analytical chemistry

Article

pubs.acs.org/ac

Capturing in Vivo Plant Metabolism by Real-Time Analysis of Low to High Molecular Weight Volatiles

César Barrios-Collado,[†,‡,§] Diego García-Gómez,[§] Renato Zenobi,[§] Guillermo Vidal-de-Miguel,[§,∥] Alfredo J. Ibáñez,[§,#,⊥] and Pablo Martinez-Lozano Sinues*[,§]

[†]Department of Energy Engineering and Fluid Dynamics, University of Valladolid, 47002 Valladolid, Spain

[‡]SEADM S.L., 28036 Madrid, Spain

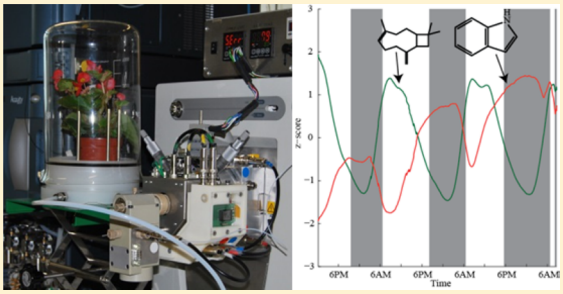
[§]Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland

[∥]Fossil Ion Technology S.L., 28036 Madrid, Spain

[#]Science Zurich — Zurich PhD Program Molecular Life Sciences, 8093 Zurich, Switzerland

Supporting Information

ABSTRACT: We have deployed an efficient secondary electrospray ionization source coupled to an Orbitrap mass analyzer (SESI-MS) to investigate the emissions of a *Begonia semperflorens*. We document how hundreds of species can be tracked with an unparalleled time resolution of 2 min during day−night cycles. To further illustrate the capabilities of this system for volatile organic compounds (VOCs) analysis, we subjected the plant to mechanical damage and monitored its response. As a result, ∼1200 VOCs were monitored displaying different kinetics. To validate the soundness of our in vivo measurements, we fully characterized some key compounds via tandem mass spectrometry (MS/MS) and confirmed their expected behavior based on prior gas chromatography/mass spectrometry (GC/MS) studies. For example, β-caryophyllene, which is directly related to photosynthesis, was found to show a periodic day−night pattern with highest concentrations during the day. We conclude that the capability of SESI-MS to capture highly dynamic VOC emissions and wide analyte coverage makes it an attractive tool to complement GC/MS in plant studies.



Plants interact with their surrounding environment releasing chemical cues. Such chemical response of plants occurs in the form of emission of volatile organic compounds (VOCs). Thus, plants send internal messages between their organs or as a response against biotic or abiotic stress.[1−3] Two well-known examples are the attraction of pollinators, or the call for insect predators when under herbivore attack. Such chemical communication allows a fast and efficient interplay between plants, and plants and other species in the vicinity. Although the study of plant VOCs has a long tradition due to its extensive use in flavorings and fragrances,[4] research aiming to understand their function in plant communication is relatively young. The availability of new mass spectrometry-based techniques has significantly improved it since the 1990s[3] and is now a growing field due to its important implications for modern agriculture. For example, the study of terpenes has helped to enhance crops endurance against abiotic stress. Thus, oxidative atmospheric conditions have been prevented or minimized by artificially spreading chemical warning signal which sets the crop "on guard", or by genetic modifications that make the plants to release it continuously.[5] Atmospheric chemistry is another important field in which plant-related VOCs play a major role.[6] For example, terpenes contribute to the formation of ozone and secondary organic aerosols in the presence of anthropogenic pollutants;[7] both ozone and aerosols are strong airway irritants.[8]

VOCs released by plants to the atmosphere comprise a wide chemical variety of molecules from 5 to 20 carbon atoms and molecular weights up to 500 Da.[9] Gas chromatography/mass spectrometry (GC/MS) has been the main analytical technique used for the analysis and characterization of such species.[10−16] However, GC/MS has some limitations. Mainly, the need to manipulate and often preconcentrate the gaseous samples makes impractical the monitoring of chemical events in the time scale of a few minutes. In addition, some studies suggest that heating—as in the case of GC/MS—significantly alters the molecular profiles of metabolites, indicating that a significant amount of the spectral data generated in GC/MS experiments may correspond to thermal degradation products.[17] In contrast, real-time mass spectrometry analysis prevents the risk of introducing artifacts associated with sample manipulation and exposure of the volatiles to high temperatures during extended periods of time. In addition, it can be crucial to capture changes in VOC emission, which in some cases occur on the order of

Received: November 24, 2015
Accepted: January 26, 2016

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX

minutes (e.g., reaction against stressful events). For this reason, a number of techniques have been developed that allow real-time monitoring of VOCs emitted by plants. Thus, current real-time VOC analysis is dominated by chemiluminescence, infrared photoacoustic spectroscopy (PA), selected ion flow tube mass spectrometry (SIFT-MS), and proton transfer reaction mass spectrometry (PTR-MS).[10,18,19] Chemiluminescence was the first real-time plant VOC analysis technique developed to study the role of isoprene. However, chemiluminescence is often limited to this particular compound and other fairly simple VOCs (e.g., ethane), limiting the investigation of complex plant responses. In addition, PA presents some limitations to resolve complex mixtures of gases, which is typically the case in plant-borne VOCs. SIFT-MS and PTR-MS are currently the most powerful techniques for real-time plant VOC analysis in terms of sensitivity and VOC coverage range. They have been successfully used to complement GC/MS.[19,20] However, their volatile metabolome coverage is still far from GC/MS capabilities, where hundreds of compounds can be detected. Other limitations in comparison to state-of-the-art mass analyzers, such as modest resolution and lack of tandem mass spectrometry (MS/MS) capabilities, limit the opportunities to perform untargeted metabolome fingerprinting and further compound identification.

