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Fast Phenotyping of LFS-Silenced (Tearless) Onions by Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

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Supporting Information

ABSTRACT: Fast MS techniques have been applied to the analysis of sulfur volatiles in *Allium* species and varieties to distinguish phenotypes. Headspace sampling by proton transfer reaction (PTR) MS and surface sampling by desorption electrospray ionization (DESI) MS were used to distinguish lachrymatory factor synthase (LFS)-silenced (tearless; LFS−) onions from normal, LFS-active (tear-inducing; LFS+), onions. PTR-MS showed lower concentrations of the lachrymatory factor (LF, **3**) and dipropyl disulfide **12** from tearless onions. DESI-MS of the tearless onions confirmed the decreased LF **3** and revealed much higher concentrations of the sulfenic acid condensates. Using DESI-MS with MS^2 could distinguish zwiebelane ions from thiosulfinate ions. DESI-MS gave reliable fast phenotyping of LFS+ versus LFS− onions by simply scratching leaves and recording the extractable ions for <0.5 min. DESI-MS leaf compound profiles also allowed the rapid distinction of a variety of *Allium* cultivars to aid plant breeding selections.

KEYWORDS: *Allium cepa, tearless onions, desorption electrospray ionization (DESI), proton transfer reaction (PTR), mass spectrometry, phenotyping, breeding, lachrymatory factor, zwiebelane, disulfide, thiosulfinates, 2,3-dimethyl-1,4-butanedithial S-oxide*

INTRODUCTION

Plants from the genus *Allium*, especially onions and garlic, have long been cultivated for their flavors and for their health effects.[1] These are both due to the unique sulfur chemistry and biochemistry of members of this genus, which store large amounts of alkylated cysteine sulfoxides plus specialized enzymes that break these down when tissues are damaged. In onions, *Allium cepa* L., the main alkylated cysteine sulfoxide is isoalliin **1** (*S*-(*E*)-1-propenylcysteine *S*-oxide (1-PRENCSO, Figure 1), which is the substrate for a series of rapid reactions upon cutting an onion. The enzyme alliinase (EC 4.4.1.4) catalyzes the conversion of **1** to (*E*)-1-propenesulfenic acid, **2**. Compound **2** is then rearranged to the volatile and highly reactive lachrymatory factor (LF) (*Z*)-propanethial *S*-oxide, **3**, by the closely associated LF synthase (LFS) (Figure 1).[2]

In addition to the onion LF, propanethial *S*-oxide, **3**, only three other natural thial *S*-oxides have been previously described: (*Z*,*Z*)-2,3-dimethyl-1,4-butanedithial *S*,*S*′-dioxide from *A. cepa*,[1] (*E*)-/(*Z*)-butanethial *S*-oxide from *Allium siculum*,[1] and (*Z*)-phenylmethanethial *S*-oxide from *Petiveria alliacea* (Phytolaccaceae).[3] While LF was first described over 45 years ago, LFS was only described in 2002.[2] The detail of the rearrangement mechanism by which the LFS mediates sulfine formation has recently been described[3] along with an analysis of the amino acids essential for the synthase activity.[4] A further study using in vitro deuterium labeling techniques has shown that the enzyme in onion is specifically an (*E*)-1-propenyl sulfenic acid isomerase.[5] The LFS in *P. alliacea* acts as a dehydrogenase and sequesters all of the sulfenic acid formed by alliinase action on its natural substrate *S*-benzyl-L-cysteine sulfoxide, and converts it entirely to (*Z*)-phenylmethanethial *S*-oxide.[3] However, in this latter example, the conversion of the sulfenic acid by LFS occurs only if the ratio of LFS to allinase is sufficiently high; otherwise, excess sulfenic acid escapes the action of the LFS and condenses with loss of water to form *S*-benzyl phenylmethanethiosulfinate.[6]

Recently, a Japanese−New Zealand collaboration announced the production of genetically modified onions with the LFS silenced, producing tearless onions.[7] The reduced production of LF **3** was demonstrated directly by GC−flame photometric detection (FPD) analyses: LF concentrations were 10−37 times lower in freshly crushed leaves from LFS− plants than control plants and 10−28 times lower in bulbs.[7] In other *Allium* species, sulfenic acids released by alliinase rapidly self-condense with loss of water to form thiosulfinates.[1] Therefore, in LFS− onions (*E*,*E*)-di-1-propenyl thiosulfinate, **4** (Figure 1), was expected to be formed.[7] The presence of thiosulfinate **4** in LFS− onions was shown indirectly by a color change (pinking) assay and by GC-MS detection of a rearrangement product assigned as a zwiebelane isomer.[7]

Aoyagi et al.[8] have investigated the chemistry of LFS− onions further. They used LC-MS to identify cepathiolane A **15** (Figure 2) in an extract of LFS− onion juice and confirmed the

