

An Overview of Plant Volatile Metabolomics, Sample Treatment and Reporting Considerations with Emphasis on Mechanical Damage and Biological Control of Weeds[†]

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ABSTRACT:

Introduction – The technology for the collection and analysis of plant-emitted volatiles for understanding chemical cues of plant–plant, plant–insect or plant–microbe interactions has increased over the years. Consequently, the *in situ* collection, analysis and identification of volatiles are considered integral to elucidation of complex plant communications. Due to the complexity and range of emissions the conditions for consistent emission of volatiles are difficult to standardise.

Objective – To discuss: evaluation of emitted volatile metabolites as a means of screening potential target- and non-target weeds/plants for insect biological control agents; plant volatile metabolomics to analyse resultant data; importance of considering volatiles from damaged plants; and use of a database for reporting experimental conditions and results.

Method – Recent literature relating to plant volatiles and plant volatile metabolomics are summarised to provide a basic understanding of how metabolomics can be applied to the study of plant volatiles.

Results – An overview of plant secondary metabolites, plant volatile metabolomics, analysis of plant volatile metabolomics data and the subsequent input into a database, the roles of plant volatiles, volatile emission as a function of treatment, and the application of plant volatile metabolomics to biological control of invasive weeds.

Conclusion – It is recommended that in addition to a non-damaged treatment, plants be damaged prior to collecting volatiles to provide the greatest diversity of odours. For the model system provided, optimal volatile emission occurred when the leaf was punctured with a needle. Results stored in a database should include basic environmental conditions or treatments. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: GC–MS; biological control agents; damage; invasive weeds; plant volatiles

Introduction

Plant secondary metabolites are non-essential chemical compounds produced by plants or microbes. They are often described as natural products used for protection against external factors such as pests, diseases and weeds (Pickett *et al.*, 2007), and for which plant-emitted volatiles have played a significant role (Dudareva *et al.*, 2006). Over the past decade, the analysis of plant-emitted volatiles for understanding chemical cues of plant–plant, plant–insect, or plant–microbe interactions has increased. This is indicated by a rise in the number of publications (Fig. 1) in professional journals or books, and is the topic of a number of reviews (Dudareva *et al.*, 2006; Ahuja *et al.*, 2010; Kaplan, 2012; Maffei *et al.*, 2012; Mithöfer and Boland, 2012; Morath *et al.*, 2012; Das *et al.*, 2013; Ponzio *et al.*, 2013). Researchers from various disciplines have investigated plant volatiles for a number of specific reasons, including integrated pest management (Ahuja *et al.*, 2010), defense against herbivores (Mithöfer and Boland, 2012; Das *et al.*, 2013), below-ground emissions (Ali *et al.*, 2012; Ghimire *et al.*, 2013), detection of disease infestation (Sankaran *et al.*, 2010; Cevallos-Cevallos *et al.*, 2011), food quality (Oms-Oliu *et al.*, 2013), chemotaxonomy (Sajewicz *et al.*, 2009; Fraga, 2012; Liu *et al.*, 2013), biological control agents (Smith and Beck, 2013; Wheeler and Schaffner, 2013) and metabolomics (Roze *et al.*, 2010; Cevallos-Cevallos *et al.*, 2011).

To add to the normal complexity of plant volatile emissions there are several other biotic and abiotic factors to be considered when investigating plants and their respective volatiles. Beyond considering the system to be studied and the question that needs to be answered, researchers also need to take into account other natural stressors that may change or alter a plant's emission profile. The following environmental conditions or stressors are examples of factors that need to be carefully considered in order to obtain reproducible emission profiles for each treatment: time of day; temperature; diurnal/nocturnal; soil; nutrient levels; water availability; fungal or

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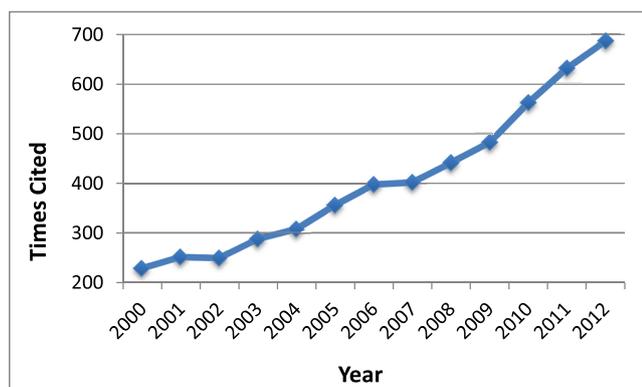


Figure 1. The number of items found from searching the terms 'plant' and 'volatiles' in abstracts – the National Agricultural Library database for the years 2000–2012. As of July 2013 there were 220 references.

endophyte presence; systemic pathogens; and mechanical or herbivore damage. (Holopainen and Gershenzon, 2010; Kusari *et al.*, 2013). If these parameters either change during an experiment or are not adequately reported, any subsequent attempt to reproduce the results may not provide corroborative data. It is the responsibility of the reporting authors to properly account for and record as many parameters as is practical and duly report them to ensure reproducibility by other researchers or the reporting of the actual volatiles for the stated plant conditions or stressors.