Our goal in this plant VOC analysis work was to achieve a broad metabolome coverage (i.e., hundreds of species with masses approaching 500 Da) as GC/MS does, yet in real time as PTR-and SIFT-MS do. To do so, we developed a method featuring a high-resolution mass spectrometer (HRMS) coupled with a secondary electrospray ionization (SESI) source, which provides sensitive online ionization.[21] SESI is a well-known soft ionization method for gas-phase analytes.[22−30] SESI-MS has been used previously for explosives,[21,31,32] drugs,[25,33,34] human metabolites,[35−42] food analysis,[43,44] and bacterial emissions.[45−47]

Here we used an optimized version of a SESI ionizer dubbed low-flow SESI (LFSESI),[48] which has been recently optimized numerically.[49] This technological development was designed as a plug-and-play add-on to preexisting atmospheric pressure ionization mass spectrometers (API-MS). Thus, by simply exchanging the standard ion source by the LFSESI the mass spectrometer turns into a sensitive vapor analyzer. Here we present the results of this new technological development for in situ analysis of VOCs released by plants. In particular, we document the real-time detection of hundreds of light- and stress-induced VOCs, suggesting that, in combination with traditional GC/MS, SESI-HRMS could provide valuable insights into plant volatile metabolism.

METHODS

Experimental Section. In this work, we enclosed a *Begonia semperflorens* within a 5 L glass beaker. The sampling air flow dragging volatiles continuously through the plant chamber was set to 1 L/min. The plant chamber featured an inlet for humid, compressed air and an outlet connected to the commercial LFSESI ion source (SEADM, S.L.).[49] The ion source was mounted in front of a Thermo-Fisher LTQ Orbitrap mass spectrometer. Note that the LFSESI source is easily exchangeable with the standard ion source and it is controlled using the MS vendor's software (i.e., no additional requirements of compatibility). Figure 1 shows a picture and a diagram of the setup.

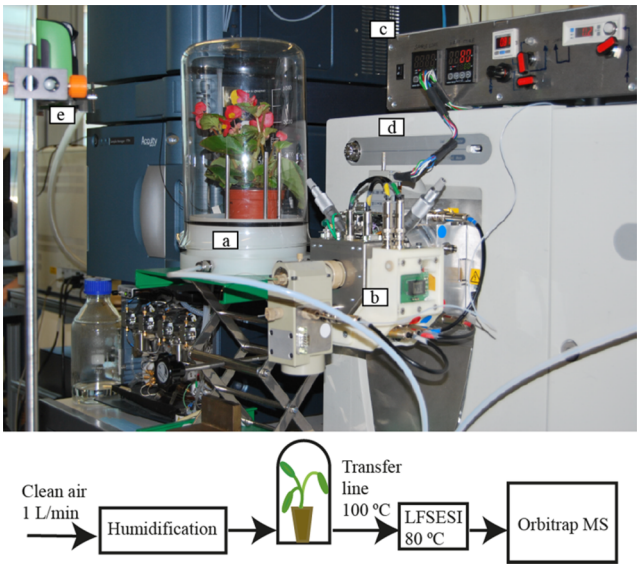


Figure 1. Experimental setup used to analyze in vivo plant volatile emissions. A commercially available secondary electrospray ionization source was interfaced with an Orbitrap mass spectrometer. Metabolites emitted by the plant were continuously dragged into the ion source and mass analyzed in real time: (a) glass beaker containing the plant; (b) SESI source; (c) SESI control module; (d) Orbitrap MS; (e) time-lapse camera.

The temperature of the transfer line connecting the chamber and the LFSESI was set to 100 °C, and the ionizer temperature was set to 80 °C (both PID controlled), limited by the boiling point of the ESI solvents (water−formic acid 0.1%). MS sweep flow was set to 2 (arbitrary units), and the inlet capillary temperature was set to 225 °C. Voltages, mechanical alignment, and electrospray tip axial position were optimized prior the experiments. All experiments were run in positive mode. We set the lock mass to four common mass spectrometry contaminants[50] (m/z 149.02332, 279.15909, 355.06994, and 371.10124 Da, $C_8H_4O_3$, $C_{15}H_{22}O_4$, and $[C_2H_6SiO]_5$ the latter two, respectively) appearing in the positive ion mode.

We ran two different experiments to illustrate the capabilities of this technique for plant VOC analysis: we sought to identify (i) light-induced emitted molecules and (ii) molecules emitted by the plant under stress due to mechanical damage. In the former case, the plant was measured nonstop during 3 entire days (Video S1). Temperature and humidity within the chamber were continuously monitored. Light levels in the lab were recorded using a luxmeter (PCE Instruments, Spain). In a second set of measurements mimicking the attack of an insect, we measured the plant under normal conditions during 90 min to establish the baseline. The chamber was then opened and the upper leaves were pierced. The response of the plant was then recorded during 120 min.

In both sets of experiments full MS scans were recorded with a time resolution of 2 min (i.e., 250 scans; 1 scan every 500 ms). The scanned m/z range was between 50 and 500 Da and resolution ranged from ∼88 000 at m/z 59 to ∼30 000 at m/z 445. In addition, we identified some key compounds observed in the full scans by performing MS/MS in real time. The fragmentation patterns were compared with standards, which were injected into the MS using a standard ESI ion source. The standards used were β-caryophillene ($C_{15}H_{24}$, Tokyo Chemical Industry), methyl jasmonate ($C_{13}H_{20}O_3$, Tokyo Chemical

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX

Industry), and(*Z*)-3-hexenyl acetate ($C_8H_{14}O_2$, Tokyo Chemical Industry).

