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In-field Volatile Analysis Employing a Hand-held Portable GC-MS: Emission Profiles Differentiate Damaged and Undamaged Yellow Starthistle Flower Heads

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ABSTRACT:

Introduction – Understanding the complex chemical signalling of plants and insects is an important component of chemical ecology. Accordingly, the collection and analysis of chemical cues from plants in their natural environment is integral to elucidation of plant–insect communications. Remote plant locations and the need for a large number of replicates make *in situ* headspace analyses a daunting logistical challenge. A hand-held, portable GC-MS system was used to discriminate between damaged and undamaged *Centaurea solstitialis* (yellow starthistle) flower heads in both a potted-plant and natural setting.

Objective – To determine if a portable GC-MS system was capable of distinguishing between undamaged and mechanically damaged plant treatments, and plant environments.

Methodology – A portable GC-MS utilising needle trap adsorbent technology was used to collect and analyse *in situ* headspace volatiles of varying yellow starthistle treatments. Principal component analysis (PCA) was used to distinguish treatments and identify biomarker volatiles. Analysis of variance (ANOVA) was used to determine differences between treatment volatile amounts.

Results – The portable GC-MS system detected 31 volatiles from the four treatments. Each GC-MS run was completed in less than 3 min. PCA showed four distinct clusters representing the four treatments – damaged and undamaged potted plant, and damaged and undamaged natural plant. Damage-specific volatiles were identified.

Conclusion – The portable GC-MS system distinguished the treatments based on their detected volatile profiles. Additional statistical analysis identified five possible biomarker volatiles for the treatments, among them cyclosativene and copaene, which indicated damaged flower heads.

Keywords: Semiochemicals; plant volatiles; *in situ*

Introduction

Recent technology has improved the ability to collect and analyse plant-emitted odours, and help understand their influential role with other plants, insects and microbes (Beck *et al.*, 2014b). In particular there have been efforts towards development of portable gas chromatography-mass spectrometry (GC-MS) systems for the separation and detection of complex volatile bouquets produced by plant–plant, plant–insect or plant–microbe interactions (Miresmailli *et al.*, 2010; Schott *et al.*, 2013; Aksenov *et al.*, 2014). While laboratory-based experiments are critical to understanding an organism under controlled conditions, it is also vital to apply this knowledge to realistic field conditions. Thus, collection of plant volatiles in their natural environment with typical biotic and abiotic stressors is integral to elucidation of multifaceted plant–insect chemical communication cues. However, this necessitates that scientists have instruments capable of operating in the field (Beck, 2012) since remote plant locations and the need for a large number of replicates can make *in situ* headspace analyses a daunting logistical challenge. Moreover, the detection of discrete plant emissions requires high instrument sensitivity. A truly portable system capable of detecting chemical cues needs to be highly sensitive, lightweight, and have ancillary power and inert gas sources built into the system; yet, it must also be relatively inexpensive to become commonly available (Bednar *et al.*, 2011).

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The identification and subsequent use of semiochemicals can help control insect pests of agricultural commodities, or determine the host-ranges of insects used as biological agents to control invasive weeds (Park *et al.*, 2013; Smith and Beck, 2013; Beck *et al.*, 2014b). Yellow starthistle is a highly invasive weed of the western United States and has been the focus of numerous investigations utilising potential insect biological control agents (Sheley *et al.*, 1999; DiTomaso *et al.*, 2006; Pitcairn *et al.*, 2006). Examples include: seed- or ovary-feeding weevils (*Bangasternus orientalis*, *Eustenopus villosus* and *Larinus curtus*) (Pitcairn *et al.*, 2008; Birdsall and Markin, 2010) and flies (*Chaetorellia succinea*, *Urophora sirunaseva*) (Balciunas and Villegas, 2007), the rosette-feeding weevil *Ceraptapion basicorne* (Smith, 2012), the phloem-feeding lace bug *Tingis grisea* (Paolini *et al.,* 2008), the rust, *Puccinia jaceae* var. *solstitialis* (Woods *et al.*, 2010) and the leaf- and seedling-feeding slug *Deroceras reticulatum* (Oster *et al.*, 2014). Several of these studies have highlighted plant volatiles as attracting the herbivores or predators of herbivores. More specifically, the study of plant volatiles from both undamaged and mechanically damaged plants can be a useful tool to evaluate host plant specificity of potential biological control agents (Smith and Beck, 2013; Beck *et al.*, 2014b).

