Planta (2018) 247:267–275
https://doi.org/10.1007/s00425-017-2782-9

ORIGINAL ARTICLE

Direct detection of surface localized specialized metabolites from *Glycyrrhiza lepidota* (American licorice) by leaf spray mass spectrometry

Dana M. Freund[1,2] · Amanda C. Martin[1,2] · Jerry D. Cohen[1,2] · Adrian D. Hegeman[1,2,3]

Received: 24 May 2017 / Accepted: 21 September 2017 / Published online: 27 September 2017

Abstract
Main conclusion **Leaf spray-MS minimizes tissue manipulation by effectively and quickly assessing in vivo specialized metabolites from intact plant tissue surfaces, including trichome metabolites.**

Intact leaves of *Glycyrrhiza lepidota* Pursh. (American licorice) were analyzed by direct electrospray leaf spray-MS, an ambient ionization technique. Comparison of metabolites detected by leaf spray-MS to those from LC–MS of bulk tissue and trichome enriched extracts showed dramatic differences. Leaf spray-MS results suggest that in specific situations this approach could complement traditional LC–MS analysis of bulk extracts. Leaf spray-MS as a metabolomics technique eliminates sample pretreatment and preparation allowing for rapid sampling in real time of living intact tissues. Specialized metabolites on the surface of tissues such as glandular trichomes metabolites are detected by leaf spray-MS.

Keywords Leaf spray mass spectrometry · *Glycyrrhiza lepidota* · Specialized metabolites · Trichomes

✉ Dana M. Freund
dfreund@umn.edu

Amanda C. Martin
corfindings@gmail.com

Jerry D. Cohen
cohen047@umn.edu

Adrian D. Hegeman
hegem007@umn.edu

[1] Department of Horticultural Science, University of Minnesota, 1970 Folwell Avenue, Saint Paul, MN 55108, USA

[2] Microbial and Plant Genomics Institute, University of Minnesota, Saint Paul, MN, USA

[3] Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN, USA

Introduction

Plants synthesize specialized metabolites that have a variety of biological roles from microbial or insect defense to pollinator attraction. Many of these compounds have biological activities that are useful for humans as medicines, dietary supplements, fragrances, etc (Cragg and Newman 2013). Specialized metabolites vary widely in physicochemical properties, necessitating a range of analytical approaches for their study. Mass spectrometry (MS) paired with extensive chromatographic separation is a highly selective and sensitive technique for detecting these compounds, which are often present at a wide range of concentrations. Typically, natural products research uses bulk extracts, which often contain multiple tissues and cell types and are generated from dried or frozen plant samples (Llewellyn et al. 2011). Bulk extracts vary in composition because they are highly dependent on extraction conditions; therefore the resulting metabolic profiles reflect averages of tissue-specific metabolite variations (Martin et al. 2014). Alternatively, direct MS techniques, such as leaf spray-MS ionize metabolites directly from plant tissue with no sample preparation and minimal adjustments to the ionization source (Cooks et al. 2006; Liu et al. 2011; Monge et al. 2013; Jarmusch and Cooks 2014; Müller and Cooks 2014; Gemperline et al. 2016). Previous studies have implemented this technique to detect pesticides on fruit and vegetables and to

measure metabolites in various plant species (Chan et al. 2011; Malaj et al. 2012; Snyder et al. 2015; Falcone and Cooks 2016; Liu et al. 2016). While these studies have shown the utility of leaf spray-MS, there are few reports of direct comparisons of leaf spray-MS with traditional LC–MS methodology or attempts to elucidate the cell types from which metabolites detected using leaf spray-MS are derived.

The focus of this study was on *Glycyrrhiza lepidota* Pursh. (American licorice), a species belonging to a genus of economically important plants containing well-documented bioactive compounds. The plant genus *Glycyrrhiza* (licorice) includes many closely related species, some of which have a long history of medicinal and industrial use globally (Dalton 2002). Leaf spray-MS results were compared with more traditional and lengthy solvent maceration extraction, referred to here as bulk extracts and trichome-enriched leaf dip extracts, to determine the degree of compound specificity of leaf spray-MS. Leaf spray-MS was considered potentially advantageous because it can yield a fairly large amount of data from fresh unmodified plant material in an extremely short amount of time.

Materials and methods

Plant collection and propagation

Glycyrrhiza lepidota seedpods were collected from established prairies from locations distributed in Minnesota. Cleaned, scarified, cold-stratified seed was greenhouse geminated and transplanted into an outdoor field plot on the Saint Paul field site and maintained without pesticides or herbicides. Four different populations of *G. lepidota* with nearly identical chemotypes were analyzed with each MS method.

Leaf spray-MS

Leaf spray-MS methods were adapted from methods developed by (Liu et al. 2011). Aerial parts were harvested and fresh leaves were selected for immediate analysis. A nano-electrospray source was modified to administer 4.5 kV via the clamp and the capillary temperature was 250 °C. An alligator clip was attached at the leaf base with the apex aimed at the MS inlet (Fig. 1). Methanol (10 µL) was applied twice during the one min acquisition. MS acquisition with full scan mass scan range 130–1000 *m/z*, polarity switching, and 70,000 resolution. A second leaf was used for fragmentation of the most abundant negative ions using normalized collision energy (NCE) of 50 and 60 with a resolution of 17,500. Compound identities were verified by matching accurate mass and fragment ions to Metlin (Smith et al. 2005) and Human metabolome databases (Wishart et al. 2013) or in silico tandem mass spectra.