Collection of plant volatiles

There are a multitude of techniques and methods for the analyses of plant matrices, which range from simple *ex situ* control experiments performed in the laboratory and under carefully controlled environmental conditions (Beck *et al.*, 2008a; Roitman *et al.*, 2011) to extremely complex, multi-treatment *in situ* experiments performed under varying abiotic conditions (Dudareva *et al.*, 2004; Zhang and Li, 2010; Bicchi and Maffei, 2012). If designed properly and the sample prepared correctly (Kim and Verpoorte, 2010), each experiment will provide sufficient volatile profiles for subsequent data analysis; however, as mentioned previously, the composition and content of volatiles emitted from plants is dependent upon numerous factors and thus selection of the volatile collection method is important. Despite the obvious pitfalls of *ex situ* experiments (i.e. the matrix out of context or no longer part of the living system) they remain an excellent starting point for the collection of volatiles under carefully controlled conditions; experimental parameters can be altered to obtain optimal conditions for a model or control system. Once optimised, an *ex situ* or laboratory-based system's complexity can be increased in order to determine possible emission patterns for more elaborate, yet controlled conditions. Additionally, results from an *ex situ* system are also more readily reproducible by another research laboratory. However, these same results should not be offered as representative of the natural system, but rather as a piece of information toward a basic understanding of the natural system. The ultimate goal of an *ex situ* or laboratory-based system should be to transfer the small piece(s) of knowledge learned to an *in situ* system with the desired treatments mimicking the natural system to be studied (Beck, 2012).

The challenge of correlating volatile production to a specific causal agent or condition is elegantly summarised and addressed in an extensive review by Aksenov *et al.* (2013). In their review the authors suggest four parameters be considered for a volatile

collection experiment to properly account for emission variability: (i) determine and account for the present biotic and abiotic stressors and their resultant volatiles; (ii) volatile emission sampling – however, in this review the authors highlighted non-invasive techniques (the authors also emphasise that some sampling techniques may themselves introduce a plant stressor and consequential volatile emissions as a result); (iii) choice of instrumentation and more importantly the detector – for instance, proton-transfer reaction mass spectrometry (PTR/MS) or selected ion flow tube mass spectrometry (SIFT/MS) have been cited as being very suitable for plant volatile analysis; (iv) the processing and interpretation of large amounts of data typically generated by investigation of volatiles from complex matrices and treatments. The use of multivariate analysis is becoming more widespread and expected in the chemical-based literature (Zhang and Li, 2010). This expectation appears to be warranted given the increase in complexity of matrices being studied.

In their review of recent developments in the analysis of volatiles from biological systems, Zhang and Li (2010) discuss several collection techniques that are applicable for both *ex situ* (steam distillation and supercritical fluid extraction) and *in situ* (solid phase microextraction (SPME) and purge and trap) experiments. As the focus of the present paper is to promote the proper design and implementation of experiments that investigate plant-produced volatiles for biological control of invasive weeds we recommend *in situ* experiments using either microextraction, that is, SPME or stir-bar sorptive (Duan *et al.*, 2011), or purge and trap collections (Aksenov *et al.*, 2013). For instance, our laboratories have utilised SPME fibres for the collection of static headspace volatiles from plant parts enclosed in customised Teflon® bags as well as purge and trap systems with Tenax® to: analyse host-plant volatiles for potential attractants for the insect pest navel orangeworm (Beck *et al.*, 2009, 2012); determine host-plant volatile emissions from the invasive weed *Ludwigia* spp. as well as the insect emissions of a potential biocontrol agent *Altica litigata* (Carruthers *et al.*, 2011); and to evaluate possible chemotaxonomical volatiles from several invasive weeds (Beck *et al.*, 2008b; Smith and Beck, 2013). As mentioned above, there are a number of instruments and detectors available for volatile analyses; however, the most commonly employed is the bench-top GC–MS, which has acceptable detection capabilities for most applications. Manufacturers of bench-top GC–MS instruments appear to be improving the sensitivity of the MS detector while maintaining the durability and reliability for heavy usage (personal communication from a GC–MS manufacturer engineer to JJB). It should be noted that portable mass spectral detectors for use in agricultural settings have made advances and may be soon applicable to simple plant volatile analyses (Beck, 2012; Aksenov *et al.*, 2013).

Plant volatile metabolomics

Plant volatiles can provide important clues as to the biochemical processes of a plant, and are also considered secondary metabolites. More importantly, volatiles can be critical chemical cues that enable a plant to communicate with its surroundings. This includes communication with insects, microbes and other plants. If one considers a definition of metabolomics to be studies that 'aim at a better understanding of biochemical processes by studying relations between...metabolites and other types of information' (Hendriks *et al.*, 2011), then we as researchers of plant volatiles are actually participating in volatile metabolomics. Accordingly, because the choice of an appropriate instrument

for analysing volatiles is important, it makes sense that the choice of a data-analysis tool to extract the relevant information is just as important.

Just as there has been a steady increase in the number of publications each year concerning plant volatiles (Fig. 1), there has also been a steady increase in the number of publications with the words 'plant' and 'metabolomics' in the abstract (Fig. 2). Even more interesting, and thus the topic of this paper and Special Issue of *Phytochemical Analysis*, is what appears to be the emerging field of plant volatile metabolomics, as seen by the rising number of publications with the words 'plant', 'volatile' and 'metabolomics' in the abstract (Fig. 3). As further evidence of this emerging field a report by Wolfender *et al.* (2013) reviews MS- and NMR-based approaches to plant metabolomics. Although the majority of this report discusses NMR-based metabolomics the authors do a very good job of addressing MS-based systems. They point out some possible difficulties that some of the larger molecular weight metabolites may present using a MS-based approach. However, this may not hold true for the smaller molecules usually seen in volatile analyses and therefore GC-MS could be a good platform for volatile metabolomics because it also separates the majority of components and may be easier to identify an appropriate biomarker; a biomarker in this usage

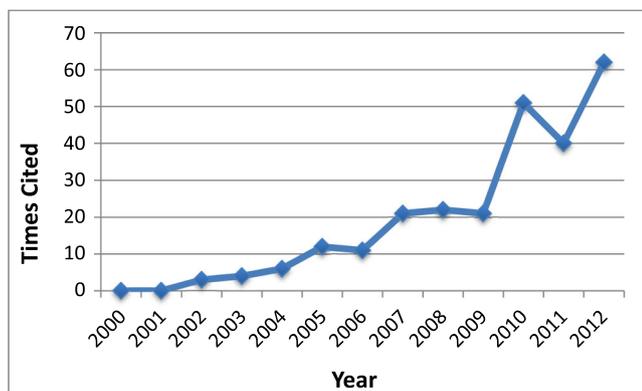


Figure 2. The number of items found from searching the terms 'plant' and 'metabolomics' in abstracts – the National Agricultural Library database for the years 2000–2012. As of July 2013 there were 25 references.

