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Real-Time Volatilomics: A Novel Approach for Analyzing Biological Samples

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The use of the 'omics techniques in environmental research has become common-place. The most widely implemented of these include metabolomics, proteomics, genomics, and transcriptomics. In recent years, a similar approach has also been taken with the analysis of volatiles from biological samples, giving rise to the so-called 'volatilomics' in plant analysis. Developments in direct infusion mass spectrometry (DI-MS) techniques have made it possible to monitor the changes in the composition of volatile flux from parts of plants, single speci- mens, and entire ecosystems in real-time. The application of these techniques enables a unique insight into the dynamic metabolic processes that occur in plants. Here, we provide an overview of the use of DI-MS in real-time volatilomics research involving plants.

Volatile Plant Metabolites: Solving Complex Problems Requires Advanced Approaches

Plants produce N100 000 different chemical compounds [1], some of which, biogenic volatile organic compounds (BVOCs; see Glossary), are emitted to the atmosphere [2]. It is estimated that terrestrial vegetation alone emits 30 000 different volatiles [3,4], both as a result of usual plant metabolism and in response to stress [5,6]. Stress can be due to abiotic factors, such as illumi- nation conditions, drought, overabundance of water, or harsh temperatures [1,7,8]. Other stressors include interactions with herbivores, fungi, bacteria, and various other biological pathogens [8–10]. Thus, volatile compounds emitted by vegetation can be considered a 'smell print' of sorts, which, when analyzed comprehensively, can reveal details regarding the condition of particular plants as well as entire ecosystems.

Plant BVOCs include volatile hormones (ethylene, methyl salicylate, and methyl jasmonate) [9,11], terpenoids (isoprene, monoterpenes, sesquiterpenes, and their derivates) [7,10,12,13], and lipoxygenase (LOX) pathway volatiles, also called 'green leaf volatiles' (GLVs), including C6 alcohols and aldehydes [5,8], flower VOCs [12], and herbivore-inducted volatiles (HIV) [2]. Major challenges in the determination of these compounds are due to their numerousness and often extremely low abundance [14], which makes it necessary to analyze them using sensitive, high-throughput analytical techniques [15]. The current gold standard in the analysis of volatile plant metabolites is gas chromatography-MS (GC-MS) [16]. This method does have certain limitations, such as relatively lengthy analysis and often elaborate sampling procedure, which might affect the composition of the gaseous mixture. These limitations have been addressed in recent years by the development of DI-MS techniques [16]. These techniques are also referred to as direct injection MS, especially when the methodology involves a rapid sample injection, rather than prolonged sampling, which is more common in metabolomics research. Unlike GC-MS, DI-MS does not involve a separation step, which facilitates real-time measurements [17] and enables the investigation of phenomena resulting from a rapid response of plants to stressors [18].
Over the past 10 years, several reviews have been published detailing the generation of BVOCs [8–10,19,20], their analysis [11,14–16,21], and their impact on the environment [2]. Here, we provide a comprehensive description of recent advances in DI-MS techniques, which are quickly developing into unique and useful tools for the analysis of volatile plant metabolites.

DI-MS Techniques: An Ideal Solution for Real-Time Measurements?

Accurate mass measurement is currently one of the best methods for the identification of chemical compounds. The closer the result is to the exact mass of the compound, the higher the likelihood of its correct identification, with an ideal case being the determination of the exact monoisotopic mass of the compound of interest. Conversely, unambiguous identification of a chemical compound or ion might be challenging without the correct determination of its exact mass. For instance, isoprene, one of the main BVOCs emitted by plants, has a molecular formula of C_5H_8 and a nominal mass of 68 Da. However, the same nominal mass is shared by furan (C_4H_4O), a compound with entirely different structure and chemical properties. Furan is emitted to the atmosphere mostly due to combustion processes [22]. Nevertheless, both isobaric com- pounds can be discerned based on their exact mass (68.0626 Da and 68.0262 Da in the case of isoprene and furan, respectively). More challenging still is the discrimination between structural isomers [23], such as isoprene and piperylene (1,3-pentadiene; C_5H_8), which have the same exact mass and have to be discerned based on additional properties. These might include reten- tion indices in the case of GC, fragmentation pattern in the case of MS, or common sense. In the above example, there is no indication that piperylene, a substrate in the synthesis of polymers [24], is a plant BVOC.