Bulk extraction

Aerial parts were dried for 3 days at 30 °C with no forced air then ground in a Thomas Wiley laboratory mill model 4 (Thomas Scientific) with a 6-mm screen. Plant material (250 mg) was extracted for 4 h with 1.5 mL of aqueous ethanol (ethanol:water, 70:30 v/v) and agitated at 700 RPM with a Geno/Grinder® (SPEX Sample Prep). Extracts were centrifuged at 11,750 × *g* for 5 min. The supernatant was diluted 1:10 with 70% ethanol prior to LC–MS analysis.

Leaf dip extraction

Leaves of similar size were harvested by cutting the petioles at the stem. Each fresh leaf was separately dipped in 1 mL methanol for 2 min and solvent was evaporated in a vacuum centrifuge (Speedvac) then re-suspended in 50 µL of 80% acetonitrile:water (v/v) followed by 2 min vortexing.

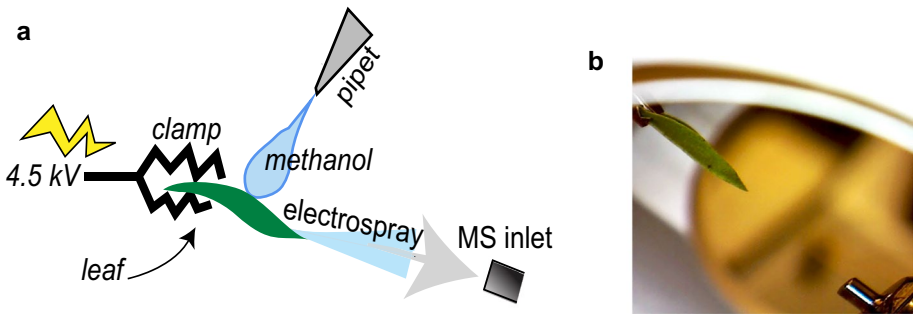

Fig. 1 Direct leaf spray ionization mass spectrometry is a metabolite profiling technique that eliminates sample pretreatment and preparation allowing for rapid sampling in real time of living intact tissue. **a** Diagram of leaf spray-MS set-up with 4.5 kV voltage and methanol applied to plant tissue. **b** *G. lepidota* leaf during leaf spray-MS analysis before methanol is placed on the adaxial leaf surface

Extracts were centrifuged at 2600 × *g* for 10 min and the supernatants were used for LC–MS analysis.

LC–MS data acquisition

Metabolic profiles were generated using C_{18}-reversed-phase ultra-performance liquid chromatography–electrospray ionization–hybrid quadrupole–orbitrap mass spectrometer (Ultimate® 3000 HPLC, Q Exactive™, Thermo Scientific). Chromatographic separation was accomplished on a reversed-phase C_{18} HSS T_3 1.8 µm particle size, 2.1 × 100 mm column (waters) with column temperature at 40 °C, flow rate 0.45 mL/min, and 1 µL injected. A 20-min gradient using mobile phases A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile was run according to the gradient elution profile: initial 15% B, 1 min 15% B, 2 min 50% B, 15 min 98% B, 16 min 98% B, 16.5 min 15% B, 20 min 15% B. The MS conditions were used: full scan mass scan range 130–1000 *m/z*, resolution 35,000 desolvation temperature 350 °C.

Data analysis and bioinformatics

Xcalibur™ software version 2.1 (Thermo Scientific) recorded the chromatograms and spectra. Raw files were converted to mzXML files with msConvert from Proteowizard (Chambers et al. 2012). The XCMS software package implemented in R was used for peak picking and alignment (Smith et al. 2006). To account for experimental variability due to differences in leaf size, the intensity of each metabolite was normalized by the total ion current (TIC). The scripts used for data processing can be found at https://github.com/HegemanLab/Leaf-Spray-Code and raw data files are deposited at http://conservancy.umn.edu/handle/11299/185430?show=full repository.

Results and discussion

Leaf spray-MS analysis

Freshly collected, field grown *G. lepidota* leaves were analyzed by leaf spray-MS (Fig. 1). Direct electrospray occurs by (1) attaching a metal clamp through which 4.5 kV is applied to a leaf and (2) manually pipetting methanol onto the adaxial surface of the leaf (Fig. 1a). The lanceolate leaf shape (Fig. 1b) was particularly well suited to electrospray ionization resulting in signal that was sustained for approximately 1 min following the initial application of methanol and a second application at 30 s to allow signal intensity to persist. Leaf spray-MS was enhanced dramatically by coupling ionization to a high-resolution, accurate-mass (HRAM) mass spectrometer, which made it possible to resolve multiple slightly differing masses. Leaf spray-MS produces a mass chronogram and dense mass spectrum after 1 min of acquisition in negative ionization mode (Fig. 2). The fast scanning capabilities of the Q Exactive™ hybrid quadrupole-Orbitrap™ Mass Spectrometer (ThermoFisher Scientific) enable polarity switching, which makes it feasible to obtain data in both positive and negative ionization modes nearly simultaneously. Figure 3 is a representative positive ion mass chronogram and mass spectrum that was concurrently acquired with the aforementioned negative ion data. A total of seven previously characterized bioactive compounds were detected by leaf spray-MS (Table 1). These compounds were putatively identified by the exact masses of the deprotonated (Fig. 2b) and protonated (Fig. 3b) molecular ions. Moreover, compound putative identities were verified with tandem mass spectra (MS/MS) by matching fragments with those predicted in publically available databases (Fig. 4). The isoflavone prunetin has been identified in *G. glabra* aerial tissues (Ammosov and Litvineko 2003). The flavanone glabranin has been isolated from *G. glabra*, *G. uralensis* and *G. lepidota* whole plants and shown to possess antimicrobial activity (Siracusa et al. 2011; Ammosov and Litvineko 2003). Glepidotin A and B are flavonols, and glepidotin D is a dihydrostilbene, each with antimicrobial, anti-inflammatory, and anti-HIV1 activity and previously isolated from *G. lepidota* leaves (Manfredi et al. 2001; Biondi et al. 2005). Licocoumarone, a benzofuran, has previously been isolated from *G. uralensis* roots and has shown efficacy as an anti-inflammatory (Hatano et al. 1989; Wu et al. 2017). The $[M - H]^-$ ion at 339.1238 *m/z* was identified as originating from both glepidotin B and licocoumarone since the MS/MS spectra contained diagnostic fragment ions for both compounds. Isolicoflavonol has previously been detected in *G. glabra* and G. *uralensis* (Da-Yuan et al. 1984; Zhang and Ye 2009). Leaf spray-MS has the potential for even higher throughput applications, as data acquisition for only a few seconds was sufficient to produce adequate signal to detect these bioactive compounds.

