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METHODS IN PLANT FOLIAR VOLATILE ORGANIC COMPOUNDS RESEARCH¹

DUŠAN MATERIC^{2,5}, DAN BRUHN^{2,6}, CLAIRE TURNER³, GERAINT MORGAN⁴, NIGEL MASON⁴,
AND VINCENT GAUCI²

²Department of Environment, Earth and Ecosystems, The Open University, Walton Hall, Milton Keynes MK7 6AA, United Kingdom; ³Department of Life, Health and Chemical Sciences, The Open University, Walton Hall, Milton Keynes MK7 6AA, United Kingdom; and ⁴Department of Physical Sciences, The Open University, Walton Hall, Milton Keynes MK7 6AA, United Kingdom

Plants are a major atmospheric source of volatile organic compounds (VOCs). These secondary metabolic products protect plants from high-temperature stress, mediate in plant–plant and plant–insect communication, and affect our climate globally. The main challenges in plant foliar VOC research are accurate sampling, the inherent reactivity of some VOC compounds that makes them hard to detect directly, and their low concentrations. Plant VOC research relies on analytical techniques for trace gas analysis, usually based on gas chromatography and soft chemical ionization mass spectrometry. Until now, these techniques (especially the latter one) have been developed and used primarily by physicists and analytical scientists, who have used them in a wide range of scientific research areas (e.g., aroma, disease biomarkers, hazardous compound detection, atmospheric chemistry). The interdisciplinary nature of plant foliar VOC research has recently attracted the attention of biologists, bringing them into the field of applied environmental analytical sciences. In this paper, we review the sampling methods and available analytical techniques used in plant foliar VOC research to provide a comprehensive resource that will allow biologists moving into the field to choose the most appropriate approach for their studies.

Key words: gas chromatography–mass spectrometry (GC-MS); leaf cuvette; plant volatile organic compound (VOC); proton transfer reaction–mass spectrometry (PTR-MS); selected ion flow tube–mass spectrometry (SIFT-MS); thermal desorption–gas chromatography–mass spectrometry (TD-GC-MS).

Plant leaves are a major source of volatile organic compounds (VOCs; for a list of abbreviations used in this article, see Appendix 1) emitted in the atmosphere. VOCs play an important role in protecting plants from high temperature stress (Sharkey and Yeh, 2001). Plants also use VOCs as a means of interacting with other plants and organisms (Baldwin et al., 2002; Ruther and Kleier, 2005; Babikova et al., 2013). Plants also, through VOCs, influence troposphere chemistry by contributing to biogenic aerosol formation and ozone production in the lower troposphere (Sharkey and Yeh, 2001).

Different plant organs (root, flowers, fruits, leaves) emit different groups of VOCs; however, in this work we focus on foliar VOCs. Among the many plant foliar VOCs, C₆ and some other compounds like methyl salicylate and jasmonic acid methyl ester are relevant to a plant's response to wounding and

plant interactions with other organisms, whereas the VOCs most relevant for climate change are isoprene, monoterpenes, and sesquiterpenes (e.g., isoprenoids, terpenoids) (Baldwin et al., 2002; Loreto et al., 2006; Babikova et al., 2013).

C₆ compounds are a group of green leaf VOCs that are emitted during physical damage of leaf tissue, following membrane denaturation (Loreto et al., 2006). The most notable example of C₆ VOC emission is the smell released after cutting grass. These C₆ aldehydes and alcohols and their oxidation products originate from linoleic and α -linolenic acids, and belong to the hexanal and hexenal families (Fall et al., 1999).

Isoprene (C₅H₈, 2-methyl-1,3-butadiene) is the most abundant VOC emitted by plants and is the focus of considerable current research because of its influence on the troposphere. The total contribution of isoprene to the atmosphere is estimated to be 500–750 Tg (440–660 Tg of carbon) per year, which exceeds carbon emissions from all sources of atmospheric methane combined (Guenther et al., 2006). Isoprene is emitted by many plant species, and a summary of isoprene-emitted taxa has been published elsewhere (Harley et al., 1999). Every major plant group is known to emit isoprene but not every plant species (Harley et al., 1999). More isoprene is emitted in tropical humid forests than any other biome because more isoprene-emitting species are present there than in other ecosystems (Sharkey et al., 2008).

Monoterpenes (C₁₀H₁₆) and sesquiterpenes (C₁₅H₂₄) are composed of two or three isoprene-like molecules (C₅H₈). Monoterpenes are major compounds in plant resin, and the main source

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⁵Author for correspondence: dusan.materic@open.ac.uk

⁶Current address: Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg East, Denmark

of monoterpene emissions is conifer needles. α - and β -pinene, together with Δ -3-carene, are the most significant and abundant monoterpenes found in conifer woodlands (Tingey et al., 1980; Räsänen et al., 2009). The most significant factors that influence monoterpene emission are a combination of vapor pressure, ambient temperature, and size of the monoterpene pool in plant tissue (Tingey et al., 1980). Sesquiterpenes are among the least-studied groups of VOCs because of their high reactivity and low vapor pressure, which make them difficult to analyze (Duhl et al., 2008).

Further details on plant VOC biosynthesis and functions of VOCs can be found elsewhere (Muhlemann et al., 2014).

Plant foliar VOC research is usually related to two main fields of study: (1) their impact on global climate change and (2) their role as mediators of interactions between plants and other organisms.

Individual VOCs can have both a positive and negative contribution to climate change. For example, when emitted in the presence of a high concentration of NO_x , isoprene contributes to the formation of ozone in the lower troposphere, which affects the quality of urban air; therefore, it is seen as a negative contribution. However, when in the presence of low NO_x abundances, as is frequently found in rural environments, isoprene keeps ozone concentrations lower than would otherwise be expected (Sharkey et al., 2008). VOCs also compete for hydroxyl radicals (OH), which are the primary atmospheric sink of methane (Peñuelas and Staudt, 2010). Thus, during their short molecular lifetime, VOCs increase tropospheric ozone formation, significantly prolong the lifetime of methane, and finally oxidize and contribute to CO_2 in the troposphere. In contrast to the role of isoprene, monoterpenes and their oxidation products contribute to aerosol and cloud formation, which potentially have a cooling effect (Peñuelas and Staudt, 2010; Riccobono et al., 2014).

Plant foliar VOCs also play a biological communication role in ecosystems (Clavijo McCormick et al., 2014; Pierik et al., 2014). They can be released from plants that are grazed by herbivores where they may function as a chemical signal of the hazard to neighboring and usually downwind plants (Baldwin et al., 2002; Ruther and Kleier, 2005). Multiple stress interactions (abiotic and biotic) and their impact on VOC emission are reviewed elsewhere (Holopainen and Gershenson, 2010).