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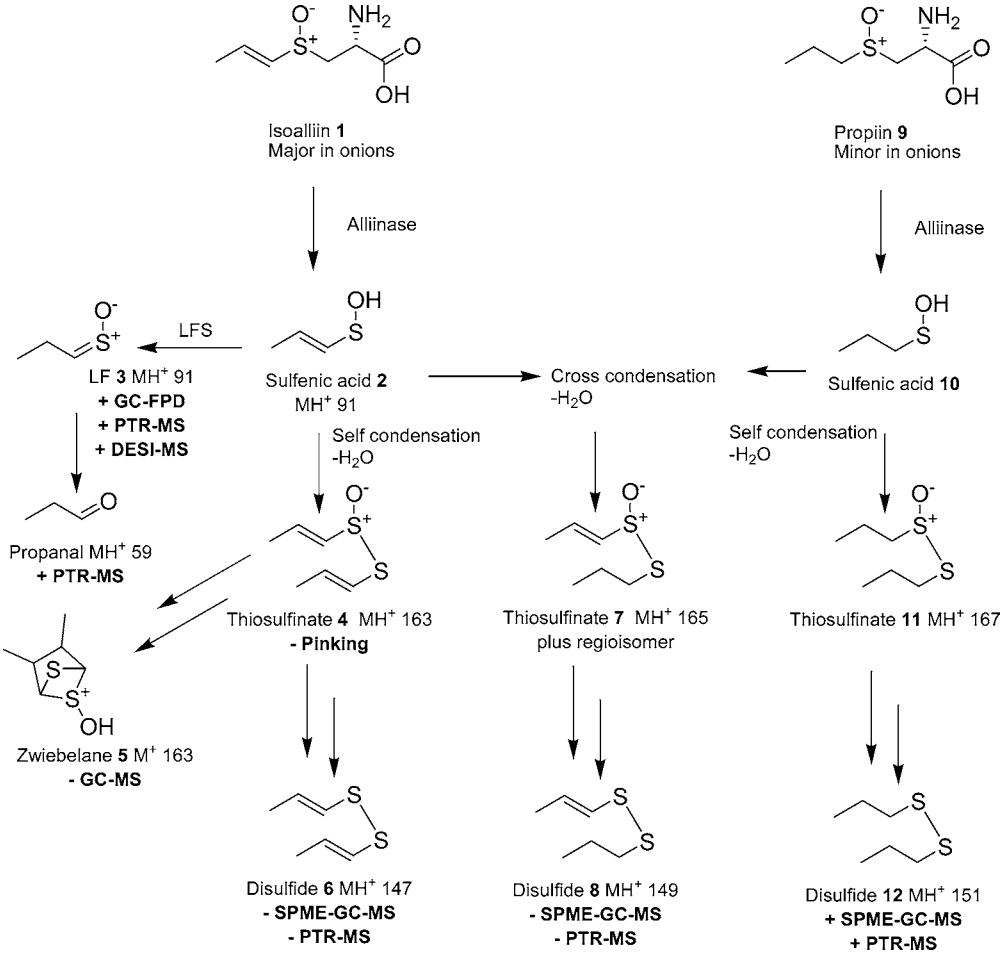

Figure 1. First stages of sulfur chemistry in cut onions showing the analytical techniques that detected different compounds and the effect of LF silencing on relative levels; + means higher in LFS+ (tearing) onions and − means high in LFS− (tearless) onions. (PTR-MS this work; GC-FPD, Pinking and SPME-GC-MS in Eady et al.;[7] and LC-MS in Aoyagi et al.[8]). Only the predominant (*E*) isomers of the thiosulfinates and disulfides are presented to simplify the schematic.

structure by purification and full structural characterization.[8] They proposed the formation of **15** via addition of sulfenic acid **2** to a transient 5,6-dimethyl-2-oxa-3,7-dithiabicycloheptane **14**, formed in two steps from (*E*,*E*)-di-1-propenyl thiosulfinate, **4** (Figure 2). Formation of **13** and **14** has been identified by stereospecific reactions of (*E*,*E*)-di-1-propenyl thiosulfinate **4**, but not from the (*Z*,*Z*) isomer.[9] Compound **5** (Figure 1) has been chemically synthesized by spontaneous cyclization of (*Z*,*Z*)-di-1-propenyl thiosulfinate, **4**.[9] The detection of zwiebelane isomers by earlier investigators was explained by compound **15** reverting to compound **14** under the high-temperature conditions of GC-MS.[8]

These enzymatic and spontaneous reactions in onions (Figures 1 and 2) are rapid: one paper states that hydrolysis of **1** was almost complete 20 s after cutting an onion[10] and another that recovery of LF **3** was at a maximum 2 min after cutting; then LF was lost due to volatilization, hydrolysis, or reduction.[11] Recent advances in MS techniques offer great promise for studying unstable *Allium* reaction products. Proton reaction transfer (PTR) MS has been recently used for real time headspace analysis of chopped onions.[12] This work showed that LF **3** and its breakdown products dominated for the first 10 min after chopping, and then propanethiol and dipropyl disulfide **12** increased in concentration.[12] A similar headspace sampling technique using selected ion flow tube (SIFT) MS applied to cut onion showed an m/z 91 ion assigned as protonated LF **3**, which dropped to <50% of the starting level within 100 s.[13] These same researchers also reported an ion at m/z 163 from crushed garlic, assigned as protonated di-2-propenyl thiosulfinate (allicin), which rose to a maximum at about 200 s and then declined. The surface sampling technique of desorption electrospray ionization (DESI) MS has also been used to detect this thiosulfinate from garlic, but no time dependency was mentioned.[14] Recently, Block and co-workers have reported applications of another surface sampling technique, direct analysis in real time (DART) MS, to *Allium* chemistry.[15] They found ions from LF **3** predominant from onion[16] and ions from di-2-propenyl thiosulfinate predominant from garlic.[17] They also reported that 2-propenesulfenic acid from garlic had a much shorter gas phase lifetime than the isomeric LF **3** from onion.[17]

