Journal of Experimental Botany Advance Access published May 11, 2015

Journal of Experimental Botany
doi:10.1093/jxb/erv219

REVIEW PAPER

Volatile organic compounds as non-invasive markers for plant phenotyping

B. Niederbacher, J.B. Winkler and J.P. Schnitzler*

Research Unit Environmental Simulation, Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, D-85764, Neuherberg, Germany

* To whom correspondence should be addressed. E-mail: jp.schnitzler@helmholtz-muenchen.de

Received 4 December 2014; Revised 9 April 2015; Accepted 15 April 2015

Editor: Tracy Lawson

Abstract

Plants emit a great variety of volatile organic compounds (VOCs) that can actively participate in plant growth and protection against biotic and abiotic stresses. VOC emissions are strongly dependent on environmental conditions; the greatest ambiguity is whether or not the predicted change in climate will influence and modify plant–pest interactions that are mediated by VOCs. The constitutive and induced emission patterns between plant genotypes, species, and taxa are highly variable and can be used as pheno(chemo)typic markers to distinguish between different origins and provenances. In recent years significant progress has been made in molecular and genetic plant breeding. However, there is actually a lack of knowledge in functionally linking genotypes and phenotypes, particularly in analyses of plant–environment interactions. Plant phenotyping, the assessment of complex plant traits such as growth, development, tolerance, resistance, etc., has become a major bottleneck, and quantitative information on genotype–environment relationships is the key to addressing major future challenges. With increasing demand to support and accelerate progress in breeding for novel traits, the plant research community faces the need to measure accurately increasingly large numbers of plants and plant traits. In this review article, we focus on the promising outlook of VOC phenotyping as a fast and non-invasive measure of phenotypic dynamics. The basic principle is to define plant phenotypes according to their disease resistance and stress tolerance, which in turn will help in improving the performance and yield of economically relevant plants.

Key words: Biomarker, non-invasive, phenotyping, plant breeding, terpenes, volatile organic compounds.

Introduction

Plants emit a great diversity of biogenic volatile organic compounds (BVOCs) from flowers, fruits, leaves, bark, and roots, as well as specialized storage structures (Kesselmeier and Staudt, 1999; Loreto and Schnitzler, 2010). VOCs are a vital element of a plant's phenotype and are a central character in the ecosystem of plants due to their role as ecological signals and their influence on atmospheric chemistry (Guenther *et al.*, 1995; Fall, 1999; Fuentes *et al.*, 2000; Guenther, 2000; Dicke and Loreto, 2010). BVOCs are very diverse and consist of various organic classes such as isoprene, terpenes, fatty acid derivatives, alcohols, alkanes, alkenes, esters, and acids, among others (e.g. Penuelas and Llusia, 2001; Pichersky and Gershenzon, 2002). These compounds are formed in various plant tissues (see Takabayashi *et al.*, 1994; Mumm and Hilker, 2006; Fineschi and Loreto, 2012) and in diverse physiological processes (Laothawornkitkul *et al.*, 2009).

The distinction between genotype and phenotype was introduced at the beginning of the 20th century by the Danish botanist Johannsen (1909). According to Johannsen, the phenotype develops under the influence of its genotype and environment.

This review will focus on the application of VOCs as biomarkers for plant phenotyping. To the best of our knowledge, VOCs have remained an overlooked trait in plant phenotyping despite their biological importance (Paré and Tumlinson, 1999; Dudareva *et al.*, 2006; Loreto and Schnitzler, 2010; Loreto *et al.*, 2014) and the strong genetic component (Degen *et al.*, 2004; War *et al.*, 2012) that enables the identification of specific chemotypic profiles among and within species (e.g. Snoeren *et al.*, 2010). The importance of plant phenotyping provides a rich ground for the development of new methods and platforms, and has been the pillar of many studies [e.g. automation and robotics, imaging technologies (hardware and software)] in environmental science (Granier *et al.*, 2006; Poorter *et al.*, 2010, 2012), agrobiology (Cabrera-Bosquet *et al.*, 2012; Panguluri and Kumar, 2013; Yang *et al.*, 2013), and ecophysiology (Regnard *et al.*, 2008; Donovan *et al.*, 2009) for studying the relationship between plant functioning and its environment, linking the function of species/varieties or study plant reactions to the atmosphere (Walter *et al.*, 2007; Lee *et al.*, 2012; Granier and Vile, 2014). Phenotyping systems are needed to illustrate the full collection of genetic factors that contribute to quantitative phenotypic variation across cells, organs, and tissues, evolving stages, years, atmospheres, species, and research platforms (Cobb *et al.*, 2013).

Thus, during the last few years, high-throughput analysis methods have emerged as state-of-the-art techniques in the life sciences (Hartmann *et al.*, 2011). An automated greenhouse system for high-throughput plant phenotyping is one of the latest developments to allow non-destructive screening of plants over particular time intervals by means of image acquisition techniques (Hartmann *et al.*, 2011).

In this review we will describe and discuss the potential and different possibilities of using mass spectrometry techniques for non-invasive VOC analysis of plant traits.

Volatile organic compound emissions

VOC emissions from plants can be either constitutive (Kesselmeier and Staudt, 1999) or induced by abiotic (Loreto and Schnitzler, 2010) and biotic (Paré and Tumlinson, 1999; Arimura *et al.*, 2005) factors, providing important information about diverse adaptation and defence processes taking place in plants and the underlying biological pathways of formation of the structurally diverse compounds (Fig. 1). Constitutive levels of VOC emissions are largely controlled by genetic and environmental conditions (Degen *et al.*, 2004; Delphia *et al.*, 2009; Splivallo *et al.*, 2012; Wason and Hunter, 2014), whereas a high phenotypic plasticity can be found regarding the emission of induced VOCs that can be significantly affected by both biotic (pathogens) (Baldwin and Preston, 1999; Walling, 2000; Cardoza *et al.*, 2002; Loreto and Schnitzler, 2010) and abiotic factors (drought, heat stress, ozone) (Tingey *et al.*, 1980; Paré and Tumlinson, 1999; Sharkey and Yeh, 2001; Arimura *et al.*, 2005).

While VOC emission patterns are genetically variable and phenotypically plastic (Ballhorn *et al.*, 2011), modern gas exchange cuvette systems and VOC analysis techniques (Tholl *et al.*, 2006) allow measurement under highly controlled and environmentally constant conditions, generating normalized emission profiles that can then be compared under a set of pre-determined conditions. Thus, VOCs can be used as phenotypic markers which can assist with a variety of forest and agricultural applications.

Genetic variability of VOC emissions

The production and emission of volatile compounds is a developmentally regulated process. Plant VOC emissions from flowers, leaves, and fruits follow more or less the same developmental patterns; for instance, volatile emissions either increased (when leaves are young and growing, fruit is not yet ripe, or when flowers are ready for pollination) or remained relatively constant or were reduced over the development of an organ (e.g. Dudareva and Pichersky, 2000; Gershenzon *et al.*, 2000). To consider these developmental constraints, it is important to understand the linkage between genotype and phenotype under different developmental and environmental conditions, which to date has been hindered by insufficient capability at both the technical and theoretical level of plant science (http://www.plant-phenotyping-network.eu/). Plant

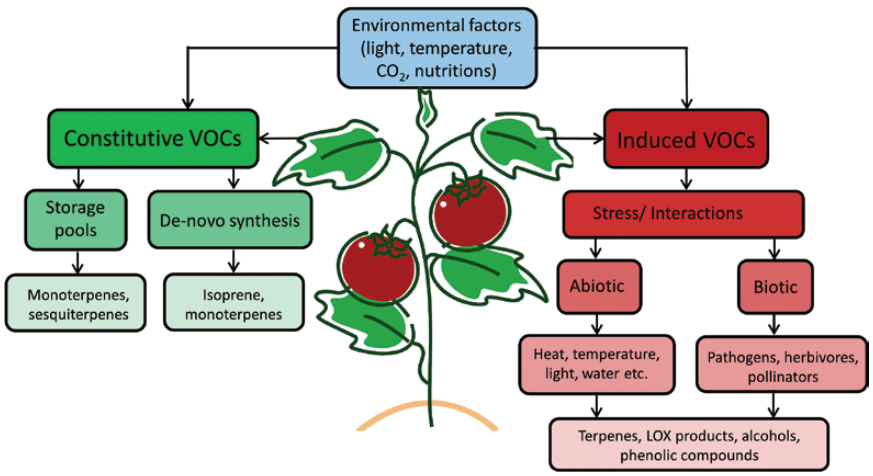

Fig. 1. Scheme of the influence of abiotic and biotic stresses on VOC emissions that in turn play an important role in plant-insect and plant-microbe interactions and also affect atmospheric processes.

growth is affected by complex interactions of genotypes with their respective environments and, for the understanding of this phenome, it is necessary to characterize the key factors that determine the structure and function of plants. Thus, quantitative analysis of these factors and traits will enable the link to the genotype and thereby contribute to the understanding of the genetic basis of the plant–environment interactions.

VOC emissions not only reflect environmental suitability, they also reflect the genetic quality of a plant (Gouinguené and Turlings, 2002). Many studies have shown that the production of VOCs is strongly regulated by genetics, making VOC emissions highly species specific (e.g. Guenther *et al.*, 1995; Kesselmeier and Staudt, 1999; Degen *et al.*, 2004; Delphia *et al.*, 2009; Splivallo *et al.*, 2012; Wason and Hunter, 2014). Also several studies have shown the variability among VOC emissions with genotypes (Table 1) of cultivated and wild plants (Takabayashi *et al.*, 1994; Gouinguene *et al.*, 2001; Degen *et al.*, 2004; Hare, 2007; Köllner *et al.*, 2008). In a recent example, Farneti and colleagues (2014) demonstrated that fruits of different apple accessions group into distinct clusters according to their VOC profiles. Similarly, the same authors (Farneti *et al.*, 2012) demonstrated earlier that the odour profile of tomato varieties allowed separation between the tomato types. Chemotypic diversity in VOC emissions is also observed in trees. In poplar hybrids collected from field sites, the genotype as well as the parental cross predicted the isoprene and monoterpene emission potential (Eller *et al.*, 2012). Also *Arabidopsis thaliana* L. accessions across Eurasia differ in quantity and blend of VOCs as well as in their response to VOC induction by herbivores or jasmonic acid (Snoeren *et al.*, 2010).