Research in the field of plant foliar VOCs is challenging for a range of reasons, the principal ones being: (1) VOCs are very reactive to other compounds in the atmosphere; (2) VOCs can react with monitoring equipment surfaces and some monitoring equipment materials can emit VOCs; (3) during the sampling, plants need to be enclosed in a special chamber, which may stress the plant (e.g., change of light condition, wounding) and result in unrepresentative emissions; (4) the measured concentrations are often very low—usually 1–100 ppb (sometimes <1 ppb)—thus challenging the detection limits of analytical instruments. These factors make plant foliar VOCs research unique with challenges in sampling and analysis. To address these challenging tasks, diverse analytical methodologies (e.g., chemical ionization mass spectrometry) and sampling techniques have recently been developed and used.

In this paper, we review available methodologies in plant foliar VOC research to provide a comprehensive resource for plant scientists with no background in physics or analytical sciences to move into the field of plant foliar VOC research and select the most appropriate analytical method and sampling approach.

ANALYTICAL METHODS

The two most commonly used techniques in quantitative and qualitative analysis of plant VOCs are: (1) gas chromatography (GC)-based techniques such as gas chromatography-mass spectrometry (GC-MS), GC with flame ionization detector (GC-FID), and thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS); and (2) mass spectrometry based on soft chemical ionization, such as selected ion flow tube-mass spectrometry (SIFT-MS) and proton transfer reaction-mass spectrometry (PTR-MS).

Gas chromatography-based methods

Method overview—GC is a well-established technique that is suitable for qualitative and quantitative analysis of plant foliar VOCs. The core part of a gas chromatograph is the column, which is placed in the oven of the instrument (Fig. 1). Generally, there are two types of columns: (1) packed and (2) capillary. Although packed columns are still in use for some applications, capillary columns are more commonly used in plant VOC research. The most important property of the column that needs to be considered is its stationary phase. Nowadays, capillary columns are coated with various stationary phases, ranging from nonpolar to polar, depending on the functionality of the target compounds. There is a range of columns that can be used for plant foliar VOC research; therefore, referring to successful published reports about a compound of interest and contacting column manufacturers are good starting points for method development. In general, for good separation of polar VOCs, a polar column is required, whereas nonpolar VOCs require a nonpolar column. Thus, general advice for the analysis of rich mixtures of foliar VOCs is to start with a 5% phenyl-substituted (polar) column. The usual length of a column for plant foliar VOCs research is between 15 and 60 m, depending on the compounds of interest.

The GC instrument consists of a temperature-controlled oven, capable of being rapidly ramped up reproducibly from room temperature to over 300°C. The instrument also houses a series of pressure control systems and provides the interfaces for the introduction of samples and the analytical detectors. Inside the oven is an open tubular column (30–60 m), containing a stationary-phase film capable of separating compounds according to their physical and chemical properties. One end of the gas chromatograph column is connected to the inlet (usually an injector), and the other end (outlet) is connected to the detector. Samples are introduced via a heated inlet and then transported by the carrier gas flow (usually helium) through the column. Each of the VOCs interacts differently with the stationary phase of the column and is therefore differentially partitioned between the stationary phase and mobile phase (helium). An increase in temperature changes the partition coefficient, ultimately resulting in the compound being completely moved into the mobile phase and being swept into the detector, via a heated transfer line. Thus, different VOCs come out of the column at different times (known as retention time), and after exiting the column, they may be identified and quantified by a mass spectrometer or other detector (Fig. 1).

The most common gas chromatograph detectors for plant VOC research are flame ionization detectors (FID) and mass spectrometers. FIDs are simple, low-cost detectors for organic compounds (VOCs, such as hydrocarbons, which can be detected when burnt). When a mass spectrometer is used as a detector, analysis of the fragmentation patterns of the ions at each point in the total ion chromatogram enables compound identification. Deconvolution software can also be used to identify/separate overlapping peaks (Colby, 1992). Compound identification is also facilitated by the use of a library of previously generated spectra, such as the National Institute of Standards and Technology (NIST) library. Characteristic ions are then selected for each compound to enable quantification through comparison with the responses determined during calibration. Ideally, an internal standard is also introduced to enable corrections for any instrument variations over time.

Modern gas chromatograph systems are highly reproducible and therefore both the characteristic retention times (known as the Kovats retention index) and the mass fragmentation pattern can be used to identify and quantify the injected compounds.

GC can be used to directly analyze plant VOCs in tissue extracts (solvent extraction of leaves). However, the concentration of plant VOCs emitted from plant surfaces is quite low and usually requires preconcentration to reach detection limits.

The samples trapped on TD tubes are thermally desorbed, over several minutes, and retrapped on a cold trap in the thermal desorption instrument (Fig. 1). The cold trap, which is a tube containing packing material, is then rapidly heated and the VOCs are released and transferred through a heated transfer line

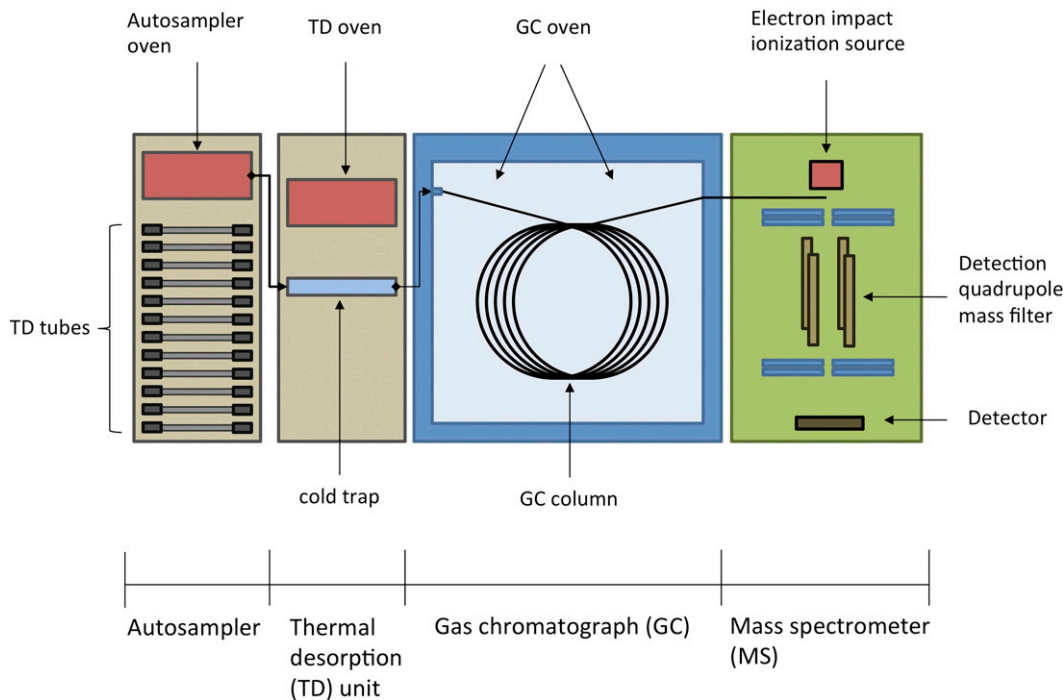


Fig. 1. Thermal desorption–gas chromatograph–mass spectrometer. VOC samples trapped onto TD tubes are introduced into the *autosampler*. The *autosampler* collects a tube and heats it up according to the setup. VOCs are then retrapped onto material in the *cold trap*. The *cold trap* heats up rapidly, and VOCs are introduced into the *GC column*. VOCs are separated in the *GC column*, thus escaping the column at different times (retention time). Escaped VOCs are analyzed by a *mass spectrometer*.