In this study the needle trap system incorporated into the Tridion-9™ portable GC-MS system was assessed for its applicability to an agricultural/chemical ecology plant–insect communication project. This unique volatile collection system was designed specifically for either dynamic or static headspace analyses (Asl-Hariri *et al.,* 2014) on an existing hand-held portable GC-MS system (Fig. 1) (Smith *et al.*, 2011). Its capability to distinguish volatile profiles emitted by undamaged and mechanically damaged plant treatments was evaluated under both cultivated and natural conditions. *In situ* flower heads of yellow starthistle were used as the target organ. Leaf volatile emissions of yellow starthistle have been well studied (Beck *et al.*, 2008; Smith and Beck, 2013; Oster *et al.*, 2014) with the resultant volatile profiles used to elucidate the chemical ecology of the plant and its associated biocontrol agents. Additionally, volatiles of the essential oil have been reported for aerial portions and flower heads of yellow starthistle (Senatore *et al.*, 2008; Kilic, 2013). Though the latter study analysed the essential oil components from *ex situ* plant flower heads, the results permitted an indirect comparison of the volatiles detected from *in situ* collections. Results from the present study provide researchers with the identity of several volatiles that may act as chemical cues for insects that attack the seeds within the flower head (Balciunas and Villegas, 2007; Birdsall and Markin, 2010).

Materials and methods

Plants

The plant *Centaurea solstitialis* L. (Asteraceae) is a winter annual plant that was chosen for study due to its importance as an invasive weed, and for the existing literature on its volatile emissions. Plant identifications were originally performed by G.F. Hrusa, California Department of Food and Agriculture Herbarium. Subsequent identifications were based upon ongoing plant studies performed by project personnel. Voucher herbarium specimens are held in the USDA-ARS Herbarium (accession no. S-452) in Albany, CA. Mature *C. solstitialis* plants in the potted treatments were grown from seed (from positively identified *C. solstitialis*) at the Albany, CA location on an outdoor table and in 15 cm diameter plastic pots. Plants volatile profiles were collected when plants were approximately 5 months old, at bolted stage with flower heads not quite in bloom (see Fig. 2A) and plants were 60–80 cm in height. Volatile profiles of naturally growing *C. solstitialis* were collected from plants in open fields at Briones Regional Park, Martinez, CA near Old Briones Road Trail (37.928710°N, 122.151645°W) within 1 day of the potted plant evaluations to ensure a similar phenological stage.

Figure 1. The hand-held portable GC-MS with case (A) and an adsorbed sample being thermally desorbed (B) onto the GC-MS from the needle trap/holder assembly.

In situ collection of volatiles

Undamaged flower heads ($n = 6$, one flower head each from six different plants) were gently sealed in a modified scintillation vial collection apparatus [Fig. 2(B)–(E)] comprised of a rubber ring sealing a Teflon® skirt on the plant stem and onto the scintillation vial with the bottom removed (Beck *et al.*, 2014a). The closed system was allowed to permeate volatiles into the headspace for 15 min, then an 18 gauge needle connected to Teflon® tubing was inserted through the septum capped vial, connected to the needle trap and a 12 VDC eccentric diaphragm pump (Schwarzer Precision, Germany) (Beck *et al.*, 2011) set to a flow of 20 mL/min, and volatiles collected for 15 min. The needle trap [Fig. 3(A)–(B)] (Torion Technologies, American Fork, UT) was inserted into the collection device (Fig. 3C) (Torion Technologies). The needle trap contained the polymer Tenax® TA (1 mg), Carboxen® 1016 (1.6 mg), and Carboxen 1003 (1.5 mg) as the adsorbent (Supelco, Bellefonte, PA). Prior to project volatile collections, breakthrough volumes were determined on *ex situ* flower head tissues at varying flow rates. A second needle at the rear of the vial was held in place