Data Analysis. Acquisition files, in Thermo-Fisher's proprietary file format.raw, were converted to .mzxml files via ProteoWizard software[51] and were subsequently postprocessed with MATLAB (R2015b). For each scan the m/z vector (50−500) was interpolated using 1×10^6 equidistant points. The raw mass spectra were converted into a peak list using 5000 counts as threshold. ^{13}C isotopes were removed from the peak list, leading as a result to around 3000 mass spectral features. The resulting time traces for each of the mass spectral features were smoothed (moving average; span = 99) and autoscaled. The resulting matrix ($m/z \times$ time) was subjected to hierarchical cluster analysis (Euclidean distance; Ward linkage algorithm) to identify distinct trends of time traces. Finally, we computed the molecular formulas (elements: C, H, O, N, and S) for the accurate masses using the approach described by Kind and Fiehn.[52]

Safety Considerations. High temperatures and high voltages are applied to the LFSESI. In order to avoid burns and electric discharges, careful operation is required. Note nevertheless that the commercial SESI ion source used in this study includes the required safeguards to minimize these risks, as listed in the European normative (Low Voltage Directive 2006/95/EC).

RESULTS AND DISCUSSION

Light-Induced Metabolism. During a three-day nonstop measurement, we evaluated which volatiles showed a clear correlation (either positive or negative) with the light levels in the room. Figure S1 shows the recorded light levels across the 3 days (time resolution = 5 min), clearly showing the day/night cycles. Our high-resolution mass spectra contained more than 3000 resolved peaks (after filtering isotopes) in the range of 50−500 Da in full scan mode during the three-day experiment. To identify those showing a correlation with day/night cycles, we subjected the time traces matrix to hierarchical cluster analysis. Essentially, we identified four distinct trends: (1) compounds whose intensity showed almost no change during the three-day experiment, (2) those which showed a decreasing trend without day−night correlation (Figure S2), (3) compounds with a constant rising trend (Figure S3 and Table S1), and (4) compounds with a clear periodic pattern correlated with the day−night cycle. Compounds of trends 1 and 2 (around 86% of all mass spectral features) were chemical noise not related with the "plant ecosystem" (defined as the plant and the soil, as we are not able to separate them), whereas the third (85 mass spectral features) and fourth (156 mass spectral features) trends were associated with the plant ecosystem. Of those showing a periodic fluctuation, we found 111 diurnal species (i.e., maximum emission rates were around midday) and 55 nocturnal species (i.e., maximum emission rates were around midnight). Parts a and b of Figure 2 show the heatmaps corresponding to the diurnal and nocturnal species, respectively. Further visual inspection of the time traces revealed 154 additional compounds which were the sum of a periodic pattern and a decreasing negative-exponential baseline as the emission rates did not reach the steady state in 3 days (see, for example, the time trace of monoterpenes in Figure 3). In a similar fashion, 74 additional nocturnal substances were identified. Figure S4 shows the complete heatmaps of the species with increased emission rates at day and night, including the compounds identified by visual inspection. The accurate

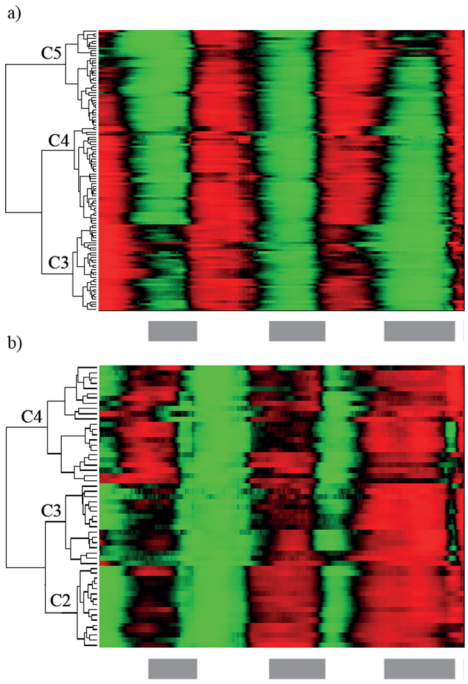


Figure 2. Capturing periodic VOC patterns during three entire days. False color heatmaps of 111 diurnal (a) and 55 nocturnal (b) species. Red and green represent relatively high and low values, respectively. Gray bars indicate periods of darkness (i.e., <1 lx). Exact masses and molecular formulas for each of the numerated clusters are listed in Tables S2 and S3.

masses and their corresponding molecular formulas are listed in Supporting Information (Tables S2 and S3). For greater clarity, the tables are organized by the different clusters revealed by cluster analysis. Interestingly, a number of a clusters identified by unsupervised cluster analysis turned out to correspond to clear chemical families. For example, among the compounds showing a systematic increase during the 3 days (irrespective of light conditions; trend 3), we identified a family of 20 compounds (cluster 5; Table S1) with molecular formulas C_xH_yS (x = 19−23; y = 31−43). Figure S5 shows an example of the experimental mass spectrum of $[C_{21}H_{37}S]^+$ (m/z 321.2616) and its match with the theoretical isotopic distribution, confirming the molecular formulas of this chemical family. This group of compounds are probably monothiophenes, which are natural wide-spectrum biocides released by the plant against parasitic fungi and nematodes.[53,54] In the case of the diurnal species (Table S2), the dominating chemical families were hydrocarbons (i.e., C_xH_y) and $C_xH_yO_z$. In contrast, a large number of nocturnal VOCs (Table S3) contained 1−2 nitrogen atoms, possibly as a result of elimination of nitrogen by oxidation during photosynthetic rest periods.[55]

To gain further confidence in the value of our observations, we followed a targeted approach focusing on key compounds that have been extensively characterized by GC/MS. Thus, monoterpenes (m/z 137.1324) and sesquiterpenes (m/z 205.1950) are two families of metabolites known to be related to photosynthesis.[3] Figure 3 shows the mass spectrum in the region at m/z 137. It shows four clearly resolved peaks separated by less than 0.1 Da (m/z 137.0248, 137.0596, 137.0958, and 137.1324). The latter corresponds to the monoterpene family $[C_{10}H_{17}]^+$. The inset shows the corresponding time traces for each of the peaks. As expected, the