to the GC. From the cold trap, the sample can be split and a portion of the sample may be saved in a TD tube for later use, which is particularly useful if the sample is rare and precious. The cold trap is also required to ensure that the compounds are transferred to the column efficiently. Optimized injection parameters are needed to ensure good chromatographic peak shapes, optimal peak resolution (i.e., how well peaks are separated), and peak capacity (i.e., how many peaks can be resolved). The interface of a commercial TD system with a gas chromatograph and mass spectrometer as a detector (TD-GC-MS) is shown in Fig. 1.

Advantages and disadvantages—The main advantages of using GC in plant foliar VOC research are: (1) the sample may be taken, stored in tubes, and analyzed later (usually within a month); (2) sampling using thermal desorption preconcentrates target compounds, achieving very high sensitivity; (3) the method can separate very similar chemical compounds (isomers such α - and β -pinene); (4) instruments can be custom-made inexpensively and designed for analysis of a particular compound (or group of compounds) (e.g., the zNose [Electronic Sensor Technology, Newbury Park, California, USA], described by Kunert et al. [2002]); and (5) the instrument can be miniaturized for fieldwork (e.g., Tridion-9 GC-MS [Torion, American Fork, Utah, USA]).

The main disadvantage of GC-based instruments is that it is not a real-time instrumental approach, thus it is not suitable for research where high time resolution is needed. Furthermore, TD-GC-MS is sometimes considered to be semi-quantitative because of the unknown effects of two-stage trapping. This is especially true when complex samples are used and where high concentrations of VOCs are absorbed. However, this should not be a problem when the instrument is calibrated correctly on the compounds of interest.

Usage—GC comes in many variations (e.g., injection method or detector type), thus usage of the technique in plant foliar VOC research is broad. Direct sampling (syringe or loop injection) of plant foliar VOCs into a gas chromatograph is usually not possible due to the low VOC concentration in the sample. However, coupling GC with a high-sensitivity detector/instrument can give good results (Materić et al., 2015). Direct injection is also suitable when estimating VOCs emission potentials by analyzing tissue (solvent) extraction (Thoss et al., 2007; Räisänen et al., 2009; Lusebrink et al., 2011; Ormeño et al., 2011).

Because sample preconcentration is usually required, TD-GC-MS is the most widely used GC technique for plant foliar VOC research. TD-GC-MS is suitable for the analysis (identification, separation, and quantification) of isomers such as terpenoids, as well as for field VOC emission estimation where real-time analysis is not currently possible (Hakola et al., 2006; Ruuskanen et al., 2007; Räisänen et al., 2009).

Usage of GC systems in plant foliar VOC research requires estimation of the detection limit and calibration. The limit of the detection (LOD) and limit of the quantification (LOQ) can easily be determined for the compound of interest by measuring 20 replicates of the background signal (Armbruster and Pry, 2008). In plant VOC research, the LOD is usually calculated as a sum of the mean and three standard deviations of the background replicates ($LOD = \text{MEAN}_{\text{blank}} + \text{SD}_{\text{blank}} * 3$). Calibration is done by loading a known concentration of the VOCs of interest before or after the sample analysis, and readings are used to generate a calibration curve. Loaded amounts of standards are usually in the range of 10–50 ng, and five known concentrations are optimal for a good calibration (Janson, 1993; Räisänen et al., 2009; Materić et al., 2015).

Selected ion flow tube–mass spectrometry

Method overview—SIFT-MS is a soft chemical ionization technique that utilizes chemical ionization of the VOC with H_3O^+ , NO^+ , and O_2^+ as precursor ions (reagent ions). SIFT-MS is a sensitive instrument suitable for real-time monitoring of plant VOCs (Smith and Španěl, 2005; Sovová et al., 2011). The basics of the instrument are explained here, but more details may be found in earlier reviews (Smith and Španěl, 2005). To generate precursor ions, the instrument uses water and air in a microwave resonator, producing many different ions. A quadrupole mass filter enables the user to select the desired precursor ion (H_3O^+ , NO^+ , or O_2^+). Precursor ions enter the flow tube (a metal cylinder), where helium is used as a carrier gas. The sample is introduced into the flow tube via a heated sampling capillary with a constant flow (usually 30 mL/min). Further down the flow tube, the precursor ions react with the sample VOCs and ionize them. The ionized VOCs are filtered by the detection quadrupole and detected by the ion detector (Fig. 2).

Using the precursor ions count and a well-established kinetics library for the flow tube, it is possible to calculate the concentrations of trace gases without further calibration of the instrument. The instrument has an option of multiple

