathogen and insect attacks (Heiden et al., 2003). Propanal formed through linolenic acid and pentane formed through linoleic acid were constantly emitted from *N. tabacum* (**Paper III**), and propanal emissions were highest at 53-54 °C (Cao et al., 2014). Linolenic acid is the precursor of the plant hormone jasmonic acid (JA) that regulates plant response to abiotic and biotic stress (Creelman & Mullet, 1995; Ruan et al., 2019). In the case of *N. tabacum*, nicotine synthesis in the roots is also regulated by jasmonic acid (Shi et al., 2006; Yin et al., 2017).

6.4. Glucosinolate breakdown products emission after heat stresses

Glucosinolates or their breakdown products are characteristic to the Brassica family and are used as chemical weapons against herbivory (Ahuja et al., 2010). It is well known, that the combination of few or structurally various breakdown products are determined by the glucosinolate profile, protein cofactors and the status of the reaction or pH (Grubb & Abel, 2006; Tsao et al., 2000).

Heat stresses applied to *B. nigra* (**Paper II**) revealed that the emission of various glucosinolate breakdown products was related to heat stress (Fig. 5d). High emissions of glucosinolate breakdown products after heat shock stress in *B. nigra* indicate cellular damage as myrosinases are released from myrosin cells (Fig. 5d). Additionally, varying release of volatile glucosinolate breakdown products in the long-term treatment and heat shock treatments may indicate activation of different biosynthesis routes (Grubb & Abel, 2006). It has been found that the major aliphatic glucosinolate in *B. nigra* is sinigrin (chemical name: 2-propenylglucosinolate) and the myrosinase degrades it to allyl isothiocyanate, which was one of the main volatile glucosinolate breakdown products in the odour of heat stressed *B. nigra* (Table 1 in **Paper II**) (Tsao et al., 2000, 2002). A study by Guo et al., (2019) indicated that *Brassica alboglabra* sprout variety with high glucosinolate content was

more tolerant to heat stress than the variety of low glucosinolate content. Yet, leaf volatiles did not correlate with the content of glucosinolates in *B. rapa* leaves, suggesting that focusing only on volatile glucosinolates in brassicaceous species is not sufficient to assess the plant resistance status (Schiestl, 2014). However, the plant heat stress response becomes clearer by looking at the positive relationship between glucosinolate breakdown products and fatty acid derived compounds in the long-term stress (**Paper II**) (Fig. 9). At 44 °C, the emission of glucosinolate breakdown products was most likely caused by the release of myrosinases from myrosin cells. It has been reported that exogenously applied allyl isothiocyanate can increase heat tolerance (Hara et al., 2013) and based on that we cannot rule out that *B. nigra* increased its thermotolerance by producing allyl isothiocyanate and maybe also methyl isothiocyanate. Considering the plant and herbivore species and the chemical communication between plants and their pests, it can be concluded, that under hot climate the brassicaceous plants may become more attractive to herbivorous insects (Fatouros et al., 2012; Mithen, 2001; Veromann et al., 2012).

6.5. GDP- and GGDP-pathway compound emissions after heat stress

In total five monoterpenes were found in the emissions of *B. nigra* (**Paper II**) and four in the emissions of *N. tabacum* (**Paper III**). Both species are low monoterpene emitters, especially when comparing the emissions from leaves to the emissions from flowers (Andersen et al., 1988; Loughrin et al., 1990; Veromann et al., 2013). In both species, α-pinene and limonene were dominating, nevertheless, no significant differences were found in total monoterpene emissions after heat treatments. In general, constitutive monoterpene emissions from vegetation are influenced by temperature and studies have shown that constitutive monoterpene emission decreases in response to heat stress following the reductions in photosynthesis (Kleist et al., 2012; Loreto et al., 1996). It has been shown, that experimental warming by 2 °C in the subarctic ecosystem doubled the total monoterpene emission (Valolahti et al., 2015).

Depending of plant species, monoterpenes are emitted constitutively from special storage structures or are synthesized *de novo* (Gershenzon et al., 2000; Taipale et al., 2011). Severe stress did not alter GDP-compound emission from glandular trichomes in *N. tabacum* (**Paper III**), but the findings of Harada et al., (2010) show that abiotic or biotic

stress response of glandular trichomes of tobacco is determined by the activation of stress responsive genes in trichomes. Clearly, in these experiments, the time for elicitation of gene-level responses was too short in the temperature curve and heat shock experiments, and thus, the responses observed primarily reflect substrate-level regulation and temperature-dependent changes in enzyme activity. Thus in temperature response curve treatment, the reduced photosynthesis rate together with decreased emission of monoterpenes suggests the inhibition of *de novo* biosynthesis of GDP-pathway volatiles in *B. nigra* leaves due to lack of substrate (**Paper II**). A similar response was found in *Prunus persica* (L.), where GDP pool size decreased after 42 °C treatment (Nogués et al., 2006). At the same time, an increase of monoterpene emissions occurred under heat shock stress (**Paper II**) as has been also reported in the heat-stressed *S. lycopersicum* (Copolovici et al., 2012). This rise of the emissions can be partly related to an increase in the activity of monoterpene synthases, although the temperatures between 40 °C to 45 °C could result in partial enzyme denaturation, as monoterpene synthases optimum temperature is considered to be 40 °C (Fischbach et al., 2000; Loreto et al., 2006; Loreto & Schnitzler, 2010). *S. lycopersicum* treated with 41 and 46 °C (as mild stress) showed decreased expression of β-phellandrene synthase gene 2 and 10 h after heat stress followed by recovery 24 h later, but 46 °C (severe stress) resulted in irrecoverable synthase gene expression (Pazouki et al., 2016). It is possible that in *B. nigra* constitutive plant defence (temperatures 25-35 °C) switched over to induced plant defence (40, 44 °C) or more likely reflected the onset of cellular damage (temperatures 45-50 °C). On the other hand, GDP-pathway compounds showed a correlation with fatty acid derived compounds in *B. nigra* (**Paper II**) and the correlation was dependent on the type of heat stress and temperature (Fig. 10). Hence, the sudden rise of monoterpene emission rate was rather caused by the collapse of cellular structures (Guidolotti et al., 2019).

In *B. nigra* GGDP-pathway (geranylgeranyl diphosphate) volatiles geranyl acetone and 6-methyl-5-hepten-2-one were in the emissions of control and heat stressed plants (**Paper II**). GGDP-pathway compounds emission decreased only in temperature response curve treatment (Fig. 5d) (**Paper II**). Both volatiles have been previously found in the aroma of tomato fruits, *B. napus* cv. Silva buds and flowers and from *A. thaliana* treated with different temperatures in the presence or absence of larvae (Simkin et al., 2004; Tieman et al., 2006; Truong et al., 2014; Veromann

et al., 2013). It is believed, that these compounds are derived from carotenoids, especially from lycopene (Gao et al., 2008; Tieman et al., 2006; Vogel et al., 2010).

6.6. Heat stress effects on cell walls and cellular metabolites

Methanol in the plant cell walls is formed as the result of pectin demethylation by the protein pectin methylesterases (Dorokhov et al., 2018). In the case of transgenic tobacco, high pectin methylesterases synthesis refers to the resistance of the cultivars against tobacco mosaic virus (Gasanova et al., 2008). Yet, in the present study, increased LOX compound (Fig. 6) and methanol emission (Fig. 7) at 52-55 °C clearly indicate the damaged cell walls and cell membranes (Dorokhov et al., 2012).

Isoprene was detected in *N. tabacum* emissions (Fig. 11) (Table in **Paper III**), although *N. tabacum* is typically considered a non-isoprene emitter. Low isoprene emissions were observed in *N. tabacum* after 35 °C treatment and it is suggested that isoprene is formed by non-enzymatic conversion of DMADP pool (dimethylallyl diphosphate) (Zuo et al., 2019). It has been previously shown that even non-isoprene emitters have a significant DMADP pool (Nogués et al., 2006). Isoprene emissions from transgenic tobacco have been shown to improve plant response to oxidative damage (Vickers et al., 2009). The highest methacrolein emission was quantified in the odour of *N. tabacum* (**Paper III**) at 53-54 °C treatments (Fig. 12). Methacrolein is one of the predominant oxidation products of isoprene (Jardine et al., 2012; Liu et al., 2013). A strong correlation between methacrolein and isoprene (Fig. 11) was also found by Pierotti et al., (1990). It is proposed, that methacrolein may protect plant cellular components from oxidative damage as ROS contents rise upon heat stress (Jardine et al., 2010). *N. tabacum* had increased ROS content after drought stress when compared with transgenic *N. tabacum* as isoprene-emitter (Ryan et al., 2014).

Higher 2-ethylfuran levels were present in the emissions of *B. nigra* treated with 40, 44, 50 °C and in *N. tabacum* treated with 53-54 °C. As ROS contents increase after the treatment with high temperature, the increased 2-ethylfuran levels may be connected to this. The biosynthesis pathway for this compound is uncertain, nevertheless, it is also present in the headspace of *Vicia faba* genotypes and damaged plant tissues (Cozzolino et al., 2016; Luca et al., 2017; Rouseff et al., 2008).

7. CONCLUSIONS

This thesis provides an overview of three different types of heat stress (temperature response curve at 20-40 °C, long-term stress at 25-44 °C and shock stress at 25 °C, 45-50 °C) impacts on *Brassica nigra* L. (**Paper II**) and severe stress (46-55 °C) on *Nicotiana tabacum* L. (**Paper III**) photosynthetic characteristics and volatile organic compound emissions. In the case of *B. nigra*, species-specific glucosinolate breakdown products were evaluated together with fatty acid derived compounds (including LOX compounds), monoterpenes and GGDP-pathway compounds. In *N. tabacum*, emissions of isoprene and monoterpenes and oxygenated volatiles including LOX pathway compounds, methanol, and methacrolein were investigated.

Different heat stress treatments on *B. nigra* and severe stress on *N. tabacum* resulted in decreased photosynthetic characteristics and changes in volatile organic compound emission through heat treatments. Both plant species experienced strongly reduced stomatal conductance and negative carbon fixation at the highest temperatures, indicating the collapse of photosynthesis and the prevalence of respiration.

In the case of *B. nigra*, long-term stress and heat shock stress caused increased emissions of species-specific glucosinolate breakdown products together with LOX volatiles, but long-term stress treatment resulted in a stronger variation in volatile bouquets of these two groups than the heat shock stress. Heat shock stress treatment at 52 °C, was the maximum threshold temperature for *N. tabacum* as volatile emissions started to show an increase, although LOX compound and methanol emissions were the highest at 54 °C. Emissions in both species contained typical stress volatiles (*Z*)-3-hexen-1-ol and (*E,E*)-2,4-hexadienal, indicating cellular damage. Increased emission of 2-methyl-2-cyclopenten-1-one from *N. tabacum* after 53 °C treatment showed clearly damaged cell walls.

Monoterpene (GDP-pathway volatiles) emissions from both species were not strongly enhanced by moderate increases in temperature. However, when a certain high temperature threshold was exceeded, for example under heat shock stress, major emission bursts of GDP volatiles were observed. Methacrolein emission from *N. tabacum* shows

the possibility of isoprene oxidation by ROS in the leaves, although *N. tabacum* is considered a non-isoprene emitter.

These results improve the understanding of species-specific responses of plant photosynthesis and volatile organic compound emissions. In particular, heat stress severity and type affect volatile organic compound synthesis pathways and emission from the leaves. Depending on the magnitude of changes in volatile emissions, stress-dependent emissions can play a key role in plant-plant, plant-insect and plant-insect-environment relationships (**Paper I**).

REFERENCES

Aartsma, Y., Bianchi, F.J.J., van der Werf, W., Poelman, E. H., & Dicke, M. (2017). Herbivore-induced plant volatiles and tritrophic interactions across spatial scales. *New Phytologist, 216*(4), 1054-1063.

Agrawal, A. A., & Kurashige, N. S. (2003). A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology, 29*(6), 1403-1415.

Ahuja, I., Rohloff, J., & Bones, A. M. (2010). Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. *Agronomy for Sustainable Development, 30*(2), 311-348.

Allakhverdiev, S. I., Kreslavski, V. D., Klimov, V. V., Los, D. A., Carpentier, R., & Mohanty, P. (2008). Heat stress: an overview of molecular responses in photosynthesis. *Photosynthesis Research, 98*(1-3), 541-550.

Allmann, S., & Baldwin, I. T. (2010). Insects betray themselves in nature to predators by rapid isomerization of green leaf volatiles. *Science, 329*(5995), 1075-1078.

Andersen, R. A., Hamiltonkemp, T. R., Loughrin, J. H., Hughes, C. G., Hildebrand, D. F., & Sutton, T. G. (1988). Green leaf headspace volatiles from *Nicotiana tabacum* lines of different trichome morphology. *Journal of Agricultural and Food Chemistry, 36*(2), 295-299.

Angadi, S. V., Cutforth, H. W., Miller, P. R., McConkey, B. G., Entz, M. H., Brandt, S. A., & Volkmar, K. M. (2000). Response of three Brassica species to high temperature stress during reproductive growth. *Canadian Journal of Plant Science, 80*(4), 693-701.

Arimura, G., Kost, C., & Boland, W. (2005). Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids, 1734*(2), 91-111.

Arimura, G., Matsui, K., & Takabayashi, J. (2009). Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant and Cell Physiology, 50*(5), 911-923.

Augustine, R., & Bisht, N. C. (2017). Regulation of glucosinolate metabolism: from model plant *Arabidopsis thaliana* to Brassica Crops. In: Mérillon JM., Ramawat K. (eds) Glucosinolates. Reference Series in Phytochemistry. Springer, Cham,, 163-199.

Backenköhler, A., Eisenschmidt, D., Schneegans, N., Strieker, M., Brandt, W., & Wittstock, U. (2018). Iron is a centrally bound cofactor of specifier proteins involved in glucosinolate breakdown. *PLoS ONE, 13*(11), e0205755.

Barba, F. J., Nikmaram, N., Roohinejad, S., Khelfa, A., Zhu, Z. Z., & Koubaa, M. (2016). Bioavailability of glucosinolates and their breakdown products: impact of processing. *Frontiers in Nutrition, 3*, *24*.

Beauchamp, J., Wisthaler, A., Hansel, A., Kleist, E., Miebach, M., Niinemets, Ü., . . . Wildt, J. (2005). Ozone induced emissions of biogenic VOC from tobacco: relationships between ozone uptake and emission of LOX products. *Plant, Cell and Environment, 28*(10), 1334-1343.

Bell, L., Oruna-Concha, M. J., & Wagstaff, C. (2015). Identification and quantification of glucosinolate and flavonol compounds in rocket salad (*Eruca sativa*, *Eruca vesicaria* and *Diplotaxis tenuifolia*) by LC-MS: Highlighting the potential for improving nutritional value of rocket crops. *Food Chemistry, 172*, 852-861.

Bidart-Bouzat, M. G., & Imeh-Nathaniel, A. (2008). Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology, 50*(11), 1339-1354.

Bilalis, D., Karkanis, A., Efthimiadou, A., Konstantas, A., & Triantafyllidis, V. (2009). Effects of irrigation system and green manure on yield and nicotine content of Virginia (flue-cured) Organic tobacco (*Nicotiana tabaccum*), under Mediterranean conditions. *Industrial Crops and Products, 29*(2-3), 388-394.

Bilalis, D., Karkanis, A., Triantafyllidis, V., Ladavos, A., Bizos, D., Patsiali, S., . . . Papatheohari, Y. (2010). Effects of organic and inorganic fertilization on growth, yield and nicotine content of flue-cured and oriental tobacco (*Nicotiana tabacum* L.) seedlings grown in organic and conventional float system. *Journal of Food Agriculture and Environment, 8*(2), 585-589.

Bischoff, A., & Hurault, B. (2013). Scales and drivers of local adaptation in *Brassica nigra* (Brassicaceae) populations. *American Journal of Botany, 100*(6), 1162-1170.

Blatt, S. E., Smallegange, R. C., Hess, L., Harvey, J. A., Dicke, M., & van Loon, J. J. A. (2008). Tolerance of *Brassica nigra* to *Pieris brassicae* herbivory. *Botany-Botanique, 86*(6), 641-648.

Bones, A. M., & Rossiter, J. T. (2006). The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry, 67*(11), 1053-1067.

Bracho-Nunez, A., Welter, S., Staudt, M., & Kesselmeier, J. (2011). Plant-specific volatile organic compound emission rates from young and mature leaves of Mediterranean vegetation. *Journal of Geophysical Research-Atmospheres, 116*, D16304.

Brilli, F., Hörtnagl, L., Bamberger, I., Schnitzhofer, R., Ruuskanen, T. M., Hansel, A., . . . Wohlfahrt, G. (2012). Qualitative and quantitative characterization of volatile organic compound emissions from cut grass. *Environmental Science and Technology, 46*(7), 3859-3865.

Brilli, F., Ruuskanen, T. M., Schnitzhofer, R., Muller, M., Breitenlechner, M., Bittner, V., . . . Hansel, A. (2011). Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction "Time-of-Flight" mass spectrometry (PTR-TOF). *PLoS ONE, 6*(5), e20419.

Brosché, M., Merilo, E., Mayer, F., Pechter, P., Puzõrjova, I., Brader, G., . . . Kollist, H. (2010). Natural variation in ozone sensitivity among *Arabidopsis thaliana* accessions and its relation to stomatal conductance. *Plant, Cell and Environment, 33*(6), 914-925.

Broz, A. K., Broeckling, C. D., De-La-Peña, C., Lewis, M. R., Greene, E., Callaway, R. M., . . . Vivanco, J. M. (2010). Plant neighbor identity influences plant biochemistry and physiology related to defense. *BMC Plant Biology, 10*, 115.

Burow, M., Bergner, A., Gershenzon, J., & Wittstock, U. (2007). Glucosinolate hydrolysis in *Lepidium sativum* - identification of the thiocyanate-forming protein. *Plant Molecular Biology, 63*(1), 49-61.

Calfapietra, C., Fares, S., & Loreto, F. (2009). Volatile organic compounds from Italian vegetation and their interaction with ozone. *Environmental Pollution, 157*(5), 1478-1486.

Camejo, D., Rodríguez, P., Morales, A., Dell'Amico, J. M., Torrecillas, A., & Alarcón, J. J. (2005). High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Journal of Plant Physiology, 162*(3), 281-289.

Cao, J., Deng, L., Zhu, X. M., Fan, Y. W., Hu, J. N., Li, J., & Deng, Z. Y. (2014). Novel approach to evaluate the oxidation state of vegetable oils using characteristic oxidation indicators. *Journal of Agricultural and Food Chemistry, 62*(52), 12545-12552.

Cartea, M. E., de Haro, A., Obregón, S., Soengas, P., & Velasco, P. (2012). Glucosinolate variation in leaves of *Brassica rapa* crops. *Plant Foods for Human Nutrition, 67*(3), 283-288.

Castro, A. M., Tapias, J., Ortiz, A., Benavides, P., & Góngora, C. E. (2017). Identification of attractant and repellent plants to coffee berry borer, *Hypothenemus hampei*. *Entomologia Experimentalis Et Applicata, 164*(2), 120-130.

Ceuppens, B., Ameye, M., Van Langenhove, H., Roldan-Ruiz, I., & Smagghe, G. (2015). Characterization of volatiles in strawberry varieties 'Elsanta' and 'Sonata' and their effect on bumblebee flower visiting. *Arthropod-Plant Interactions, 9*(3), 281-287.

Cevher-Keskin, B., Yildizhan, Y., Yüksel, B., Dalyan, E., & Memon, A. R. (2019). Characterization of differentially expressed genes to Cu stress in *Brassica nigra* by Arabidopsis genome arrays. *Environmental Science and Pollution Research, 26*(1), 299-311.

Chang, C. R., Bowman, J. L., & Meyerowitz, E. M. (2016). Field guide to plant model systems. *Cell, 167*(2), 325-339.

Chantal, K., Shao, X. H., Jing, B. B., Yuan, Y. B., Hou, M. M., & Liao, L. X. (2013). Effects of effective microorganisms (EM) and bio-organic fertilizers on growth parameters and yield quality of flue-cured tobacco (*Nicotiana tabacum*). *Journal of Food Agriculture and Environment, 11*(2), 1212-1215.

Chen, F., Tholl, D., Bohlmann, J., & Pichersky, E. (2011). The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant Journal, 66*(1), 212-229.

Chen, W. R., Zheng, J. S., Li, Y. Q., & Guo, W. D. (2012). Effects of high temperature on photosynthesis, chlorophyll fluorescence, chloroplast ultrastructure, and antioxidant activities in fingered citron. *Russian Journal of Plant Physiology, 59*(6), 732-740.

Chutteang, C., Booker, F. L., Na-Ngern, P., Burton, A., Aoki, M., & Burkey, K. O. (2016). Biochemical and physiological processes associated with the differential ozone response in ozone-tolerant and sensitive soybean genotypes. *Plant Biology, 18*, 28-36.

Colgan, R., Atkinson, C. J., Paul, M., Hassan, S., Drake, P. M. W., Sexton, A. L., . . . Ma, J. K. C. (2010). Optimisation of contained *Nicotiana*

tabacum cultivation for the production of recombinant protein pharmaceuticals. *Transgenic Research, 19*(2), 241-256.

Copolovici, L., Kännaste, A., Pazouki, L., & Niinemets, Ü. (2012). Emissions of green leaf volatiles and terpenoids from *Solanum lycopersicum* are quantitatively related to the severity of cold and heat shock treatments. *Journal of Plant Physiology, 169*, 664-672.

Copolovici, L., & Niinemets, Ü. (2010). Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. *Plant, Cell and Environment, 33*(9), 1582-1594.

Courtois, E. A., Paine, C. E. T., Blandinieres, P. A., Stien, D., Bessiere, J. M., Houel, E., . . . Chave, J. (2009). Diversity of the volatile organic compounds emitted by 55 species of tropical trees: a survey in French Guiana. *Journal of Chemical Ecology, 35*(11), 1349-1362.

Cozzolino, R., Martignetti, A., Pellicano, M. P., Stocchero, M., Cefola, M., Pace, B., & De Giulio, B. (2016). Characterisation of volatile profile and sensory analysis of fresh-cut "Radicchio di Chioggia" stored in air or modified atmosphere. *Food Chemistry, 192*, 603-611.

Creelman, R. A., & Mullet, J. E. (1995). Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *Proceedings of the National Academy of Sciences of the United States of America, 92*(10), 4114-4119.

de Gouw, J. A., Howard, C. J., Custer, T. G., & Fall, R. (1999). Emissions of volatile organic compounds from cut grass and clover are enhanced during the drying process. *Geophysical Research Letters, 26*(7), 811-814.

Delory, B. M., Delaplace, P., Fauconnier, M.-L., & du Jardin, P. (2016). Root-emitted volatile organic compounds: can they mediate belowground plant-plant interactions? *Plant and Soil, 402*(1-2), 1-26.

Dicke, M. (2016). Plant phenotypic plasticity in the phytobiome: a volatile issue. *Current Opinion in Plant Biology, 32*, 17-23.

Dicke, M., & Loreto, F. (2010). Induced plant volatiles: from genes to climate change. *Trends in Plant Science, 15*(3), 115-117.

Dorokhov, Y. L., Komarova, T. V., Petrunia, I. V., Frolova, O. Y., Pozdyshev, D. V., & Gleba, Y. Y. (2012). Airborne signals from a wounded leaf facilitate viral spreading and induce antibacterial resistance in neighboring plants. *PLoS Pathogens, 8*(4), 19.

Dorokhov, Y. L., Sheshukova, E. V., & Komarova, T. V. (2018). Methanol in plant life. *Frontiers in Plant Science, 9*, 1623.

Du, Y. C., Nose, A., & Wasano, K. (1999). Effects of chilling temperature on photosynthetic rates, photosynthetic enzyme activities and metabolite levels in leaves of three sugarcane species. *Plant, Cell and Environment, 22*(3), 317-324.

Duke, J. A. (1983). Handbook of energy crops. NewCROPS web site, Purdue University.https://www.hort.purdue.edu/newcrop/duke_energy/dukeindex.html

Evans, J. R., & von Caemmerer, S. (2013). Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. *Plant, Cell and Environment, 36*(4), 745-756.

Evans, S. M., Vergés, A., & Poore, A. G. B. (2017). Genotypic diversity and short-term response to shading stress in a threatened seagrass: does low diversity mean low resilience? *Frontiers in Plant Science, 8*, 1417.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., . . . Huang, J. L. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science, 8*, 1417.

Fall, R., & Benson, A. A. (1996). Leaf methanol - the simplest natural product from plants. *Trends in Plant Science, 1*(9), 296-301.

Farag, M. A., & Paré, P. W. (2002). C-6-green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry, 61*(5), 545-554.

Farré-Armengol, G., Peñuelas, J., Li, T., Yli-Pirilä, P., Filella, I., Llusia, J., & Blande, J. D. (2016). Ozone degrades floral scent and reduces pollinator attraction to flowers. *New Phytologist, 209*(1), 152-160.

Fatouros, N. E., Lucas-Barbosa, D., Weldegergis, B. T., Pashalidou, F. G., van Loon, J. J. A., Dicke, M., . . . Huigens, M. E. (2012). Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. *PLoS ONE, 7*(8), e43607.

Filella, I., Wilkinson, M. J., Llusia, J., Hewitt, C. N., & Penuelas, J. (2007). Volatile organic compounds emissions in Norway spruce (*Picea abies*) in response to temperature changes. *Physiologia Plantarum, 130*(1), 58-66.

Fischbach, R. J., Zimmer, I., Steinbrecher, R., Pfichner, A., & Schnitzler, J. P. (2000). Monoterpene synthase activities in leaves of *Picea abies* (L.) Karst. and *Quercus ilex* L. *Phytochemistry, 54*(3), 257-265.

Fischer, R., & Emans, N. (2000). Molecular farming of pharmaceutical proteins. *Transgenic Research, 9*(4-5), 279-299.

Folkers, A., Hüve, K., Ammann, C., Dindorf, T., Kesselmeier, J., Kleist, E., . . . Wildt, J. (2008). Methanol emissions from deciduous tree species: dependence on temperature and light intensity. *Plant Biology, 10*(1), 65-75.

Fuentes, J. D., Lerdau, M., Atkinson, R., Baldocchi, D., Bottenheim, J. W., Ciccioli, P., . . . Stockwell, W. (2000). Biogenic hydrocarbons in the atmospheric boundary layer: A review. *Bulletin of the American Meteorological Society, 81*(7), 1537-1575.

Gao, H. Y., Zhu, H. L., Shao, Y., Chen, A. J., Lu, C. W., Zhu, B. Z., & Luo, Y. B. (2008). Lycopene accumulation affects the biosynthesis of some carotenoid-related volatiles independent of ethylene in tomato. *Journal of Integrative Plant Biology, 50*(8), 991-996.

García-Plazaola, J. I., Portillo-Estrada, M., Fernández-Marín, B., Kännaste, A., & Niinemets, Ü. (2017). Emissions of carotenoid cleavage products upon heat shock and mechanical wounding from a foliose lichen. *Environmental and Experimental Botany, 133*, 87-97.

Gasanova, T. V., Skurat, E. V., Frolova, O. Y., Semashko, M. A., & Dorokhov, Y. L. (2008). Pectin methylesterase as a factor of plant transcriptome stability. *Molecular Biology, 42*(3), 421-429.

Gerardin, T., Douthe, C., Flexas, J., & Brendel, O. (2018). Shade and drought growth conditions strongly impact dynamic responses of stomata to variations in irradiance in *Nicotiana tabacum*. *Environmental and Experimental Botany, 153*, 188-197.

Gershenzon, J., McConkey, M. E., & Croteau, R. B. (2000). Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology, 122*(1), 205-213.

Gielen, B., Vandermeiren, K., Horemans, N., D'Haese, D., Serneels, R., & Valcke, R. (2006). Chlorophyll a fluorescence imaging of ozone-stressed *Brassica napus* L. plants differing in glucosinolate concentrations. *Plant Biology, 8*(5), 698-705.

Girón-Calva, P. S., Li, T., & Blande, J. D. (2016). Plant-plant interactions affect the susceptibility of plants to oviposition by pests but are disrupted by ozone pollution. *Agriculture Ecosystems and Environment, 233*, 352-360.

Girón-Calva, P. S., Li, T., Koski, T.-M., Klemola, T., Laaksonen, T., Huttunen, L., & Blande, J. D. (2014). A role for volatiles in intra- and inter-plant interactions in birch. *Journal of Chemical Ecology, 40*(11-12), 1203-1211.

Goff, S. A., & Klee, H. J. (2006). Plant volatile compounds: sensory cues for health and nutritional value? *Science, 311*(5762), 815-819.

Gols, R., Witjes, L. M. A., van Loon, J. J. A., Posthumus, M. A., Dicke, M., & Harvey, J. A. (2008). The effect of direct and indirect defenses in two wild brassicaceous plant species on a specialist herbivore and its gregarious endoparasitoid. *Entomologia Experimentalis Et Applicata, 128*(1), 99-108.

Gomaa, N. H., Sherif, E. A. A., Hegazy, A. K., & Hassan, M. O. (2012). Floristic diversity and vegetation analysis of *Brassica nigra* (L.) Koch communities. *Egyptian Journal of Biology, 14*, 63-72.

Gratani, L., Pesoli, P., Crescente, M. F., Aichner, K., & Larcher, W. (2000). Photosynthesis as a temperature indicator in *Quercus ilex* L. *Global and Planetary Change, 24*(2), 153-163.

Grubb, C. D., & Abel, S. (2006). Glucosinolate metabolism and its control. *Trends in Plant Science, 11*(2), 89-100.

Guidi, L., Degl'Innocenti, E., Martinelli, F., & Piras, M. (2009). Ozone effects on carbon metabolism in sensitive and insensitive *Phaseolus cultivars*. *Environmental and Experimental Botany, 66*(1), 117-125.

Guidolotti, G., Pallozzi, E., Gavrichkova, O., Scartazza, A., Mattioni, M., Loreto, F., & Calfapietra, C. (2019). Emission of constitutive isoprene, induced monoterpenes, and other volatiles under high temperatures in Eucalyptus camaldulensis: A C-13 labelling study. *Plant, Cell and Environment, 42*(6), 1929-1938.

Guo, R. F., Wang, X. R., Han, X. Y., Li, W. J., Liu, T., Chen, B. X., . . . Wang-Pruski, G. (2019). Comparative transcriptome analyses revealed different heat stress responses in high- and low-GS *Brassica alboglabra* sprouts. *BMC Genomics, 20*, 269.

Gutbrodt, B., Dorn, S., & Mody, K. (2012). Drought stress affects constitutive but not induced herbivore resistance in apple plants. *Arthropod-Plant Interactions, 6*(2), 171-179.

Guy, C. L., Haskell, D. W., Kaplan, F., & Sung, D. Y. (2003). Emerging basic and applied advances for research on acquired tolerances of plants exposed to temperature extremes. *Acta Horticulturae*, *618*, 17-29.

Haldimann, P., & Feller, U. (2004). Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens* L.) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase. *Plant, Cell and Environment, 27*(9), 1169-1183.

Halkier, B. A., & Du, L. (1997). The biosynthesis of glucosinolates. *Trends in Plant Science, 2*(11), 425-431.

Han, B. Y., & Chen, Z. M. (2002). Composition of the volatiles from intact and mechanically pierced tea aphid-tea shoot complexes and their attraction to natural enemies of the tea aphid. *Journal of Agricultural and Food Chemistry, 50*(9), 2571-2575.

Hara, M., Harazaki, A., & Tabata, K. (2013). Administration of isothiocyanates enhances heat tolerance in *Arabidopsis thaliana*. *Plant Growth Regulation, 69*(1), 71-77.

Harada, E., Kim, J. A., Meyer, A. J., Hell, R., Clemens, S., & Choi, Y. E. (2010). Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. *Plant and Cell Physiology, 51*(10), 1627-1637.

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences, 14*(5), 9643-9684.

Heiden, A. C., Kobel, K., Langebartels, C., Schuh-Thomas, G., & Wildt, J. (2003). Emissions of oxygenated volatile organic compounds from plants - part I: Emissions from lipoxygenase activity. *Journal of Atmospheric Chemistry, 45*(2), 143-172.

Hossain, M. S., Ye, W., Hossain, M. A., Okuma, E., Uraji, M., Nakamura, Y., . . . Murata, Y. (2013). Glucosinolate degradation products, isothiocyanates, nitriles, and thiocyanates, induce stomatal closure accompanied by peroxidase-mediated reactive oxygen speciesp production in *Arabidopsis thaliana*. *Bioscience Biotechnology and Biochemistry, 77*(5), 977-983.

Hu, Z., Zhang, H., Leng, P., Zhao, J., Wang, W., & Wang, S. (2013). The emission of floral scent from Lilium 'siberia' in response to light intensity and temperature. *Acta Physiologiae Plantarum, 35*(5), 1691-1700.

Hu, Z. H., Shen, Y. B., Luo, Y. Q., Shen, F. Y., Gao, H. B., & Gao, R. F. (2008). Aldehyde volatiles emitted in succession from mechanically damaged leaves of poplar cuttings. *Journal of Plant Biology, 51*(4), 269-275.

Hüve, K., Bichele, I., Kaldmäe, H., Rasulov, B., Valladares, F., & Niinemets, Ü. (2019). Responses of aspen leaves to heatflecks: both damaging and non-damaging rapid temperature excursions reduce photosynthesis. *Plants-Basel, 8*(6), 145.

Hüve, K., Bichele, I., Rasulov, B., & Niinemets, Ü. (2011). When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and H_2O_2 formation. *Plant, Cell and Environment, 34*(1), 113-126.

Ishida, M., Hara, M., Fukino, N., Kakizaki, T., & Morimitsu, Y. (2014). Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. *Breeding Science, 64*(1), 48-59.

Intergovernmental Panel on Climate Change. (2014). Foreword. In Climate Change 2014 – Impacts, Adaptation and Vulnerability: Part A: Global and Sectoral Aspects: Working Group II Contribution to the IPCC Fifth Assessment Report (pp. Vii-Viii). Cambridge: Cambridge University Press.

Jansson, S., & Douglas, C. J. (2007). Populus: A model system for plant biology. *Annual Review of Plant Biology, 58*, 435-458.

Jardine, K., Abrell, L., Kurc, S. A., Huxman, T., Ortega, J., & Guenther, A. (2010). Volatile organic compound emissions from *Larrea tridentata* (creosotebush). *Atmospheric Chemistry and Physics, 10*(24), 12191-12206.

Jardine, K. J., Monson, R. K., Abrell, L., Saleska, S. R., Arneth, A., Jardine, A., . . . Huxman, T. (2012). Within-plant isoprene oxidation confirmed by direct emissions of oxidation products methyl vinyl ketone and methacrolein. *Global Change Biology, 18*(3), 973-984.

Joó, É., Dewulf, J., Amelynck, C., Schoon, N., Pokorska, O., Šimpraga, M., . . . Van Langenhove, H. (2011). Constitutive versus heat and biotic stress induced BVOC emissions in *Pseudotsuga menziesii*. *Atmospheric Environment, 45*(22), 3655-3662.

Jud, W., Fischer, L., Canaval, E., Wohlfahrt, G., Tissier, A., & Hansel, A. (2016). Plant surface reactions: an opportunistic ozone defence mechanism impacting atmospheric chemistry. *Atmospheric Chemistry and Physics, 16*(1), 277-292.

Junker, R. R., Kuppler, J., Amo, L., Blande, J. D., Borges, R. M., van Dam, N. M., . . . Köllner, T. G. (2017). Covariation and phenotypic integration in chemical communication displays: biosynthetic constraints and eco-evolutionary implications. *The New Phytologist*, *220*(3), 739-749.

Kanagendran, A., Pazouki, L., Li, S., Liu, B., Kännaste, A., & Niinemets, Ü. (2018). Ozone-triggered surface uptake and stress volatile emissions in *Nicotiana tabacum* 'Wisconsin'. *Journal of Experimental Botany, 69*(3), 681-697.

Karlsson, P. E., Medin, E. L., Wallin, G., Sellden, G., & Skarby, L. (1997). Effects of ozone and drought stress on the physiology and growth of two clones of Norway spruce (*Picea abies*). *New Phytologist, 136*(2), 265-275.

Kask, K., Kännaste, A., Talts, E., Copolovici, L., & Niinemets, Ü. (2016). How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*. *Plant, Cell and Environment, 39*(9), 2027-2042.

Kelly, P. J., Bones, A., & Rossiter, J. T. (1998). Sub-cellular immunolocalization of the glucosinolate sinigrin in seedlings of *Brassica juncea*. *Planta, 206*(3), 370-377.