The need to discriminate between chemical compounds based on their exact mass is particularly acute in the case of DI-MS techniques due to the lack of a sample separation step, which makes it necessary to rely solely on the accurately measured ion mass. While DI-MS techniques appear to be tailored for the analysis of VOCs in general, and BVOCs in particular, for successful application they need to be characterized by the following: (i) a versatile and well-researched ionization mechanism; (ii) soft ionization conditions limiting the fragmentation of chemical compounds; (ii) high sensitivity and mass resolution necessary for trace analysis and determination of the exact mass of the ion; (iv) straightforward sampling; and (v) possibility to conduct the measurement over an extended period of time.

One of the most prominent incarnations of DI-MS used in real-time measurements is proton transfer reaction MS (PTR-MS). It has been rapidly developed since its introduction during the mid-1990s [25] and is used in numerous fields, such as environmental monitoring [26], medical diagnostics [27], and food analysis [28]. The technique was, from its inception, developed with the analysis of BVOCs in mind. One of its earliest implementations was the assessment of the emission of volatile metabolites, such as methanol, acetaldehyde, ethanol, acetic acid, isoprene, and methyl ethyl ketone (MEK), from leaves [29]. The coupling of PTR-MS with time-of-flight MS (TOFMS) opened a new chapter in the monitoring of volatile metabolites. It introduced exceptional resolution, detection thresholds in the ppt range, and high acquisition rates [30].

Currently PTR-MS is the method of choice for real-time BVOC emission monitoring, owing to its ability to ionize most volatile plant metabolites while not ionizing the main constituents of atmospheric air [31]. There is then no need to use a separate carrier gas, which would otherwise complicate sampling and dilute the BVOC flux. This is a particular advantage over another DI-MS technique, namely selected-ion flow-tube MS (SIFT-MS) [32]. Owing to the relatively well- established kinetics of the proton transfer reaction, it is possible to estimate the concentration of monitored BVOCs [33]. A simplified overview of the said reaction is shown in Figure 1.

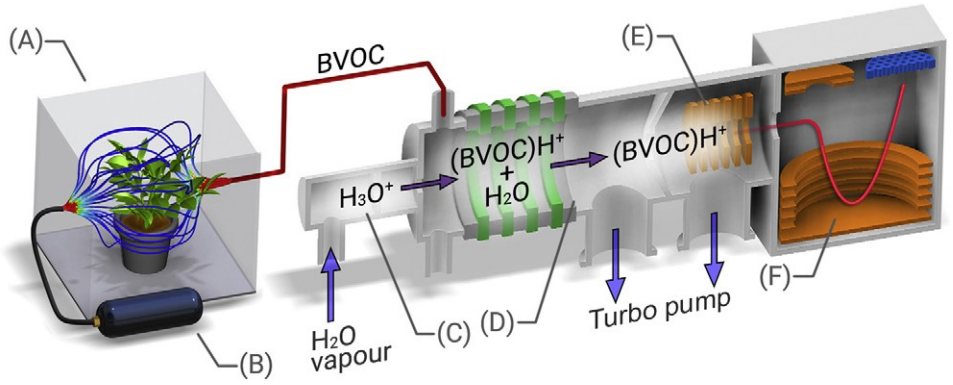

Figure 1. Principle of Operation of Proton Transfer Reaction Mass Spectrometry (PTR-MS). In the most common configuration, the proton transfer reaction occurs between hydronium ions (H_3O^+) and volatile organic compounds (VOCs) and, in most cases, yields $(VOC)H^+$ ions [37]. Most VOCs, with the exception of those characterized by a lower proton affinity than water, such as short alkanes, are subject to this mechanism. However, some compounds, such as alcohols, are ionized due to dehydration [38]. A hydronium ion is generated during a hollow cathode discharge and is subsequently directed to the drift chamber, where it reacts with the analytes. The product ions are then separated and reach the detector (usually a microchannel plate; MCP) [39]. When using PTR-MS, it is difficult to monitor the concentration of structural isomers, such as monoterpenes [40,41] or methyl vinyl ketone (MVK) and methacrolein [42,43], all of which are commonly occurring biogenic VOCs (BVOCs). Thus the concentration of structural isomers is often reported as the total value. The elements of the diagram are: sampling enclosure (A), ambient air VOC filter (B), hollow cathode (C), drift chamber (D), transfer lenses (E), and time-of-flight mass analyzer (F).