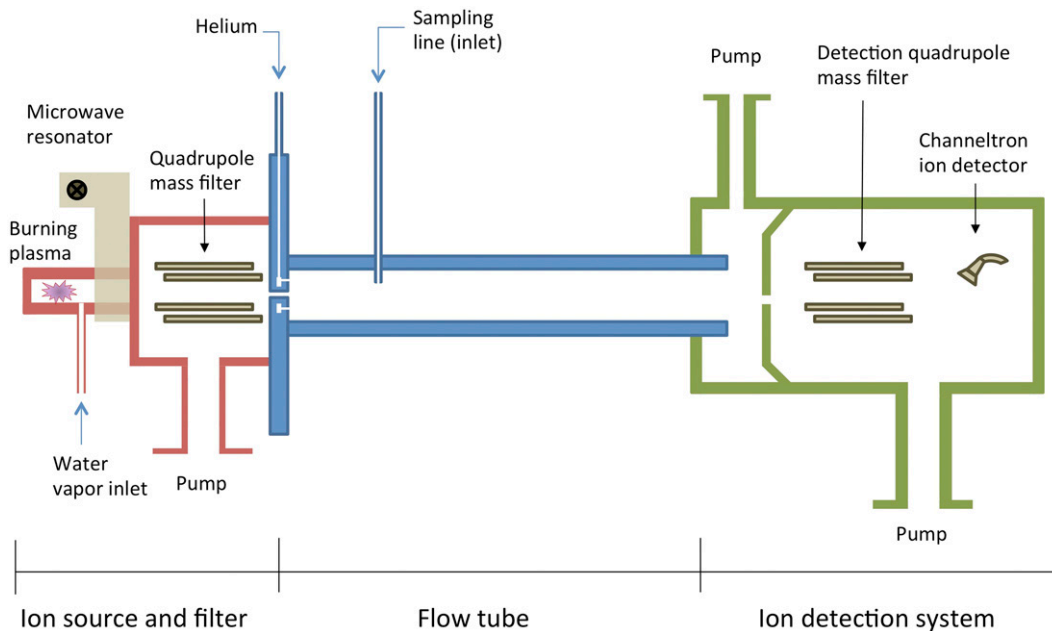


Fig. 2. Selected ion flow tube–mass spectrometer. VOCs are introduced into the *flow tube* of the instrument via the *inlet*, where they are ionized by a beam of precursor ions (H_3O^+ , NO^+ , or O_2^+) generated by the ion source. Ionized VOCs, their fragments, and water clusters are separated by the *detector quadrupole mass filter* and detected by a *channeltron ion detector*.

ion monitoring (MIM) mode, which is particularly useful for online monitoring of certain compounds (such as isoprene, methanol, and monoterpenes). Repeated sampling enables the user to average the concentration during a desired time slot.

Advantages and disadvantages—Advantages of using SIFT-MS in plant foliar VOC research are the following: (1) SIFT-MS is suitable for online (real-time) VOC measurements; (2) different precursor ions are available, which increases the qualitative performance of the instrument (identification of the gas); (3) SIFT-MS is direct and quantitative, so concentrations in parts per billion may be obtained automatically; (4) the instrument does not require calibration against gas standards.

The main disadvantages of SIFT-MS when applied to plant foliar VOC research are: (1) isoprene and monoterpenes cannot be measured simultaneously. Isoprene- H^+ , which is generated through the reaction of isoprene with the H_3O^+ precursor, has a mass-to-charge ratio (m/z) of 69, which overlaps with that of protonated methanol linked with two water molecules ($\text{CH}_3\text{OH}\cdot\text{H}^+(\text{H}_2\text{O})_2$). For this reason, isoprene should be analyzed using the NO^+ precursor for analytical accuracy (Smith and Španěl, 2005). (2) SIFT-MS is less sensitive than PTR-MS (Milligan et al., 2007; Blake et al., 2009). (3) Compared to GC-MS, differentiating isomers is extremely difficult for both SIFT-MS and PTR-MS. (4) Current SIFT-MS instruments use a quadrupole, so the time and mass resolution is quite low compared to PTR-TOF-MS (see below).

Usage—SIFT-MS is suitable for online monitoring of the major VOCs emitted from plant leaves (Smith and Španěl, 2011; Amelynck et al., 2013; Akpolat and Barringer, 2015). It can be used in different ionization modes (H_3O^+ , NO^+ , or O_2^+), allowing different qualitative applications. Most of the common foliar VOCs are well studied and the kinetics of these compounds are well known, so quantification is straightforward, directly given by the software. However, the limit of the detection should be estimated as described earlier. It should be pointed out that humidity can affect the concentration readings and condensate water must not enter the instrument.

For VOC monitoring over time, the MIM mode is most suitable. The operator should set the instrument to measure all water cluster ions, the analytes of interest, and their fragments. Generally, the number of ions monitored by the instrument in MIM mode should be set as low as possible (up to 10), but sufficient for any particular experiment, as they compete for quadrupole time in a measurement cycle.

Proton transfer reaction–mass spectrometry

Method overview—PTR-MS is the most sensitive real-time technique that uses soft ionization of hydronium ions (H_3O^+) to ionize VOCs in a drift tube. PTR-MS is suitable for online VOC monitoring with high sensitivity and high resolution. The basics of the technique are explained here (see Fig. 3), but a more extensive explanation may be found elsewhere (Blake et al., 2009; Ellis and Mayhew, 2014).

PTR-MS uses a hollow-cathode discharge source combined with a source drift region to generate hydronium ions with a purity of more than 99.5%. The ions enter the drift tube, which is a series of metal rings (electrodes) insulated from one another. The gas sample is introduced close to the beginning of the drift tube, where chemical ionization occurs. The ionized VOCs are pulled out of the drift tube by the field generated by electrodes, and focused toward the detection part of the instrument. It is possible to manipulate the voltage across the drift tube, which changes the ionization conditions inside the tube.

The ionized VOCs are separated either by a quadrupole (QUAD) or by a time-of-flight (TOF) mass spectrometer, and counted by a detector. The instrument gives raw data in counts per second (cps), which need to be converted to give concentrations in parts per billion (ppb).

Advantages and disadvantages—The main advantages of using PTR-MS in plant foliar VOC research are: (1) PTR-MS is the most sensitive instrument for real-time VOC research; (2) PTR-MS instruments come equipped with either quadrupole (PTR-QUAD-MS, or just PTR-MS) or time-of-flight mass spectrometers (PTR-TOF-MS); (3) instruments can be equipped to have different precursor ions, which increase the analytical power of the instrument; (4) the ion-molecule collision energy in a drift tube (more precisely, E/N) can easily be changed by changing the electric field parameters on the electrodes, and hence improving the analytical power of the instrument (Blake et al., 2009; Ellis and Mayhew, 2014); (5) PTR-MS could be coupled with fastGC for isomer analysis (Materić et al., 2015).

The main disadvantages of PTR-MS are: (1) the method requires calibration gas standards to perform quantitative analysis; (2) the instrument is not suitable for qualitative analysis of isomers because it gives the concentration of all compounds of the same molecular weight; and (3) unlike the SIFT-MS, where switching the reagent ions is achieved in seconds, such switching in PTR-MS is not that straightforward and requires a stabilization period of several minutes.