Khaling, E., Li, T., Holopainen, J. K., & Blande, J. D. (2016). Elevated ozone modulates herbivore-induced volatile emissions of *Brassica nigra* and alters a tritrophic interaction. *Journal of Chemical Ecology, 42*(5), 368-381.

Khokon, M. A. R., Jahan, M. S., Rahman, T., Hossain, M. A., Muroyama, D., Minami, I., . . . Murata, Y. (2011). Allyl isothiocyanate (AITC) induces stomatal closure in *Arabidopsis*. *Plant, Cell and Environment, 34*(11), 1900-1906.

Kigathi, R. N., Weisser, W. W., Reichelt, M., Gershenzon, J., & Unsicker, S. B. (2019). Plant volatile emission depends on the species composition of the neighboring plant community. *BMC Plant Biology, 19*, 58.

Kigathi, R. N., Weisser, W. W., Veit, D., Gershenzon, J., & Unsicker, S. B. (2013). Plants suppress their emission of volatiles when growing with conspecifics. *Journal of Chemical Ecology, 39*(4), 537-545.

Kim, K., & Portis, A. R. (2005). Temperature dependence of photosynthesis in *Arabidopsis* plants with modifications in Rubisco activase and membrane fluidity. *Plant and Cell Physiology, 46*(3), 522-530.

Kinley, R. (2017). Climate change after Paris: from turning point to transformation. *Climate Policy, 17*(1), 9-15.

Kirkegaard, J. A., & Sarwar, M. (1998). Biofumigation potential of brassicas - I. Variation in glucosinolate profiles of diverse field-grown brassicas. *Plant and Soil, 201*(1), 71-89.

Klaiber, J., Dorn, S., & Najar-Rodriguez, A. J. (2013). Acclimation to elevated CO_2 increases constitutive glucosinolate levels of Brassica plants and affects the performance of specialized herbivores from contrasting feeding guilds. *Journal of Chemical Ecology, 39*(5), 653-665.

Kleist, E., Mentel, T. F., Andres, S., Bohne, A., Folkers, A., Kiendler-Scharr, A., . . . Wildt, J. (2012). Irreversible impacts of heat on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species. *Biogeosciences, 9*(12), 5111-5123.

Kliebenstein, D. J., Kroymann, J., Brown, P., Figuth, A., Pedersen, D., Gershenzon, J., & Mitchell-Olds, T. (2001). Genetic control of natural variation in Arabidopsis glucosinolate accumulation. *Plant Physiology, 126*(2), 811-825.

Klopsch, R., Witzel, K., Artemyeva, A., Ruppel, S., & Hanschen, F. S. (2018). Genotypic variation of glucosinolates and their breakdown products in leaves of *Brassica rapa*. *Journal of Agricultural and Food Chemistry*, *66*(22), 5481-5490.

Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D. J., & Gershenzon, J. (2001). The Arabidopsis epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell, 13*(12), 2793-2807.

Lange, B. M., & Turner, G. W. (2013). Terpenoid biosynthesis in trichomes-current status and future opportunities. *Plant Biotechnology Journal, 11*(1), 2-22.

Lehrman, A., Boddum, T., Stenberg, J. A., Orians, C. M., & Björkman, C. (2013). Constitutive and herbivore-induced systemic volatiles differentially attract an omnivorous biocontrol agent to contrasting Salix clones. *AoB Plants, 5*, plt005.

Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature, 529*, 84-87.

Li, S., Tosens, T., Harley, P. C., Jiang, Y., Kanagendran, A., Grosberg, M., . . . Niinemets, Ü. (2018). Glandular trichomes as a barrier against atmospheric oxidative stress: relationships with ozone uptake, leaf damage and emission of LOX products across a diverse set of species. *Plant, Cell and Environment*, *41* (6), 1263-1277.

Lichtenthaler, H. K. (1996). Vegetation stress: An introduction to the stress concept in plants. *Journal of Plant Physiology, 148*(1-2), 4-14.

Lichtenthaler, H. K. (1998). The stress concept in plants: An introduction. *Stress of Life: from Molecules to Man, 851*, 187-198.

Liu, Y. J., Herdlinger-Blatt, I., McKinney, K. A., & Martin, S. T. (2013). Production of methyl vinyl ketone and methacrolein via the hydroperoxyl pathway of isoprene oxidation. *Atmospheric Chemistry and Physics, 13*(11), 5715-5730.

Loreto, F., Barta, C., Brilli, F., & Nogués, I. (2006). On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. *Plant, Cell and Environment, 29*(9), 1820-1828.

Loreto, F., Ciccioli, P., Cecinato, A., Brancaleoni, E., Frattoni, M., & Tricoli, D. (1996). Influence of environmental factors and air composition on the emission of alpha-pinene from *Quercus ilex* leaves. *Plant Physiology, 110*(1), 267-275.

Loreto, F., Förster, A., Durr, M., Csiky, O., & Seufert, G. (1998). On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant, Cell and Environment, 21*(1), 101-107.

Loreto, F., Pinelli, P., Manes, F., & Kollist, H. (2004). Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves. *Tree Physiology, 24*(4), 361-367.

Loreto, F., Pollastri, S., Fineschi, S., & Velikova, V. (2014). Volatile isoprenoids and their importance for protection against environmental constraints in the Mediterranean area. *Environmental and Experimental Botany, 103*, 99-106.

Loreto, F., & Schnitzler, J. P. (2010). Abiotic stresses and induced BVOCs. *Trends in Plant Science, 15*(3), 154-166.

Loughrin, J. H., Hamiltonkemp, T. R., Andersen, R. A., & Hildebrand, D. F. (1990). Headspace compounds from flowers of *Nicotiana tabacum* and related species. *Journal of Agricultural and Food Chemistry, 38*(2), 455-460.

Luca, A., Kjær, A., & Edelenbos, M. (2017). Volatile organic compounds as markers of quality changes during the storage of wild rocket. *Food Chemistry, 232*, 579-586.

Lucas-Barbosa, D., Dicke, M., Kranenburg, T., Aartsma, Y., van Beek, T. A., Huigens, M. E., & van Loon, J. J. A. (2017). Endure and call for help: strategies of black mustard plants to deal with a specialized caterpillar. *Functional Ecology, 31*(2), 325-333.

Macdonald, R. C., & Fall, R. (1993). Detection of substantial emissions of methanol from plants to the atmosphere. *Atmospheric Environment. Part A. General Topics, 27*(11), 1709-1713.

Mainali, K. P., Heckathorn, S. A., Wang, D., Weintraub, M. N., Frantz, J. M., & Hamilton, E. W. (2014). Impact of a short-term heat event on C and N relations in shoots vs. roots of the stress-tolerant C-4 grass, *Andropogon gerardii*. *Journal of Plant Physiology, 171*(12), 977-985.

Maron, J. L. (1998). Insect herbivory above- and belowground: Individual and joint effects on plant fitness. *Ecology, 79*(4), 1281-1293.

Matsui, K. (2006). Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Current Opinion in Plant Biology, 9*(3), 274-280.

Matsui, K., Sugimoto, K., Mano, J. i., Ozawa, R., & Takabayashi, J. (2012). Differential metabolisms of green leaf volatiles in injured and intact parts of a wounded leaf meet distinct ecophysiological requirements. *PLoS ONE, 7*(4): e36433.

Mejia-Garibay, B., Palou, E., & López-Malo, A. (2015). Composition, diffusion, and antifungal activity of black mustard (*Brassica nigra*) essential oil when applied by direct addition or vapor phase contact. *Journal of Food Protection, 78*(4), 843-848.

Mengiste, T., Chen, X., Salmeron, J., & Dietrich, R. (2003). The BOTRYTIS SUSCEPTIBLE1 gene encodes an R2R3MYB transcription factor protein that is required for biotic and abiotic stress responses in Arabidopsis. *Plant Cell, 15*(11), 2551-2565.

Mithen, R. F. (2001). Glucosinolates and their degradation products. *Advances in Botanical Research, 35*, 213-262.

Mittler, R., Finka, A., & Goloubinoff, P. (2012). How do plants feel the heat? *Trends in Biochemical Sciences, 37*(3), 118-125.

Mumm, R., Burow, M., Bukovinszkine'kiss, G., Kazantzidou, E., Wittstock, U., Dicke, M., & Gershenzon, J. (2008). Formation of simple nitriles upon glucosinolate hydrolysis affects direct and indirect defense against the specialist herbivore, *Pieris rapae*. *Journal of Chemical Ecology, 34*(10), 1311-1321.

Nagata, T., Nemoto, Y., & Hasezawa, S. (1992). Tobacco BY-2 cell line as the "HeLa" cell in the cell biology of higher plants. *International Review of Cytology, 132*, 1-30.

Nankishore, A., & Farrell, A. D. (2016). The response of contrasting tomato genotypes to combined heat and drought stress. *Journal of Plant Physiology, 202*, 75-82.

Nayidu, N. K., Tan, Y., Taheri, A., Li, X., Bjorndahl, T. C., Nowak, J., . . . Gruber, M. Y. (2014). *Brassica villosa*, a system for studying non-glandular trichomes and genes in the Brassicas. *Plant Molecular Biology, 85*(4-5), 519-539.

Newton, E. L., Bullock, J. M., & Hodgson, D. J. (2009). Glucosinolate polymorphism in wild cabbage (*Brassica oleracea*) influences the structure of herbivore communities. *Oecologia, 160*(1), 63-76.

Niinemets, Ü. (2010). Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in Plant Science, 15*(3), 145-153.

Niinemets, Ü., Kännaste, A., & Copolovici, L. (2013). Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. *Frontiers in Plant Science, 4*, 1-15.

Niinemets, Ü., Kuhn, U., Harley, P. C., Staudt, M., Arneth, A., Cescatti, A., . . . Peñuelas, J. (2011). Estimations of isoprenoid emission capacity from enclosure studies: measurements, data processing, quality and standardized measurement protocols. *Biogeosciences, 8*(8), 2209-2246.

Nogués, I., Brilli, F., & Loreto, F. (2006). Dimethylallyl diphosphate and geranyl diphosphate pools of plant species characterized by different isoprenoid emissions. *Plant Physiology, 141*(2), 721-730.

Olivas, N. H. D., Frago, E., Thoen, M. P. M., Kloth, K. J., Becker, F. F. M., van Loon, J. J. A., . . . Dicke, M. (2017). Natural variation in life history strategy of *Arabidopsis thaliana* determines stress responses to drought and insects of different feeding guilds. *Molecular Ecology, 26*(11), 2959-2977.

Ortiz, C., & Cardemil, L. (2001). Heat-shock responses in two leguminous plants: a comparative study. *Journal of Experimental Botany, 52*(361), 1711-1719.

Pashalidou, F. G., Frago, E., Griese, E., Poelman, E. H., van Loon, J. J. A., Dicke, M., & Fatouros, N. E. (2015). Early herbivore alert matters: plant-mediated effects of egg deposition on higher trophic levels benefit plant fitness. *Ecology Letters, 18*(9), 927-936.

Pashalidou, F. G., Gols, R., Berkhout, B. W., Weldegergis, B. T., van Loon, J. J. A., Dicke, M., & Fatouros, N. E. (2015). To be in time: egg deposition enhances plant-mediated detection of young caterpillars by parasitoids. *Oecologia, 177*(2), 477-486.

Pazouki, L., Kanagendran, A., Li, S., Kännaste, A., Memari, H. R., Bichele, R., & Niinemets, Ü. (2016). Mono- and sesquiterpene release from tomato (*Solanum lycopersicum*) leaves upon mild and severe heat stress and through recovery: From gene expression to emission responses. *Environmental and Experimental Botany, 132*, 1-15.

Perdomo, J. A., Capó-Baucà, S., Carmo-Silva, E., & Galmés, J. (2017). Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. *Frontiers in Plant Science, 8*, 490.

Pereira, A. (2016). Plant abiotic stress challenges from the changing environment. *Frontiers in Plant Science, 7*, 1123.

Pierotti, D., Wofsy, S. C., Jacob, D., & Rasmussen, R. A. (1990). Isoprene and its oxidation products: Methacrolein and methyl vinyl ketone. *Journal of Geophysical Research-Atmospheres, 95*(D2), 1871-1881.

Piesik, D., Delaney, K. J., Wenda-Piesik, A., Sendel, S., Tabaka, P., & Buszewski, B. (2013). *Meligethes aeneus* pollen-feeding suppresses, and oviposition induces, *Brassica napus* volatiles: beetle attraction/repellence to lilac aldehydes and veratrole. *Chemoecology, 23*(4), 241-250.

Pollastri, S., Jorba, I., Hawkins, T. J., Llusià, J., Michelozzi, M., Navajas, D., . . . Loreto, F. (2019). Leaves of isoprene-emitting tobacco plants maintain PSII stability at high temperatures. *New Phytologist, 223*(3), 1307-1318.

Ponzio, C., Gols, R., Weldegergis, B. T., & Dicke, M. (2014). Caterpillar-induced plant volatiles remain a reliable signal for foraging wasps during dual attack with a plant pathogen or non-host insect herbivore. *Plant, Cell and Environment, 37*(8), 1924-1935.

Ponzio, C., Papazian, S., Albrectsen, B. R., Dicke, M., & Gols, R. (2017). Dual herbivore attack and herbivore density affect metabolic profiles of *Brassica nigra* leaves. *Plant, Cell and Environment, 40*(8), 1356-1367.

Portillo-Estrada, M., Kazantsev, T., Talts, E., Tosens, T., & Niinemets, Ü. (2015). Emission timetable and quantitative patterns of wound-induced volatiles across different leaf damage treatments in aspen (*Populus tremula*). *Journal of Chemical Ecology, 41*(12), 1105-1117.

Rask, L., Andreasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B., & Meijer, J. (2000). Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Molecular Biology, 42*(1), 93-113.

Redovniković, I. R., Glivetić, T., Delonga, K., & Vorkapić-Furač, J. (2008). Glucosinolates and their potential role in plant. *Periodicum Biologorum, 110*(4), 297-309.

Rejeb, I. B., Pastor, V., & Mauch-Mani, B. (2014). Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants, 15*(3), 458-475.

Rodríguez, V. M., Soengas, P., Alonso-Villaverde, V., Sotelo, T., Cartea, M. E., & Velasco, P. (2015). Effect of temperature stress on the early vegetative development of *Brassica oleracea* L. *BMC Plant Biology, 15*, 145.

Rosa, E. A. S., & Rodrigues, P. M. F. (1998). The effect of light and temperature on glucosinolate concentration in the leaves and roots of cabbage seedlings. *Journal of the Science of Food and Agriculture, 78*(2), 208-212.

Rouseff, R. L., Onagbola, E. O., Smoot, J. M., & Stelinski, L. L. (2008). Sulfur volatiles in guava (*Psidium guajava* L.) leaves: Possible defense mechanism. *Journal of Agricultural and Food Chemistry, 56*(19), 8905-8910.

Ruan, J. J., Zhou, Y. X., Zhou, M. L., Yan, J., Khurshid, M., Weng, W. F., . . . Zhang, K. X. (2019). Jasmonic acid signaling pathway in plants. *International Journal of Molecular Sciences, 20*(10), 2479.

Ryan, A. C., Hewitt, C. N., Possell, M., Vickers, C. E., Purnell, A., Mullineaux, P. M., . . . Dodd, I. C. (2014). Isoprene emission protects photosynthesis but reduces plant productivity during drought in transgenic tobacco (*Nicotiana tabacum*) plants. *New Phytologist, 201*(1), 205-216.

Salas, J. J., García-Gonzàlez, D. L., & Aparicio, R. (2006). Volatile compound biosynthesis by green leaves from an *Arabidopsis thaliana* hydroperoxide lyase knockout mutant. *Journal of Agricultural and Food Chemistry, 54*(21), 8199-8205.

Salvucci, M. E., & Crafts-Brandner, S. J. (2004). Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum, 120*(2), 179-186.

Sarker, S., & Lim, U. T. (2018). Extract of *Nicotiana tabacum* as a potential control agent of *Grapholita molesta* (Lepidoptera: Tortricidae). *PLoS ONE, 13*(8).

Sarwar, M., & Kirkegaard, J. A. (1998). Biofumigation potential of brassicas - II. Effect of environment and ontogeny on glucosinolate production and implications for screening. *Plant and Soil, 201*(1), 91-101.

Scafaro, A. P., Haynes, P. A., & Atwell, B. J. (2010). Physiological and molecular changes in *Oryza meridionalis* Ng., a heat-tolerant species of wild rice. *Journal of Experimental Botany, 61*(1), 191-202.

Scala, A., Allmann, S., Mirabella, R., Haring, M. A., & Schuurink, R. C. (2013). Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences, 14*(9), 17781-17811.

Schiestl, F. P. (2014). Correlation analyses between volatiles and glucosinolates show no evidence for chemical defense signaling in *Brassica rapa*. *Frontiers in Ecology and Evolution, 2*, 10.

Schillberg, S., Raven, N., Fischer, R., Twyman, R. M., & Schiermeyer, A. (2013). Molecular farming of pharmaceutical proteins using plant suspension cell and tissue cultures. *Current Pharmaceutical Design, 19*(31), 5531-5542.

Schiller, D., Contreras, C., Vogt, J., Dunemann, F., Defilippi, B. G., Beaudry, R., & Schwab, W. (2015). A dual positional specific lipoxygenase functions in the generation of flavor compounds during climacteric ripening of apple. *Horticulture Research, 2*, 15003.

Sharkey, T. D. (2005). Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell and Environment, 28*(3), 269-277.

Shen, J., Tieman, D., Jones, J. B., Taylor, M. G., Schmelz, E., Huffaker, A., . . . Klee, H. J. (2014). A 13-lipoxygenase, TomloxC, is essential for synthesis of C5 flavour volatiles in tomato. *Journal of Experimental Botany, 65*(2), 419-428.

Shen, Q. P., Xu, X. M., Li, L., Zhao, W., Xiang, N. J., Yang, G. Y., . . . Liu, Z. H. (2016). Sesquiterpenes from the leaves of *Nicotiana tabacum* and their anti-tobacco mosaic virus activity. *Chinese Chemical Letters, 27*(5), 753-756.

Shi, Q. M., Li, C. J., & Zhang, F. S. (2006). Nicotine synthesis in *Nicotiana tabacum* L. induced by mechanical wounding is regulated by auxin. *Journal of Experimental Botany, 57*(11), 2899-2907.

Shiojiri, K., Kishimoto, K., Ozawa, R., Kugimiya, S., Urashimo, S., Arimura, G., . . . Takabayashi, J. (2006). Changing green leaf volatile biosynthesis in plants: An approach for improving plant resistance against both herbivores and pathogens. *Proceedings of the National Academy of Sciences of the United States of America, 103*(45), 16672-16676.

Siebert, S., Ewert, F., Rezaei, E. E., Kage, H., & Grass, R. (2014). Impact of heat stress on crop yield-on the importance of considering canopy temperature. *Environmental Research Letters, 9*(4), 044012.

Sierro, N., Battey, J. N. D., Ouadi, S., Bakaher, N., Bovet, L., Willig, A., . . . Ivanov, N. V. (2014). The tobacco genome sequence and its comparison with those of tomato and potato. *Nature Communications, 5*, 3833.

Simkin, A. J., Schwartz, S. H., Auldridge, M., Taylor, M. G., & Klee, H. J. (2004). The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles beta-ionone, pseudoionone, and geranylacetone. *Plant Journal, 40*(6), 882-892.

Singsaas, E. L., Laporte, M. M., Shi, J. Z., Monson, R. K., Bowling, D. R., Johnson, K., . . . Sharkey, T. D. (1999). Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (*Quercus rubra*) leaves. *Tree Physiology, 19*(14), 917-924.

Singsaas, E. L., & Sharkey, T. D. (2000). The effects of high temperature on isoprene synthesis in oak leaves. *Plant, Cell and Environment, 23*(7), 751-757.

Sinsawat, V., Leipner, J., Stamp, P., & Fracheboud, Y. (2004). Effect of heat stress on the photosynthetic apparatus in maize (*Zea mays* L.) grown at control or high temperature. *Environmental and Experimental Botany, 52*(2), 123-129.

Sønderby, I. E., Geu-Flores, F., & Halkier, B. A. (2010). Biosynthesis of glucosinolates - gene discovery and beyond. *Trends in Plant Science, 15*(5), 283-290.

Song, Y. P., Chen, Q. Q., Ci, D., Shao, X. N., & Zhang, D. Q. (2014). Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC Plant Biology, 14*, 111.

Sotelo, T., Velasco, P., Soengas, P., Rodríguez, V. M., & Cartea, M. E. (2016). Modification of leaf glucosinolate contents in *Brassica oleracea* by divergent selection and effect on expression of genes controlling glucosinolate pathway. *Frontiers in Plant Science, 7*, 1012.

Srba, M., Černiková, A., Opatrný, Z., & Fischer, L. (2016). Practical guidelines for the characterization of tobacco BY-2 cell lines. *Biologia Plantarum, 60*(1), 13-24.

Staudt, M., Bertin, N., Hansen, U., Seufert, G., Ciccioli, P., Foster, P., . . . Fugit, J. L. (1997). Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. *Atmospheric Environment, 31*, 145-156.

Street, N. R., Tallis, M. J., Tucker, J., Brosché, M., Kangasjarvi, J., Broadmeadow, M., & Taylor, G. (2011). The physiological, transcriptional and genetic responses of an ozone- sensitive and an ozone tolerant poplar and selected extremes of their F-2 progeny. *Environmental Pollution, 159*(1), 45-54.

Sung, D. Y., Kaplan, F., Lee, K. J., & Guy, C. L. (2003). Acquired tolerance to temperature extremes. *Trends in Plant Science, 8*(4), 179-187.

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist, 203*(1), 32-43.

Svenning, J. C., Gravel, D., Holt, R. D., Schurr, F. M., Thuiller, W., Münkemüller, T., . . . Normand, S. (2014). The influence of interspecific interactions on species range expansion rates. *Ecography, 37*(12), 1198-1209.

Taipale, R., Kajos, M. K., Patokoski, J., Rantala, P., Ruuskanen, T. M., & Rinne, J. (2011). Role of de novo biosynthesis in ecosystem scale monoterpene emissions from a boreal Scots pine forest. *Biogeosciences, 8*(8), 2247-2255.

Talukder, A., McDonald, G. K., & Gill, G. S. (2014). Effect of short-term heat stress prior to flowering and early grain set on the grain yield of wheat. *Field Crops Research, 160*, 54-63.

Tholl, D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology, 9*(3), 297-304.

Tieman, D. M., Zeigler, M., Schmelz, E. A., Taylor, M. G., Bliss, P., Kirst, M., & Klee, H. J. (2006). Identification of loci affecting flavour volatile emissions in tomato fruits. *Journal of Experimental Botany, 57*(4), 887-896.

Tietel, Z., Porat, R., Weiss, K., & Ulrich, D. (2011). Identification of aroma-active compounds in fresh and stored 'Mor' mandarins. *International Journal of Food Science and Technology, 46*(11), 2225-2231.

Toome, M., Randjärv, P., Copolovici, L., Niinemets, Ü., Heinsoo, K., Luik, A., & Noe, S. M. (2010). Leaf rust induced volatile organic compounds signalling in willow during the infection. *Planta, 232*(1), 235-243.

Truong, D.-H., Delory, B. M., Brostaux, Y., Heuskin, S., Delaplace, P., Francis, F., & Lognay, G. (2014). *Plutella xylostella* (L.) infestations at varying temperatures induce the emission of specific volatile blends by *Arabidopsis thaliana* (L.) Heynh. *Plant Signaling and Behavior, 9*(11), e973816.

Tsao, R., Yu, Q., Friesen, I., Potter, J., & Chiba, M. (2000). Factors affecting the dissolution and degradation of oriental mustard-derived sinigrin and allyl isothiocyanate in aqueous media. *Journal of Agricultural and Food Chemistry, 48*(5), 1898-1902.

Tsao, R., Yu, Q., Potter, J., & Chiba, M. (2002). Direct and simultaneous analysis of sinigrin and allyl isothiocyanate in mustard samples by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 50*(17), 4749-4753.

Turlings, T. C. J., Loughrin, J. H., McCall, P. J., Röse, U. S. R., Lewis, W. J., & Tumlinson, J. H. (1995). How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America, 92*(10), 4169-4174.

Usano-Alemany, J., Pála-Paúl, J., & Herráiz-Peñalver, D. (2014). Temperature stress causes different profiles of volatile compounds in two chemotypes of *Salvia lavandulifolia* Vahl. *Biochemical Systematics and Ecology, 54*, 166-171.

Valolahti, H., Kivimäenpää, M., Faubert, P., Michelsen, A., & Rinnan, R. (2015). Climate change-induced vegetation change as a driver of increased subarctic biogenic volatile organic compound emissions. *Global Change Biology, 21*(9), 3478-3488.

van Dam, N. M., Witjes, L., & Svatoš, A. (2004). Interactions between aboveground and belowground induction of glucosinolates in two wild *Brassica* species. *New Phytologist, 161*(3), 801-810.

Vega, F. E., Simpkins, A., Miranda, J., Harnly, J. M., Infante, F., Castillo, A., . . . Cossé, A. (2017). A potential repellent against the coffee berry borer (Coleoptera: Curculionidae: Scolytinae). *Journal of Insect Science, 17*(6), 122.

Velikova, V., Tsonev, T., Barta, C., Centritto, M., Koleva, D., Stefanova, M., . . . Loreto, F. (2009). BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature. *Environmental Pollution, 157*(10), 2629-2637.

Veromann, E., Metspalu, L., Williams, I. H., Hiiesaar, K., Mand, M., Kaasik, R., . . . Luik, A. (2012). Relative attractiveness of *Brassica napus*, *Brassica nigra*, *Eruca sativa* and *Raphanus sativus* for pollen beetle (*Meligethes aeneus*) and their potential for use in trap cropping. *Arthropod-Plant Interactions, 6*(3), 385-394.

Veromann, E., Toome, M., Kännaste, A., Kaasik, R., Copolovici, L., Flink, J., . . . Niinemets, Ü. (2013). Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. *Crop Protection, 43*, 79-88.

Vickers, C. E., Possell, M., Cojocariu, C. I., Velikova, V. B., Laothawornkitkul, J., Ryan, A., . . . Hewitt, C. N. (2009). Isoprene synthesis protects transgenic tobacco plants from oxidative stress. *Plant, Cell and Environment, 32*(5), 520-531.

Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B-Biological Sciences, 275*(1635), 649-659.

Vogel, J. T., Tieman, D. M., Sims, C. A., Odabasi, A. Z., Clark, D. G., & Klee, H. J. (2010). Carotenoid content impacts flavor acceptability in tomato (*Solanum lycopersicum*). *Journal of the Science of Food and Agriculture, 90*(13), 2233-2240.

von Caemmerer, S., & Farquhar, G. D. (1981). Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta, 153*, 376-387.

von Caemmerer, S., & Evans, J. R. (2015). Temperature responses of mesophyll conductance. *Plant, Cell and Environment, 38*(4), 629-637.

Vos, J., Evers, J. B., Buck-Sorlin, G. H., Andrieu, B., Chelle, M., & de Visser, P. H. B. (2010). Functional-structural plant modelling: a new versatile tool in crop science. *Journal of Experimental Botany, 61*(8), 2101-2115.

Vuorinen, T., Nerg, A. M., & Holopainen, J. K. (2004). Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signalling. *Environmental Pollution, 131*(2), 305-311.

Waters, E. R., & Schaal, B. A. (1996). Heat shock induces a loss of rRNA-encoding DNA repeats in *Brassica nigra*. *Proceedings of the National Academy of Sciences of the United States of America, 93*(4), 1449-1452.

Westman, A. L., & Kresovich, S. (1999). Simple sequence repeat (SSR)-based marker variation in *Brassica nigra* genebank accessions and weed populations. *Euphytica, 109*(2), 85-92.

Wu, Y. R., Deng, Z. Y., Lai, J. B., Zhang, Y. Y., Yang, C. P., Yin, B. J., . . . Xie, Q. (2009). Dual function of Arabidopsis ATAF1 in abiotic and biotic stress responses. *Cell Research, 19*(11), 1279-1290.

Xing, Z. L., Liu, Y. Q., Cai, W. Z., Huang, X. Z., Wu, S. Y., & Lei, Z. R. (2017). Efficiency of trichome-based plant defense in *Phaseolus vulgaris* depends on insect behavior, plant ontogeny, and structure. *Frontiers in Plant Science, 8*, 2006.

Yan, N., Xu, X. F., Wang, Z. D., Huang, J. Z., & Guo, D. P. (2013). Interactive effects of temperature and light intensity on photosynthesis and antioxidant enzyme activity in *Zizania latifolia* Turcz. plants. *Photosynthetica, 51*(1), 127-138.

Yan, X. F., & Chen, S. X. (2007). Regulation of plant glucosinolate metabolism. *Planta, 226*(6), 1343-1352.

Yang, L. Y., Yang, S. L., Li, J. Y., Ma, J. H., Pang, T., Zou, C. M., . . . Gong, M. (2018). Effects of different growth temperatures on growth, development, and plastid pigments metabolism of tobacco (*Nicotiana tabacum* L.) plants. *Botanical Studies, 59*(1), 5.

Ye, M., Veyrat, N., Xu, H., Hu, L. F., Turlings, T. C. J., & Erb, M. (2018). An herbivore-induced plant volatile reduces parasitoid attraction by changing the smell of caterpillars. *Science Advances, 4*(5), eaar4767.

Yin, G. Y., Wang, W. J., Niu, H. X., Ding, Y. Q., Zhang, D. Y., Zhang, J., . . . Zhang, H. B. (2017). Jasmonate-sensitivity-assisted screening and characterization of nicotine synthetic mutants from activation-tagged population of tobacco (*Nicotiana tabacum* L.). *Frontiers in Plant Science, 8*, 157.

Yoshida, T., Mogami, J., & Yamaguchi-Shinozaki, K. (2014). ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Current Opinion in Plant Biology, 21*, 133-139.

Zhang, R., Cruz, J. A., Kramer, D. M., Magallanes-Lundback, M. E., Dellapenna, D., & Sharkey, T. D. (2009). Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves. *Plant, Cell and Environment, 32*(11), 1538-1547.

Zhang, T. G., Mo, J. N., Zhou, K., Chang, Y., & Liu, Z. G. (2018). Overexpression of *Brassica campestris* BcICE1 gene increases abiotic stress tolerance in tobacco. *Plant Physiology and Biochemistry, 132*, 515-523.

Zhao, Z., Zhang, W., Stanley, B. A., & Assmann, S. M. (2008). Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. *Plant Cell, 20*(12), 3210-3226.

Zuo, Z. J., Weraduwage, S. M., Lantz, A. T., Sanchez, L. M., Weise, S. E., Wang, J., . . . Sharkey, T. D. (2019). Isoprene acts as a signaling molecule in gene networks important for stress responses and plant growth. *Plant Physiology, 180*(1), 124-152.

SUMMARY IN ESTONIAN

Doktoritöö annab ülevaate, kuidas kuumastress mõjutab musta kapsasrohu (*Brassica nigra* L.) (**II artikkel**) ja vääristubaka (*Nicotiana tabacum* L.) (**III artikkel**) lenduvühendite emissiooni ja fotosünteesi. Musta kapsasrohtu töödeldi kolme erineva kuumastressiga: temperatuurikõver 20-40 °C, pikaajaline stress 25-44 °C ja šokistress 25 °C, 45-50 °C. Vääristubakale tekitati kuumastress kõrgete temperatuuridega vahemikus 46-55 °C. Mustal kapsasrohul uuriti lenduvühenditest liigile omaseid glükosinolaatide laguprodukte koos rasvhapete sünteesirajast tekkivate ühenditega (sh lipoksügenaasirajast tekkivaid ehk rohulõhna komponente), monoterpeene ja võimalikke karotenoidide laguprodukte. Vääristubaka puhul uuriti isopreeni, oksüdeerunud ühendite (sh rohulõhna komponentide), metanooli ja metakroleiini emissiooni.

Töö hüpoteesid:
1) tugev kuumastress stress põhjustab glükosinolaatide laguproduktide ilmnemist emissiooni ning lenduvühendite emissioon on kvantitatiivselt rohkem varieeruv pikaajalise stressi korral (**II artikkel**)
2) kui kriitiline kuumastressi taluvuse lävi on saavutatud, siis halvenevad fotosünteesi näitajad, kuid samas suurenevad peamiste stressi näitavate lenduvühendite kogused ka sellistel temperatuuridel, kus fotosünteetilised protsessid on tugevasti kahjustunud (**III artikkel**)

Erinevad kuumastressid põhjustasid katsetes mustal kapsasrohul ja vääristubakal fotosünteesinäitajate langust ja lenduvühendite emissioonide muutust. Mõlemal taimeliigil esines kõrgematel temperatuuridel tugev õhulõhede juhtivuse langus ja negatiivne süsihappegaasi omastamine, mis viitab fotosünteesi protsesside ulatuslikule kahjustusele ja hingamise ülekaalule.

Musta kapsasrohu puhul suurendasid pikaajaline ja šokistress liigile spetsiifiliste glükosinolaatide laguproduktide ning rohulõhna komponentide emissiooni, kuid pikaajalise stressi tulemusel oli individuaalsete lenduvühendite varieeruvus mitmekesisem võrreldes šokistressiga. Temperatuurid 46-51 °C ei tekitanud vääristubakal suuri lenduvühendite emissioonide muutusi, kuid 52 °C oli maksimaalne temperatuuritaluvuse lävi, millest alates lenduvühendite emissioon suurenes hüppeliselt. Kõige suurem oli vääristubaka poolt emiteeritud

metanooli ja rohulõhna komponentide emissioon 54 °C juures. Mõlemad taimed emiteerisid nn stressiühendeid (rohulõhna komponente) nagu näiteks (*Z*)-3-hekseen-1-ool ja (*E,E*)-2,4-heksadienaal, mille esinemine viitab rakkude kahjustusele. Seda kinnitab ka vääristubaka suurenenud 2-metüül-2-tsüklopenteen-1-oon-i emissioon pärast 53 °C töötlust.

Mõõdukas temperatuuritõus ei mõjutanud vääristubaka ja musta kapsasrohu monoterpeenide emissiooni lehtedest. Küll aga emiteeris must kapsasrohi monoterpeene hüppeliselt rohkem šokikatsetes. Vääristubakal ei esinenud sellist reaktsiooni ka kõige kõrgematel temperatuuridel. Metakroleiini esinemise põhjuseks vääristubaka lenduvühendite emissioonis võib olla reaktiivsete hapnikuühendite poolt põhjustatud isopreeni oksüdatsioon, kuigi vääristubakat peetakse üldjoontes isopreeni mitte emiteerivaks liigiks.

Doktoritöös esitatud tulemused parendavad üldist arusaamist liigispetsiifilisest fotosünteesist ja lenduvühendite emissioonist. Iseäranis mõjutavad lenduvühendite sünteesiradasid ja lenduvühendite emissiooni lehtedest kuumastressi tugevus ja tüüp. Lenduvühenditel on tähtis roll taimede omavahelises suhtluses, taimede ja putukate vahelises suhtluses ning taimede, putukate ja keskkonna omavahelises koosmõjus (**I artikkel**) ning stress (nt kuumastress) võib neid interaktsioone mõjutada olulisel määral.

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I

ORIGINAL PUBLICATIONS

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Emission of volatile organic compounds as a signal of plant stress

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Plant volatiles

During growth and development plants produce around 100 000 chemical products out of which 1700 are known to be volatile (Dicke and Loreto, 2010). Plant volatile organic compounds (VOCs) are released by various plant organs such as flowers (Colquhoun et al., 2013), fruits (Laothawornkitkul et al., 2009), roots (Köllner et al., 2004; Crespo et al., 2012) and leaves (Owen et al. 2001). Among all the VOC-s, isoprene (Laothawornkitkul et al., 2008; Sharkey et al., 2008; Darbah et al., 2010; Li and Sharkey, 2013), mono- and sesquiterpenes (Chen and Pawliszyn, 2003; Martin et al., 2003; Tholl, 2006; Chen et al., 2011; Rajabi Memari et al., 2013), fatty acid cleavage products known also as green leaf volatiles (GLV-s)(Hatanaka, 1993) and aromatic volatiles (e.g., methyl benzoate and methyl salicylate) (Dudareva et al., 2000; Cardoza et al., 2002; Dudareva et al., 2004; Zhao et al., 2010; Holopainen et al., 2013) are the most studied ones. The rate of plant volatile release can depend on environmental drivers including temperature (Guenther et al., 1993; Bertin et al., 1997; Staudt and Bertin, 1998; Niinemets et al., 2010b; Hu et al., 2013) and light (Guenther et al., 1993; Staudt et al., 1997; Niinemets et al., 2010b; Kesselmeier and Staudt, 1999; Hu et al., 2013; Monson, 2013) and atmospheric CO_2 concentration (Vourinen et al., 2004; Räisänen et al., 2008; Velikova et al., 2009; Rasulov et al., 2009; Monson, 2013; Sun et al., 2012) and endogenous drivers including circadian rhythms (Wilkinson et al., 2006; Loivamäki et al., 2007), leaf age (Mayrhofer et al., 2005; Guenther et al., 2006; Sun et al., 2009; Niinemets et al., 2010a; Sun et al., 2012) and plant age (Shiojiri and Karban, 2006).