Furthermore, such determination is characterized by a high temporal resolution, enabling the detection of labile and incidentally released BVOCs. However, PTR-MS cannot be used on its own for the identification of chemical compounds, since the mass of the parent ion is insufficient for conclusive determination, as indicated in the Metabolomics Standards Initiative (MSI) guidelines [34]. Although this can be remedied to some extent by complementary use of GC [17], the introduction of the lengthy separation step hinders real-time measurements. A more recent approach is to improve BVOC identification by using alternative ionization sources coupled with tandem MS (MS/MS) [35,36].

Due to its limitations in the identification of unknown compounds, PTR-MS is predominantly used for monitoring of real-time changes of known entities or for fingerprinting [16]. In both cases, the analytical information contained in the obtained mass spectra can be extracted by proper data analysis. The first step in the data-mining process is usually preprocessing of the raw mass spectra. It involves postanalysis internal calibration of the m/z axis, which is necessary to reach the mass accuracy sufficient for assigning molecular formulas to particular spectrometric peaks. It is also necessary to remove the baseline signal, such as by subtracting the mass spectra of the background (usually ambient air or sample before stress-induced release of BVOCs). After eliminating ions that are not related to the sample (O^{2+}, NO^{+}, H_2O clusters, etc.) [44], relevant peaks are selected based on a combination of criteria such as a concentration threshold, corre- lation with reference data, or common sense. The target species can then be identified based on the isotope ratios [45], and the exact mass. In the case of fingerprinting, the preprocessing of the raw data is followed by additional data-mining stages. First, it is important to reduce the number of variables (m/z signals) by selecting only those that are relevant in a given scenario. The selected variables are also transformed and normalized. If the chosen fingerprinting approach is based on unsupervised multivariate statistics, as is often the case in 'omics research, the transformed data matrix can then be subjected to principal component analysis (PCA), or to several other unsupervised algorithms, such as the hierarchical cluster analysis (HCA).

Box 1. Novel Leaf Cutter for Real-Time Monitoring of BVOCs

Sampling of BVOCs from particular plant parts, such as leaves, can be challenging. To limit the background effect, it is desirable to build the sampling enclosures from chemically inert materials, such as steel, glass, polytetrafluoroethylene (PTFE), or polyaryletheretherketone (PEEK). Furthermore, material properties other than the effect, or lack thereof, on the VOC composition should also be considered. For instance, sampling with translucent enclosures in direct sunlight can lead to a significant increase in the internal temperature and, thus, affect the plant metabolism [54], while opaque ma- terials, such as steel, have limited applicability in studies in which PAR is a factor. Care should also be taken when fastening the sampling chamber around the selected plant part, since creating an airtight seal might lead to compression damage and emission of wound-induced BVOCs, altering the headspace of the sample [12].

However, in some studies, the effect of wounding due to natural phenomena or to the activity of chewing and piercing her- bivores on the composition of volatiles emitted by plants is the main focus. The use of DI-MS techniques allows for real- time monitoring of rapidly released plant stress volatiles. However, their applicability is limited by the need to interrupt the sampling to perform the wounding manually, by smearing of the signal due to inertia in large sampling enclosures, and by the lack of precision when operating the cutting implement in a relatively tight space. A novel, within-chamber leaf cutter has been developed to address these issues and to facilitate real-time monitoring of leaf-wounding responses [18]. A steel cutting blade is mounted within the 4×2-cm sampling head of the Walz GFS-3000 gas-exchange system (Walz GmbH, Effeltrich, Germany). It can be operated through a rod passed through a stainless tube sealed with PTFE stoppers. The leaf that is placed in the sampling chamber is cut by turning the operating rod 180°, resulting in 14-mm incisions. The modified gas exchange system is coupled directly to a PTR-TOFMS through a short PTFE interface to minimize the dead volume. By using this setup, it was possible to detect a hitherto unnoticed initial burst of monoterpene emission within seconds of the wounding of a *Eucalyptus globulus* leaf. This would likely not be possible if the wounding was performed out- side the gas sampling chamber, or if the chamber was opened to perform the incision and subsequently sealed, with a lag in stabilization of the carrier gas flow [18].