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX

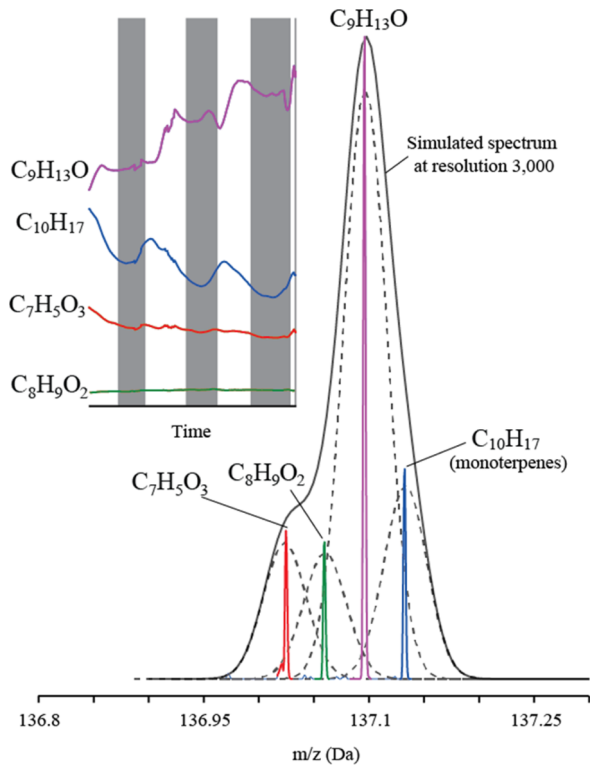


Figure 3. High resolution is crucial in real-time analysis of complex VOCs mixtures. Four different species were detected at $m/z \sim 137$. The inset shows their corresponding time profiles, indicating that the two minor ions were chemical noise. The monoterpenes showed the expected periodic pattern. The dominant $[C_9H_{13}O]^+$ ion trace indicates that it also stems from the plant. For reference, a simulated spectrum at resolution 3000 is overlaid, suggesting that the monitoring of monoterpenes would have been compromised under these conditions.

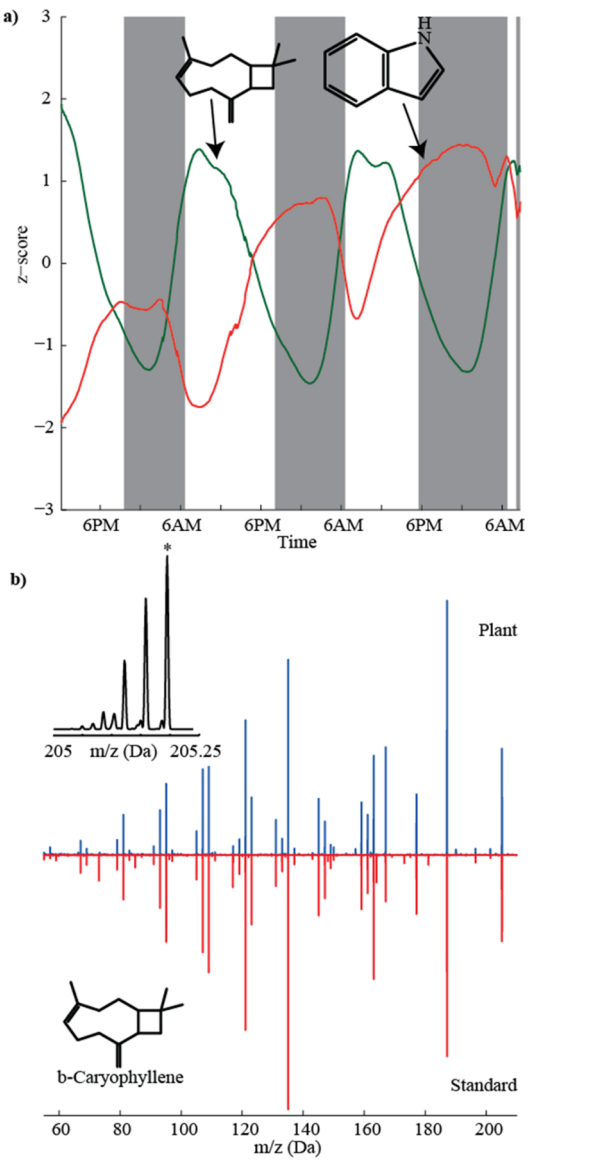


Figure 4. Identified compounds show the expected day−night periodic behavior. (a) Time traces for β-caryophyllene $[C_{15}H_{24}]^+$ and indole $[C_8H_7N]^+$ illustrate a typical diurnal and nocturnal pattern, respectively. (b) MS/MS confirmation of β-caryophyllene. The inset shows the mass spectrum in the vicinity of β-caryophyllene (marked with an asterisk).

monoterpenes show a periodic diurnal behavior.[3,56] The most abundant compound at m/z 137.0958 $[C_9H_{13}O]^+$ showed a sort of periodic pattern with an accumulation tendency during the 3 days. In contrast, the two minor species at m/z 137.0248 and 137.0596 showed a flat or falling tendency, indicating that they were just chemical noise not related to the plant ecosystem. This example illustrates the importance of using high-resolution mass analyzers for real-time analysis of complex mixtures. For comparison, Figure 3 along with the experimental mass spectrum, shows the simulated spectrum at resolution 3000 for these four species. This is the typical resolution of a high-end PTR-TOF system.[57] Clearly, at this resolving power, the four species would have gone unresolved and the identification and quantification of the monoterpenes would have been seriously compromised.

