Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/cbst20

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Accepted author version posted online: 24 May 2013.Published online: 15 Aug 2013.

To cite this article: Lincoln Smith & John J. Beck (2013) Effect of mechanical damage on emission of volatile organic compounds from plant leaves and implications for evaluation of host plant specificity of prospective biological control agents of weeds, Biocontrol Science and Technology, 23:8, 880-907, DOI: 10.1080/09583157.2013.807908

To link to this article: http://dx.doi.org/10.1080/09583157.2013.807908

Biocontrol Science and Technology, 2013
Vol. 23, No. 8, 880–907, http://dx.doi.org/10.1080/09583157.2013.807908

RESEARCH ARTICLE

Effect of mechanical damage on emission of volatile organic compounds from plant leaves and implications for evaluation of host plant specificity of prospective biological control agents of weeds

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(*Received 14 March 2013; returned 17 April 2013; accepted 20 May 2013*)

Assessment of host plant specificity is a critical step in the evaluation of classical biological control agents of weeds which is necessary for avoiding possible damage to non-target plants. Volatile organic compounds (VOCs) emitted by plants likely play an important role in determining which plants attract and are accepted by a prospective arthropod agent. However, current methods to evaluate host plant specificity usually rely on empirical choice and no-choice behavioural experiments, with little knowledge about what chemical or physical attributes are stimulating the insect. We conducted experiments to measure the quantitative and qualitative effects on emission of VOCs caused by simple mechanical damage to leaves of plants known to differ in suitability and attractiveness to a prospective agent. More VOCs were detected from damaged than from undamaged leaves for all three species tested. Discriminant analysis was able to correctly distinguish the taxonomic identity of all plants based on their VOC profiles; however, the VOCs that discriminated species among undamaged leaves were completely different from those that discriminated among damaged leaves. Thus, damaged and undamaged plants present different VOC profiles to insects, which should be considered when conducting host plant specificity experiments. An unacceptable non-target plant, *Centaurea cineraria*, emitted all except one of the VOCs that were emitted by its preferred host plant, *Centaurea solstitialis*, indicating the importance of compounds that are repellant in host plant specificity. *Centaurea cyanus* emitted fewer VOCs than *C. solstitialis*, which suggests that it lacked some VOCs important for host plant recognition.

Keywords: biological control; host plant specificity; volatile organic compound; GC–MS

Introduction

Determination of host plant specificity is critical for assessing the direct non-target risk of prospective arthropod agents for classical biological control of weeds, which must be done before they can be approved for release (Horner 2004; Louda, Arnett, Rand, & Russell, 2003a; Louda, Pemberton, Johnson, & Follett, 2003b; Sheppard, van Klinken, & Heard, 2005). Host plant specificity is usually evaluated by performing choice or no-choice behavioural assays using plants in the laboratory or under field conditions (Briese, 2004; Cullen, 1990; Spafford Jacob & Briese, 2003;

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van Klinken, 2000; Withers, Barton Browne, & Stanley, 1999). The results of such experiments are empirical in the sense that the results indicate which plants are acceptable or suitable for a prospective agent under the experimental conditions; however, they do not address the question why some plants are acceptable and others are not. In situations where a non-target plant may be suitable for development of a stenophagous arthropod in laboratory experiments, but the plant is not attacked under normal field conditions (e.g., Bredow, Pedrosa-Macedo, Medal, & Cuda, 2007; Cristofaro, De Biase, & Smith, 2013; Smith, 2007; Smith et al., 2006), understanding why the herbivore is so selective in the field could help improve our ability to assess its potential risk to the non-target plant (Briese, 2005; Sheppard et al., 2005). Key factors that determine whether a plant is likely to be attacked by an arthropod include habitat preference, seasonal synchrony and attractancy and suitability of the plant (Bernays & Chapman, 1994; Schoonhoven, van Loon, & Dicke, 2006). Secondary metabolites can be an important component of attractancy or acceptance of a plant, and thus knowledge about which compounds are present in plants may provide an important explanation why some are attacked while others are not. Thus, study of the secondary metabolite composition of target and non-target plants may help to complement the currently widely accepted centrifugal phylogenetic approach for choosing which plants to test (Wapshere, 1974; Wheeler, 2012; Wheeler & Schaffner, 2013) by revealing the more proximate cause for attractancy or acceptance of host plants.

Some volatile organic compounds (VOC) emitted from plants are known to act as attractants or repellants to phytophagous insects (e.g., Heil, 2004; Junker & Blüthgen, 2008; Kessler & Baldwin, 2002; Mitchell, 1994; Moyes & Raybould, 2001; Otálora-Luna, Hammock, Alessandro, Lapointe, & Dickens, 2009; Piesik, Wenda-Piesik, Kotwica, Lyszczarz, & Delaney, 2011; van Tol, Visser, & Sabelis, 2002; Visser, 1986; Zhang & McEvoy, 1995). VOCs emitted by plants can act as kairomones that attract herbivores, allomones that repel herbivores, or as synomones that attract parasitoids or predators of attacked plants in tritrophic interactions (e.g., Hare, 2011; Kugimiya, Shimoda, Tabata, & Takabayashi, 2010; Mumm & Dicke, 2010; Tumlinson, 1991; Turlings, McCall, Alborn, & Tumlinson, 1993; Turlings, Tumlinson, & Lewis, 1990; van Dam, Qiu, Hordijk, Vet, & Jansen, 2010). Much of the literature is concentrated on the latter interactions, or on attractancy of gregarious species such as bark beetles (Scolytinae; e.g., Amin et al., 2012). It is well known that feeding by phytophagous insects or mites can cause a plant to emit specific VOCs, which is important for understanding how a plant may be able to respond to such attack (e.g., Gosset et al., 2009; Kikuta et al., 2011; Neveu, Grandgirard, Nenon, & Cortesero, 2002; Raghava, Puja, Rajendra, & Anil, 2010). Most recent studies are of herbivore-induced plants rather than of uninjured plants, often focusing either on attraction of predators or parasitoids or on deterrence of herbivores. However, a prospective weed biological control agent, which is typically stenophagous, must be well adapted to finding its host plant (Dickens, 1999; Jermy, 1984), regardless of whether it is previously damaged. For example, the ragwort flea beetle, *Longitarsus jacobaeae*, which has been shown to respond to odours of its host plant in a wind tunnel (Zhang & McEvoy, 1995), continues to find isolated patches of its host plant long after the weed's population declined in Oregon and California. However, only recently have scientists begun to study the role of secondary plant chemistry for classical biological control of weeds (Andreas, Schwarzlander, Ding, & Eigenbrode, 2008; Padovan, Keszei, Köllner, Degenhardt, & Foley, 2010; Park,

Schwarzländer, & Eigenbrode, 2012; Rapo et al., 2012; Smith, Beck, & Gaskin, 2012; Wheeler, 2005, 2012; Wheeler & Schaffner, 2013). These studies have typically involved undamaged plants; however, the effects of prior mechanical damage on qualitative and quantitative emission of VOCs is usually unknown and assumed to be unimportant (Arnett & Louda, 2002; Heard & van Klinken, 2004; Palmer, 1999; Smith, 2012). Furthermore, both olfactory and gustatory stimuli may be important in host plant selection (Bernays & Chapman, 1994; Chapman, 2003; Courtney & Kibota, 1990; Heard, 2000), but studies of only undamaged plants may overlook secondary metabolites that are released only after damage, which would typically occur at the gustatory stage. While it is known that insect-induced secondary metabolites can differ from those induced by mechanical damage (Bricchi et al., 2010), we will first focus on mechanical damage because that is expected to produce the fastest change in VOCs (Baldwin, 1994; Turlings, Lengwiler, Bernasconi, & Wechsler, 1998) and because it is the type of damage most likely to occur in host specificity experiments on prospective biological control agents.

The purpose of this study is to explore the effects of physical damage on emission of VOCs from several plants that have been previously determined to be either acceptable or unacceptable to the prospective biological control agent, *Ceratapion basicorne* (Illiger), which is a weevil that develops internally in an invasive alien weed, yellow starthistle (*Centaurea solstitialis* L., Asteraceae) (Smith, 2007, 2012). Adults emerging from winter diapause search for host plants in the spring and feed on and oviposit in foliage of yellow starthistle (Smith & Drew, 2006). Larvae tunnel down the leaf midrib and complete development in the root crown, in which they pupate. A congener of this weevil, *C. onopordi*, is known to use olfactory cues to help find its host plant (Müller & Nentwig, 2011); however, little is known about *C. basicorne*'s responsiveness to VOCs. This study is a preliminary step towards improving our understanding of how oligophagous insects choose their host plants.

Methods and materials

Experiments were conducted with *C. solstitialis* and two congeners that differed in acceptability to *C. basicorne* (Smith, 2007). *Centaurea cineraria* L., dusty miller, was chosen because it is not acceptable to *C. basicorne* and because of the high number of VOCs that it emits (Beck, Smith, & Merrill, 2008). *Centaurea cyanus* L., bachelor's button, was chosen because *C. basicorne* can oviposit and develop on it, although preference is lower than for *C. solstitialis* (Smith, 2007; Smith, 2012). Plants were grown from seed in flower pots outdoors and were tested indoors in the rosette stage of development, at five weeks of development or older. Origins of the plants were: *C. cineraria* variety 'Colchester white' from a local commercial nursery (accession s-277), *C. cyanus* from locally produced seed (s-382), and *C. solstitialis* from a field population near Woodland, California (s-452). Plant identifications were confirmed by Dr G. F. Hrusa, California Department of Food and Agriculture Herbarium, and voucher specimens were deposited at the USDA-ARS, WRRC Herbarium.