Zoom in: A Look at the Details

To investigate the impact of different extraneous factors, such as wounding or illumination, on volatile plant metabolites, it is often necessary to study them in isolation, targeting particular plant parts for sampling. This has the added benefit of reducing the dead volume of the sampling enclosures, which prevents unwanted dilution of the gaseous mixture with ambient air, reducing signal acquisition delay and blurring.

The effect of mechanical damage on the emission of plant BVOCs is commonly investigated by testing the leaf lamina. Wounding causes a quick release of fatty acids from plant membranes, which initiates a sequence of reactions leading to emission of GLVs [46]. Direct damage to the leaf (e.g., through cutting with a blade or puncturing) causes immediate oxidation of the contents of cytoplasm and vacuoles by the atmospheric air (Box 1). This triggers reactions of the LOX path- way as well as the emission of its products and volatile compounds from potential reservoirs. For instance, the increased emission of methanol after wounding is partly due to the release of the products of pectin demethylation accumulated in the liquid phase of leaves. Leaf lamina damage can also be the result of squeezing, for instance due to pinching and consumption by herbivores or flexible bending under strong winds, or of the activity of leaf miners, in which case the release of volatiles occurs through the stomata [47]. Benzenoids, jasmonates, and isoprenoids (isoprene, mono-, di-, sesquiterpenes, and their derivatives) emitted in such a way are regarded as infochemicals for herbivorous parasites. The nature of the damage can be inferred from the composition of the released gaseous mixture, with punctures in leaves producing higher relative amounts of benzenoids, jasmonates, and induced isoprenoids, while squeezing results in a higher proportion of hexanal family LOX products. The initial, rapid response to wounding leads mostly to the emission of simple molecules containing a few carbon atoms, of which methanol, hexenal, and acetaldehyde are preeminent [47,48].

Temporal variations in the composition of the gaseous mixture emitted from leaves are also due to their senescence. For instance, PTR-MS was used to measure the emission of BVOCs from maize (*Zea mays* L.) leaves at different development stages. The relative concentration of methanol, the most abundant VOC, varied from 61% to 13% to 26% for growing, mature, and senescent leaves, respectively [49]. The flux composition showed the greatest variety in the case of senescent leaves. The variability of the emission from leaves at different development stages is further compounded by additional factors, such as the photosynthetically active radiation (PAR) and temperature [50,51]. This highlights the particular applicability of DI-MS techniques in real-time monitoring of the changes in the BVOC emissions from leaves under rapidly changing illumination conditions, also during field measurements [51]. Aside from the diurnal fluctuations, seasonal changes in the emission of volatiles from parts of plants, further impacted by drought periods, can also be measured on-line, such as by enclosing entire tree branches during field campaigns and sampling the gas exchange using PTR-MS [50]. The applications of DI-MS techniques in the analysis of volatile com- pounds emitted from plants are not limited to their aboveground parts. They can also be selectively applied to roots, for instance to assess the impact of symbiotic fungi on the behavior of soil-dwelling herbivores [51–53].

Blind Men and an Elephant: Let Us Widen the Scope

While the monitoring of BVOC emissions using enclosures encompassing parts of plants can deliver valuable information, the sealing of the system can itself lead to additional stress and obscure the results [21]. An alternative approach is to encompass the entire plant and direct the flow of ambient gas through the sampling chamber and into the DI-MS instrument. The enclosures can range from glass jars [55], beakers [56], and polymer containers [50] to sophisticated systems that enable a comprehensive analysis of volatile plant metabolites [57,58].

One of the most challenging aspects of monitoring plant BVOC emissions is maintaining the desired measurement conditions. Changes in variables such as temperature, relative humidity, or atmospheric pressure, together with extraneous factors, can affect the emission of volatile metabolites. Addressing this issue requires comprehensive solutions for reliable control of the environment of the plant. Recent developments in this area include dedicated research platforms, such as the system developed at the Albert-Ludwigs-University in Freiburg (ALU) [57] and the VOC-SCREEN (Helmholtz Zentrum München, HMGU) [58]. These systems are situated in rooms with tightly controlled climate conditions and comprise three basic units: a clean air supply, gas-tight plant enclosure chambers, and a measurement unit. Such comprehensive setups facilitate simultaneous, real-time monitoring of atmospheric conditions, the determination of volatile plant metabolites (e.g., using PTR-MS), and of gases, such as CO_2 and H_2O, using an infrared gas analyzer. The instrumentation within the chamber can be supplemented, for instance with ^{13}C or NO_X analyzers. The chambers can also be fitted with sorbent tube samplers for subsequent GC-MS analysis, allowing for comprehensive determination of emitted BVOCs [17]. The VOC-SCREEN can also be used for photogrammetry-based leaf area estimation.