Figure 4a shows two examples of compounds whose emissions were oppositely correlated with the day−night cycle. The first species peaked around 8:30−9:00 a.m. to then decline until it reached a minimum around 1 a.m., when it bounced back to recover again the previous day's levels (i.e., diurnal behavior). In contrast, the second example shown in Figure 4a has a maximum around 1 a.m. and its levels drop until 8−9 a.m., when it reaches a minimum (i.e., nocturnal behavior). In this case, although the period pattern is clearly preserved across the 3 days, it shows an accumulation trend. This results in highest concentration values during the third night. In addition, the profile shows a plateau, rather than a well-defined maximum (as the diurnal compound), suggesting different kinetics. The fact that SESI can be interfaced with virtually any API-MS, enabled us to take full advantage of the Orbitrap mass analyzer to perform real-time MS/MS for further characterization. The diurnal compound was identified as β-caryophyllene (Figure 4b). The isolation mass window (1 Da) is shown in the inset. Obviously, as a result of performing real-time measurements, the fragmentation spectrum is populated with fragments stemming from all the species within the isolation mass window. In this particular case, β-caryophyllene was the most abundant species and fragments easily. As a result, the MS/MS spectrum is dominated by its fragments, as evidenced by the perfect match with the standard. β-Caryophyllene is one of the most important sesquiterpenes, which is directly related to photosynthesis.[58] Consistent with previous GC studies investigating different plants, we found that β-caryophyllene's

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX

emission rate is increased during the day.[3,56,58] The nocturnal compound has been tentatively assigned to indole based on its accurate mass and isotopic distribution. Indole is usually related to plant defense against herbivore attack[59] and is also used as an inhibitor for the germination of seeds of other plant species.[56] The correlation of indole emission with light is unclear in literature where examples of plants with maximum emissions both at day[60] or night[61] can be found.

To the best of our knowledge, this is the first study reporting nearly 400 light-induced species captured in vivo simultaneously. This is in contrast with prior efforts to monitor plant VOCs in real time, for example, with PTR-MS and PA, where no more than 20 volatiles have been reported.[62,63] This wide analyte coverage is actually comparable to GC/MS analysis, where typically a few hundred compounds per run are detected.[13,14]

Stress-Induced Metabolism. To further illustrate the potential of our vapor analyzer to study plant metabolism, we subjected the plant to a stress event and examined its response. We first monitored the plant around 1.5 h under normal conditions. We then opened the glass bell jar, pierced some upper leafs (i.e., mimicking an insect attack event), closed the glass bell jar, and observed the plant's instantaneous response during 2 h after wounding the leaves. As a result of the mechanical damage, we observed a rise in the emission of 1224 compounds. Interestingly, 996 of them were not previously detected in the three-days experiment. This suggests that this wide range of molecules was associated with stress-induced metabolism. Figure 5 shows the heatmap of the 1224 species

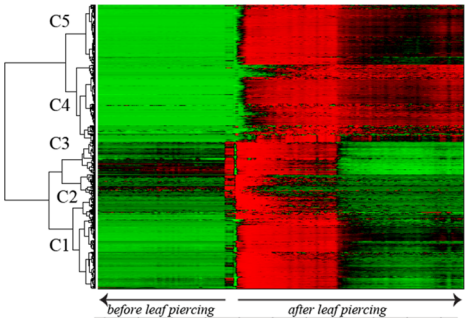


Figure 5. Plant response to mechanical damage. Heatmap of VOCs time profiles providing an overview of the 1224 species whose emission rose upon piercing the upper leaves. Table S4 lists their m/z values and molecular formulas.

whose emission rose upon leaf piercing, providing an overview of the plant response. Table S4 lists the associated m/z and molecular formulas. Table S5 lists the 996 species associated exclusively to the stress-induced metabolism. The kinetics were similar for all the species, but with some subtle differences. Figure 6a displays five selected examples. Two of them were identified by MS/MS as methyl jasmonate and hexenyl acetate (Figure 6, parts b and c).The third one is a sesquiterpene or probably a mixture of them (i.e., isomeric structures), and the rest are two unidentified molecules detected exclusively during mechanical damage. Methyl jasmonate is a chemical alarm for herbivore attack and an insect digestive inhibitor, and (Z)-3-hexenyl acetate is an attractant to predatory insects.[64] Both are related through the oxylipin pathway and rose under stressful conditions, as expected. Their time profiles, as well as the sesquiterpenes, were actually very similar, with a sharp increase

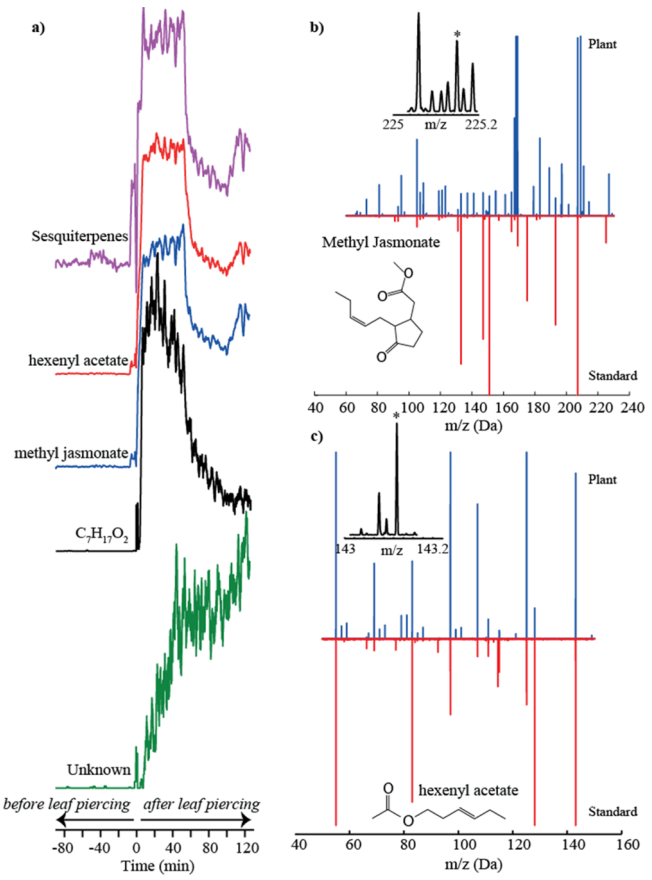


Figure 6. (a) Different VOCs kinetics suggest a compound-dependent response to mechanical damage. (b and c) MS/MS spectra of methyl jasmonate $[C_{13}H_{20}O_3]^+$ and (Z)-3-hexenyl acetate $[C_8H_{14}O_2]^+$ for the plant and pure standards, respectively. As expected, these compounds rose as a response to leaves piercing.