Wason and Hunter (2014) investigated the intraspecific variability among VOC emissions in natural populations of plants (e.g. Delphia *et al.*, 2009; Kariyat *et al.*, 2012; Steinbrecher *et al.*, 2013). In order to consider the intraspecific variation in VOC emissions and their role in plant defence, Wason and Hunter (2014) studied naturally growing and common garden milkweed plants (*Asclepias syriaca* L.). The genetic variation from a single population of native milkweeds in terms of total concentration and blend of (constitutive and induced) VOCs appear to unite into similar phenotypes with regard to magnitude of induction and natural enemy attraction. Species and genotypic variations in VOC blends are not only restricted to plants. Splivallo *et al.* (2012) reported in fungi (truffles) that variation in C8-VOC biosynthesis (2-butanone; 1-octen-3-ol, 3-octanone, 3-octanol, 2-octen-1-ol, and trans-2-octenal) is associated with genotypic variation. Following this, a recent study (Müller *et al.*, 2013) described the possibility of identifying the functional groups of root-associated fungi (ectomycorrhizal, pathogenic, and saprophytic species) based on VOC (mainly sesquiterpenes) emissions.

Constitutive VOCs

Constitutive VOC emissions are those which are always expressed in plants regardless of the experience of any stress, and the emission rates change significantly with basic environmental conditions, such as light or temperature (Dicke and Loreto, 2010; Holopainen, 2011; Holopainen and Blande, 2012). Constitutive VOC emission rates vary from leaf to leaf, plant to plant, season, age, and between community level, genotypes, and so on (e.g. Dicke, 1999; Paré and Tumlinson, 1999; Holopainen *et al.*, 2010; Niinemets *et al.*, 2010; Grote *et al.*, 2013; Alves *et al.*, 2014). Well known examples are the glandular trichomes of mints or the resin ducts of pines which store large amounts of mono- and sesquiterpenes (Farmer and Ryan, 1990; Langenheim, 1994). Depending on leaf temperature and compound volatility, there is a constant release of VOCs from these organs (Monson *et al.*, 2012; Grote *et al.*, 2013), varying within the diurnal course (Pio *et al.*, 2005; Grabmer *et al.*, 2006). The presence or absence of storage pools affects the tissue-internal concentration of VOCs, and the diffusive resistance for VOC species. This diffusive resistance, however, depends largely on leaf anatomy (Niinemets *et al.*, 2004) and is important for the release from internal pools such as resin ducts rather than from external compartments (such as glandular trichomes) (Peñuelas and Llusia, 2001).

Beside these plants with storage structures, there are many plant species with no storage structures nevertheless emitting large quantities of VOCs from their foliage. These species, in particular shrubs and trees, release isoprenoids (isoprene and monoterpenes) from their leaves in response to light intensity and leaf temperature, and closely linked to the photosynthetic activity (Ghirardo *et al.*, 2011; Li and Sharkey, 2013; Monson, 2013).

Methanol, another constitutive VOC, is an excellent non-invasive marker for monitoring cell (plant) growth (Nemecek-Marshall *et al.*, 1995; Hüve *et al.*, 2007). It is predominantly emitted during the degradation and formation of cell wall pectins, such as (i) during cell expansion in all types of plant tissue and seeds; and (ii) during leaf abscission, senescence, and seed maturation (Table 2).

Constitutively emitted volatiles, from either solely temperature-dependent or active light-dependent biochemical processes, can be released at any time irrespective of whether or not plants experience stress, making them possible markers for genotypic variability. In conifers, isoprenoids (in particular monoterpenes) stored in resin ducts are common markers of chemotypic variability (Nerg *et al.*, 1994; Powell and Raffa, 1999). The emissions of monoterpenes from storage pools play an important role for plant host selection by beetles (Kelsey and Joseph, 1997; Mita *et al.*, 2002). Plant species lacking storage pools, such as poplars (Brilli *et al.*, 2009) and some tropical tree species (Kuhn *et al.*, 2004), are also known to emit constitutive monoterpenes from young leaves that do not experience biotic or abiotic stresses. It has also been found that in mature poplar leaves monoterpene emissions appeared to be switched to isoprene emissions (Brilli *et al.*, 2009; Ghirardo *et al.*, 2011), which in turn indicates that the amount of carbon invested towards isoprenoid biosynthesis increases with leaf ontogeny.

Stress-induced VOCs

Induced VOC emissions are those which are triggered in a plant following stress or damage (Trowbridge and

Table 1. *Variability of VOCs among genotypes in different species*

Examples showing that constitutive or induced VOCs—quantity and blends—might serve as markers for specific genotypes or accessions. VOCs emitted from diverse organs (leaves or fruits) were determined by either GC-MS or PTR-MS.

Species	Focus of research	Induced/ constitutive	VOC measurement technique	Result	References
Mousear cress (*Arabidopsis thaliana* L.).	Nine accessions	Induced by herbivores and hormone (jasmonic acid)	Headspace (Tenax) GC-MS	Differences in quantity and blend between accessions and in response to induction; 73 compounds identified	Snoeren *et al.* (2010)
Sacred thorn-apple (*Datura wrightii* Regel)	Eight genetic lines of plants used and backcrossed for three generations	Induced by herbivores and methyl jasmonate	GC-MS	Overall plant emitted at least 17 compounds and most of them were sesquiterpenes. The increment in VOC emissions varied from 3.6- to >32-fold among *D. wrightii* genotypes. The most abundant compound was (*E*)-β-caryophyllene.	Hare (2007)
Milkweed (*Asclepias syriaca* L.)	Intraspecific variation in natural populations; seven genotypes, field collection and greenhouse common garden	Constitutive and herbivore-induced, interannual variation	GC-MS	Genetic variation in both total concentration and blend of constitutive and induced plant VOCs; similar response of all genotypes to herbivore induction	Wason and Hunter (2014)
Poplars (*Populus trichocarpa* Torr. & Gray ex Hook, *Populus deltoides* Bartram ex Marshall, *Populus nigra* L.)	30 genotypes, field collection	Constitutive and induced by elevated CO_2 (isoprene, methanol, monoterpenes)	PTR-MS	Genotype and parental cross predicted isoprene and monoterpene emissions. Genotypes differed in response to elevated CO_2	Eller *et al.* (2012)
Apple (*Malus×domestica* Borkh.)	190 accessions, fruits	Constitutive, aroma characterization, VOC fingerprinting	Headspace, PTR-MS, GC-MS	Accessions were grouped into six clusters based on concentration of four VOC clusters (esters, alcohols, carbonyl compounds, and general fragments)	Farneti *et al.* (2014)
Tomato (*Solanum lycopersicum* L.)	14 tomato varieties, fruit at red stage	Aromatic profile, 15 volatiles (mainly aldehydes and ketones)	Headspace (SPME) GC-MS (fruit powder) PTR-MS (intact or half-cut fruits)	Varieties can be separated, with a clear fingerprinting separation between tomato types (round truss, cocktail, and cherry tomatoes). Also changes in volatile profiles during post-harvest ripening and storage were monitored by PTR-MS	Farneti *et al.* (2012)
Truffles (*Tuber uncinatum* L.)	Different scales; genotype, fruiting body maturity, and geographical origin	Constitutive, aroma variability (C8-VOCs)	Headspace (SPME) GC-MS	Aroma variation (C8-VOCs concentration) is linked to genotypic variation	Splivallo *et al.* (2012)

Stoy, 2013). These emissions vary greatly depending on the plant functional type, species, and genotype, as well as on tissue, developmental stage, and past and current environmental and biological conditions (Niinemets *et al.*, 2010; Spinelli *et al.*, 2011; Way *et al.*, 2011). Plant responses to abiotic stresses are dynamic and multifaceted (Cramer, 2010; Skirycz and Inzé, 2010); they can be both elastic (reversible) and plastic (irreversible). The exponential increase in the emissions of

Table 2. *Examples of VOCs as possible non-invasive phenotyping markers for different processes such as growth, ripening, senescence, storage, interactions, and stress (abiotic and biotic)*

Process/stress/interactions	Volatile compounds (constitutive/induced)	References
Growth	Methanol	Nemecek-Marshall *et al.* (1995); Hüve *et al.* (2007)
Ripening	Ethylene	Dugardeyn and Van Der Straeten (2008)
Senescence	Ethylene	Dugardeyn and Van Der Straeten (2008)
Internal (resin ducts) and external (e.g. glands, trichomes) storage tissues	Monoterpenes and sesquiterpenes	Shao *et al.* (2001); Tarvainen *et al.* (2004); Hakola *et al.* (2005)
Abiotic factors		
High temperature	Isoprene, monoterpenes	Loreto *et al.* (1996); Sharkey and Yeh (2001)
Anoxia (roots)	Ethanol, methanol, acetaldehydes	Bracho-Nunez *et al.* (2012)
Ozone (air pollution)	Benzoids, methyl jasmonate, methyl salicylate, indole, phenol, LOX products. etc.	Beauchamp *et al.* (2005); Iriti and Faoro (2009)
Biotic interactions		
Herbivores	Terpenes (*E*)-β-ocimene, (*Z*)-ocimene, α-farnesene, linalool, DMNT, methyl salicylate, methyl jasmonate	Pare and Tumlinson (1999); Arimura *et al.* (2005); Vuorinen *et al.* (2007); Howe and Jander (2008); Dicke and Baldwin (2010); Schmidt *et al.* (2011)
Pathogens	α-Farnesene, methyl salicylate	Zhu and Park (2005); Iriti and Faoro (2009); Dicke and Baldwin (2010); Schmidt *et al.* (2011); Müller *et al.* (2013)
Saprophytes	Sesquiterpenes: y-selinene, α-muurolene	Gioacchini *et al.* (2008); Agger *et al.* (2009); Müller *et al.* (2013)
Ectomycorrhizal (EM) fungi	3,5-Dimethylanisole, 3-cyclohepten-1-one and 1,4aβ-dimethyl-7-isopropyl-1,2,3,4,4a,9,10,10aα-octahydrophenanthrene	Müller *et al.* (2013)

VOCs in response to temperature has been well documented (Tingey *et al.*, 1980), particularly for isoprene (Sharkey and Yeh, 2001), monoterpenes (Loreto *et al.*, 1996), and sesquiterpenes (Duhl *et al.*, 2008). Depending on the severity, duration, and developmental timing of the stress, plant volatiles experience variability to high temperature (Wahid *et al.*, 2007). Water availability, scarcity (Ebel *et al.*, 1995; Vallat *et al.*, 2005), or flooding (Kreuzwieser *et al.*, 2000), salt stress (Loreto and Delfine, 2000), and oxidative stress via ozone treatment (Heiden *et al.*, 1999; Vuorinen *et al.*, 2004) may result in elevated VOC emissions and an altered emission pattern which can also be species specific. A study on the effect of high salinity on *A. thaliana* has been recently published (Lee and Seo, 2014), reporting salt-induced VOC emissions [especially terpenes such as isoprene, mono- and sesquiterpenes, lipoxygenase (LOX) products, and methanol] from *Arabidopsis* plants, which are relevant in priming stress tolerance and triggering induction of high salt resistance in neighbouring plants (Lee and Seo, 2014). Another recent study (Egigu *et al.*, 2014) showed that a rise of 10 °C in the ambient temperature increased the isoprene emission rate from *Cordeauxis edulis* Hemsl. leaves, by a factor of 4, and by a factor of 2–5 in the emission of monoterpenes, sesquiterpenes, and total isoprenoids with a 5 °C rise in the ambient temperature.