The multifaceted approach to the analysis of plant BVOCs emissions, such as by combining PTR- MS measurements, ^{13}C labeling, and statistical analysis, enables the discovery of metabolic pathways of less studied plant volatiles and screening for phenotypic traits. For instance, the solution developed by HMGU was used for the untargeted analysis of BVOC emissions from barley exposed to synthetic elicitor (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analog of salicylic acid (SA) [59]. SA is, in turn, a well-known signaling molecule: several volatile compounds emitted in stress conditions originate from SA-dependent pathways [60]. The exposure to BTH did not affect methanol emission, which varied diurnally. However, the emission of methanethiol was strikingly high compared with untreated plants, which indicated that this chemical is a marker of SA-induced stress. This revelation would not have been possible if the study followed a targeted approach focused on the main BVOCs. Another untargeted approach is shown in Figure 2 (KeyFigure).

The Global Perspective: There Is more to See

Unlike under laboratory conditions, the emission of volatile plant metabolites affects not only the particular specimen, but also the entire ecosystem, even at the global level (e.g., by contributing to climate change). It is estimated that the total annual emission rate of terrestrial BVOCs is 1×10^{12} kg of carbon equivalent [62]. As such, the monitoring of BVOC emissions is important not only in the context of biochemical processes or plant responses to stimuli, but also as an indicator of environmental quality. For instance, isoprene, one of the main volatile plant metabo- lites, undergoes radical reactions in the atmosphere, leading to the generation of harmful tropospheric ozone. The estimated annual global emission of isoprene constitutes approximately half of the total global emission of BVOCs [62]. A detailed description of the possible radical reac- tion pathways of atmospheric isoprene was provided by Wennberg *et al.* [63]. This plethora of complex chemical reactions can be monitored using DI-MS methods (Figure 3).

A Novel Real-Time Metabolomics Approach Using Direct Infusion Mass Spectrometry (DI-MS) Techniques in Whole-Plant Biogenic Volatile Organic Compound (BVOC) Flux Analysis

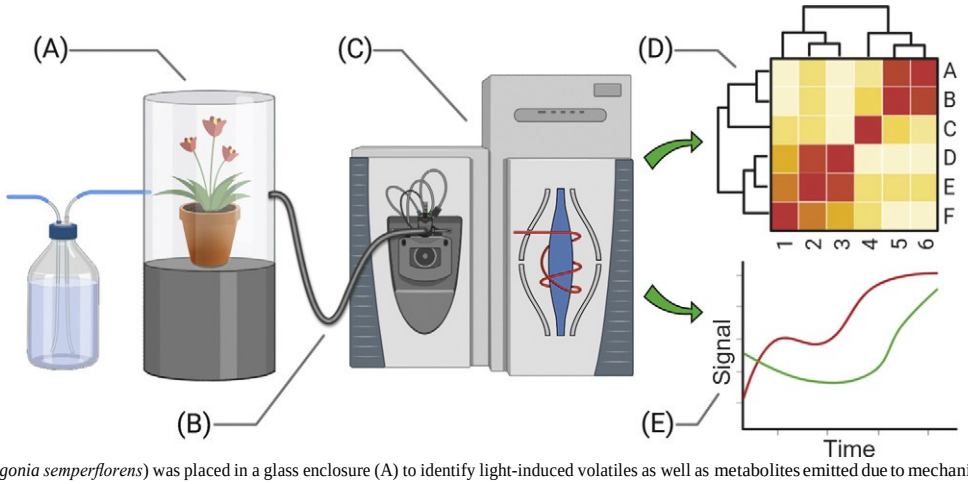

Figure 2. The plant (*Begonia semperflorens*) was placed in a glass enclosure (A) to identify light-induced volatiles as well as metabolites emitted due to mechanical damage. The sampled flux was transferred through a line heated to 100°C (B). The experimental setup was designed in such a way as to mitigate one of the main shortcomings of proton transfer reaction mass spectrometry (PTR-MS), namely difficulties in qualitative, nontargeted analysis in metabolomics (top-down approach) resulting from the lack of analyte fragmentation. The solution was to use secondary electrospray ionization (SESI) with a high-resolution Orbitrap mass spectrometer (C), which was used to obtain tandem MS (MS/MS) spectra. The approach to data analysis was twofold: the targeted approach involved the monitoring of BVOC traces (D), while heatmaps were used for untargeted data analysis (E). The study highlights the potential of DI-MS techniques in untargeted plant metabolite profiling. Figure created using BioRender(https://biorender.com/).