Leaf spray-MS and LC–MS method comparison

Metabolite profiles were produced for three different sample preparation methods: traditional bulk extraction, leaf dip, and leaf spray-MS. Bulk extraction is a long process with heating, drying, and/or extended extraction times, which can alter and degrade endogenous compounds. Alternatively, the leaf dip method involves a 2-min extraction of a single leaf and was developed to enrich for trichome metabolites, however, it also likely extracts metabolites from other surface cells (Ghosh et al. 2013). Leaf surfaces often have accumulated specialized metabolites that are frequently of interest because of their benefits to plants and potential uses; bulk extractions can modify and dilute these compounds.

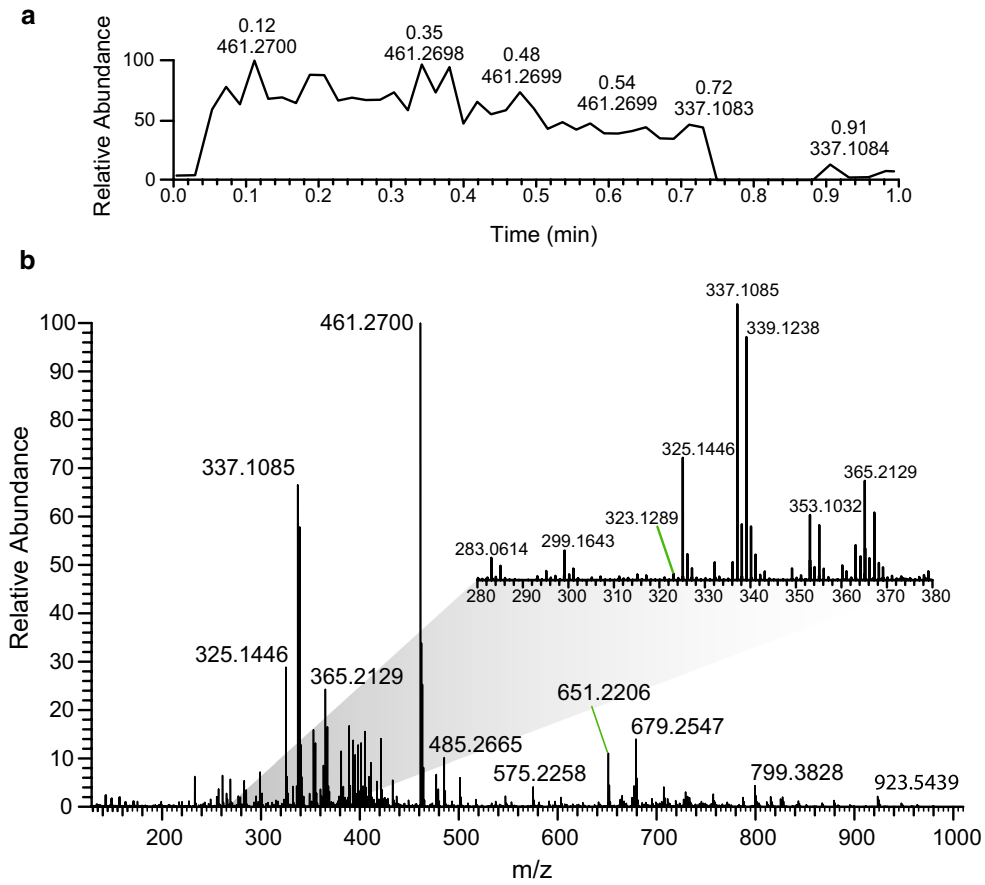

Fig. 2 Metabolite profiling of *G. lepidota* by leaf spray-MS with negative ionization. **a** Leaf spray-MS TIC mass chronagram **b** Leaf spray-MS metabolite profile of *G. lepidota* mass spectrum. Inset displays 280–380 *m/z*. Accurate masses reported out to four decimal places with error < 2 ppm

Comparative chemical profiles were generated using a 20-min LC–MS gradient method for both bulk and leaf dip extracts. We used this relatively long gradient to collect exhaustive chemical profiles to determine if leaf spray-MS was robust enough to produce comparable data and to thoroughly assess the relative concentrations of bioactive compounds of interest.