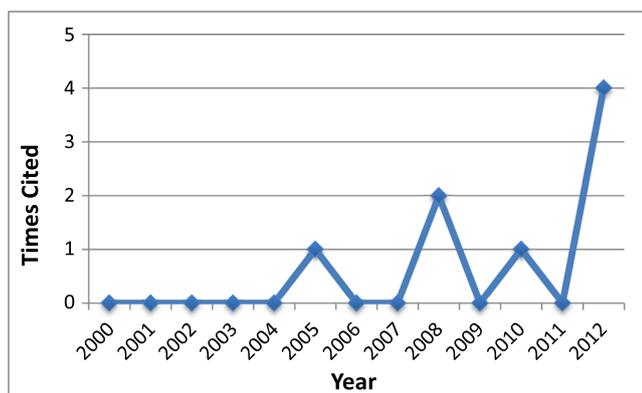


Figure 3. The number of items found from searching the terms 'plant' and 'volatiles' and 'metabolomics' in abstracts – the National Agricultural Library database for the years 2000–2012. As of July 2013 there was 1 reference.

being a volatile or volatiles that are indicative of a defined physiological state of the plant (e.g. diseased, or other damage). Indeed, it has been stated that the 'majority of plant metabolomics studies have been performed by GC-MS' (Wolfender *et al.*, 2013). The review by Wolfender *et al.* (2013) also discusses the importance of biomarkers and how they play a critical role in translating the metabolomics data into usable biological knowledge. The authors are quick to point out the challenges of plant metabolomics due to the frequency of species-specific metabolites. However, with volatiles this may not be a critical issue given that many plants emit relatively similar classes of compounds (e.g. green leaf, benzenoids, terpenoids).

A second and more recent review regarding current metabolomics was performed by Putri *et al.* (2013). The authors provide a good overview of the current technological advances and discuss the advantages of GC-MS, such as availability of fragment libraries for peak identification and detection of a large number of metabolites (peak capacity), and recognise electron ionisation (EI) MS as a highly repeatable and robust detector. To highlight the infancy of volatile metabolomics the authors discussed derivatisation and analysis of non-volatile compounds, but did not mention the applicability of GC-MS to metabolic profiling of low molecular weight (50–240 amu) or low boiling point compounds.

Other publications using GC-MS for plant metabolomics studies (metabolite profiling) include: a chemotaxonomic study of three *Curcuma* spp. (Xiang *et al.*, 2011), where the authors used essential oils to discriminate among the cultivars; quality control of a common medicinal herb (Tianniam *et al.*, 2010) and the use of principal component partial least-square discriminant analysis to visually discriminate among samples; phenological differences in leaves of *Vitis vinifera* (Weingart *et al.*, 2012), in which the authors used retention indices, high-match factors and multivariate statistics to differentiate the two samples; and metabolic differences between disease-infected leaves of citrus (Cevallos-Cevallos *et al.*, 2011) by volatile headspace analysis.

The above examples highlight well the use of GC-MS as an analytical platform for plant metabolites, but more importantly its applicability to the emerging field of plant volatile metabolomics. The recognition of GC-MS as the primary analytical platform for plant volatile metabolomics will contribute extensively to the promising trend noted in Fig. 3.

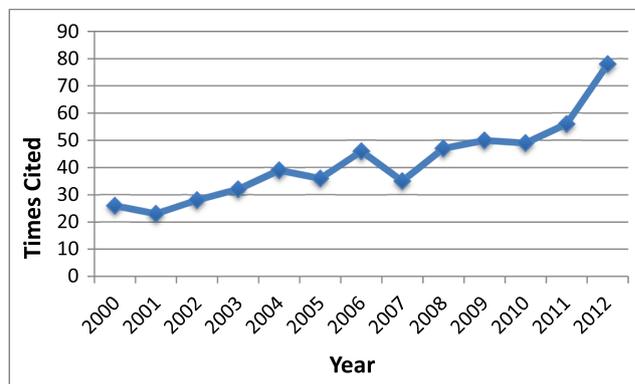


Figure 4. The number of items found from searching the terms 'volatiles' and 'biological control' in abstracts – the National Agricultural Library database for the years 2000–2012. As of August 2013 there were 26 references.