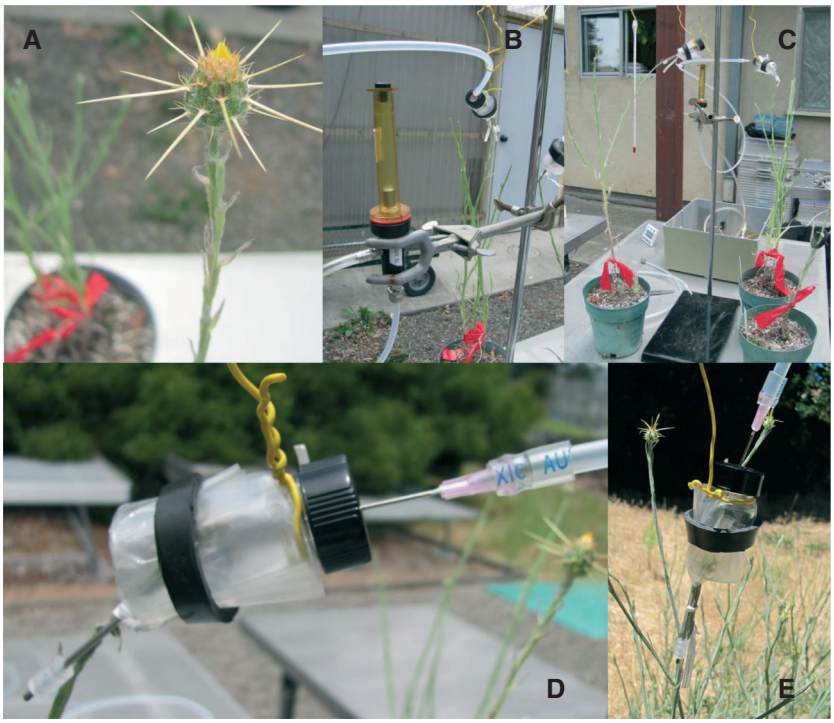

Figure 2. The flower head of *Centaurea solstitialis* from a potted plant (A), the collection system showing the needle trap/holder assembly and collection vial enveloping the flower head (B), replicate analysis of the potted treatments (C), close-up of the collection vial enveloping the flower head of the potted treatment (D), and headspace volatiles being collected from the natural treatment flower head (E) at Briones Regional Park.

parallel to the plant stem to allow entry of ambient air to flow across the flower head (bottom left of Fig. 2D). Collections of volatiles from a blank system were performed to determine background and contaminant volatiles, which were subsequently subtracted during analyses. Once the volatiles were collected from each undamaged flower head, the vials were gently removed. The flower heads were allowed to rest in ambient air for 15 min, and then punctured 10× symmetrically around the side of the flower head using a sterile 22 gauge needle to mechanically damage the tissue. The vials were gently replaced, the Teflon® paper re-sealed, and the volatiles collected using the same parameters as the undamaged experiments. The average temperature during collection of volatiles of the potted treatments (Albany, CA) was ca. 20°C (range of 19 to 21°C) with sunny skies. The average temperature for the natural treatments (Briones Regional Park) was ca. 26°C (range of 25 to 27°C) with sunny skies.

Analysis of volatiles – portable GC-MS

Adsorbed volatiles on the needle trap were thermally desorbed by inserting the needle trap into the injection port of the portable GC-MS (Fig. 1B), which was set at 270°C. The injector assembly was modified to accommodate the needle trap and allow for proper flow of helium through the needle trap during desorption. Other instrument parameters were as follows: transfer line, 250°C; column, initial temperature 50°C, hold 10 s, ramp 2°C/s, end temperature 270°C, final hold time 10 s; helium carrier gas flow, 0.3 mL/min; mass spectrometer trap heater, 150°C, scan time between 0.015 and 60 ms. Column parameters: Restek MTX-5 (5 m × 0.100 mm × 4 μm) bundled into a low thermal mass (LTM) bundle (Agilent, Palo Alto, CA). Fragmentation patterns from the toroidal mass spectrometer (TMS) were compared with those of the benchtop GC-MS and available standards were used to

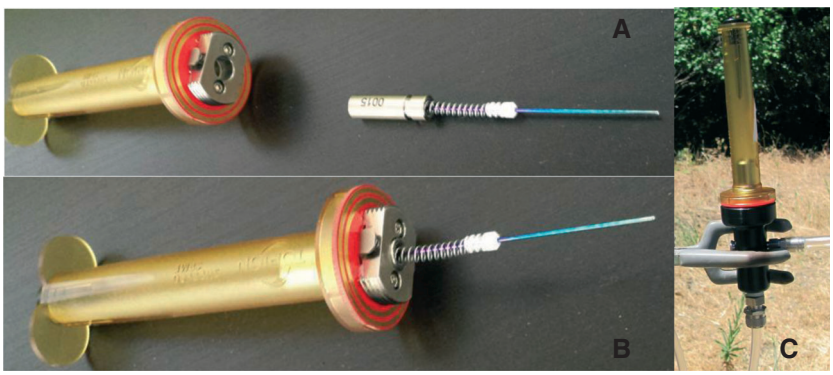

Figure 3. Needle trap assembly components and collection system showing quick connect when disconnected (A), connected to holder (B), and needle trap/holder assembly inserted into collection apparatus to adsorb volatiles from flower heads (C) of *Centaurea solstitialis*.

Table 1. Relative amounts of each signal for the treatments potted control and damage, and natural control and damage