Up to this point, we have focused mostly on the impact of abiotic factors, but biotic factors (herbivores, pollinators, microbes, and fungal pathogens) are equally or—with respect to plant phenotyping—more important in determining VOC emissions, (e.g. Baldwin and Preston, 1999; Walling, 2000; Cardoza *et al.*, 2002) (Table 2). It has been repeatedly shown (for a global overview, see Loreto *et al.*, 2014) that herbivore-induced plant volatiles (HIPVs), especially green leaf volatiles (GLVs) and mono- and sesquiterpenes (e.g. Dudareva *et al.*, 2006; Ghirardo *et al.*, 2012; Holopainen and Blande, 2013), are synthesized and emitted in plants in response to herbivory. Large VOC emissions (terpenes) following herbivore attack are also due to the mechanical rupturing of specialized storage structures, such as resin ducts or glandular trichomes (Turlings and Tumlinson, 1992; Paré and Tumlinson, 1997). Additionally, GLVs are produced by the autolytic oxidative breakdown of membrane lipids, and are emitted upon mechanical damage to the leaves. The patterns of induced emissions have been analysed both in the field (e.g. Markovic *et al.*, 1996; Tollsten and Müller, 1996) and under greenhouse conditions (e.g. Mattiacci *et al.*, 1994; Takabayashi *et al.*, 1994). While some VOCs are immediately released following mechanical damage (i.e. monoterpenes from storage reservoirs and GLVs from membrane peroxidation), others are induced *de novo* by herbivore feeding, and are emitted not only from damaged tissue, but also from undamaged leaves following a systemic response (De Moraes *et al.*, 2000). The emissions of these compounds [such as the monoterpenes β-pinene and (*E*)-β-ocimene; the sesquiterpenes α-humulene and (*E,E*)-α-farnesene; the aldoxime phenylacetaldoxime; and the nitrile benzyl cyanide, etc.] can be delayed by minutes, hours, days, and potentially seasons as a result of *de novo* synthesis in damaged organs or in nearby undamaged organs (e.g. Paré and Tumlinson, 1997, 1999; Clavijo McCormick *et al.*, 2014; Irmisch *et al.*, 2014). These emissions can be genotype specific, as shown for pedunculate oak (*Quercus robur* L.) where green oak leaf roller-sensitive oaks subjected to stress emit predominantly (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E*)-β-ocimene, while the emission spectrum of tolerant plants is dominated by the sesquiterpenes α-farnesene and germacrene D (Ghirardo *et al.*, 2012).

HIPV emissions can also act as markers that indicate the presence of herbivore damage. Several environmental factors are known to influence how changes in leaf damage translate to different HIPV emissions. These include light intensity and water stress (Dicke and Baldwin, 2010). In addition to herbivory, increases in VOC emissions (monoterpenes, sesquiterpenes, or GLVs) have also been observed following oviposition (Mumm *et al.*, 2003). Furthermore, pathogen attack is also well known to result in increased VOC emissions [3-octanone, methyl salicylate, α-farnesene, and 4,8-dimethylnona-1,3,7-triene (DMNT)]; for example, fungal or bacterial invasion or application of pathogen elicitors enrich VOC emissions (Huang *et al.*, 2003; Leitner *et al.*, 2008; Yi *et al.*, 2009; Table 2).

Up to this point, our aim was to demonstrate that plant VOC emissions are not only induced by environmental factors but can also be a function of plant ontogeny, and the emission patterns vary between genotypes, species, and taxa (Table 1). Therefore, the compound-specific features allow the use of VOCs as ideal (stress) markers in plant phenotyping.

Recent systems for measurement of plant VOC emissions

The variations in the emission of VOCs resulting from the complex interactions of plants with the environment and the factors which control and influence these emissions are only partly understood (Monson *et al.*, 1995). Cuvette systems are used to measure gas exchange and VOC emissions between plants and the atmosphere at the leaf or branch scale. Measurements of plant emissions can be conducted at different levels, namely from the leaf to whole-plant and canopy level (Harley *et al.*, 1996; Guenther, 2000; Matsunaga *et al.*, 2009; Andrade-Sanchez *et al.*, 2013; Bracho-Nunez *et al.*, 2013; Grote *et al.*, 2013). For these measurements, enclosure chambers fitting the size of the plants or the parts of interest (leaves, branches, flowers, fruits, or roots) are commonly used. However, since these methods give the emission parameters at the leaf to tree scale, the subsequent up-scaling (or down-scaling on the leaf level) can introduce large uncertainties into the results. Besides architecture, aerodynamics, and material, the chamber environment, mixing of the air inside the chamber, and an effective seal are also important for the functionality of the chambers. Emissions of various plant species have been measured with cuvette techniques (e.g. Tingey *et al.*, 1979; Geron *et al.*, 2000; Boissard *et al.*, 2001). Enclosure methods are an important tool for VOC screening from different plant species and for studying the dependencies of VOC emissions on environmental parameters. The need for gas exchange systems has been reported/discussed frequently in the past by various authors (Grabmer *et al.*, 2006; Brilli *et al.*, 2009; Bracho-Nunez *et al.*, 2013; Bourtsoukidis *et al.*, 2014; Egigu *et al.*, 2014). Numerous studies were conducted at the leaf level for calculating the isoprene emissions related to leaf development, light environment, canopy position, and temperature history (e.g. Harley *et al.*, 1996; Sharkey *et al.*, 1996; Geron *et al.*, 2000; Singsaas and Sharkey, 2000; Petron *et al.*, 2001). Many researchers have constructed their own cuvette systems that are suitable for quantitative measurements of VOC emissions from their specific plants (Geron *et al.*, 2000; Singsaas and Sharkey, 2000; Brilli *et al.*, 2009). These cuvettes, constructed in such a way that they can control temperature and light levels, allow for testing of the VOC emissions over a range of conditions (Brilli *et al.*, 2009; Bracho-Nunez *et al.*, 2013). The cuvette system used by Bracho-Nunez *et al.* (2013) was made of fully light-permeable (FEP) Teflon foil and was flushed with ozone-free air. All the sample tubing was made of Teflon and heated at a constant temperature of 45 °C in order to avoid condensation in the lines. The gas exchange systems can be either closed (no purge flow) (Hewitt and Street, 1992) or open (flow-through) (Hayward *et al.*, 2004). In closed systems, due to the photosynthetic uptake, the CO_2 concentration is often decreasing and air temperature can increase dramatically because of the greenhouse heating, which leads to artificial conditions and in turn affects the VOC emissions (Niinemets *et al.*, 2011). Therefore, closed systems should be used only for very short enclosure times. For more accurate VOC emission analysis, an open enclosure system should be used where circulation of air is possible around the leaf, which keeps the climatic conditions such as temperature, water vapour, CO_2 etc., rather constant and closer to ambient levels (Tholl *et al.*, 2006).

For measuring strong leaf-level VOC emissions (e.g. isoprene), the use of commercial portable photosynthesis systems with built-in carbon dioxide and water vapour gas sensors with a compatible leaf chamber with temperature and light control might be the preferred method (e.g. LI-6400 instrument, Li-COR, USA; GFS-3000, Walz, D, Ciras3, PP-systems, US, LCi-SD Photosynthesis system, ADC BioScientific Ltd, UK). Leaf-level cuvette measurements are of biological importance; however, the enclosure time of the leaves should not be longer than several minutes to a few hours, depending on the cuvette size. Leaf enclosure measurements are not suitable for phenotyping responses of genotypes to stress during longer periods (up to several days). Ideally the sampling time with these systems is kept as short as possible in order to avoid changes in plant physiological status during sampling (Niinemets *et al.*, 2011). Besides this, these systems do not enclose the leaves automatically, and are therefore somewhat labour intensive. Therefore, they are not convenient for integration into (automated) phenotyping platforms.

Measurements at the plant level gives a view of the dynamics in the overall plant response under changing climatic conditions or to biotic stress and also permits calculation of a wide range of parameters such as net CO_2, H_2O, VOC emissions, etc. of the whole plant or plant–soil system (Ortega and Helmig, 2008). For measuring plant-level emission rates, chambers are used in which the entire plant can be enclosed. Consequently, enclosure capacities can be very high, which require high flow rates (e.g. 475–525 l min^{-1}) (Kempf *et al.*, 1996) for exchange of the air volume in adequate time, and further attention has to be paid to avoid low biomass/volume ratios which might result in low VOC concentrations. Again, this condition makes quantitative chemical analysis of VOC

emissions more difficult. Another point to consider is that VOC release from the soil can contribute to the overall emissions. To avoid this type of contamination, branch enclosures or whole plant enclosure systems excluding soil and belowground organs must be used. Ghirardo and colleagues (2012) described a cuvette system made of Perspex glass where only the shoot (above ground) was enclosed in the cuvette and the root system (below ground) was left outside to avoid the emissions from soil or roots. The tightening of these cuvettes is rather time consuming (A. Ghirardo, personnel communication) and therefore again not suitable for phenotyping many plants or for the very fast (within minutes) responses to biotic stress application for which the cuvettes have to be opened. However, use of plant cuvettes also allows for application of herbivores within the cuvettes, which is not possible in leaf-level cuvettes. Phenotyping on the plant level would be very slow when performed only with a single cuvette or a set of a few cuvettes; therefore, for medium- to high-throughput VOC phenotyping, development of multiple cuvette systems is recommended.