Table 1. Examples of volatile differences of undamaged, mechanically damaged or herbivore damaged plants

Plant studied	Damage type		Reference
	Mechanical	Herbivore	
<i>Solanum lycopersicon</i> (tomato)	Pattern wheel	<i>Spodoptera littoralis</i> (African cotton leafworm)	Zebelo <i>et al.</i> , 2012
<i>Brassica oleracea</i> (cabbage plants)	Hole punch	<i>Plutella xylostella</i> (diamondback moth)	Girling <i>et al.</i> , 2011
<i>Mentha aquatica</i> (watermint)	Pattern wheel	<i>Chrysolina herbacea</i> (mint leaf beetle)	Zebelo <i>et al.</i> , 2011
<i>Phaseolus lunatus</i> (lima bean)	Mecrowm; pattern wheel	<i>Spodoptera littoralis</i> (African cotton leafworm)	Bricchi <i>et al.</i> , 2010
<i>Triticum aestivum</i> (wheat)	Leaves scratched	<i>Oulema melanopus</i> (cereal leaf beetle)	Piesik <i>et al.</i> , 2010
<i>Avena sativa</i> (oat)	Leaves scratched	<i>Oulema melanopus</i> (cereal leaf beetle)	Piesik <i>et al.</i> , 2010
<i>Hordeum vulgare</i> (barley)	Leaves scratched	<i>Oulema melanopus</i> (cereal leaf beetle)	Piesik <i>et al.</i> , 2010
<i>Brassica oleracea</i> (brussels sprout)	Miniature drill	<i>Pieris brassicae</i> (cabbage white caterpillar)	Connor <i>et al.</i> , 2007
<i>Centaurea nigra</i> (knapweed)	1 cm leaf tip cut off	<i>Uroleucon jaceae</i> (large knapweed aphid)	Pareja <i>et al.</i> , 2007

Undamaged: 8 main volatiles, green leaf, terpenoid, and fatty acid breakdown. *Mechanical damage:* 15 main volatiles with 8 equivalent to control, the remaining 7 primarily terpenoid. *Herbivore damage:* 16 main volatiles, nearly all statistically greater than mechanically damaged volatiles

Undamaged: 100 volatiles, sulphides, terpenoids, benzenoids. *Mechanical damage:* 106 volatiles, 5 different than undamaged. *Herbivore damage:* 125 volatiles, 16 more than undamaged

The undamaged and mechanically damaged volatile profiles were essentially equivalent in terms of major component terpenoids

Herbivore damage: resulted in statistically greater amounts of the major terpenoids with the exception of one monoterpene

The continuous damage performed by the MecWorm was able to elicit a similar response to that of the insect. Single mechanical damage elicited a smaller response

Control: trace amounts of (Z)-3-hexenal; (Z)-3-hexenol; and (Z)-3-hexenyl acetate. *Mechanical damage:* significant increase in control volatiles. *Herbivore damage:* significant increase in mechanical damage volatiles and increase in compounds emitted (e.g. monoterpenes, benzenoids, sesquiterpenes)

Control: essentially no volatiles emitted. *Mechanical damage:* green leaf volatiles similar to wheat. *Herbivore damage:* significant increase in mechanical damage volatiles and increase in compounds emitted (e.g. monoterpenes, benzenoids, sesquiterpenes)

Undamaged: trace amounts of (Z)-3-hexenal; (Z)-3-hexenol; and (Z)-3-hexenyl acetate. *Mechanical damage:* significant increase compared to control volatiles. *Herbivore damage:* significant increase in mechanical damage volatiles and increase in compounds emitted (e.g. monoterpenes, benzenoids, sesquiterpenes)

Undamaged: no volatiles; bioassay only. Authors note similar volatile profiles between mechanical and herbivore damage treatments

Undamaged: green leaf volatile and terpenoids. *Mechanical damage:* non-quantified increases in some long-chain alkenes and sesquiterpenes during first 24 h. *Herbivore damage:* no significant increase in composition or amounts relative to control when aphid present

Analysis of plant volatile metabolomics data

With the advent of metabolomics there has been a plethora of software tools/packages available for GC–MS-based metabolite experiments and many have been reviewed or reported in recent literature. For instance, Putri *et al.* (2013) provide a summary a various data-processing programs for GC–MS analyses and their corresponding references. Some examples include: Metab R, which automates the processing of AMDIS files (Aggio *et al.*, 2011); MetaboliteDetector, automatic analysis from raw MS data, which determines quantification ions and integrates them (Hiller *et al.*, 2009); MetaQuant, a Java-based program for quantitation of metabolites, and exports NetCDF files as CSV or XLS (Bunk *et al.*, 2006); MET-IDEA, extracts semi-quantitative data from raw files and provides a data matrix to work from (Broeckling *et al.*, 2006); MSFACTs, aligns integrated peaks and reads from ASCII data (Duran *et al.*, 2003); SIMCA, an algorithm to obtain more peaks from unknowns in GC–MS data (Tsugawa *et al.*, 2011); TagFinder, facilitates analysis of EI/TOF/MS fragment ions (Luedemann *et al.*, 2008); and TargetSearch, sophisticated normalisation strategy to deal with missing data and numerous parameters for quality control (Cuadros-Inostroza *et al.*, 2009).

Other recent reports provide a good overview of and guidance for the processing of metabolomics or large volatile files. For researchers well versed in statistics, Kwon (2013) reviews and discusses when and how to use certain methods for the

interpretation of the relationship between metabolomics data and phenotype differences. The methods are listed as six different categories: correlation based, dimension reduction, regression based, discrimination, clustering and self-organising maps. The review is geared toward metabolomics in general, but the author stresses some important points that can most likely be applied to plant volatile metabolomics – pre-processing data is important and that one single method should not be singled out, but rather more than one approach should be applied to the data for proper interpretation of the results. In the review, the author makes a perceptive statement regarding the use of GC–MS as an analytical platform – ‘GC–MS has an advantage to separate volatile metabolites and available libraries are abundant...’.