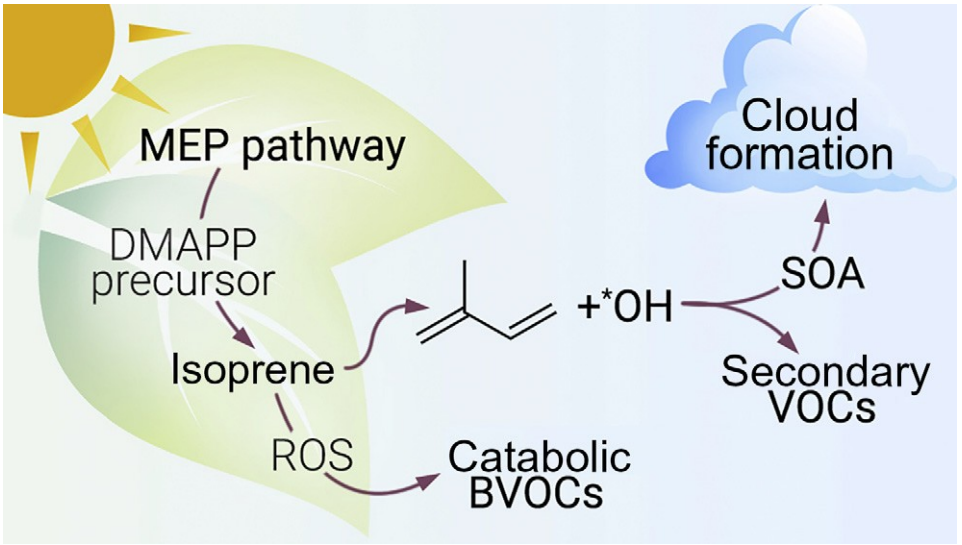

Figure 3. An Overview of the Generation, Emission, and Secondary Atmospheric Reactions of Isoprene. Isoprene is generated from a dimethylallyl pyrophosphate (DMAPP) precursor following the methylerythritol-4-phosphate (MEP) pathway [7,42]. Isoprene can be emitted by the plant immediately, or can react with reactive oxygen species (ROS) in the leaves (see Box 2). What biochemists would consider the final product of plant metabolic pathways is, for atmosphere chemists, the substrate for several processes taking place in the atmosphere. The measurement of the emission of biogenic volatile organic compounds (BVOCs) on an ecosystem scale is at the interface of both fields. Most BVOCs are unstable in the atmosphere, which leads to difficulties with their determination. However, direct infusion mass spectrometry (DI-MS) methods can be used for *in situ* measurements of emission in real time, facilitating research into the local and global effects of volatiles of plant origin in the atmosphere.

The presence of plant BVOCs, including isoprenoids, in the atmosphere can lead to the produc- tion of secondary organic aerosols (SOA), which in turn impacts cloud condensation nuclei production, increasing the albedo of the Earth [64]. In environmental chemistry, this phenomenon is known as the cooling feedback mechanism [65].

Due to the challenges associated with field studies, BVOC measurements at the scale of ecosystems are usually limited to the monitoring of changes in the concentration of selected metabolites, such as isoprenoids, methanol, ketones, or aldehydes. Said challenges result from changing atmospheric conditions (e.g., illumination, precipitation, shifting wind direction, and speed), seasonal variations, changing solar zenith angle [66] and interference from anthropogenic emissions, among others, making the organization of the sampling site crucial (Box 2). While collecting samples from selected plants (e.g., using branch enclosure systems [67]) can provide valuable information re- garding BVOC emissions, the results of such measurements are, by their very nature, approximate. An alternative approach is to sample the volatiles above the canopies, which is often performed using flux towers. These towers are usually equipped with an array of instruments for measuring me- teorological variables (e.g., thermometers, anemometers, and PAR meters), gas analyzers [68] or active sorbent tube samplers [69]. One of the better-known flux towers, or rather a system of several towers, is the Amazon Tall Tower Observatory (ATTO) located in the heart of the Amazon forest in Brazil [69]. The tallest structure of the complex stands at 325 m. The infrastructure of the observatory was previously used to take vertical profile measurements (at 12 m and 24 m, i.e., below the top of the canopy, which is at an average height of 30 m) of diurnal, seasonal, and vertical variations in chemical speciation of VOCs [69]. Similar flux towers were used for BVOC emission measurements in, among others, a bamboo forest in China [70], an oak-hickory forest in Missouri [68], a mixed forest in Michigan [71], and a mixed oak and hornbeam forest in Italy [72]. In each case, a DI-MS technique (i.e., PTR-MS), was used to monitor the volatile plant metabolites.