		Potted		Natural				
	Calculated	Control	Damaged	Control	Damaged		Portable	Benchtop
RT (s)	RI[a]	Average (± SEM)	Average (± SEM)	Average (± SEM)	Average (± SEM)	Identity[b]	MS fragments[c]	MS fragments[c]
11.39	478	0.0 ± 0.0	0.0 ± 0.0	244.2 ± 116.1	809.9 ± 355.7	Unknown	45,43,57	
31.79	731	112.9 ± 112.9	0.0 ± 0.0	110.7 ± 70.1	0.0 ± 0.0	Unknown	43,69,53	
34.93	768	157.2 ± 100.3	92.5 ± 66.5	94.4 ± 50.4	0.0 ± 0.0	2-Hexanone	43,101,58,57,85,71	43,58,41,57,100
36.42	789	929.1 ± 495.2	1261.4 ± 466.7	237.8 ± 93.0	448.0 ± 158.2	Hexanal	56,83,57,44,101	44,56,41,43,57
41.06	845	104.3 104.3	1948.2 ± 1,093.4	0.0 ± 0.0	112.8 ± 112.8	Unknown	56,69,43,85	
42.05	859	779.4 ± 625.8	122.6 ± 50.0	185.6 ± 163.7	564.9 ± 245.1	Hexanol	56,55,43,69,41	56,43,55,41,42
42.17	860	1099.0 ± 675.7	3002.2 ± 1,026.2	986.4 ± 502.1	647.1 ± 434.9	(3*Z*)-Hexen-1-ol	67,82,83,55,69	67,41,82,55,69
43.09	871	191.5 ± 126.5	405.0 ± 279.3	69.0 ± 46.7	262.7 ± 193.9	(2*Z*)-Hexen-1-ol	57,67,82,43,55	57,41,67,82,43
50.23	960	0.0 ± 0.0	733.2 ± 265.6	0.0 ± 0.0	419.0 ± 234.4	α-Pinene	93,91,77,67,105	93,91,92,77,79
51.90	979	26.5 ± 26.5	397.9 ± 397.9	0.0 ± 0.0	0.0 ± 0.0	Unknown	57,45,43,83,69	
55.63	1025	248.1 ± 103.8	325.5 ± 140.2	100.9 ± 64.9	138.3 ± 73.2	Octanal	56,69,57,43,55	41,43,57,56,84
55.83	1027	275.8 ± 184.1	233.1 ± 133.5	490.7 ± 232.0	108.8 ± 108.8	(3*Z*)-Hexenyl acetate	67,43,83,82,143	67,43,82,41,55
56.43	1036	173.9 ± 94.8	254.9 ± 169.6	638.9 ± 277.0	469.2 ± 161.1	α-Phellandrene	93,91,77,136,92	93,91,77,92,136
57.93	1054	32.0 ± 32.0	84.0 ± 55.0	25.0 ± 25.0	0.0 ± 0.0	2-Ethylhexanol	57,43,55,70,83	57,41,43,55,70
58.13	1056	51.8 ± 41.8	43.5 ± 24.6	37.8 ± 28.3	30.9 ± 20.0	*p*-Cymene	119,134,91,117	119,134,91,117,77
60.17	1082	70.0 ± 70.0	95.5 ± 95.5	25.2 ± 25.2	16.5 ± 16.5	Unknown	69,95,67	
62.29	1110	0.0 ± 0.0	0.0 ± 0.0	368.6 ± 83.8	405.9 ± 90.9	Unknown	105,77,51	
64.39	1134	452.2 ± 128.7	599.8 ± 189.4	146.7 ± 104.8	255.4 ± 179.2	Nonanal	57,43,70,67,81	57,41,70,82,98
65.29	1145	329.4 ± 116.6	601.8 ± 195.2	285.1 ± 142.6	39.8 ± 39.8	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	69,79,81,43,135	69,41,79,81,53
70.68	1212	0.0 ± 0.0	0.0 ± 0.0	462.0 ± 121.5	433.1 ± 55.4	(3*Z*)-Hexenyl butyrate	67,82,71,43,55	67,82,71,43,41
72.51	1238	438.1 ± 69.6	476.1 ± 86.9	33.7 ± 33.7	0.0 ± 0.0	Decanal	57,55,43,82,67	57,41,43,55,82
78.62	1309	0.0 ± 0.0	861.7 ± 314.6	0.0 ± 0.0	121.7 ± 77.0	1-Tridecene	55,69,97,57,43	55,69,41,83,43
85.60	1401	0.0 ± 0.0	0.0 ± 0.0	121.2 ± 55.1	1155.7 ± 391.7	Tetradecane	71,85,57,99,72	57,43,71,85,99
86.59	1408	86.9 ± 86.9	2216.4 ± 392.8	0.0 ± 0.0	1055.3 ± 347.3	Cyclosativene/α-Copaene[d]	105,119,161,91,93	
87.47	1419	423.8 ± 289.9	2018.2 ± 451.9	230.9 ± 94.8	1885.7 ± 576.0	Unk. sesquiterpene	81,123,161,91,105	
90.04	1451	325.9 ± 172.4	1918.9 ± 336.8	53.7 ± 35.0	0.0 ± 0.0	Caryophyllene	91,105,133,93,67	93,133,91,79,105
92.31	1479	39.6 ± 39.6	323.0 ± 160.1	95.3 ± 44.0	13,457.5 ± 5,210.2	1-Pentadecene	55,83,97,69,57	83,55,97,41,69
94.01	1501	2557.1 ± 1,417.5	19,299.4 ± 3,218.6	0.0 ± 0.0	988.6 ± 519.3	Germacrene D	161,105,91,119,79	161,105,91,119,79
94.99	1512	115.9 ± 115.9	1347.8 ± 423.0	79.0 ± 50.8	128.1 ± 128.1	Bicyclogermacrene (tentative)	121,93,107,161,79	121,93,105,107,91
100.04	1575	36.4 ± 36.4	76.3 ± 76.3	174.1 ± 60.0	83.0 ± 83.0	Unknown	57,43,69,83	
112.33	1726	0.0 ± 0.0	0.0 ± 0.0	517.5 ± 167.6	293.5 ± 146.1	Unknown	120,138,121,57	