For an overview of the same material, but geared more towards large amounts of data from volatile analyses, Aksenov *et al.* (2013) do a good job of discussing the material, and are more understandable to non-statistically-inclined scientists. Again, the idea of pre-processing is emphasised for GC–MS-based data in order to remove unwanted data (i.e. SPME, column or solvent contaminants), with resolution of co-eluting peaks and alignment of the remaining peaks provided as examples (see also Cuadros-Inostroza *et al.*, 2009). The authors then suggest normalisation of the data via an internal standard to account for sample-to-sample variations, but also point out the challenge of using an internal standard when the data are collected via a gas phase (i.e. SPME). The authors go on to

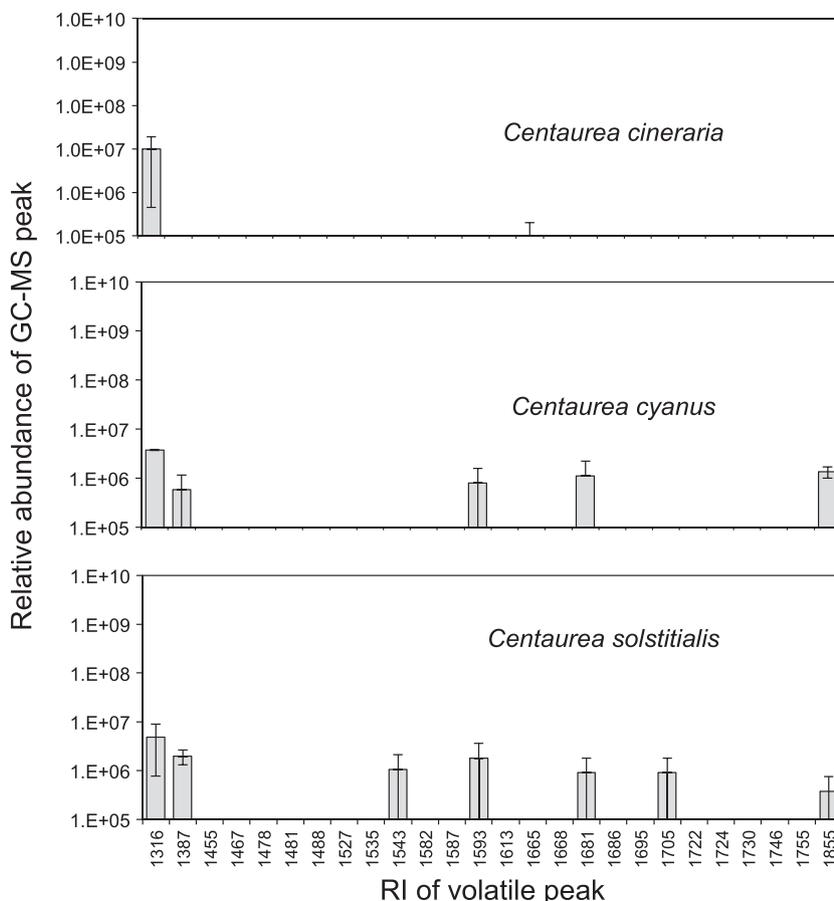


Figure 5. Volatile profiles from undamaged leaves of *Centaurea cineraria* (unsuitable host plant), *C. cyanus* (less preferred host plant) and *C. solstitialis* (preferred host plant) of the weevil, *Ceratopion basicorne* (data from Smith and Beck, 2013).

discuss other data-analysis techniques, similar to those discussed by Kwon (2013), and in a reader-friendly manner. A final step suggested by Aksenov and co-workers is final validation of the data when a biomarker is being ascribed to a particular biological state. This can be done by what the authors term the 'hold-out' strategy, which relies on a single split of the data to assess the risk (Arlot and Celisse, 2010).

Lastly, retention indices (RI) are considered one of the most important parameters for proper and reliable peak assignment (Lisec *et al.*, 2006). The use of RI and mass-fragmentation patterns is a powerful tool for compound identification. Zhang *et al.* (2013) outline and discuss strategies for identifying unknown compounds using RIs and fragmentation patterns.

Overall, the majority of these software packages appear to be readily available for free, at a low cost, or included with instruments, and thus incorporation into the analyses of GC-MS-produced data should not be too great a burden for most groups. Many of the free tools were developed to evaluate large-scale experiments and work well for a specific application, or can be tailored for specific needs. A good way to ensure proper data analysis of plant volatile metabolomics is to enlist the help of another scientist or statistician conversant in these programs.

Database of results from plant volatile metabolomics studies

In their review of plant metabolomics, Wolfender *et al.* (2013) provide the following statement 'Although great efforts have been made to create databases for researchers, these repositories remain incomplete in plant science, particularly in terms of secondary metabolites', which summarises very well the current need for an informative, thorough, and readily available database for plant volatile metabolomics. This call for a database is not new. In 2011 Skogerson *et al.* also made a similar statement with regard to volatile secondary metabolites 'In past decades, sampling methods and instrumentation for the analysis of complex volatile mixtures have improved; however, design and implementation of database tools to process and store the complex datasets have lagged behind'.

This is not to imply that databases are not available or none have been explored. Skogerson *et al.* (2011) addressed the need for database by making the BinBase Database system available online and appear to be making progress with plant metabolites (Stein, 2012). Zhang *et al.* (2013) also discuss the construction of a database based on RIs and mass-fragmentation patterns. Other experts in the field (Hur *et al.*, 2013; Wolfender *et al.*, 2013) are aware of the need to understand plant natural-product metabolism and are calling for metabolomics data to be stored and 'curated in publicly available metabolomics databases' (Hur *et al.*, 2013). Additionally, Hur and co-workers (2013) make two bold statements that may prove to be prophetic if a database of plant volatiles is to truly work. With regard to maximising the quality of a database and the metadata within, Hur and co-workers assert that 'the members of each research group that contribute data must be responsible for ensuring submission is complete and clear data and metadata'. They go on to advocate the idea that metabolomics data and metadata undergo review prior to publication to ensure completeness. This would require an obvious change in infrastructure that perhaps an ad hoc committee of several experts could consider.