Box 2. Anabolic and Katabolic BVOCs: The Effect of Drought

Both isoprene and methanol are anabolic BVOCs [42]. Isoprene is generated in chloroplasts in the methylerythritol-4- phosphate (MEP) and mevalonate (MVA) pathways, while methanol originates from pectin demethylation in plant cells [77]. Both can be transformed into other BVOCs as the result of abiotic stresses, such as drought, due to within-leaf oxidation reactions between anabolic BVOCs and reactive oxygen species (ROS) [77]. Catabolic BVOCs are the products of these reactions. Oxidation of isoprene produces, for example, methacrolein (MACR), methyl vinyl ketone (MVK), and isoprene hydroxy hydroperoxides (ISOPOOH) [78], while methanol degradation yields formaldehyde [79]. Reactions of other anabolic BVOCs can produce, among others, acetaldehyde and GLV. The monitoring of the con- centration changes of both anabolic and catabolic plant volatiles, as well as establishing a correlation between their generation, can lead to valuable insight into the resilience of the plant to environmental stress and the nature of inter- actions between vegetation and the atmosphere.

A study of the effect of drought on the emission of these compounds was carried out at an experimental site equipped with a rainfall exclusion device (i.e., an awning unfolded during precipitation) [67]. This allowed the simulation of the 30% reduction in rainfall induced by climate change by the year 2100, based on the Intergovernmental Panel on Climate Change (IPCC) forecast [80]. The measurements were performed using a PTR-TOFMS instrument coupled with dynamic branch enclosures. It was shown that the emission of anabolic and catabolic BVOCs was highly seasonal and decreased in sum- mertime. The correlation between the emission of isoprene and of its catabolic products was established (R^2=0.86–0.96), while the correlation between isoprene and methanol was not evident (R^2=0.25–0.74). This suggests that the total concentration of MACR, MVK, and ISOPOOH is a good indicator of oxidative pressure during drought.

A different approach to the assessment of the BVOC emission budget of extensive swaths of forests is to mount DI-MS devices on board an aircraft, as was the case with isoprene concentration measurements above the Amazon rainforest [73]. The emission rate of the canvased area was estimated using the Model of Emissions of Gases and Aerosols from Nature (MEGAN). BVOC emission fluxes depend strongly on wind conditions, with eddies and direction shifts causing incidental changes in concentration at the sampling site. The effect of air turbulence can be to some extent accounted for, such as by using eddy covariance techniques [68,72]. Moreover, forest BVOCs are not exclusively products of plant metabolism; some originate from the soil and the forest floor [74].

While the research into the emission of plant volatile metabolites at the ecosystem scale is focused on areas with natural vegetation, only a handful of studies have targeted analogous scenarios with agricultural land. Although plantations are a significant source of BVOCs [75], the environmental impact of agricultural plant volatile emissions remains largely unknown. A re- cent field study demonstrated that the flux of methanol from wheat fields is an order of magnitude higher than the flux of isoprene [76].

Concluding Remarks and Future Perspectives

The advances in DI-MS techniques have opened new vistas in the analysis of plant BVOCs, facilitating real-time *in situ* determination of volatile metabolites. They have also enabled the discovery of new metabolic pathways with particular stages lasting several seconds, which was hitherto difficult to achieve using, for example, GC-MS. Improvements in analytical instrumentation, such as the fitting of PTR-MS with TOF analyzers, are being combined with novel sampling methods that enable not only the isolation of emissions from parts of plants or from whole specimens, but also the analysis of the BVOC flux from entire ecosystems. This enables unprecedented control over the experiment and provides insight into the impact of a plethora of variables on the metabolism of the plant. The introduction of DI-TOFMS in particular has made it possible to conduct untargeted, screening analysis of BVOC flux, making it a valuable tool in plant metabolomics.