[a]Retention indices (RIs) as calculated by the portable GC-MS software.
[b]Peak identity based upon comparison of retention times (RTs), RI values, and fragmentation patterns of injected standards on benchtop and portable system.
[c]Top five fragments based on relative abundance in fragmentation patterns.
[d]Compounds co-eluted on portable, but identified by resolved peaks on benchtop.
SEM, standard error of the mean.

confirm retention times and TMS fragmentation patterns. Compound identities not verified by a commercial or available standard were marked as tentative in Table 1.

Analysis of volatiles – benchtop GC-MS

To assist with compound identification using established retention indices, additional volatile collection experiments were performed on the undamaged and damaged flower head treatments and injected into a modified injection port equipped with a custom methyl deactivated liner installed on an HP-6890 GC coupled to an HP-5973 MSD (Palo Alto, CA), and outfitted with an Agilent DB-1MS UI column (30 m × 0.250 mm × 0.25 μm). Volatiles were analysed via the following method parameter: injection and inlet temperatures 200°C, splitless mode, constant flow of 1.2 mL/min, oven initial temperature 40°C, hold time 0 min, ramp 10°C/min, final temperature 260°C, hold 0 min. Retention indices (RIs) were calculated using a homologous series of *n*-alkanes on the DB-1MS UI column. RIs were used to assist with initial identification and identities were further confirmed by comparison to retention times and fragmentation patterns of standards. Compound identities not verified on both instruments with a commercial or other available standard were marked as tentative in Table 1.

Statistical analysis

Peak areas for volatiles ($n = 31$) were log transformed (base 10) for all statistical comparisons. Principal component analysis (PCA) was performed using BioNumerics. Peak areas of select volatiles were compared using analysis of variance (ANOVA) with a Bonferroni *t*-test in Sigmaplot. Peaks areas were considered different among treatments ($P < 0.05$).

Table 2. Examples of some commercially available miniature or portable mass spectrometry-based GC-MS systems

Manufacturer	Model	Type
1st Detect	MMS-1000	desktop
Advion	expression	desktop
Flir Systems	Griffin 400	field-ready
Flir Systems	Griffin 824	desktop
Microsaic Systems	4000 MiD	desktop
Smith Detection	Guardion	field-ready
Torion Technologies	Tridion-9	field-ready
Syft Technologies	Voice200	vehicle-ready

Results and discussion

Mass spectrometry-based technology has allowed for the advancement of numerous miniature GC-MS systems (Table 2) ranging from small desktop models to field-ready systems with the necessary ancillary items (helium, electrical power) built into the unit. While each of the systems listed in Table 2 may have their individual benefits or features, the intent of the current article is not a comparison of features or sensitivities, but rather to utilise a needle trap system recently incorporated into the Tridion-9™ and its applicability to an agricultural/chemical ecology plant–insect communication project. Moreover, a field-ready system that is capable of either static [e.g. capture of volatiles on solid-phase microextraction (SPME) with no flow of air] or dynamic headspace (e.g. capture of volatiles via needle trap and a flow of air) collections will help advance in-depth studies of plant–insect interactions in remote locations.

The portable GC-MS system detected a total of 31 plant-emitted volatiles from the four treatments (Fig. 4). The replicated volatile profiles of each treatment showed segregation when analysed via PCA (Fig. 5), and also showed good clustering among the replicates. The separation of treatments and good clustering of replicates was despite the inclusion of data from a low volatile collection replicate from both the potted undamaged and potted damaged. These replicates, demarcated by the black and blue arrows in Fig. 5, showed vastly decreased number of signals (number of peaks and amounts detected), perhaps due to an improper seal at time of collection. The diminished volatile amounts collected from these two particular replicates ranged between 2 and 10% of the other replicates' corresponding volatile amounts collected.