during ~8 min to then reach a plateau until min 50, followed by a decline until min 100 and when they recovered again part of the signal during the last 20 min. In contrast, the $[C_7H_{17}O_2]^+$ species rose sharply after the mechanical damage to peak 20 min later. It then constantly declined during 80 min and stabilized during the last 20 min. A totally different behavior was shown by the unknown compound at m/z 134.0808, which increased constantly during the 2 h following the leaves piercing. Such distinct behavior and rapid changes in VOCs profiles illustrate the benefit of using real-time techniques to capture such events with enough time resolution.

Real-time techniques are particularly beneficial to study stress-induced metabolism in plants if they are able to capture fast changes in metabolite levels with sufficient resolution. For example, it has been recently shown that indole is an important priming agent in maize.[65] This GC-based study has shown that, upon wounding maize plants, five major families of VOCs were induced. The first, second, and third measured time points were 45, 90, and 180 min after elicitation. While the general trends may well be captured using this time resolution, some important details might gone undetected. For example, Figure S6a shows the time traces for the eight species tracked in the study by Erb et al.[65] It shows that indole rises immediately after leaf wounding, but around 45 min later declines sharply to reach the same levels prior to elicitation. Different dynamics were observed for other species in the range of 45 min, suggesting that, indeed, real-time mass spectrometry-based

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX

methods may be necessary to understand in greater detail complex response of plants to stimuli. This notion is reinforced by other PTR-MS studies suggesting that dramatic changes in emitted volatiles occur in as short as 10 min after mechanical damage.[62] In another GC/MS example, a number of compounds with a defensive function against herbivores have been proposed.[66] In this case, volatiles were trapped during 7 h. Figure 6b displays the time traces for the compounds identified by Kessler and Baldwin,[66] suggesting again that SESI-MS could complement GC/MS methods to capture with a finer time resolution plants' response to herbivore infestation.

CONCLUSIONS

In conclusion, we presented an analytical platform that combines the sensitivity and selectivity required to detect hundreds of VOCs covering typical GC/MS methods range (i.e., 50−500 Da), yet with unparalleled time resolution and no sample preparation. This platform combines an efficient vapor ionizer and a high-resolution Orbitrap mass spectrometer. To illustrate the capabilities of such system in the field of plant metabolomics, we investigated the volatiles emitted by a *B. semperflorens* in real time and in vivo. In a first set of measurements, we proved the stability of the system by monitoring continuously the plant during three entire days. Around 400 species were found to correlate with light levels (i.e., diurnal and nocturnal), capturing its behavior with a time resolution of 2 min. Some of these species were identified by MS/MS and confirmed an overlap between the species typically investigated by GC/MS and the VOCs detected by SESI-MS. In a second round of experiments we investigated the response of the *B. semperflorens* to mechanical damage. We detected more than 1200 VOCs emitted by the plant as result of mechanical damage. As in the case of light-induced metabolites, we found a large overlap with compounds described in the literature. However, we observed significant VOC changes on the time scale of minutes, implying that these details would have gone undetected by off-line methods without the required sampling frequency. We therefore conclude that SESI-MS could provide valuable complementary real-time chemical information on plant metabolomics to GC/MS, which in addition is still indispensable for accurate chemical identification isomeric species. Future developments including an ion mobility cell could add real-time separation capabilities.[30]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b04452.

Time-lapse of the light-induced experiment (ZIP)

Light levels during the light-induced experiment, examples of substances not associated with light-induced metabolism, extended heatmaps of light-induced volatiles metabolites, isotopic distribution of monothiophene, additional examples of substances emitted upon leave mechanical damage, and tables listing *m*/*z* and proposed formulas for light- and damage-induced emission of volatiles (PDF)

AUTHOR INFORMATION

Corresponding Author

*Phone: +41 44 632 4838. Fax: +41 44 632 1292. E-mail: pablo.mlsinues@org.chem.ethz.ch.

Present Address

⊥(A.J.I.) Pontificia Universidad Católica del Perú — Instituto de Ciencias Ómicas y Biotecnología Aplicada, Lima 32, Perú

Notes

The authors declare the following competing financial interest(s): César Barrios-Collado, Guillermo Vidal-de-Miguel, and Pablo Martinez-Lozano Sinues work in the framework of a project (Analytical Chemistry Instrumentation Development, FP7-2013-IAPP, 609691), one of whose main objectives is to develop the commercial ion source used in this study.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Juan Zhang (Novartis AG) for the donation of the LTQ Orbitrap instrument used in this study and the European Community's Seventh Framework Programme (FP7-2013-IAPP) for funding the project "Analytical Chemistry Instrumentation Development" (609691). We are indebted to Christoph Bärtschi (ETH workshop) for his assistance machining the ion source and plant chamber. We also thank Myriam Macía for assisting during the development phase. We are indebted to Professor Juan Fernandez de la Mora (Yale) and Mr. Gonzalo Fernandez de la Mora (SEADM) for their continuous support during the development of the ion source used in this study. Professor Asaph Aharoni is gratefully acknowledged for his encouragement to pursue this study.

REFERENCES

(1) Gershenzon, J. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 5257−5258.