Collection of volatiles

Volatiles were collected *in situ* from leaf samples in an identical manner to that of Beck et al. (2008). Briefly, a leaf from each plant was enclosed in a Teflon® bag (SKC

West, Inc., Fullerton, CA), and VOCs were collected by solid-phase microextraction (SPME) (Supelco, Bellefonte, PA; 100 μm, polydimethylsiloxane fibre) adsorption. The bag was gently sealed onto the stem/leaves of the plant by the use of a twist tie and the SPME inserted into the portal of the bag. For comparison of the relative quantities of detected peaks from each treatment (Romeo, 2009) the VOC collections were kept consistent by the following standardised parameters: *P,* permeation time, amount of time leaf is encased in the collection bag prior to VOC collection; *E,* exposure time, amount of time the SPME fibre is exposed to the permeated volatiles; *S,* storage time, length of time the volatiles are stored on the fibre prior to injection onto the gas chromatography (GC); and *T,* thermal desorption, amount of time the fibre and SPME are kept on the GC injector port. For all VOC analyses, the volatile collection parameters were $P = 5$ min, $E = 55$ min, $S = 30$ s and $T = 15$ min. Background analyses were performed on a SPME fibre exposed in a Teflon bag filled with ambient air.

Analysis of collected volatiles

For all experiments, the collected volatiles were analysed via gas chromatography–mass spectrometry (GC–MS) using identical methods previously published (Beck et al., 2008). National Institute of Standards and Technology (NIST) and Wiley databases were used for fragmentation pattern identification. The retention indices (RIs) were calculated using a homologous series of *n-alkanes* on DB-Wax and DB-1 columns. Volatile identifications were verified by injection of authentic samples and/or comparison of retention times and fragmentation patterns. Each experiment was performed in duplicate on each GC column for a total of four replicates.

Data from GC–MS analyses were transferred to Microsoft Excel for comparison of retention times and compound identification for same-column analysis. Calculated RIs were used to assist in compound identification and to perform comparison of DB-1 to DB-Wax column results. Compounds consistent through all replicates are included in Table 1. The GC–MS data were error-checked by plotting the area of each identified peak from DB-Wax versus from DB-1. Outliers from the regression line were reviewed for errors of interpretation or transcription and were corrected when appropriate. The area of the peak on DB-Wax was used as the response variable because it was generally larger than that on DB-1 (on average by a factor of 2.1).

Leaf damage

The effect of mechanical damage on production of VOCs by leaves was evaluated on all three species by use of four treatments: no damage, puncturing, cutting and scratching a leaf on a plant (Beck et al., 2008). For each type of treatment, only one leaf on a plant was treated and sampled for VOC emission. For the puncture treatment, the leaf was punctured 10 times with a sterile 22-gauge needle inserted through the injection port of the collection bag. For the cut treatment, the petiole was cut so that both the severed leaf blade and the intact petiole remained in bag. For the scratched treatment, the leaf blade was scratched by a small spatula inserted into the bag's opening. An undamaged leaf was sampled before any of the damage treatments (check1) and after all the damage treatments (check2). The treatments were all performed in the same order: check1, puncture, cut, scratch, check2 on one

Table 1. Identification of VOCs adsorbed on SPME that were detected by GC–MS.

		RT – DB-wax		RI	
Compound	Class[a]	Obs.	Auth.	Calc.	Auth.
(*Z*)-3-hexenyl acetate	glv	13.41	13.10	1316	1320
(*Z*)-3-hexen-1-ol	glv	15.63	15.18	1387	1387
α-cubebene	ses	17.71	17.41	1455	1460
δ-elemene (tentative)	mon	18.09	–	1467	–
cyclosativene	ses	18.43	18.02	1478	1480
unknown sesquiterpene A	ses	18.59	–	1481	–
α-copaene	ses	18.74	18.43	1488	1493
α-gurjunene	ses	19.89	19.82	1527	1535
β-cubebene	ses	20.15	19.83	1535	1540
1-pentadecene		20.37	20.03	1543	1547
(*E*)-α-bergamotene	ses	21.56	21.20	1582	1586
calarene	ses	21.70	21.30	1587	1589
β-caryophyllene	ses	21.87	21.49	1593	1596
unknown sesquiterpene B	ses	22.45	–	1613	–
(*E*)-β-farnesene	ses	23.93	23.49	1665	1666
α-humulene	ses	23.93	23.55	1665	1668
unknown sesquiterpene C	ses	24.00	–	1668	–
unknown sesquiterpene D	ses	24.37	–	1681	–
γ-muurolene (tentative)	ses	24.52	–	1686	–
unknown sesquiterpene E	ses	24.77	–	1695	–
germacrene-D	ses	25.05	24.65	1705	1707
α-muurolene (tentative)	ses	25.52	–	1722	–
unknown sesquiterpene F	ses	25.58	–	1724	–
bicylcogermacrene	ses	25.76	–	1730	–
(*E*,*E*)-α-farnesene	ses	26.20	25.81	1746	1749
δ-cadinene	ses	26.43	26.03	1755	1757
γ-cadinene	ses	26.43	26.03	1755	1757
geranyl acetone	ses	29.08	28.64	1855	1853

[a]glv, green leaf volatile; mon, monoterpene; ses, sesquiterpene.
RT, retention time on DB-wax column (min); RI, retention index; Obs., observed value; Auth., authentic sample; Calc., calculated from observed values.

plant during one day. Thus, a difference between check1 and check2 would indicate a systemic response of the plant within 4 h of initial damage (puncture treatment). Immediately after application of each type of damage, the bag was closed with a twist tie. The experiment was repeated on two plant replicates for each species.

Statistical analysis

For all statistical analyses, the area of GC–MS peaks was transformed by ln(area + 1) to improve normality by reducing skewness and kurtosis, which also reduced the coefficient of variation (CV = SD/mean); however, all graphs present means and SEs of untransformed data plotted on a log scale. The significance of plant species and type of damage was analysed by 2-way ANOVA followed by Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) to compare means (SAS Institute, 2003).

Discriminant analysis was performed to determine if any combination of the VOCs could be used to discriminate between the three plant species *C. cineraria*, *C. cyanus* and *C. solstitialis* (Statsoft, 1998). It was also used to examine differences between the treatments within each plant species. Some VOCs were highly correlated, which would create an ill-conditioned matrix, so we had to select a subset of the total VOCs to use for analysis. When two variables were highly correlated, we chose the one with the lowest CV, within species, to include in the analysis. We used forward stepwise analysis using a minimum tolerance of 0.01. Undamaged leaves (check1, check2) were analysed separately from damaged leaves (cut, punctured, scratched) because the VOC profiles for these two groups of damage treatment were so different.

Results

A total of 28 compounds were detected among the three plant species (Table 1). These included 2 green leaf volatiles, 1 monoterpene and 24 sesquiterpenes. Characteristics of the six unknown sesquiterpenes that could not be identified are presented in Table 2. In addition, screen shots of the GC traces (total ion chromatogram) and corresponding mass fragmentation patterns (electron impact) for the unknown sesquiterpenes are shown in Figures A1–A3.

Leaf damage

Overall analysis of the amount of VOCs emitted indicated significant main effects for plant species (ANOVA, $F_{(2,\ 795)} = 30.5$, $P < 0.0001$) and type of damage ($F_{(4,\ 795)} = 96.9$, $P < 0.0001$), and a significant interaction ($F_{(8,\ 795)} = 7.9$, $P < 0.0001$). The amount of VOCs differed between species, with *C. cyanus* having lower levels of VOCs (relative abundance of $4.69 \times 10^6 \pm 1.13 \times 10^6$ [SE]) than the other two species: *C. cineraria* ($2.52 \times 10^7 \pm 7.43 \times 10^6$) or *C. solstitialis* ($1.44 \times 10^7 \pm 5.53 \times 10^6$). Undamaged leaves had the lowest amount of VOCs, and there was no difference between the initial and final readings from undamaged leaves (check1 vs. check2; Figure 1). The highest amounts of VOCs were from scratched leaves, although this was not always significantly greater than that of punctured leaves. Cut leaves usually had amounts intermediate between undamaged and punctured or scratched leaves.

For *C. cineraria*, only one VOC peak (at RI 1316 = (*Z*)-3-hexenyl acetate) was detected in both undamaged treatments (check1 and check2), whereas the three types

Table 2. Characteristics of the unknown sesquiterpenes detected from the three *Centaurea* species and the major fragments (m/*z*) from electron impact mass spectral detection. The fragment abundances relative to the base peak are shown in parentheses.