Other important considerations

Various roles of plant volatiles

In Fig. 1 the growing trend for the use of plant volatiles as the main analytes for various investigations is shown. Indeed, the literature demonstrates the varying roles in which plant volatiles play a role. Some of these roles include: as a semiochemical for plant-plant, plant-insect, plant-fungal and fungal-insect communication either as a two-way interaction or a more complex interaction (e.g. plant-insect-microbe); plant health; chemotaxonomy; biological control for protecting agricultural commodities using plants (push/pull systems) or insects (predators or parasitoids of plant herbivores); biological control of invasive weeds; cultivar quality of agricultural commodities; or metabolomics, which is highlighted here as an emerging field of study.

A sampling of examples and reviews is provided as an overview of recent research of plant volatile emissions and their various roles. A discussion of less recent, but classic reviews of plant volatiles is provided in a recent report on volatiles from damaged plant leaves (Smith and Beck, 2013). In their recent review, Das *et al.* (2013) discuss plant volatiles as a form of communication in a broad context covering several interactions – plant-insect-microbe – and the many classes of compounds typical of these interactions across varying species. These

Table 2. Compound identities and corresponding retention indices based on *n*-alkanes on a DB-Wax column (data from Smith and Beck, 2013)

RI	Compound
1316	(Z)-3-hexenyl acetate
1387	(Z)-3-hexen-1-ol
1455	α -Cubebene
1467	δ -Elemene (tentative)
1478	Cyclosativene
1481	Unknown sesquiterpene
1488	α -Copaene
1527	α -Gurjunene
1535	β -Cubebene
1543	1-Pentadecene
1566	(Z)- α -bergamotene (tentative)
1582	(E)- α -bergamotene
1587	Calarene
1593	β -Caryophyllene
1613	Unknown sesquiterpene
1665	(E)- β -farnesene
1665	α -Humulene
1668	Unknown sesquiterpene
1681	Unknown sesquiterpene
1686	γ -Muurolole (tentative)
1695	Unknown sesquiterpene
1705	Germacrene-D
1722	α -Muurolole (tentative)
1724	Unknown sesquiterpene
1730	Bicylcogermacrene (tentative)
1746	(E,E)- α -farnesene
1755	δ -Cadinene
1755	γ -Cadinene
1855	Geranyl acetone

classes of compounds include terpenoids, benzenoids and green-leaf volatiles. Ponzio *et al.* (2013) explore the complexity of field-realistic conditions by discussing multiple interactions of plants with herbivores and pathogens and the resultant volatiles emitted as a function of what pathway is activated within the plant – salicylic acid, jasmonic acid or ethylene. Plant volatiles also can be used to determine the health of a plant. While reviewing techniques for detecting plant diseases, Sankaran *et al.* (2010) drew attention to several studies that used volatiles to distinguish between healthy or diseased trees under field conditions or post-harvest fruits and vegetables.

The use of plant-emitted volatiles for chemotaxonomic studies has been documented to a limited extent. Liu *et al.* (2013) studied the volatile profiles of *Citrus* and related genera to assess their chemotaxonomical classification. However, a cursory search of the literature suggests essential oils appear to be studied for chemotaxonomy more than plant volatile emissions. In addition to plants, the taxa of fungi have also been evaluated based on their volatile profiles. Aliferis *et al.* (2013) used GC–MS, metabolite profiling and multivariate analysis to examine different isolates of *Rhizoctonia solani* cultures.

Volatiles and biological control have also seen a steady rise in use over the past decade (Fig. 4). However, a vast majority of these studies pertain to fungal biological control. The use of

plant-emitted volatiles appears to be gaining ground with a recent perspective on the topic (Kaplan, 2012) and the idea of ‘attract and reward’ for conservation biological control (Orre Gordon *et al.*, 2013). Yet just a few years earlier, a review by Morin *et al.* (2009) discussed weed biological control agents, and in which they provide a flow chart with respect to approval of the biocontrol agent for release. In their review no mention is made regarding the use of volatiles as a means to assist in the decision-making process surrounding the release of biological control agents for invasive weeds. However, the importance of understanding chemical ecology has been more recently recognised (Wheeler and Schaffner, 2013). The present authors of this overview see this as an opportunity to explore more thoroughly the use of plant volatile metabolomics to assist researchers in determining what plants a potential biological control agent may or may not choose as a host (Smith and Beck, 2013).

Emission of plant volatiles as a function of treatment

The emission of volatiles from a plant is highly dependent upon numerous biotic and abiotic conditions (Holopainen and Gershenson, 2010; Maffei *et al.*, 2011; Král'ová *et al.*, 2012). The plethora of conditions or stressors that can affect plant volatile emissions include: scotophase/photophase (Chamberlain *et al.*,