We now present the results from the headspace sampling technique of PTR-MS[18] and compare them to those from the surface sampling technique DESI-MS,[19] applied for rapid comparative analyses of normal and LFS- (tearless) onion bulbs. We also report the application of DESI-MS for fast phenotyping of the offspring from LFS- onions based on direct leaf analyses and as a tool for the rapid analysis of sulfur volatiles from other *Allium* spp. and varieties.

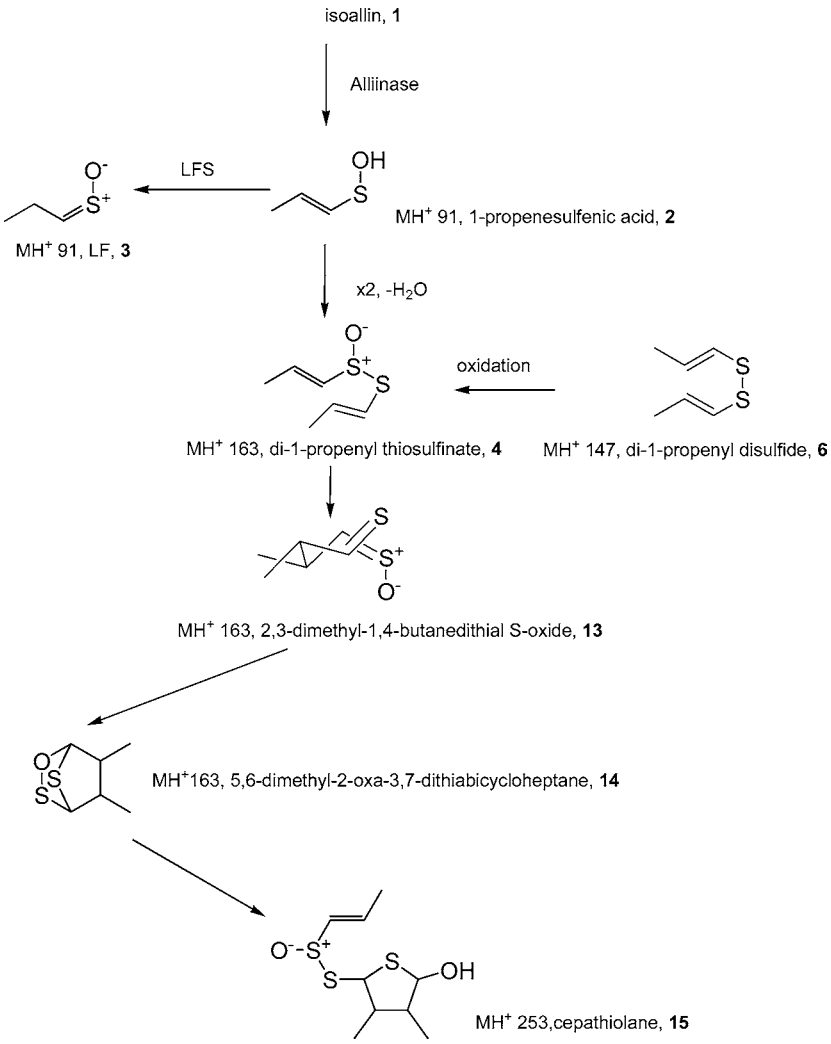

Figure 2. Sulfur chemistry in cut onions based on Aoyagi et al.[8].

MATERIALS AND METHODS

Plant Material. Transgenic green fluorescent protein (GFP) positive, LFS− dehydration onion line D2[7] was crossed with its original nontransgenic dehydration parent, and a second cross was made to a brown intermediate day length Pukekohe Longkeeper (PLK) type. The F_1 progeny from these crosses produced GFP-positive and GFP-negative individuals, as observed by fluorescence microscopy, indicating that LFS− and LFS+ progeny had been produced. The cross with the parent onion produced white dehydration type onions, whereas the cross with the PLK onion produced visually brown fresh market type onions. All bulbs contained isoalliin **1** with a concentration range as previously reported.[7] F_1 bulb material was couriered overnight to the University of Otago for PTR-MS analysis or used in-house for DESI-MS. The visually brown type F_1 onions were grown to maturity and selfed to produce an F_2 population of brown and white onions, which segregated for GFP-positive and -negative offspring again indicating inheritance, or not, of the LFS− phenotype. This material was grown in a Biotron growth chamber (Lincoln University), with temperature and daylight conditions changed every 3 months to reflect external conditions to produce mature bulbs. Each individual transgenic plant was coded, and records of the status of each were maintained by the biosafety compliance team at The New Zealand Institute for Plant and Food Research Limited. Actively growing young leaves from these were used for DESI-MS analyses.