Challenges of VOC phenotyping

The accurate analysis of VOC emission rates is technically very sensitive, and depends on the analytical instrumentation, the enclosure design, and also on the inertness of materials used for tubing and cuvettes (Tholl *et al.*, 2006; Brilli *et al.*, 2009). Determining which technique to use for detection and analysis of VOCs generally depends on the biological importance and the plant species being investigated. There are several techniques available for measuring plant VOC emissions, from the very simple to the highly advanced (Bicchi and Maffei, 2012). The most popular existing detectors for routine analysis of VOCs are mass spectrometers. In most standard gas chromatographs–mass spectrometers, compounds leaving the gas chromatography (GC) column are ionized by an electron impact (EI) ionization source, resulting in positively charged volatiles, and their fragments are designated according to their mass-to-charge (m/z) ratio by entering a quadrupole ion trap or a quadrupole mass filter. Total ion count chromatograms (TICs) are obtained, which provide information on the retention time of each compound and its mass spectrum consisting of a characteristic ion fragmentation pattern. Moreover, advances in automated analysis of VOCs have allowed the monitoring of fast changes in VOC emissions and facilitated *in vivo* studies of VOC biosynthesis (Tholl *et al.*, 2006).

In comparison, proton-transfer-reaction mass spectrometry (PTR-MS) instruments (Hansel *et al.*, 1995) utilize chemical ionization with swarm-based techniques to enable quantification of trace species with rapid response times and minimizing fragmentation, which results in a less complicated mass spectrum and makes quantification of individual compounds simpler when analysing a mixture of many compounds (Lindinger and Jordan, 1998; Lindinger *et al.*, 1998; Blake *et al.*, 2009). Electronic noses can also achieve rapid sensing of VOCs. Electronic noses (e-noses) are instruments which mimic the sense of smell (Loutfi *et al.*, 2015). Typically, these devices are arrays of sensors that are used to detect and distinguish odours precisely, and at a low cost, in complex samples. VOCs can also be detected by the use of the so-called 'zNose'. The zNose® has a fast analysis time (~3 min for a complete cycle) (Miresmailli *et al.*, 2013) and can deliver measurements with the precision and accuracy of GC. One of the main limitations of the zNose® is that it has a very short column length (1 m), which results in poor peak resolution in samples containing high numbers of compounds (Miresmailli *et al.*, 2010).

The following sections will provide a brief introduction to the different techniques [gas chromatography–mass spectrometry (GC-MS; offline VOC analysis) and PTR-MS (online VOC analysis)] and will discuss the suitability and limitations of each technique for plant VOC phenotyping.

Offline VOC analysis by gas chromatography–mass spectrometry

The detailed quantitative analysis of VOCs in air samples generally depends on GC with flame ionization detection (FID) or with MS. The GC-FID method has been realized comprehensively in the past for quantitative analysis of VOCs in both gaseous and liquid matrices. The more developed GC-MS technique is a powerful tool, as it allows both qualitative and quantitative analysis of VOCs released by plants in different ecological and biological conditions at the same time (Ezquerro *et al.*, 2004; Samuelsson *et al.*, 2006; Demeestere *et al.*, 2008).

The quantification of VOCs by GC-MS can be improved with the help of the following five major approaches (Demeestere *et al.*, 2007; Aeppli *et al.*, 2008): direct injection, immobilized sorbent, cryogenic trapping, solvent extraction, and membrane absorption. The determination of VOCs can be enabled further by headspace (HS), and purge and trap methods (Golfinopoulos *et al.*, 2001; Mounchili *et al.*, 2005; Ojala *et al.*, 2006). One of the benefit of the HS method, assisted by the immobilized sorbent, is that it can efficiently capture extracted compounds from both the gas and liquid phases. The HS method is very valuable as it shortens the pre-treatment process. The HS method has several advantages and can be applied in various ways such as HS–solid phase microextraction (HS-SPME) and HS–solvent microextraction (HS-SME) (Vas and Vékey, 2004; Tholl *et al.*, 2006; Pawliszyn, 2012). The essential considerations to take into account for the development of the SPME technique are the extraction time, the extraction temperature, the amount of sample, and the desorption time and temperature (Isidorov *et al.*, 2003; Sousa *et al.*, 2006). In order to identify VOCs in GC-MS analysis, recommendations can be acquired from standard mass spectral libraries such as Wiley and NIST MS databases.

The sampling and analytical procedures of GC methods can be very time-consuming (Tholl *et al.*, 2006), and mostly they do not allow the simultaneous, time-resolved monitoring of different VOC classes (i.e. isoprenoid hydrocarbons,

C_1–C_2 alcohols and aldehydes, GLVs, and volatile benzoic acid derivatives). Because of these limitations, these techniques are not optimal to assess the high-throughput screening of VOC emissions from a great number of genotypes (e.g. apples, fruits, flowers, and leaf samples in vials) caused by biotic stresses. Therefore, proton-transfer-reaction time of flight mass spectrometry (PTR-TOF-MS) can be used for this purpose, a technique allowing rapid, online detection of trace gases from various chemical groups in the order of seconds at (sub) parts per billion (ppb) levels (Hansel *et al.*, 1995; Boamfa *et al.*, 2004; Blake *et al.*, 2009).

Online VOC analysis by proton-transfer-reaction mass spectrometry

The need for real-time detection and analysis of VOCs has prompted the use of PTR-MS. This is a very sensitive online, non-invasive method, which allows the sensitive assessment (ppt/ppb concentrations) of plant VOCs in real time at high throughput. PTR-MS has become an established technique for the analysis of traces of VOCs and offers many advantages over other conventional analytical methods, such as real-time high-throughput analysis, reduced sample preparation, very low detection limits, high selectivity, and very short response times (Hansel *et al.*, 1995; Lindinger and Jordan, 1998; Tholl *et al.*, 2006).

By combining a compact high resolution time-of-flight detector with the ion source, PTR-MS allows complete and rapid detection of VOCs with a time resolution of <1 s (Graus *et al.*, 2010). A detailed description of PTR-TOF can be found in Jordan *et al.* (2009). It has also been reported that the PTR-MS technique is very successful in validating the time- and light-dependent data (emissions) of various compounds (terpenes, GLVs, methanol, etc.; Graus *et al.*, 2004; Tholl *et al.*, 2006).

However, there are limitations when using the PTR-MS technique; in particular, obtaining speciation for molecules with the same molecular weight (e.g. monoterpenes, *m/z* 137 or sesquiterpenes, *m/z* 205) is not possible because these compounds are identified according to their molecular masses. Furthermore, without enhancement or GC separation, PTR-MS will typically not be as sensitive as what can be attained with 'offline' GC methods. GC techniques are still the most suitable tool for speciated analysis.

Another recent development in PTR-MS analysis of VOCs is the coupling of a multicapillary column (MCC), also known as fast GC, in order to improve the identification of isomers (e.g. ketones, monoterpenes, and sesquiterpenes) (Ruzsanyi *et al.*, 2013). The idea of coupling a PTR-MS instrument to a commercial GC system has been implemented by several other groups (Lindinger *et al.*, 2005). The principle of operation of the MCC coupling with PTR-MS is described elsewhere (Ruzsanyi *et al.*, 2013). The VOC analysis using MCC is carried out in a few minutes, which can be comparable with the efficiency of conventional high-resolution GC on the same matrices (Matisova and Domotorova, 2003). The application of MCC could be considered for high-throughput analyses in VOC metabolite profiling. Nevertheless, it requires some additional non-standard instrumentation to support, for example, fast temperature changes of the column and high inlet pressures (Tholl *et al.*, 2006).

Example of a cuvette system design for plant VOC phenotyping

A phenotyping platform for screening of VOCs as non-invasive indicators of abiotic and biotic stresses requires multiple cuvette systems made of inert material such as glass or special transparent plastics (e.g. Teflon) that (i) avoid chemical contamination from outgassing (Bracho-Nunez *et al.*, 2013; Trowbridge *et al.*, 2014); (ii) prevent losses due to uptake and chemical reactions; and (iii) allow a standardized and defined cultivation of plants and a high-resolution determination of the spatial and temporal induction of VOC emissions (Fig. 2). Since no material is completely inert, it is essential to determine the background emissions from empty cuvettes prior either to and/or after the experiment or simultaneously using an empty cuvette. Here we describe the technical design of a VOC phenotyping platform (Fig. 2) with respect to the actual technical boundaries. Such a potential multiple cuvette system should be gas-tight and a defined (adjustable) gas inflow (and outflow) should be regulated accurately by mass flow controllers (MFCs) before air enters the cuvettes. Flow rates at the outlet of the cuvette towards the detector should be controlled (e.g. by gas flow controllers or rotameters) to check for system leakage. It is recommended to check the cuvette system using standard gas mixtures for measuring the possible uptake of VOCs by the cuvette material and tubing (de Gouw and Warneke, 2007). For such a system, long tubing is needed, which can lead to memory effects and VOC contamination. To reduce memory effects, heating or flushing of the lines/tubing will be preferablem as used by Ghirardo *et al.* (2011). On a smaller scale, Ghirardo *et al.* (2011) performed an experiment with poplar trees using four cuvettes that were run in parallel and the outlet of each cuvette (~750 ml volume) was connected to electronic valves switching automatically between the cuvettes. The airflow (synthetic, VOC-free and humidified air) in this set-up was 2 l min^{-1} for each cuvette and all the tubing used for the experiment was made of Teflon. The Teflon line/tubing used in this system was isolated and, to reduce or avoid memory effects, the data of the first few seconds were omitted and the remaining data were used for the averages (Ghirardo *et al.*, 2011). Bracho-Nunez and colleagues (2013) used two identical branch cuvettes system. One cuvette was operated as the reference 'empty' cuvette and the second one contained the complete above-ground plant. These authors used two different sizes of the cuvette systems (volumes of 9 l and 100 l), depending on the plant size. The respective flow for the cuvettes was adjusted to 10 l min^{-1} for small cuvettes and 20–40 l min^{-1} for the larger cuvettes.