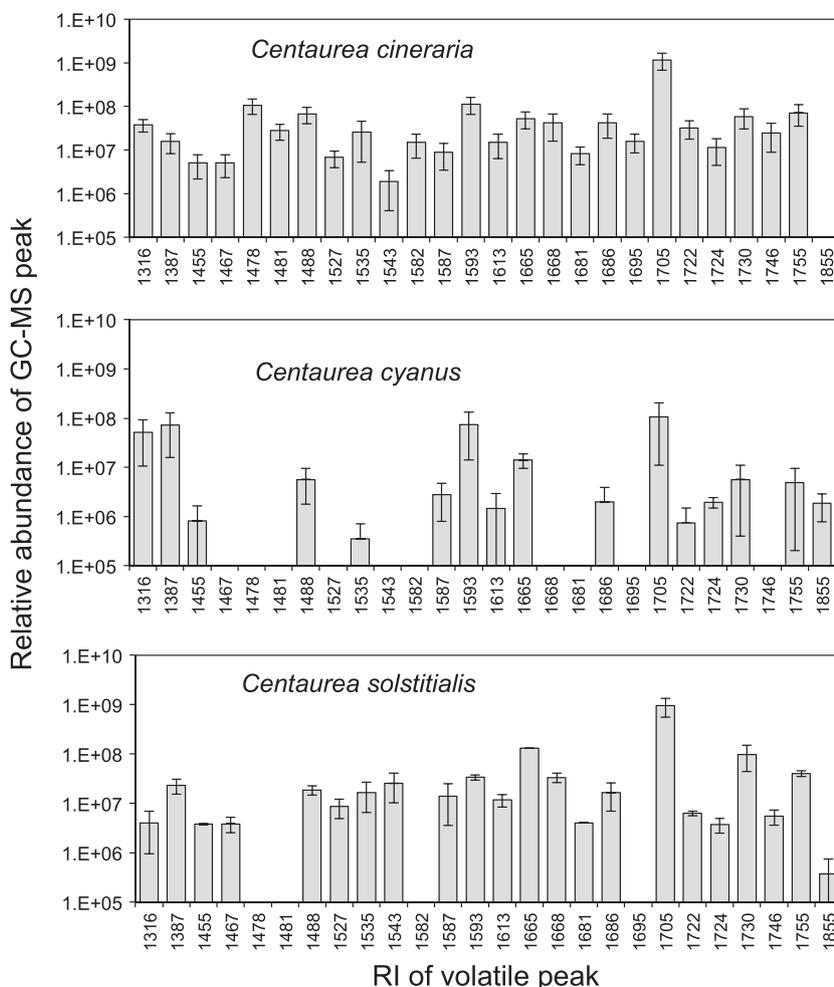


Figure 6. Volatile profiles emitted from punctured leaves of *Centaurea cineraria* (unsuitable host plant), *C. cyanus* (less preferred host plant) and *C. solstitialis* (preferred host plant) of the weevil, *Ceratopion basicorne* (data from Smith and Beck, 2013).

2006; Webster *et al.*, 2010); phenology (Beck *et al.*, 2009); infections by fungi (Piesik *et al.*, 2013), endophytes (Kusari *et al.*, 2013; Pańka *et al.*, 2013); or pathogens (Sankaran *et al.*, 2010; Ponzio *et al.*, 2013); drought (Šimpraga *et al.*, 2011); increased carbon dioxide (Yuan *et al.*, 2009); and invertebrate herbivore damage (Paré and Tumlinson, 1999; Das *et al.*, 2013) to name a few. It has also been shown that plant volatile amounts can increase due to foliar disturbance by simply walking through them (Barney *et al.*, 2009).

Several reviews and reports have discussed the complexities of herbivore-induced plant volatiles (i.e. Turlings *et al.*, 1990; Dicke and Baldwin, 2010; Mithöfer and Boland, 2012; Peñafior and Bento, 2013), and the following section 'Plant volatiles for weed biological control' also provides specific examples. Table 1 provides an overview of investigations that explored the volatile emissions from various mechanical versus herbivore damage treatments. In general, plants that sustained mechanical damage emitted more volatile compounds than undamaged plants, but those damaged by herbivores often emitted higher quantities as well as additional compounds.

Plant volatiles for weed biological control

Secondary chemical compounds often act as feeding deterrents and can be toxic to herbivores (Wheeler and Schaffner, 2013); however, species of herbivores that have evolved to exploit a specific host plant not only can avoid or detoxify such compounds, but they often use them to help recognise their host (e.g. Wheeler, 2005; Padovan *et al.*, 2010). Several studies have shown that stenophagous arthropod herbivores can be attracted by volatiles emitted from their host plant. For example, the ragwort flea beetle, *Longitarsus jacobaeae*, responds to odours of its host plant in a wind tunnel by moving upwind (Zhang and McEvoy, 1995). *Mogulones cruciger*, a host-specific beetle that is a biological control agent of houndstongue, *Cynoglossum officinale*, preferred odours of its host plant in a Y-tube olfactometer (Park *et al.*, 2013). The weevil, *Ceratapion onopordi*, preferred odours of its host plant over those of an empty chamber in a four-field olfactometer (Müller and Nentwig, 2011). These studies have typically involved undamaged plants, and the effects of prior mechanical damage on qualitative and quantitative emission of volatiles is usually unknown and assumed to be unimportant (Palmer, 1999; Arnett and Louda, 2002; Heard and Van Klinken, 2004).

Many studies have shown that feeding damage by herbivorous arthropods can cause plants to emit volatiles, and that these odours can be used by predators or parasitoids to help find their herbivorous hosts (e.g. Turlings *et al.*, 1990, 1993; Tumlinson, 1991; Choudhary *et al.*, 2008; van Dam *et al.*, 2010; Kugimiya *et al.*, 2010; Mumm and Dicke, 2010; Hare, 2011). However, non-specific mechanical damage can also cause some changes in volatile emissions, and these can differ from insect-induced secondary metabolites (Table 1). A recent example has shown how dramatic the differences can be (Beck *et al.*, 2008b; Smith and Beck, 2013). The herbivorous weevil, *Ceratapion basicorne*, prefers yellow starthistle (*Centaurea solstitialis*) (Smith, 2007, 2012). This weevil can also attack the congeneric plant bachelor's button (*C. cyanus*), but never attacks dusty miller (*C. cineraria*). Very few volatiles were emitted by undamaged plants of any of these species, as detected by GC-MS (Fig. 5, Table 2), and there was very little difference among the volatile profiles of these plants. However, physically puncturing or scratching

leaves caused plants to emit many more volatiles, which greatly increased differences among the plant species (Fig. 6, Table 2). Discriminant analysis of volatiles from these plants provided differences based on specific volatiles, further demonstrating the importance of undamaged and damaged treatments and identifying the resultant volatile profiles or individual key volatiles (Fig. 7, Table 2). These results indicate that undamaged host plants emit quite different volatile profiles from damaged ones. How well this insect responds to damaged versus undamaged plants is not yet known. Furthermore, gustatory stimuli are likely to interact with olfactory ones in the process of host-plant selection (Courtney and Kibota, 1990; Bernays and Chapman, 1994; Heard, 2000; Chapman, 2003). In any case, studies of only undamaged plants may overlook secondary metabolites that are released only after damage, which would typically occur at the gustatory stage.