Young leaves from garden-grown *Allium* spp., sourced from local garden centers and colleagues, of *Allium schoenoprasum* L. (chives), *Allium fistulosum* L. (spring onion), *A. cepa* (onion, cv. 'Pukekohe Longkeeper'), *Allium sativum* L. (garlic), *Allium tuberosum* Rottler ex Spreng. (Chinese chives), *A. cepa* var. *vivaparum* (Egyptian tree onion), *Allium ampeloprasum* L. (elephant garlic), *A. cepa* var. *aggregatum* (shallot), and *Allium porrum* L. (leek) were used for comparative DESI-MS analyses. Leaf samples (10−20 cm long) were harvested into plastic bags and stored on ice for up to 8 h prior to analysis.

PTR-MS Analyses. A white dehydration type F_1 LFS− onion (GFP-positive, 05J0116) and an F_1 LFS+ (GFP-negative, 05J0207) white dehydration type were used for analysis. Onions were peeled and cut into quarters, and separate quarters were used for PTR-MS analyses. Quarters were chopped for 10 s into approximately 5 mm sized pieces using a hand-held chopper. PTR-MS data collection began at the start of the chopping, and then the chopped sample was transferred to a Schott bottle (100 mL), which was screwed into a cap with inlet and outlet tubing (0.25 mm diameter Teflon) attached. Air (BOC, New Zealand) flow into the bottle was 500 mL/min with the outlet connected to a glass T-splitter, and flow into the PTR-MS set to 100 mL/min. The PTR-MS instrument (Ionicon Analytik, Innsbruck, Austria) had inlet tubing and chamber maintained at 30−60 °C. The water vapor flow into the ion source was controlled at 8 mL/min and 29 kPa, corresponding to a reaction chamber pressure range of 0.18−0.21 kPa. Detector voltage was 3400 V, and a drift voltage of 525 V was used for all samples. All measurements were carried out under drift tube conditions of 120−130 Td (1 Townsend = 10^{-17} cm^2 V^{-1}) over a mass range of 21−261 Da and a dwell time of 0.1 s/Da, giving a cycle time of 24 s. Data were collected over 2 h, and ion intensities

were converted to concentration (ppb) and then corrected for fresh weight sample mass. Background air scans of 10 cycles were conducted from an empty bottle before each sample measurement.

DESI-MS Analyses. An F_1 LFS- (GFP-positive, 05J0100) white dehydration type daughter and an F_1 LFS+ (GFP-negative, 05J0279) daughter normal onion were used for bulb analyses. An F_1 LFS− (GFP-positive, 05J0402) white dehydration type daughter and an F_1 LFS+ (GFP-negative, 05J0292) daughter normal onion, were used for the first leaf analyses, and garden-grown *Allium* samples (see above) were used for the second leaf analyses.

The DESI source was built based on a published description[20] and consisted of microscope stage components, micro-LC fittings (Upchurch Scientific), and modified mass spectrometer source parts. DESI was performed with a spray tip angle (α) of 55°, tip to surface (d1) of 2 mm, tip to MS inlet (d2) of 4.5 mm, MS inlet to sample (d3) of 1−2 mm, collection angle (β) of 10°, spray tip voltage of 3.5 kV, N_2 pressure of ≈110 psi, and solvent flow of 5 μL/min of methanol/water (1:1) containing 0.1% formic acid (Figure 3). The mass spectrometer

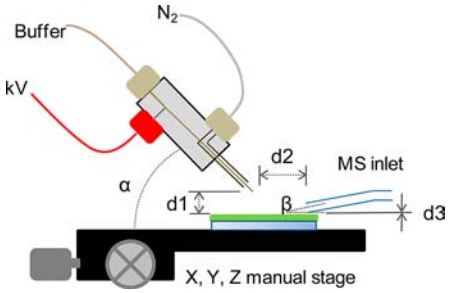

Figure 3. DESI-MS source schematic, with *Allium* tissue (shown as green) samples mounted on a glass slide with double-sided tape.

(LTQ, 2D linear ion-trap, Thermo-Finnigan, San Jose, CA, USA) was tuned with a 10 μg/mL solution of *S*-methyl-L-cysteine in methanol/water (1:1) by direct infusion, optimizing m/z 135 $[M + H]^+$ prior to analysis with a capillary inlet temperature of 275 °C.

Sections of onion bulb scales (10−20 mm × 20 mm) or leaves (2−20 mm natural width × 25 mm long) were mounted with double-sided tape on a standard glass slide so that the tissue was positioned in line with the spray tip and MS inlet. The DESI source and MS were operating and collecting data before surface cells were ruptured. Cells were ruptured by scraping with a scalpel blade held at 45° to the tissue surface.

Initially, data of LFS± onion bulb were recorded for up to 15 min while the sample stage was moved in a serpentine manner across and down the surface of ruptured cells. After the initial 3 min, the sample was left standing and analysis continued periodically until 15 min. Rapid analysis of onion seedlings to identify LFS− versus LFS+ plants from leaf blade could be performed within 30 s from small areas of ruptured cells with minimal need to move the stage.