Plant stress factors and defenses

During the growth, plants have to cope with various abiotic and biotic stress factors (Mittler, 2006; Loreto and Schnitzler, 2010; Niinemets, 2010a; Niinemets, 2010b; Copolovici et al., 2011). Among these, herbivores (Fatouros et al., 2012), plant viruses (Eigenbrode et al., 2002) and pathogens (Huang et al., 2012) are the key biotic stressors, while temperature

(Velikova and Loreto, 2005; Possell and Loreto, 2013; Sun et al., 2013), drought and flooding (Rennenberg et al. 2006; Kreuzwieser and Rennenberg, 2013), light (Loreto et al. 2006), ozone (Beauchamp and Wisthaler, 2005; Pinto et al., 2010; Calfapietra et al., 2013) and nutrient availability (Lopéz-Bucio et al., 2003) are the main abiotic stress factors. In addition, the severity of stress can be importantly modified by elevated atmospheric CO_2 concentrations (Vourinen et al., 2004; Räisänen et al., 2008; Calfapietra et al., 2013; Sun et al., 2013).

Multiple stress factors can affect plants´ resistance simultaneously or consecutively. In canopy top high leaf temperature and radiance can cause leaf necrosis, which in turn endangers the survival of low-growing trees (Valladares and Pearcy, 1995). Meanwhile in areas of low soil nutrient availability development of root system of young seedlings is blocked and seedlings experience drought stress (Oliet *et al*., 2013). Additionally soil nitrogen content affects frost injury of plants, because nitrogen contributes the biosynthesis of anti-freeze proteins in apoplast and prevents ice crystal formation (Lambers et al. 2008), otherwise breakage of cell membranes triggers immediate release of GLVs (Copolovici *et al*., 2012).

Plants have developed physical and chemical defense systems to protect themselves against stressors. Among physical defense systems, spines, thorns and hardened leaves play a major role in several plants species (Milewski *et al.*, 1991; Cooper and Ginnet, 1998; Hanley *et al.*, 2007), while enhanced investment in waxes and secondary plant metabolites is a common direct defense response across plants (Halitschke *et al.*, 2000; Arimura *et al.*, 2005; Leitner *et al.*, 2005; Howe and Schaller, 2008; Kessler and Heil, 2011). In contrast, stress-induced volatile compounds that can be attractants of predators and parasitoids belong to indirect defense, when the attacked plant is „calling for help“ against herbivores (Dicke, 1994; Dicke *et al.* 2009; Dicke and Baldwin, 2010; Holopainen and Gershenzon, 2010; Fatouros *et al.* 2012). There are numerous recent studies demonstrating that indirect chemical defense systems do increase the fitness of attacked plants (for reviews see Dicke and Baldwin, 2010; Trowbridge and Stoy, 2013), and thereby constitute an important rapidly induced defense system.

Induced stress volatiles

Volatiles are biosynthesized mainly via four biochemical pathways: the lipoxygenase pathway for green leaf volatiles (GLV-s) (Hatanaka, 1993), shikimic acid pathway for aromatic volatiles (Paré and Tumlinson, 1996), methylerythritol pathway (MEP) for isoprene and monoterpenoids (Pichersky *et al.*, 2006; Rajabi Memari *et al.*, 2013) and mevalonic acid

pathway (MVA) for volatile sesquiterpenoids (Taveira *et al.*, 2009; Rajabi Memari *et al.*, 2013; Rosenkranz and Schnitzler, 2013) (Figure 1).

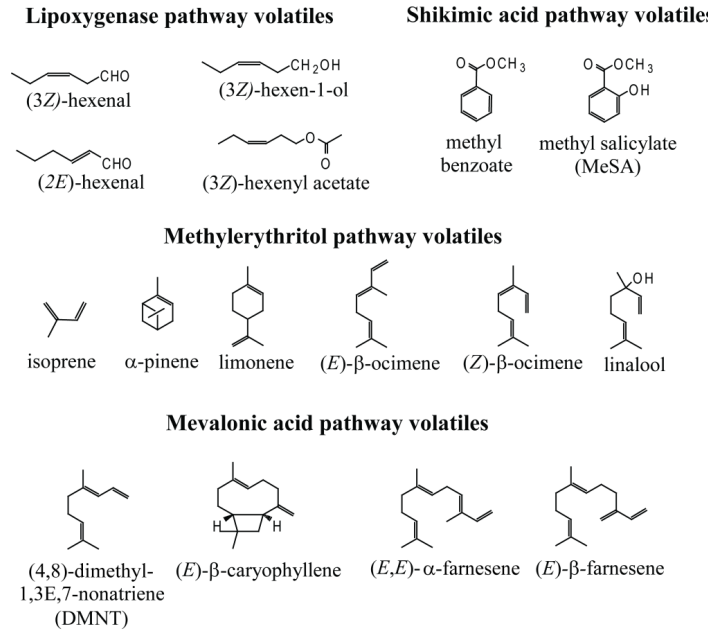

Figure 1. Molecular structures of plant volatiles in the emission of a stressed plant. Fatty acid cleavage products known also as green leaf volatiles (GLV-s) are biosynthesized via lipoxygenase pathway (Hatanaka, 1993), aromatic volatiles such as methyl salicylate (MeSA) are volatile products of shikimic acid pathway (Dudareva et al., 2000), isoprene and monoterpenoids are produced via methylerythritol (MEP) pathway (Pichersky et al., 2006), homoterpene (4,8)-dimethyl-1,3E,7-nonatriene (DMNT) and sesquiterpenes are produced via mevalonic acid (MVA) pathway (Taveira et al., 2009).

Typical stress emissions consist of green leaf volatiles such as (*E*)-2-hexenal, (*Z*)-3-hexenol, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexenyl acetate, volatile mono- and sesquiterpenoids such as linalool, ocimene isomers, farnesene isomers, (*E*)-*β*-caryophyllene, methyl jasmonate, methyl salicylate (MeSA), and methanol (Geervliet et al., 1997; Lerdau and Gray, 2003; Holopainen, 2004; Baldwin et al., 2006; Maffei, 2010; Raghava et al., 2010; Copolovici et al., 2011; Spinelli et al., 2011; Copolovici et al., 2012; Pinto-Zevallos et al., 2013). Any stress factor can potentially change the rate of volatile release and alter the bouquet of VOCs and thereby affect the relationships between living organisms (Dicke and Baldwin, 2010; Holopainen and Gershenzon, 2010; Niinemets et al., 2013). For example, leaf damage increases the emission of sesquiterpenes and increases alkaloid content of nectar, and hence,

can alter pollinator preference (Adler et al., 2006; Theis et al., 2009). In addition, (Z)-3-hexenol, which is a signaling volatile of herbivore attack or mechanical wounding, induces the stress reaction in neighboring intact plants (Wei and Kang, 2011). On the other, the bouquet of volatiles can importantly depend on stressed plant species (Llusià et al., 2010a; Llusià et al., 2010b; Holopainen et al., 2013; Llusià et al., 2013) or a stressor (Känanste et al., 2009, Takabayashi et al., 1991).

Role of volatile organic compounds in plant-plant-, plant-insect- or plant-insect-environment relationships

The question of why plants emit VOC-s has been posed over and over again, and the role of many volatiles in abiotic stress tolerance, including thermotolerance of photosynthesis and reduced oxidative stress, has been highlighted (Sharkey and Singsaas, 1995; Singsaas et al., 1997; Loreto et al., 1998; Loreto and Velikova, 2001; Velikova et al., 2004; Copolovici et al., 2005; Llusià et al., 2005; Velikova et al., 2005; Vickers et al., 2009; Possell and Loreto, 2013; Sun et al., 2013). Moreover in the 21th century global climate is predicted to change drastically (IPPC, 2007). For example today we know that water availability affects the content and emission of secondary metabolites in plants and different plant species respond to water deficit differently (Kainulainen et al., 1992; Turtola et al., 2003; Peñuelas et al., 2009; Lusebrink et al., 2011; Kännaste et al., 2013). Yet in future in relation to climate change the existence and prolongation of drought may increase the attack of pines of low vitality by the mountain pine beetle *Dendroctonus ponderosae* MPB (Lusebrink et al., 2011).

VOC-s are essential in plant-plant and plant-insect (Baldwin et al., 2002; Duhl et al., 2008; Dicke and Baldwin, 2010; Fatouros et al., 2012; Holopainen et al., 2013; Trowbridge and Stoy, 2013). In plant-herbivore interactions, the volatiles can act as attractants or repellents to herbivores (Laothawornkitkul et al., 2008; Loivamäki et al., 2008). For instance, the monoterpene α-pinene released by wounded Scots pine (*Pinus sylvestris* L.) acts as attractant to large pine weevil (*Hylobius abietis*), and thus, previous damage of a conifer can increase herbivory damage. Yet attraction of *H. abietis* can be reduced by repelling limonene (Nordlander, 1991). Due to the increasing emissions of allylisothiocyanate heat stressed *Brassica nigra* plants may become attractive to spezialized feeders of Brassicaceae (Figure 2) (Mithen, 2001).

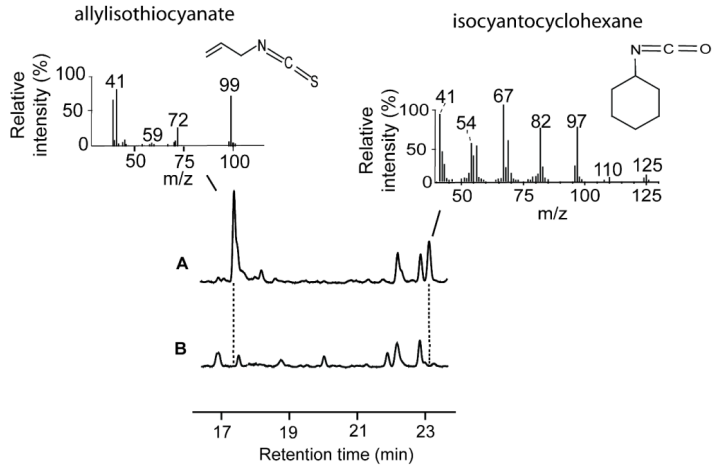

Figure 2. Volatile glucosinolate biodegradation products of *Brassica nigra* released at leaf temperature of 25 °C (A) and 44 °C (B).

For example volatiles emitted from *Tetranychus urticae*-infested lima bean (*Phaseolus lunatus* L.) can activate genes encoding pathogenesis-related proteins and phenylalanine ammonia-lyase in leaves of non-infested neighboring plants', as well GLV-s can serve as signal compounds in plant-plant communication (Arimura et al., 2001; Bate, Rothstein, 1998). This kind of info "sharing" depends on the diffusion and convection of the volatile info between the sender and the receiver plant (Baldwin et al., 2002).

Conclusions

Overall, this information summarized here emphasizes the rich spectrum of stress-triggered volatile emissions and underscores the importance of volatiles in stress responses, stress tolerance and plant interactions with other plants and organisms. While a lot of basic information on plant volatile emissions has accumulated during the last years, we still lack quantitative understanding of how the emission rate scales with the severity of stresses, how far the stress-elicited volatiles travel in the atmosphere and what are the relationships between the strength of the emitted signal and receiver plants' and other receiver organisms' responses. There is encouraging evidence that the strength of the emission signal can be quantitatively related to the severity of both abiotic and biotic stresses (Niinemets et al., 2013) and we argue that future work should be devoted towards filling these important gaps in knowledge.

References

Adler, L. S., Wink, M., Distl, M., Lentz, A. J. 2006. Leaf herbivory and nutrients increase nectar alkaloids. Ecology Letters 9(8), 960-967. doi:10.1111/j.1461-0248.2006.00944.x

Arimura, G., Kost, C., Boland, W. 2005. Herbivore-induced, indirect plant defences. Biochimica et Biophysica Acta 1734(2), 91-111.

Arimura, G., Ozawa, R., Horiuchi, J., Nishioka, T., Takabayashi, J. 2001. Plant–plant interactions mediated by volatiles emitted from plants infested by spider mites. Biochemical Systematics and Ecology 29(10), 1049-1061.

Baldwin, I. T., Halitschke, R., Paschold, A., von Dahl, C. C., Preston, C. A. 2006. Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. Science, 311(5762), 812-815.

Baldwin, I., Kessler, A., Halitschke, R. 2002. Volatile signaling in plant–plant–herbivore interactions: what is real? Current Opinion in Plant Biology 5(4), 351-354.

Bate, N. J., Rothstein, S. J. 1998. C6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. Plant Journal, 16(5), 561-569.

Beauchamp, J., Wisthaler, A. 2005. Ozone induced emissions of biogenic VOC from tobacco: relationships between ozone uptake and emission of LOX products. Plant, Cell and Environment 28(10), 1334-1343.

Bertin, N., Staudt, M., Hansen, U., Seufert, G., Ciccioli, P., Foster, P., Fugit, J. L., Torres, L. 1997. Diurnal and seasonal course of monoterpene emissions from *Quercus ilex* (L.) under natural conditions - applications of light and temperature algorithms. Atmospheric Environment 31, 135-144.

Calfapietra, C., Pallozzi, E., Lusini, I., Velikova, V. 2013. "Modification of BVOC emissions by changes in atmospheric [CO_2] and air pollution," in "Biology, Controls and Models of Tree Volatile Organic Compound Emissions", eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 253-284

Cardoza, Y. J., Alborn, H. T., Tumlinson, J. H. 2002. In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. Journal of Chemical Ecology 28(1), 161-174.

Chen, Y., Pawliszyn, J. 2003. Time-weighted average passive sampling with a solid-phase microextraction device. Analytical Chemistry 75(9), 2004-2010.

Chen, F., Tholl, D., Bohlmann, J., Pichersky, E. 2011. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. Plant Journal: for Cell and Molecular Biology 66(1), 212-229.

Colquhoun, T. A., Schwieterman, M. L., Gilbert, J. L., Jaworski, E. A., Langer, K. M., Jones, C. R., Rushing, G.V. Hunter, T. M., Olmstead, J. C., David G. D., Folta, K. M. 2013. Light modulation of volatile organic compounds from petunia flowers and select fruits. Postharvest Biology and Technology 86, 37-44.

Cooper, S. M., Ginnet, T. F. 1998. Spines protect plants against browsing by small climbing mammals. Oecologia 113(2), 219-221.

Copolovici, L., Filella, I., Llusià, J., Niinemets, Ü., Peñuelas, J. 2005. The capacity for thermal protection of photosynthetic electron transport varies for different monoterpenes in *Quercus ilex*. Plant Physiology 139(1), 485-496.

Copolovici, L., Kännaste, A., Pazouki, L., Niinemets, Ü. 2012. Emissions of green leaf volatiles and terpenoids from *Solanum lycopersicum* are quantitatively related to the severity of cold and heat shock treatments. Journal of Plant Physiology 169(7), 664-672.

Copolovici, L., Kännaste, A., Remmel, T., Vislap, V., Niinemets, Ü. 2011. Volatile emissions from *Alnus glutinosa* induced by herbivory are quantitatively related to the extent of damage. Journal of Chemical Ecology 37(1), 18-28.

Crespo, E., Hordijk, C. A, de Graaf, R. M., Samudrala, D., Cristescu, S. M., Harren, F. J. M., van Dam, N. M. 2012. On-line detection of root-induced volatiles in *Brassica nigra* plants infested with *Delia radicum* L. root fly larvae. Phytochemistry 84, 68-77.

Darbah, J. N. T., Sharkey, T. D., Calfapietra, C., Karnosky, D. F. 2010. Differential response of aspen and birch trees to heat stress under elevated carbon dioxide. Environmental Pollution 158(4), 1008-1014.

Dicke, M. 1994. Why do plants "talk"? Chemoecology 165, 159-165.

Dicke, M., Baldwin, I. T. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. Trends in Plant Science 15(3), 167-175.

Dicke, M., van Loon, J. J. A., Soler, R. 2009. Chemical complexity of volatiles from plants induced by multiple attack. Nature Chemical Biology 5, 317-324.

Dicke, M., Loreto, F. 2010. Induced plant volatiles: from genes to climate change. Trends in Plant Science 15(3), 115-117.

Dudareva, N., Murfitt, L. M., Mann, C. J., Gorenstein, N., Kolosova, N., Kish, C. M., Bonham, C., Wood, K. 2000. Developmental regulation of methyl benzoate biosynthesis and emission in snapdragon flowers. The Plant Cell 12(6), 949-961.

Dudareva, N., Pichersky, E., Gershenzon, J. 2004. Biochemistry of plant volatiles. Plant Physiology 135, 1893-1902.

Duhl, T. R., Helmig, D., Guenther, A. 2008. Sesquiterpene emissions from vegetation: a review. Biogeosciences 5(3), 761-777.

Eigenbrode, S. D., Ding, H., Shiel, P., Berger, P. H. 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proceedings. Biological sciences / The Royal Society 269(1490), 455-460.

Fatouros, N. E., Lucas-Barbosa, D., Weldegergis, B. T., Pashalidou, F. G., van Loon, J. J. A., Dicke, M., Harvey, J. A., Gols, R., Huigens, M. E. 2012. Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. Plos one 7(8), e43607.

Geervliet, J. B. F., Posthumus, M. A., Vet, L. E. M., Dicke, M. 1997. Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris* species. Journal of Chemical Ecology 23(12), 2935-2954.

Guenther, A., Karl, T., Harley, P., Wiedinmyer, C., Palmer, P. I., Geron, C. 2006. Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). Atmospheric Chemistry and Physics 6, 3181-3210.

Guenther, A. B., Zimmerman, P.R., Harley, P. C., Monson, R.K., Fall, R. 1993. Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. Journal of Geophysical Research: Atmospheres 98 (1984–2012), D7, 12609-12617.

Halitschke, R., Kessler, A., Kahl, J., Lorenz, A., Baldwin, I. T. 2000. Ecophysiological comparison of direct and indirect defenses in *Nicotiana attenuata*. Oecologia 124(3), 408-417.

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., Rafferty, C. M. 2007. Plant structural traits and their role in anti-herbivore defence. Perspectives in Plant Ecology, Evolution and Systematics 8(4), 157-178.

Hatanaka, A. 1993. The biogeneration of green odour by green leaves. Phytochemistry 34(5), 1201-1218.

Holopainen, J. K. 2004. Multiple functions of inducible plant volatiles. Trends in Plant Science 9(11), 529-533.

Holopainen, J. K., Gershenzon, J. 2010. Multiple stress factors and the emission of plant VOCs. Trends in Plant Science 15, 176-184.

Holopainen, J. K., Nerg, A.-M., Blande, J. D. 2013. “Multitrophic signalling in polluted atmospheres,” in “Biology, Controls and Models of Tree Volatile Organic Compound Emissions”, eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 285-314

Howe, G. A., Schaller, A. 2008. “Direct defenses in plants and their induction by wounding and insect herbivores,” in “Induced plant resistance to herbivory”, ed Schaller A. Springer, Berlin, pp 7-29

Hu, Z., Zhang, H., Leng, P., Zhao, J., Wang, W., Wang, S. 2013. The emission of floral scent from *Lilium* “siberia” in response to light intensity and temperature. Acta Physiologiae Plantarum 35(5), 1691-1700.

Huang, M., Sanchez-Moreiras, A. M., Abel, C., Sohrabi, R., Lee, S., Gershenzon, J., Tholl, D. 2012. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (*E*)-*β*-caryophyllene, is a defense against a bacterial pathogen. New Phytologist 193(4), 997-1008.

IPCC (Intergovernmental Panel on Climate Change) 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovern- mental Panel on Climate Change. Cambridge University Press, U.K., p. 996

Kainulainen, P., Oksanen, J., Palomäki, V., Holopainen, J. K., Holopainen, T. 1992. Effect of drought and waterlogging stress on needle monoterpenes of *Picea abies*. Canadian Journal of Botany, 70(8), 1613–1616.

Kellomäki, S., Wang, K., Lemettinen, M. 2000. Controlled environment chambers for investigating tree response to elevated CO_2 and temperature under boreal conditions. Photosynthetica 38(1), 69-81.

Kesselmeier, J., Staudt, M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. Journal of Atmospheric Chemistry 33(1), 23-88.

Kessler, A., Heil, M. 2011. The multiple faces of indirect defences and their agents of natural selection. Functional Ecology 25(2), 348-357.

Kreuzwieser, J., Rennenberg, H. 2013. “Flooding-driven emissions from trees,” in “Biology, Controls and Models of Tree Volatile Organic Compound Emissions”, eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 237-252

Köllner, T. G., Schnee, C., Gershenzon, J., Degenhardt, J. 2004. The sesquiterpene hydrocarbons of maize (*Zea mays*) form five groups with distinct developmental and organ-specific distributions. Phytochemistry 65(13), 1895-1902.

Kännaste, A., Copolovici, L., Pazouki, L., Suhhorutšenko, M., Niinemets, Ü. 2013. Highly variable chemical signatures over short spatial distances among Scots pine (*Pinus sylvestris*) populations. Tree Physiology, 33(4), 374–87.

Kännaste, A., Nordenhem, H., Nordlander, G., & Borg-Karlson, A.-K. 2009. Volatiles from a mite-infested spruce clone and their effects on pine weevil behavior. Journal of Chemical Ecology, *35*(10), 1262–1271.

Lambers, H., Chapin, F.S., Pons, T.L. 2008 "Plant physiological ecology", 2nd edn. Springer, New York. Pp. 4.

Laothawornkitkul, J., Paul, N. D., Vickers, C. E., Possell, M., Taylor, J. E., Mullineaux, P. M., Hewitt, C. N. 2008. Isoprene emissions influence herbivore feeding decisions. Plant, Cell and Environment 31(10), 1410-1415.

Laothawornkitkul, J., Taylor, J. E., Paul, N. D., Hewitt, C. N. 2009. Biogenic volatile organic compounds in the Earth system. New Phytologist 183(1), 27-51.

Leitner, M., Boland, W., Mithöfer, A. 2005. Direct and indirect defences induced by piercing-sucking and chewing herbivores in *Medicago truncatula*. New Phytologist 167(2), 597-606.

Lerdau, M., Gray, D. 2003. Ecology and evolution of light-dependent and light-independent phytogenic volatile organic carbon. New Phytologist 157(2), 199-211.

Li, Z., Sharkey, T. D. (2013). "Molecular and pathway controls on biogenic volatile organic compound emission," in "Biology, Controls and Models of Tree Volatile Organic Compound Emissions", eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 119-151.

Llusià, J., Peñuelas, J., Alessio, G. A., Ogaya, R. 2010a. Species-specific, seasonal and inter-annual changes in foliar terpene emission rates in *Phillyrea latifolia* L. and *Quercus ilex* L. submitted to rain exclusion in the *Prades mountains* (Catalonia). Russian Journal of Plant Physiology 58(1), 126-132.

Llusià, J., Peñuelas, J., Asensio, D., Munné-Bosch, S. 2005. Airborne limonene confers limited thermotolerance to *Quercus ilex*. Physiologia Plantarium 123(1), 40-48.

Llusià, J., Peñuelas, J., Guenther, A., Rapparini, F. 2013. Seasonal variations in terpene emission factors of dominant species in four ecosystems in NE Spain. Atmospheric Environment 70, 149-158.

Llusià, J., Peñuelas, J., Sardans, J., Owen, S. M., Niinemets, Ü. 2010b. Measurement of volatile terpene emissions in 70 dominant vascular plant species in Hawaii: aliens emit more than natives. Global Ecology & Biogeography 19(6), 863-874.

Loivamäki, M., Louis, S., Cinege, G., Zimmer, I., Fischbach, R. J., Schnitzler, J.-P. 2007. Circadian rhythms of isoprene biosynthesis in grey poplar leaves. Plant Physiology 143(1), 540-551.

Loivamäki, M., Mumm, R., Dicke, M., Schnitzler, J.-P. 2008. Isoprene interferes with the attraction of bodyguards by herbaceous plants. Proceedings of the National Academy of Sciences of the United States of America 105(45), 17430-17435.

Loreto, F., Barta, C., Brilli, F., Nogues, I. 2006. On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant, Cell and Environment 29(9), 1820-1828.

Loreto, F., Förster, A., Dürr, M., Csiky, O., Seufert, G. 1998. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. Plant Cell and Environment 21(1), 101-107.

Loreto, F., Schnitzler, J.-P. 2010. Abiotic stresses and induced BVOCs. Trends in Plant Science 15(3), 154-166.

Loreto, F., Velikova, V. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology 127, 1781-1787.

Lusebrink, I., Evenden, M. L., Blanchet, F. G., Cooke, J. E. K., Erbilgin, N. 2011. Effect of water stress and fungal inoculation on monoterpene emission from an historical and a new pine host of the mountain pine beetle. Journal of Chemical Ecology, 37(9), 1013–26.

Maffei, M. E. 2010. Sites of synthesis, biochemistry and functional role of plant volatiles. South African Journal of Botany 76(4), 612-631.

Martin, D., Gershenzon, J., Bohlmann, J. 2003. Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. Plant Physiolgy 132(3), 1586-1599.

Mayrhofer, S., Teuber, M., Zimmer, I., Louis, S., Fischbach, R. J., Schnitzler, J.-P. 2005. Diurnal and seasonal variation of isoprene biosynthesis-related genes in grey poplar leaves. Plant Physiology 139(1), 474-484.

Milewski, A.V., Young, T. P., Madden, D. 1991. Thorns as induced defenses: experimental evidence. Oecologia 86(1), 70-75.

Mithen, R.F. 2001. Glucosinolates and their degradation products. Advances in Botanical Research 35, 213-232.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science 11(1), 15-19.

Monson, R. K. 2013. “Metabolic and gene expression controls on the production of biogenic volatile organic compounds,” in “Biology, Controls and Models of Tree Volatile Organic Compound Emissions”, eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 153-179

Niinemets, Ü. 2010a. Mild versus severe stress and BVOCs: thresholds, priming and consequences. Trends in Plant Science 15(3), 145-153.

Niinemets, Ü. 2010b. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. Forest Ecology and Management 260(10), 1623-1639.

Niinemets, Ü., Arneth, A., Kuhn, U., Monson, R. K., Peñuelas, J., Staudt, M. 2010a. The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses. Biogeosciences 7, 2203-2223.

Niinemets, Ü., Kännaste, A., Copolovici, L. 2013. Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. Frontiers in Plant Science 4, 262.

Nordlander, G.. 1991. Host finding in the pine weevil *Hylobius abietis*: effects of conifer volatiles and added limonene. Entomologia Experimentalis et Applicata 59(3), 229-237.

Oliet, J. A., Puértolas, J., Planelles, R., Jacobs, D. F. 2013. Nutrient loading of forest tree seedlings to promote stress resistance and field performance: a Mediterranean perspective. New Forests, 44(5), 649–669.

Owen, S., Boissard, C., Hewitt, C. 2001. Volatile organic compounds (VOCs) emitted from 40 mediterranean plant species: VOC speciation and extrapolation to habitat scale. Atmospheric Environment 35(32), 5393–5409.

Paré, P., Tumlinson, J. 1996. Plant volatile signals in response to herbivore feeding. Florida Entomologist 79, 93-103.

Peñuelas, J., Filella, I., Seco, R., Llusià, J. 2009. Increase in isoprene and monoterpene emissions after re-watering of droughted *Quercus ilex* seedlings, Biologia Plantarum, 53(2), 351–354.

Pinto, D. M., Blande, J. D., Souza, S. R., Nerg, A.-M., Holopainen, J. K. 2010. Plant volatile organic compounds (VOCs) in ozone (O_3) polluted atmospheres: The ecological effects. Journal of Chemical Ecology 36(1), 22-34.

Pinto-Zevallos, D. M., Hellén, H., Hakola, H., van Nouhuys, S., Holopainen, J. K. 2013. Induced defenses of *Veronica spicata*: Variability in herbivore-induced volatile organic compounds. Phytochemistry Letters 6(4), 653-656.

Pichersky, E., Noel, J., Dudareva, N. 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science 311(5762), 808-811.

Possell, M., Loreto, F. 2013. “The role of volatile organic compounds in plant resistance to abiotic stresses: responses and mechanisms,” in: “Biology, Controls and Models of Tree Volatile Organic Compound Emissions”, eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 209-235

Raghava, T., Ravikumar, P., Hegde, R., Kush, A. 2010. Spatial and temporal volatile organic compound response of select tomato cultivars to herbivory and mechanical injury. Plant Science 179(5), 520-526.

Rajabi Memari, H., Pazouki, L., Niinemets, Ü. 2013. “The biochemistry and molecular biology of volatile messengers in trees,” in “Biology, Controls and Models of Tree Volatile Organic Compound Emissions”, eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 47-93

Rasulov, B., Hüve, K., Välbe, M., Laisk, A., Niinemets, Ü. 2009. Evidence that light, carbon dioxide and oxygen dependencies of leaf isoprene emission are driven by energy status in hybrid aspen. Plant Physiology 151(1), 448-460.

Rennenberg, H., Loreto, F., Polle, A, Brilli, F., Fares, S., Beniwal, R. S., Gessler, A. 2006. Physiological responses of forest trees to heat and drought. Plant Biology 8(5), 556-571.

Rosenkranz, M., Schnitzler, J.-P. (2013). “Genetic engineering of BVOC emissions from trees,” in “Biology, Controls and Models of Tree Volatile Organic Compound Emissions”, eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 95-118

Räisänen, T., Ryyppö, A., Kellomäki, S. 2008. Effects of elevated CO_2 and temperature on monoterpene emission of Scots pine (*Pinus sylvestris* L.). Atmospheric Environment 42(18), 4160-4171.

Sharkey, T. D., Singsaas, E. 1995. Why plants emit isoprene? Nature 374, 769.

Sharkey, T. D., Wiberley, A. E., Donohue, A. R. 2008. Isoprene emission from plants: why and how. Annals of Botany 101(1), 5-18.

Shiojiri, K., Karban, R. 2006. Plant age, communication, and resistance to herbivores: young sagebrush plants are better emitters and receivers. Oecologia 149(2), 214-220.

Singsaas, E. L., Lerdau, M., Winter, K., Sharkey, T. D. 1997. Isoprene increases thermotolerance of isoprene-emitting species. Plant Physiology 115(4), 1413-1420.

Spinelli, F., Cellini, A., Marchetti, L. 2011. “Emission and function of volatile organic compounds in response to abiotic stress”, in Agricultural and Biological Sciences “Abiotic Stress in Plants - Mechanisms and Adaptations”, eds A. Shanker and B. Venkateswarlu.

Staudt, M., Bertin, N. 1998. Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. Plant, Cell and Environment 21(4), 385-395.

Staudt, M., Bertin, N., Hansen, U. 1997. Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. Atmospheric Environment 31(97), 145-156.

Sun, Z., Copolovici, L., Niinemets, Ü. 2012. Can the capacity for isoprene emissions acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? Journal of Plant Research 125, 263-274.

Sun, Z., Hüve, K., Vislap, V., Niinemets, Ü. 2013. Elevated growth [CO_2] magnifies isoprene emissions under heat, alters environmental responses and improves thermal resistance in hybrid aspen. Journal of Experimental Botany, in Press.

Sun, Z., Niinemets, Ü., Copolovici, L. 2009. Foliar isoprene emission during autumn senescence in aspen (*Populus tremula*). Geochimica et Cosmochimica Acta 73:A1295

Takabayashi, J., Dicke, M., Posthumus, M. 1991. Variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: Relative influence of plant and herbivore. Chemoecology, 2(1), 1–6.

Taveira, M., Fernandes, F., Guedes de Pinho, P., Andrade, P. B., Pereira, J. A., Valentão, P. 2009. Evolution of *Brassica rapa* var. *rapa* L. volatile composition by HS-SPME and GC/IT-MS. Microchemical Journal 93(2), 140-146.

Theis, N., Kesler, K., Adler, L. S. 2009. Leaf herbivory increases floral fragrance in male but not female *Cucurbita pepo* subsp. *texana* (Cucurbitaceae) flowers. American Journal of Botany 96(5), 897-903.

Tholl, D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. Current Opinion in Plant Biology 9(3), 297-304.

Trowbridge, A. M., Stoy, P. C. 2013. "BVOC mediated plant-herbivore interactions," in "Biology, Controls and Models of Tree Volatile Organic Compound Emissions", eds Ü. Niinemets and R. K. Monson. Springer, Berlin, pp 21-46.

Turtola, S., Manninen, A. M., Rikala, R., Kainulainen, P. 2003. Drought stress alters the concentration of wood terpenoids in Scots pine and Norway spruce seedlings. Journal of Chemical Ecology, 29(9), 1981–1995.

Valladares, F.,Pearcy, R. W. 1997. Interactions between water stress, sun-shade acclimation, heat tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. Plant, Cell and Environment, 20(1), 25–36.

Velikova, V., Edreva, A., Loreto, F. 2004. Endogenous isoprene protects *Phragmites australis* leaves against singlet oxygen. Physiolgia Plantarium 122(2), 219-225.

Velikova, V., Loreto, F. 2005. On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat. Plant, Cell and Environment 28(3) 318-327.

Velikova, V., Pinelli, P., Pasqualini, S., Reale, L., Ferranti, F., Loreto, F. 2005. Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. New Phytologist 166(2), 419-425.

Velikova, V., Tsonev, T., Barta, C., Centritto, M., Koleva, D., Stefanova, M., Busheva, M. Loreto, F. 2009. BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature. Environmental Pollution 157(10), 2629-2637.

Vickers, C. E., Possell, M., Cojocariu, C. I., Velikova, V. B., Laothawornkitkul, J., Ryan, A., Mullineaux, P. M., Hewitt, C. N. 2009. Isoprene synthesis protects transgenic tobacco plants from oxidative stress. Plant, Cell and Environment 32, 520-53.

Wei, J., Kang, L. 2011. Roles of (*Z*)-3-hexenol in plant-insect interactions. Plant Signaling & Behavior 6(3), 369-371.

Wilkinson, M. J., Owen, S. M., Possell, M., Hartwell, J., Gould, P., Hall, A., Vickers, C., Hewitt, C. N. 2006. Circadian control of isoprene emissions from oil palm (*Elaeis guineensis*). Plant Journal 47(6), 960-968.

Zhao, N., Guan, J., Ferrer, J.-L., Engle, N., Chern, M., Ronald, P., Tschaplinski, T. J., Chen, F. 2010. Biosynthesis and emission of insect-induced methyl salicylate and methyl benzoate from rice. Plant Physiology and Biochemistry 48, 279-289.

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Original Article

How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*

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ABSTRACT

Brassicales release volatile glucosinolate breakdown products upon tissue mechanical damage, but it is unclear how the release of glucosinolate volatiles responds to abiotic stresses such as heat stress. We used three different heat treatments, simulating different dynamic temperature conditions in the field to gain insight into stress-dependent changes in volatile blends and photosynthetic characteristics in the annual herb *Brassica nigra* (L.) Koch. Heat stress was applied by either heating leaves through temperature response curve measurements from 20 to 40 °C (mild stress), exposing plants for 4 h to temperatures 25–44 °C (long-term stress) or shock-heating leaves to 45–50 °C. Photosynthetic reduction through temperature response curves was associated with decreased stomatal conductance, while the reduction due to long-term stress and collapse of photosynthetic activity after heat shock stress were associated with non-stomatal processes. Mild stress decreased constitutive monoterpene emissions, while long-term stress and shock stress resulted in emissions of the lipoxygenase pathway and glucosinolate volatiles. Glucosinolate volatile release was more strongly elicited by long-term stress and lipoxygenase product released by heat shock. These results demonstrate that glucosinolate volatiles constitute a major part of emission blend in heat-stressed *B. nigra* plants, especially upon chronic stress that leads to induction responses.

Key-words: Brassicales; glucosinolate breakdown products; heat shock; high temperature; lipoxygenase pathway; terpenoid emission; volatile organic compounds.