The first principal component on the *x*-axis was responsible for separating the natural (right side) and potted (left side) treatments. The relative distance of the green and red ellipses (on the far right of the plot in Fig. 5) from the black and blue ellipses (far left), indicate the difference of volatile emission profiles between the natural and potted treatments, respectively. On the *y*-axis, the second principal component was responsible for separating the undamaged (top) and damaged (bottom) treatments. The relative overlap distance between the green and red ellipses compared to that of the black and blue ellipses highlight the difference in effect of

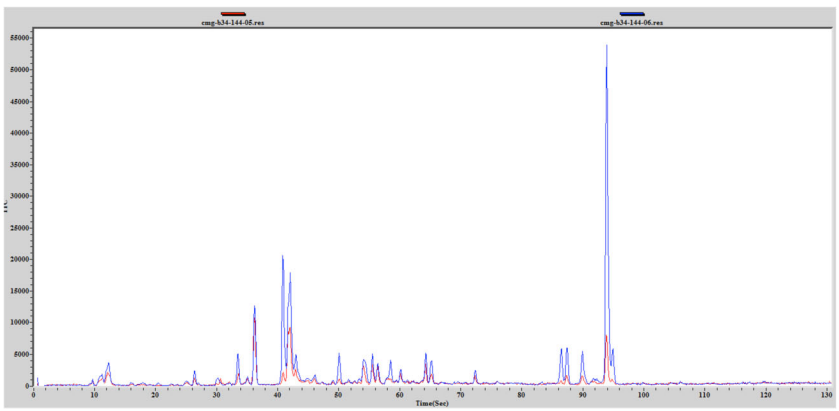

Figure 4. An example of the GC trace screen display as provided by the software loaded on a lap top computer. The red trace is from the potted control replicate and the blue trace is from the potted damaged yellow starthistle flower head.

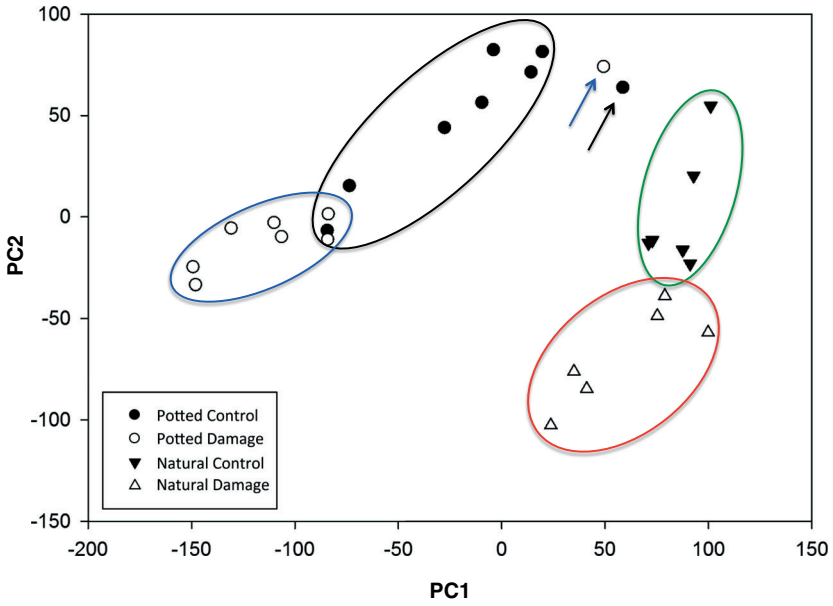

Figure 5. Principal component analysis (PCA) plot of data from the eight (potted) and six (natural) replicates of the four treatments. Potted control treatments are shown in black ellipse, potted damaged in blue ellipse, natural control in green ellipse, and natural damage in red ellipse. The replicates denoted by a black and blue arrow were essentially blank compared to the other seven replicates.

damage on volatile emission profiles of natural plants versus potted plants.

A loading factor plot from the PCA (Fig. 6) enabled identification of the volatiles responsible for distinguishing the treatments. The volatiles in the lower half of the plot represented odours produced as a result of damage to the flower heads. For example, the compounds cyclosativene and copaene, which co-eluted on the portable system (86.59 s), 1-pentadecene (92.31 s), α-pinene (50.23 s), and an unknown sesquiterpene (87.47 s) were released in greater amounts from the damaged versus undamaged treatments of both the natural and potted plants (Fig. 7). In a different study when *ex situ*, dried and crushed yellow starthistle flower heads were extracted by hydrodistillation, the compound cyclosativene was detected in minor amounts, but not copaene (Senatore *et al.*, 2008). In contrast, copaene was detected in relatively large amounts from *in situ* damaged yellow starthistle leaves (Smith and Beck, 2013), but cyclosativene was not. This suggests that cyclosativene is released by damaged flower heads, but not by damaged or undamaged leaves of yellow starthistle. This warrants further investigation of cyclosativene as a possible semiochemical related to flower head damage, despite limited reports of its semiochemical behaviour (Beck *et al.*, 2008).