For the leaf samples of the various *Allium* spp., data were collected for 0−3 min with repeated cell rupture and measurement across and down each leaf, to capture a broad range of initial and reacted species. The large cell structure and hydration of onion bulb provided strong ion signals and allowed areas of ruptured cells to sit and react before measurement. Therefore, a kinetic profile could be recorded for onion bulb scale surface cells. In contrast, the low hydration of leaf cells gave good initial signals but depleted quickly and was only suitable for rapid measurements. Full scan precursor and MS^2 product ions spectra were recorded over various mass ranges, from m/z 20 to 2000 for onion bulb studies and from m/z 89 to 350 in positive ion mode for leaf phenotyping. Data were processed with the aid of Xcalibur 2.05 software (Thermo Electron Corp.).

RESULTS AND DISCUSSION

Plant Material. The original LFS− onion work[3] produced six transgenic onion lines, but only two of these gave seed and only one, D2, produced sufficient seed to enable this research. The dehydration phenotype was not representative of fresh market onions, so crossing onto fresh market PLK type onions was initiated. F_1 white dehydration type and brown "fresh market" type onions were produced. The brown F_1 onions were selfed to produce white and brown F_2 onions.

PTR-MS. For initial PTR-MS analyses we used F_1 white dehydration type quarters of one LFS+ tearing onion (05J0207) and one LFS− onion (05J0116).[7] Timing was started as soon as the onions were chopped, but there was a 1−2 min delay before headspace molecules reached the MS, while the chopped onion was loaded into the sample bottle, attached to the instrument and headspace sampling begun. MS data were collected for 120 min, giving very large data sets. Principal component analysis clearly separated the MS compositions of the LFS+ samples from the LFS− sample (data not shown), but did not help to pick out the distinguishing ions. This was achieved by visual inspection of overlaid plots of the 241 ions monitored, picking out ions for which levels changed consistently over time and distinguished LFS+ and LFS− samples.

The major ion in the early PTR-MS of the LFS+ replicates was at m/z 91, at >10 times the level from the LFS− sample (Figure 4). The level of this ion dropped to half its highest level within 2 min (Figure 4 inset), in accord with previous results on production of LF **3**.[11−13] Ions at m/z 73 and 63 showed decay curves parallel to that of the m/z 91 ion in the LFS+ samples, which we interpret as neutral losses of H_2O and C_2H_4 from protonated LF **3**. Another ion that was high in LFS+ but low in LFS− samples was at m/z 59 (Figure 4). This ion builds up and decays more slowly than m/z 91 (Figure 4 inset), and we suggest that it is due to protonated propanal (Figure 1) from decomposition of LF **3** in the presence of water,[1] previously noted in SIFT-MS[13] and PTR-MS[12] for LFS+ onions.

We had expected the LFS− onion to show a strong m/z 163 ion due to production of thiosulfinate **4** and/or zwiebelane isomer **5** (Figure 1) because both of these compounds were detected in our previous work on LFS− onions.[7] However, PTR-MS detected only very low concentrations of m/z 163 ions and only 20 min after chopping (data not shown). We suggest that **4** and **5** were not sufficiently volatile to enter the headspace or that they reacted rapidly between chopping and securing the headspace bottle to the PTR drift tube.

Other less intense ions that distinguished between the LFS+ and LFS− onions were those for MH^+ of the disulfides: m/z 147 from di-1-propenyl disulfide, **6**; m/z 149 from propyl 1-propenyl disulfide, **8**; and m/z 151 from dipropyl disulfide, **12** (Figures 1 and 4). The relative intensities match the earlier report on SPME-GC-MS analyses:[7] LFS− onions produced much less dipropyl disulfide, **12**, and more of **6** and **8**. Dipropyl disulfide, **12**, is one of the characteristic onion aroma compounds, produced by reaction of thiosulfinate **11**, itself derived from the minor onion component propiin, **9**, with thiols from thiosulfinate decomposition[1] (Figure 1). The production of dipropyl disulfide, **12**, in LFS+ onions was similar to that just reported in another PTR-MS study.[12] Intermediate sulfenic acid **10** is not a substrate for LFS, so it can self-condense to thiosulfinate **11**. However, in LFS− onions, there are high concentrations of sulfenic acid **2**, which can react with sulfenic acid **10** to give thiosulfinate **7** and then disulfide **8**, thus reducing production of **12** (Figure 1). Alternatively, exchange reactions between sulfenic acids **2** and