INTRODUCTION

Among abiotic stresses, heat stress is one of the most deleterious factors resulting in major cellular damage once the heat stress threshold has been exceeded (Bidart-Bouzat & Imeh-Nathaniel 2008). Such deleterious heat effects are manifested in ubiquitous stress responses such as collapse of leaf photosynthetic activities and formation of reactive oxygen species in leaf tissues (Vacca *et al.* 2004; Hüve *et al.* 2011) and elicitation of release of lipoxygenase (LOX) pathway volatiles (Maccarrone *et al.* 1992; Copolovici *et al.* 2012). Nevertheless, even mild to moderate heat stress that does not result in visible lesions can result in significant reductions in leaf photosynthetic activities (Sharkey 2005; Zhang & Sharkey 2009; Zhang *et al.* 2009) and modifications in volatile emission profiles (Loreto *et al.* 1998; Kleist *et al.* 2012; Possell & Loreto 2013). In fact, release of several constitutive and induced volatiles can be extremely temperature sensitive and only moderate increases in temperature, even in the range of 30–38 °C can result in major changes in the emissions (Hartikainen *et al.* 2009; Kleist *et al.* 2012; Hu *et al.* 2013; Farré-Armengol *et al.* 2014).

Apart from ubiquitous stress responses elicited in a wide range of species in response to practically any severe stress, several plant taxonomic groups have specialized volatile defence pathways (Karban 2011). Glucosinolates constitute the unique secondary metabolites in the order Brassicales (Fahey *et al.* 2001; Redovniković *et al.* 2008; Ishida *et al.* 2014), and so far the occurrence of more than 130 natural glucosinolates has been documented (Agneta *et al.* 2014). Depending on their molecular structure, they can be divided among aliphatic-, aromatic- or indole glucosinolates (Hopkins *et al.* 1997; Ishida *et al.* 2014). Members of each group are biosynthesized from different precursors via slightly different pathways. Yet, in general, the biosynthesis starts with the chain elongation of an amino acid, continues with the creation of glucosinolate basic structure and ends up with the transformation of the core structure into the final glucosinolate molecule (Ishida *et al.* 2014). Glucosinolates are hydrolyzed to toxic volatile products by myrosinases that are released from specialized cells upon mechanical wounding, for example, upon insect herbivory (Barth & Jander 2006; Wittstock & Burow 2010; Najar-Rodriguez *et al.* 2015). These breakdown products can be isothiocyanates, thiocyanates, nitriles, epithionitriles and oxazolidines (Bones & Rossiter 2006; Kos *et al.* 2012), disulfides and thiols (Olivier *et al.* 1999; Agrawal & Kurashige 2003; Crespo *et al.* 2012) shown to significantly reduce herbivory by omnivorous insects (Hopkins *et al.* 2009), but also performance of specialist herbivores (Bruinsma *et al.* 2007; de Vos *et al.* 2008).

Apart from mechanical damage due to herbivory, multiple stress factors including heat stress can lead to cellular damage with potential release of myrosinases, but there is surprisingly little information about the relationship of abiotic stressors and volatile glucosinolate degradation products in brassicoid species (Wittstock & Burow 2010). Provided myrosinases are

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indeed released as the permeability of cellular membranes increases upon developing heat stress, release of glucosinolate breakdown products is likely, and the release of these specialized volatiles might importantly contribute to the total heat-triggered volatile blend next to ubiquitous emissions of LOX products. Furthermore, there is recent evidence that exogenously applied glucosinolate volatiles, isothiocyanates, enhanced the development of heat-tolerance of *Arabidopsis thaliana* (Hara *et al.* 2013). Thus, heat-dependent induction of glucosinolate volatile emissions might contribute to development of induced abiotic stress resistance, but which temperature conditions lead to the release of glucosinolate volatiles and how these potential emissions are related to ubiquitous stress responses is not known.

During their lifetime, plants can be exposed to a wide variety of heat episodes differing in duration and temperature during the stress, including short-term to mid-term excursions of leaf temperature to high values upon light flecks and upon clearing up the sky when shaded leaves are suddenly exposed to strong beam irradiance (Singsaas & Sharkey 1998; Sharkey 2005; Behnke *et al.* 2007; Way *et al.* 2011), as well as during heat waves that are predicted to become more common in the future (Ameye *et al.* 2012). Given this variety, it is relevant to consider that the heat stress threshold is determined by the heat dose (heat sum) that is dependent on both the actual temperature and the duration of the heat episode (Bilger *et al.* 1984; Niinemets 2010a) as well as on possible increases of heat stress resistance due to acclimation and priming responses occurring through the heat wave (Niinemets 2010b). Thus, modifications in the volatile blend triggered by heat stress can depend on the type of heat stress that ultimately determines whether the stress threshold for physiological damage is exceeded and whether acclimation or priming responses can occur.

The goal of the present study was to investigate how foliage photosynthetic characteristics and emissions of constitutive and ubiquitous and specialized stress-elicited volatiles respond to heat-stress of various types in black mustard (*B. nigra* (L.) Koch, Brassicaceae). To our knowledge, there is no information about the relationships between the volatile glucosinolate degradation products and volatiles of other biosynthetic pathways through abiotic stress treatments. Hence, next to the volatile glucosinolates, we also studied the heat responses of emissions of lipoxygenase, terpenoid and shikimate pathway products. We used three different heat treatments simulating dynamic temperature conditions in field environments that can occur during short-term heat episodes and longer-term heat waves to gain an insight how the share of different volatiles changes in dependence on reversible and irreversible stress conditions of different duration. We hypothesized that severe heat stress leads to elicitation of glucosinolate volatiles and that the emissions are quantitatively more significant upon long-term stress due to elicitation of induction responses.

B. nigra is a fast-growing 1- to 2-m-tall annual herb native to the southern Mediterranean region of Europe, growing over a broad temperature range and therefore classified as a stress tolerant species (Duke 1983). It is occasionally cultivated for its seeds (Rajamurugan *et al.* 2012) as well as for leaves, extracts of which have allelopathic effects due to its secondary plant chemicals (Turk & Tawaha 2003). However, as a rapidly growing plant, *B. nigra* has become an aggressive weed in temperate Europe where it colonizes old fields (Gomaa *et al.* 2012). Because of extensive spread, more complex genome and greater stress tolerance, it has become next to *A. thaliana,* an additional brassicoid model system in studies on plant biology, ecology and plant-insect interactions (Dicke & van Loon 2000; Fatouros *et al.* 2012).

MATERIALS AND METHODS

Plant material

Plants of *B. nigra* were grown from the seeds provided by the Department of Entomology, University of Wageningen, the Netherlands. This standardized seed-lot corresponds to a wild-grown Dutch *B. nigra* population that has been used in multiple studies on plant-insect interactions (Bruinsma *et al.* 2008; Khaling *et al.* 2015; Pashalidou *et al.* 2015). The seeds were sown in 0.8 L plastic pots filled with a mixture of commercial garden soil with slow-release nutrients (Biolan Oy, Eura, Finland) and quartz sand. Day length was 12 h, and light intensity at plant level of 400 μmol m^{-2} s^{-1} was provided by metal halide lamps (HPI-T Plus 400 W, Philips, Eindhowen, The Netherlands). Day/night temperatures were maintained at 24/20 °C and relative humidity of 60%. The plants were watered every other day to soil field capacity. Five-to six-weeks-old non-bolted plants with at least three fully developed leaves were used in the experiments. Temperature response curves were measured, and two different heat stress treatments were conducted in three to seven replications with different plants (Fig. 1 for entire experimental protocol). New plants were used for individual temperatures within heat stress treatments, and each plant was stressed and analysed only once. Hence, emissions from 57 plants were analysed with GC-MS, and 65 plants were used in gas-exchange measurements (8 volatiles samples were lost due to malfunctioning of GC-MS cartridge autosampler, but nevertheless, the sample size was never below three for individual treatments).

Long-term heat stress treatment

For long-term stress application, the potted plants were placed in a Percival growth chamber (model E-36HO, Percival Scientific, Inc., Perry, IA, USA) under controlled conditions of light intensity at plant level of 400 μmol m^{-2} s^{-1} provided for 16 h day^{-1} (6:00–22:00 h), 60% of humidity and day/night temperature of 25/21 °C. Before the start of the heat stress treatment, the plants were acclimated for 24 h under these growth chamber conditions. After the acclimation, the heat stress was applied in two heat waves, one in the evening between 20:00–22:00 h and the second in the following morning between 06:00–8:00 h, providing a total treatment period of 4 h, but intervened with a night-time non-stressed period at 21 °C to allow for a recovery and induction of volatile stress responses. Although the increase of temperature to preset conditions took ~0.5 h, the chamber cool down to 21 °C after turning off the lights in the evening took ~1 h. Thus, the total stress period

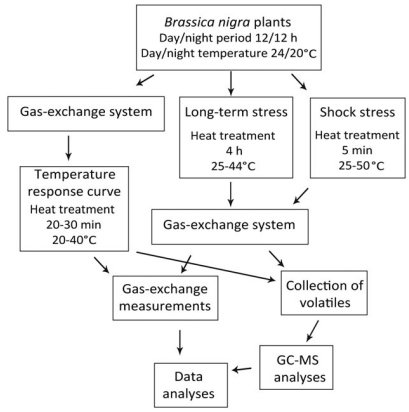

Figure 1. A schematic representation of the study experimental design. *Brassica nigra* plants were subjected to three different heat treatments: moderate exposure through stepwise raising temperatures through temperature response curve measurements, long-term heat stress and shock stress both achieved by stepwise increase in temperature, but differing in duration and treatment temperature. Plants were placed in a gas exchange system to collect volatiles and measure foliage net assimilation and transpiration rates. Collected volatiles were analysed with a GC-MS system followed by data analyses. In the case of temperature response curve measurements, the treatment and physiological measurements occurred simultaneously at the treatment temperature, while in the case of the two other experimental protocols, the physiological measurements were performed after the heat stress treatment at 25 °C.

was somewhat longer than 4 h. The temperatures applied in the growth chamber in individual stress experiments were 25 °C (control), 30, 35, 40 and 44 °C. After completion of the stress experiment at the given temperature, individual plants were enclosed in the custom-made gas-exchange system, and foliage photosynthesis, transpiration and volatile organic compound emission measurements were immediately carried out at 25 °C as described later (Fig. 1).

Heat shock stress

Shock stress treatment started at 9:00 in the morning, approximately 1 h after the light regime was automatically turned on in the plant room. Heat shock treatments followed the protocol of Copolovici *et al.* (2012). A temperature-controlled glass vessel equipped with a magnetic stirrer (Heidolph MR Hei-Standard with an EXT Hei-Con temperature sensor, Heidolph, Schwabach, Germany) was used. In the glass vessel, distilled water was heated to the desired temperature, and the plant's uppermost part with three fully developed leaves was inserted in the water with given temperature for 5 min. After the treatment, leaves were left to air-dry for approximately 5 min and then measured for gas-exchange and volatile emissions using the custom-made gas-exchange system at 25 °C as described later (Fig. 1). The heat shock response was studied at temperatures of 25 (control), 45, 48 and 50 °C, and individual plants were used for each treatment.

Gas-exchange measurement system

Foliage gas-exchange rates were measured with a custom-made open gas-exchange system described in detail in Copolovici & Niinemets (2010). The system has a temperature-controlled 1.2 L chamber made of double-walled glass and stainless steel bottom ring specially designed for volatile compound measurements. The chamber temperature is controlled by circulating thermostated water between the chamber walls (Copolovici & Niinemets 2010). An infra-red dual-channel gas analyzer operated in differential mode (CIRAS II, PP-Systems, Amesbury, MA, USA) was used to measure CO_2 and H_2O concentrations at the chamber inlets and outlets (Copolovici & Niinemets 2010). The ambient air was drawn from outside, passed through a 10 L buffer volume and an HCl-activated copper tubing to scrub ozone and was humidified to ~60% humidity using a custom-made humidifier. After passing the ozone scrubber, ozone concentrations were less than 1 nmol mol^{-1} (Sun *et al.* 2012). The chamber CO_2 concentration was 380–400 μmol mol^{-1} in these experiments.

Gas-exchange measurements and volatile sampling in heat-stressed leaves

For plants subjected to long-term heat stress, at least three top leaves, and for heat shock treated leaves all treated leaves were inserted into the leaf chamber (approximately 80–100 cm^2 leaf area), and standard conditions of light intensity of 800 μmol m^{-2} s^{-1} and chamber temperature of 25 °C (leaf temperature was within ± 1 °C of chamber temperature) were established. The measurements of net assimilation and transpiration rates were taken immediately after the gas flows had stabilized in the system, typically in 10–20 min after plant enclosure.

After the gas flows had stabilized, volatiles were collected onto multi-bed stainless steel cartridges filled with three different carbon-based adsorbents for optimal adsorption of all volatiles between C3–C17 (Kännaste *et al.* 2014) (for details of cartridges). A portable 210-1003MTX air sampling pump (SKC Inc., Houston, TX, USA) was used for sampling the chamber outlet air with a constant flow rate of 200 mL min^{-1}. The sampling time was 20 min, and thus, 4 L of air was sampled. Blank samples from the empty cuvette were taken before the plant measurements.

Measurements of temperature response curves of net assimilation and volatile release

Temperature response curve measurements started at 9:00 in the morning, 1 h after automatic turn-on of the light in the plant room. Three upper leaves were enclosed in the leaf chamber and stabilized at the reference conditions of light intensity at leaf level of 800 μmol m^{-2} s^{-1}, chamber temperature of 20 °C, CO_2

concentration of 380–400 μmol mol^{-1} and air humidity of 60% until stomata opened and gas-exchange rates stabilized, typically in 20–30 min since leaf enclosure. After reaching the steady-state, net assimilation and transpiration rates were recorded and volatile organic compounds were collected for 20 min as described earlier (Fig. 1). The chamber temperature was raised to the next higher temperature, the plant was conditioned again at this temperature for 20–30 min, and gas-exchange rates were recorded and volatiles collected. Foliage gas-exchange rates were measured at temperatures 20, 25, 30, 35 and 40 °C, while volatile organic compounds were collected at 20, 25, 30, and 40 °C.

GC-MS analyses

The cartridges with adsorbed volatiles were analysed with a combined Shimadzu TD20 automated cartridge desorber connected to a Shimadzu 2010 Plus GC–MS system (Shimadzu Corporation, Kyoto, Japan). Adsorbent cartridges were back flushed with high purity He (99,9999% AGA, Linde Group, Tallinn, Estonia) during thermal desorption with the following TD20 parameters: He purge flow rate of 40 mL min^{-1}, primary desorption temperature of 250 °C, primary desorption time of 6 min, the second stage trap temperature during primary desorption of −20 °C, the second stage trap desorption temperature of 280 °C, hold time of 6 min. The compounds were separated on a Zebron ZB-624 fused silica capillary column (0.32 mm i.d., 60 m length, 1.8 μm film thickness, Phenomenex, Torrance, CA, USA) using He with a flow rate of 1.48 mL min^{-1} as the carrier gas. The following GC oven programme was employed: 40 °C for 1 min, 9 °C min^{-1} to 120 °C, 2 °C min^{-1} to 190 °C, 20 °C min^{-1} to 250 °C for 5 min. The Shimadzu QP2010 Plus mass spectrometer was operated in the electron impact mode. The transfer line temperature was 240 °C and ion-source temperature 150 °C. The GC-MS system was calibrated as explained in Kännaste *et al.* (2014) and the compound quantification follows Copolovici *et al.* (2009). The compounds were identified by comparing the mass-spectra of volatiles with the spectra of authentic standards of the highest purity purchased from Sigma-Aldrich (St. Louis, MO, USA, GC purity, most of the standards) and Fluka (Buchs, Switzerland, GC purity, 1-hexanol and methyl salicylate). For quantification of emissions, the GC-MS system was calibrated with standard compounds in hexane solution. Six concentrations of each compound (range 0.1–1 μL/mL) were prepared, and 1 μL of each sample was injected into the adsorbent cartridge. The cartridge was back flushed with a stream of N_2 at 200 mL min^{-1} to simulate conditions during sampling of volatiles. Ultimately, the calibration factor for each compound was estimated as the slope of the GC chromatogram peak area versus compound mass concentration.

We grouped the volatile compounds released according to their formation pathways (Table 1) as fatty acid derived compounds (lipoxygenase volatiles, also called green leaf volatiles) (Matsui 2006), geranyl diphosphate (GDP) derived volatiles (GDP-pathway, various monoterpenoids synthesized from GDP) (Maffei 2010), geranylgeranyl diphosphate (GGDP) derived volatiles (homoterpenes such as DMNT and some carotenoid breakdown products such as geranyl acetone) (Arimura *et al.* 2009), shikimate pathway volatiles (different benzenoids such as methyl salicylate) (Wahid *et al.* 2007; Betz *et al.* 2009) and glucosinolate breakdown compounds (various sulphur- and nitrogen-containing compounds, often containing the CN functional group) (Sønderby *et al.* 2010; Ishida *et al.* 2014). It is primarily these latter compounds that give the plants from Brassicaceae the characteristic 'cabbage smell' (Buttery *et al.* 1976). No sesquiterpenes were observed in the emission blends in these experiments.

Data analyses

Net assimilation rate (A) and stomatal conductance (g_s) per leaf area and intercellular CO_2 concentration (C_i) were calculated according to von Caemmerer & Farquhar (1981) and the volatile emission rates according to Niinemets *et al.* (2011).

For normalization of data and residuals, logarithmic data transformation was used, and average values of gas-exchange and volatile emission rates at different temperatures were compared with one-way ANOVA followed by Tukey's *post-hoc* test. In addition, linear- and non-linear regression analyses were conducted to explore the relationships among gas-exchange and volatile emission rates and among the emission rates of different volatile compound classes. (StatSoft Inc., Tulsa, OK, USA) was used in these analyses. Heat stress effects on volatile bouquets and changes in the volatile bouquets through the temperature response curve were evaluated by principal component analysis (PCA) (Wold *et al.* 1987). Loading and score plots were derived after mean-centering and logarithmic data transformation. Redundancy data analysis was also used to test for the differences in bouquets among stress treatments. Monte-Carlo permutation tests were used to evaluate the statistical significance of the model. Multivariate data analyses were performed with CANOCO 5.0 software (ter Braak and Smilauer, Biometris Plant Research International, the Netherlands). All statistical tests were considered statistically significant at $P < 0.05$.

RESULTS

Effects of heat stress on foliage photosynthetic characteristics

Temperature response curve measurements indicated that leaf net assimilation rate (A) of *Brassica nigra* had a broad temperature optimum between 20 and 30 °C (Fig. 2a). Net assimilation rate declined over temperatures 35–40 °C, reaching ~4-fold lower values at the highest measurement temperature than at 20 °C (Fig. 2a). Stomatal conductance to water vapor (g_s) decreased with increasing temperature through the entire temperature range from on average (±SE) 99 ± 13 mmol m^{-2} s^{-1} at 20 °C to 13 ± 4 mmol m^{-2} s^{-1} at 40 °C (Fig. 2b). Thus, the intercellular CO_2 concentration (C_i) decreased with increasing temperature to low values of 60–100 μmol mol^{-1} at the highest measurement temperature (data not shown).

Exposure of plants to long-term heat stress (4 h exposure to given temperature, measurements of photosynthetic characteristics at 25 °C) was associated with minor modifications in A and g_s over the treatment temperature range of 25–35 °C, but

Table 1. Average ±SE emission rates (pmol $m^{-2} s^{-1}$) of different volatiles released from leaves of *Brassica nigra* in response to three different heat treatments grouped according to the compound formation pathways

No	Compound	Temperature response curve				Long-term stress	
		20 °C *(3)*	25 °C *(3)*	30 °C *(3)*	40 °C *(3)*	25 °C *(4)*	30 °C *(5)*
				Fatty acid derived compounds			
1	*(E)*-3-Hexen-1-ol						
2	*(E, E)*-2, 4-Hexadienal						
3	*(Z)*-3-Hexen-1-ol				0.7[#]	10	2.9 ± 1.4 a*
4	*(Z)*-3-Hexenyl acetate						6
5	*(Z)*-3-Hexenyl formate						
6	1-Hexanol		0.17			1.5 ± 0.6 a	1.2 ± 0.7 a
7	1-Pentanol						3.1
8	1-Penten-3-ol						
9	1-Penten-3-one						
10	2-Ethylfuran						
11	2-Pentanone					2.7	2.2 ± 0.7 a
12	Heptanal	3.8 ± 2.0 a	0.8	1.15 ± 0.14 a	0.71 ± 0.11 a	10.0 ± 4.2 ab	3.8 ± 1.9 ab
13	Hexanal	5.8 ± 4.2 a	6.4 ± 0.9 a	2.8 ± 0.9 a	0.96 ± 0.34 a	17 ± 5 ab	3.5 ± 1.1 a
14	Nonanal	22 ± 14 a	4.9 ± 3.7 a	6.8 ± 2.9 a	3.6 ± 3.0 a	54 ± 22 ab	20 ± 10 ab
15	Octanal	7.6 ± 4.7 a	0.9 ± 0.7 a	3.5 ± 1.4 a	1.5 ± 1.0 a	22 ± 9 ab	87.6 ± 3.9 ab
				GDP-pathway			
16	3-Carene	6.0 ± 0.6 a	2.55 ± 0.20 a	2.509 ± 0.032 a	0.103 ± 0.024 b	6.9 ± 2.8 a	2.4 ± 0.9 a
17	Camphene					0.66 ± 0.32 a	0.27 ± 0.08 a
18	Limonene	0.63 ± 0.25 a	0.42 ± 0.10 a	0.45 ± 0.09 a		1.6 ± 0.5 a	0.26 ± 0.16 a
19	α-Pinene	10.9 ± 2.1 a	3.75 ± 0.27 a	2.604 ± 0.023 a	0.272 ± 0.021 b	17 ± 8 a	1.8 ± 1.0 a
20	β-Pinene					0.54 ± 0.17 a	0.302 ± 0.021 a
				Shikimate pathway			
21	Methyl salicylate						0.031
				Glucosinolate breakdown products			
22	Tetramethylthiourea					0.47 ± 0.20 a	0.42
23	2-Propenenitrile		12	9		14 ± 10 a	8.9 ± 1.5 ab
24	Allyl isothiocyanate		20			2.6	
25	Cyclohexyl isocyanate					2.1	3.4
26	Cyclohexyl isothiocyanate	1.3 ± 0.6 a	1.2 ± 0.6 a	0.91		4.7 ± 2.1 a	0.7
27	Methanethiol					0.28	0.6
28	Methyl isothiocyanate						
29	Tetramethylurea					2.7 ± 0.9 a	1.5 ± 1.2 a
				GGDP-pathway			
30	6-Methyl-5-hepten-2-one	11 ± 8 a	7.29 ± 0.7 a	2.57	0.36 ± 0.20 a	7.5 ± 3.9 a	2.2 ± 2.0 a
31	Geranyl acetone	20 ± 6 a	23.3 ± 3.5 a	3.0 ± 2.6 a	6.7 ± 2.6 a	1.35 ± 0.27 ab	2.43 ± 1.02 ab

Different stress treatments as outlined in Fig. 1. Five primary compound groups were distinguished on the basis of compound synthesis pathways: fatty acid derived volatiles (products of lipoxygenase pathway, also called green leaf volatiles), geranyl diphosphate (GDP) derived volatiles (GDP-pathway, various monoterpenoids), geranylgeranyl diphosphate (GGDP) derived volatiles (homoterpenes such as DMNT and some carotenoid breakdown products including geranyl acetone), and shikimate pathway volatiles (different benzenoids such as methyl salicylate) and glucosinolate breakdown compounds (various sulphur- and nitrogen-containing volatiles). The compound number corresponds to the number in the PCA factor loading plot (Fig. 4). Number of replicates (individual plants) is shown in parenthesis below each stress temperature.
[#]for emissions of compounds, which were above the detection limit in only one of the replicate experiments, no SE values could be calculated.
*different letters indicate statistical significance at $P < 0.05$.

further increases in temperature were associated with both reduced A (4.5-fold reduction at 44 °C compared with the value at 25 °C) and g_s (2.8-fold reduction), from 69 to 24 mmol $m^{-2} s^{-1}$ (Fig. 2a, b, respectively). Intercellular CO_2 concentration was similar through temperatures 25–40 °C (192 ± 16 μmol mol^{-1}), but there was a significant increase in C_i at 44 °C (334 ± 30 μmol mol^{-1}, $P < 0.01$ for the difference among the means). Heat shock treatment (exposure for 5 min to given temperature, measurements of photosynthetic characteristics at 25 °C) was associated with major reductions in both A and g_s, with barely positive rates of net assimilation observed after 45 °C treatment, and negative net assimilation rates observed at treatment temperatures 48 and 50 °C (Fig. 2a). Intercellular CO_2 concentration was greater in heat shock treated than in control leaves ($P < 0.005$).

Temperature response curves of volatile emission

Total emission of fatty acid derived compounds was low and weakly affected by temperatures through the temperature response curves (Fig. 3a). Among C6-volatiles, only 1-hexanol and *(Z)*-3-hexen-1-ol were above the detection limit at 25 and 40 °C, and the rest of the emissions were due to aliphatic aldehydes (Table 1). Total emission of monoterpenoids (GDP-

Table 1. Average ±SE emission rates (pmol $m^{-2} s^{-1}$) of different volatiles released from leaves of *Brassica nigra* in response to three different heat treatments grouped according to the compound formation pathways

No	Long-term stress			Shock stress			
	35 °C *(6)*	40 °C *(6)*	44 °C *(7)*	25 °C *(3)*	45 °C *(4)*	48 °C *(3)*	50 °C *(7)*
			Fatty acid derived compounds				
1							3900 ± 1600
2		6					45 ± 17
3	9.4 ± 2.2 a	20 ± 13 a	300 ± 170 a	18 ± 18 a	1.1 ± 0.8 a	14	130 ± 50 a
4	14 ± 5 a		17 ± 9 a				
5							36
6	0.587 ± 0.042 a	7.6 ± 3.6 a	6.3 ± 3.2 a	2.7	0.087 ± 0.027 a		64 ± 30 b
7		14 ± 10 a	23 ± 10 a				17 ± 5
8		21	73 ± 19				180 ± 90
9			66				43 ± 8
10		2.613 ± 0.012 a	11 ± 7a	5.0			133 ± 47
11		16 ± 8 b					
12	3.9 ± 1.7 a	49 ± 13 b	14 ± 6 ab	1.8 ± 0.7 a	3.6 ± 1.0 a	15.9 ± 1.3 a	6.0 ± 3.1 a
13	6.0 ± 1.9 a	106 ± 41 b	44 ± 19 ab	5.2 ± 2.0 a	6.8 ± 2.0 a	31.4 ± 2.6 a	22 ± 9 a
14	7.8 ± 4.6 a	53 ± 16 b	55 ± 22 b	11.7 ± 3.0 a	11.4 ± 2.2 a	54 ± 33 a	21 ± 5 a
15	4 ± 1.9 a	43 ± 13 b	27 ± 11 b	3.9 ± 2.0 a	4.1 ± 0.6 a	22 ± 6 b	7.8 ± 20 a
			GDP-pathway				
16	5.1 ± 2.3 a	5.5 ± 1.6 a	5.4 ± 1.5 a	0.61 ± 0.44 a	6.9 ± 2.9 a	9.5 ± 4.1 a	13.9 ± 4.7 a
17	0.66 ± 0.12 a	0.23 ± 0.11 a	0.19	9	0.29 ± 0.19 a	1.17 ± 0.20 a	2.17 ± 1.02 a
18	1.4 ± 0.7 a	2.9 ± 1.1 a	4.7 ± 4.4 a	6 ± 6 a	1.7 ± 0.9 a	3.3 ± 2.2 a	6.23 ± 1.03 a
19	6.1 ± 3.0 a	4.6 ± 1.3 a	4.6 ± 2.3 a	3.0 ± 1.0 a	8 ± 6 a	27 ± 6 ab	38 ± 10 a
20	1.06 ± 0.22 a	0.47 ± 0.20 a	0.31 ± 0.23 a		0.85 ± 0.14 a		1.69 ± 0.10 a
			Shikimate pathway				
21		0.134 ± 0.042 a	0.118 ± 0.031 a				
			Glucosinolate breakdown products				
22	0.6	14 ± 7 a	110 ± 80 a		96 ± 38 a	178.9 ± 0.7 ab	400 ± 90 b
23	14.7 ± 4.2 ab	30 ± 8 ab	81 ± 25 b			19	
24		12.3 ± 4.8 a	1300 ± 700 b		2.5		250 ± 15
25	1.6	14.8 ± 0.8 a	38 ± 22 a		22.4 ± 4.3 a	17 ± 5 a	10.5 ± 3.1 a
26	0.35	28	110 ± 80 a		0.86 ± 0.30 a	10 ± 9 a	2.7 ± 0.5 a
27		3.3 ± 1.2 a	13 ± 8 a				
28		3.9	21 ± 11				15.1 ± 2.2
29	3.1	30 ± 24 a	63 ± 26 a			10 ± 6 a	14 ± 6 a
			GGDP-pathway				
30	3.2 ± 1.7 a	3.6 ± 1.3 a	3.1 ± 1.3 a	1.60 ± 0.27 a	1.4 ± 0.5 a	3.4 ± 1.5 a	5.1 ± 1.9 a
31	0.94 ± 0.70 a	5.7 ± 1.8 b	2.01 ± 0.5 ab	7.5 ± 0.9 a	4.7 ± 1.9 a	4.9 ± 3.4 a	8.6 ± 3.2 a

Different stress treatments as outlined in Fig. 1. Five primary compound groups were distinguished on the basis of compound synthesis pathways: fatty acid derived volatiles (products of lipoxygenase pathway, also called green leaf volatiles), geranyl diphosphate (GDP) derived volatiles (GDP-pathway, various monoterpenoids), geranylgeranyl diphosphate (GGDP) derived volatiles (homoterpenes such as DMNT and some carotenoid breakdown products including geranyl acetone), and shikimate pathway volatiles (different benzenoids such as methyl salicylate) and glucosinolate breakdown compounds (various sulphur- and nitrogen-containing volatiles). The compound number corresponds to the number in the PCA factor loading plot (Fig. 4). Number of replicates (individual plants) is shown in parenthesis below each stress temperature.
#for emissions of compounds, which were above the detection limit in only one of the replicate experiments, no SE values could be calculated.
*different letters indicate statistical significance at $P < 0.05$.

pathway compounds) decreased considerably from 20 to 40 °C (Fig. 3b). Among GDP-pathway compounds, α-pinene followed by 3-carene was the dominating volatiles at all temperatures (Table 1). Similarly to LOX-compounds, total emission of glucosinolate breakdown products was low and not affected by temperature (Fig. 3c). Allyl isothiocyanate and 2-propenenitrile were rare volatiles at only at 20 °C, while the emissions of cyclohexyl isothiocyanate were not affected by temperature (Table 1). Total emission of GGDP-pathway volatiles (carotenoid breakdown products) was overall low, and the emissions were dominated by geranyl acetone (Table 1). Similarly to GDP-pathway volatiles, GGDP-pathway volatiles decreased from 0.0309 ± 0.0027 nmol $m^{-2} s^{-1}$ at 20–25 °C to 0.0043 ± 0.0012 nmol $m^{-2} s^{-1}$ at 30–40 °C (Fig. 3d).

Effects of long-term heat stress on volatile emissions

Long-term heat stress had no significant effect on LOX-compounds over the treatment temperatures 25–35 °C, but the emissions were strongly enhanced upon exposure

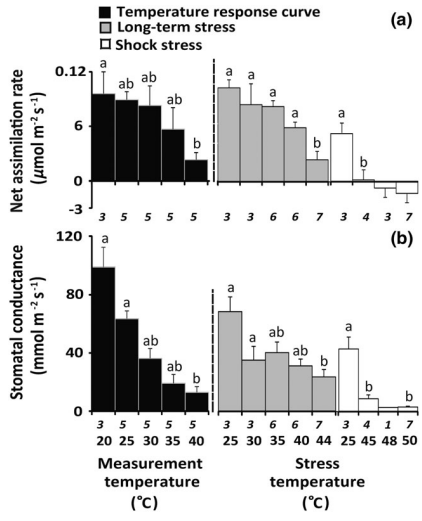

Figure 2. Effects of three different heat treatments on mean (±SE) net assimilation rate (A) and stomatal conductance to water vapor (B) in leaves of *Brassica nigra*. The treatments included raising temperatures through temperature response curve measurements (mild stress, total exposure for 1 h to given moderately high temperature), long-term stress (chronic stress, 4 h treatment with given temperature), and short-term heat shock stress (severe stress, exposure for 5 min to potentially lethal temperature). In the case of long-term and heat shock stress treatments, the measurements were conducted at 25 °C after heat exposure. Three to seven replicate plants were used for each treatment temperature (in each case, the number of biological replicates is shown above the temperature values). For statistical analyses, data were log-transformed and compared with one-way ANOVA followed by Tukey's *post-hoc* test. Different letters indicate statistically significant differences at $P < 0.05$.

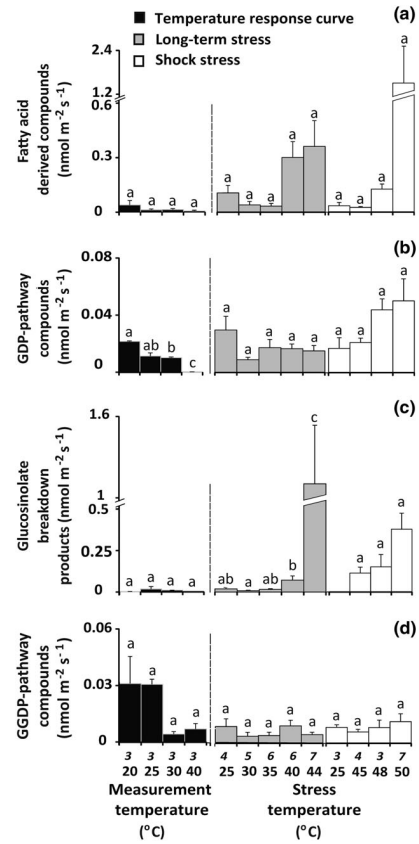

Figure 3. Rates of emission (nmol m^{-2} s^{-1}, mean ± SE) of fatty acid derived compounds (A), GDP-pathway compounds (B), glucosinolate breakdown products (C) and GGDP-pathway compounds (D) from the foliage of *Brassica nigra* in three different temperature stress treatments – temperature response curve (black bars), long-term (grey bars), and shock stress (white bars). Number of biological replicates is shown above the temperature values. Definition of compound groups and emissions of individual volatiles within each group is demonstrated in Table 1. Stress application, statistical analysis and data presentation as in Figs 1 and 2.

to 40 °C and 44 °C (Fig. 3a). Moreover, the emission composition significantly changed as at 40 °C treatment, the plants began to release 2-ethylfuran and 1-penten-3-ol and at 44 °C treatment, the emission of these volatiles increased even more (Table 1). At 44 °C treatment, the LOX bouquet was dominated by *(Z)*-3-hexen-1-ol, 1-penten-3-ol and 1-penten-3-one and aliphatic aldehydes hexanal, nonanal and octanal (Table 1). Heat stress effects on the release of glucosinolate breakdown products followed the same pattern as that observed for LOX-compounds (Fig. 3c). At treatment temperatures of 25–35 °C, total emission of glucosinolate breakdown products remained at a low level of 0.0087–0.0193 nmol m^{-2} s^{-1} (Fig. 3c) but increased somewhat already at 40 °C treatment, and a major emission burst of 1.10 ± 0.43 nmol m^{-2} s^{-1} was observed at the highest applied temperature treatment (Fig. 3c). At this temperature treatment, allyl isothiocyanate was the dominating volatile followed by tetramethylthiourea, cyclohexyl isothiocyanate and 2-propenenitrile (Table 1). Emissions of GDP-pathway and GGDP-pathway compounds were not affected by treatment temperature (Figs. 3b, d), but emissions of the benzenoid and methyl salicylate were detected after higher temperature treatments (Table 1).