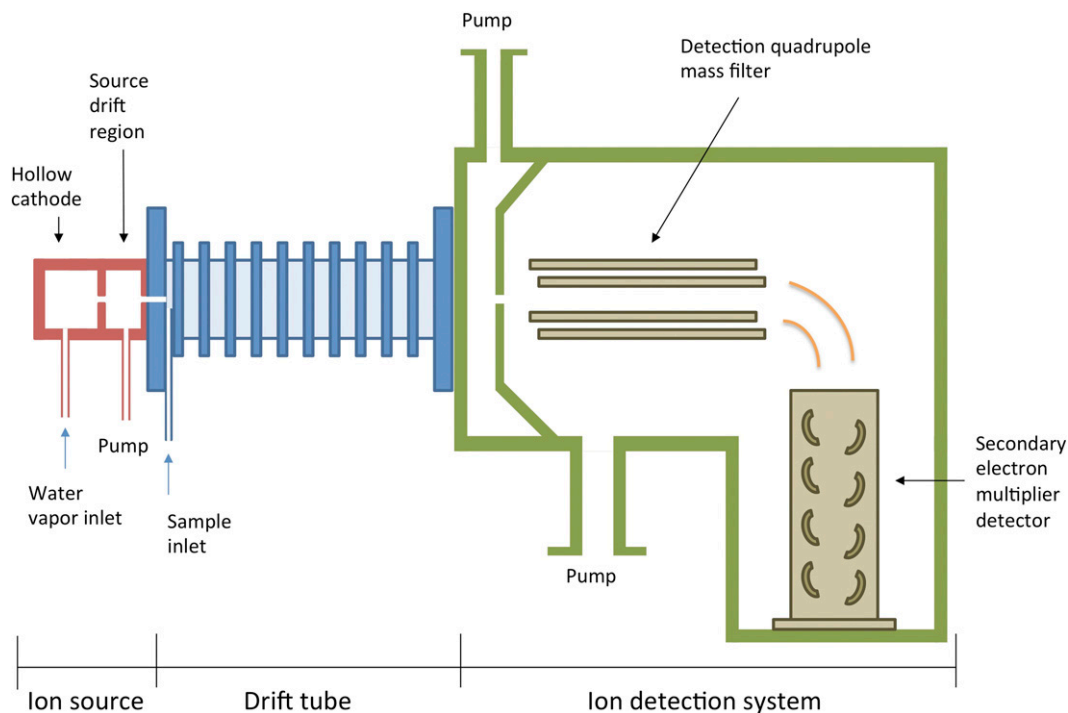


Fig. 3. Proton transfer reaction–mass spectrometer. VOCs are introduced into the *drift tube* of the instrument via a *sample inlet*, where they are ionized by a beam of reagent ions (H_3O^+) generated by the ion source. Ionized VOCs and their fragments are separated by the *detector quadrupole mass filter* (or time-of-flight) and detected by the *detector*.

Usage—PTR-MS is the most sensitive technique for real-time plant foliar VOC analysis (Fall et al., 1999; Tani et al., 2003; Ruuskanen et al., 2005; Loreto et al., 2006; Brilli et al., 2011). PTR-TOF-MS is suitable for real-time experiments with high time (<1 s) and mass resolutions. PTR-QUAD-MS has similar applications to SIFT-MS. If specific isomers need to be analyzed, the instrument should be run in parallel with GC-FID/MS (Lindinger et al., 2005; Ruuskanen et al., 2005). Furthermore, recent developments in coupling PTR-MS with fastGC showed successful monoterpene separation at low concentrations, applicable in plant foliar VOC research (Materić et al., 2015).

To convert raw data (cps) into absolute concentrations, the instrument needs to be calibrated with a mixture of standard gases that have a known concentration. Ideally, the VOC of interest should be included in the mixture; otherwise, the mixture should contain standard gases with different molecular masses that cover the instrument's available mass range.

There are several solutions available for instrument calibration. The most popular one is to use gas cylinders containing several gases with known concentrations (usually 1 ppm) and make a sequence of dilutions using flow controllers. Alternatively, permeation tubes or a diffusion source could be used as a cheaper solution (Maria et al., 2002; Tumbiolo et al., 2005; Thompson and Perry, 2009).

Usually, a scripting language such Perl, MATLAB, R, or Python is essential to use to process large amounts of raw data and convert them into concentrations and emission rates (Müller et al., 2013; Materić and Gonzalez, 2014).

SAMPLING METHODS

The choice of sampling method for plant foliar VOC research mainly depends on the spatial scale at which the experiment is meant to operate (Hewitt et al., 2011). The most common sampling approaches are leaf/branch enclosures and tower-based measurements (Dabberdt et al., 1993; Hewitt et al., 2011). In addition, solvent extraction of plant foliar VOCs from leaf tissue is a common approach to estimate VOC reservoirs and essential oils (Thoss et al., 2007; Räisänen et al., 2009; Lusebrink et al., 2011; Ormeño et al., 2011; Bicchi and Maffei, 2012).

However, in this paper, only the leaf/branch enclosure systems are reviewed because they are the most common in plant foliar research. Other systems, including a flower enclosure, are described elsewhere (Tholl et al., 2006; Kallenbach et al., 2014).

Chamber materials—Leaf cuvettes and branch chambers (Fig. 4) are usually custom-made, using VOC semineutral materials such as: polytetrafluoroethylene (PTFE/Teflon), stainless steel, brass, glass, and perfluoroalkoxy (PFA) (Tholl et al., 2006; Hewitt et al., 2011). Although there is no completely ideal material, the best available is PTFE (Teflon). An adapted photosynthesis leaf cuvette can also be used for some experimental setups, but this is not the best solution because some standard materials (such as rubber and plastics) in these cuvettes may emit or adsorb VOCs.

Chamber design—Chambers or leaf cuvettes vary in design from the very simple (a bag with internal support to prevent contact of the plant tissue with the chamber itself) to quite complex chambers (a chamber with a fan, temperature and humidity sensors, and controls, controlled by a computer), including robotic systems (Tholl et al., 2006; Kolari et al., 2012). At least one wall of the chamber should be transparent, allowing uninterrupted photosynthesis, as emission of some VOCs is light dependent (Schuh et al., 1997; Sharkey and Yeh, 2001; Duhl et al., 2008).

Leaf cuvettes should be designed in such a way that good air mixing is achieved during flushing, and in the case of a large-volume branch chamber, the use of a fan should be considered. If a fan needs to be used, the best solution would be to situate the fan's motor outside of the chamber. A fan should be used carefully because high speed and turbulence may cause plant stress and thus artificially induce VOC emission. However, good air mixing is vital to ensure homogeneous gas concentrations and stable temperature within the cuvette.