Name	RI[a]	Fragments							
A	1481	105 (100)	161 (82)	204 (55)	93 (53)	106 (42)	119 (36)	133 (36)	189 (27)
B	1613	161 (100)	105 (30)	91 (25)	119 (20)	204 (16)	120 (15)	162 (14)	133 (12)
C	1668	161 (100)	204 (23)	105 (22)	91 (17)	119 (14)	162 (14)	133 (9)	147 (5)
D	1681	69 (100)	93 (84)	133 (58)	161 (55)	120 (45)	41 (45)	79 (40)	204 (12)
E	1695	123 (100)	94 (45)	121 (32)	93 (30)	107 (20)	161 (11)	189 (11)	204 (8)
F	1724	93 (100)	119 (88)	69 (48)	107 (40)	91 (40)	79 (36)	105 (33)	204 (9)

[a]DB-wax retention index.

of damage produced similar profiles of 25 peaks, although many of these were not consistently detected in cut leaves (Figure 2, where error bars drop to 10^5). Check2 had five more peaks than check1, suggesting a possible systemic effect from the preceding damage treatments (ANOVA contrast of check1 vs. check2: $F_{(1, 26)} = 4.49$, $P = 0.04$). However, none of these peaks was consistently detected.

For *C. cyanus*, four VOC peaks were detected in both undamaged treatments (1316 = (*Z*)-3-hexenyl acetate, 1387 = (*Z*)-3-hexen-1-ol, 1593 = β-caryophyllene, 1855 = geranyl acetone), with three additional peaks in check2 and one in check1 that were not consistently detected (Figure 3). The three types of damage produced similar profiles of 11 peaks. Several additional VOCs were emitted by punctured or

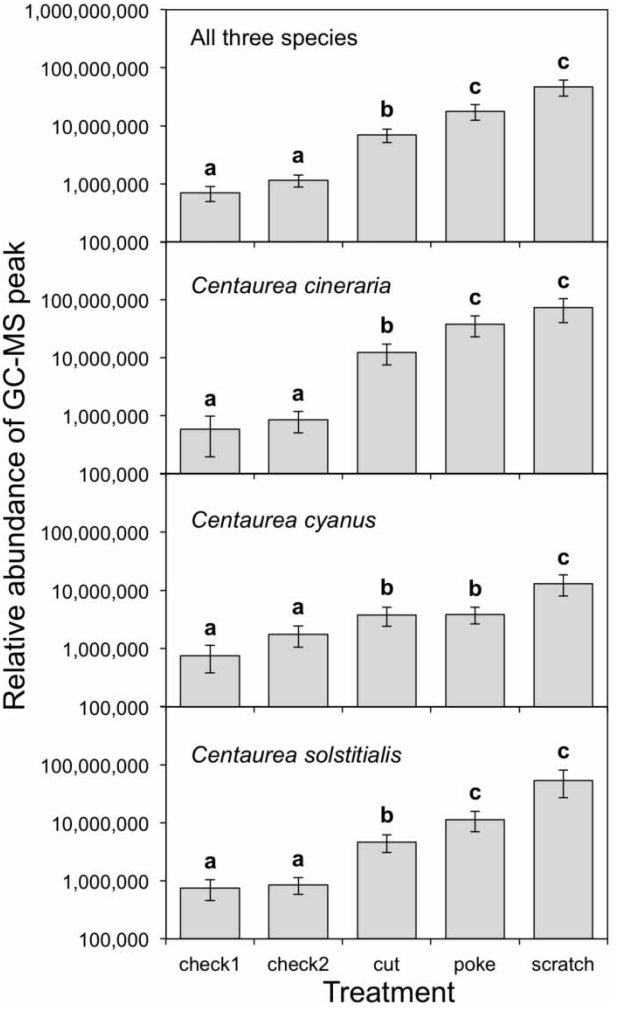

Figure 1. Effect of leaf damage on quantity of total VOCs emitted from leaves of three plant species.

Notes: Means with the same letter are not significantly different (REGWQ multiple comparisons, $\alpha = 0.05$). Check1 is the collection of VOCs from an undamaged leaf before any damage, and Check2 is from an undamaged leaf after all types of damage were performed.

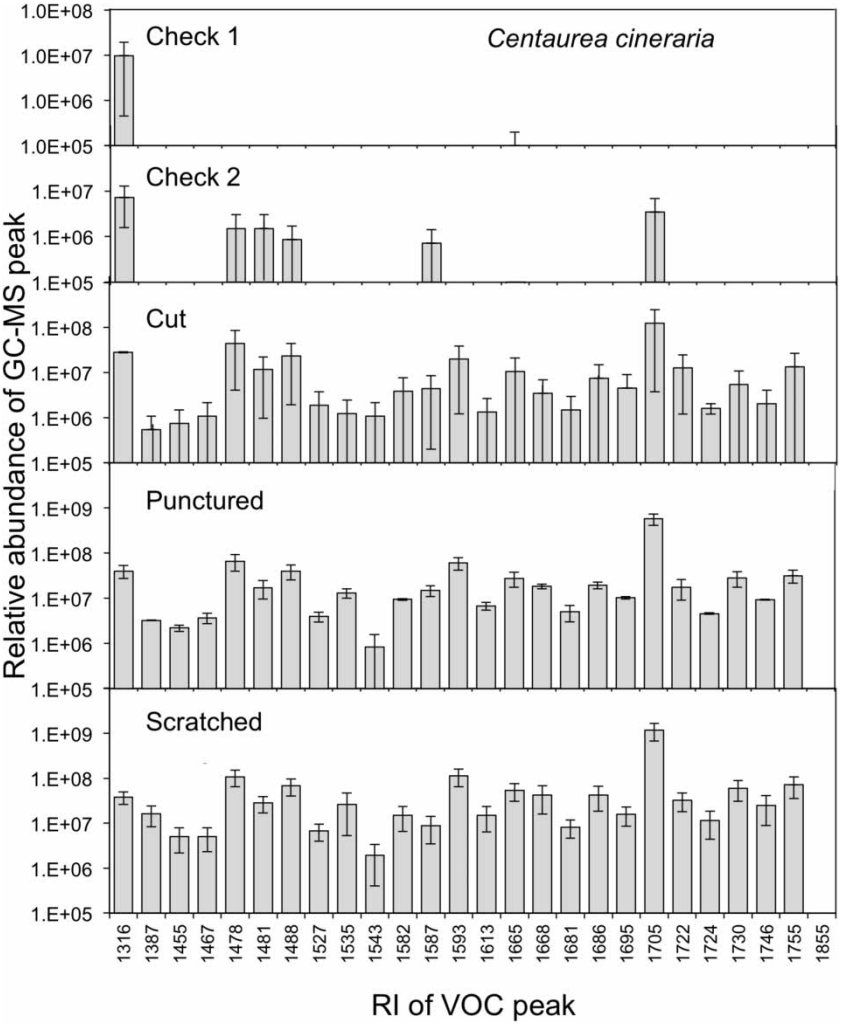

Figure 2. Effect of leaf damage on quantity of VOCs emitted from leaves of *C. cineraria*. Notes: Compounds are labelled by RI_{calc} (see Table 1 for compound names). Check1 is the collection of VOCs from an undamaged leaf before any damage, and Check2 is from an undamaged leaf after all types of damage were performed ('1.E + 05' = 10^5).

scratched leaves, although they were not always detected. The most VOCs were emitted by scratched leaves. There was no indication of a systemic effect of damage (ANOVA contrast of check1 vs. check2: $F_{(1,\ 26)} = 1.83$, $P = 0.18$).

For *C. solstitialis*, five peaks were detected in both undamaged treatments (1316 = (*Z*)-3-hexenyl acetate, 1387 = (*Z*)-3-hexen-1-ol, 1593 = β-caryophyllene, 1705 = germacrene-D, 1855 = geranyl acetone), with two additional VOCs in check1 and one in check2, although they were not consistently detected (Figure 4). The three types of damage produced similar profiles of 16 peaks, and punctured and scratched leaves emitted an additional six VOCs (1455 = α-cubebene, 1467 = δ-elemene, 1613 = unknown sesquiterpene B, 1722 = α-muurolene, 1724 = unknown sesquiterpene