Influences of heat shock stress on volatile release

Short-term exposure of leaves to severe heat stress increased total emissions of all volatiles at heat shock temperatures higher than 48 °C and for glucosinolate breakdown products already at 45 °C treatment (Fig. 3). A particularly strong enhancement was observed for LOX volatiles that increased from 0.036 ± 0.019 nmol $m^{-2} s^{-1}$ at 25 °C treatment to 1.4 ± 1.0 nmol $m^{-2} s^{-1}$ at 50 °C treatment (Fig. 3a). (*E*)-3-hexen-1-ol was released at temperature treatments of 45 and 48 °C as well, but at 50 °C treatment, the plants started to release additional C5-volatiles and C6-volatiles such as (*E,E*)-2,4-hexadienal, (*Z*)-3-hexenyl formate, 1-pentanol, 1-penten-3-ol and 1-penten-3-one (Table 1). The total emission of GDP-pathway volatiles rose from 0.013 ± 0.008 nmol $m^{-2} s^{-1}$ at 25 °C to 0.048 ± 0.016 nmol $m^{-2} s^{-1}$ at 50 °C treatment, mainly due to enhanced emissions of α-pinene and 3-carene (Table 1, Fig. 3b). Glucosinolate breakdown products were not detected at 25 °C treatment (Fig. 3c), but their emission increased from 0.113 ± 0.035 nmol $m^{-2} s^{-1}$ at 45 °C treatment to 0.37 ± 0.09 nmol $m^{-2} s^{-1}$ at 50 °C treatment (Fig. 3c). Methyl isothiocyanate was detected only at 50 °C treatment and tetramethylthiourea together with allyl isothiocyanate were the dominating volatiles at 50 °C treatment (Table 1). Total emission of GGDP-pathway volatiles remained similarly low as in the long-term stress (0.006 to 0.012 nmol $m^{-2} s^{-1}$), and it was not significantly different among the heat shock treatments (Fig. 3d).

Changes in emission blends among different temperature treatments and among different temperatures within treatments

Principal component analysis demonstrated that the emission blends in three different treatments (temperature response curve measurements constituting a mild-short stress, long-term heat stress and heat shock stress) differed significantly from each other (Fig. 4. Monte-Carlo permutation test, $P < 0.05$). A certain plant-to-plant variability was observed in the release of LOX volatiles, (*E*)-3-hexen-1-ol, (*Z*)-3-hexenyl formate and 1-penten-3-one (Table 1), upon heat stress. The plants emitting these volatiles, and experiencing a severe stress under the imposed conditions were grouped in the upper right corner of PCA score plot (Fig. 4b). In the case of other stressed plants, C5-volatiles such as 1-pentanol, 1-penten-3-ol and 2-ethylfuran and some glucosinolate degradation products such as methyl isothiocyanate constituted a stress signal of heat-stressed *B. nigra*. In the case of emissions higher than approximately 10 pmol $m^{-2} s^{-1}$ (*Z*)-3-hexen-1-ol, tetramethylthiourea, isocyanides, methyl isothiocyanate and tetramethylurea became stress signals (Table 1 and Fig. 4a, b). In the case of allyl isothiocyanate, the limit of emission for classification the plant as stressed in the PCA plot was about 200 pmol $m^{-2} s^{-1}$ (Table 1 and Fig. 4a, b).

In the case of emissions observed at different temperatures through the temperature response curve, the blend of emissions at 25 °C did not differ from that at 20 °C (Fig. 4b). Analogously, the emission blends at higher temperatures (30 and 40 °C) did not differ from those at 20 and 25 °C (Fig. 4b). In contrast, in long-term stress, emissions after treatments at 40 and 44 °C differed significantly from the control treatment (Fig. 4b). Analogously, heat shock of 45–50 °C resulted in major changes in the emission blend compared with the controls (Fig. 4b).

As emissions of LOX products and glucosinolate breakdown products were low through the entire temperature response curve, 20–40 °C (Fig. 3a, c), all temperature response curve data were distributed close to the control plants for long-term – and heat shock stresses in the upper corner of the left side of PCA score plot (Fig. 4b). High emission of glucosinolate breakdown

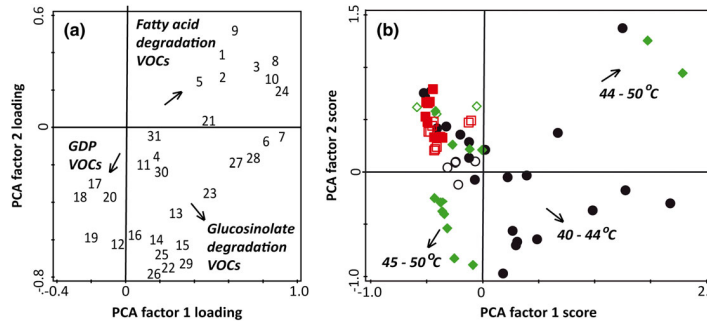

Figure 4. Loading-plot (A) and score-plot (B) of PCA analysis based on the emissions of volatiles released (Table 1 for the emission rates) from non-stressed and heat-stressed *Brassica nigra* plants. In the loading plot, the numbers represent different volatiles (Table 1 for the coding of individual compounds), while in the score plot, each symbol represents an individual non-stressed (empty symbols) or heat-stressed plant (filled symbols). Red symbols correspond to temperature response curve measurements, black symbols to long-term heat stress and green symbols to heat shock stress (Fig. 1 for the details of heat shock treatments and Fig. 3 for the heat stress effects on key volatile groups). In the loading plot, the impact of chemical compounds on PCA increases with the distance from the origin of the co-ordinate system.

volatiles (Fig. 4a), elicitation of methyl salicylate emissions and changes in the composition of GDP-pathway (e.g. induction of camphene emissions) were characteristic to plants exposed to long-term stress at 40 and 44 °C (Fig. 4b). Finally, heat shock treatments differed from long-term heat stress by greater elicitation of LOX-compounds and GDP-pathway volatiles and lower induction of glucosinolate breakdown products (Figs 3 & 4).

Correlations among emissions of different volatile compound classes and among emissions and photosynthetic characteristics

Through the temperature response curves, the emissions of GDP-pathway compounds (Fig. 3b) were positively correlated with A ($r = 0.71$) and g_s ($r = 0.83$, $P < 0.05$ for both), but low-level emissions of LOX-compounds (Fig. 3a) and glucosinolate breakdown products (Fig. 3c) were not correlated with foliage photosynthetic characteristics.

In long-term stress treatment, emissions of glucosinolate breakdown products and LOX-compounds were positively correlated through treatment temperatures of 25 to 40 °C, but the correlation was lost in leaves at 44 °C treatment where the increase in the emission of glucosinolate breakdown products was much stronger than that in LOX-compounds (Fig. 5). Emission of GDP-pathway compounds was also positively correlated with LOX-compound emission, but the slopes differed for treatment temperature range 25–35 °C and 40 and 44 °C, reflecting the stronger increase of LOX-compounds over this temperature range (Fig. 6a). Foliage photosynthetic characteristics were negatively correlated with emissions of glucosinolate breakdown products (Fig. 7a, b) and LOX-compounds (Fig. 7c, d), whereas the correlations were strongly non-linear (Figs 7 & 8).

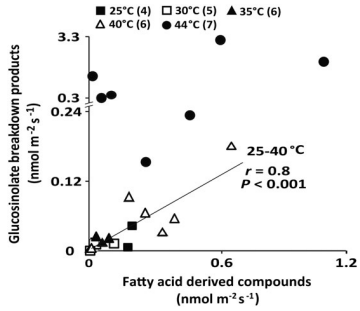

Figure 5. Emission of glucosinolate breakdown products in relation to emission of fatty acid derived compounds in *Brassica nigra* in the long-term stress treatment. The plants were exposed for 4 h to given temperature, and volatile release was measured after the treatment at 25 °C. Number of biological replicates is shown after the temperature values. Each symbol corresponds to an individual plant (Table 1 for stress effects on average emissions). Data over 25–40 °C were fitted by a linear regression.

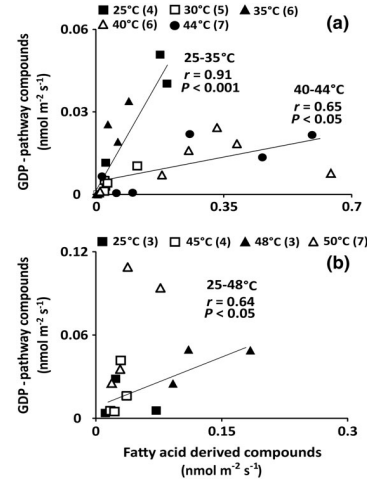

Figure 6. Emission of GDP-pathway compounds (various monoterpenoids, Table 1) in relation to the emission of fatty acid derived compounds in *Brassica nigra* in long-term (A) and shock (B) stress treatments (Fig. 3 for average emission rates). The emission rates were measured after heat treatments at 25 °C (heat stress treatments as in Fig. 1). Number of biological replicates is shown after the treatment temperature values. Each symbol corresponds to an individual replicate experiment. Data fitting as in Fig. 5.

In the heat shock treatments, glucosinolate breakdown products and LOX emission were not correlated ($P > 0.8$), although the slope was shallower than that observed for long-term heat treatments due to greater elicitation of emissions of LOX-compounds in heat shock treatments (cf. Figs 5 & 3). The emissions of GDP-pathway volatiles and LOX-compounds were positively correlated over the temperature range of 25 to 48 °C (Fig. 6b), but the treatment at 50 °C was characterized by much stronger elicitation of LOX-compounds (Fig. 3). Analysis of relationships among photosynthetic characteristics and emissions of glucosinolate and LOX-compounds, indicated that photosynthetic activity was lost earlier than stress volatile emissions were elicited (cf. Figs 2 & 3).

DISCUSSION

How different types of heat stress affect leaf photosynthetic characteristics in *B. nigra*

High temperature stress alters a plethora of plant physiological functions ranging from cellular to organ and whole plant processes (Ludwig-Müller *et al.* 2000; Sung *et al.* 2003; Loreto *et al.* 2006; Velikova *et al.* 2009; Usano-Alemany *et al.* 2014).

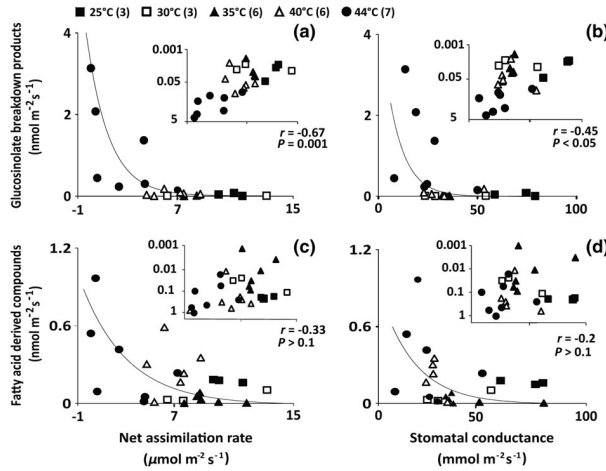

Figure 7. Correlations of emissions of glucosinolate breakdown products (A, B) and fatty acid derived compounds with net assimilation rate (A, C) and stomatal conductance to water vapor (B, D) in *Brassica nigra* in the long-term stress treatment (Fig. 2 for average values of photosynthetic characteristics and Fig. 3 for average emission rates). Heat stress treatment consisted of a 4 h exposure of plants to given temperature, after which the volatile release was measured at 25 °C. The insets demonstrate emissions with the *y*-scale reversed and log-transformed. Individual symbols stand for replicate experiments. Additionally number of biological replicates is shown after the temperature values. Data were fitted by non-linear regressions.

In the current study high temperature resistance of *B. nigra* was studied by three sets of experiments with differing severity of heat stress, including measurements of temperature responses where temperature was raised up to 40 °C (mild stress), long-term moderate heat stress where plant temperature was raised up to 44 °C for 4 h and heat shock stress where leaves were exposed to sublethal to lethal temperatures of 45–50 °C for 5 min. Given that Brassicaceae have a specialized defense system constituting of high constitutive levels of glucosinolates and release of glucosinolate breakdown products, the key aim of the study was to gain insight into the relationships among ubiquitous stress responses and brassicoid-specific stress responses through the different heat stress treatments.

Among the ubiquitous stress responses, foliage net assimilation rate (A) and stomatal conductance (g_s) decreased in all heat stress treatments (Fig. 2), but the mechanism of photosynthetic decline differed among the different types of heat treatment (Figs 2 & 8a). In temperature response curve measurements, the temperature-dependent reduction in A resulted from reduced intercellular CO_2 concentration (C_i) due to a reduction in g_s (Fig. 2). Closure of stomata is often observed at higher temperatures (Cui *et al.* 2006; Hüve *et al.* 2006; Hüve *et al.* 2011; Copolovici *et al.* 2012), and this response reduces water loss in conditions of higher vapor pressure deficit typical to high temperature (Shinohara & Leskovar 2014). However, after long-term heat stress at 40 and 44 °C and heat shock stress at 45–50 °C, C_i actually increased, indicating that heat stress resulted in stronger reductions in leaf photosynthetic capacity than in stomatal conductance. This result is in agreement with previous studies indicating heat dose dependent reductions in foliage photosynthetic capacity after a certain threshold heat dose has been exceeded (Kadir *et al.* 2007; Hüve *et al.* 2011). Such decreases in photosynthetic capacity might reflect inactivation of foliage photosynthetic electron transport processes due to increased leakiness of membranes and enhanced non-photochemical quenching (Havaux 1993; Lu & Zhang 2000; Zhang & Sharkey 2009; Zhang *et al.* 2009), but they might also result from irreversible cellular damage (Hüve *et al.* 2011). As the result of sustained inhibition of photosynthetic activity or cellular damage, foliage photosynthetic activity does not recover upon return to lower temperatures as was also observed in our study after long-term heat stress at 44 °C and heat shock treatments between 45–50 °C (Figs 2 & 8a).

Different heat stresses have varying effects on lipoxygenase pathway volatiles

The release of LOX volatiles in low amounts from flowers, leaves or fruits is a widespread phenomenon (Bengtsson *et al.* 2001; Ceuppens *et al.* 2015). In our study, characteristic C6 LOX volatiles such as 1-hexanol and *(Z)*-3-hexen-1-ol were emitted in small quantities, close to the analytical detection limit, at low temperatures (Table 1). In addition, aliphatic saturated aldehydes hexanal, heptanal, octanal and nonanal were consistently emitted at low level through all three sets of experiments (Table 1). Although in the literature, the LOX-pathway

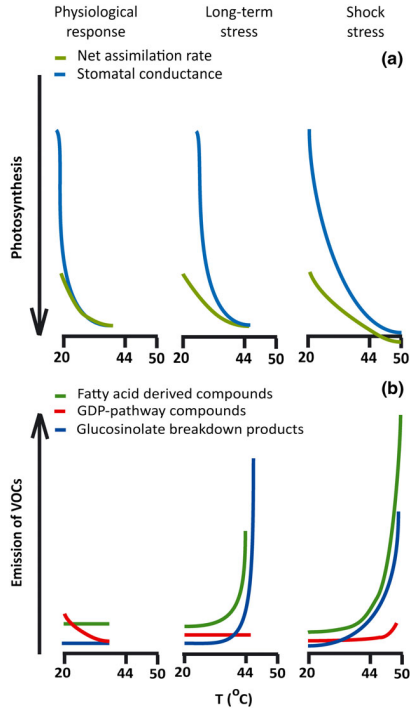

Figure 8. A comparative scheme of the main effects of three different heat stress applications (physiological temperature response, long-term and shock heat stress) on photosynthetic characteristics (A) and on total emission of main classes of emitted volatiles (B).

is primarily associated with the emission of C6 aldehydes and their derivatives (Wildt *et al.* 2003), longer chain length aldehydes are often found in plant emissions, including emissions from *Brassica rapa* var. *rapa* (Taveira *et al.* 2009), tomato (*Solanum lycopersicum*) (Wang *et al.* 2001) and hybrid poplar (*Populus simonii* x *Populus pyramidalis*) (Hu *et al.* 2011), and there is evidence that activation of LOX-pathway is responsible for the emissions of all these aliphatic aldehydes (Hu *et al.* 2009; Hu *et al.* 2011).

In addition to the low-level emissions of LOX volatiles in non-stressed conditions, a major burst of LOX volatiles upon severe stress constitutes a key ubiquitous stress response (Matsui 2006; Copolovici *et al.* 2012). Multiple LOXs are constitutively active in leaves, and thus, the release of volatile LOX-compounds occurs rapidly as soon as the substrate, polyunsaturated fatty acids, becomes available because of membrane lesions (Feussner & Wasternack 2002; Liavonchanka & Feussner 2006; Andreou & Feussner 2009). Accordingly, elicitation of emissions of LOX-compounds constitutes a classic indicator of cellular damage (Matsui 2006; Jansen *et al.* 2009; Matsui *et al.* 2012). In our study, long-term heat stress at 40 and 44 °C and heat shock treatment at 48 and 50 °C resulted in a major increase in lipoxygenase pathway volatiles, while the lipoxygenase volatile emissions remained low through the temperature response curve measurements (Figs 3a & 8, Table 1). At these high temperatures in both heat stress treatments, the plants began to release next to C6-volatiles also various C5-volatiles such as 1-pentanol, 1-penten-3-ol, 1-penten-3-one, and C7-volatile (*Z*)-3-hexenyl formate (Table 1) that are also formed through the LOX-pathway (Shen *et al.* 2014) and are emitted upon several other stresses (de Gouw *et al.* 1999; Brilli *et al.* 2012).

Previous studies indicate that upon short-term heat pulses as those applied in the heat shock treatments, LOX product emissions are elicited between temperatures 46–49 °C according to a highly non-linear switch-type response (Loreto *et al.* 2006; Copolovici *et al.* 2012) as was also observed in our study (Fig. 3a). This temperature range corresponds to major increases in plasmalemma membrane permeability and time-dependent reductions in foliage photosynthetic activity upon return to lower temperature (Hüve *et al.* 2011). However, photosynthesis rate of *B. nigra* strongly decreased upon heat shock at 45 °C as well (Fig. 2), but no significant elicitation of LOX-compounds was observed (Fig. 3a). This discrepancy suggests that the reduction in photosynthetic activity at this temperature likely reflected enhanced engagement of non-photochemical processes or impaired photochemistry without direct membrane-level damage.

Similar to our study (Fig. 3a), long-term exposure, from several hours to days, to moderately high temperatures of 35–45 °C resulted in elicitation of LOX product emissions that was accompanied by reduced *A* (Fig. 7c) in several tree species (Kleist *et al.* 2012). This evidence together with our observations further underscores that heat stress impact on cellular processes is dose-dependent, and even moderately high sustained heat waves can result in major cellular lesions progressively leading to the cessation of photosynthetic activity.

Constitutive terpenoid release upon heat stress

Several plant species emit GDP-pathway compounds, mainly monoterpenes, constitutively. Constitutive monoterpene synthesis occurs in plastids where the terminal enzymes and monoterpene synthases are located (Tholl 2006; Chen *et al.* 2011; Rajabi Memari *et al.* 2013). Constitutive monoterpene emissions either come from specialized storage tissues or from immediate *de novo* synthesis (Grote *et al.* 2013). In the latter case, the emissions are strongly related to foliage photosynthetic characteristics, and thus, reduction in foliage photosynthetic rate upon heat stress typically also leads to reduction in constitutive monoterpene emissions (Loreto *et al.* 1998; Peñuelas & Llusià 2002; Kleist *et al.* 2012). In *B. nigra*, α-pinene and 3-carene followed by limonene were the main monoterpenes emitted under moderate temperatures in heat stress treatments and through the temperature response curve measurements (Table 1). Through the temperature response curve

measurements, the rates of total monoterpene emission and net assimilation were positively correlated, and the emissions decreased with increasing temperature parallel to photosynthesis (Figs 2a & 3b), suggesting that these emissions resulted from *de novo* synthesis.

However, sustained heat stress can result in the induction of monoterpene synthesis (Staudt & Bertin 1998), although not always (Kleist *et al.* 2012). These induced monoterpene emissions typically consist of different monoterpenes than constitutive emissions, reflecting expression of new terpene synthases (Staudt & Bertin 1998; Niinemets *et al.* 2010a,b; Copolovici & Niinemets 2016). In *B. nigra*, the total emission rate of monoterpenes was not affected by long-term heat treatment, but the emission rates of GDP volatiles correlated with the emissions of LOX-compounds (Fig. 6a). These correlations differed for different treatment temperature ranges, suggesting that the constitutive plant defense was gradually replaced by induced plant defense as the treatment temperature raised.

Heat shock treatment was associated with a significant increase of monoterpene emissions (Figs 3b & 8b) as has been observed in tomato (*S. lycopersicum*) (Copolovici *et al.* 2012), but the mechanism of this increase is unclear. Enhanced substrate availability for monoterpene synthases due to disruption of other metabolic pathways consuming isopentenyl diphosphate and dimethylallyl diphosphate such as carotenoid synthesis could provide an explanation for the increase of monoterpene emission. It can also reflect a certain storage capacity of monoterpenoids in idioblasts, also called the myrosin cells or 'mustard oil bombs' (Ahuja *et al.* 2009; Borgen *et al.* 2012), or non-specific storage of monoterpenes in cellular membranes as is common in constitutive *de novo* monoterpene emitters (Niinemets & Reichstein 2002; Niinemets *et al.* 2010b). Thus, the release of these compounds, especially the release of α-pinene (Table 1) upon heat shock can occur due to cellular damage. A positive correlation between LOX-compounds and monoterpene emissions through the heat shock treatments (Fig. 6b) suggests that this is a plausible explanation, although the correlation collapsed at 50 °C where the increase in LOX emissions vastly exceeded that in monoterpene emission.

Release of specialized brassicoid volatiles as a major trait differentiating among heat stress treatments

Synthesis of glucosinolates and formation of their volatile toxic hydrolysis products by myrosinases constitute the characteristic defence system in Brassicales (Halkier & Du 1997; Raybould & Moyes 2001; Wang *et al.* 2011). Formation of glucosinolates primarily occurs in vascular tissues (Li *et al.* 2011), while myrosinases are stored in myrosin cells diffusely distributed through plant tissues (Kelly *et al.* 1998; Burow *et al.* 2007; Zhao *et al.* 2008; Misra *et al.* 2015). Thus, the release of myrosinases upon damage of myrosin cells is the first step required for the formation of glucosinolate volatiles (Winde & Wittstock 2011), whereas the blend of volatiles released depends on the mixture of structurally different glucosinolates, reaction conditions and protein cofactors (Ahuja *et al.* 2009; Borgen *et al.* 2012). In *B. nigra,* we observed eight different glucosinolate breakdown products (Table 1). As with the emissions of LOX volatiles, emissions of glucosinolate breakdown products was enhanced at 40–44 °C in long-term stress and at 45–50 °C in heat shock treatments (Figs 3c & 8b). Moreover, in long-term stress treatments, the emissions of LOX volatiles and glucosinolate breakdown products were correlated over temperatures of 25 to 40 °C (Fig. 5). Similar elicitation of LOX volatiles and volatile glucosinolate products suggests that both reflect the propagation of lesions with increasing the severity of heat stress.

However, long-term and heat shock stresses importantly differed in the quantitative relationship between LOX volatiles and glucosinolate breakdown products (Figs 3a, c & 8b). In particular, long-term heat stress led to a much stronger elicitation of glucosinolate volatiles than the heat shock stress and at the highest long-term heat treatment temperature of 44 °C, glucosinolate volatile production exceeded LOX volatile production (Figs 3a,c & 8b). This evidence suggests that while the release of glucosinolate volatiles upon heat shock reflects a release of myrosinases upon disruption of plant cells, a certain induction process is activated upon long-term heat stress. In fact, there is evidence that myrosinase expression can be enhanced by different biotic and abiotic stresses (Jost *et al.* 2005; Pan *et al.* 2014; del Carmen Martinez-Ballesta & Carvajal 2015). In addition, reactive oxygen species such as H_2O_2 can directly enhance myrosinase expression (Pan *et al.* 2014). As heat stress leads to a major burst of H_2O_2 (Hüve *et al.* 2011), heat stress dependent enhancement of myrosinase activity is likely. On the other hand, there is still limited information of tissue-specific expression of different isoforms of myrosinases as well as stress effects on the synthesis of glucosinolates. For example, methanethiol, that has been previously observed in *Brassica* upon tissue damage (Tulio *et al.* 2002; van Dam *et al.* 2012) was mostly detected in long-term stress experiment, suggesting a certain reprogramming of glucosinolate synthesis. Furthermore, given the spatial separation of myrosinases and glucosinolates, any structural or physiological change that reduces the degree of separation, for example, expression of a different myrosinase closer to the site of synthesis of glucosinolates or vice versa is also expected to enhance the release of glucosinolate breakdown products.

Clearly, the release of glucosinolate volatiles constitutes a stress marker in Brassicaceae, but there is also evidence that glucosinolate volatiles may also play a key signalling role. In particular, exposure to allyl isothiocyanate has been shown to enhance thermotolerance of *A. thaliana* (Hara *et al.* 2013). Application of allyl isothiocyanate has also been shown to lead to reactive oxygen species formation and activation of a signalling cascade leading to the closure of stomata (Khokon *et al.* 2011; Hossain *et al.* 2013). In our study, stomatal conductance and glucosinolate release were correlated through the long-term heat treatment (Fig. 7b), however, given that stomatal closure also occurred through temperature response curves where glucosinolate volatile release was minimal (Fig. 3c), the correlation in Fig. 7b likely is not causal, but part of the heat stress syndrome.

Heat stress effects on benzenoids and geranylgeranyl diphosphate pathway volatiles

In addition to LOX-pathway, GDP-pathway and glucosinolate volatiles, heat treatments were associated with differences in emissions of two other volatile compound classes. The only benzenoid, methyl salicylate (MeSA), was detected in long-term stress treatment at temperatures 30, 40 and 44 °C (Table 1). MeSA is a common plant stress volatile, activating multiple biochemical pathways upon biotic and abiotic stresses (Arimura *et al.* 2005; Zhao *et al.* 2010), and its release has been observed in several cases upon long-term exposure to moderately high temperatures (Karl *et al.* 2008; Kleist *et al.* 2012). MeSA can be *de novo* synthesized upon stress, but it can also be released from a glycosidically bonded form (Blažević & Mastelić 2009). Given that no MeSA release was observed upon heat shock treatment, the release of MeSA upon long-term stress suggests that it was *de novo* synthesized.

In the case of geranylgeranyl diphosphate (GGDP) pathway volatiles, we observed emissions of geranyl acetone and 6-methyl-5-hepten-2-one that are suggested to result from oxidative cleavage of carotenoids (Buttery *et al.* 1988; Goff & Klee 2006; Tieman *et al.* 2006). Both volatiles, geranyl acetone (Taveira *et al.* 2009; Truong *et al.* 2014) and 6-methyl-5-hepten-2-one (Geervliet *et al.* 1997) have been observed in emissions from Brassicaceae species. In *B. nigra*, only a reduction of emission with raising temperature was observed in temperature response curve measurements similarly to changes in GDP-pathway volatiles (Fig. 3d). Analogously, geranyl acetone emissions decreased in *A. thaliana* with increasing temperature (Truong *et al.* 2014). As both GDP-pathway and GGDP-pathway are confined to plastids (Rajabi Memari *et al.* 2013), the release of these volatiles might be associated with turnover of carotenoids that occurs as part of everyday plant metabolism (Beisel *et al.* 2010). If so, inhibition of the release of GGDP-volatiles with raising temperatures might indicate reversible inhibition of carotenoid synthesis.

CONCLUSIONS

Overall, the results indicated that different types of heat treatment are associated with major variation in photosynthetic and volatile responses in *B. nigra* (Figs 2–4 & 8). Temperature response curve measurements constituted a mild, physiological stress that led to reductions in constitutively synthesized volatiles associated with immediate photosynthetic metabolism. Both long-term and heat shock stress resulted in elicitation of lipoxygenase and glucosinolate volatiles once the threshold heat dose was achieved. However, these two types of stresses primarily differed in the extent to which glucosinolate volatile emission was induced relative to LOX product release (Fig. 8). In particular, long-term heat stress was associated with much stronger elicitation of glucosinolate emissions than the heat shock response. In addition, methyl salicylate emissions were only induced by long-term heat stress. Although both long-term and short-term shock stress resulted in major raises of stress volatile emissions, sustained moderate heat stress resulted in the engagement of induced metabolic defense systems that did not occur upon short severe stress. As the result, different types of heat stress, mild, chronic and shock stress, are associated with different volatile fingerprints (Fig. 4). These different volatile blends could play important roles in heat-elicited signalling responses as well as in multitrophic interactions in natural stressful environments (Hopkins *et al.* 2009; Copolovici *et al.* 2014). Further work is needed to gain insight into the role of induction of glucosinolate volatiles in heat resistance and into how different types of heat stress affect plant–insect interactions in Brassicaceae.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Agneta R., Lelario F., De Maria S., Möllers C., Bufo S.A. & Rivelli A.R. (2014) Glucosinolate profile and distribution among plant tissues and phenological stages of field-grown horseradish. *Phytochemistry* **106**, 178–187.

Agrawal A.A. & Kurashige N.S. (2003) A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology* **29**, 1403–1415.

Ahuja I., Rohloff J. & Bones A.M. (2009) Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. *Agronomy for Sustainable Development* **30**, 311–348.

Ameye M., Wertin T.M., Bauweraerts I., McGuire M.A., Teskey R.O. & Steppe K. (2012) The effect of induced heat waves on *Pinus taeda* and *Quercus rubra* seedlings in ambient and elevated CO_2 atmospheres. *The New Phytologist* **196**, 448–461.

Andreou A. & Feussner I. (2009) Lipoxygenases – structure and reaction mechanism. *Phytochemistry* **70**, 1504–1510.

Arimura G., Kost C. & Boland W. (2005) Herbivore-induced, indirect plant defences. *Biochimica et Biophysica Acta-Molecular and Cell Biology of Lipids* **1734**, 91–111.

Arimura G., Matsui K. & Takabayashi K. (2009) Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant and Cell Physiology* **50**, 911–923.

Barth C. & Jander G. (2006) Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. *The Plant Journal* **46**, 549–562.

Behnke K., Ehlting B., Teuber M., Bauerfeind M., Louis S., Hänsch R. & Schnitzler J.-P. (2007) Transgenic, non-isoprene emitting poplars don't like it hot. *The Plant Journal* **51**, 485–499.

Beisel K.G., Jahnke S., Hofmann D., Köppchen S., Schurr U. & Matsubara S. (2010) Continuous turnover of carotenes and chlorophyll *a* in mature leaves of Arabidopsis revealed by $^{14}CO_2$ pulse-chase labeling. *Plant Physiology* **152**, 2188–2199.

Bengtsson M., Bäckman A.C., Liblikas I., Ramirez M.I., Borg-Karlson A.K., Ansebo L. & Witzgall P. (2001) Plant odor analysis of apple: antennal response of codling moth females to apple volatiles during phenological development. *Journal of Agricultural and Food Chemistry* **49**, 3736–3741.

Betz G.A., Gerstner E., Stich S., Winkler B., Welzl G., Kremmer E., … Ernst D. (2009) Ozone affects shikimate pathway genes and secondary metabolites in saplings of European beech (*Fagus sylvatica* L.) grown under greenhouse conditions. *Trees* **23**, 539–553.

Bidart-Bouzat M.G. & Imeh-Nathaniel A. (2008) Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology* **50**, 1339–1354.

Bilger H.W., Schreiber U. & Lange O.L. (1984) Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. *Oecologia* **63**, 256–262.

Blažević I. & Mastelić J. (2009) Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus* L.) *Food Chemistry* **113**, 96–102.

Bones A.M. & Rossiter J.T. (2006) The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* **67**, 1053–1067.

Borgen B.H., Ahuja I., Thangstad O.P., Honne B.I., Rohloff J., Rossiter J.T. & Bones A.M. (2012) 'Myrosin cells' are not a prerequisite for aphid feeding on oilseed rape (*Brassica napus*) but affect host plant preferences. *Plant Biology* **14**, 894–904.

Brilli F., Hörtnagl L., Bamberger I., Schnitzhofer R., Ruuskanen T.M., Hansel A., Loreto F. & Wohlfahrt G. (2012) Qualitative and quantitative characterization of volatile organic compound emissions from cut grass. *Environmental Science & Technology* **46**, 3859–3665.

Bruinsma M., van Dam N.M., van Loon J.J.A. & Dicke M. (2007) Jasmonic acid-induced changes in *Brassica oleracea* affect oviposition preference of two specialist herbivores. *Journal of Chemical Ecology* **33**, 655–668.

Bruinsma M., Ijdema H., van Loon J.J.A. & Dicke M. (2008) Differential effects of jasmonic acid treatment of *Brassica nigra* on the attraction of pollinators, parasitoids, and butterflies. *Entomologia Experimentalis et Applicata* **128**, 109–116.

Burow M., Rice M., Hause B., Gershenzon J. & Wittstock U. (2007) Cell- and tissue-specific localization and regulation of the epithiospecifier protein in *Arabidopsis thaliana*. *Plant Molecular Biology* **64**, 173–185.

Buttery R.G., Guadagni D.G., Ling L.C., Seifert R.M. & Lipton W. (1976) Additional volatile components of cabbage, broccoli, and cauliflower. *Journal of Agricultural and Food Chemistry* **24**, 829–832.

Buttery R.G., Teranishi R., Ling L.C., Flath R.A. & Stern D.J. (1988) Quantitative studies on origins of fresh tomato aroma volatiles. *Journal of Agricultural and Food Chemistry* **36**, 1247–1250.

Ceuppens B., Ameye M., Van Langenhove H., Roldan-Ruiz I. & Smagghe G. (2015) Characterization of volatiles in strawberry varieties 'Elsanta' and 'Sonata' and their effect on bumblebee flower visiting. *Arthropod-Plant Interactions* **9**, 281–287.

Chen F., Tholl D., Bohlmann J. & Pichersky E. (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal* **66**, 212–229.

Copolovici L., Kännaste A. & Niinemets Ü. (2009) Gas chromatography-mass spectrometry method for determination of monoterpene and sesquiterpene emissions from stressed plants. *Studia Universitatis Babes-Bolyai, Chemia* **54**, 329–339.

Copolovici L., Kännaste A., Pazouki L. & Niinemets Ü. (2012) Emissions of green leaf volatiles and terpenoids from Solanum lycopersicum are quantitatively related to the severity of cold and heat shock treatments. *Journal of Plant Physiology* **169**, 664–672.

Copolovici L., Kännaste A., Remmel T. & Niinemets Ü. (2014) Volatile organic compound emissions from Alnus glutinosa under interacting drought and herbivory stresses. *Environmental and Experimental Botany* **100**, 55–63.

Copolovici L. & Niinemets Ü. (2010) Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. *Plant, Cell and Environment* **33**, 1582–1594.

Copolovici L. & Niinemets Ü. (2016) Environmental impacts on plant volatile emission. In *Deciphering Chemical Language of Plant Communication* (eds Blande J. & Glinwood R.), Springer, Berlin.

Crespo E., Hordijk C.A., de Graaf R.M., Samudrala D., Cristescu S.M., Harren F. J.M. & van Dam N.M. (2012) On-line detection of root-induced volatiles in *Brassica nigra* plants infested with *Delia radicum* L. root fly larvae. *Phytochemistry* **84**, 68–77.

Cui L.J., Li J.L., Fan Y.M., Xu S. & Zhang Z. (2006) High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. *Botanical Studies* **47**, 61–69.

de Gouw J.A., Howard C.J., Custer T.G. & Fall R. (1999) Emissions of volatile organic compounds from cut grass and clover are enhanced during the drying process. *Geophysical Research Letters* **26**, 811–814.

de Vos M., Kriksunov K.L. & Jander G. (2008) Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. *Plant Physiology* **146**, 916–926.

del Carmen Martinez-Ballesta M. & Carvajal M. (2015) Myrosinase in Brassicaceae: the most important issue for glucosinolate turnover and food quality. *Phytochemistry Reviews* **14**, 1045–1051.

Dicke M. & van Loon J.J.A. (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata* **97**, 237–249.

Duke J.A. (1983) Handbook of energy crops. In: *NewCROP*. Purdue University, Department of Horticulture and Landscape Architecture, 625 Agriculture Mall Drive West Lafayette, IN 47907-2010.

Fahey J.W., Zalcmann A.T. & Talalay P. (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* **56**, 5–51.

Farré-Armengol G., Filella I., Llusià J., Niinemets Ü. & Peñuelas J. (2014) Changes in floral bouquets from compound-specific responses to increasing temperatures. *Global Change Biology* **20**, 3660–3669.

Fatouros N.E., Lucas-Barbosa D., Weldegergis B.T., Pashalidou F.G., van Loon J. J.A., Dicke M. & Huigens M.E. (2012) Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. *PloS One* **7**, e43607.

Feussner I. & Wasternack C. (2002) The lipoxygenase pathway. *Annual Review of Plant Biology* **53**, 275–297.

Geervliet J.B.F., Posthumus M.A., Vet L.E.M. & Dicke M. (1997) Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris* species. *Journal of Chemical Ecology* **23**, 2935–2954.

Goff S.A. & Klee H.J. (2006) Plant volatile compounds: sensory cues for health and nutritional value? *Science* **311**, 815–819.