The airflow established through the enclosure is critical for regulating temperature and relative humidity and ensuring photosynthetic gas exchange between the plant tissue and the surrounding air. In order to avoid cold spots or condensation

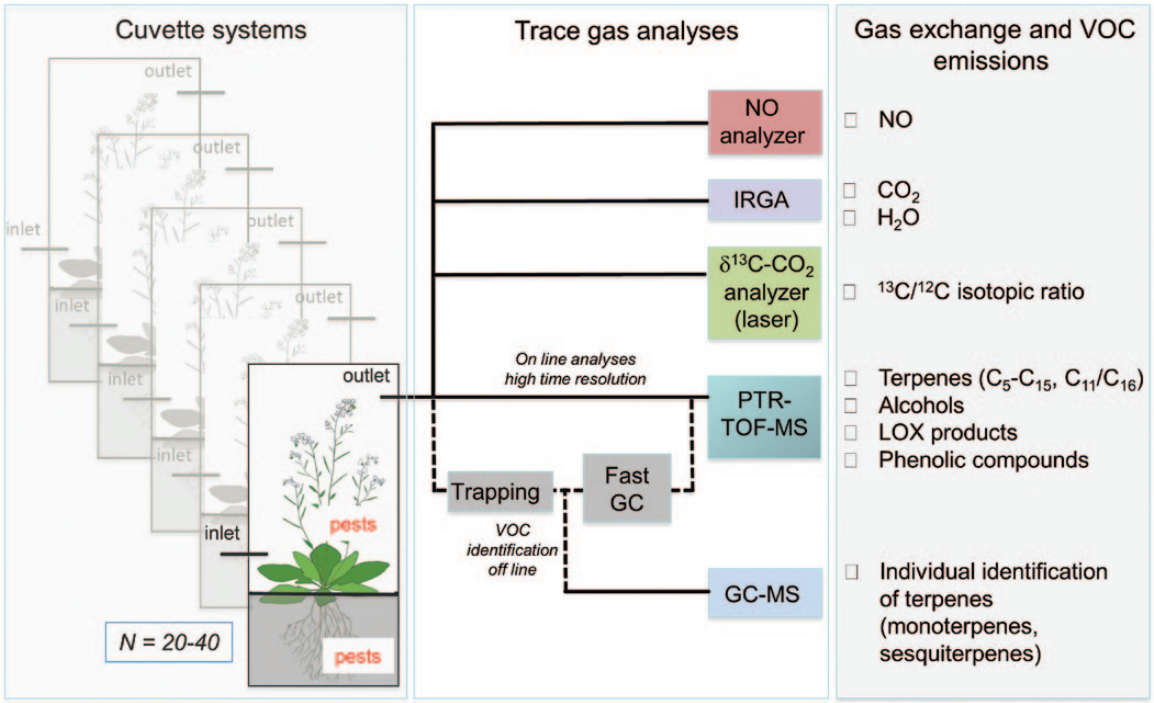

Fig. 2. Schematic illustration of a potential plant VOC phenotyping platform: a medium-throughput (20–40) multiple cuvette system (MCS) where time series (2 h time resolution) measurements in real time of many plants can be possible for VOC emissions, photosynthetic CO_2/H_2O gas exchange, temperature, and humidity (air and soil).

in the cuvette, sufficient flow is needed to refresh the gas content in a relatively short time, preventing air stagnation and CO_2 depletion. Higher flow rates are also needed to reduce a possible overheating of the ambient cuvette air from the irradiation system. For the adjustment of the ideal flow rates for the recording of VOC emission, the cuvette size is the critical parameter. For homogenous gas flow inside the cuvette, it might be useful to install fans circulating the air to ensure adequate mixing and uniform VOC concentrations within the cuvettes (Bracho-Nunez *et al.*, 2013; Trowbridge *et al.*, 2014). However, operating a fan inside the cuvette will result in difficulties in estimating the correct VOC mixing ratios since the material from which fans are usually made also emits VOCs. To overcome this problem, Teflon-coated fans can be used. In the proposed design, homogeneous air mixing is achieved by a porous ring releasing a homogenous airflow around the plant. It is essential to monitor the environmental parameters air temperature, relative humidity, and irradiance. These sensors must be placed inside the cuvette, but here also care has to be taken as the material of which the sensors are made can also emit VOCs. Additionally the use of controlled climate chambers or greenhouse cabins might be beneficial to place the VOC phenotyping system under controlled and reproducible climatic conditions. When using lamps for plant irradiation, overheating can be avoided by placing filters or a layer of water in transparent containers between the lamp system and the cuvette blocks to reduce infrared radiation.

The whole plant's gas exchange (e.g. VOCs, CO_2, H_2O, and NOx, depending on the gas of interest) can be achieved by parallel online analysis of air samples with different gas analysers. In parallel to PTR-MS (coupled with fast GC) for measuring the VOCs, an infrared gas analyser (IRGA) can be used to monitor CO_2/H_2O exchange for the determination of net CO_2 assimilation, transpiration, and stomatal conductance. The additional analysis of NO emissions (Wildt *et al.*, 2012) also provides information on plant atmosphere gas exchange that can be gained from phenotypes with different sensitivities to (a)biotic stressors. By additional VOC sampling using solid adsorbent and subsequent offline determination by GC-MS (qualitatively and quantitatively), the information on the phenotype VOC emission will be amended (Tholl *et al.*, 2006). Such a multiple cuvette system (Fig. 2) will be beneficial for medium- (a dozens to a hundred individuals) throughput plant VOC phenotyping. High-throughput phenotyping (>100 individuals) of plant VOCs can be performed by collecting biological samples prior to the measurement and transferring them into steady-state vials (e.g. apples, flowers, and leaf samples) where VOCS can accumulate in the headspace. However, there are certain limitations with phenotyping platforms, such as the number of samples that can be measured in parallel, speed, and costs (PTR-MS, GC-MS) (e.g. Farneti *et al.*, 2014).

Outlook

The present review illustrates the potential of the analysis of plant VOC emissions for suitability in plant phenotyping. Plant VOC emissions are highly variable within and between species, making them interesting as non-invasive markers, in particular in response to abiotic and biotic constraints. Moreover, plant VOC profiles differ significantly between organs (shoots, leaves, flowers, or fruits), possibly allowing the

screening of distinct traits, such as odour profiles of flowers and fruits or different responses of leaves to biotic stressors.

However, the biggest challenge for using VOCs as biomarkers for plant phenotyping under biotic stress is the high temporal and spatial variability of these emissions and the influence of the abiotic environment (temperature, light intensity, water and nutrient availability, etc.) on it.

Phenotypic variations in VOC emissions might be masked by differences in growth conditions or the presence of undetected diseases (e.g. below-ground; Peñuelas *et al.*, 2014) or endophytes (Li *et al.*, 2014) influencing the overall plant response and VOC emissions.

Therefore, it is necessary to combine plant VOC phenotyping with other non-invasive imaging tools (i.e. fluorescence spectroscopy, visible–infrared spectroscopy, fluorescence imaging, and hyperspectral imaging; Rascher *et al.*, 2011; Fiorani *et al.*, 2012; Mahlein *et al.*, 2012a, b) under highly controlled conditions to make sure that the observed phenotypic variations in VOC emissions are not affected by changes in the environment. Gratani (2014) cited the phenotypic plasticity (physiological, anatomical, and morphological) as the key factor that allows plants to grow under miscellaneous environmental conditions.

Acknowledgements

This work was performed within the German-Plant-Phenotyping Network (Deutsches-Pflanzen-Phänotypisierung-Netzwerk; DPPN), which is funded by the German Federal Ministry of Education and Research BMBF (project identification no. 031A053). We thank Amy Trowbridge for constructive comments on the manuscript.

References

Aeppli C, Berg M, Hofstetter TB, Kipfer R, Schwarzenbach RP. 2008. Simultaneous quantification of polar and non-polar volatile organic compounds in water samples by direct aqueous injection-gas chromatography/mass spectrometry. *Journal of Chromatography A* **1181,** 116–124.

Agger S, Lopez-Gallego F, Schmidt-Dannert C. 2009. Diversity of sesquiterpene synthases in the basidiomycete *Coprinus cinereus*. *Molecular Microbiology* **72,** 1181–1195.

Alves EG, Harley P, Goncalves JFD, Moura CED, Jardine K. 2014. Effects of light and temperature on isoprene emission at different leaf developmental stages of *Eschweilera coriacea* in central Amazon. *Acta Amazonica* **44,** 9–18.

Andrade-Sanchez P, Gore MA, Heun JT, Thorp KR, Carmo-Silva AE, French AN, Salvucci ME, White JW. 2013. Development and evaluation of a field-based high-throughput phenotyping platform. *Functional Plant Biology* **41,** 68–79.

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

Baldwin IT, Preston CA. 1999. The eco-physiological complexity of plant responses to insect herbivores. *Planta* **208,** 137–145.

Ballhorn DJ, Kautz S, Jensen M, Schmitt I, Heil M, Hegeman AD. 2011. Genetic and environmental interactions determine plant defences against herbivores. *Journal of Ecology* **99,** 313–326.

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: relationships between ozone uptake and emission of LOX products. *Plant, Cell and Environment* **28,** 1334–1343.

Bicchi C, Maffei M. 2012. The plant volatilome: methods of analysis. *Methods in Molecular Biology* **918,** 289–310.

Blake RS, Monks PS, Ellis AM. 2009. Proton-transfer reaction mass spectrometry. *Chemical Reviews* **109,** 861–896.

Boamfa EI, Steeghs MML, Cristescu SM, Harren FJM. 2004. Trace gas detection from fermentation processes in apples; an intercomparison study between proton-transfer-reaction mass spectrometry and laser photoacoustics. *International Journal of Mass Spectrometry* **239,** 193–201.

Boissard C, Cao XL, Juan CY, Hewitt CN, Gallagher M. 2001. Seasonal variations in VOC emission rates from gorse (*Ulex europaeus*). *Atmospheric Environment* **35,** 917–927.

Bourtsoukidis E, Bonn B, Noe SM. 2014. On-line field measurements of BVOC emissions from Norway spruce (*Picea abies*) at the hemiboreal SMEAR-Estonia site under autumn conditions. *Boreal Environment Research* **19,** 153–167.

Bracho-Nunez A, Knothe NM, Costa WR, Maria Astrid LR, Kleiss B, Rottenberger S, Piedade MTF, Kesselmeier J. 2012. Root anoxia effects on physiology and emissions of volatile organic compounds (VOC) under short- and long-term inundation of trees from Amazonian floodplains. *SpringerPlus* **1,** 9.

Bracho-Nunez A, Knothe NM, Welter S, Staudt M, Costa WR, Liberato MAR, Piedade MTF, Kesselmeier J. 2013. Leaf level emissions of volatile organic compounds (VOC) from some Amazonian and Mediterranean plants. *Biogeosciences* **10,** 5855–5873.