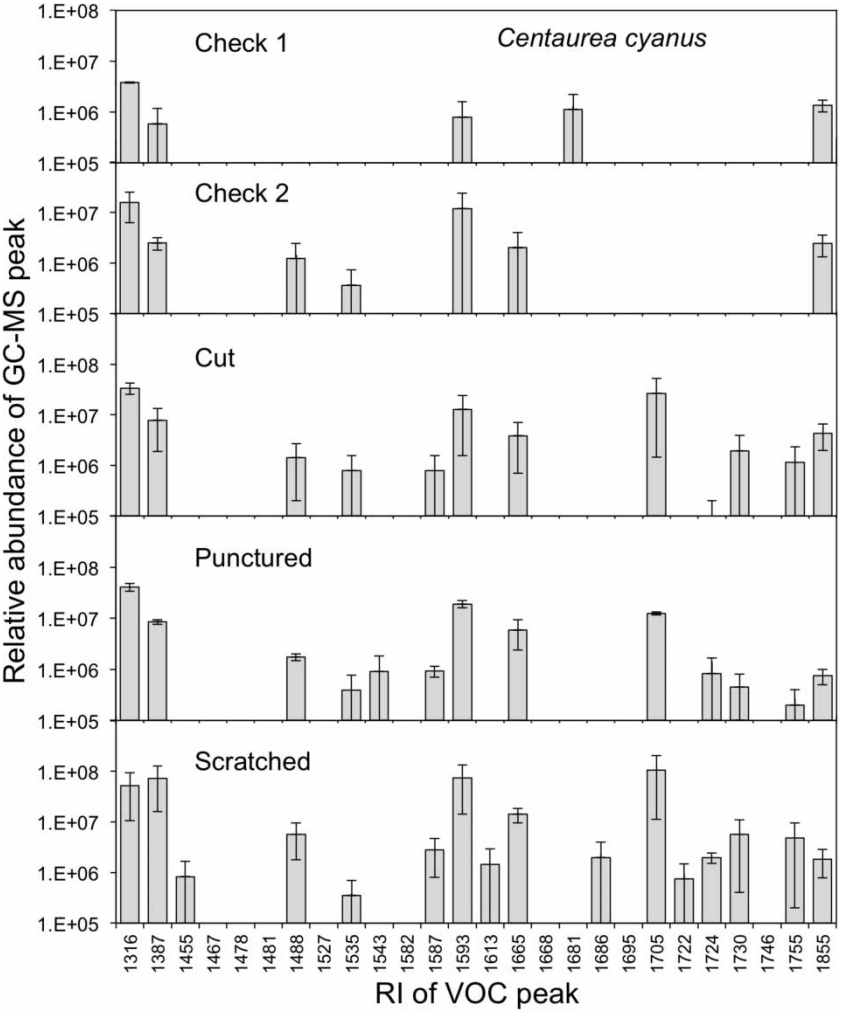

Figure 3. Effect of leaf damage on quantity of VOCs emitted from leaves of *C. cyanus*.
Notes: Compounds are labelled by RI_{calc} (see Table 1 for compound names). Check1 is the collection of VOCs from an undamaged leaf before any damage, and Check2 is from an undamaged leaf after all types of damage were performed ('1.E + 05' = 10^5).

F, 1746 = (*E*,*E*)-α-farnesene). There was no indication of a systemic effect of damage (ANOVA contrast of check1 vs. check2: $F_{(1,\ 26)} = 0.18$, $P = 0.68$).

Discriminant analysis was conducted separately on undamaged and damaged leaves because of the large differences in VOC profiles (Figures 2–4).

Undamaged leaves

Both the check treatments were included in the analysis of undamaged leaves, and six VOCs were selected for analysis: 1316, 1387, 1488, 1593, 1705 and 1855. These VOCs were relatively uncorrelated to the other VOCs (correlation coefficients <0.65; Table A1). The best model included four VOCs: 1316, 1387, 1705 and 1855 (Table 3), and it

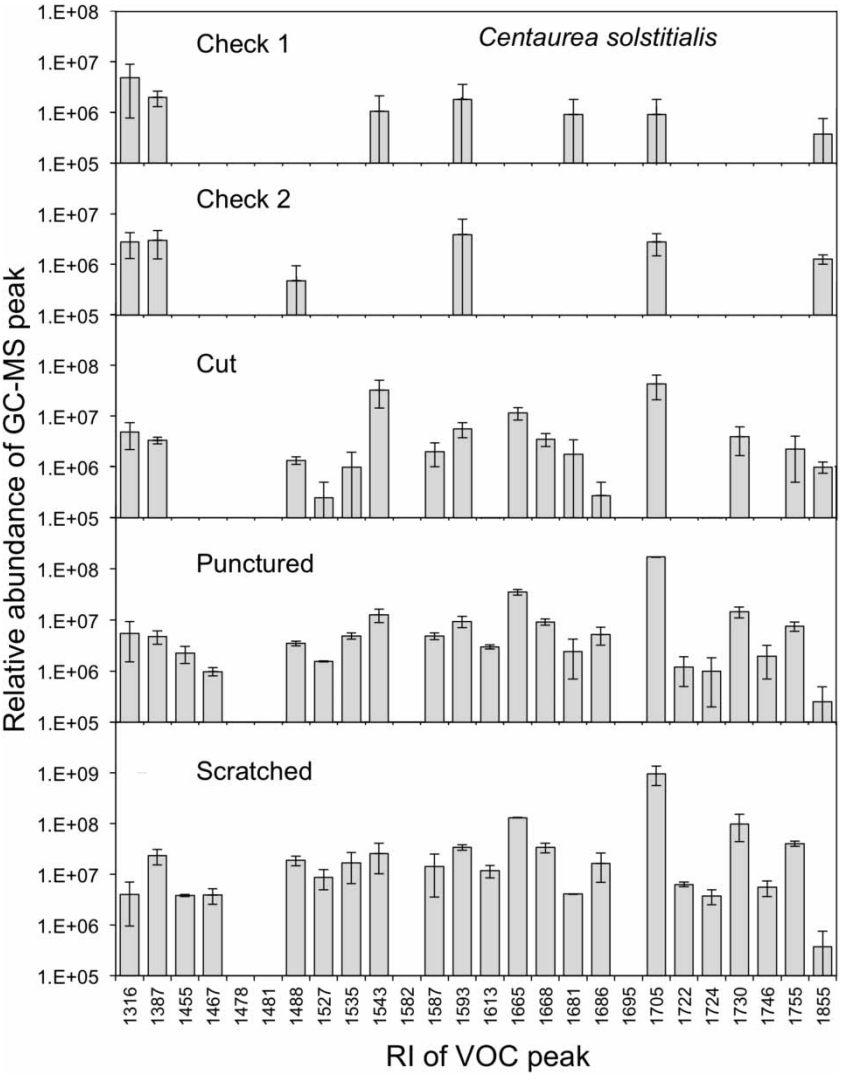

Figure 4. Effect of leaf damage on quantity of VOCs emitted from leaves of *C. solstitialis.* Notes: Compounds are labelled by RI_{calc} (see Table 1 for compound names). Check1 is the collection of VOCs from an undamaged leaf before any damage, and Check2 is from an undamaged leaf after all types of damage were performed ('1.E + 05' = 10^5).

successfully classified all undamaged samples (Figure 5a). However, the distances between the three clusters were not very much larger than the distances between points within each cluster. The first discriminant function, Root 1, is primarily influenced by positive correlation (1.19) to the abundance of 1855 and negative correlation (−0.91) to 1705, which distinguished all three species (Table 4). Root 2 is primarily influenced by negative correlation (−1.05 and −0.72) to the abundance of 1705 and 1387 and positive correlation (0.68) to 1316, which further distinguished between *C. solstitialis* and the other two species. Both *C. solstitialis* and *C. cyanus*

had (*Z*)-3-hexen-1-ol (1387), β-caryophyllene (1593) and geranyl acetone (1855), whereas *C. cineraria* lacked these compounds when undamaged. Germacrene-D (1705) was usually detected in undamaged *C. solstitialis*, only once in *C. cineraria*, but never in *C. cyanus*. All three plants emitted (Z)-3-hexenyl acetate (1316).

All damaged leaves

For discriminant analysis of all damaged leaves, which included cut, punctured and scratched treatments, 16 VOCs were selected for analysis: 1316, 1387, 1467, 1478, 1488, 1527, 1535, 1543, 1582, 1587, 1593, 1613, 1665, 1724, 1755 and 1855. Thirty-eight combinations of VOCs had correlation coefficients greater than 0.8 (Table A1), so many of the selected VOCs were correlated to others that were not selected. The best model included four VOCs (Table 5), and it successfully classified all damaged samples (Figure 5b). Root 1 is primarily influenced by positive correlation (3.32) to the abundance of 1478 and negative correlation to 1582 (-3.06), which distinguished *C. cineraria* from the other two species (Table 4). Root 2 is primarily influenced by negative correlation (-1.59) to the abundance of 1582 and positive correlation (1.07 and 0.98) to 1543 and 1527, which distinguished between *C. cyanus* and *C. solstitialis*. All the *C. cyanus* points were closely clustered except for one outlier in which 1543 was detected, and one *C. solstitialis* outlier lacked 1527. *C. cineraria* was the only species that had cyclosativene (1478), unknown sesquiterpene A (1481), (*E*)-α-bergamotene (1582), and unknown sesquiterpene E (1695). *C. solstitialis* was distinguished from *C. cyanus* by having α-gurjunene (1527), 1-pentadecene (1543), unknown sesquiterpene C (1668), unknown sesquiterpene D (1681) and (*E*,*E*)-α-farnesene (1746).

Damaged leaf blades

When cut leaves were excluded from the set of damaged plants, the best discriminant analysis model included seven VOCs (Table 6). This model grouped each of the species into extremely small clusters that are widely separated from each other (Figure 5c). The first root is primarily influenced by negative correlation (-12.95, -7.95 and -2.43) to the abundance of 1665, 1543 and 1724 and positive correlation (6.17, 5.53 and 1.77) to 1467, 1387 and 1527, which separated all three species. The second root is primarily influenced by positive correlation (1.95) to the abundance of 1467 and negative correlation (-1.89, -1.84, -1.33 and -1.17) to 1665, 1543, 1724 and 1478, which further separated the three species. *C. cineraria* was the only species that had cyclosativene (1478), unknown sesquiterpene A (1481), (*E*)-α-bergamotene (1582) and unknown sesquiterpene E (1695). *C. solstitialis* was distinguished from *C. cyanus* by having δ-elemene (1467), α-gurjunene (1527), 1-pentadecene (1543), unknown sesquiterpene C (1668), unknown sesquiterpene D (1681) and (*E*,*E*)-α-farnesene (1746), and more α-cubebene (1455), β-cubebene (1535), unknown sesquiterpene B (1613), γ-muurolene (1686), α-muurolene (1722), bicylcogermacrene (1730) and γ-cadinene (1755).