(2) Holopainen, J. K.; Gershenzon, J. *Trends Plant Sci.* **2010**, *15*, 176−184.

(3) Loreto, F.; Schnitzler, J. P. *Trends Plant Sci.* **2010**, *15*, 154−166.

(4) Bicchi, C.; Cordero, C.; Rubiolo, P. *J. Chromatogr. Sci.* **2004**, *42*, 402−409.

(5) Vickers, C. E.; Possell, M.; Cojocariu, C. I.; Velikova, V. B.; Laothawornkitkul, J.; Ryan, A.; Mullineaux, P. M.; Nicholas Hewitt, C. *Plant, Cell Environ.* **2009**, *32*, 520−531.

(6) Li, X.; Zhou, Z.; Huang, Z.; Gao, W.; Li, M.; Li, L. *Emerging Cont.* **2015**, *1*, 33−38.

(7) Atkinson, R. *Atmos. Environ.* **2000**, *34*, 2063−2101.

(8) Wolkoff, P.; Clausen, P. A.; Wilkins, C. K.; Nielsen, G. D. *Indoor Air* **2000**, *10*, 82−91.

(9) Skogerson, K.; Wohlgemuth, G.; Barupal, D. K.; Fiehn, O. *BMC Bioinf.* **2011**, *12*, 321.

(10) Tholl, D.; Boland, W.; Hansel, A.; Loreto, F.; Rose, U. S. R.; Schnitzler, J. P. *Plant J.* **2006**, *45*, 540−560.

(11) Davies, N. W. *J. Chromatogr.* **1990**, *503*, 1−24.

(12) Isidorov, V.; Zenkevich, I.; Sacharewicz, T. *Chem. Anal. (Warsaw)* **1997**, *42*, 627−634.

(13) Soto, V. C.; Maldonado, I. B.; Jofre, V. P.; Galmarini, C. R.; Silva, M. F. *Microchem. J.* **2015**, *122*, 110−118.

(14) Fiehn, O.; Kopka, J.; Trethewey, R. N.; Willmitzer, L. *Anal. Chem.* **2000**, *72*, 3573−3580.

(15) Fukusaki, E.; Jumtee, K.; Bamba, T.; Yamaji, T.; Kobayashi, A. *Z. Naturforsch., C: J. Biosci.* **2006**, *61*, 267−272.

(16) Boggia, L.; Sgorbini, B.; Bertea, C. M.; Cagliero, C.; Bicchi, C.; Maffei, M. E.; Rubiolo, P. *BMC Plant Biol.* **2015**, *15*, 1−13.

(17) Fang, M.; Ivanisevic, J.; Benton, H. P.; Johnson, C. H.; Patti, G. J.; Hoang, L. T.; Uritboonthai, W.; Kurczy, M. E.; Siuzdak, G. *Anal. Chem.* **2015**, *87*, 10935−10941.

(18) Harren, F. J. M.; Cristescu, S. M. *AoB Plants* **2013**, *5*, plt003.