Brilli F, Ciccioli P, Frattoni M, Prestininzi M, Spanedda AF, Loreto F. 2009. Constitutive and herbivore-induced monoterpenes emitted by *Populus×euroamericana* leaves are key volatiles that orient *Chrysomela populi* beetles. *Plant, Cell and Environment* **32,** 542–552.

Cabrera-Bosquet L, Crossa J, von Zitzewitz J, Serret MD, Luis Araus J. 2012. High-throughput phenotyping and genomic selection: the frontiers of crop breeding converge. *Journal of Integrative Plant Biology* **54,** 312–320.

Cardoza YJ, Alborn HT, Tumlinson JH. 2002. *In vivo* volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage. *Journal of Chemical Ecology* **28,** 161–174.

Clavijo McCormick AL, Irmisch S, Reinecke R, Boeckler GA, Veit D, Reichelt M, Hansson BS, Gershenzon J, Köllner TG, Unsicker SB. 2014. Herbivore-induced volatile emission in black poplar—regulation and role in attracting herbivore enemies. *Plant, Cell and Environment* **37,** 1909–1923.

Cobb J, DeClerck G, Greenberg A, Clark R, McCouch S. 2013. Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. *Theoretical and Applied Genetics* **126,** 867–887.

Cramer GR. 2010. Abiotic stress and plant responses from the whole vine to the genes. *Australian Journal of Grape and Wine Research* **16,** 86–93.

Degen T, Dillmann C, Marion-Poll F, Turlings TCJ. 2004. High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. *Plant Physiology* **135,** 1928–1938.

De Gouw J, Warneke C. 2007. Measurements of volatile organic compounds in the earth's atmosphere using proton-transfer-reaction mass spectrometry. *Mass Spectrometry Reviews* **26,** 223–257.

Delphia CM, Rohr JR, Stephenson AG, De Moraes CM, Mescher MC. 2009. Effects of genetic variation and inbreeding on volatile production in a field population of horsenettle. *International Journal of Plant Sciences* **170,** 12–20.

Demeestere K, Dewulf J, De Roo K, De Wispelaere P, Van Langenhove H. 2008. Quality control in quantification of volatile organic compounds analysed by thermal desorption-gas chromatography-mass spectrometry. *Journal of Chromatography A* **1186,** 348–357.

Demeestere K, Dewulf J, De Witte B, Van Langenhove H. 2007. Sample preparation for the analysis of volatile organic compounds in air and water matrices. *Journal of Chromatography A* **1153,** 130–144.

De Moraes CM, Lewis WJ, Tumlinson JH. 2000. Examining plant–parasitoid interactions in tritrophic systems. *Anais da Sociedade Entomológica do Brasil* **29,** 189–203.

Dicke M. 1999. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Experimentalis et Applicata* **96,** 131–142.

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.

Donovan LA, Ludwig F, Rosenthal DM, Rieseberg LH, Dudley SA. 2009. Phenotypic selection on leaf ecophysiological traits in Helianthus. *New Phytologist* **183,** 868–879.

Dudareva N, Negre F, Nagegowda DA, Orlova I. 2006. Plant volatiles: recent advances and future perspectives. *Critical Reviews in Plant Sciences* **25,** 417–440.

Dudareva N, Pichersky ED. 2000. Biochemical and molecular genetic aspects of floral scents. *Plant Physiology* **122,** 627–633.

Dugardeyn J, Van Der Straeten D. 2008. Ethylene: fine-tuning plant growth and development by stimulation and inhibition of elongation. *Plant Science* **175,** 59–70.

Duhl TR, Helmig D, Guenther A. 2008. Sesquiterpene emissions from vegetation: a review. *Biogeosciences* **5,** 761–777.

Ebel RC, Mattheis JP, Buchanan DA. 1995. Drought stress of apple trees alters leaf emissions of volatile compounds. *Physiologia Plantarum* **93,** 709–712.

Egigu MC, Ibrahim MA, Riikonen J, Yahya A, Holopainen T, Julkunen-Tiitto R, Holopainen JK. 2014. Effects of rising temperature on secondary compounds of Yeheb (*Cordeauxia edulis* Hemsley). *American Journal of Plant Sciences* **5,** 517–527.

Eller ASD, de Gouw J, Graus M, Monson RK. 2012. Variation among different genotypes of hybrid poplar with regard to leaf volatile organic compound emissions. *Ecological Applications* **22,** 1865–1875.

Ezquerro O, Ortiz G, Pons B, Tena MT. 2004. Determination of benzene, toluene, ethylbenzene and xylenes in soils by multiple headspace solid-phase microextraction. *Journal of Chromatography A* **1035,** 17–22.

Fall R. 1999. Biogenic emissions of volatile organic compounds from higher plants In: Hewitt CN, ed. *Reactive hydrocarbons in the atmosphere* . New York: Academic Press, 41–96.

Farmer EE, Ryan CA. 1990. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences, USA* **87,** 7713–7716.

Farneti B, Cristescu SM, Costa G, Harren FJM, Woltering EJ. 2012. Rapid tomato volatile profiling by using proton-transfer reaction mass spectrometry (PTR-MS). *Journal of Food Science* **77,** 551–559.

Farneti B, Khomenko I, Cappellin L, Ting V, Romano A, Biasioli F, Costa G, Costa F. 2014. Comprehensive VOC profiling of an apple germplasm collection by PTR-ToF-MS. *Metabolomics* (in press).

Fineschi S, Loreto F. 2012. Leaf volatile isoprenoids: an important defensive armament in forest tree species. *IForest* **5,** 13–17.

Fiorani F, Rascher U, Jahnke S, Schurr U. 2012. Imaging plants dynamics in heterogenic environments. *Current Opinion in Biotechnology* **23,** 227–235.

Fuentes JD, Lerdau M, Atkinson R, *et al*. 2000. Biogenic hydrocarbons in the atmospheric boundary layer: a review. *Bulletin of the American Meteorological Society* **81,** 1537–1575.

Geron C, Guenther A, Sharkey T, Arnts RR. 2000. Temporal variability in basal isoprene emission factor. *Tree Physiology* **20,** 799–805.

Gershenzon J, McConkey ME, Croteau RB. 2000. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiology* **122,** 205–214.

Ghirardo A, Gutknecht J, Zimmer I, Brüggemann N, Schnitzler JP. 2011. Biogenic volatile organic compound and respiratory CO_2 emissions after ^{13}C-labeling: online tracing of C translocation dynamics in poplar plants. *PLoS One* **6,** e17393.

Ghirardo A, Heller W, Fladung M, Schnitzler JP, Schroeder H. 2012. Function of defensive volatiles in pedunculate oak (*Quercus robur*) is tricked by the moth *Tortrix viridana*. *Plant, Cell and Environment* **35,** 2192–2207.

Gioacchini AM, Menotta M, Guescini M, Saltarelli R, Ceccaroli P, Amicucci A, Barbieri E, Giomaro G, Stocchi V. 2008. Geographical traceability of Italian white truffle (Tuber magnatum Pico) by the analysis of volatile organic compounds. *Rapid Communications in Mass Spectrometry* **22,** 3147–3153.

Golfinopoulos SK, Lekkas TD, Nikolaou AD. 2001. Comparison of methods for determination of volatile organic compounds in drinking water. *Chemosphere* **45,** 275–284.

Gouinguene S, Degen T, Turlings TCJ. 2001. Variability in herbivore-induced odour emissions among maize cultivars and their wild ancestors (teosinte). *Chemoecology* **11,** 9–16.

Gouinguené SP, Turlings TCJ. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology* **129,** 1296–1307.

Grabmer W, Kreuzwieser J, Wisthaler A, Cojocariu C, Graus M, Rennenberg H, Steigner D, Steinbrecher R, Hansel A. 2006. VOC emissions from Norway spruce (*Picea abies* L. Karst) twigs in the field—results of a dynamic enclosure study. *Atmospheric Environment* **40,** S128–S137.

Granier C, Aguirrezabal L, Chenu K, *et al*. 2006. PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytologist* **169,** 623–635.

Granier C, Vile D. 2014. Phenotyping and beyond: modelling the relationships between traits. *Current Opinion in Plant Biology* **18,** 96–102.

Gratani L. 2014. Plant phenotypic plasticity in response to environmental factors. *Advances in Botany ID* **208747,** 17.

Graus M, Müller M, Hansel A. 2010. High resolution PTR-TOF: quantification and formula confirmation of VOC in real time. *Journal of the American Society for Mass Spectrometry* **21,** 1037–1044.

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.

Grote R, Monson R, Niinemets Ü. 2013. Leaf-level models of constitutive and stress-driven volatile organic compound emissions. In: Niinemets Ü, Monson RK, eds. *Biology, controls and models of tree volatile organic compound emissions* . Dordrecht: Springer, 315–355.

Guenther A. 2000. Natural emissions of non-methane volatile organic compounds, carbon monoxide, and oxides of nitrogen from North America. *Atmospheric Environment* **34,** 2205–2230.

Guenther A, Hewitt CN, Erickson D, *et al*. 1995. A global model of natural volatile organic compound emissions. *Journal of Geophysical Research: Atmospheres* **100,** 8873–8892.

Hakola H, Tarvainen V, Bäck J, Ranta H, Bonn B, Rinne J, Kulmala M. 2005. Seasonal variation of mono- and sesquiterpene emission rates of Scots pine. *Biogeosciences Discussions* **2,** 1697–1717.

Hansel A, Jordan A, Holzinger R, Prazeller P, Vogel W, Lindinger W. 1995. Proton transfer reaction mass spectrometry: on-line trace gas analysis at the ppb level. *International Journal of Mass Spectrometry and Ion Processes* **149–150,** 609–619.

Hare JD. 2007. Variation in herbivore and methyl jasmonate-induced volatiles among genetic lines of *Datura wrightii*. *Journal of Chemical Ecology* **33,** 2028–2043.

Harley P, Guenther A, Zimmerman P. 1996. Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. *Tree Physiology* **16,** 25–32.

Hartmann A, Czauderna T, Hoffmann R, Stein N, Schreiber F. 2011. HTPheno: an image analysis pipeline for high-throughput plant phenotyping. *BMC Bioinformatics* **12,** 9.

Hayward S, Tani A, Owen SM, Hewitt CN. 2004. Online analysis of volatile organic compound emissions from Sitka spruce (*Picea sitchensis*). *Tree Physiology* **24,** 721–728.

Heiden AC, Hoffmann T, Kahl J, *et al*. 1999. Emission of volatile organic compounds from ozone-exposed plants. *Ecological Applications* **9,** 1160–1167.