Discriminant analysis of three types of damage treatment ['undamaged' (check1 and check2), cut, and 'damaged' (punctured and scratched)] indicated that one of the cut leaves of *C. cineraria* and *C. cyanus* produced a VOC profile like that of damaged leaves (Figure 6, arrows). Otherwise, the profiles of undamaged, cut and damaged leaves were very distinct for each of the three species. Model parameters are presented in Tables A2–A4.

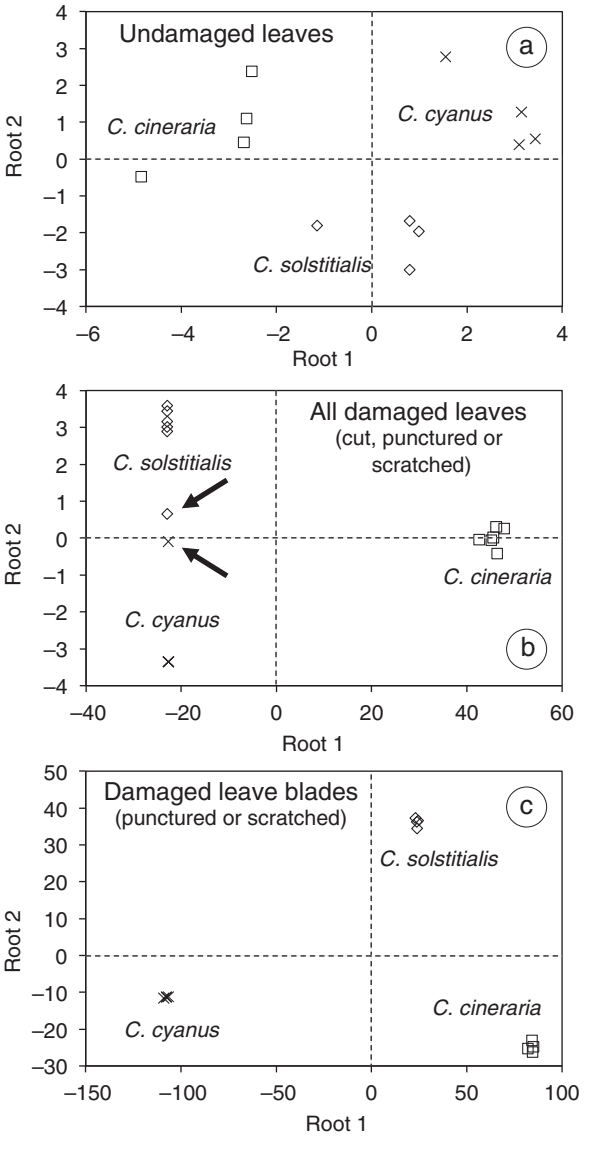

Figure 5. Discriminant analysis of VOCs from three species of plants.
Notes: Plots contain unstandardized canonical scores for the first two roots of the discriminant function. Undamaged leaves were classified by 4 VOCs (panel a; Table 3), all damaged leaves by 4 VOCs (panel b; Table 5), and damaged leaf blades by 7 VOCs (panel c; Table 6). None of VOCs retained in the latter two models were retained in the model for undamaged leaves. Arrows indicate outliers.

Discussion

There was substantial variation in the amounts of VOCs among plants of the same species. This was especially common in the undamaged and cut leaf treatments, which had large error bars (Figures 2–4). Differences in VOC profiles have been

Table 3. Discriminant analysis of undamaged leaves (including check1 and check2 treatments).

VOC RI					Standardised coefficients	
	Partial lambda[a]	$F_{(2,6)}$	P	Tolerance	Root 1	Root 2
1855	0.2654	8.30	0.0187	0.5411	1.1931	0.3501
1705	0.2869	7.46	0.0236	0.4586	−0.9077	−1.0470
1387	0.4644	3.46	0.1001	0.9718	0.4272	−0.7199
1316	0.7450	1.03	0.4136	0.7379	0.0581	0.6756
Eigenvalue					8.02	3.01
Cumulative proportion					0.727	1.000

[a]The lower the partial lambda, the greater the contribution to discrimination.
Wilks' Lambda: 0.0237, $F_{(8,\ 12)} = 8.24$, $P < 0.0007$.

found among inbred lines of maize (Degen, Dillmann, Marion-Poll, & Turlings, 2004), and such differences may occur among individuals of an invasive plant population (Padovan et al., 2010; Wheeler & Schaffner, 2013), especially those with known biotypes. Future studies should further examine intraspecific variability in VOC emission, which could be affected by genetics or environmental factors (Wheeler & Schaffner, 2013). It is possible that such VOC variation could be related to variation in the intraspecific acceptability of plants (e.g., Haines et al., 2004; Padovan et al., 2010; Wheeler, Pratt, Giblin-Davis, & Ordung, 2007), although this is apparently not true for *Tyria jacobaeae* (L.) on tansy ragwort (*Senecio jacobaea* L.) (Macel, Klinkhamer, Vrieling, & van der Meijden, 2002).

Overall, we found that VOC amounts below about 1×10^6 could not be reliably detected by our methods, which is indicated by the frequent occurrence of SE bars that extend down to the x-axis (1×10^5) in Figures 2–4 for peaks measured in this range. If insects are able to detect amounts less than this, then it means that we may be missing the presence of some VOCs that might affect insect behaviour. Quantitation of the classes of compounds detected in this study was not performed; however, comparison of quantitation results from our other experiments gives an approximate value of 9 ng/h of a sesquiterpene (e.g., α-humulene) corresponding to a GC–MS peak area of 1×10^6. The adult dock leaf beetle, *Gastrophysa viridula* Deg. (Coleoptera: Chrysomelidae) is attracted to 300 ng/h of (Z)-3-hexenal and 300 –

Table 4. Means of canonical variables from the three different discrimination models for VOCs from undamaged and damaged leaves.

	Undamaged leaves[a]		Cut, punctured, scratched[b]		Punctured, scratched[c]	
Species	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2
C. cineraria	−3.1681	0.8641	45.5999	0.0089	83.8986	−24.8369
C. cyanus	2.8061	1.2480	−22.6912	−2.8069	−107.6954	−11.3523
C. solstitialis	0.3620	−2.1120	−22.9086	2.7979	23.7968	36.1892

[a]This corresponds to Table 3 and Figure 5a.
[b]This corresponds to Table 5 and Figure 5b.
[c]This corresponds to Table 6 and Figure 5c.

Table 5. Discriminant analysis of all damaged leaves (including cut, punctured and scratched treatments).

					Standardised coefficients	
VOC RI	Partial lambda[a]	$F_{(2,\ 6)}$	P	Tolerance	Root 1	Root 2
1478	0.0038	1559.72	0.0001	0.0900	3.3250	0.1638
1582	0.1305	39.99	0.0001	0.0754	−3.0609	−1.5856
1543	0.4685	6.81	0.0106	0.5343	−0.0237	1.0736
1527	0.6004	3.99	0.0468	0.4822	−0.0603	0.9780
Eigenvalue					1247.61	6.28
Cumulative proportion					0.995	1.000

[a]The lower the partial lambda, the greater the contribution to discrimination.
Wilks' lambda = 0.0001, $F_{(8,\ 24)} = 283.08$, $P < 0.00001$.

1500 ng/h of (Z)-3-hexen-1-yl acetate, in a Y-tube olfactometer, which was in the range of induced concentrations emitted by a single injured leaf (ca. 450–550 ng/h; Piesik, Wenda-Piesik, Ligor, Buszewski, & Delaney, 2012). However, the method of calculating the concentration of volatiles emitted from impregnated filter paper for the Y-tube olfactometer was not described, and volatiles from plants were collected in Super-Q from air aspirated at 0.8 L/min for three hours, so it is not clear how comparable the concentrations are to our results. In comparison, electroantennogram studies of the Colorado potato beetle (*Leptinotarsa decemlineata* L. [Say]) have shown antennal responsiveness at concentrations of 300×10^{-9} ng (Z)-3-hexen-1-ol/mL in air (Schütz et al., 2000). The pine shoot beetle *Tomicus destruens* (Wollaston) responded to headspace concentrations of 0.02–92.3 μg/h of VOCs in an olfactometer (Faccoli, Anfora, & Tasin, 2008). The latter two examples suggest that it is possible that we did not detect all the VOC peaks that might be detected by a stenophagous herbivore of these plants.

Table 6. Discriminant analysis of damaged leaf blades (including punctured and scratched treatments).