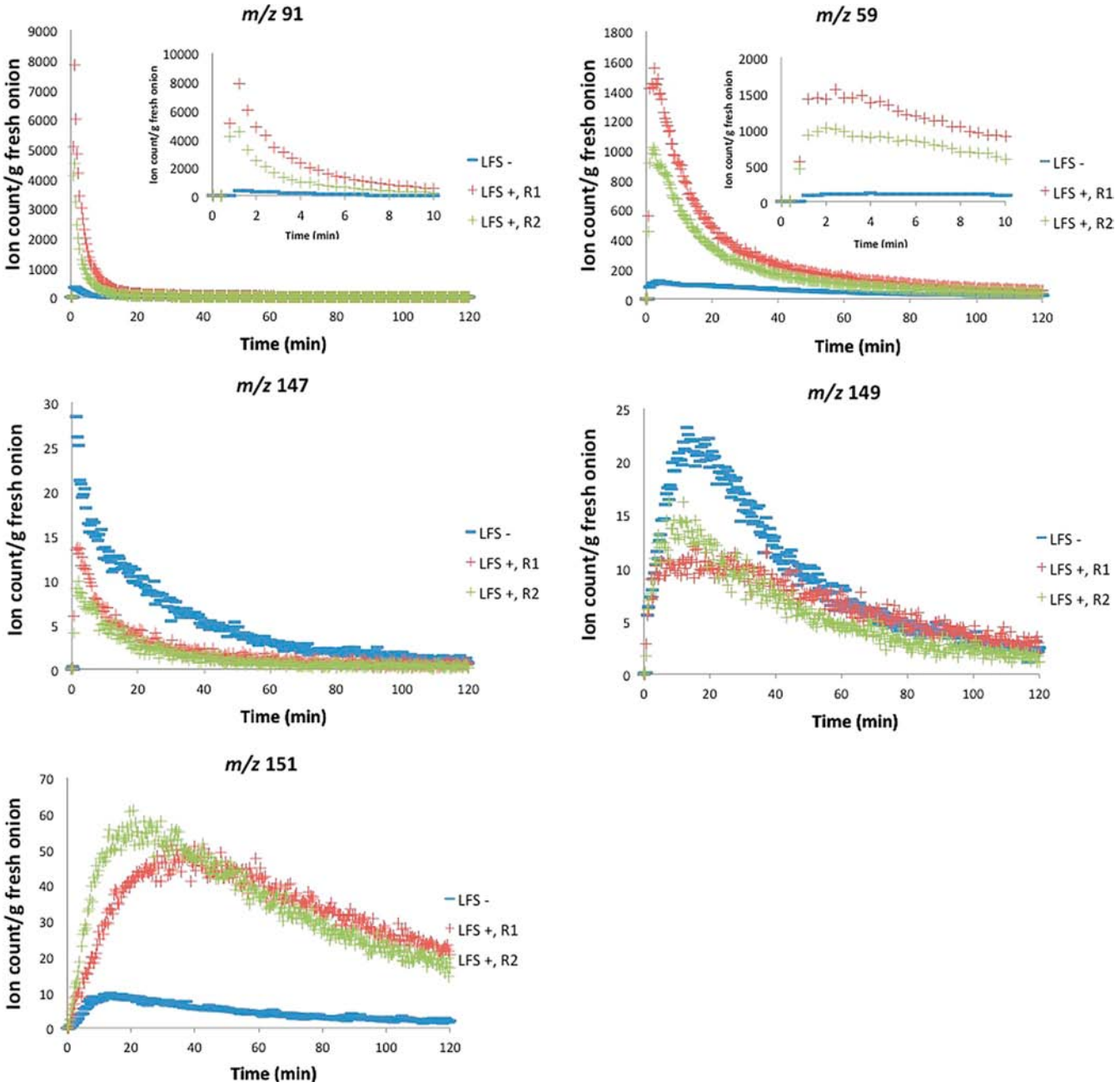

Figure 4. Time course for major ions in PTR-MS analyses of the headspace of LFS+ active (tearing, two replicates) and LFS− onion bulb samples.

10 and thiosulfinates **4** and **11** could be occurring, as has been shown in vitro.[21] We previously noted that LFS− onions had different, sweeter aromas than their nontransgenic counterparts.[7] Methiin (*S*-methyl-L-cysteine *S*-oxide) is the second most abundant alkylated cysteine sulfoxide in onion,[1] but only trace amounts of the corresponding reaction products, dimethyl thiosulfinate (m/z 111) and dimethyl disulfide (m/z 95), were found in our study and in the other[12] PTR-MS study. However, these compounds may have important aroma contributions.

DESI-MS. DESI operates by depositing solvent onto the sample surface, allowing dissolution of sample surface phase analytes into this liquid. The secondary droplets formed from continued spraying then capture the analytes by liquid−liquid phase transfer. These highly charged secondary microdroplets undergo evaporation to form a charged gas phase ion, which is presented to the MS inlet (Figure 3).[22] This design gives a much shorter transit between sample and MS than does PTR-MS, so we hoped to capture more of the very early release compounds from onions, plus the less volatile thiosulfinates.

Two scales each from one F_1 LFS+ (05J0279) tearing phenotype onion and one F_1 LFS− onion (05J0100) were analyzed. In LFS+ onion bulb the m/z 91 ion, $[M + H]^+$ LF **3**, maximized within 0.5 min and was not detectable after 3 min (Figure 5). The m/z 91 ion was significantly weaker in the precursor scan from LFS− onion bulb.