Although much of the material in this overview is applicable to numerous types of analyses of volatiles, we would like to emphasise the use of plant volatiles as a means to 'screen' target- and non-target plants that may be considered by prospective biological control agents. Similar to the use of volatiles to control insect pests, the use of volatiles to screen biological control agents (insects) must also account for numerous and variable factors associated with plant-insect interactions. An important consideration of a biological control agent is that it remains host-specific and does not shift to a non-target or beneficial plant (Pearson and Callaway, 2005). However, other key factors to consider in terms of volatiles emissions include allelopathic compounds from undamaged plants (Glinwood *et al.*, 2011),

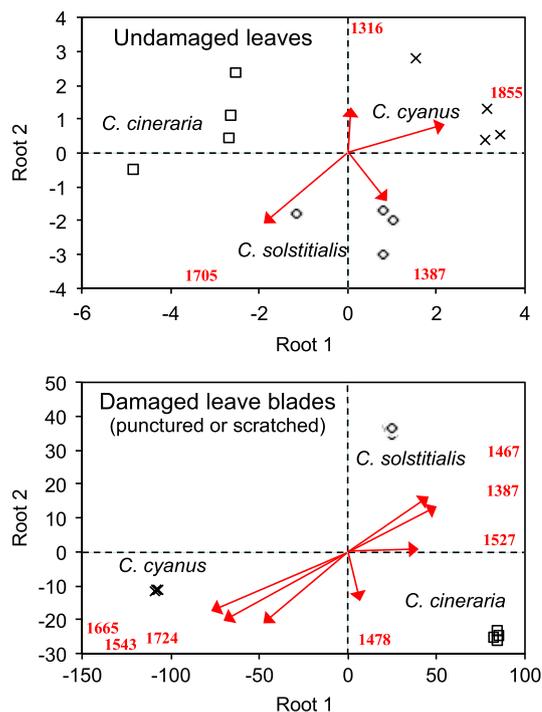


Figure 7. Discriminant analysis of volatiles from three species of plants. Plots contain unstandardised canonical scores for the first two roots of the discriminant function. Undamaged leaves were classified by four volatiles, and by damaged leaf blades by seven volatiles. None of volatiles retained in the latter model were retained in the model for undamaged leaves. Arrows indicate direction of influence of the respective volatiles denoted by RI (Table 2; data from Smith and Beck, 2013).

Table 3. Examples of experimental conditions for reporting plant volatiles and results to a database

Plant and abiotic	Biotic	Collection conditions/results
Plant (family, genus, species)	Damage type (i.e. puncture, cut)	<i>In situ</i> or <i>ex situ</i>
Developmental stage (phenology)	Damage duration	Field or laboratory
Plant part (i.e. fruit, flower, leaf)	Time since damage	Static or dynamic
Water conditions or stressors	Degree of damage	Flow rate or duration of exposure
Air and soil humidity	Microbe type	Ab/adsorbent media (Tenax, SPME, Twister)
Temperature	Systemic or localised infection	Detector
Light source, intensity, photoperiod	Herbivore type	Column parameters
Geographical details	Herbivory location (i.e., root, leaf)	Retention indices
Nutrients, fertilisers or pesticides		Fragmentation patterns

distances that plant volatiles may travel (Braasch and Kaplan, 2012) and if the insect of interest is flying or walking (Webster, 2012), spatial- and landscape-level variances (Kaplan, 2012), and genetic variability of some invasive plants and thus resultant differences of volatile emissions (Mendes *et al.*, 2011).

Reporting considerations for plant volatiles

The design of an experiment is highly dependent upon the information desired. In the 'Collection of plant volatiles' section we discussed briefly the multitude of techniques and methods for the collection of plant volatiles. In the 'Database of results from plant volatile metabolomics studies' section we also discussed the need for a public database for the dissemination of volatiles by plant and by treatment. In this section, we want to provide the reader with the vast assortment of conditions, both biotic and abiotic (Gouinguene and Turlings, 2002; Loreto and Schnitzler, 2010; Niinemets *et al.*, 2013) that should be considered and reported. Not all conditions are applicable to every experiment, but because of the large number of possible stressors that affect the volatile emissions from a plant we wanted to provide a list of conditions (Table 3) that if applicable should be considered when reporting plant volatile emissions. In addition to the conditions, particular results should be consistently reported to ensure ease of data mining by other users. These items are also included in Table 3.

Summary

This overview touched upon many examples from the plethora of uses and applications in which plant volatiles play a role. And, in light of the evidence provided herein, there exists a good niche for the use of plant volatiles and plant volatile metabolomics for the screening of target- and non-target hosts for prospective biological control agents. As researchers involved in volatiles or biological control we would recommend the evaluation of both mechanically damaged and undamaged plant volatiles in order to determine a general profile from the target- and non-target host plants. These volatile profiles should then be listed in a publically accessible database for other researchers to view and add subsequent results.

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