					Standardised coefficients	
VOC RI	Partial lambda[a]	$F_{(2,6)}$	P	Tolerance	Root 1	Root 2
1478	0.0043	342.64	0.0003	0.5425	0.6817	−1.1713
1467	0.0714	19.51	0.0191	0.0221	6.1746	1.9518
1665	0.0657	21.34	0.0168	0.0055	−12.9476	−1.8949
1543	0.0561	25.22	0.0133	0.0142	−7.9530	−1.8353
1387	0.1741	7.12	0.0726	0.0263	5.5307	0.9027
1724	0.2455	4.61	0.1217	0.0980	−2.4339	−1.3338
1527	0.4513	1.82	0.3032	0.1652	1.7692	0.4385
Eigenvalue					8534.92	913.51
Cumulative proportion					0.903	1.000

[a]The lower the partial lambda, the greater the contribution to discrimination.
Wilks' lambda = 0.000001, $F_{(14,\ 6)} = 1{,}197.0$, $P < 0.00001$.

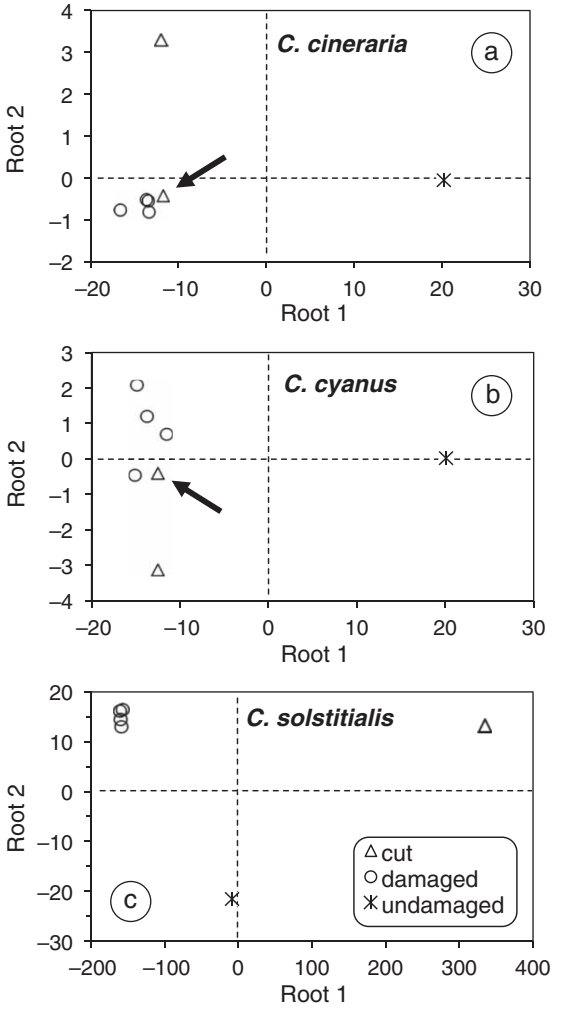

Figure 6. Discriminant analysis of three types of damage treatments (cut, damaged [punctured & scratched], and undamaged [check1 and check2]) on each of three species of plants.

Notes: Plots contain unstandardized canonical scores for the first two roots of the discriminant function. Arrows indicate cut leaves that had VOC profiles very similar to that of damaged leaves.

Effect of damage

Given that VOC peaks lower than about 1×10^6 were usually not consistently detected, undamaged plants of the three species differed very little. All three species shared one peak, (*Z*)-3-hexenyl acetate (1316), a common 'green leaf odor' (Piesik et al., 2012; Turlings et al., 1998; Visser, 1986). *C. solstitialis* and *C. cyanus* shared three additional peaks: the green leaf volatile (*Z*)-3-hexen-1-ol (1387) and two sesquiterpenes: β-caryophyllene (1593) and geranyl acetone (1855). *C. solstitialis* had one additional peak of germacrene D (1705), which appeared only once in undamaged

C. cineraria. Although there was enough difference in VOC profiles of undamaged leaves to reliably distinguish the three species using discriminant analysis, the number of VOCs emitted by damaged leaves was usually much greater. VOC profiles were similar for cut, punctured and scratched leaves, within each species; however, cut leaves usually had fewer VOCs than the other two treatments. Abscised leaves with their petioles inserted in water vials are sometimes used for testing host plant specificity of prospective weed biological control agents (e.g., Arnett & Louda, 2002; Palmer, 1999; Smith, 2007). Based on our results, such leaves may be expected to release VOC profiles that are intermediate between undamaged intact leaves and leaves that have been damaged, either mechanically or by insect feeding. The additional VOCs emitted from damaged leaves may include some compounds that are important for gustatory discrimination of host plant suitability. Although stenophagous beetles are known to nibble on non-target plants under experimental conditions, there are few data showing how important gustation is to determining host plant acceptance (Bernays & Chapman, 1994; Chapman, 2003; Courtney & Kibota, 1990; Heard, 2000; Heisswolf, Gabler, Obermaier, & Müller, 2007). *C. basicorne* adults feed by making small holes in the leaf and chewing on cells within reach of their rostrum, damage which is physically most similar to the puncture or scratch treatments (Smith & Drew, 2006). Damaged *C. cyanus* did not have any VOCs that were not present in *C. solstitialis*, so it is not likely to have any repellants for this insect. However, given that *C. cyanus* is less attractive to *C. basicorne* than *C. solstitialis* (Smith, 2012), the lower number of VOCs in *C. cyanus* suggests that it lacks some important components of an attractive profile (Bruce, Wadhams, & Woodcock, 2005). On the other hand, *C. cineraria* had four VOCs not shared by *C. solstitialis*: cyclosativene (1478), unknown sesquiterpene A (1481), (*E*)-α-bergamotene (1582) and unknown sesquiterpene E (1695), which could possibly act as repellants to *C. basicorne*, which does not accept this plant (Smith, 2007).

Other studies have reported a positive relationship between the extent of physical damage and the amount of volatile emissions (Copolovici, Kännaste, Remmel, Vislap, & Niinemets, 2011) and particularly differences between undamaged and damaged plants (Kikuta et al., 2011; Pareja, Moraes, Clark, Birkett, & Powell, 2007; Pearse, Gee, & Beck, 2013; Piesik et al., 2010a, 2010b; Raghava et al., 2010; Turlings et al., 1998). Some stenophagous herbivorous insects have been shown to prefer mechanically damaged host plants to undamaged plants. For example, the vine weevil, *Otiorhynchus sulcatus*, not only preferred undamaged *Euonymus fortunei* cv. Dart's Blanket to clean air in an olfactometer but also preferred mechanically damaged to undamaged euonymus (van Tol et al., 2002). Although digestive secretions from insect mouthparts can stimulate emission of additional VOCs (Halitschke, Kessler, Kahl, Lorenz, & Baldwin, 2000; Heil, 2008; Röse & Tumlinson, 2005; Turlings et al., 1993, 1998), as can sustaining mechanical damage for a longer period (Bricchi et al., 2010), this has not been tested yet with *C. basicorne*. Plants reacting to such phytophage-specific stimuli can make the plant less attractive and less suitable for development of some stenophagous insects (Bruinsma, van Dam, van Loon, & Dicke, 2007; Karban & Baldwin, 1997; Viswanathan, Narwani, & Thaler, 2005), which should also be a concern to practitioners of classical biological control of weeds.

Relevance to assessing host plant specificity

Behavioural host specificity experiments are conducted to evaluate the potential risk of a prospective biological control agent damaging non-target plants. The experiments are usually conducted under artificial conditions, but the inference is expected to pertain to the environment in which the insects will be released (Briese, 2005; van Klinken, 2000). If an agent is incapable of surviving on a non-target plant species, then there is usually little risk that it would substantially damage such a species in the wild. However, agents that can develop on a non-target plant under laboratory conditions are more difficult to assess, because their behaviour in the wild may significantly reduce risk to the non-target plant. For many stenophagous species, selection of the host plant by ovipositing females may be the most critical stage for limiting the host range (Bernays & Chapman, 1994; Schoonhoven et al., 2006). Understanding how stenophagous insects find and accept a host plant is critical to improving our ability to design experiments and interpret their results for risk assessment. VOCs likely play a critical role in the host plant specificity, so understanding how the experimental conditions affect emission of VOCs and the behavioural responses of the insects to them, will help advance our science. A few olfactometer experiments have been performed on prospective biological control agents of weeds (Park et al., 2012); however, more studies should be done to explore the importance of volatile versus gustatory stimuli.

Although there is a general awareness that cut foliage may have different characteristics than uncut foliage, especially regarding suitability for development of immature insects (Palmer, 1999), there have been few studies that compare the relative attractiveness of cut versus uncut foliage (Loreto, Barta, Brilli, & Nogues, 2006). Palmer argued that responses induced by mechanical damage would require days to appear (Karban & Baldwin, 1997); however, more recent studies, including this one, indicate that mechanical damage causes immediate changes, and that herbivore-induced changes can appear in as soon as 2–4 hours (Turlings et al., 1998). Although immediately released compounds are often considered to be generic 'green leaf' volatiles (Baldwin, 1994; Dudareva, Negre, Nagegowda, & Orlova, 2006; Turlings et al., 1998) we observed a wide range of compounds that were released immediately (within 1 hr). Pearse et al. (2013) found that such mechanical damage was critical for using VOCs to distinguish among oak (*Quercus*) species. The fact that some *C. cineraria* and some *C. cyanus* cut leaves had VOC profiles that resembled those of damaged (punctured or scratched) leaves while others had distinct 'cut' leaf profiles suggests that the attractiveness of cut leaves could be more variable than previously assumed.