Leaf spray-MS is likely extracting metabolites from glandular trichomes, other epidermal cells, and cuticle waxes. Plants secrete and store specialized metabolites in glandular trichomes, which are a type of specialized epidermal cell (Schilmiller et al. 2008). *G. uralensis* has been characterized histochemically and shown to have glandular trichomes on the adaxial surface (Peng and Hu 2007) containing flavonoids, polysaccharides and lipophilic compounds, although flavonoids were observed only in the mature trichomes. Therefore, the identification of flavonoids in leaf spray-MS may be an indication that the method is preferentially ionizing trichome metabolites and more specifically mature trichomes. Thus, these data indicate that leaf spray-MS may be a rapid method to enrich for glandular trichome metabolites in fresh tissue. Our current understanding of the distribution of metabolites in specific plant tissue and cell types is greatly under-explored. It is not surprising, therefore, that Fig. 5a indicates differences in the observed chemical profiles for plant tissues processed with different extraction/analysis methods. Chemical profiles derived from the leaf spray-MS method were more similar to those derived from leaf dip as contrasted to bulk extraction. Both were more rapid extraction procedures than bulk extraction and we assume that they are primarily extracting surface metabolites. From the principal components analysis (PCA) scores plot, the bulk extracts are separated from the two surface extraction methods on PC1. The chemical profiles derived from the two surface extraction methods essentially align on PC1. The variation between these two extraction methods is explained by PC2, which has a total of only 18% of the detected variation. The PC1 loadings, which demonstrate the specific *m/z* and corresponding features that account for the sample variation, revealed the very abundant $[M - H]^-$ ions of the detected compounds from leaf spray-MS

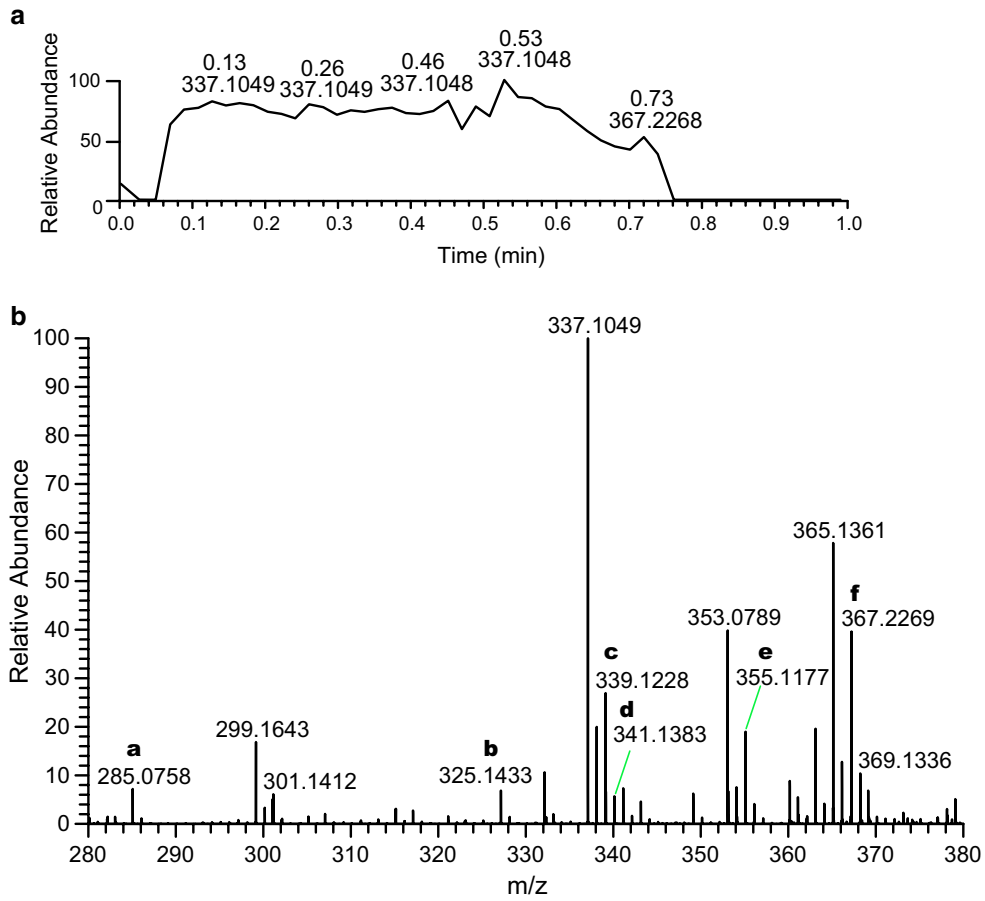

Fig. 3 Metabolite profiling of *G. lepidota* by leaf spray-MS with positive ionization. **a** Leaf spray-MS TIC mass chronogram. **b** Metabolite profile of *G. lepidota* mass spectrum. Metabolites detected in *Glycyrrhiza lepidota* by leaf spray-MS in positive ionization mode. The $[M + H]^+$ ions were detected for the following compounds: prunetin (*a*), glabranin (*b*), glepidotin A (*c*), glepidotin B and licocoumarone (*d*), isolicoflavonol (*e*), and glepidotin D (*f*). The mass range of 280–380 *m/z* is displayed although data was acquired from 130 to 1000 *m/z*. Accurate masses reported out to four decimal places with error < 2 ppm