Hewitt CN, Street RA. 1992. A qualitative assessment of the emission of non-methane hydrocarbon compounds from the biosphere to the atmosphere in the U.K.: present knowledge and uncertainties. *Atmospheric Environment. Part A. General Topics* **26,** 3069–3077.

Holopainen JK. 2011. Can forest trees compensate for stress-generated growth losses by induced production of volatile compounds? *Tree Physiology* **31,** 1356–1377.

Holopainen JK, Blande JD. 2012. Molecular plant volatile communication. *Advances in Experimental Medicine and Biology* **739,** 17–31.

Holopainen JK, Blande JD. 2013. Where do herbivore-induced plant volatiles go? *Frontiers in Plant Science* **4,** 185.

Holopainen JK, Heijari J, Oksanen E, Alessio GA. 2010. Leaf volatile emissions of *Betula pendula* during autumn coloration and leaf fall. *Journal of Chemical Ecology* **36,** 1068–1075.

Howe GA, Jander G. 2008. Plant immunity to insect herbivores. *Annual Review of Plant Biology* **59,** 41–66.

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.

Hüve K, Christ MM, Kleist E, Uerlings R, Niinemets Ü, Walter A, Wildt J. 2007. Simultaneous growth and emission measurements demonstrate an interactive control of methanol release by leaf expansion and stomata. *Journal of Experimental Botany* **58,** 1783–1793.

Iriti M, Faoro F. 2009. Chemical diversity and defence metabolism: how plants cope with pathogens and ozone pollution. *International Journal of Molecular Sciences* **10,** 3371–3399.

Irmisch S, Clavijo McCormick A, Günther J, Schmidt A, Boeckler GA, Gershenzon J, Unsicker SB, Köllner TG. 2014. Herbivore-induced poplar cytochrome P450 enzymes of the CYP71 family convert aldoximes to nitriles which repel a generalist caterpillar. *The Plant Journal* **80,** 1095–1107.

Isidorov VA, Vinogorova VT, Rafalowski K. 2003. HS-SPME analysis of volatile organic compounds of coniferous needle litter. *Atmospheric Environment* **37,** 4645–4650.

Johannsen W. 1909. *Elemente der exakten erblichkeitslehre. Deutsche wesentlich erweiterte ausgabe in fünfundzwanzig vorlesungen, von W. Johannsen* . Jena: G. Fischer.

Jordan A, Haidacher S, Hanel G, Hartungen E, Mark L, Seehauser H, Schottkowsky R, Sulzer P, Mark TD. 2009. A high resolution and high sensitivity proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF-MS). *International Journal of Mass Spectrometry* **286,** 122–128.

Kariyat RR, Mauck KE, De Moraes CM, Stephenson AG, Mescher MC. 2012. Inbreeding alters volatile signalling phenotypes and influences tri-trophic interactions in horsenettle (*Solanum carolinense* L.). *Ecology Letters* **15,** 301–309.

Kelsey R, Joseph G. 1997. Ambrosia beetle host selection among logs of Douglas fir, Western hemlock, and Western red cedar with different ethanol and α-pinene concentrations. *Journal of Chemical Ecology* **23,** 1035–1051.

Kempf K, Allwine E, Westberg H, Claiborn C, Lamb B. 1996. Hydrocarbon emissions from spruce species using environmental chamber and branch enclosure methods. *Atmospheric Environment* **30,** 1381–1389.

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

Köllner TG, Held M, Lenk C, Hiltpold I, Turlings TCJ, Gershenzon J, Degenhardt J. 2008. A maize (E)-beta-caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *The Plant Cell* **20,** 482–494.

Kreuzwieser J, Kuhnemann F, Martis A, Rennenberg H, Urban W. 2000. Diurnal pattern of acetaldehyde emission by flooded poplar trees. *Physiologia Plantarum* **108,** 79–86.

Kuhn U, Rottenberger S, Biesenthal T, Wolf A, Schebeske G, Ciccioli P, Brancaleoni E, Frattoni M, Tavares TM, Kesselmeier J. 2004. Seasonal differences in isoprene and light-dependent monoterpene emission by Amazonian tree species. *Global Change Biology* **10,** 663–680.

Langenheim JH. 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. *Journal of Chemical Ecology* **20,** 1223–1280.

Laothawornkitkul J, Taylor JE, Paul ND, Hewitt CN. 2009. Biogenic volatile organic compounds in the Earth system. *New Phytologist* **183,** 27–51.

Lee K, Seo PJ. 2014. Airborne signals from salt-stressed Arabidopsis plants trigger salinity tolerance in neighboring plants. *Plant Signaling and Behavior* **9,** e28392.

Lee SJ, Warnick TA, Leschine SB, Hazen SP. 2012. High-throughput phenotyping in plants. *Methods in Molecular Biology* **918,** 341–349.

Leitner M, Kaiser R, Rasmussen MO, Driguez H, Boland W, Mithofer A. 2008. Microbial oligosaccharides differentially induce volatiles and signalling components in *Medicago truncatula*. *Phytochemistry* **69,** 2029–2040.

Li T, Blande JD, Gundel PE, Helander M, Saikkonen K. 2014. Epichloë endophytes alter inducible indirect defences in host grasses. *PLoS One* **9,** e101331.

Li Z, Sharkey T. 2013. Molecular and pathway controls on biogenic volatile organic compound emissions. In: Niinemets Ü, Monson RK, eds. *Biology, controls and models of tree volatile organic compound emissions* . Dordrecht: Springer, 119–151.

Lindinger W, Hansel A, Jordan A. 1998. On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) medical applications, food control and environmental research. *International Journal of Mass Spectrometry and Ion Processes* **173,** 191–241.

Lindinger W, Jordan A. 1998. Proton-transfer-reaction mass spectrometry (PTR–MS): on-line monitoring of volatile organic compounds at pptv levels. *Chemical Society Reviews* **27,** 347.

Lindinger C, Pollien P, Ali S, Yeretzian C, Blank I, Mark T. 2005. Unambiguous identification of volatile organic compounds by proton-transfer reaction mass spectrometry coupled with GC/MS. *Analytical Chemistry* **77,** 4117–4124.

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,** 267–275.

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 TCJ. 2014. Plant volatiles and the environment. *Plant, Cell and Environment* **37,** 1905–1908.

Loreto F, Schnitzler JP. 2010. Abiotic stresses and induced BVOCs. *Trends in Plant Science* **15,** 154–166.

Loutfi A, Coradeschi S, Mani GK, Shankar P, Rayappan JBB. 2015. Electronic noses for food quality: a review. *Journal of Food Engineering* **144,** 103–111.

Mahlein AK, Oerke EC, Steiner U, Dehne HW. 2012*a*. Recent advances in sensing plant diseases for precision crop protection. *European Journal of Plant Pathology* **133,** 197–209.

Mahlein AK, Steiner U, Hillnhutter C, Dehne HW, Oerke EC. 2012*b*. Hyperspectral imaging for small-scale analysis of symptoms caused by different sugar beet diseases. *Plant Methods* **8,** 1–13.

Markovic I, Norris DM, Phillips JK, Webster FX. 1996. Volatiles involved in the nonhost rejection of *Fraxinus pennsylvanica* by *Lymantria dispar* larvae. *Journal of Agricultural and Food Chemistry* **44,** 929–935.

Matisova E, Domotorova M. 2003. Fast gas chromatography and its use in trace analysis. *Journal of Chromatography A* **1000,** 199–221.

Matsunaga SN, Guenther AB, Greenberg JP, Potosnak M, Papiez M, Hiura T, Kato S, Nishida S, Harley P, Kajii Y. 2009. Leaf level emission measurement of sesquiterpenes and oxygenated sesquiterpenes from desert shrubs and temperate forest trees using a liquid extraction technique. *Geochemical Journal* **43,** 179–189.

Mattiacci L, Dicke M, Posthumus M. 1994. Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. *Journal of Chemical Ecology* **20,** 2229–2247.

Miresmailli S, Bradbury R, Isman MB. 2010. Qualitative assessment of an ultra-fast portable gas chromatograph (zNose®) for analyzing volatile organic chemicals and essential oils in laboratory and greenhouses. *Arthropod-Plant Interactions* **4,** 175–180.

Miresmailli S, Zeri M, Zangerl AR, Bernacchi CJ, Berenbaum MR, DeLucia EH. 2013. Impacts of herbaceous bioenergy crops on atmospheric volatile organic composition and potential consequences for global climate change. *Global Change Biology Bioenergy* **5,** 375–383.

Mita E, Tsitsimpikou C, Tsiveleka L, Petrakis P V, Ortiz A, Vagias C, Roussis V. 2002. Seasonal variation of oleoresin terpenoids from *Pinus halepensis* and *Pinus pinea* and host selection of the scale insect *Marchalina hellenica* (Homoptera, Coccoidea, Margarodidae, Coelostonidiinae). *Holzforschung* **56,** 572–578.

Monson R. 2013. Metabolic and gene expression controls on the production of biogenic volatile organic compounds. In: Niinemets Ü, Monson RK, eds. *Biology, controls and models of tree volatile organic compound emissions* . Dordrecht: Springer, 153–179.

Monson RK, Grote R, Niinemets U, Schnitzler JP. 2012. Modeling the isoprene emission rate from leaves. *New Phytologist* **195,** 541–559.

Monson RK, Lerdau MT, Sharkey TD, Schimel DS, Fall R. 1995. Biological aspects of constructing volatile organic compound emission inventories. *Atmospheric Environment* **29,** 2989–3002.

Mounchili A, Wichtel JJ, Bosset JO, Dohoo IR, Lmhof M, Altieri D, Mallia S, Stryhn H. 2005. HS-SPME gas chromatographic characterization of volatile compounds in milk tainted with off-flavour. *International Dairy Journal* **15,** 1203–1215.

Müller A, Faubert P, Hagen M, Zu Castell W, Polle A, Schnitzler JP, Rosenkranz M. 2013. Volatile profiles of fungi—chemotyping of species and ecological functions. *Fungal Genetics and Biology* **54,** 25–33.

Mumm R, Hilker M. 2006. Direct and indirect chemical defence of pine against folivorous insects. *Trends in Plant Science* **11,** 351–358.

Mumm R, Schrank K, Wegener R, Schulz S, Hilker M. 2003. Chemical analysis of volatiles emitted by *Pinus sylvestris* after induction by insect oviposition. *Journal of Chemical Ecology* **29,** 1235–1252.

Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski CL, Fall R. 1995. Methanol emission from leaves (enzymatic detection of gas-phase methanol and relation of methanol fluxes to stomatal conductance and leaf development). *Plant Physiology* **108,** 1359–1368.

Nerg A, Kainulainen P, Vuorinen M, Hanso M, Holopainen JK, Kurkela T. 1994. Seasonal and geographical variation of terpenes, resin acids and total phenolics in nursery grown seedlings of Scots pine (*Pinus sylvestris* L.). *New Phytologist* **128,** 703–713.

Niinemets U, Arneth A, Kuhn U, Monson RK, Penuelas J, Staudt M. 2010. 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.

Niinemets U, Kuhn U, Harley PC, *et al*. 2011. Estimations of isoprenoid emission capacity from enclosure studies: measurements, data processing, quality and standardized measurement protocols. *Biogeosciences* **8,** 2209–2246.

Niinemets U, Loreto F, Reichstein M. 2004. Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends in Plant Science* **9,** 180–186.

Ojala S, Lassi U, Keiski RL. 2006. Testing VOC emission measurement techniques in wood-coating industrial processes and developing a cost-effective measurement methodology. *Chemosphere* **62,** 113–120.

Ortega J, Helmig D. 2008. Approaches for quantifying reactive and low-volatility biogenic organic compound emissions by vegetation enclosure techniques–Part A. *Chemosphere* **72,** 343–364.

Panguluri SK, Kumar AA. 2013. *Phenotyping for plant breeding: applications of phenotyping methods for crop improvement* . New York: Springer.

Paré PW, Tumlinson JH. 1997. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology* **114,** 1161–1167.

Paré PW, Tumlinson JH. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiology* **121,** 325–331.

Pawliszyn J. 2012. Theory of solid-phase microextraction. In: Pawliszyn J, ed. *Handbook of solid phase microextraction* . Oxford: Elsevier 13–59.

Peñuelas J, Asensio D, Tholl D, Wenke K, Rosenkranz M, Piechulla B, Schnitzler JP. 2014. Biogenic volatile emissions from the soil. *Plant, Cell and Environment* **37,** 1866–1891.

Peñuelas J, Llusia J. 2001. The complexity of factors driving volatile organic compound emissions by plants. *Biologia Plantarum* **44,** 481–487.

Petron G, Harley P, Greenberg J, Guenther A. 2001. Seasonal temperature variations influence isoprene emission. *Geophysical Research Letters* **28,** 1707–1710.

Pichersky E, Gershenzon J. 2002. The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology* **5**. 237–243.

Pio CA, Silva PA, Cerqueira MA, Nunes TV. 2005. Diurnal and seasonal emissions of volatile organic compounds from cork oak (*Quercus suber*) trees. *Atmospheric Environment* **39,** 1817–1827.

Poorter H, Fiorani F, Stitt M, *et al*. 2012. The art of growing plants for experimental purposes: a practical guide for the plant biologist. *Functional Plant Biology* **39,** 821–838.

Poorter H, Niinemets Ü, Walter A, Fiorani F, Schurr U. 2010. A method to construct dose–response curves for a wide range of environmental factors and plant traits by means of a meta-analysis of phenotypic data. *Journal of Experimental Botany* **61,** 2043–2055.

Powell JS, Raffa KF. 1999. Sources of variation in concentration and composition of foliar monoterpenes in tamarack (*Larix laricina*) seedlings: roles of nutrient availability, time of season, and plant architecture. *Journal of Chemical Ecology* **25,** 1771–1797.

Rascher U, Blossfeld S, Fiorani F, *et al*. 2011. Non-invasive approaches for phenotyping of enhanced performance traits in bean. *Functional Plant Biology* **38,** 968–983.

Regnard JL, Ducrey M, Porteix E, Segura V, Costes E. 2008. Phenotyping apple progeny for ecophysiological traits: how and what for? *Acta Horticulturae* **772,** 151–158.

Ruzsanyi V, Fischer L, Herbig J, Ager C, Amann A. 2013. Multi-capillary-column proton-transfer-reaction time-of-flight mass spectrometry. *Journal of Chromatography A* **1316,** 112–118.

Samuelsson R, Nilsson C, Burvall J. 2006. Sampling and GC-MS as a method for analysis of volatile organic compounds (VOC) emitted during oven drying of biomass materials. *Biomass and Bioenergy* **30,** 923–928.

Schmidt A, Nagel R, Krekling T, Christiansen E, Gershenzon J, Krokene P. 2011. Induction of isoprenyl diphosphate synthases, plant hormones and defense signalling genes correlates with traumatic resin duct formation in Norway spruce (*Picea abies*). *Plant Molecular Biology* **77,** 577–590.

Sharkey TD, Singsaas EL, Vanderveer PJ, Geron C. 1996. Field measurements of isoprene emission from trees in response to temperature and light. *Tree Physiology* **16,** 649–654.

Sharkey T, Yeh S. 2001. Isoprene emission from plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **52,** 407–436.

Shao M, Czapiewski KV, Heiden AC, Kobel K, Komenda M, Koppmann R, Wildt J. 2001. Volatile organic compound emissions from Scots pine: mechanisms and description by algorithms. *Journal of Geophysical Research* **106,** 20483.

Singsaas EL, Sharkey TD. 2000. The effects of high temperature on isoprene synthesis in oak leaves. *Plant, Cell and Environment* **23,** 751–757.

Skirycz A, Inzé D. 2010. More from less: plant growth under limited water. *Current Opinion in Biotechnology* **21,** 197–203.

Snoeren TAL, Kappers IF, Broekgaarden C, Mumm R, Dicke M, Bouwmeester HJ. 2010. Natural variation in herbivore-induced volatiles in *Arabidopsis thaliana*. *Journal of Experimental Botany* **61,** 3041–3056.

Sousa ET, Rodrigues FD, Martins CC, de Oliveira FS, Pereira PAD, de Andrade JB. 2006. Multivariate optimization and HS-SPME/GC-MS analysis of VOCs in red, yellow and purple varieties of *Capsicum chinense* sp peppers. *Microchemical Journal* **82,** 142–149.

Spinelli F, Cellini A, Marchetti L. 2011. Emission and function of volatile organic compounds in response to abiotic stress. In: Shanker A, ed. *Abiotic stress in plants—mechanisms and adaptations* . InTech.

Splivallo R, Valdez N, Kirchhoff N, Ona MC, Schmidt JP, Feussner I, Karlovsky P. 2012. Intraspecific genotypic variability determines concentrations of key truffle volatiles. *New Phytologist* **194,** 823–835.

Steinbrecher R, Contran N, Gugerli F, Schnitzler JP, Zimmer I, Menard T, Günthardt-Goerg MS. 2013. Inter- and intra-specific variability in isoprene production and photosynthesis of Central European oak species. *Plant Biology* **15,** 148–156.

Takabayashi J, Dicke M, Posthumus MA. 1994. Volatile herbivore-induced terpenoids in plant–mite interactions: variation caused by biotic and abiotic factors. *Journal of Chemical Ecology* **20,** 1329–1354.

Tarvainen V, Hakola H, Hellén H, Bäck J, Hari P, Kulmala M. 2004. Temperature and light dependence of the VOC emissions of Scots pine. *Atmospheric Chemistry and Physics Discussions* **4,** 6691–6718.

Tholl D, Boland W, Hansel A, Loreto F, Rose USR, Schnitzler JP. 2006. Practical approaches to plant volatile analysis. *The Plant Journal* **45,** 540–560.

Tingey DT, Manning M, Grothaus LC, Burns WF. 1979. The influence of light and temperature on isoprene emission rates from live oak. *Physiologia Plantarum* **47,** 112–118.

Tingey DT, Manning M, Grothaus LC, Burns WF. 1980. Influence of light and temperature on monoterpene emission rates from slash pine. *Plant Physiology* **65,** 797–801.

Tollsten L, Müller PM. 1996. Volatile organic compounds emitted from beech leaves. *Phytochemistry* **43,** 759–762.

Trowbridge AM, Daly RW, Helmig D, Stoy PC, Monson RK. 2014. Herbivory and climate interact serially to control monoterpene emissions from pinyon pine forests. *Ecology* **95,** 1591–1603.

Trowbridge AM, Stoy PC. 2013. BVOC-mediated plant–herbivore interactions. In: Niinemets Ü, Monson RK, eds. *Biology, controls and models of tree volatile organic compound emissions* . Dordrecht: Springer, 21–46.

Turlings TC, Tumlinson JH. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proceedings of the National Academy of Sciences, USA* **89,** 8399–8402.

Vallat A, Gu HN, Dorn S. 2005. How rainfall, relative humidity and temperature influence volatile emissions from apple trees *in situ*. *Phytochemistry* **66,** 1540–1550.

Vas G, Vékey K. 2004. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. *Journal of Mass Spectrometry* **39,** 233–254.

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

Vuorinen T, Nerg A-M, Syrjälä L, Peltonen P, Holopainen JK. 2007. Epirrita autumnata induced VOC emission of silver birch differ from emission induced by leaf fungal pathogen. *Arthropod-Plant Interactions* **1,** 159–165.

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

Walling LL. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* **19,** 195–216.

Walter A, Scharr H, Gilmer F, *et al*. 2007. Dynamics of seedling growth acclimation towards altered light conditions can be quantified via GROWSCREEN: a setup and procedure designed for rapid optical phenotyping of different plant species. *New Phytologist* **174,** 447–455.

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavior* **7,** 1306–1320.

Wason E, Hunter M. 2014. Genetic variation in plant volatile emission does not result in differential attraction of natural enemies in the field. *Oecologia* **174,** 479–491.

Way DA, Schnitzler JP, Monson RK, Jackson RB. 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, Kley D, Rockel A, Rockel P, Segschneider HJ. 2012. Emission of NO from several higher plant species. *Journal of Geophysical Research–Atmospheres* **102,** 5919–5927.

Yang W, Duan L, Chen G, Xiong L, Liu Q. 2013. Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. *Current Opinion in Plant Biology* **16,** 180–187.

Yi HS, Heil M, Adame-Alvarez RM, Ballhorn DJ, Ryu CM. 2009. Airborne induction and priming of plant defenses against a bacterial pathogen. *Plant Physiology* **151,** 2152–2161.

Zhu J, Park KC. 2005. Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator Coccinella septempunctata. *Journal of Chemical Ecology* **31,** 1733–1746.