Besides mechanical damage, other environmental factors that may affect VOC profiles include light intensity, soil fertility and drought stress (Kigathi et al., 2009; Loreto et al., 2006; Peñuelas & Llusià, 2001; Wang, Owen, Li, & Peñuelas, 2007). Furthermore, the mixing of VOCs from different plants in the same arena may mask the presence of a host plant even when repellency of another plant is not evident (Jermy, Szentesi, & Horváth, 1988). We have previously been alerted to the possible influences of all these factors on the results of laboratory host specificity tests (Keller, 1999; Marohasy, 1998; van Klinken, 2000); however, analysis of headspace volatiles may be an effective way to assess some of them quantitatively.

Acknowledgements

The authors would like to thank Glory Merrill, Wai Gee and M. Irene Wibawa (USDA-ARS) for their technical assistance and Ikju Park (University of Idaho) and Gregory S. Wheeler (USDA-ARS) for comments on a draft of this paper. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

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Appendix

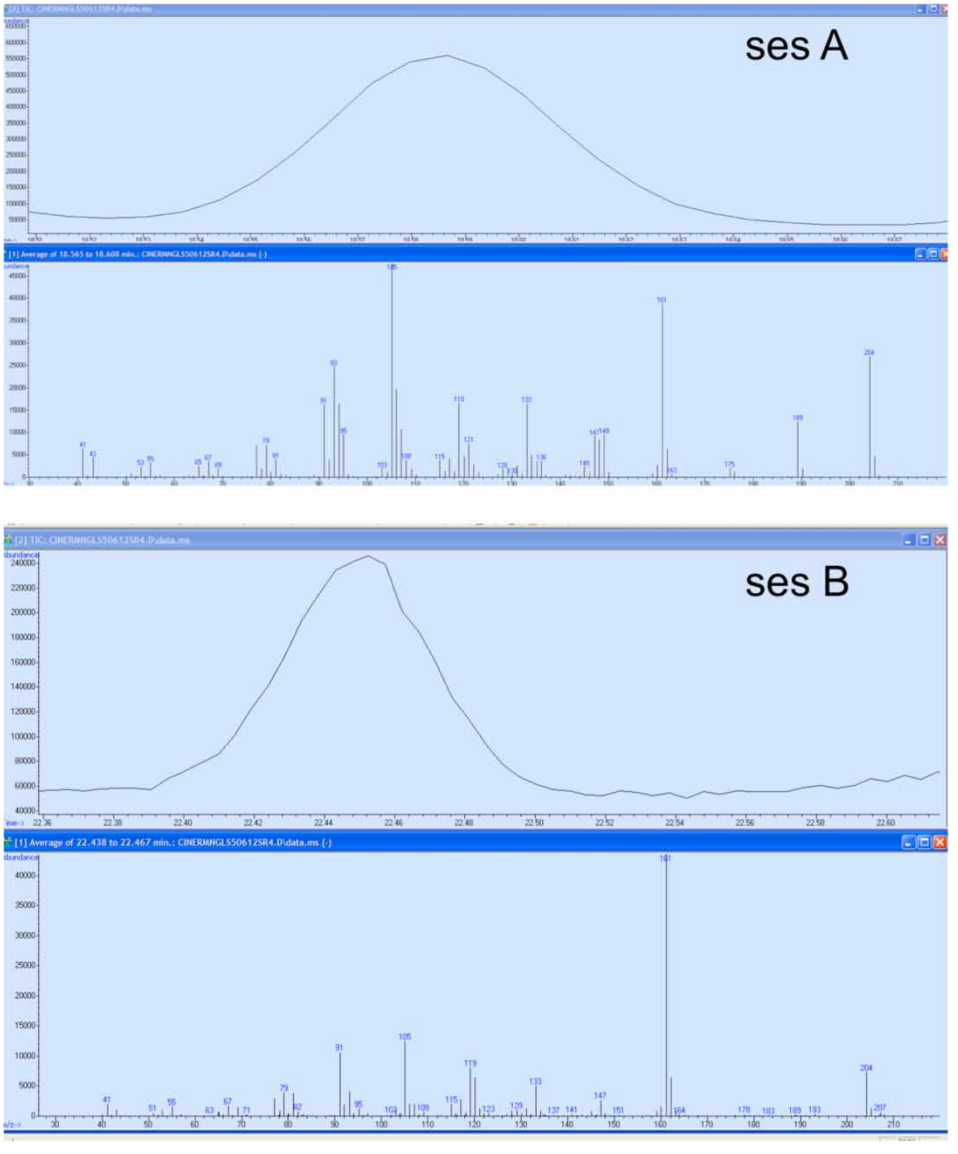

Figure A1. GC peaks and MS fragmentation patterns for unknown sesquiterpenes A and B.

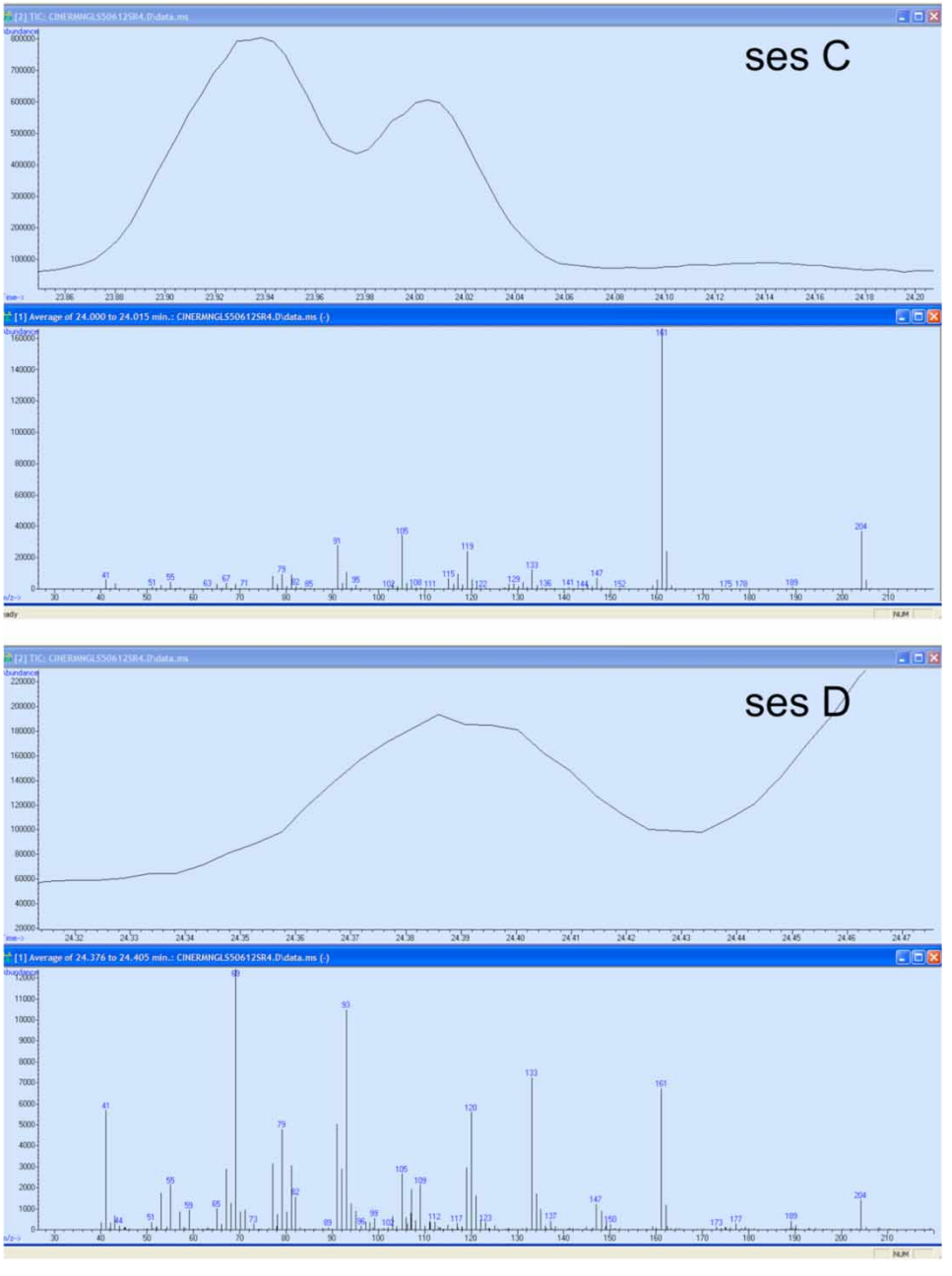

Figure A2. GC peaks and MS fragmentation patterns for unknown sesquiterpenes C and D.