Any plant enclosure brings some changes in abiotic factors that could affect VOC emission (see Fig. 5), such as: mechanical wounding, a shift in CO_2 concentration, increased humidity, changes in temperature, changes in ozone concentration, and changes in light conditions. Thus, the enclosure system should be designed in a way that reduces these factors to a minimum. Most of these

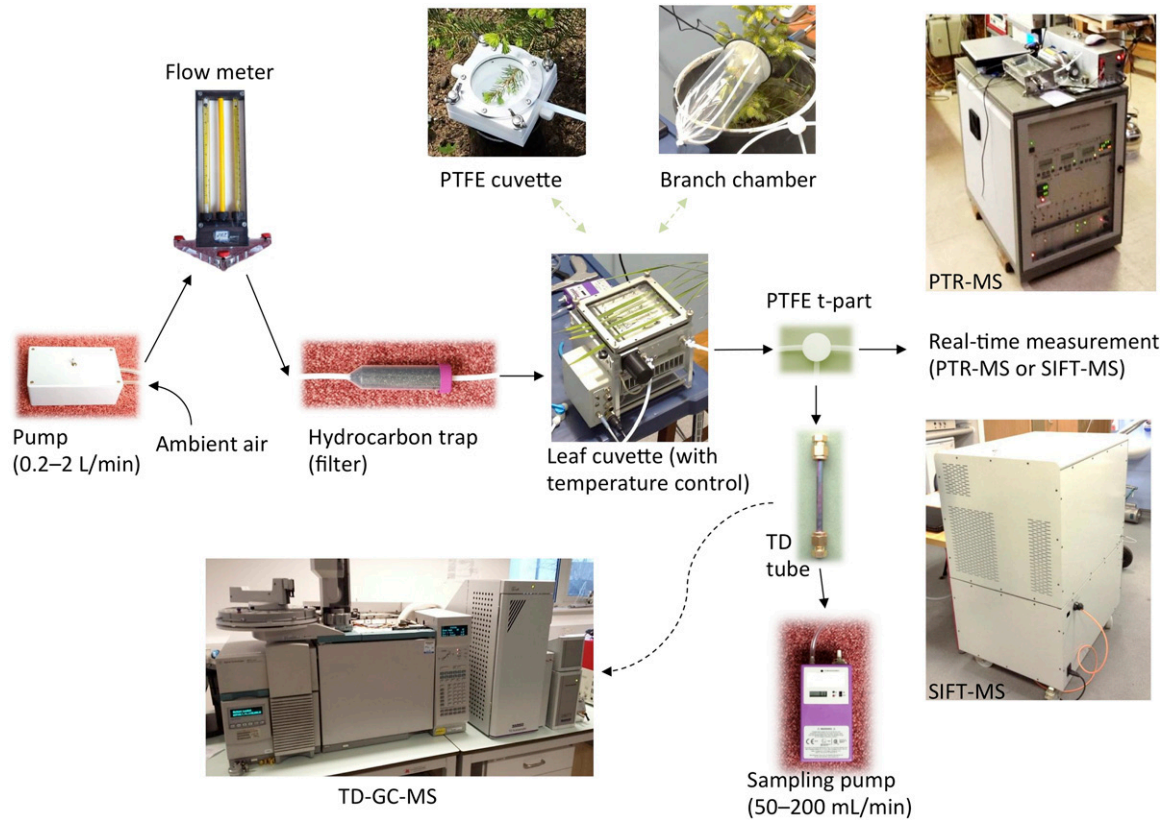


Fig. 4. Plant VOC sampling system. Ambient air is used to generate VOC-free air, and the flow is measured by a flow meter. Various cuvettes/chambers could be used, preferably made of PTFE. The VOCs are directly measured by a real-time instrument and/or trapped on TD tubes for later GC-MS analysis.

changes (CO₂, humidity, temperature, and ozone concentrations) can be overcome by increasing the airflow of the system. Wounding can occur when plant tissues are in contact with the sampling equipment. The plant–chamber connection can be made using a PTFE-coated sponge and/or PTFE tape. To reduce the effect of enclosure-induced stress, enclosed plants should be flushed with clean air over a period ranging from several hours to several days, depending on the enclosure type, compound of interest, and plant species.

Chamber types—There are two main types of chamber when considering air flushing: static and dynamic chambers.

1. Static chambers—These chambers have a long tradition in plant–soil gas exchange analysis providing measurements of CO₂, CH₄, and N₂O and flower VOC studies (Matson, 1991; Matson and Harriss, 1995; Kallenbach et al., 2014). In such systems with no airflow, a plant/branch/leaf is enclosed in a chamber where the concentration of the compounds changes over time. A sample of the air is taken at several time points, usually by syringe or a solid-phase microextraction (SPME) needle, and subsequently analyzed by an instrument (usually GC). Polydimethylsiloxane (PDMS), in the form of silicon laboratory tubing, can also be used when passive adsorption sampling with low time resolution is sufficient (Kallenbach et al., 2014). Care should be taken when using the syringe sampling approach, as this could generate low pressure in a sealed chamber system, resulting in several disadvantages such as: artificial VOC diffusion from soil, ambient air, or plant tissue, and change in photosynthesis parameters.

In static chamber experiments, change in concentration of the specific VOC of interest over time is used to calculate the flux (emission or uptake rate). In plant foliar VOC analysis, this is not a commonly used technique because the enclosure introduces a significant change in abiotic factors and thus artificial VOC fluxes. A more detailed description of the method may be found elsewhere (Tholl et al., 2006; Hewitt et al., 2011; Kallenbach et al., 2014).

2. Dynamic chambers—Dynamic chambers are the most commonly used chambers in plant foliar VOC analysis. In a dynamic system, part of a plant with a known leaf surface area is enclosed in a chamber. A typical chamber is made of low-VOC-emitting/adsorbing materials, has an inlet and outlet, and is continuously flushed with clean air.

The inlet is connected to a clean air supply, which flushes the system at a known flow rate (usually between 100–2000 mL/min). At the outlet, a portion of the air is collected onto a trap (such as thermal desorption tubes, described later) or directly introduced into an instrument capable of real-time measurement (PTR-MS or SIFT-MS). To calculate the flux (emission or uptake rate), the flow should also be measured accurately during the experiment. Dynamic chamber systems involve the following components/considerations:

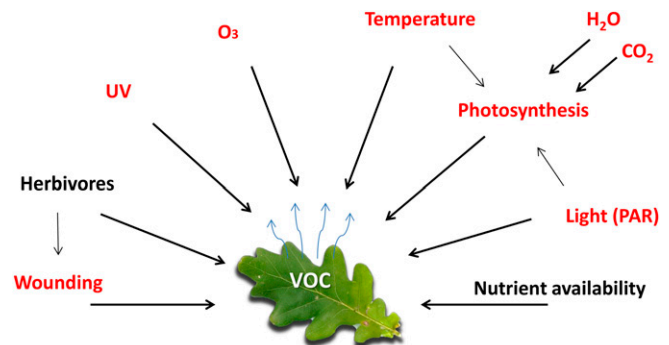


Fig. 5. Impact of abiotic and biotic factors on plant VOC emission. The factors in red are affected by any plant enclosure.