Gomaa N.H., Al Sherif E.A., Hegazy A.K. & Hassan M.O. (2012) Floristic diversity and vegetation analysis of Brassica nigra (L.) Koch communities. *Egyptian Journal of Biology* **14**, 63–72.

Grote R., Monson R.K. & Niinemets Ü. (2013) Leaf-level models of constitutive and stress-driven volatile organic compound emissions. In *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* (eds Niinemets Ü. & Monson R.K.), pp. 315–355. Springer, Berlin.

Halkier B.A. & Du L.C. (1997) The biosynthesis of glucosinolates. *Trends in Plant Science* **2**, 425–431.

Hara M., Harazaki A. & Tabata K. (2013) Administration of isothiocyanates enhances heat tolerance in *Arabidopsis thaliana*. *Plant Growth Regulation* **69**, 71–77.

Hartikainen K., Nerg A.-M., Kivimäenpää M., Kontunen-Soppela S., Mäenpää M., Oksanen E., Rousi M. & Holopainen T. (2009) Emissions of volatile organic compounds and leaf structural characteristics of European aspen (*Populus tremula*) grown under elevated ozone and temperature. *Tree Physiology* **29**, 1163–1173.

Havaux M. (1993) Characterization of thermal damage to the photosynthetic electron transport system in potato leaves. *Plant Science* **94**, 19–33.

Hopkins R.J., Birch A.N.E., Griffiths D.W., Baur R., Städler E. & McKinlay R.G. (1997) Leaf surface compounds and oviposition preference of turnip root fly *Delia floralis*: The role of glucosinolate and nonglucosinolate compounds. *Journal of Chemical Ecology* **23**, 629–643.

Hopkins R.J., van Dam N.M. & van Loon J.J.A. (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology* **54**, 57–83.

Hossain M.S., Ye W., Hossain M.A., Okuma E., Uraji M., Nakamura Y., Mori I.C. & Murata Y. (2013) Glucosinolate degradation products, isothiocyanates, nitriles, and thiocyanates, induce stomatal closure accompanied by peroxidase-mediated reactive oxygen species production in *Arabidopsis thaliana*. *Bioscience, Biotechnology, and Biochemistry* **77**, 977–983.

Hu Z.-H., Leng P.-S., Shen Y.-B. & Wang W.-H. (2011) Emissions of saturated C_6–C_{10} aldehydes from poplar (*Populus simonii* × *P. pyramidalis* '*O*pera 8277') cuttings at different levels of light intensity. *Journal of Forestry Research* **22**, 223–238.

Hu Z.-H., Shen Y.-B. & Su X.-H. (2009) Saturated aldehydes C_6-C_{10} emitted from ashleaf maple (*Acer negundo* L.) leaves at different levels of light intensity, O_2, and CO_2. *Journal of Plant Biology* **52**, 289–298.

Hu Z., Zhang H., Leng P., Zhao J., Wang W. & Wang S. (2013) The emission of floral scent from *Lilium* 'siberia' in response to light intensity and temperature. *Acta Physiologiae Plantarum* **35**, 1691–1700.

Hüve K., Bichele I., Rasulov B. & Niinemets Ü. (2011) When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and H_2O_2 formation. *Plant, Cell and Environment* **34**, 113–126.

Hüve K., Bichele I., Tobias M. & Niinemets Ü. (2006) Heat sensitivity of photosynthetic electron transport varies during the day due to changes in sugars and osmotic potential. *Plant, Cell and Environment* **29**, 212–228.

Ishida M., Hara M., Fukino N., Kakizaki T. & Morimitsu Y. (2014) Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. *Breeding Science* **64**, 48–59.

Jansen R.M.C., Miebach M., Kleist E., van Henten E.J. & Wildt J. (2009) Release of lipoxygenase products and monoterpenes by tomato plants as an indicator of *Botrytis cinerea*-induced stress. *Plant Biology* **11**, 859–868.

Jost R., Altschmied L., Bloem E., Bogs J., Gershenzon J., Hänel U., … Hell R. (2005) Expression profiling of metabolic genes in response to methyl jasmonate reveals regulation of genes of primary and secondary sulfur-related pathways in *Arabidopsis thaliana*. *Photosynthesis Research* **86**, 491–508.

Kadir S., Von Weihe M. & Al-Khatib K. (2007) Photochemical efficiency and recovery of photosystem II in grapes after exposure to sudden and gradual heat stress. *Journal of the American Society for Horticultural Science* **132**, 764–769.

Kännaste A., Copolovici L. & Niinemets Ü. (2014) Gas chromatography-mass spectrometry method for determination of biogenic volatile organic compounds emitted by plants. In: *Plant Isoprenoids: Methods and Protocols* (ed M. Rodríguez-Concepción), pp. 161—169. Springer Science + Business Media, New York.

Karban R. (2011) The ecology and evolution of induced resistance against herbivores. *Functional Ecology* **25**, 339–347.

Karl T., Guenther A., Turnipseed A., Patton E.G. & Jardine K. (2008) Chemical sensing of plant stress at the ecosystem scale. *Biogeosciences* **5**, 1287–1294.

Kelly P.J., Bones A. & Rossiter J.T. (1998) Sub-cellular immunolocalization of the glucosinolate sinigrin in seedlings of *Brassica juncea*. *Planta* **206**, 370–377.

Khaling E., Papazian S., Poelman E.H., Holopainen J.K., Albrectsen B.R. & Blande J.D. (2015) Ozone affects growth and development of *Pieris brassicae* on the wild host plant *Brassica nigra*. *Environmental Pollution* **199**, 119–129.

Khokon M.A.R., Jahan M.S., Rahman T., Hossain M.A., Muroyama D., Minami I., … Murata Y. (2011) Allyl isothiocyanate (AITC) induces stomatal closure in *Arabidopsis*. *Plant, Cell and Environment* **34**, 1900–1906.

Kleist E., Mentel T.F., Andres S., Bohne A., Folkers A., Kiendler-Scharr A., … Wildt J. (2012) Irreversible impacts of heat on the emissions of monoterpenes, sesquiterpenes, phenolic BVOC and green leaf volatiles from several tree species. *Biogeosciences* **9**, 5111–5123.

Kos M., Houshyani B., Achhami B.B., Wietsma R., Gols R., Weldegergis B.T., … van Loon J.J.A. (2012) Herbivore-mediated effects of glucosinolates on different natural enemies of a specialist aphid. *Journal of Chemical Ecology* **38**, 100–115.

Li J., Kristiansen K.A., Hansen B.G. & Halkier B.A. (2011) Cellular and subcellular localization of flavinmonooxygenases involved in glucosinolate biosynthesis. *Journal of Experimental Botany* **62**, 1337–1346.

Liavonchanka A. & Feussner N. (2006) Lipoxygenases: occurrence, functions and catalysis. *Journal of Plant Physiology* **163**, 348–357.

Loreto F., Barta C., Brilli F. & Nogues I. (2006) On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. *Plant, Cell and Environment* **29**, 1820–1828.

Loreto F., Förster A., Dürr M., Csiky O. & Seufert G. (1998) On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. *Plant, Cell and Environment* **21**, 101–107.

Lu C.M. & Zhang J.H. (2000) Heat-induced multiple effects on PSII in wheat plants. *Journal of Plant Physiology* **156**, 259–265.

Ludwig-Müller J., Krishna P. & Forreiter C. (2000) A glucosinolate mutant of *Arabidopsis* is thermosensitive and defective in cytosolic Hsp90 expression after heat stress. *Plant Physiology* **123**, 949–958.

Maccarrone M., Veldink G.A. & Vliegenthart J.F. (1992) Thermal injury and ozone stress affect soybean lipoxygenases expression. *FEBS Letters* **309**, 225–230.

Maffei M.E. (2010) Sites of synthesis, biochemistry and functional role of plant volatiles. *South African Journal of Botany* **76**, 612–631.

Matsui K. (2006) Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. *Current Opinion in Plant Biology* **9**, 274–280.

Matsui K., Sugimoto K., Mano J.i., Ozawa R. & Takabayashi J. (2012) Differential metabolisms of green leaf volatiles in injured and intact parts of a wounded leaf meet distinct ecophysiological requirements. *PloS One* **7**, e36433.

Misra B.B., Acharya B.R., Granot D., Assmann S.M. & Chen S. (2015) The guard cell metabolome: functions in stomatal movement and global food security. *Frontiers in Plant Science* **6**, 1–13.

Najar-Rodriguez A.J., Friedli M., Klaiber J. & Dorn S. (2015) Aphid-deprivation from *Brassica* plants results in increased isothiocyanate release and parasitoid attraction. *Chemoecology* **25**, 303–311.

Niinemets Ü. (2010a) Mild versus severe stress and BVOCs: thresholds, priming and consequences. *Trends in Plant Science* **15**, 145–153.

Niinemets Ü. (2010b) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management* **260**, 1623–1639.

Niinemets Ü., Arneth A., Kuhn U., Monson R.K., Peñuelas J. & Staudt M. (2010a) The emission factor of volatile isoprenoids: stress, acclimation, and developmental responses. *Biogeosciences* **7**, 2203–2223.

Niinemets Ü., Kuhn U., Harley P.C., Staudt M., Arneth A., Cescatti A., … Peñuelas J. (2011) Estimations of isoprenoid emission capacity from enclosure studies: measurements, data processing, quality and standardized measurement protocols. *Biogeosciences* **8**, 2209–2246.

Niinemets Ü., Monson R.K., Arneth A., Ciccioli P., Kesselmeier J., Kuhn U., … Staudt M. (2010b) The leaf-level emission factor of volatile isoprenoids: caveats, model algorithms, response shapes and scaling. *Biogeosciences* **7**, 1809–1832.

Niinemets Ü. & Reichstein M. (2002) A model analysis of the effects of nonspecific monoterpenoid storage in leaf tissues on emission kinetics and composition in Mediterranean sclerophyllous *Quercus* species. *Global Biogeochemical Cycles* **16**, 1110.

Olivier C., Vaughn S.F., Mizubuti E.S.G. & Loria R. (1999) Variation in allyl isothiocyanate production within *Brassica* species and correlation with fungicidal activity. *Journal of Chemical Ecology* **25**, 2687–2701.

Pan Y., Xu Y.-Y., Zhu X.-W., Liu Z., Gong Y.-Q., Xu L., Gong M.-Y. & Liu L.-W. (2014) Molecular characterization and expression profiles of myrosinase gene (*RsMyr2*) in radish (*Raphanus sativus* L.) *Journal of Integrative Agriculture* **13**, 1877–1888.

Pashalidou F.G., Fatouros N.E., van Loon J.J.A., Dicke M. & Gols R. (2015) Plant-mediated effects of butterfly egg deposition on subsequent caterpillar and pupal development, across different species of wild Brassicaceae. *Ecological Entomology* **40**, 440–450.

Peñuelas J. & Llusiá J. (2002) Linking photorespiration, monoterpenes and thermotolerance in *Quercus*. *The New Phytologist* **155**, 227–237.

Possell M. & Loreto F. (2013) The role of volatile organic compounds in plant resistance to abiotic stresses: responses and mechanisms. In *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* (eds Niinemets Ü. & Monson R.K.), pp. 209–235. Springer, Berlin.

Rajabi Memari H., Pazouki L. & Niinemets Ü. (2013) The biochemistry and molecular biology of volatile messengers in trees. In *Biology, Controls and Models of Tree Volatile Organic Compound Emissions* (eds Niinemets Ü. & Monson R. K.), pp. 47–93. Springer, Berlin.

Rajamurugan R., Suyavaran A., Selvaganabathy N., Ramamurthy C.H., Reddy G.P., Sujatha V. & Thirunavukkarasu C. (2012) *Brassica nigra* plays a remedy role in hepatic and renal damage. *Pharmaceutical Biology* **50**, 1488–1497.

Raybould A.F. & Moyes C.L. (2001) The ecological genetics of aliphatic glucosinolates. *Heredity* **87**, 383–391.

Redovniković I.R., Glivetić T., Delonga K. & Vorkapić-Furač J. (2008) Glucosinolates and their potential role in plant. *Periodicum Biologorum* **110**, 297–309.

Sharkey T.D. (2005) Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell and Environment* **28**, 269–277.

Shen J., Tieman D., Jones J.B., Taylor M.G., Schmelz E., Huffaker A., … Klee H. J. (2014) A 13-lipoxygenase, TomloxC, is essential for synthesis of C5 flavour volatiles in tomato. *Journal of Experimental Botany* **65**, 519–428.

Shinohara T. & Leskovar D.I. (2014) Effects of ABA, antitranspirants, heat and drought stress on plant growth, physiology and water status of artichoke transplants. *Scientia Horticulturae* **165**, 225–234.

Singsaas E.L. & Sharkey T.D. (1998) The regulation of isoprene emission responses to rapid leaf temperature fluctuations. *Plant, Cell and Environment* **21**, 1181–1188.

Sønderby I.E., Geu-Flores F. & Halkier B.A. (2010) Biosynthesis of glucosinolates-gene discovery and beyond. *Trends in Plant Scince* **15**, 283–290.

Staudt M. & Bertin N. (1998) Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (*Quercus ilex* L.) leaves. *Plant, Cell and Environment* **21**, 385–395.

Sun Z., Copolovici L. & Niinemets Ü. (2012) Can the capacity for isoprene emission acclimate to environmental modifications during autumn senescence in temperate deciduous tree species *Populus tremula*? *Journal of Plant Research* **125**, 263–274.

Sung D.Y., Kaplan F., Lee K.J. & Guy C.L. (2003) Acquired tolerance to temperature extremes. *Trends in Plant Science* **8**, 179–187.

Taveira M., Fernandes F., de Pinho P.G., Andrade P.B., Pereira J.A. & Valentão P. (2009) Evolution of *Brassica rapa* var. *rapa* L. volatile composition by HS-SPME and GC/IT-MS. *Microchemical Journal* **93**, 140–146.

Tholl D. (2006) Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology* **9**, 297–304.

Tieman D.M., Zeigler M., Schmelz E.A., Taylor M.G., Bliss P., Kirst M. & Klee H.J. (2006) Identification of loci affecting flavour volatile emissions in tomato fruits. *Journal of Experimental Botany* **57**, 887–896.

Truong D.-H., Delory B.M., Brostaux Y., Heuskin S., Delaplace P., Francis F. & Lognay G. (2014) *Plutella xylostella* (L.) infestations at varying temperatures

induce the emission of specific volatile blends by *Arabidopsis thaliana* (L.) Heynh. *Plant Signaling & Behavior* **9**, 1–11.

Tulio A.Z., Yamanaka H., Ueda Y. & Imahori Y. (2002) Formation of methanethiol and dimethyl disulfide in crushed tissues of broccoli florets and their inhibition by freeze-thawing. *Journal of Agricultural and Food Chemistry* **50**, 1502–1507.

Turk M.A. & Tawaha A.M. (2003) Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.) *Crop Protection* **22**, 673–677.

Usano-Alemany J., Palá-Paúl J. & Herráiz-Peñalver D. (2014) Temperature stress causes different profiles of volatile compounds in two chemotypes of *Salvia lavandulifolia* Vahl. *Biochemical Systematics and Ecology* **54**, 166–171.

Vacca R.A., de Pinto M.C., Valenti D., Passarella S., Marra E. & De Gara L. (2004) Production of reactive oxygen species, alteration of cytosolic ascorbate peroxidase, and impairment of mitochondrial metabolism are early events in heat shock-induced programmed cell death in tobacco bright-yellow 2 cells. *Plant Physiology* **134**, 1100–1112.

van Dam N.M., Samudrala D., Harren F.J.M. & Cristescu S.M. (2012) Real-time analysis of sulfur-containing volatiles in *Brassica* plants infested with root-feeding *Delia radicum* larvae using proton-transfer reaction mass spectrometry. *AoB* 1–12.

Velikova V., Tsonev T., Barta C., Centritto M., Koleva D., Stefanova M., Busheva M. & Loreto F. (2009) BVOC emissions, photosynthetic characteristics and changes in chloroplast ultrastructure of *Platanus orientalis* L. exposed to elevated CO_2 and high temperature. *Environmental Pollution* **157**, 2629–2637.

von Caemmerer S. & Farquhar G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387.

Wahid A., Gelani S., Ashraf M. & Foolad M.R. (2007) Heat tolerance in plants: an overview. *Environmental and Experimental Botany* **61**, 199–223.

Wang C.L., Xing J.S., Chin C.K., Ho C.T. & Martin C.E. (2001) Modification of fatty acids changes the flavor volatiles in tomato leaves. *Phytochemistry* **58**, 227–232.

Wang H., Wu J., Sun S., Liu B., Cheng F., Sun R. & Wang X. (2011) Glucosinolate biosynthetic genes in *Brassica rapa*. *Gene* **487**, 135–142.

Way D.A., Schnitzler J.-P., Monson R.K. & Jackson R.B. (2011) Enhanced isoprene-related tolerance of heat- and light-stressed photosynthesis at low, but not high, CO_2 concentrations. *Oecologia* **166**, 273–282.

Wildt J., Kobel K., Schuh-Thomas G. & Heiden A.C. (2003) Emissions of oxygenated volatile organic compounds from plants – part II: Emissions of saturated aldehydes. *Journal of Atmospheric Chemistry* **45**, 173–196.

Winde I. & Wittstock U. (2011) Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. *Phytochemistry* **72**, 1566–1575.

Wittstock U. & Burow M. (2010) Glucosinolate breakdown in *Arabidopsis*: mechanism, regulation and biological significance. *The Arabidopsis Book* **8**, 1–14.

Wold S., Esbensen K. & Geladi P. (1987) Principal component analysis. *Chemometrics and Intelligent Laboratory Systems* **2**, 37–52.

Zhang R., Cruz J.A., Kramer D.M., Magallanes-Lundback M.E., DellaPenna D. & Sharkey T.D. (2009) Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves. *Plant, Cell and Environment* **32**, 1538–1547.

Zhang R. & Sharkey T.D. (2009) Photosynthetic electron transport and proton flux under moderate heat stress. *Photosynthesis Research* **100**, 29–43.

Zhao N., Guan J., Ferrer J.-L., Engle N., Chern M., Ronald P., Tschaplinski T.J. & Chen F. (2010) Biosynthesis and emission of insect-induced methyl salicylate and methyl benzoate from rice. *Plant Physiology and Biochemistry* **48**, 279–289.

Zhao Z., Zhang W., Stanley B.A. & Assmann S.M. (2008) Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. *Plant Cell* **20**, 3210–3226.

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RESEARCH PAPER

Lethal heat stress-dependent volatile emissions from tobacco leaves: what happens beyond the thermal edge?

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Abstract

Natural vegetation is predicted to suffer from extreme heat events as a result of global warming. In this study, we focused on the immediate response to heat stress. Photosynthesis and volatile emissions were measured in the leaves of tobacco (*Nicotiana tabacum* cv. Wisconsin 38) after exposure to heat shock treatments between 46 °C and 55 °C. Exposure to 46 °C decreased photosynthetic carbon assimilation rates (*A*) by >3-fold. Complete inhibition of *A* was observed at 49 °C, together with a simultaneous decrease in the maximum quantum efficiency of PSII, measured as the F_v/F_m ratio. A large increase in volatile emissions was observed at 52 °C. Heat stress resulted in only minor effects on the emission of monoterpenes, but volatiles associated with membrane damage such as propanal and (*E*)-2-hexenal+(*Z*)-3-hexenol were greatly increased. Heat induced changes in the levels of methanol and 2-ethylfuran that are indicative of modification of cell walls. In addition, the oxidation of metabolites in the volatile profiles was strongly enhanced, suggesting the acceleration of oxidative processes at high temperatures that are beyond the thermal tolerance limit.

Keywords: Chlorophyll fluorescence, heat stress, isoprene, lipoxygenase pathway, methacrolein, methanol, monoterpenes, photosynthesis, stress severity.

Introduction

Biogenic volatile organic compounds (BVOCs) are messengers in plant to plant, plant to herbivore, plant to pollinator, and plant to seed disperser communication (Arimura *et al.*, 2005; Gershenzon and Dudareva, 2007; Dicke and Baldwin, 2010; Vickers *et al.*, 2014; Vucetic *et al.*, 2014; Cozzolino *et al.*, 2015; Mishyna *et al.*, 2015). Additionally, BVOCs participate in atmospheric processes by contributing to the formation of tropospheric ozone, cloud condensation nuclei, and secondary aerosols (Guenther, 2000; Huff Hartz *et al.*, 2005; Librando and Tringali, 2005; Fuentes *et al.*, 2007; Dicke and Loreto, 2010; Kulmala *et al.*, 2013).

The emission of BVOCs is either constitutive or induced by different stress factors (Niinemets, 2010; Niinemets *et al.*, 2013; Loreto *et al.*, 2014; Copolovici and Niinemets, 2016). Wild tobacco plants are non-isoprene emitters, but, for example, in non-stressed conditions several other plant species constitutively release isoprene and terpenoids (Guenther *et al.*, 1994; Kesselmeier and Staudt, 1999; Vickers *et al.*, 2009; Fineschi *et al.*, 2013; Pollastri *et al.*, 2014). While only a few species are strong constitutive emitters, induced volatile emissions caused by herbivores, pathogens, drought, low or high temperature, salinity, ozone, and high light have been demonstrated in both

strong and low constitutive emitters (Loreto and Delfine, 2000; Beauchamp *et al.*, 2005; Loreto *et al.*, 2006; Hartikainen *et al.*, 2009; Lavoir *et al.*, 2009; Dicke and Baldwin, 2010; Niinemets, 2010; Copolovici *et al.*, 2011, 2012; Staudt and Lhoutellier, 2011; Jiang *et al.*, 2016, 2018; Kask *et al.*, 2016; Kanagendran *et al.*, 2018*a*, *b*).

Among different abiotic factors, high temperature is one of the most frequent and harmful stressors (Atkin *et al.*, 2015; Heyduk *et al.*, 2016; O'Sullivan *et al.*, 2017; Niinemets, 2018; Zhu *et al.*, 2018) and, due to global warming, its impact on vegetation is expected to progressively increase worldwide (IPCC, 2013). In a natural setting, heat stress often occurs during heatwaves. In addition, sudden rises in leaf temperature to >50 °C can occur during lightflecks when shaded leaves are exposed to a bright light penetrating the canopy gaps (Singsaas *et al.*, 1999; Singsaas and Sharkey, 2000; Valladares and Niinemets, 2007).

Plant physiological and molecular responses to heat stress depend on the severity of heat stress (Singsaas *et al.*, 1999; Niinemets, 2010; Atkin *et al.*, 2015; Ruiz-Vera *et al.*, 2015; O'Sullivan *et al.*, 2017; Niinemets, 2018; Zhu *et al.*, 2018). For example, mild heat stress inhibits PSII activity, but, upon return to a lower temperature, mild stress-driven changes are typically fully reversible (Lu and Zhang, 2000; Zhang and Sharkey, 2009; Zhang *et al.*, 2009; Hüve *et al.*, 2011). Further increases in temperature lead to damaged PSII, membrane leakiness, production of various reactive oxygen species (ROS), and propagation of damage, ultimately leading to massive cellular death (Hüve *et al.*, 2011; Grover *et al.*, 2013; Pospíšil and Prasad, 2014; Kask *et al.*, 2016; O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018). Plants cope with heat stress by up-regulating heat shock proteins and by scavenging ROS via enzymatic and/or non-enzymatic reactions involving antioxidative molecules (Vacca *et al.*, 2004; Hasanuzzaman *et al.*, 2013; Qu *et al.*, 2013; Pospíšil and Prasad, 2014), but, once the heat stress threshold is exceeded, ROS accumulation progressively increases and an apoptosis-like process is initiated (Bernardi *et al.*, 1999; Jones, 2000; Dutilleul *et al.*, 2003; Wang *et al.*, 2015), which in turn leads to the time-dependent reductions in the rate of photosynthesis even after return to the lower temperature (Hüve *et al.*, 2011).

Characteristic plant stress volatiles released rapidly upon different stresses are short-chained oxygenated compounds such as methanol, acetone, acetaldehyde, and various lipoxygenase (LOX) pathway-derived compounds also known as green leaf volatiles (GLVs) (Heiden *et al.*, 2003; Loreto *et al.*, 2006). Many of these volatiles are signal molecules, which activate regulatory genes involved in plant defense, stress tolerance, and apoptosis (Kost and Heil, 2006; Scala *et al.*, 2013; Ameye *et al.*, 2018). Stress-dependent release of methanol occurs due to modifications in cell wall pectins (Körner *et al.*, 2009; Li *et al.*, 2017). LOX pathway volatiles are synthesized from linolenic and linoleic acids in a cascade of reactions (Baur and Yang, 1969; Gigot *et al.*, 2010). Their synthesis is initiated by cell membrane breakdown followed by the release of free linoleic and linolenic acids and further sequential action of LOXs, hydroperoxide lyases, alcohol dehydrogenases, and acetyltransferases (ul Hassan *et al.*, 2015). Due to the constitutive activity of these enzymes, the emissions of LOX pathway-derived volatiles occur rapidly as soon as their substrates become available (Feussner and Wasternack, 2002; Liavonchanka and Feussner, 2006; Rasulov *et al.* 2019). Next to GLVs, plant stress may trigger or increase the emission of terpenoids (McConkey *et al.*, 2000; Dudareva *et al.*, 2006; Niinemets *et al.*, 2013; Kanagendran *et al.*, 2018*b*), but, because their release requires expression of corresponding synthases, the stress emissions typically occur with a time delay (Pazouki *et al.*, 2016). Heat stress alters the emission of monoterpenes, and these emissions might increase or decrease depending on the duration and severity of stress and on whether terpenes are released from storage structures or whether they are *de novo* synthesized (Loreto *et al.*, 1996, 1998; Copolovici *et al.*, 2012; Kleist *et al.*, 2012).

The amount of different volatiles scales positively with the severity of heat stress (Copolovici *et al.*, 2012; Kask *et al.*, 2016). However, in previous studies, signaling and antioxidative controls on the heat stress response of transgenic tobacco (*Nicotiana tabacum*) have been studied only at temperatures below 50 °C (Wang *et al.*, 2017; Xu *et al.*, 2014; Liu *et al.*, 2017). Yet, mitochondrial processes are functional even at higher temperatures (Hüve *et al.*, 2011, 2012; O'Sullivan *et al.*, 2013, 2017), and such high temperatures do occur in nature. Thus, studies of higher temperatures are relevant, especially considering the vegetation responses to future heat waves that probably increase the BVOC emission from vegetation (Farré-Armengol *et al.*, 2014; Jardine *et al.*, 2015). Leaf temperature can be lower than the air temperature in windy conditions in actively transpiring plants, especially in small-leaved species, but leaf temperature can also be higher than air temperature in calm air and/or in water-stressed plants, especially in large-leaved species such as tobacco (Crawford *et al.*, 2012; Yu *et al.*, 2015; Urban *et al.*, 2017; Wright *et al.* 2017; Blonder and Michaletz, 2018). Thus, we have used a water-bath methodology to apply highly controlled heat shock treatments to the leaves (Frolec *et al.*, 2008; Copolovici *et al.*, 2012; Kask *et al.*, 2016; Pazouki *et al.*, 2016).

The goal of the present study was to evaluate the effects of heat shock varying from moderate (46 °C) to extremely severe (55 °C) temperature. We hypothesized that once the critical heat stress threshold is reached, foliage photosynthetic characteristics are impaired, while key stress volatile emissions increase even above the temperatures that lead to the collapse of the photosynthetic machinery. Additionally we show that upon the onset of an apoptosis-like process, plant leaves release a very high and surprisingly diverse blend of volatiles and that the release of stress volatiles also has a certain temperature optimum.

Materials and methods

Plant growth conditions and experimental material

Tobacco (*N. tabacum* cv. Wisconsin 38) seeds were germinated in Kekkilä garden soil (Kekkilä Group, Vantaa, Finland). After germination, individual seedlings were replanted in 4 liter plastic pots and cultivated in the same substrate in a plant growth room at day/night temperatures of 27/23 °C and at a relative humidity of 60%. The day length was 14 h and illumination of 400–500 μmol m^{-2} s^{-1} at the plant level was achieved by Philips HPI-T Plus 400 W metal halide lamps (Eindhoven, The Netherlands). Plants were watered daily and fertilized with 0.5% fertilizer solution [Baltic Agro, Lithuania; NPK content ratio, 5:5:6; and

micronutrients, B (0.01%), Cu (0.03%), Fe (0.06%), Mn (0.028%), and Zn (0.007%)] on a weekly basis.

In the experiments, fully mature non-senescent leaves of 9- to 10-week-old and 40–60 cm tall plants were used. All measurements were conducted with attached leaves. Due to short and winged leaf petioles of tobacco leaves, it was impossible to tighten the gas exchange chamber at the leaf to petiole junction (discussed below). Hence, 2–3 d prior to the stress treatments, part of the axial leaf tissue was removed so that the remaining portion of the leaf of 25–40 cm^2 could be air-tightly sealed in the gas exchange chamber (Fig. 1). The integrity of major veins was preserved such that the photosynthetic activity of the remaining portion of the leaf was not significantly different from that of the intact leaf. Leaf wounding elicited a significant release of LOX pathway-derived volatiles, which was short-lived and lasted <30 min (Brilli *et al.*, 2011, 2012; Portillo-Estrada *et al.*, 2015; Rasulov *et al.*, 2019). At the time of the heat stress treatments, no LOX pathway-derived or other stress volatiles were observed (Kanagendran *et al.*, 2018*a*; Li *et al.*, 2018) and there were also no quantitative or qualitative differences in the bouquets of base-level volatile emissions among mechanically wounded and intact leaves (data not shown).

Heat stress application

Heat shock was applied as characterized in earlier papers (Frolec *et al.*, 2008; Copolovici *et al.*, 2012). Briefly, a temperature-controlled water bath (MB-5, Julabo GmbH, Germany) was heated to the desired temperature and the selected leaf was immersed in water for 10 min (see Fig. 1 for the experimental set-up); longer than 10 min would have caused lethal injuries to the leaf and thus the emissions would have been dominated by unsaturated C6-aldehydes, alcohols, and esters. Then, during 30 s, the excess water was gently removed with a soft tissue paper and the leaf was enclosed in the gas exchange system for the measurements of photosynthetic characteristics and BVOCs. Each experimental plant was measured twice. At first, the photosynthetic characteristics and BVOCs of controls leaves were evaluated (immersed in 25 °C water), and then the same procedure was repeated at 46, 47, 48, 49, 50, 51, 52, 53, 54, and 55 °C. For every different heat shock temperature, separate leaves were used (Fig. 1).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were conducted before heat stress and 30 min after the given heat treatment using a Maxi-Imaging-PAM-fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Before the measurements, the attached leaves were dark-adapted for 15 min. First, the minimum Chl *a* fluorescence (F_0) was measured, and then a saturating pulse of blue light of 2800 µmol m^{-2} s^{-1} for 0.8 s was given to measure the maximum fluorescence yield (F_m). The maximum quantum yield of PSII in the dark-adapted state was calculated as F_v/F_m, where F_v is equal to F_m–F_0 (Schreiber, 2004).

In our study, F_v/F_m of the control leaves was on average (±SE) 0.749±0.005 (~88% of theoretical maximum), which was somewhat lower than the theoretical maximum. The lower F_v/F_m reflects the circumstance that the maximum flash intensity of the imaging PAM was not fully saturating to yield the true F_m value, and thus the maximum F_v/F_m value was moderately underestimated (5–15%) (Masclaux-Daubresse *et al.*, 2007; Ehlert and Hincha, 2008; Woo *et al.*, 2008). Despite some underestimation, stress-dependent changes in F_v/F_m constitute an accurate measure of stress effects on leaf photochemistry and overall stress status (Ehlert and Hincha, 2008; Woo *et al.*, 2008).

Gas exchange measurements

The treated leaves were enclosed in a custom-made gas exchange system (Copolovici and Niinemets, 2010; Kask *et al.*, 2016) with a modified leaf chamber. The leaf chamber consisted of a stainless steel cylindrical bottom part (2.5 cm height) and a double-layered glass upper part (50 cm^2 window area). The gas flow rate through the system was 1.26 l min^{-1} and, given the chamber volume of 0.22 liter, the resulting chamber response half-time was 7.3 s (calculated for first-order decay kinetics according to Niinemets, 2012). The chamber temperature was controlled by water that circulated between the double glass layers of the chamber window. Thus, the temperature difference between the leaf surface and the temperature-controlled chamber was <0.5 °C. The leaf temperature was measured with a thermocouple attached to the lower leaf surface. Four halogen lamps (50 W, Philips) provided a light intensity of 500 µmol m^{-2} s^{-1} at the leaf surface. Ambient air drawn from outside by a pump passed through a 10 liter buffer volume, a charcoal filter, and a custom-made humidifier. CO_2 concentration was 380–400 µmol mol^{-1} and humidity was ~60%. CO_2 and H_2O concentrations at the inlet and outlet of the chamber were measured with a LI-7000 infra-red CO_2/H_2O analyzer (Li-Cor, Inc., Lincoln, NE, USA). The analyzer was operated in the absolute mode and the readings were taken relative to the reference cell that was flushed with pure N_2. The measurement cell of the analyzer was switched between the chamber inlet (ingoing air) and outlet (outgoing air) with electronic valves.

Non-treated leaves were stabilized under standard measurement conditions for 10–20 min. When stomata fully opened and gas exchange rates stabilized, the gas exchange rates were recorded. In the case of heat stress treatments and control leaves immersed in 25 °C water, the measurements were always recorded 30 min after the stress application. The net assimilation rate (A), stomatal conductance to water vapor (g_s), and CO_2 concentration in substomatal cavities (C_i) were calculated according to von Caemmerer and Farquhar (1981).

Volatile sampling and GC-MS analyses

Collection of volatiles was performed via the gas exchange cuvette outlet with a flow rate of 200 ml min^{-1} for 15 min after heat treatments.

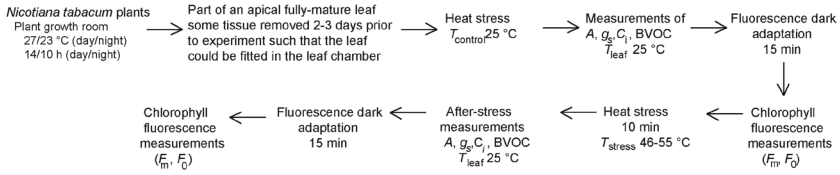

Fig. 1. Description of the workflow for tobacco (*Nicotiana tabacum* cv. Wisconsin 38) heat stress experiment. Two to three days prior to the experimental treatment, a part of the leaf tissue was removed such that the leaf could be fitted in the gas exchange system. Heat treatments were applied for 10 min by immersion of leaves in water at a given temperature (T_{stress}; control leaves were immersed in water at 25 °C and heat stressed leaves in water at temperatures between 46 °C and 55 °C). After treatments, the physiological leaf characteristics were measured at 25 °C (T_{leaf}=25 °C). The treated leaf was inserted in the cuvette of the gas exchange system, and foliage photosynthetic characteristics were measured (net assimilation rate, stomatal conductance to water vapor, and intercellular CO_2) and volatiles (BVOCs) were collected. After these measurements, the leaf was dark-adapted for 15 min for chlorophyll fluorescence measurements. Each individual leaf was treated only once with one of the heat shock temperatures.

A constant flow air sample pump (1003-SKC, SKC Inc., Houston, TX, USA) was used to draw the cuvette air through the multi-bed stainless steel cartridges, which were filled with Carbotrap C (20–40 mesh), Carbopack B (40–60 mesh), and Carbotrap X (20–40 mesh) adsorbents (Supelco, Sigma-Aldrich) (see Kännaste *et al.*, 2014 for details). A blank (background) sample was taken from the empty cuvette prior to the measurement of each control leaf.

A Shimadzu 2010 Plus GC-MS system with a Shimadzu TD20 automated cartridge desorber (Shimadzu Corporation, Kyoto, Japan) was used for the desorption and quantification of volatiles (Kännaste *et al.*, 2014). The system was calibrated (Kask *et al.*, 2016) with commercially available high-purity standards of LOX pathway products and terpenoids (Sigma-Aldrich). Volatiles were identified by comparing their retention times and mass spectra with the spectra of authentic standards and with the spectra in the NIST database (NIST05). The emission rates of volatiles were calculated according to Niinemets *et al.* (2011). In the current study, we focused mainly on the emissions of individual LOX pathway-derived volatiles (2,4-hexadienal, hexanal, hexane, 1-hexanol, (*E*)-hexenal+(*Z*)-3-hexenol, pentane, 1-penten-3-one, and propanal) and some 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway (MEP/DOXP pathway) volatiles. Additionally, we studied emissions of acetaldehyde, acetone, ethanol, 2-ethylfuran, 2-methyl-2-cyclopenten-1-one, and methanol (Table 1). The detection limit of volatiles was better than 0.2 pmol m^{-2} s^{-1}. Emission rates of sesquiterpenes, which are elicited several hours after the stress (Beauchamp *et al.*, 2005; Pazouki *et al.*, 2016; Kanagendran *et al.*, 2018*a*, b), remained below the detection limit in these experiments.