The long-chain alkene 1-pentadecene (92.31 s) was produced in significantly greater amounts in the natural damaged treatment relative to the potted damaged or both undamaged treatments (Fig. 6), and was the highest emitted volatile compared to all

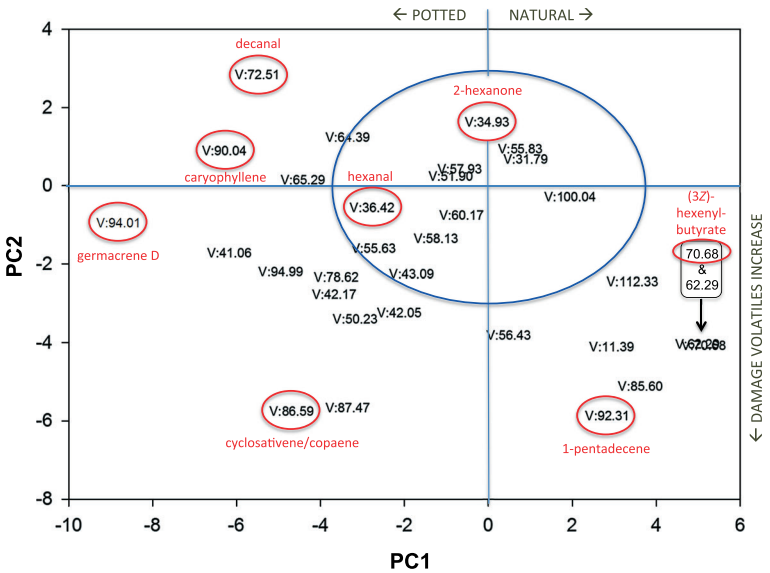

Figure 6. Loading factor plot from the principal component analysis (PCA) of the four treatments. Each factor is listed as their corresponding retention time. Volatiles within the blue ellipse are likely to be statistically equivalent among treatments, and volatiles within red ellipses were further analysed via analysis of variance (ANOVA) and are displayed in Fig. 7.

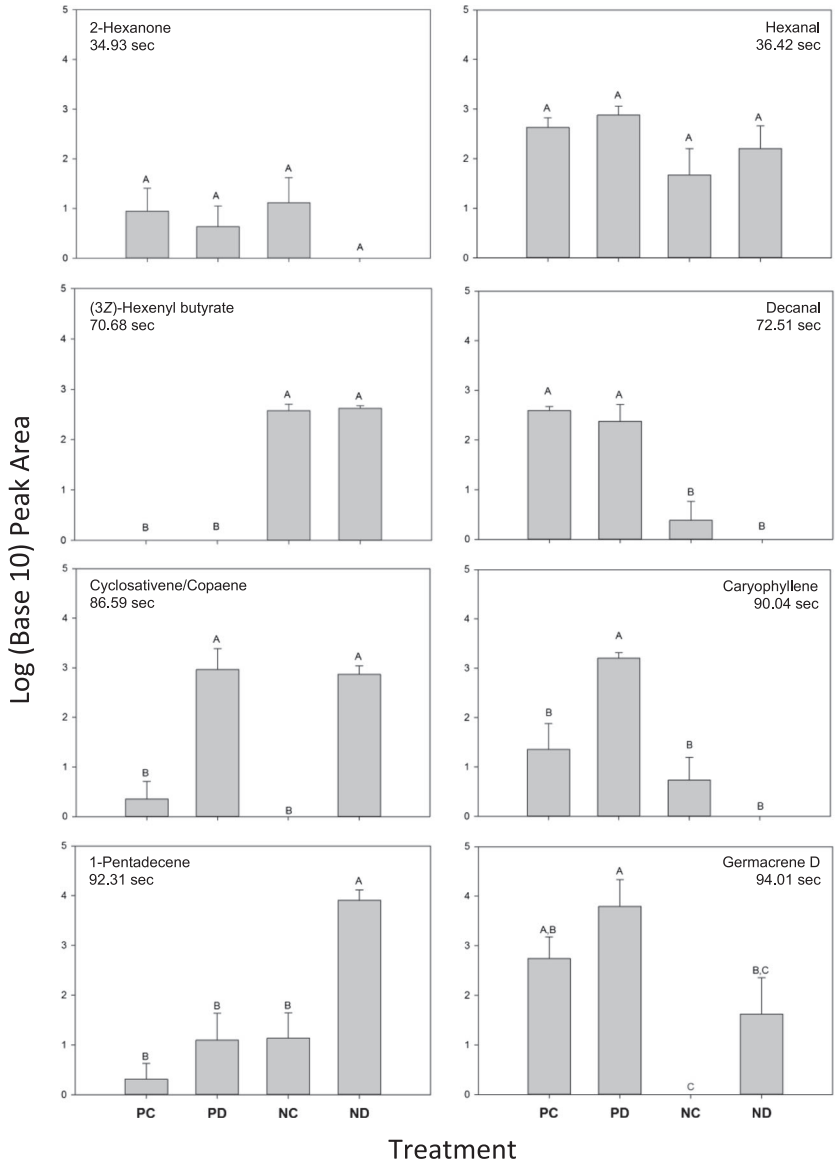

Figure 7. Graphical representation of the peak areas (log base 10) of select volatiles demarcated in red in Fig. 6. Treatments with different letters within each compound are significantly different (ANOVA, Bonferroni *t*-test, $P < 0.05$). PC, potted control (undamaged); PD, potted damaged; NC, natural control (undamaged); ND, natural damaged.

volatiles emitted in the damaged treatment. 1-Pentadecene was detected from *in situ* yellow starthistle leaf in another study (Smith and Beck, 2013), and is also known as a semiochemical of ants (Co *et al.*, 2003) and beetles (Keville and Kannowski, 1975). Considering its relatively large emission from naturally damaged flower heads and history of semiochemical behaviour, 1-pentadecene could be considered as a candidate for signalling to potential biological control agents of yellow starthistle flower heads (e.g. possible chemotaxonomic biomarker).