A. Pumps—All dynamic chamber systems require (1) vacuum pumps or compressors and (2) clean air. Vacuum pumps are used to pull the air into the analytical instrument or into a VOC trap. The mass spectrometric techniques PTR-MS and SIFT-MS have a negative pressure at the inlet, so a vacuum pump is not needed for sampling. However, a low-flow vacuum pump (usually 50–200 mL/min) is needed for TD-GC-MS. Air compressors are used to ensure a constant flushing flow of air over the enclosed branch/leaf. It is crucial to maintain constant airflow, and hence to monitor it during the experiment. Thus, a flow meter is usually part of the sampling system (Fig. 4). However, a voltmeter/data-logger can be used instead, because the flow rate generated by a pump will linearly correlate with its operating voltage, when applied to a given length of the pipe with a specific conductance. Thus, a simple calibration should be performed at three to five different voltages on a given sampling system measuring the exact flow, generating a calibration line that can be used subsequently.

B. Zero air—Dynamic sampling systems usually use incoming VOC-free air (called zero air), which can be obtained in several ways. The simplest way of obtaining zero air, useful for both laboratory and fieldwork, is to use filters packed with a hydrocarbon trap (called scrubbers), commonly used in GC, SIFT-MS, and PTR-MS laboratories. For some applications, such as low (sub-ppb) measurements, hydrocarbon traps might not give sufficiently pure air, which can result in a low signal-to-noise ratio and hence high limits of detection. Thus, more advanced, high-temperature-based zero air generators need to be used.

For the smaller laboratory applications, zero air can be supplied from a gas cylinder and used as such, or mixed with nitrogen, oxygen, CO₂, and water vapor to make the desired composition of synthetic air. Alternatively, zero air generators can be used to generate a high amount of VOC-free air, which can be useful for work in laboratories and field stations (Kolari et al., 2012).

If ambient air must be used, the foliar VOCs should be measured/sampled from both the enclosure inlet and outlet. This may be challenging in an online measurement design (for example using SIFT-MS or PTR-MS), as appropriate automatic solenoid valves or switchers need to be inserted in the piping to control the direction of the flows in the system and enable the instrument to measure inlet and outlet VOC concentrations simultaneously, in time sequence.

C. Sample preconcentration—To reach detection limit of particular VOCs, in some cases sample preconcentration is required. Preconcentration is achieved either by using a sorbent material(s) or by cold trapping. Sorbent material (such as Tenax TA [Tenax Corporation, Baltimore, Maryland, USA]) is packed in a glass-coated stainless steel tube, known as a thermal desorption tube. An exact amount of air (<10 L) is pumped through the tube at a low rate (usually 50–200 mL/min), allowing adsorption of VOCs onto the trapping material.

DISCUSSION

How to choose the analytical technique—The choice of instrument in plant foliar VOC research depends mainly on three factors: the research question, instrument availability, and budget. The major consideration to take into account is the trade-off between time resolution and separation. Real-time instruments cannot separate isomers (unless coupled with GC with the time cost), and the best-optimized GC system cannot achieve time resolutions of less than a couple of minutes (Fig. 6) (Blake et al., 2009; Misztal et al., 2012; Shen et al., 2012; Ellis and Mayhew, 2014; Materić et al., 2015).

If the goal of the research does not require real-time VOC measurement, then GC-based techniques are suitable and less expensive (Figs. 6 and 7). GC sampling can be accomplished through direct air injection (usually via a loop) or preconcentration (usually by TD). Direct injection is suitable only if the compound of interest has a suitable concentration, which usually is not the case when analyzing plant foliar VOCs. Preconcentration, on the other hand, leads to higher sensitivities, but the approach brings additional variables to the quantification (trapping, re trapping, desorption), which could make the method only semiquantitative. While performing experiments based on TD-GC instruments, adequate calibration of the VOCs of interest is crucial for quantification. If the experiment is not

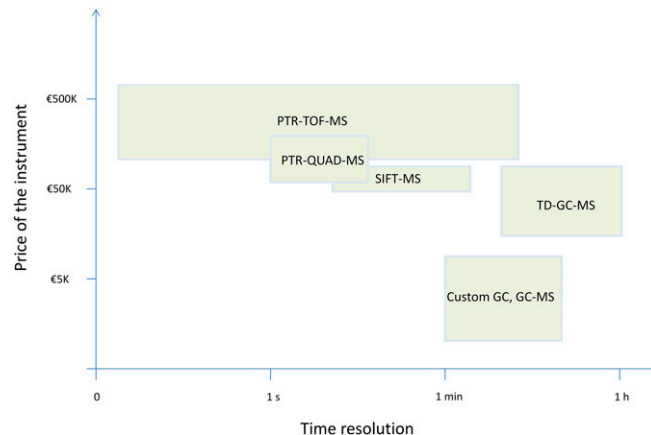


Fig. 6. Relation between instrument cost and time resolution.

to be conducted in real time or the results need to be qualitative, then TD-GC-MS is the gold standard. On the other hand, real-time experiments require PTR-MS or SIFT-MS (Smith and Španěl, 2005, 2011; Blake et al., 2009; Ellis and Mayhew, 2014). There are several reasons why PTR-MS is used more often than SIFT-MS: (1) PTR-MS is more sensitive; (2) it comes as either a quadrupole or time-of-flight mass spectrometer; and (3) the instrument is produced by several companies, which results in it being more readily available.

PTR-TOF-MS is the most expensive and the best instrument for real-time plant VOC research, particularly if the compounds of interest are well characterized (Fig. 7). The technique is evolving every year, so sensitivity and resolution are constantly increasing. Current PTR-TOF-MS systems have time resolution <1 s, mass resolution between 1500 and 10,000 m/Δm full width at half maximum (FWHM), and a detection limit less than 1 part per trillion by volume (pptv) (Sulzer et al., 2014). Thus, PTR-TOF-MS is needed in experiments where time and mass resolution are crucial (e.g., plant signaling, emission rate monitoring of many compounds). However, such high acquisition rates raise issues of storage and data analysis, because the instrument can easily generate 1 TB of data per operational day.

PTR-QUAD-MS is more affordable and the most sensitive instrument on the market (<1 pptv). However, novel PTR-TOF-MS upgrades are obtaining this sensitivity, showing the way for future PTR-MS development (Sulzer et al., 2014). Despite this, quadrupole PTR-MS still has its place in long-term online VOC monitoring, and is well suited to fieldwork, as it is more compact, lighter, and easier in terms of data storage and analysis.

SIFT-MS may be applied to plant VOC research in a similar way to PTR-QUAD-MS, with some advantages and disadvantages. To its advantage, it is a truly analytical instrument with a well-established kinetics library, so it does not require calibration (Smith and Španěl, 2005). On the other hand, it has a lower sensitivity and the disadvantage that the instrument cannot simultaneously measure isoprene and monoterpene concentrations using the H₃O⁺ precursor ion (as explained earlier) (Milligan et al., 2007). However, an analytical method has been developed to analyze these two compounds simultaneously using NO⁺ as the precursor ion (Wang et al., 2003).