Table 1 Metabolites detected in *Gycyrrhiza lepidota* by leaf spray-MS

Compound	Class	Molecular formula	Measured monoisotopic mass *m/z* [M − H]	ppm error	Fragments
Prunetin	Isoflavone	$C_{16}H_{12}O_5$	283.0614	0.7	149, 211
Glabranin	Flavone	$C_{20}H_{20}O_4$	323.1291	0.6	65, 173, 219, 279
Glepidotin A	Flavonol	$C_{20}H_{18}O_5$	337.1085	1.2	281, 293
Glepidotin B	Flavonol	$C_{20}H_{20}O_5$	339.1238	0.0	65, 91, 133, 203, 219, 321
Licocoumarone	Benzofuran	$C_{20}H_{20}O_5$	339.1238	0.0	65, 91, 265, 293, 321
Isolicoflavonol	Flavonol	$C_{20}H_{18}O_6$	353.1032	0.3	253, 283, 335
Glepidotin D	Dihydrostilbene	$C_{24}H_{30}O_5$	365.2129	1.6	241, 253

mainly contributed to the separation of chemical profiles derived from leaf spray-MS and leaf dip extracts from those derived from bulk extracts. These included exact mass measurements for prunetin, licocoumarone, glepidotin A, B, and D, as well as other, unidentified, ions. Prunetin, isolicoflavonol, and glepidotin D were completely undetectable in the LC–MS bulk extracts. Glabranin was detected exclusively in the leaf spray-MS spectra and the other six compounds were also present in the leaf dip method. The similarity observed between the leaf spray-MS and leaf dip method results suggests that the types of surface compounds and intensity of ions detected were

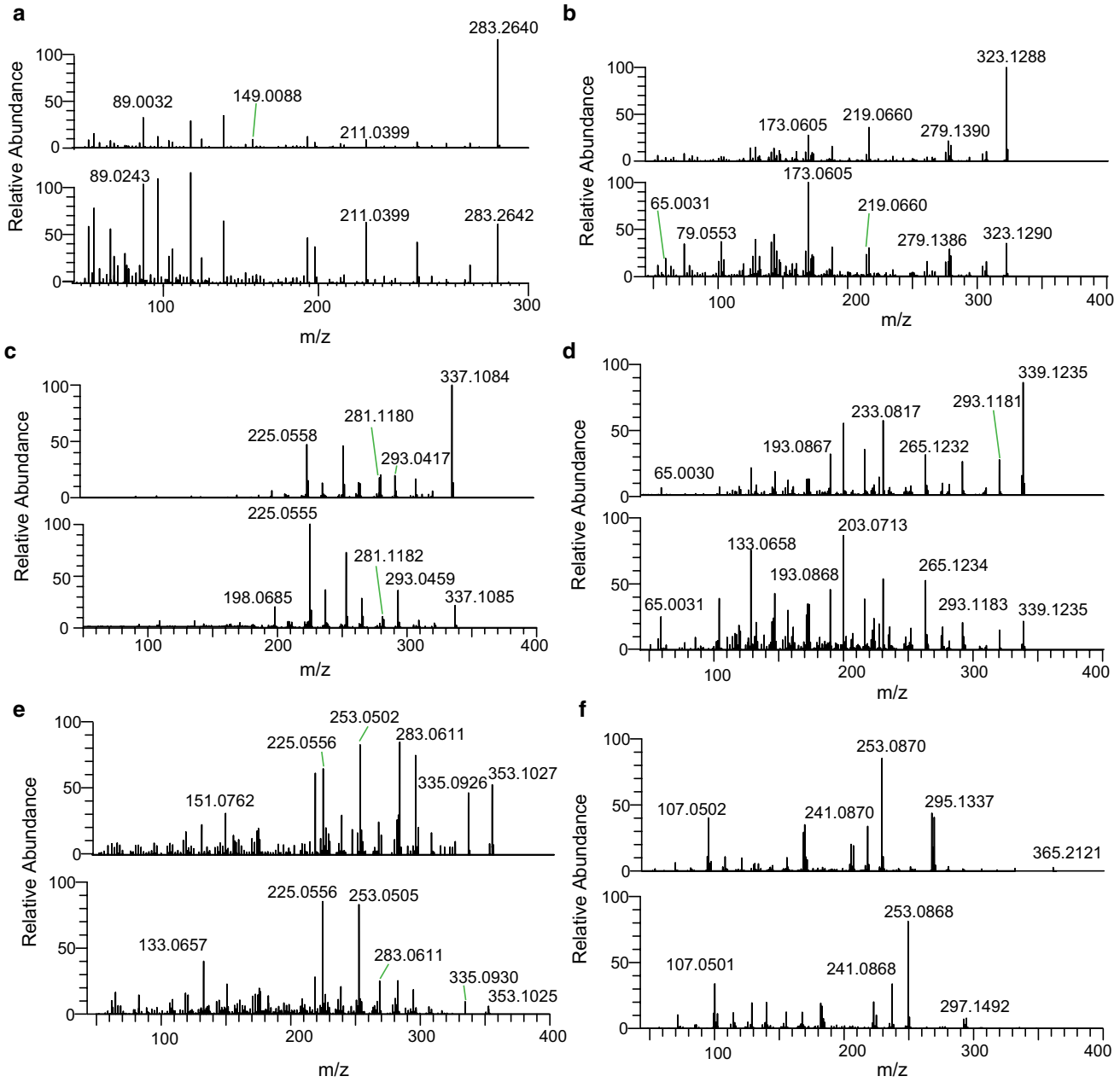

Fig. 4 Leaf spray-MS negative ionization tandem mass spectra from *Glycyrrhiza lepidota*. Putative identifications of compounds made with accurate mass and predicted mass fragmentation for the following: prunetin (**a**), glabranin (**b**), glepidotin A and B (**c**), licocoumarone (**d**), isolicoflavonol (**e**), glepidotin D (**f**). Top spectra are NCE 50 and bottom spectra are NCE 60, respectively