Despite the clear advantages of using DI-MS in the analysis of plant volatiles, the technique still has some limitations. In particular, it does not enable a definitive identification of chemical compounds, such as structural isomers, requiring a complementary use of GC-MS for reliable qualitative analysis. This issue might be addressed in the future by developing DI-MS instruments equipped with MS/MS detectors sufficiently portable for field use. Since DI-MS methods are relatively new and being developed rapidly, their use in routine screening analyses is hindered by the lack of easily implementable, open-access databases of plant metabolites, containing, for example, the mass of the primary ion, fragmentation pattern, proton transfer reaction rate constant, and proton affinity. Such databases, akin to the well-established GC-MS libraries, would greatly expedite the interpretation of the results of experiments (see Outstanding Questions).

The current trend is to integrate DI-MS with 'omics platforms through coupling with other high- throughput techniques, which will allow for comprehensive use of metabolomics, genomics, proteomics, and so on [81–83], and will provide unprecedented insights into the complex processes occurring at each stage of plant development. Furthermore, despite the clear advantages of using PTR-MS for the analysis of plant volatiles, researchers are already investigating alternative ionization sources compatible with high-resolution mass spectrometers, potentially enabling sub-ppt sensitivity [35,56]. Such developments in instrumentation might spur the more widespread use of DI-MS in applications, in which the analytes are at trace concentration levels, such as in the monitoring of BVOC flux in the atmosphere of entire ecosystems. Here, a widespread monitoring network could be used to supplement and refine the databases of global emission budgets, which are based on remote-sensing methods [84]. A similar, commercial implementation could be envisioned, focused on the monitoring of extensive farmlands and agricultural areas, providing an overview of the state of vegetation and early warning in cases of pest infestation.

Glossary

Biogenic volatile organic compounds (BVOCs): chemical compounds produced in plants during the course of vegetative growth, reproduction, and as a result of defence mechanisms. They also act as a means of communication between plants and their surrounding environment. They are also referred to as 'volatile plant metabolites'. Green leaf volatiles (GLVs): chemical compounds emitted due to plant tissue damage. Wounding causes a rapid release of free fatty acids from plant membranes, which triggers a chain reaction of lipoxygenase (LOX), leading to the emission of volatile GLV products. These products mostly include C_6 aldehydes, esters, and alcohols. Their emission in response to stress is prevalent in green plants.

Metabolomics Standards Initiative (MSI): an initiative inaugurated in 2005 during a workshop hosted by the US National Institutes of Health (NIH) and the Metabolomics Society. Its aim is to promulgate reporting standards for metabolomics and to facilitate effective sharing of experimental data through metabolomics data set repositories, such as the MetabolomeXchange[i].

Microchannel plate (MCP): a type of detector often used in MS, especially in TOFMS. It operates by multiplying the electrons that were initially emitted due to ion impact, thus strengthening the signal. It has a multitude of in-plane channels, each acting as an independent electron multiplier.

'Omics: a generic term for techniques using a holistic approach to the analysis of the totality of molecules that constitute a given biological sample, be it a cell, tissue, or entire organism. In particular, metabolomics focuses on a quantitative measurement of the dynamic changes in concentration of a multitude of chemical compounds in the living organisms, often in response to external stimuli.

Tandem mass spectrometry (MS/MS): the coupling of two or more mass spectrometers to determine additional structure features other than the molecular mass. The initial mass determination is followed by the selection of particular ions and subjecting them to fragmentation, with additional information gained from the analysis of both the masses and the fragmentation pattern. The combinations of analyzers include, among others, triple quadrupole (Q-q-Q) and hybrid quadrupole TOF (Q-q-TOF).

Highlights

Direct infusion mass spectrometry (DI-MS) techniques are a valuable tool for real-time monitoring of the plant volatilome.

DI-MS techniques are particularly useful in detecting brief episodes of increased biogenic volatile organic compound emissions caused, for instance, by herbi- vore attacks.

Current studies on the use of DI-MS techniques for the determination of plant volatiles are conducted at several scales, from parts of plants and whole specimens to entire ecosystems.

Proton transfer reaction MS is the pre- eminent technique used for the monitor- ing of volatile plant metabolites.

Current developments in DI-MS solu- tions are focused on the application of tandem MS.

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