How to choose the sampling method—Apart from instrument availability, the main factors to be considered in choosing

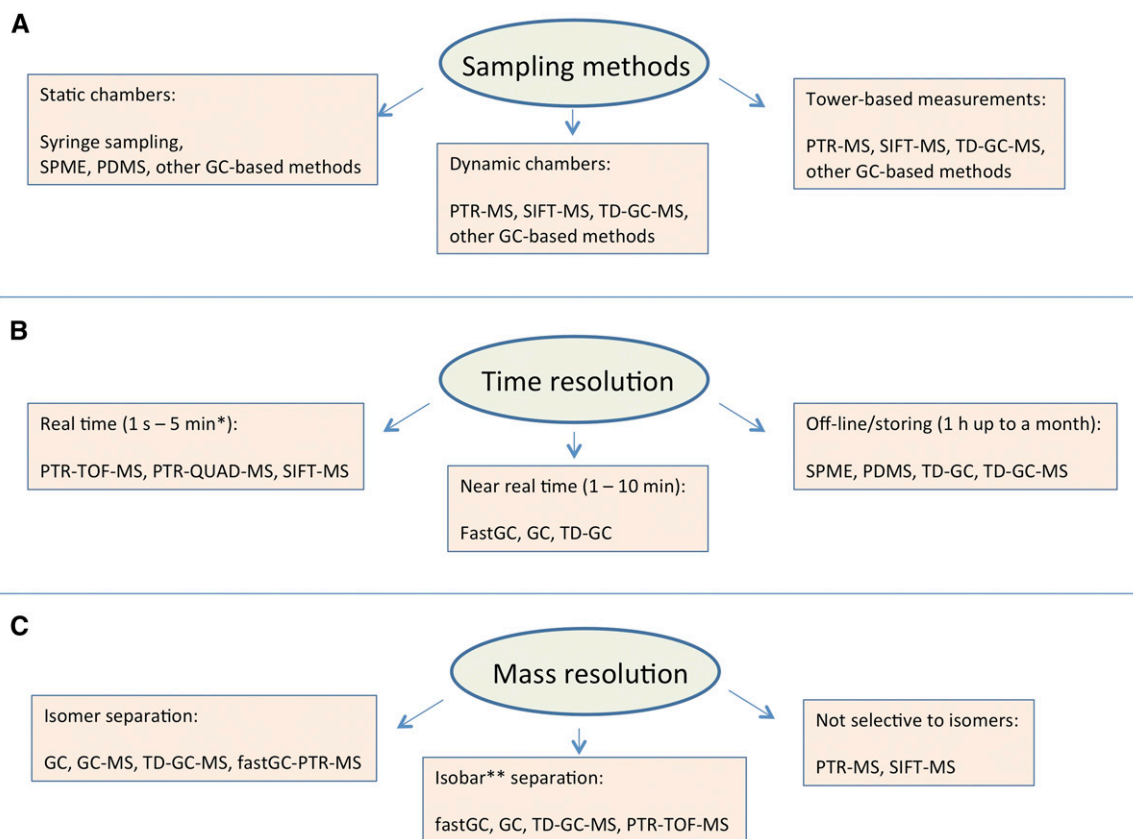


Fig. 7. Instrument choice with respect to (A) required sampling method, (B) time resolution, and (C) mass resolution. *Cumulative measurement by a real-time instrument. **Molecules that have the same molecular mass but are composed from different atoms.

the sampling method are: (1) the concentration of the foliar VOCs in the system, (2) the time resolution required, and (3) the level of VOC separation required (Fig. 7).

Real-time experiments, which have a time resolution of around 1 s (see the sections on SIFT-MS and PTR-MS), require a minimum flow rate of approximately 30 mL/min, and thus require a dynamic chamber (Räisänen et al., 2009; Kolari et al., 2012). Sample dilution in a dynamic system is usually not a problem for these instruments, as they have sub-ppb sensitivities (refer to manufacturer's specifications: IONICON Analytik, Innsbruck, Austria; KORE Technology, Cambridgeshire, United Kingdom; Syft Technologies, Middleton, New Zealand).

Near-real-time and offline experiments, with time resolutions of a couple of minutes to a couple of hours (see section on TD-GC-MS), can be used in static and dynamic chambers. If the concentrations of the VOCs are high enough, which is usually not the case when measuring foliar VOC emissions, direct GC sampling can be used. However, when the VOC concentration is low, sample preconcentration must be performed. Sorbent tube/cartridge preconcentrations require an airflow, so a dynamic chamber is required as described in Janson (1993). On the other hand, passive (static) sorption/adsorption can be applied in static systems using PDMS and SPME (Kallenbach et al., 2014). However, quantification of VOCs in such a system is difficult and would require a calibration method development similar to Bouvier-Brown et al. (2007).

Future (and current) developments in methods in plant foliar VOCs are linked to development of instrumentation/methodology

that can analyze samples with low concentration of VOCs (sub-ppb levels), but with high time resolution (up to 5 min) and high analytical power (isomer separation). This goal led to the development of a group of “cloned” methods, which lie between GC, SIFT-MS, and PTR-MS, such as: (1) fastGC-PTR-MS—a method with GC time resolution of less than 2 min with all of the sensitivity benefits of PTR-MS (Romano et al., 2014; Materić et al., 2015); (2) SIFT–drift tube–MS—a method developed to increase analytical performance of SIFT-MS by varying E/N (Spesyvyi and Španěl, 2015); (3) switchable reagent ion–PTR-MS—a method that, apart from H_3O^+ , utilizes NO^+ , O_2^+ , and Ar^+ for better analytical performance (Jordan et al., 2009; Lanza et al., 2013); (4) quadrupole-interfaced PTR-MS—a method that uses a quadrupole in front of the drift tube, in order to get better instrument sensitivity (Sulzer et al., 2014). Miniaturization of the instruments and use of different sampling methods and detectors have made GC methodology the only portable measurement solution so far (Overton et al., 2001; Bicchi and Maffei, 2012). Thus, more synergistic methods and approaches are expected for plant foliar VOC research in the future.

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APPENDIX 1. List of abbreviations.

FID: flame ionization detectors
GC: gas chromatography
GC-MS: gas chromatography–mass spectrometry
LOD: limit of detection
MS: mass spectrometry
PDMS: polydimethylsiloxane
PTFE: polytetrafluoroethylene
PTR-MS: proton transfer reaction–mass spectrometry
PTR-TOF-MS: proton transfer reaction–time-of-flight–mass spectrometry
QUAD: quadrupole
SIFT-MS: selected ion flow tube–mass spectrometry
SPME: solid-phase microextraction
TD: thermal desorption
TD-GC-MS: thermal desorption–gas chromatography–mass spectrometry
TOF: time of flight
VOC: volatile organic compound