more similar according to extraction method rather than MS analysis method, LC–MS or leaf spray-MS. Therefore, indicating that leaf spray-MS may be a rapid method to enrich for glandular trichome metabolites in fresh tissue. Furthermore, this comparison suggests the added time required for chromatographic separation was unnecessary to detect these known licorice bioactive compounds and that leaf spray-MS can be a viable technique for rapid qualitative assessment of bioactive compounds of interest.

To comprehensively visualize the differences in chemical classes detected by the various methods, van Krevelen diagrams were generated. These are two dimensional plots of the ratios of major elements (hydrogen, carbon, and oxygen) in molecules (Kim et al. 2003). The hydrogen to carbon ratio (H:C) and the oxygen to carbon (O:C) ratio are based on the chemical formula calculated from the exact mass. Elemental compositions were calculated from spectra to facilitate the determination of elemental ratios. Figure 5b shows the

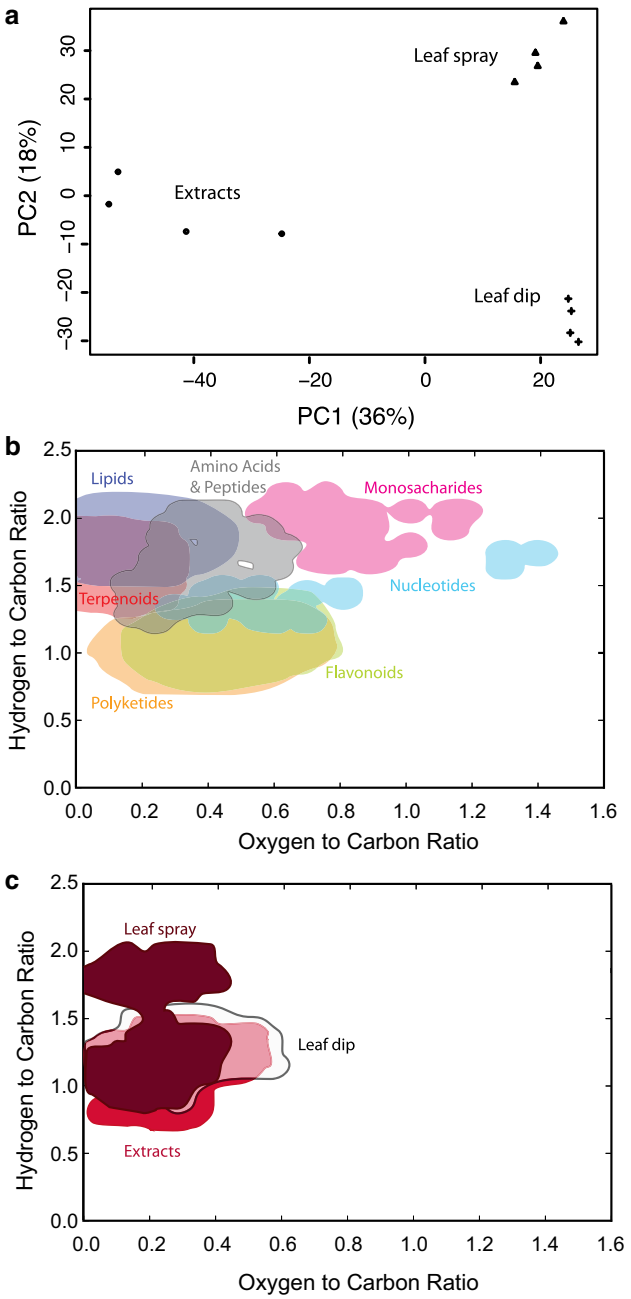

Fig. 5 **a** Principal component analysis (PCA) of *G. lepidota* metabolite profiles obtained from bulk extracts, leaf dip extraction, and leaf spray-MS. The largest metabolite differences occur along PC1 between the 'Extracts' as compared to 'Leaf spray' and 'Leaf dip'. 'Leaf spray' and 'Leaf dip' profiles are separated along PC2, which displays a smaller level of variation than PC1. Variation between biological replicates is relatively small as seen by the tight clustering within each experimental method. Percentage of variance for each PC is shown parenthetically. Analysis includes negative ions only. **b** Chemical map of known compounds from Biological Magnetic Resonance Data Bank database (http://www.bmrb.wisc.edu/). **c** Negative ions detected from leaf spray-MS (dark red), LC–MS of leaf dip (white), and LC–MS of bulk extracts (red). The leaf spray-MS method has a greater density of lipids and terpenoids than the LC–MS methods (leaf dip and extracts)