Methanol emission measurements

As methanol cannot be quantitatively trapped with the GC cartridges used, its emission was monitored with a proton transfer reaction mass spectrometer equipped with a quadrupole detector (high sensitivity version PTR-QMS, Ionicon GmbH, Innsbruck, Austria) by connecting the inlet of the PTR-QMS to the gas exchange system. The flow drawn by PTR-QMS from the gas exchange system was 10 ml min^{-1}. Methanol was detected as the protonated parent ion at an m/z of 33 (Copolovici and Niinemets, 2010). The PTR-QMS system was calibrated with a standard certified gas mixture (Ionimed Analytik, Innsbruck, Austria). The methanol emission rate was calculated from the difference between the measurements with a leaf and empty chamber according to Niinemets *et al.* (2011). Ultimately, the average methanol emission for 15 min after heat stress was calculated.

Statistical analyses

Treatments of 46, 47, 49, and 53 °C were repeated at least three times, and treatments of 48, 50–52, 54, and 55 °C were repeated four times. Hence, together with the blank and control measurements, 70 analyses were done. Foliage photosynthetic characteristics and emission rates of volatiles were expressed as average ±SE. Log-transformed data were used to study the impact of temperature on foliage physiological characteristics by ANOVA followed by a Tukey test [IBM SPSS Statistics 22 (IBM Corp, Armonk, NY)]. Additionally, the relationships between the volatiles, or photosynthetic characteristics and volatiles were evaluated by Pearson correlation (Kännaste *et al.*, 2014; Kask *et al.*, 2016) and non-linear regression analyses (SigmaPlot ver. 12.5, Systat Software, Inc., San Jose CA, USA).

Results

Heat effects on Chl a fluorescence

In the temperature range of 46–48 °C, the maximum dark-adapted quantum efficiency of PSII (F_v/F_m) diminished initially relatively slowly, but at temperatures of 48–50 °C, it decreased rapidly to a value of 0.198±0.019 (average ±SE; i.e. by >70%) compared with the control leaves (Fig. 2A). With further increases in heat stress severity, F_v/F_m gradually declined, reaching at 55 °C a value of ~3% of that in control leaves (Fig. 2A).

Given that the minimum Chl *a* fluorescence F_0 did not change much until 49 °C, the first initial gradual change of F_v/F_m between 46 °C and 48 °C reflected primarily the reduction of F_m (e.g. a 43% reduction from 0.661±0.031 in the control to 0.379±0.014 in leaves exposed to 46 °C; Fig. 2A). At 49 °C, F_0 started to increase (Fig. 2A) and in all data, there was a negative correlation between F_v/F_m and F_0 (Fig. 2B).

Changes in photosynthetic rate and stomatal conductance upon heat exposure

Moderate heat stress at 46 °C, which had a relatively minor effect on photochemistry (Fig. 2A), already reduced the net assimilation rate (A) by almost 80% from 12.5±0.7 μmol m^{-2} s^{-1} (control treatment) to 2.60±0.33 μmol m^{-2} s^{-1}. A further declined with increasing heat stress severity, reaching close to zero values after 48 °C treatment and negative values at higher treatment temperatures (Fig. 3A). In contrast to A, stomatal conductance to water vapor (g_s) also decreased in response to heat stress of 50 °C, but at higher temperatures g_s gradually increased (Fig. 3B). Although g_s initially decreased together with A, the intercellular CO_2 concentration (C_i) actually slightly increased in high temperature-treated leaves (Fig. 3C) and the C_i to ambient CO_2 concentration (C_a) ratio (C_i/C_a) behaved analogously (data not shown).

Emission of volatiles

The emissions of hexane and pentane, acetaldehyde, acetone, ethanol, and terpenoids were unaffected by the heat stress (Table 1), but 2-ethylfuran, 2,4-hexadienal, hexanal, 1-hexanol, (*E*)-2-hexenal+(*Z*)-3-hexenol, methacrolein, 2-methyl-2-cyclopenten-1-one, and propanal increased with increasing treatment temperature after the heat stress threshold was exceeded (Table 1). Particularly massive emissions were observed at temperatures of 52–54 °C, but at 55 °C the emissions declined again (Table 1).

In control plants and in plants exposed to 46–51 °C, the total emission of LOX pathway-derived volatiles was low (Fig. 4A). Starting from 52 °C, LOX pathway-derived volatiles began to increase and at 54 °C the total emission attained an average value of 14±5 nmol m^{-2} s^{-1}. After reaching the maximum, the LOX emission declined to 2.8±1.6 nmol m^{-2} s^{-1} at 55 °C (Fig. 4A). Heat stress also enhanced the emission of methanol that increased from 2.9±0.6 nmol m^{-2} s^{-1} in control treatments to 270±50 nmol m^{-2} s^{-1} in plants exposed to 54 °C (Fig. 4B). In contrast to LOX pathway-derived volatile emissions (Fig. 4A), the elicited methanol emission stayed at a high level even at the hottest temperature of 55 °C (Fig. 4B).

Tobacco plants also released the hemiterpene isoprene and monoterpenes α-pinene, β-pinene, (*Z*)-β-ocimene, and limonene (Table 1). In control and heat-stressed plants, the emission rates of individual volatile isoprenoids remained low (Table 1). Yet, the total emission of monoterpenes correlated positively with the treatment temperature through 46 °C to 55 °C (r=0.41, P<0.05, data not shown).

Table 1. *Average (±SE) emission rates (pmol m^{-2} s^{-1}) of detected volatiles in heat-stressed* Nicotiana tabacum *after exposure to different heat shock treatments*

No	Compounds	Treatment temperature (°C)										
		25	46	47	48	49	50	51	52	53	54	55
	Lipoxygenase pathway volatiles											
1	2,4-Hexadienal	1.74 (0.25) **a***	0.85 (0.30) **a**	7.1 (3.8) **ab**	1.85 (0.68) **a**	0.59 (0.43) **a**	1.16 (0.57) **a**	2.5 (1.9) **a**	13 (9) **ab**	130 (70) **bc**	220 (90) **b**	24 (22) **ac**
2	Hexanal	63 (13) **a**	40 (20) **a**	138 (58) **abc**	58 (19) **a**	28 (17) **a**	78 (33) **ab**	110 (33) **ab**	230 (130) **abc**	1260 (550) **bc**	2170 (840) **c**	310 (200) **abc**
3	Hexane	440 (110) a	480 (360) a	1600 (1400) a	460 (200) a	110 (65) a	190 (130) a	260 (110) a	540 (220) a	620 (310) a	350 (150) a	400 (120) a
4	1-Hexanol	1.03 (0.19) **a**	1.05 (0.46) **a**	2.4 (1.6) **ab**	1.56 (0.77) **ab**	2.2 (1.8) **ab**	3.0 (2.5) **ab**	4.3 (1.5) **abc**	9 (6) **abc**	20 (6) **bc**	54 (21) **c**	5.7 (3.8) **ab**
5	(*E*)-2-Hexenal +(*Z*)-3-hexenol	5.68 (1.79) **a**	1.75 (0.69) **ab**	5.0 (3.6) **ab**	3.9 (1.8) **ab**	0.434 (0.091) **a**	15 (15) **ab**	14 (7) **ab**	80 (61) **abc**	600 (530) **bc**	1980 (1270) **c**	33 (22) **ab**
6	Pentane	70 (15) a	54 (18) a	54.0 (2.1) a	87 (45) a	33 (12) a	37 (6) a	43 (12) a	165 (103) a	130 (30) a	100 (15) a	106 (19) a
7	1-Penten-3-one	9.0 (1.8) **a**	3.3 (2.6) **a**	4.1 (1.8) **ab**	9 (8) **ac**	5.4 (2.9) **ab**	6.0 (4.1) **ab**	3.34 (0.80) **ab**	5.1 (2.7) **ab**	180 (68) **b**	130 (41) **bc**	66 (39) **ab**
8	Propanal	72 (7) **a**	54 (7) **a**	103 (42) **a**	96 (25) **a**	97 (53) **a**	107 (35) **a**	88 (22) **a**	200 (100) **a**	2830 (1180) **b**	3110 (1450) **b**	720 (480) **ab**
	MEP/DOXP pathway volatiles											
9	Isoprene	55 (12) a	50 (18) a	47 (12) a	59 (24) a	26 (13) a	31 (13) a	36 (21) a	88 (43) a	100 (55) a	65 (25) a	57 (17) a
10	Limonene	87 (20) a	35 (23) a	17.2 (4.6) a	47 (33) a	60 (49) a	17.2 (4.5) a	23 (11) a	73 (41) a	140 (65) a	47 (13) a	117 (70) a
11	Methacrolein	41 (5) **a**	27 (6) **ab**	65 (38) **ab**	33 (15) **ab**	43 (22) **ab**	33 (9) **ab**	24 (5) **ab**	43 (15) **ab**	240 (100) **b**	140 (80) **ab**	93 (64) **ab**
12	(*Z*)-β-Ocimene	24.9 (3.0) a	18 (9) a	13.7 (4.4) a	22 (7) a	11 (5) a	26 (14) a	26 (9) a	29 (10) a	27 (11) a	23 (7) a	23 (5) a
13	α-Pinene	29.8 (3.6) a	21 (9) a	22 (5) a	58 (30) a	29 (18) a	34 (12) a	25 (6) a	36 (14) a	29 (9) a	26.7 (3.8) a	28.1 (3.7) a
14	β-Pinene	6.5 (2.7) a	2.1 (1.2) a	9 (5) a	1.7 (0.9) a	4.8 (4.3) a	1.49 (0.64) a	2.6 (0.6) a	9 (8) a	4.6 (1.3) a	3.07 (0.73) a	5.3 (3.7) a
	Volatiles of other pathways											
15	Acetaldehyde	1030 (260) a	1380 (1190) a	660 (340) a	860 (670) a	1020 (890) a	460 (155) a	1760 (1310) a	1880 (1110) a	3870 (1795) a	3080 (630) a	1930 (1310) a
16	Acetone	630 (120) a	310 (160) a	1050 (445) a	500 (260) a	470 (315) a	810 (360) a	400 (80) a	1050 (480) a	845 (200) a	350 (50) a	370 (200) a
17	Ethanol	1530 (540) a	970 (960) a	1620 (1610) a	1260 (1260) a	1600 (1600) a	230 (220) a	580 (520) a	1600 (1580) a	2880 (2770) a	760 (520) a	2340 (2330) a
18	2-Ethylfuran	14.8 (2.3) **a**	8.1 (2.7) **a**	8.5 (2.1) **a**	10.6 (1.8) **a**	10.8 (1.9) **a**	14 (9) **a**	45 (36) **ac**	57 (22) **ac**	2620 (1260) **b**	1840 (550) **b**	700 (440) **bc**
19	2-Methyl-2-cyclopenten-1-one	4.65 (0.74) **a**	5.6 (2.4) **ab**	5.3 (3.2) **ab**	18.1 (2.7) **ab**	6.3 (4.4) **ab**	26 (22) **ab**	100 (80) **ab**	130 (100) **bc**	2030 (880) **cd**	4430 (1450) **d**	440 (390) **bc**

Leaves were exposed to each different treatment temperature for 10 min, transferred to 25 °C, and, at this temperature, volatiles were collected on adsorbent cartridges for 15 min after the treatment and analyzed by GC-MS (Fig. 1). Independent leaves were used for every temperature.
[a] Statistical difference between the log-transformed emissions of volatiles was tested with one-way ANOVA followed by Tukey test. Letters in bold indicate statistically significant differences at a *P*-value of 0.05.

Relationships of key BVOCs with photosynthetic characteristics and among different BVOCs

Relationships between all the volatiles derived via the LOX pathway, photosynthetic characteristics (A and F_v/F_m) and methanol were evaluated via regression analysis. In the heat stress treatments, the total LOX pathway-derived volatile emission rate showed a negative threshold-type non-linear relationship with F_v/F_m ($P<0.0001$) (Fig. 5A) and A ($P<0.002$) (Fig. 5B). A non-linear positive relationship was observed ($P<0.0001$) between the total LOX pathway volatiles and methanol emission rates (Fig. 5C).

In control and heat-stressed plants, the isoprene emission rate correlated positively with the emission rate of pentane ($r=0.67$, $P<0.001$ for control plants and $r=0.59$, $P<0.01$ for stressed plants; data not shown). In addition, the emission rates of methacrolein and isoprene (Fig. 6), and methacrolein and 2-ethylfuran were positively correlated, especially at 50–55 °C (Fig. 7A, B). Finally, significant positive relationships existed

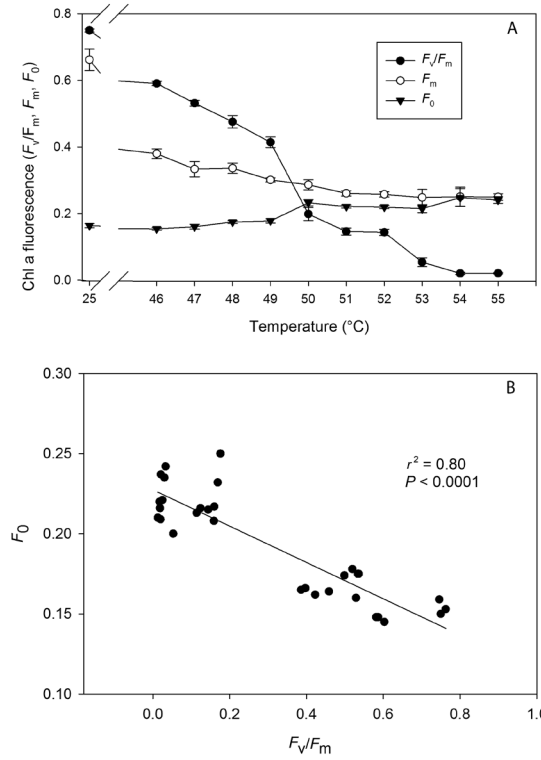

Fig. 2. Minimum (F_0) and maximum (F_m) dark-adapted Chl *a* fluorescence yield and maximum dark-adapted quantum yield of PSII (F_v/F_m; A), and correlation between F_0 and F_v/F_m (B) in control (25 °C) and heat-stressed tobacco (*Nicotiana tabacum* cv. Wisconsin 38) leaves (10 different heat stress treatments). In (A), data correspond to averages ±SE of 3–4 replicates, while values for individual leaves fitted by linear regression are plotted in (B). The heat stress treatment lasted for 10 min, and gas exchange measurements and volatile collection took 15 min (Fig. 1). Thus, chlorophyll fluorescence measurements were conducted in ~30 min after the heat treatment at the control conditions (25 °C). Different leaves were used for each heat treatment.

between two other LOX pathway-derived volatiles, pentane and propanal (r>0.48 and P<0.01 for controls or plants stressed at 50–55 °C; data not shown). In most cases, the strength of these correlations and slopes were different for different treatment temperature ranges (Figs 6, 7).

Discussion

Modifications of foliage photosynthetic characteristics due to moderate to extreme heat stress

Heat stress reduces leaf photosynthetic activity by inhibiting multiple processes and damaging key components of the photosynthetic machinery (Law and Crafts-Brandner, 1999; Tóth *et al.*, 2005; Murata *et al.*, 2007; Ashraf and Harris, 2013; Yan *et al.*, 2013). Moderate heat stress inactivates PSII, ATP synthase, Rubisco, and Rubisco synthase activities, and typically also causes stomatal closure such that reduction in the rate of photosynthesis is due to both non-stomatal and stomatal factors, yet, upon return to lower temperatures, this rate characteristically recovers (Law and Crafts-Brandner, 1999; Tóth *et al.*, 2005; Murata *et al.*, 2007; Hüve *et al.*, 2011; Ashraf and Harris, 2013; Kask *et al.*, 2016). Onset of the rapid rise in F_0 during continuous heating indicates a critical temperature for the start of irreversible damage of PSII (O'Sullivan *et al.*, 2017; Zhu *et al.*, 2018). From a mechanistic point of view, the rise of

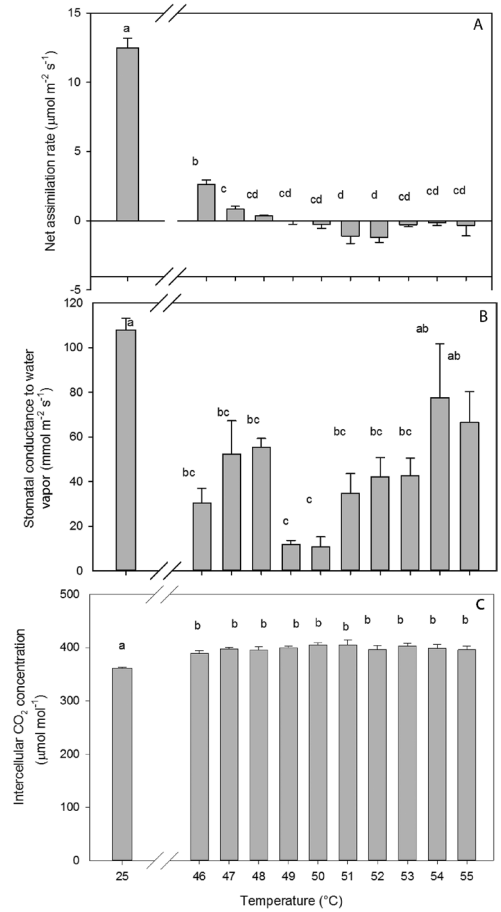

Fig. 3. Heat stress effects on foliage net assimilation rate (A), stomatal conductance to water vapor (B), and intercellular CO_2 concentration (C) in *N. tabacum* cv. Wisconsin 38. Photosynthetic characteristics were measured 30 min after the heat treatment at 25 ºC (see Fig. 2 for heat treatments). Data are means of 3–4 replicate measurement at each temperature. Different letters indicate statistical difference (ANOVA followed by Tukey test, $P<0.05$).

F_0 level has been explained by dissociation of light-harvesting Chl *a*/*b* protein complexes from the reaction center complex of PSII, and an excessive reduction of the plastoquinone pool, ultimately leading to damaged PSII reaction centers (Briantais *et al.*, 1996). Although in our study the F_0 level was assessed after the heat treatment at 25 °C (Fig. 1), we argue that this reflects a sustained damage at PSII, analogously to a sustained photoinhibition (Havaux, 1992; Demmig-Adams and Adams, 2006). Continued decrease of F_v/F_m and F_0 at 50–55 °C (Fig. 2) could be attributed to the propagation of lesions as the result of severe membrane damage, protein denaturation, and complete impairment of repair processes due to ROS accumulation

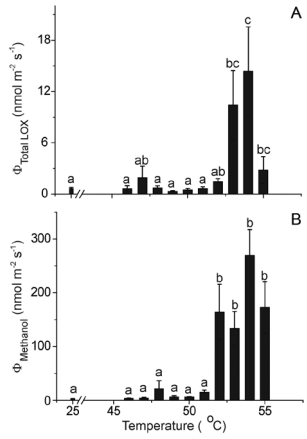

Fig. 4. Influence of heat stress on the emissions of volatile products of the lipoxygenase (LOX) pathway compounds (A) and methanol (B) in leaves of *N. tabacum* cv. Wisconsin 38. The emission rates correspond to the average values for a period of 15 min after heat stress and were measured with GC-MS (A) and PTR-QMS (B). Data reporting and statistical analysis are as in Fig. 3.

(Suzuki and Mittler, 2006; Allakhverdiev *et al.*, 2008; Hüve *et al.*, 2011; Ambastha *et al.*, 2015).

Concomitant with reductions in F_v/F_m, A also decreased (Fig. 3A). A complete cessation of A observed at 49 °C was followed by negative values of A at even higher temperatures (Fig. 3A), implying that the dark respiration rate was greater than the rate of gross photosynthesis. Although g_s decreased under heat stress temperatures of up to 50 °C and then slightly increased (Fig. 3B), C_i concentration actually increased throughout the entire heat stress range (Fig. 3C). This indicates that a decline in A was not immediately associated with the decrease in g_s, as found in other heat stress studies (Hüve *et al.*, 2011; Jie *et al.*, 2012). The reduction in A after exposure to temperatures of 46–48 °C was stronger than that in F_v/F_m, suggesting that dark reactions of photosynthesis were initially more strongly suppressed than the light reactions, possibly as the result of deactivation of Rubisco and activities of other Calvin cycle enzymes (Crafts-Brandner and Salvucci, 2000; Kurek *et al.*, 2007). However, once the A was completely inhibited at 49 °C, the activity of light reactions was also rapidly lost (Fig. 2).

Heat-triggered emissions of lipoxygenase pathway volatiles from tobacco leaves: a relationship with an optimum

A number of studies have demonstrated LOX pathway-derived volatile emissions in response to wounding, herbivore attack, heat, and other abiotic stresses at the immediate sites of stress/injury as well as in plant parts distant from the immediate site of damage (Major and Thomas, 1972; Kessler and Baldwin, 2002; Farag *et al.*, 2006; Matsui, 2006; Wei *et al.*, 2007; Adamas

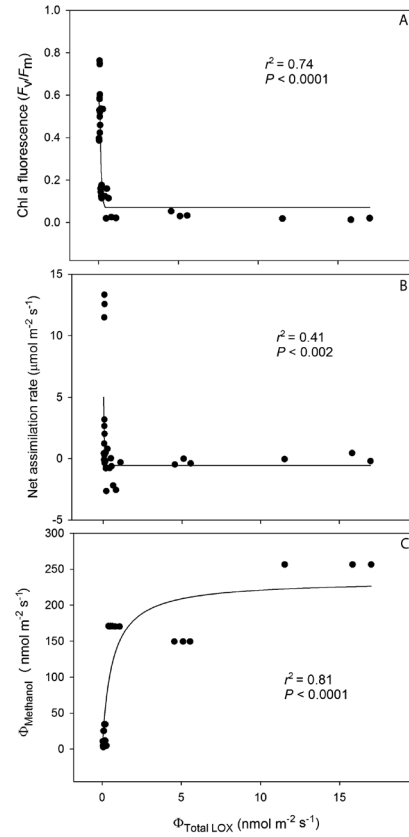

Fig. 5. Relationship between the dark-adapted quantum yield of PSII (A), net assimilation rate (B), and methanol emission rate (C) and the emission rate of lipoxygenase (LOX) pathway volatiles during the heat stress treatments in *N. tabacum* cv. Wisconsin 38. Data correspond to individual heat-stressed leaves. Heat stress effects on individual characteristics are shown in Fig. 2A (chlorophyll fluorescence), Fig. 3A (net assimilation rate), and Fig. 4 (LOX pathway compounds and methanol). Data were fitted by non-linear regressions in the form: $y=a_1+b_1/(1+\exp[-(x-c_1)/d_1)]^{e1}$ for (A); $y=a_2+b_2\exp[-\exp(-(x-c_2)/d_2)]$ for (B); $y=a_3x/(b_3+c_3x)$ for (C).

et al., 2011; Blom *et al.*, 2011; Wei and Kang, 2011; Copolovici *et al.*, 2012; Rambla *et al.*, 2016; Jiang *et al.*, 2017; Rasulov *et al.* 2019). The release of LOX pathway-derived volatiles plays an essential role in induced direct and indirect plant defenses including tri-trophic interactions and even in apoptosis, because the amount of LOX pathway-derived volatiles and

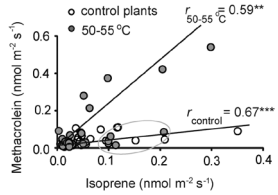

Fig. 6. Linear regressions between the emissions of isoprene and methacrolein from control (white circles) and 50–55 °C treated (gray circles) leaves of *N. tabacum* cv. Wisconsin 38. Stressed plants, which lacked the high emission of methacrolein (within the ellipse), were excluded from the regression test of heat-stressed plants (50–55 °C). The relationships of control and stressed plants were statistically significant at $P<0.001$ (**) and $P<0.01$ (***), respectively.

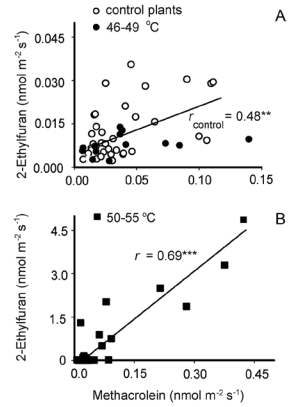

Fig. 7. Linear regressions between the emissions of methacrolein and 2-ethylfuran in *N. tabacum* cv. Wisconsin 38 in control plants and in plants treated with 46–49 °C (A) and 50–55 °C (B). The relationships of control and stressed plants were statistically significant at $P<0.01$ (**) and $P<0.001$ (***), respectively.

ROS produced by any stress determines the type of signaling or plant response they will induce (Geervliet *et al.*, 1997; Engelberth *et al.*, 2004; Ruther and Kleier, 2005; Kishimoto *et al.*, 2008; Bruinsma *et al.*, 2009; Wei and Kang, 2011; von Arx *et al.*, 2012; Bhattacharjee, 2012; Tieman *et al.*, 2012). There is evidence that the amount of LOX pathway-derived volatiles scales quantitatively with the severity of stress (Jiang *et al.*, 2017), but there is little information on whether this scaling is valid for extreme stress levels that can also inhibit LOX activity together with the cessation of entire plant metabolism. On the other hand, stress-generated ROS could directly oxidize plant volatiles and potentially also lead to the release of oxidized volatiles from other cell compartments including cell walls and membranes (Jardine *et al.*, 2012, 2013). That kind of a non-enzymatic oxygenated volatile release might scale with stress severity without any signs of decline at extreme stress levels.

In the present study, the emission of LOX pathway-derived volatiles was induced at higher temperatures according to a switch-type response (Table 1) analogously to heat-stressed tomato (*Solanum lycopersicum*) (Copolovici *et al.*, 2012) and black mustard (*Brassica nigra*) plants (Kask *et al.*, 2016). Total LOX pathway-derived volatile emission, F_v/F_m, and A were related according to a threshold-type response (Fig. 5), which is in accordance with the fact that the rise of LOX volatile emission did not start before the heat shock temperature was raised to at least 53 °C (Fig. 4A). At that temperature, F_v/F_m attained almost a minimum value and A was completely inhibited. This indicates that massive damage at the membrane level was absent until very high temperatures were reached (Suzuki *et al.*, 2011). However, the decline of LOX pathway-derived volatile emissions at 55 °C suggests that the heat resistance of LOX enzymes or enzymes downstream of LOX was compromised.

Constant emission of pentane and hexane from heat-stressed plants (Table 1) is similar to the findings of Croft *et al.* (1993), where the release of pentane was not related to the heat stress of tobacco. On the other hand, the release of saturated aliphatic compounds, including propanal (Table 1) under heat stress, has been attributed to by-products of lipid peroxidation (Anderson, 1994). Pentane is derived from linoleic acid (Kunert and Tappel, 1983), but propanal is derived from linolenic acid (Lieberman and Kunishi, 1967) albeit other, yet unknown pathways such as the ethylene pathway, can be responsible for the biosynthesis of saturated aldehydes (Anderson, 1994; Morgan and Drew, 1997; Wildt *et al.*, 2003); however, ethylene could not be detected by our analytical set-up.

The overall composition of emitted LOX pathway-derived volatiles differed at different temperatures (Fig. 4), and this might indicate differences in the activation of different LOX pathway enzymes, in the sites of LOX action, and in heat resistance of different processes downstream of LOX and possible non-enzymatic reactions. Further studies are needed to gain an insight into processes governing the blend of LOX pathway-derived emissions under heat stress.

Heat stress effects on volatiles from cell walls in tobacco

Severe abiotic stress such as ozone stress leads to major destruction of cell walls and thus to a complete loss of internal leaf structure (Matyssek *et al.*, 1991; Günthardt-Goerg *et al.*, 1997). So far, there are limited data on the cell wall-associated volatile 2-methyl-2-cyclopentene-1-one. Yet, in our study, its enhanced emission at 53–54 °C provided clear evidence that heat stress causes the decomposition of cell walls (Table 1). 2-Methyl-2-cyclopentene-1-one is suggested to originate from hemicellulose (Carrier *et al.*, 2012), and methanol (Fig. 4B) from cell wall pectins as the result of activation of pectin methylesterases and demethylation of pectins (Pelloux *et al.*, 2007).

There is much more information on stress-dependent methanol emissions (Anderson, 1994; Graus *et al.*, 2004; Beauchamp *et al.*, 2005; Peñuelas *et al.*, 2005; Cojocariu *et al.*, 2006; Loreto *et al.*, 2006; Copolovici and Niinemets, 2010; Brilli *et al.*, 2011; Li

et al., 2017; Niinemets *et al*., 2018). Pectin-released methanol is considered as a signaling compound, which triggers systemic resistance processes in neighboring plants and also in non-stressed parts of a stressed plant (Dorokhov *et al*.,1999; Komarova, 2014). However, in our study, methanol emissions reached extremely high levels and, differently from LOX pathway-derived volatiles, methanol emissions were not inhibited in leaves exposed to 55 °C (Fig. 4). This suggests that pectin methylesterases were probably more heat resistant than all symplastic processes that became completely inhibited at the highest temperature.

Heat stress effects on the release of volatile terpenoids from tobacco leaves

Nicotiana tabacum is a low-level constitutive emitter of monoterpenes due to their presence in glandular trichomes on the leaf surface (Kanagendran *et al*., 2018*a*, b). Thus, severe stress can lead to enhanced terpene emission due either to the breakage of trichomes or to the increased permeability of glandular trichome outer surfaces (Guenther *et al*., 1993; Jansen *et al*., 2009). Such an enhanced emission response has been observed in heat shock-treated tomato (*S. lycopersicum*) (Copolovici *et al*., 2012; Pazouki *et al*., 2016). In our study, the effect of heat stress on the emission of monoterpenes remained small throughout the entire temperature range (Table 1), but still a significant positive relationship with heat shock temperature was observed according to regression analyses. Although this positive correlation suggests that heat stress might have changed the permeability of glandular trichomes, emission of terpenes changed much less compared with the changes in the leaf photosynthetic apparatus and cellular oxidative status.

Different biotic and abiotic stresses lead to an induction of terpene synthesis and release from mesophyll cells (Loreto and Schnitzler, 2010; Niinemets, 2010). The stress-induced terpene emissions depend both on the induction of expression of corresponding terpene synthases and on substrate availability (Chern *et al*., 2013; Pazouki *et al*., 2016). In *N. tabacum*, enhancement of mono- and sesquiterpene release has been observed in response to both pathogen attacks (Huang *et al*., 2003) and ozone stress (Kanagendran *et al*., 2018*a*, b). However, due to the delays associated with gene expression and synthesis of relevant terpene synthase proteins, there is generally a time delay between the stress impact and the start of the release of terpenes (Pazouki *et al*., 2016). Nevertheless, we cannot rule out that elicitation of *de novo* terpene emissions did contribute to the positive relationship between heat shock temperature and terpene emission rate.

Isoprene, methacrolein, and 2-ethylfuran release from heat-stressed tobacco leaves

Tobacco lacks the key terminal enzyme, isoprene synthase, but transgenic isoprene-emitting *N. tabacum* have been constructed and their oxidative stress resistance has been studied (Vickers *et al*., 2009; Jardine *et al*., 2012). Thus, it was surprising to find isoprene, albeit at a low level, in the emissions of control and heat-stressed wild-type tobacco plants (Table 1; Fig. 6). Lack of isoprene synthase in tobacco raises the question of what was the source of isoprene emissions in these plants. Non-enzymatic formation of isoprene from plastidial or cytosolic pools, especially under heat stress, could lead to release of some isoprene. Tobacco smoke contains large amounts of isoprene (Sleiman *et al*., 2014), which suggests non-enzymatic isoprene formation from tobacco metabolites. The emission of isoprene may also be associated with the presence of polyisoprenoid solanesol (C45, i.e. nine isoprene units) in tobacco leaves (Yan *et al*., 2017) and its decomposition under heat stress. On the other hand, many plant species contain hemiterpene glycosides (Ward *et al*., 2011; Ono *et al*., 2015; Lihavainen *et al*., 2016), including the Solanaceae sp. (Ono *et al*., 2015), and thus isoprene release as the result of specific glycosidases is possible. Next to isoprene, methacrolein is also present in tobacco smoke (Sleiman *et al*., 2014). Differently from isoprene, significantly elevated emissions of methacrolein at high temperatures (Table 1; Figs 6, 7) could be related to the within-plant isoprene oxidation by ROS (Jardine *et al*., 2012) or other/additional sources for methacrolein (Cappellin *et al*., 2019). In fact, the co-existence of 2-ethylfuran and methacrolein also indicates the overall rise of leaf ROSs in heat-stressed plant leaves (Fig. 7). The exact biosynthesis pathway of 2-ethylfuran is unclear but, according to the literature, the biosynthesis of furans may be related to the oxidation of phenolic compounds and even isoprene (Atkinson *et al*., 1989; Krause *et al*., 2014). Finally, 2-ethylfuran was recently detected in pathogen-infected leaves of grapevine (*Vitis vinifera*) (Lazazzara *et al*., 2018).

Conclusions

Tobacco is an important agricultural plant and a model in plant biology, and thus the results obtained contribute to the understanding of the mechanisms of stress signaling and plant response to heat stress. This study indicates that in heat-stressed tobacco leaves, net assimilation rate (*A*) is inhibited first, followed by cessation of light reactions of photosynthesis, and ultimately by enhancement of stress volatile emissions, whereas elicitation of foliage stress volatiles occurs according to a switch-type response. The critical heat shock temperature leading to a rapid increase of stress volatile emissions was 52 °C. In contrast to methanol, emissions of which increased even at the highest studied temperature, the emissions of most stress volatiles such as membrane-derived LOX pathway-derived volatiles, cell wall-derived methyl-2-cyclopentene-1-one, and oxidation products of cellular volatiles and/or non-volatiles initially increased with increasing severity of heat stress, but decreased at the most severe heat stress of 55 °C. This indicates that extreme heat stress resulted in the collapse of leaf metabolism, including the capacity to form ROS and respond to stress via volatiles. Although the total volatile emission rates decreased at the most severe heat stress treatment, the composition of volatiles was altered, providing an unique fingerprint of this extreme temperature. Regarding the emission and synthesis of terpenes, further studies are needed to understand how elicitation of terpenoid synthases scales with the severity of heat stress and how heat stress affects the accumulation and/or degradation of terpenoids such as diterpenoids and solanesol. Overall, monitoring emissions of stress volatiles during heat stress may be used as a non-invasive tool for quantifying heat stress resistance in phenotyping studies in other plant species.

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References

Adamas A, Bouckaert C, Van Lancker F, De Meulenaer B, De Kimpe N. 2011. Amino acid catalysis of 2-alkylfuran formation from lipid oxidation-derived α,β-unsaturated aldehydes. Journal of Agricultural and Food Chemistry **59**, 11058–11062.

Allakhverdiev SI, Kreslavski VD, Klimov VV, Los DA, Carpentier R, Mohanty P. 2008. Heat stress: an overview of molecular responses in photosynthesis. Photosynthesis Research **98**, 541–550.

Ambastha V, Tripathy BC, Tiwari BS. 2015. Programmed cell death in plants: a chloroplastic connection. Plant Signaling & Behavior **10**, e989752.

Ameye M, Allmann S, Verwaeren J, Smagghe G, Haesaert G, Schuurink RC, Audenaert K. 2018. Green leaf volatile production by plants: a meta-analysis. New Phytologist **220**, 666–683.

Anderson JA. 1994. Production of methanol from heat-stressed pepper and corn leaf disks. Journal of the American Society of Horticultural Science **119**, 468–472.

Arimura G, Kost C, Boland W. 2005. Herbivore-induced, indirect plant defences. Biochimica et Biophysica Acta **1734**, 91–111.

Ashraf M, Harris PJC. 2013. Photosynthesis under stressful environments: an overview. Photosynthetica **51**, 163–190.

Atkin OK, Bloomfield KJ, Reich PB, *et al*. 2015. Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. New Phytologist **206**, 614–636.

Atkinson R, Aschmann SM, Tuazon EC, Arey J, Zielinska B. 1989. Formation of 3-methylfuran from the gas-phase reaction of OH radicals with isoprene and the rate constant for its reaction with the OH radical. International Journal of Chemical Kinetics **21**, 593–604.

Baur A, Yang SF. 1969. Ethylene production from propanal. Plant Physiology **44**, 189–192.