The most clear-cut difference between the DESI-MS of LFS+ and that of LFS− onion was the high concentration of the precursor ion m/z 163 $[M + H]^+$ from cell rupture of LFS− bulb tissue (Figure 5). This distinct ion can also be clearly seen in precursor scans by DESI-MS from the bulbs (Figure 6) and from seedling leaves (Supporting Information, Figure S10). Precursor m/z 163 is a sulfenic acid condensate of one or more

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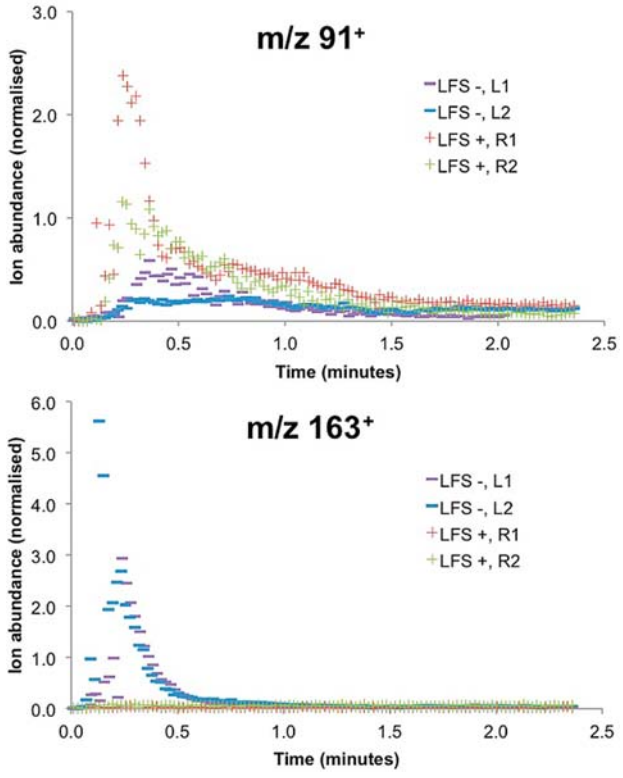

Figure 5. Time course for major precursor ions in DESI-MS analyses of LFS+, active (tearing, two replicates), and LFS− (tearless, two replicates) onion bulb samples.

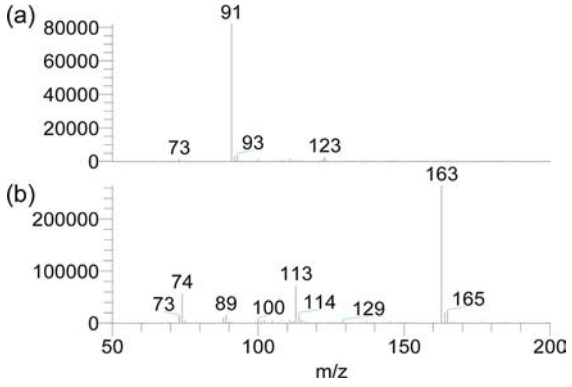

Figure 6. DESI-MS initial cell rupture traces (0−3 min average) from bulbs of F_1 LFS+ (05J0279) tearing phenotype onion (a) and F_1 LFS− onion (05J0100) (b).

of compounds **4**, **5**, **13**, and/or **14** (Figures 1 and 2). MS^2 fragmentation of m/z 163 from LFS− onion leaf showed a neutral loss of 50 Da (SH_2O), forming product m/z 113 (Figure 7). By contrast, MS^2 fragmentation of m/z 163 from garlic leaf showed a loss of 42 Da, forming product m/z 121 (Figure 7). This agrees with previously reported DESI-MS/MS for allicin from garlic[14] and predictive interpretation following the mechanism of charge site rearrangement to form product m/z 121 (Figure 8).

Predictive fragmentation, based on the previously suggested reactive onion chemistry (Figure 1) and the recently proposed chemistry for LFS− onion (Figure 2), was used to identify the distinctive precursor ion m/z 163^+. This was unlikely to be the thiosulfinate **4** as that cannot lose SH_2O to give the product ion m/z 113. Compounds **5** and **14** would require the energy of

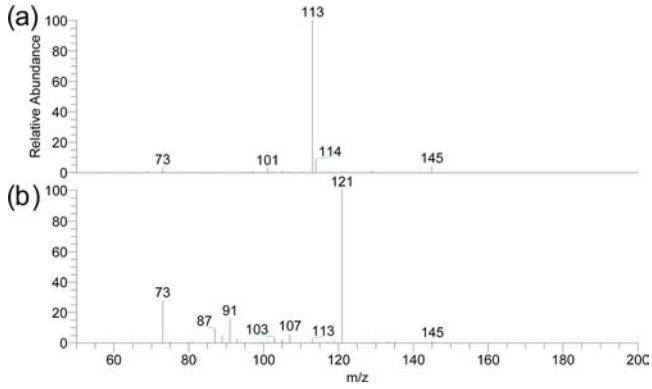

Figure 7. MS^2 product ions of m/z 163 $[M + H]^+$ from of LFS− onion leaf (a) and garlic leaf (b).

inductive cleavage to lose SH_2O, which is less favored than the charge site rearrangement needed by compound **13** to lose SH_2O (Figure 8). Therefore, we propose that DESI-MS of LFS− onions directly detects the production of (*Z*)-2,3-dimethyl-1,4-butanedithial *S*-oxide, **13**, proposed as the key intermediate in the formation of cepathiolane, **15**.[1] Cepathiolane, **15**, was not detected via DESI-MS of ruptured cells of LFS− onions monitored for up to 15 min. However, this was not unexpected as the formation of **15** would require in-solution speciation conditions to aid stepwise formation of sulfenic acid products, which are not favored by the open air ambient volatile conditions used here.[8] The detection of methiin-derived thiosulfinates was limited to trace amounts of m/z 111 due to dimethyl thiosulfinate and m/z 137 due to allyl methyl thiosulfinate in all onion samples. However, these were major ions in the DESI-MS profile of Chinese chives (see below and Supporting Information).