areas of highest density produced by grouping features plotted with H:C and O:C ratios that are within 0.2 of each other (representing ~ 15% of the total features). Compounds from the Biological Magnetic Resonance Data Bank were plotted to generate an empirical chemical map to warrant comparison of compound classes detected experimentally (Fig. 5b) (Ulrich et al. 2008). Figure 5c displays the densest areas of compounds present according to each extraction and analysis method. All methods detected differences in compound classes including those that are formulaically similar to polyketides and flavonoids. However, distinct molecular populations unique to each method were observed. The most profound difference was the presence of two distinct high-density zones for leaf spray-MS compared to only one zone for the bulk and leaf dip extracts. This second zone of high-density in leaf spray-MS aligned with areas corresponding to lipids and terpenoids on the chemical map. Both bulk and leaf dip extracts had overlapping density for these compound classes compared to the chemical map, however, leaf spray had a much larger area suggesting lipids, terpenoids, amino acids and peptides were more readily detected via leaf spray-MS analyses. Leaf spray-MS was conducted at a fairly high voltage, which may result in the formation of methanol reactive species resulting in atmospheric pressure chemical ionization (APCI) (Wang et al. 2010). Therefore, the additional compounds detected by leaf spray-MS could be the result of APCI occurring along with ESI. Nonetheless, these data suggest leaf spray-MS preferentiality ionizes compounds from specific chemical classes and because it is a rapid analysis of fresh tissue it is likely obtaining a more accurate representation of the in vivo metabolite content.

Conclusions

As analytical technology advances novel plant metabolites continue to be discovered. These discoveries provide insights into plant metabolism, may lead to the development of new and useful biologically active compounds, and may open windows into better understanding the spatial complexity of plant natural products. Novel compounds may be present at very low concentrations, enriched in specific tissues or cells, and modified by traditional extraction techniques. Therefore, it is desirable to develop low-impact techniques capable of tissue and even cellular level resolution when assessing chemical content. Leaf spray-MS minimizes tissue manipulation by effectively and quickly assessing the in vivo chemical content from intact plant tissue surfaces. In this study, *G. lepidota* was used to demonstrate the usefulness of this technique for the rapid assessment of bioactive metabolites to complement LC–MS methods. Quantitation of metabolites with leaf spray-MS can benefit from use of standards in the solvent that is applied to the plant

tissue (Pereira et al. 2016). Furthermore, the use of stable isotope labeled standards or metabolically labeled plant tissues would allow for more accurate quantitation (Freund and Hegeman 2017). Leaf spray-MS can also be coupled to portable mass spectrometers to allow for on-site screening of plant metabolites without need to transport material to the laboratory (Pulliam et al. 2015; Lawton et al. 2017).

Author contribution statement DMF and ACM conceived, designed, and conducted experiments. DMF analyzed data and wrote the manuscript. All authors read, edited, and approved the manuscript.

Acknowledgements This work was funded by the NSF Plant Genome Research Program Grants IOS-0923960, IOS-1238812, and Postdoctoral Fellowship in Biology IOS-1400818. The NSF Graduate Research Fellowship Program (00006595), and the UNCF/Merck Science Initiative. We also thank Dr. Don Wyse for the assistance in establishing and maintaining the field plot, Eric Roden for assistance in the development of the R scripts for data analysis, and Stephen Brockman for the van Krevelan diagram chemical map. We greatly appreciate the donation of a nano-electrospray source from Dr. Jessica Prenni and the Proteomics and Metabolomics facility at Colorado State University.

References

Ammosov AS, Litvineko VI (2003) Triterpenoids of plant of *Glycyrrhiza* L. and *Meristotropis* Fisch. et Mey Genuses. Pharm Chem J 37:83–94

Biondi DM, Rocco C, Ruberto G (2005) Dihydrostilbene derivatives from *Glycyrrhiza glabra* leaves. J Nat Prod 68:1099–1102. doi:10.1021/np050034q

Chambers MC, Maclean B, Burke R, Amodei D, Ruderman DL, Neumann S, Gatto L, Fischer B, Pratt B, Egertson J (2012) A cross-platform toolkit for mass spectrometry and proteomics. Nat Biotechnol 30:918–920

Chan SL-F, Wong MY-M, Tang H-W et al (2011) Tissue-spray ionization mass spectrometry for raw herb analysis. Rapid Commun Mass Spectrom 25:2837–2843. doi:10.1002/rcm.5177

Cooks RG, Ouyang Z, Takats Z, Wiseman JM (2006) Ambient mass spectrometry. Science 80(311):1566–1570

Cragg G, Newman D (2013) Natural products: a continuing source of novel drug leads. Biochim Biophys Acta 1830:3670–3695

Dalton L (2002) Licorice. Root is used worldwide as a flavor and a medicine. Chem Eng News 80:37

Da-Yuan ZHU, Fu-Xiang SG-QJ, Xing-Ruo C, Wu-Bao GUO (1984) Studies on chemical constituent of *Glycyrrhiza uralensis* Fisch: the structures of isolicoflavonol and glycycomarin. Acta Chim Sin 10:10

Falcone CE, Cooks RG (2016) Molecular recognition of emerald ash borer infestation using leaf spray mass spectrometry. Rapid Commun Mass Spectrom 30:1304–1312. doi:10.1002/rcm.7561

Freund DM, Hegeman AD (2017) Recent advances in stable isotope-enabled mass spectrometry-based plant metabolomics. Curr Opin Biotechnol 43:41–48. doi:10.1016/j.copbio.2016.08.002

Gemperline E, Keller C, Li L (2016) Mass spectrometry in plant-omics. Anal Chem 88:3422–3434. doi:10.1021/acs.analchem.5b02938

Ghosh B, Westbrook TC, Jones AD (2013) Comparative structural profiling of trichome specialized metabolites in tomato (*Solanum lycopersicum*) and *S. habrochaites*: acylsugar profiles revealed by UHPLC/MS and NMR. Metabolomics. doi:10.1007/s11306-013-0585-y