The compound germacrene D (94.01 s) on the left side of the plot (Fig. 6) was emitted by the potted treatments and natural damaged treatment, but absent in the natural undamaged treatment (Fig. 7). Germacrene D was the highest overall volatile emitted when its average relative abundance is summed across all treatments. It has also been observed in high abundance in other studies of yellow starthistle (Beck *et al.*, 2008; Senatore *et al.*, 2008; Kilic, 2013; Smith and Beck, 2013; Oster *et al.*, 2014). Germacrene D is a common plant volatile with noted semiochemical characteristics (El-Sayed, 2015). However, given its high emission level it could be considered a necessary background sesquiterpene that enhances the effect of other key semiochemicals (Beck *et al.*, 2008; Schröder and Hilker, 2008).

The emission of caryophyllene (90.04 s, Fig. 7) from both undamaged and damaged leaves of yellow starthistle is consistent with the previous *in situ* leaf study (Beck *et al.*, 2008), and caryophyllene was also a major component of flower heads in the Senatore *et al.* (2008) *ex situ* study. Thus, its absence from the natural damaged treatment emissions in our study was surprising. The emission of decanal (72.51 s) had a similar pattern: it was emitted from both potted plant treatments and the undamaged natural treatment, but not the damaged natural treatment. Decanal was not detected in the *in situ* leaf study, but was detected in a relatively large amount in the *ex situ* flower head study and thus appears to be a flower head-specific volatile. The compound 2-hexanone (34.93 s) was detected in the same type of pattern as caryophyllene and decanal, but in lower relative amounts (Fig. 7). 2-Hexanone has

been reported as a plant volatile that elicits mild electrophysiological responses from aphids (Visser *et al.*, 1996).

The esterified green leaf volatile (3*Z*)-hexenyl butyrate (70.68 s, Fig. 7) was representative of a handful of volatiles that were detected exclusively from the natural treatments. The other compounds include tetradecane (85.60 s) and two other unknown compounds (62.29 and 112.33 s). Hexenyl butyrate is a known semiochemical for several arthropods (El-Sayed, 2015). Interestingly, other green leaf volatiles have been detected from previous *in situ* yellow starthistle leaf emission studies (Beck *et al.*, 2008; Smith and Beck, 2013), but to our knowledge the butyl ester analogue has not been reported from any other *in situ* tissues of this plant. The alkane tetradecane has been reported from *ex situ* yellow starthistle flower heads and is a known semiochemical for numerous arthropods (El-Sayed, 2015).

Finally, the compounds hexanal (36.42 s) and 2-hexanone (34.93 s) highlighted in Figs. 6 and 7 represent compounds that were detected in all treatments and exemplify compounds whose relative abundances are statistically equivalent across all treatments. These and other similarly emitted volatiles are shown inside the blue ellipse in Fig. 6.

The hand-held, self-contained portable GC-MS system successfully discriminated between volatile profiles of *in situ* undamaged versus damaged flower heads of yellow starthistle, in both a potted and natural setting. The amount of separation of the potted treatments from their natural counterpart was not fully anticipated. Some separation was expected given the different environmental conditions between the two treatments; e.g. where the potted plants were grown under cooler conditions than the field conditions, 20°C versus 26°C, respectively. The compounds 1-pentadecene (92.31 s), cyclosativene, and copaene (both at 86.59 s), tetradecane (85.60 s), and an unknown sesquiterpene (87.47 s) were identified as probable biomarker volatiles indicating damaged flower head tissue. These compounds warrant further investigation into their possible role as semiochemicals for herbivores of flower heads.

While truly portable GC-MS systems are becoming more readily available (Aksenov *et al.*, 2014), there remain limitations to be overcome by the industry (Bednar *et al.*, 2011). For example, extensive internal databases need to be generated on each system using authentic standards for in-field identifications. Additionally, an in-depth comparison of sensitivities of portable systems could be performed to establish average or acceptable sensitivities. These, as well as the usual obstacles (i.e. calibration curves, method development for optimal separations) necessary for any GC-MS study remain a necessity. What is important is that portable gas chromatography and mass spectrometry-based in-field analyses, and differentiation between discrete treatments, is becoming a practical reality for use in the chemical ecology or agricultural setting.

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