DESI-MS for Fast Phenotyping of LFS− Onions. The unique production of 2,3-dimethyl-1,4-butanedithial *S*-oxide, **13** (m/z 163), in tearless onion leaves (see above and Table S1 and Figures S9 and S10 in the Supporting Information) allowed rapid DESI-MS screening of plants for confirmation of the LFS− phenotype. DESI-MS analyses (blinded) of 18 F_2 leaf blades from white and brown LFS+ and LFS− onions resulted in correct identification, according to GFP inheritance, of all LFS− material (data not shown). Some indication of silencing strength could be inferred on the basis of the abundance of ion m/z 163, which correlated with visual assessment of relative GFP activity. However, this correlation requires further confirmation of gene copy number/expression by Q-RT-PCR.

This rapid assay worked independently of the background onion phenotype (white or brown) and could be used in an automated high-throughput assay of nontransgenic onions (e.g., a tilling population) to identify conventionally produced LFS-reduced onions.

DESI-MS of Other *Allium* Varieties and Species. The fast phenotyping "scratch and sniff" DESI-MS method was extended to analyze the leaves of other *Allium* varieties and species (see Supporting Information, Table S1 and Figures S9−S18). Garden-grown *Allium* leaf analysis indicated the formation of 2,3-dimethyl-1,4-butanedithial *S*-oxide, **13**, with mild and varying abundance relative to thiosulfinates **7** and **11** in *A. schoenoprasum* (chive) and *A. porrum* (leek), suggesting a weaker presence of LFS. As expected, *A. sativum* (garlic) and *A. ampeloprasum* (elephant garlic) had similar profiles with di-2-propenyl thiosulfinate (Figure 8) as the major component

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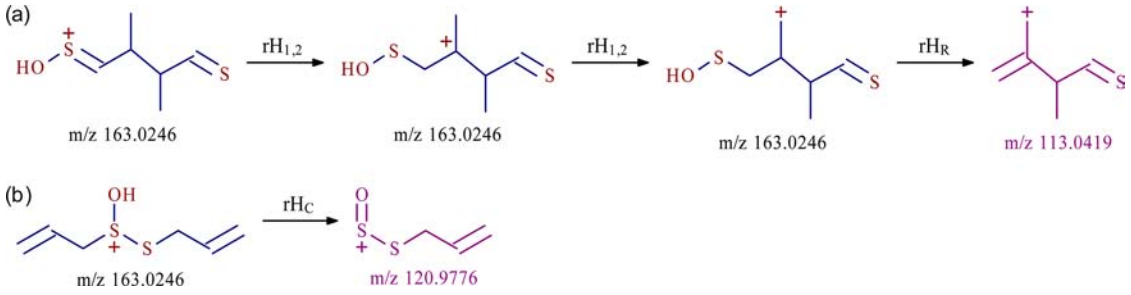

Figure 8. Predicted MS^2 products (HighChem, Mass Frontier, V4SR2.5) of m/z 163, $[M + H]^+$ based on 2,3-dimethyl-1,4-butanedithial *S*-oxide **13** formed from LFS− onion (a) and allicin (di-2-propenyl thiosulfinate) from garlic (b).

released after cell rupture ($[M + H]^+$ m/z 163, MS^2 fragment product m/z 121 indicating a 2-propenyl loss). *A. fistulosum* (spring onion) was similar to *A. cepa* (onion), as expected.[1] *A. tuberosum* (Chinese chives) stood out due to m/z 137 and 111 ions. This species has high concentrations of methiin,[1] resulting in the formation of dimethyl thiosulfinate and methyl allyl thiosulfinate.[12] *A. cepa* var. *aggregatum* (shallot) and *A. cepa* var. *vivaparum* (Egyptian tree onion) spectra showed a strong aminoacrylic acid presence due to hydrolysis producing ammonia to form the adduct precursor m/z 180 $[C_6H_{10}S_2O + NH_4]^+$.[17]

In conclusion, DESI-MS gave reliable fast phenotyping of LFS+ versus LFS− onions by simply scratching leaves and recording the extractable ions for <0.5 min. The leaf compound profiles also allowed the rapid distinction of a variety of *Allium* cultivars to aid plant breeding selections.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Allium leaf analysis by DESI-MS summary of major precursor and product ions, Table S1; DESI-MS average leaf precursor ion spectra, Figures S9−S18. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

PTR-MS, proton transfer reaction−mass spectrometry; DESI-MS desorption electrospray ionization−mass spectrometry; LFS, lachrymatory factor synthase; LF, lachrymatory factor; PRENCSO, *trans*-*S*-1-propenyl-L-cysteine sulfoxide; GC, gas chromatography; FPD, flame photometric detection; GC-MS, gas chromatography−mass spectrometry; LC-MS, liquid chromatography−mass spectrometry; SIFT, selected ion flow tube; DART, direct analysis in real time; MS, mass spectrometry; GFP, green fluorescent protein; PLK, Pukekohe Longkeeper; GM, genetically modified; SPME-GC-MS, solid phase microextraction−gas chromatography−mass spectrometry; Q-RT-PCR, quantitative reverse transcription polymerase chain reaction

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