Hatano T, Yasuhara T, Fukuda T et al (1989) Phenolic constituents of licorice. II. Structures of licopyranocoumarin, licoarylcoumarin and glisoflavone, and inhibitory effects of licorice phenolics on xanthine oxidase. Chem Pharm Bull (Tokyo) 37:3005–3009

Jarmusch AK, Cooks RG (2014) Emerging capabilities of mass spectrometry for natural products. Nat Prod Rep 31:730–738. doi:10.1039/c3np70121b

Kim S, Kramer RW, Hatcher PG (2003) Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the Van Krevelen diagram. Anal Chem 75:5336–5344

Lawton ZE, Traub A, Fatigante WL et al (2017) Analytical validation of a portable mass spectrometer featuring interchangeable. Ambient Ioniz Sour. doi:10.1007/s13361-016-1562-2

Liu J, Wang H, Cooks RG, Ouyang Z (2011) Leaf spray: direct chemical analysis of plant material and living plants by mass spectrometry. Anal Chem 83:7608–7613. doi:10.1021/ac2020273

Liu J, Gu Z, Yao S et al (2016) Rapid analysis of *Callicarpa* L. using direct spray ionization mass spectrometry. J Pharm Biomed Anal 124:93–103. doi:10.1016/j.jpba.2016.02.030

Llewellyn AM, Lewis J, Miller SJ, Corol DI, Beale MH, Ward JL (2011) Tissue preparation using *Arabidopsis*. In: Hardy N, Hall R (eds) Plant metabolomics. Methods in molecular Biology (methods and protocols), vol 860. Humana Press

Malaj N, Ouyang Z, Sindona G, Cooks RG (2012) Analysis of pesticide residues by leaf spray mass spectrometry. Anal Methods 4:1913. doi:10.1039/c2ay25222h

Manfredi KP, Vallurupalli V, Demidova M et al (2001) Isolation of an anti-HIV diprenylated bibenzyl from *Glycyrrhiza lepidota*. Phytochemistry 58:153–157. doi:10.1016/S0031-9422(01)00177-7

Martin AC, Pawlus AD, Jewett EM et al (2014) Evaluating solvent extraction systems using metabolomics approaches. RSC Adv 4:26325–26334. doi:10.1039/c4ra02731k

Monge ME, Harris GA, Dwivedi P, Fernández F (2013) Mass spectrometry: recent advances in direct open air surface sampling/ionization. Chem Rev 113:2269–2308

Müller T, Cooks RG (2014) Differential rapid screening of phytochemicals by leaf spray mass spectrometry. Bull Korean Chem Soc 35:919–924

Peng L, Hu ZH (2007) Morphogenesis and histochemical investigation of peltate glandular trichomes on *Glycyrrhiza uralensis* Fisch. leaves. Fen Zi Xi Bao Sheng Wu Xue Bao (J Mol Cell Biol) 40:395–402

Pereira I, Rodrigues S, de Carvalho TC et al (2016) Rapid screening of agrochemicals by paper spray ionization and leaf spray mass spectrometry: which technique is more appropriate? Anal Methods 8:6023–6029

Pulliam CJ, Bain RM, Wiley JS et al (2015) Mass spectrometry in the home and garden. J Am Soc Mass Spectrom 26:224–230. doi:10.1007/s13361-014-1056-z

Schilmiller AL, Last RL, Pichersky E (2008) Harnessing plant trichome biochemistry for the production of useful compounds. Plant J 54:702–711. doi:10.1111/j.1365-313X.2008.03432.x

Siracusa L, Saija A, Cristani M et al (2011) Phytocomplexes from liquorice (*Glycyrrhiza glabra* L.) leaves—chemical characterization and evaluation of their antioxidant, anti-genotoxic and anti-inflammatory activity. Fitoterapia 82:546–556

Smith CA, O'Maille G, Want EJ et al (2005) METLIN: a metabolite mass spectral database. Ther Drug Monit 27:747–751

Smith CA, Want EJ, O'Mallie G et al (2006) XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem 78:779–787

Snyder DT, Schilling MC, Hochwender G, Kaufman AD (2015) Analytical methods profiling phenolic glycosides in *Populus*

deltoides and *Populus grandidentata* by leaf spray ionization tandem mass spectrometry. Anal Methods 7:870–876. doi:10.1039/C4AY02639J

Ulrich EL, Akutsu H, Doreleijers JF et al (2008) BioMagResBank. Nucleic Acids Res 36:402–408. doi:10.1093/nar/gkm957

Wang H, Liu J, Cooks RG, Ouyang Z (2010) Paper spray for direct analysis of complex mixtures using mass. Angew Chem Int Ed 49:877–880. doi:10.1002/anie.200906314

Wishart DS, Jewison T, Guo AC et al (2013) HMDB 3.0—the human metabolome database in 2013. Nucleic Acids Res 41:801–807. doi:10.1093/nar/gks1065

Wu L, Fan Y, Fan C et al (2017) Licocoumarone isolated from *Glycyrrhiza uralensis* selectively alters LPS-induced inflammatory responses in RAW 264.7 macrophages. Eur J Pharmacol 801:46–53. doi:10.1016/j.ejphar.2017.02.049

Zhang Q, Ye M (2009) Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). J Chromatogr A 1216:1954–1969. doi:10.1016/j.chroma.2008.07.072