Beauchamp J, Wisthaler A, Hansel A, Kleist E, Miebach M, Niinemets Ü, Schurr U, Wildt J. 2005. Ozone induced emissions of biogenic VOC from tobacco: relations between ozone uptake and emission of LOX products. Plant, Cell & Environment **28**, 1334–1343.

Bernardi P, Scorrano L, Colonna R, Petronilli V, Di Lisa F. 1999. Mitochondria and cell death. Mechanistic aspects and methodological issues. European Journal of Biochemistry **264**, 687–701.

Bhattacharjee S. 2012. The language of reactive oxygen species signaling in plants. Journal of Botany **2012**, article ID 985298.

Blom D, Fabbri C, Connor EC, Schiestl FP, Klauser DR, Boller T, Eberl L, Weisskopf L. 2011. Production of plant growth modulating volatiles is widespread among rhizosphere bacteria and strongly depends on culture conditions. Environmental Microbiology **13**, 3047–3058.

Blonder B, Michaletz ST. 2018. A model for leaf temperature decoupling from air temperature. Agricultural and Forest Meteorology **262**, 354–360.

Briantais JM, Dacosta J, Goulas Y, Ducruet JM, Moya I. 1996. Heat stress induces in leaves an increase of the minimum level of chlorophyll fluorescence, Fo: a time-resolved analysis. Photosynthesis Research **48**, 189–196.

Brilli F, Hörtnagl L, Bamberger I, Schnitzhofer R, Ruuskanen TM, Hansel A, Loreto F, Wohlfahrt G. 2012. Qualitative and quantitative characterization of volatile organic compound emissions from cut grass. Environmental Science & Technology **46**, 3859–3865.

Brilli F, Ruuskanen TM, Schnitzhofer R, Müller M, Breitenlechner M, Bittner V, Wohlfahrt G, Loreto F, Hansel A. 2011. Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction 'time-of-flight' mass spectrometry (PTR-TOF). PLoS One **6**, e20419.

Bruinsma M, Posthumus MA, Mumm R, Mueller MJ, van Loon JJ, Dicke M. 2009. Jasmonic acid-induced volatiles of *Brassica oleracea* attract parasitoids: effects of time and dose, and comparison with induction by herbivores. Journal of Experimental Botany **60**, 2575–2587.

Cappellin L, Loreto F, Biasioli F, Pastore P, McKinney K. 2019. A mechanism for biogenic production and emission of MEK from MVK decoupled from isoprene biosynthesis. Atmospheric Chemistry and Physics **19**, 3125–3135.

Carrier M, Loppinet-Serani A, Absalon C, Aymonier C, Mench M. 2012. Degradation pathways of holocellulose, lignin and α-cellulose from *Pteris vittata* fronds in sub- and super critical conditions. Biomass Bioenergy **43**, 65–71.

Chern LY, Shukor MY, Muse R. 2013. Monoterpenes in plants: a mini review. Asian Journal of Plant Biology **1**, 15–19.

Cojocariu C, Solomon SJ, Burrows J, Hewitt CN. 2006. Effect of ozone fumigation on isoprene and methanol emission by the leaves of Grey poplar (*Populus* × *canescens*). Geophysical Research Abstracts **8**, 03615.

Copolovici L, Kännaste A, Pazouki L, Niinemets Ü. 2012. Emissions of green leaf volatiles and terpenoids from *Solanum lycopersicum* are quantitatively related to the severity of cold and heat shock treatments. Journal of Plant Physiology **169**, 664–672.

Copolovici L, Kännaste A, Remmel T, Vislap V, Niinemets Ü. 2011. Volatile emissions from *Alnus glutionosa* induced by herbivory are quantitatively related to the extent of damage. Journal of Chemical Ecology **37**, 18–28.

Copolovici L, Niinemets Ü. 2010. Flooding induced emissions of volatile signalling compounds in three tree species with differing waterlogging tolerance. Plant, Cell & Environment **33**, 1582–1594.

Copolovici L, Niinemets Ü. 2016. Environmental impacts on plant volatile emission. In Blande J, Glinwood R, eds. Deciphering chemical language of plant communication. Berlin: Springer International Publishing, 35–59.

Cozzolino S, Fineschi S, Litto M, Scopece G, Trunschke J, Schiestl FP. 2015. Herbivory increases fruit set in *Silene latifolia*: a consequence of induced pollinator-attracting floral volatiles? Journal of Chemical Ecology **41**, 622–630.

Crafts-Brandner SJ, Salvucci ME. 2000. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proceedings of the National Academy of Sciences, USA **97**, 13430–13435.

Crawford AJ, McLachlan DH, Hetherington AM, Franklin KA. 2012. High temperature exposure increases plant cooling capacity. Current Biology **22**, R396–R397.

Croft K, Juttner F, Slusarenko AJ. 1993. Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv *phaseolicola*. Plant Physiology **101**, 13–24.

Demmig-Adams B, Adams WW 3rd. 2006. Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytologist **172**, 11–21.

Dicke M, Baldwin IT. 2010. The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. Trends in Plant Science **15**, 167–175.

Dicke M, Loreto F. 2010. Induced plant volatiles: from genes to climate change. Trends in Plant Science **15**, 115–117.

Dorokhov YL, Mäkinen K, Frolova OY, Merits A, Saarinen J, Kalkkinen N, Atabekov JG, Saarma M. 1999. A novel function for a ubiquitous plant enzyme pectin methylesterase: the host-cell receptor for the tobacco mosaic virus movement protein. FEBS Letters **461**, 223–228.

Dudareva N, Negre F, Nagegowda DA, Orlova I. 2006. Plant volatiles: recent advances and future perspectives. Critical Reviews in Plant Sciences **25**, 417–440.

Dutilleul C, Garmier M, Noctor G, Mathieu C, Chétrit P, Foyer CH, de Paepe R. 2003. Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. The Plant Cell **15**, 1212–1226.

Ehlert B, Hincha DK. 2008. Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in Arabidopsis leaves. Plant Methods **4**, 12.

Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH. 2004. Airborne signals prime plants against insect herbivore attack. Proceedings of the National Academy of Sciences, USA **101**, 1781–1785.

Farag MA, Ryu CM, Sumner LW, Paré PW. 2006. GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry **67**, 2262–2268.

Farré-Armengol G, Filella I, Llusià J, Niinemets Ü, Peñuelas J. 2014. Changes in floral bouquets from compound-specific responses to increasing temperatures. Global Change Biology **20**, 3660–3669.

Feussner I, Wasternack C. 2002. The lipoxygenase pathway. Annual Review of Plant Biology **53**, 275–297.

Fineschi S, Loreto F, Staudt M, Peñuelas J. 2013. Diversification of volatile isoprenoid emissions from trees: evolutionary and ecological perspectives In: Niinemets Ü, Monson RK, eds. Biology, controls and models of tree volatile organic compound emissions. Berlin: Springer, 1–20.

Frolec J, Ilik P, Krchnak P, Sušila P, Nauš J. 2008. Irreversible changes in barley leaf chlorophyll fluorescence detected by the fluorescence temperature curve in a linear heating/cooling regime. Photosynthetica **46**, 537–546.

Fuentes JD, Wang D, Bowling DR, Potosnak M, Monson RK, Goliff WS, Stockwel R. 2007. Biogenic hydrocarbon chemistry within and above a mixed deciduous forest. Journal of Atmospheric Chemistry **56**, 165–185.

Geervliet JBF, Posthumus MA, Vet LEM, Dicke M. 1997. Comparative analysis of headspace volatiles from different caterpillar-infested and uninfested food plants of *Pieris* species. Journal of Chemical Ecology **23**, 2935–2954.

Gershenzon J, Dudareva N. 2007. The function of terpene natural products in the natural world. Nature Chemical Biology **3**, 408–414.

Gigot C, Ongena M, Fauconnier ML, Wathelet JP, du Jardin P, Thonart P. 2010. The lipoxygenase metabolic pathway in plants: potential for industrial production of natural green leaf volatiles. Biotechnology, Agronomy, Society and Environment **14**, 451–460.

Graus M, Schnitzler JP, Hansel A, Cojocariu C, Rennenberg H, Wisthaler A, Kreuzwieser J. 2004. Transient release of oxygenated volatile organic compounds during light–dark transitions in Grey poplar leaves. Plant Physiology **135**, 1967–1975.

Grover A, Mittal D, Negi M, Lavania D. 2013. Generating high temperature tolerant transgenic plants: achievements and challenges. Plant Science **205–206**, 38–47.

Guenther AB. 2000. Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. Atmospheric Environment **34**, 2205–2230.

Guenther AB, Zimmerman PR, Harley PC, Monson RK, Fall R. 1993. Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. Journal of Geophysical Research **98**, 12609–12617.

Guenther AB, Zimmerman PR, Wildermuth M. 1994. Natural volatile organic compound emission rates for U.S. woodland landscapes. Atmospheric Environment **28**, 1197–1210.

Günthardt-Goerg MS, McQuattie CJ, Scheidegger C, Rhiner C, Matyssek R. 1997. Ozone induced cytochemical and ultrastructural changes in leaf mesophyll cell walls. Canadian Journal of Forest Research **27**, 453–463.

Hartikainen K, Nerg AM, Kivimäenpää M, Kontunen-Soppela S, Mäenpää M, Oksanen E, Rousi M, Holopainen T. 2009. Emissions of volatile organic compounds and leaf structural characteristics of European aspen (*Populus tremula*) grown under elevated ozone and temperature. Tree Physiology **29**, 1163–1173.

Hasanuzzaman M, Nahar K, Alam MM, Roychowdhury R, Fujita M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. International Journal of Molecular Sciences **14**, 9643–9684.

Havaux M. 1992. Stress tolerance of photosystem II in vivo: antagonistic effects of water, heat, and photoinhibition stresses. Plant Physiology **100**, 424–432.

Heiden AC, Kobel K, Langebartels C, Schuh-Thomas G, Wildt H. 2003. Emissions of oxygenated volatile organic compounds from plants. Part I: emissions from lipoxygenase activity. Journal of Atmospheric Chemistry **45**, 143–172.

Heyduk K, Burrell N, Lalani F, Leebens-Mack J. 2016. Gas exchange and leaf anatomy of a C_3–CAM hybrid, *Yucca gloriosa* (Asparagaceae). Journal of Experimental Botany **67**, 1369–1379.

Huang J, Cardoza YJ, Schmelz EA, Raina R, Engelberth J, Tumlinson JH. 2003. Differential volatile emissions and salicylic acid levels from tobacco plants in response to different strains of *Pseudomonas syringae*. Planta **217**, 767–775.

Huff Hartz KE, Rosenorn T, Ferchak SR, Raymond TM, Bilde M, Donahue NM, Pandis SN. 2005. Cloud condensation nuclei activation of monoterpene and sesquiterpene secondary organic aerosol. Journal of Geophysical Research – Atmospheres **110**, D14208.

Hüve K, Bichele I, Ivanova H, Keerberg O, Pärnik T, Rasulov B, Tobias M, Niinemets Ü. 2012. Temperature responses of dark respiration in relation to leaf sugar concentration. Physiologia Plantarum **144**, 320–334.

Hüve K, Bichele I, Rasulov B, Niinemets Ü. 2011. When it is too hot for photosynthesis: heat-induced instability of photosynthesis in relation to respiratory burst, cell permeability changes and H_2O_2 formation. Plant, Cell & Environment **34**, 113–126.

IPPC. 2013: The climate change 2013. The physical science basis. In: Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, UK and New York, USA: Cambridge University Press, 1535.

Jansen RM, Miebach M, Kleist E, van Henten EJ, Wildt J. 2009. Release of lipoxygenase products and monoterpenes by tomato plants as an indicator of *Botrytis cinerea*-induced stress. Plant Biology **11**, 859–868.

Jardine KJ, Chambers JQ, Holm J, *et al*. 2015. Green leaf volatile emissions during high temperature and drought stress in a Central Amazon Rainforest. Plants **4**, 678–690.

Jardine KJ, Meyers K, Abrell L, Alves EG, Yanez Serrano AM, Kesselmeier J, Karl T, Guenther A, Chambers JQ, Vickers C. 2013. Emissions of putative isoprene oxidation products from mango branches under abiotic stress. Journal of Experimental Botany **64**, 3697–3708.

Jardine KJ, Monson RK, Abrell L. *et al*. 2012. Within-plant isoprene oxidation confirmed by direct emissions of oxidation products methyl vinyl ketone and methacrolein. Global Change Biology **18**, 973–984.

Jiang Y, Veromann-Jürgenson LL, Ye J, Niinemets Ü. 2018. Oak gall wasp infections of *Quercus robur* leaves lead to profound modifications in foliage photosynthetic and volatile emission characteristics. Plant, Cell & Environment **41**, 160–175.

Jiang Y, Ye J, Li S, Niinemets Ü. 2017. Methyl jasmonate-induced emission of biogenic volatiles is biphasic in cucumber: a high-resolution analysis of dose dependence. Journal of Experimental Botany **68**, 4679–4694.

Jiang Y, Ye J, Veromann LL, Niinemets Ü. 2016. Scaling of photosynthesis and constitutive and induced volatile emissions with severity of leaf infection by rust fungus (*Melampsora larici-populina*) in *Populus balsamifera* var. *suaveolens*. Tree Physiology **36**, 856–872.

Jie Z, Xiaodong J, Tianlai L, Zaiqiang Y. 2012. Effect of moderately-high temperature stress on photosynthesis and carbohydrate metabolism in tomato (*Lycopersicon esculentum* L.) leaves. African Journal of Agricultural Research **7**, 487–492.

Jones A. 2000. Does the plant mitochondrion integrate cellular stress and regulate programmed cell death? Trends in Plant Science **5**, 225–230.

Kanagendran A, Pazouki L, Li S, Liu B, Kännaste A, Niinemets Ü. 2018*a*. Ozone-triggered surface uptake and stress volatile emissions in *Nicotiana tabacum* 'Wisconsin'. Journal of Experimental Botany **69**, 681–697.

Kanagendran A, Pazouki L, Niinemets Ü. 2018*b*. Differential regulation of volatile emission from *Eucalyptus globulus* leaves upon single and combined ozone and wounding treatments through recovery and relationships with ozone uptake. Environmental and Experimental Botany **145**, 21–38.

Kännaste A, Copolovici L, Niinemets Ü. 2014. Gas chromatography–mass spectrometry method for determination of biogenic volatile organic compounds emitted by plants. In: Rodríguez-Concepción M, ed. Plant isoprenoids: methods and protocols. New York: Humana Press, 161–169.

Kask K, Kännaste A, Talts E, Copolovici L, Niinemets Ü. 2016. How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*. Plant, Cell and Environment **39**, 2027–2042.

Kesselmeier J, Staudt M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. Journal of Atmospheric Chemistry **33**, 23–88.

Kessler A, Baldwin IT. 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annual Reviews of Plant Biology **53**, 299–328.

Kishimoto K, Matsui K, Ozawa R, Takabayashi J. 2008. Direct fungicidal activities of C_6-aldehydes are important constituents for defense responses in *Arabidopsis* against *Botrytis cinerea*. Phytochemistry **69**, 2127–2132.

Kleist E, Mentel TF, Andres S, Bohne A, Folkers A, Kiendler-Scharr A, Rudich Y, Springer M, Tillmann R, Wildt J. 2012. Irreversible impacts of heat on the emissions of monoterpenes, sesquiterpenes, phenolic

BVOC and green leaf volatiles from several tree species. Biogeosciences **9**, 5111–5123.

Komarova TV, Sheshukova EV, Dorokhov YL. 2014. Cell wall methanol as a signal in plant immunity. Frontiers in Plant Science **5**, 101.

Körner E, von Dahl CC, Bonaventure G, Baldwin IT. 2009. Pectin methylesterase NaPME1 contributes to the emission of methanol during insect herbivory and to the elicitation of defence responses in *Nicotiana attenuata*. Journal of Experimental Botany **60**, 2631–2640.

Kost C, Heil M. 2006. Herbivore-induced plant volatiles induce an indirect defence in neighbouring plants. Journal of Ecology **94**, 619–628.

Krause T, Tubbesing C, Benzing K, Schöler HF. 2014. Model reactions and natural occurrence of furans from hypersaline environments. Biogeosciences **11**, 2871–2882.

Kulmala M, Nieminen T, Chellapermal R, Makkonen R, Bäck J, Kerminen VM. 2013. Climate feedbacks linking the increasing atmospheric CO_2 concentration, BVOC emissions, aerosols and clouds in forest ecosystems. In: Niinemets Ü, Monson RK, eds. Biology, controls and models of tree volatile organic compound emissions. Berlin: Springer, 489–508.

Kunert KJ, Tappel AA. 1983. The effect of vitamin C on in vivo lipid peroxidation in guinea pigs as measured by pentane and ethane evolution. Lipids **18**, 271–274.

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G. 2007. Enhanced thermostability of Arabidopsis Rubisco activase improves photosynthesis and growth rates under moderate heat stress. The Plant Cell **19**, 3230–3241.

Lavoir AV, Staudt M, Schnitzler JP, Landais D, Massol F, Rocheteau A, Rodriguez R, Zimmer I, Rambal S. 2009. Drought reduced monoterpene emissions from the evergreen Mediterranean oak *Quercus ilex*: results from a through fall displacement experiment. Biogeosciences **6**, 1167–1180.

Law RD, Crafts-Brandner SJ. 1999. Inhibition and acclimation of photosynthesis to heat stress is closely correlated with activation of ribulose-1,5-bisphosphate carboxylase/oxygenase. Plant Physiology **120**, 173–181.

Lazazzara V, Bueschl C, Parich A, Pertot I, Schuhmacher R, Perazzolli M. 2018. Downy mildew symptoms on grapevines can be reduced by volatile organic compounds of resistant genotypes. Scientific Reports **8**, 1618.

Li S, Harley PC, Niinemets Ü. 2017. Ozone-induced foliar damage and release of stress volatiles is highly dependent on stomatal openness and priming by low-level ozone exposure in *Phaseolus vulgaris*. Plant, Cell & Environment **40**, 1984–2003.

Li S, Tosens T, Harley PC, Jiang Y, Kanagendran A, Grosberg M, Jaamets K, Niinemets Ü. 2018. Glandular trichomes as a barrier against atmospheric oxidative stress: relationships with ozone uptake, leaf damage, and emission of LOX products across a diverse set of species. Plant, Cell & Environment **41**, 1263–1277.

Liavonchanka A, Feussner I. 2006. Lipoxygenases: occurrence, functions and catalysis. Journal of Plant Physiology **163**, 348–357.

Librando V, Tringali G. 2005. Atmospheric fate of OH initiated oxidation of terpenes. Reaction mechanism of alpha-pinene degradation and secondary organic aerosol formation. Journal of Environmental Management **75**, 275–282.

Lieberman M, Kunishi AT. 1967. Propanal may be a precursor of ethylene in metabolism. Science **158**, 938.

Lihavainen J, Keinänen M, Keski-Saari S, Kontunen-Soppela S, Sõber A, Oksanen E. 2016. Artificially decreased vapour pressure deficit in field conditions modifies foliar metabolite profiles in birch and aspen. Journal of Experimental Botany **67**, 4367–4378.

Liu ZM, Yue MM, Yang DY, Zhu SB, Ma NN, Meng QW. 2017. Overexpression of SlJA2 decreased heat tolerance of transgenic tobacco plants via salicylic acid pathway. Plant Cell Reports **36**, 529–542.

Loreto F, Barta C, Brilli F, Nogues I. 2006. On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant, Cell & Environment **29**, 1820–1828.

Loreto F, Ciccioli P, Brancaleoni E, Cecinato A, Frattoni M, Sharkey TD. 1996. Different sources of reduced carbon contribute to form three classes of terpenoid emitted by *Quercus ilex* L. leaves. Proceedings of the National Academy of Sciences, USA **93**, 9966–9969.

Loreto F, Delfine S. 2000. Emission of isoprene from salt-stressed *Eucalyptus globulus* leaves. Plant Physiology **123**, 1605–1610.

Loreto F, Dicke M, Schnitzler JP, Turlings TC. 2014. Plant volatiles and the environment. Plant, Cell & Environment **37**, 1905–1908.

Loreto F, Förster A, Dürr M, Csiky O, Seufert G. 1998. On the monoterpene emission under heat stress and on the increased thermotolerance of leaves of *Quercus ilex* L. fumigated with selected monoterpenes. Plant, Cell and Environment **21**, 101–107.

Loreto F, Schnitzler JP. 2010. Abiotic stresses and induced BVOCs. Trends in Plant Science **15**, 154–166.

Lu CM, Zhang JH. 2000. Heat-induced multiple effects on PSII in wheat plants. Journal of Plant Physiology **156**, 259–265.

Major RT, Thomas M. 1972. Formation of 2-hexenal from linolenic acid by macerated Ginkgo leaves. Phytochemistry **11**, 611–617.

Masclaux-Daubresse C, Purdy S, Lemaitre T, Pourtau N, Taconnat L, Renou JP, Wingler A. 2007. Genetic variation suggests interaction between cold acclimation and metabolic regulation of leaf senescence. Plant Physiology **143**, 434–446.

Matsui K. 2006. Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. Current Opinion in Plant Biology **9**, 274–280.

Matyssek R, Günthardt-Goerg MS, Keller T, Scheidegger C. 1991. Impairment of gas exchange and structure in birch leaves (*Betula pendula*) caused by low ozone concentrations. Trees **5**, 5–13.

McConkey ME, Gershenzon J, Croteau RB. 2000. Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. Plant Physiology **122**, 215–224.

Mishyna M, Laman N, Prokhorov V, Maninang JS, Fujii Y. 2015. Identification of octanal as plant growth inhibitory volatile compound released from *Heracleum sosnowskyi* fruit. Natural Product Communications **10**, 771–774.

Morgan PW, Drew MC. 1997. Ethylene and plant responses to stress. Physiologia Plantarum **100**, 620–630.

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. 2007. Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta **1767**, 414–421.

Niinemets Ü. 2010. Mild versus severe stress and BVOCs: thresholds, priming and consequences. Trends in Plant Science **15**, 145–153.

Niinemets Ü. 2012. Whole plant photosynthesis. In: Flexas J, Loreto F, Medrano H, eds. Terrestrial photosynthesis in a changing environment. A molecular, physiological and ecological approach, Cambridge: Cambridge University Press, 399–423.

Niinemets Ü. 2018. When leaves go over the thermal edge. Plant, Cell & Environment **41**, 1247–1250.

Niinemets Ü, Bravo LA, Copolovici L. 2018. Changes in photosynthetic rate and stress volatile emissions through desiccation–rehydration cycles in desiccation-tolerant epiphytic filmy ferns (Hymenophyllaceae). Plant, Cell & Environment **41**, 1605–1617.

Niinemets Ü, Kännaste A, Copolovici L. 2013. Quantitative patterns between plant volatile emissions induced by biotic stresses and the degree of damage. Frontiers in Plant Science **4**, 262.

Niinemets Ü, Kuhn U, Harley PC, *et al*. 2011. Estimation of isoprenoid emission factors from enclosure studies: measurements, data processing, quality and standardized measurement protocols. Biogeosciences Discussions **8**, 4633–4725.

Ono M, Yasuda S, Shiono Y, Furusawa C, Inaba S, Tanaka T, Ikeda T, Nohara T. 2015. A new hemiterpene glycoside from the ripe tomatoes. Natural Product Research **29**, 262–267.

O'Sullivan OS, Heskel MA, Reich PB, *et al*. 2017. Thermal limits of leaf metabolism across biomes. Global Change Biology **23**, 209–223.

O'Sullivan OS, Weerasinghe KW, Evans JR, Egerton JJ, Tjoelker MG, Atkin OK. 2013. High-resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*) reveal high-temperature limits to respiratory function. Plant, Cell & Environment **36**, 1268–1284.

Pazouki L, Kanagendran A, Li S, Kännaste A, Memari HR, Bichele R, Niinemets Ü. 2016. Mono- and sesquiterpene release from tomato (*Solanum lycopersicum*) leaves upon mild and severe heat stress and through recovery: from gene expression to emission responses. Environmental and Experimental Botany **132**, 1–15.

Pelloux J, Rustérucci C, Mellerowicz EJ. 2007. New insights into pectin methylesterase structure and function. Trends in Plant Science **12**, 267–277.

Peñuelas J, Filella I, Stefanescu C, Llusià J. 2005. Caterpillars of *Euphydryas aurinia* (Lepidoptera: Nymphalidae) feeding on *Succisa pratensis* leaves induce large foliar emissions of methanol. New Phytologist **167**, 851–857.

Pollastri S, Tsonev T, Loreto F. 2014. Isoprene improves photochemical efficiency and enhances heat dissipation in plants at physiological temperatures. Journal of Experimental Botany **65**, 1565–1570.

Portillo-Estrada M, Kazantsev T, Talts E, Tosens T, Niinemets Ü. 2015. Emission timetable and quantitative patterns of wound-induced volatiles across different leaf damage treatments in aspen (*Populus tremula*). Journal of Chemical Ecology **41**, 1105–1117.

Pospíšil P, Prasad A. 2014. Formation of singlet oxygen and protection against its oxidative damage in Photosystem II under abiotic stress. Journal of Photochemistry and Photobiology. B, Biology **137**, 39–48.

Qu AL, Ding YF, Jiang Q, Zhu C. 2013. Molecular mechanisms of the plant heat stress response. Biochemical and Biophysical Research Communications **432**, 203–207.

Rambla JL, Trapero-Mozos A, Diretto G, Rubio-Moraga A, Granell A, Gómez-Gómez L, Ahrazem O. 2016. Gene–metabolite networks of volatile metabolism in airen and tempranillo grape cultivars revealed a distinct mechanism of aroma bouquet production. Frontiers in Plant Science **7**, 1619.

Rasulov B, Talts E, Niinemets Ü. 2019. A novel approach for real-time monitoring of leaf wounding responses demonstrates unprecedently fast and high emissions of volatiles from cut leaves. Plant Science **283**, 256–265.

Ruiz-Vera UM, Siebers MH, Drag DW, Ort DR, Bernacchi CJ. 2015. Canopy warming caused photosynthetic acclimation and reduced seed yield in maize grown at ambient and elevated [CO2]. Global Change Biology **21**, 4237–4249.

Ruther J, Kleier S. 2005. Plant–plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (Z)-3-hexen-1-ol. Journal of Chemical Ecology **31**, 2217–2222.

Scala A, Allmann S, Mirabella R, Haring MA, Schuurink RC. 2013. Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. International Journal of Molecular Sciences **14**, 17781–17811.

Schreiber U. 2004. Pulse-amplitude (PAM) fluorometry and saturation pulse method: an overview. In: Papageorgiou GC, Govindjee, eds. Chlorophyll a fluorescence: a signature of photosynthesis. Dordrecht: Springer, 279–319.

Singsaas EL, Laporte MM, Shi JZ, Monson RK, Bowling DR, Johnson K, Lerdau M, Jasentuliytana A, Sharkey TD. 1999. Kinetics of leaf temperature fluctuation affect isoprene emission from red oak (*Quercus rubra*) leaves. Tree Physiology **19**, 917–924.

Singsaas EL, Sharkey TD. 2000. The effects of high temperature on isoprene synthesis in oak leaves. Plant, Cell & Environment **23**, 751–757.

Sleiman M, Logue JM, Luo W, Pankow JF, Gundel LA, Destaillats H. 2014. Inhalable constituents of thirdhand tobacco smoke: chemical characterization and health impact considerations. Environmental Science & Technology **48**, 13093–13101.

Staudt M, Lhoutellier L. 2011. Monoterpene and sesquiterpene emissions from *Quercus coccifera* exhibit interacting responses to light and temperature. Biogeosciences **8**, 2757–2571.

Suzuki N, Miller G, Morales J, Shulaev V, Torres MA, Mittler R. 2011. Respiratory burst oxidases: the engines of ROS signaling. Current Opinion in Plant Biology **14**, 691–699.

Suzuki N, Mittler R. 2006. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiologia Plantarum **126**, 45–51.

Tieman D, Bliss P, McIntyre LM, *et al*. 2012. The chemical interactions underlying tomato flavor preferences. Current Biology **22**, 1035–1039.

Tóth SZ, Schansker G, Kissimon J, Kovács L, Garab G, Strasser RJ. 2005. Biophysical studies of photosystem II-related recovery processes after a heat pulse in barley seedlings (*Hordeum vulgare* L.). Journal of Plant Physiology **162**, 181–194.

ul Hassan MN, Zainal Z, Ismail I. 2015. Green leaf volatiles: biosynthesis, biological functions and their applications in biotechnology. Plant Biotechnology Journal **13**, 727–739.

Urban J, Ingwers MW, McGuire MA, Teskey RO. 2017. Increase in leaf temperature opens stomata and decouples net photosynthesis from stomatal conductance in *Pinus taeda* and *Populus deltoides* × *nigra*. Journal of Experimental Botany **68**, 1757–1767.

Vacca RA, de Pinto MC, Valenti D, Passarella S, Marra E, De Gara L. 2004. Production of reactive oxygen species, alteration of cytosolic ascorbate peroxidase, and impairment of mitochondrial metabolism are early events in heat shock-induced programmed cell death in tobacco Bright-Yellow 2 cells. Plant Physiology **134**, 1100–1112.

Valladares F, Niinemets Ü. 2007. The architecture of plant crowns: from design rules to light capture and performance. In: Pugnaire FI, Valladares F, eds. Handbook of functional plant ecology. Boca Raton, FL: CRC Press, 101–149.

Vickers CE, Bongers M, Liu Q, Delatte T, Bouwmeester H. 2014. Metabolic engineering of volatile isoprenoids in plants and microbes. Plant, Cell & Environment **37**, 1753–1775.

Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J, Ryan A, Mullineaux PM, Nicholas Hewitt C. 2009. Isoprene synthesis protects transgenic tobacco plants from oxidative stress. Plant, Cell & Environment **32**, 520–531.

von Arx M, Schmidt-Büsser D, Guerin PM. 2012. Plant volatiles enhance behavioral responses of grapevine moth males, *Lobesia botrana* to sex pheromone. Journal of Chemical Ecology **38**, 222–225.

von Caemmerer S,Farquhar GD. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153**, 376–387.

Vucetic A, Dahlin I, Petrovic-Obradovic O, Glinwood R, Webster B, Ninkovic V. 2014. Volatile interaction between undamaged plants affects tritrophic interactions through changed plant volatile emission. Plant Signaling & Behavior **9**, e29517.

Wang J, Wu B, Yin H, Fan Z, Li X, Ni S, He L, Li J. 2017. Overexpression of CaAPX induces orchestrated reactive oxygen scavenging and enhances cold and heat tolerances in tobacco. BioMed Research International **2017**, 4049534.

Wang P, Zhao L, Hou H, Zhang H, Huang Y, Wang Y, Li H, Gao F, Yan S, Li L. 2015. Epigenetic changes are associated with programmed cell death induced by heat stress in seedling leaves of *Zea mays*. Plant & Cell Physiology **56**, 965–976.

Ward JL, Baker JM, Llewellyn AM, Hawkins ND, Beale MH. 2011. Metabolomic analysis of Arabidopsis reveals hemiterpenoid glycosides as products of a nitrate ion-regulated, carbon flux overflow. Proceedings of the National Academy of Sciences, USA **108**, 10762–10767.

Wei J, Kang L. 2011. Roles of (Z)-3-hexenol in plant–insect interactions. Plant Signaling & Behavior **6**, 369–371.

Wei J, Wang L, Zhu J, Zhang S, Nandi OI, Kang L. 2007. Plants attract parasitic wasps to defend themselves against insect pests by releasing hexenol. PLoS One **2**, e852.

Wildt J, Kobel K, Schuh-Thomas G, Heiden AC. 2003. Emissions of oxygenated volatile organic compounds from plants. Part II: emissions of saturated aldehydes. Journal of Atmospheric Chemistry **45**, 173–196.

Woo NS, Badger MR, Pogson BJ. 2008. A rapid, non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. Plant Methods **4**, 27.

Wright IJ, Dong N, Maire V, *et al*. 2017. Global climatic drivers of leaf size. Science **357**, 917–921.

Xu Q, Xu X, Shi Y, Xu J, Huang B. 2014. Transgenic tobacco plants overexpressing a grass PpEXP1 gene exhibit enhanced tolerance to heat stress. PLoS One **9**, e100792.

Yan K, Chen P, Shao H, Shao C, Zhao S, Brestic M. 2013. Dissection of photosynthetic electron transport process in sweet sorghum under heat stress. PLoS One **8**, e62100.

Yan N, Liu Y, Zhang H, Du Y, Liu X, Zhang Z. 2017. Solanesol biosynthesis in plants. Molecules **22**, 510.

Yu MH, Ding GD, Gao GL, Sun BP, Zhao YY, Wan L, Wang DY, Gui ZY. 2015. How the plant temperature links to the air temperature in the desert plant *Artemisia ordosica*. PLoS One **10**, e0135452.

Zhang R, Cruz JA, Kramer DM, Magallanes-Lundback ME, Dellapenna D, Sharkey TD. 2009. Moderate heat stress reduces the pH component of the transthylakoid proton motive force in light-adapted, intact tobacco leaves. Plant, Cell & Environment **32**, 1538–1547.

Zhang R, Sharkey TD. 2009. Photosynthetic electron transport and proton flux under moderate heat stress. Photosynthesis Research **100**, 29–43.

Zhu L, Bloomfield KJ, Hocart CH, Egerton JJG, O'Sullivan OS, Penillard A, Weerasinghe LK, Atkin OK. 2018. Plasticity of photosynthetic heat tolerance in plants adapted to thermally contrasting biomes. Plant, Cell & Environment **41**, 1251–1262.

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LIST OF PUBLICATIONS

I. Turan S., **Kask K.**, Kanagendran A., Li S., Anni R., Talts E., Rasulov B., Kännaste A., Niinemets Ü. (2019) Lethal heat stress-dependent volatile emissions from tobacco leaves: what happens beyond the thermal edge? *Journal of Experimental Botany, 70*, Issue 18, 5017–5030.

II. **Kask, K**.; Kännaste, A.; Talts, E.; Copolovici, L.; Niinemets, Ü. (2016). How specialized volatiles respond to chronic and short-term physiological and shock heat stress in *Brassica nigra*. *Plant, Cell and Environment, 39* (9), 2027−2042.

III. **Kask, K**.; Kännaste, A.; Niinemets, Ü. (2013). Emission of volatile organic compounds as a signal of plant stress. *Scientific Bulletin of ESCORENA, 8*, 79−93.

IV. **Kask, K.** (2012). Maheviljeluses kasvanud kännasmustika (*Vaccinium corymbosum* L.) viljade kvaliteet sõltuvalt genotüübist. Talveakadeemia ... : teaduslikud lühiartiklid : kogumik (104).. Tallinn: Talveakadeemia. In Estonian.

V. Mander, Ü.; Soosaar, K.; Burdun, I.; Kännaste, A.; **Kask, K.**; Krasnov, D.; Krasnova, A.; Kriiska, K.; Kurvits, T.; Machacova, K.; Maddison, M.; Morozov, G.; Noe, S.; Ostonen, I.; Püssa, K.; Repp, K.; Schindler, T.; Suija, A.; Veber, G.; Niinemets, Ü. (2018). Forest ecosystem response to sudden flooding in the middle of growing season: The FluxGAF experiment. *Geophysical Research Abstracts, 20*: EGU General Assembly 2018, Vienna. Copernicus Gesellschaft Mbh,.

VIIS VIIMAST KAITSMIST

RISTO RAIMETS
EFFECTS OF SYNTHETIC AND BIOLOGICAL PESTICIDE EXPOSURE ON HONEY BEES AND BUMBLE BEES
SÜNTEETILISTE JA BIOLOOGILISTE PESTITSIIDIDE MÕJUD MEEMESILASTELE JA KIMALASTELE
Professor **Marika Mänd**, **Reet Karise**
26. september 2019

PEETER PADRIK
FACTORS INFLUENCING THE QUALITY OF SEMEN FROM ESTONIAN HOLSTEIN AI BULLS, AND RELATIONSHIPS BETWEEN SEMEN QUALITY PARAMETERS AND IN VIVO FERTILITY
EESTI HOLSTEINI TÕUGU SUGUPULLIDE SPERMA KVALITEET, SEDA MÕJUTAVAD TEGURID NING SEOS IN VIVO VILJAKUSEGA
Professor **Ülle Jaakmaa**, professor **Olev Saveli**
01. november 2019

TARMO NIINE
IMPACT OF GASTROINTESTINAL PROTOZOAN INFECTIONS ON THE ACUTE PHASE RESPONSE IN NEONATAL RUMINANTS
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21. november 2019

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BIRGIT AASMÄE
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