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX

(19) Smith, D.; Spanel, P. *TrAC, Trends Anal. Chem.* **2011**, *30*, 945−959.
(20) Maleknia, S. D.; Bell, T. L.; Adams, M. A. *Int. J. Mass Spectrom.* **2007**, *262*, 203−210.
(21) Martínez-Lozano, P.; Rus, J.; Fernández de la Mora, G.; Hernández, M.; Fernández de la Mora, J. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 287−294.
(22) Kiselev, P.; Fenn, J. B. In *Proceedings of the 49th ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, Illinois, May 27−31, 2001; American Society for Mass Spectrometry: Santa Fe, NM, 2001.
(23) Fuerstenau, S.; Kiselev, P.; Fenn, J. B. In *Proceedings of the 47th ASMS Conference on Mass Spectrometry*, Dallas, TX, 1999; American Society for Mass Spectrometry: Santa Fe, NM, 1999.
(24) Whitehouse, C. M.; Levin, F.; Meng, C. K.; Fenn, J. B. In *Proceedings of the 34th ASMS Conference*, 1986; American Society for Mass Spectrometry: Cincinnati, Ohio, 1986.
(25) Wu, C.; Siems, W. F.; Hill, H. H. *Anal. Chem.* **2000**, *72*, 396−403.
(26) Steiner, W. E.; Clowers, B. H.; Haigh, P. E.; Hill, H. H. *Anal. Chem.* **2003**, *75*, 6068−6076.
(27) Fernandez de la Mora, J. *Int. J. Mass Spectrom.* **2011**, *300*, 182−193.
(28) Reynolds, J. C.; Blackburn, G. J.; Guallar-Hoyas, C.; Moll, V. H.; Bocos-Bintintan, V.; Kaur-Atwal, G.; Howdle, M. D.; Harry, E. L.; Brown, L. J.; Creaser, C. S.; Thomas, C. L. P. *Anal. Chem.* **2010**, *82*, 2139−2144.
(29) Vidal-De-Miguel, G.; Herrero, A. *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 1085−1096.
(30) Martinez-Lozano Sinues, P.; Criado, E.; Vidal, G. *Int. J. Mass Spectrom.* **2012**, *313*, 21−29.
(31) Tam, M.; Hill, H. H. *Anal. Chem.* **2004**, *76*, 2741−2747.
(32) Aernecke, M. J.; Mendum, T.; Geurtsen, G.; Ostrinskaya, A.; Kunz, R. R. *J. Phys. Chem. A* **2015**, *119*, 11514−11522.
(33) Meier, L.; Berchtold, C.; Schmid, S.; Zenobi, R. *J. Mass Spectrom.* **2012**, *47*, 555−559.
(34) Li, X.; Martinez-Lozano Sinues, P.; Dallmann, R.; Bregy, L.; Hollmen, M.; Proulx, S.; Brown, S. A.; Detmar, M.; Kohler, M.; Zenobi, R. *Angew. Chem., Int. Ed.* **2015**, *54*, 7815−7818.
(35) García-Gómez, D.; Martínez-Lozano Sinues, P.; Barrios-Collado, C.; Vidal-De-Miguel, G.; Gaugg, M.; Zenobi, R. *Anal. Chem.* **2015**, *87*, 3087−3093.
(36) He, J.; Martinez-Lozano Sinues, P.; Hollmén, M.; Li, X.; Detmar, M.; Zenobi, R. *Sci. Rep.* **2014**, *4*, 5196.
(37) Martínez-Lozano, P.; Fernandez de la Mora, J. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1060−1063.
(38) Martínez-Lozano, P. *Int. J. Mass Spectrom.* **2009**, *282*, 128−132.
(39) Martinez-Lozano, P.; Fernandez de la Mora, J. *Anal. Chem.* **2008**, *80*, 8210−8215.
(40) Reynolds, J. C.; Jimoh, M. A.; Guallar-Hoyas, C.; Creaser, C. S.; Siddiqui, S.; Thomas, C. L. P. *J. Breath Res.* **2014**, *8*, 037105.
(41) Berchtold, C.; Meier, L.; Steinhoff, R.; Zenobi, R. *Metabolomics* **2014**, *10*, 1−11.
(42) García-Gómez, D.; Bregy, L.; Barrios-Collado, C.; Vidal-de-Miguel, G.; Zenobi, R. *Anal. Chem.* **2015**, *87*, 6919−6924.
(43) Martínez-Lozano Sinues, P.; Alonso-Salces, R. M.; Zingaro, L.; Finiguerra, A.; Holland, M. V.; Guillou, C.; Cristoni, S. *Anal. Chim. Acta* **2012**, *755*, 28−36.
(44) Bean, H. D.; Mellors, T. R.; Zhu, J. J.; Hill, J. E. *J. Agric. Food Chem.* **2015**, *63*, 4386−4392.
(45) Zhu, J. J.; Bean, H. D.; Jimenez-Diaz, J.; Hill, J. E. *J. Appl. Physiol.* **2013**, *114*, 1544−1549.
(46) Zhu, J. J.; Jimenez-Diaz, J.; Bean, H. D.; Dapthary, N. A.; Aliyeva, M. I.; Lundblad, L. K. A.; Hill, J. E. *J. Breath Res.* **2013**, *7*, 037106.
(47) Ballabio, C.; Cristoni, S.; Puccio, G.; Kohler, M.; Sala, M. R.; Brambilla, P.; Martinez-Lozano Sinues, P. *J. Clin. Pathol.* **2014**, *67*, 743−746.
(48) Vidal-de-Miguel, G.; Macia, M.; Pinacho, P.; Blanco, J. *Anal. Chem.* **2012**, *84*, 8475−8479.
(49) Barrios-Collado, C.; Vidal-de-Miguel, G.; Martinez-Lozano Sinues, P. *Sens. Actuators, B* **2016**, *223*, 217−225.
(50) Keller, B. O.; Sui, J.; Young, A. B.; Whittal, R. M. *Anal. Chim. Acta* **2008**, *627*, 71−81.
(51) Kessner, D.; Chambers, M.; Burke, R.; Agus, D.; Mallick, P. *Bioinformatics* **2008**, *24*, 2534−2536.
(52) Kind, T.; Fiehn, O. *BMC Bioinf.* **2007**, *8*, 105.
(53) Ketel, D. H. *J. Exp. Bot.* **1987**, *38*, 322−330.
(54) Sütfeld, R. *Planta* **1982**, *156*, 536−540.
(55) Klepper, L. *Atmos. Environ.* **1979**, *13*, 537−542.
(56) Roshchina, V. V.; Roshchina, V. D. *The Excretory Function of Higher Plants*; Springer: Berlin, Germany, 1993; pp VII, 314 S.
(57) Herbig, J.; Müller, M.; Schallhart, S.; Titzmann, T.; Graus, M.; Hansel, A. *J. Breath Res.* **2009**, *3*, 027004.
(58) Hansen, U.; Seufert, G. *J. Geophys Res-Atmos* **2003**, *108*, 4801.
(59) D'Alessandro, M.; Held, M.; Triponez, Y.; Turlings, T. C. *J. Chem. Ecol.* **2006**, *32*, 2733−2748.
(60) Pare, P. W.; Tumlinson, J. H. *Plant Physiol.* **1999**, *121*, 325−331.
(61) Bischoff, M.; Jurgens, A.; Campbell, D. R. *Ann. Bot.* **2014**, *113*, 533−544.
(62) Brilli, F.; Ruuskanen, T. M.; Schnitzhofer, R.; Muller, M.; Breitenlechner, M.; Bittner, V.; Wohlfahrt, G.; Loreto, F.; Hansel, A. *PLoS One* **2011**, *6*, e20419.
(63) Hirschmann, C. B.; Koivikko, N. S.; Raittila, J.; Tenhunen, J.; Ojala, S.; Rahkamaa-Tolonen, K.; Marbach, R.; Hirschmann, S.; Keiski, R. L. *Sensors* **2011**, *11*, 5270−5289.
(64) Chehab, E. W.; Kaspi, R.; Savchenko, T.; Rowe, H.; Negre-Zakharov, F.; Kliebenstein, D.; Dehesh, K. *PLoS One* **2008**, *3*, e1904.
(65) Erb, M.; Veyrat, N.; Robert, C. A. M.; Xu, H.; Frey, M.; Ton, J.; Turlings, T. C. J. *Nat. Commun.* **2015**, *6*, 6273.
(66) Kessler, A.; Baldwin, I. T. *Science* **2001**, *291*, 2141−2144.

DOI: 10.1021/acs.analchem.5b04452
Anal. Chem. XXXX, XXX, XXX−XXX