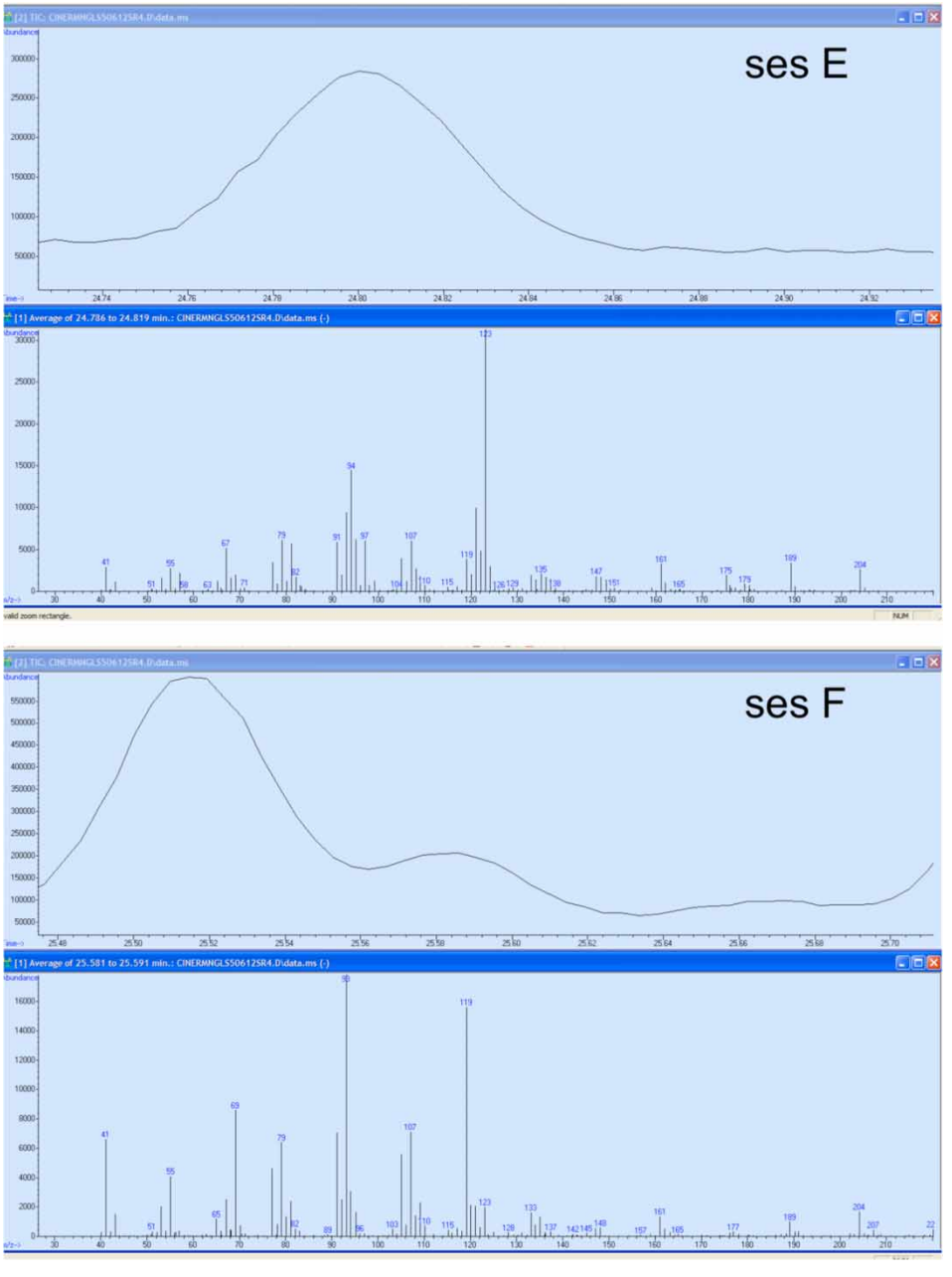

Figure A3. GC peaks and MS fragmentation patterns for unknown sesquiterpenes E and F.

Table A1. Correlation coefficients of response variables. Compounds are labelled by RI (DB-wax). An "x" indicates that the variable was selected to include in the corresponding discriminant analysis.

	1316	1387	1455	1467	1478	1481	1488	1527	1535	1543	1582	1587	1593	1613	1665	1668	1681	1686	1695	1705	1722	1724	1730	1746	1755	1855
Undamaged plants	x	x					x						x							x						x
"All damaged" plants	x	x		x	x		x	x	x	x	x	x	x	x	x							x			x	x
Cut and punctured plants	x	x		x	x			x	x	x	x	x	x	x	x							x			x	x
1316	1.00	-0.08	-0.08	-0.20	0.42	0.42	0.16	-0.38	-0.34	-0.53	0.39	-0.05	0.33	-0.81	-0.34	-0.46	-0.45	-0.18	0.39	-0.15	0.03	0.03	-0.21	-0.17	-0.21	0.20
1387	-0.08	1.00	0.31	0.20	-0.33	-0.32	0.22	0.20	0.27	0.24	0.07	-0.01	0.61	0.16	0.73	0.21	0.21	0.27	0.07	0.44	-0.10	0.01	0.69	0.20	0.16	0.20
1455	-0.08	0.31	1.00	0.90	0.43	0.43	0.83	0.81	0.20	0.51	0.56	0.04	0.66	0.37	0.74	0.71	0.71	0.75	0.56	0.89	0.91	0.60	0.63	0.90	0.67	-0.47
1467	-0.20	0.20	0.90	1.00	0.52	0.53	0.80	0.92	0.30	0.65	0.64	-0.02	0.52	0.41	0.70	0.83	0.84	0.66	0.64	0.84	0.84	0.55	0.59	1.00	0.62	-0.62
1478	0.42	-0.33	0.43	0.52	1.00	1.00	0.70	0.45	0.01	0.15	0.91	0.05	0.36	-0.43	0.02	0.38	0.41	0.45	0.91	0.40	0.64	0.43	0.08	0.55	0.37	-0.44
1481	0.42	-0.32	0.43	0.53	1.00	1.00	0.70	0.46	0.01	0.15	0.91	0.04	0.37	-0.43	0.03	0.38	0.41	0.46	0.91	0.41	0.64	0.43	0.08	0.55	0.37	-0.44
1488	0.16	0.22	0.83	0.80	0.70	0.70	1.00	0.72	0.21	0.42	0.79	0.11	0.83	0.01	0.66	0.64	0.65	0.67	0.79	0.90	0.83	0.68	0.68	0.81	0.72	-0.49
1527	-0.38	0.20	0.81	0.92	0.45	0.46	0.72	1.00	0.39	0.74	0.58	0.04	0.40	0.53	0.73	0.91	0.93	0.73	0.58	0.84	0.73	0.54	0.63	0.92	0.65	-0.69
1535	-0.34	0.27	0.20	0.30	0.01	0.01	0.21	0.39	1.00	0.13	0.13	0.22	0.20	0.30	0.44	0.30	0.31	0.33	0.13	0.37	0.07	0.45	0.41	0.30	0.18	-0.24
1543	-0.53	0.24	0.51	0.65	0.15	0.15	0.42	0.74	0.13	1.00	0.30	0.06	0.22	0.53	0.60	0.87	0.86	0.58	0.30	0.61	0.38	0.00	0.62	0.64	0.61	-0.57
1582	0.39	0.07	0.56	0.64	0.91	0.91	0.79	0.58	0.13	0.30	1.00	0.05	0.59	-0.38	0.32	0.52	0.55	0.59	1.00	0.58	0.59	0.40	0.36	0.67	0.43	-0.38
1587	-0.05	-0.01	0.04	-0.02	0.05	0.04	0.11	0.04	0.22	0.06	0.05	1.00	0.11	-0.02	0.06	0.05	0.06	0.36	0.04	0.13	0.04	0.18	0.31	-0.03	0.26	-0.14
1593	0.33	0.61	0.66	0.52	0.36	0.37	0.83	0.40	0.20	0.22	0.59	0.11	1.00	-0.13	0.70	0.33	0.33	0.51	0.59	0.78	0.51	0.49	0.77	0.53	0.57	-0.17
1613	-0.81	0.16	0.37	0.41	-0.43	-0.43	0.01	0.53	0.30	0.53	-0.38	-0.02	-0.13	1.00	0.50	0.49	0.49	0.20	-0.38	0.36	0.24	0.21	0.34	0.38	0.30	-0.38
1665	-0.34	0.73	0.74	0.70	0.02	0.03	0.66	0.73	0.44	0.60	0.32	0.06	0.70	0.50	1.00	0.72	0.72	0.60	0.32	0.87	0.46	0.49	0.91	0.70	0.59	-0.31
1668	-0.46	0.21	0.71	0.83	0.38	0.38	0.64	0.91	0.30	0.87	0.52	0.05	0.33	0.49	0.72	1.00	1.00	0.76	0.52	0.78	0.61	0.34	0.65	0.83	0.66	-0.55
1681	-0.45	0.21	0.71	0.84	0.41	0.41	0.65	0.93	0.31	0.86	0.55	0.06	0.33	0.49	0.72	1.00	1.00	0.77	0.55	0.79	0.62	0.36	0.65	0.84	0.66	-0.58
1686	-0.18	0.27	0.75	0.66	0.45	0.46	0.67	0.73	0.33	0.58	0.59	0.36	0.51	0.20	0.60	0.76	0.77	1.00	0.59	0.76	0.66	0.39	0.62	0.67	0.64	-0.37
1695	0.39	0.07	0.56	0.64	0.91	0.91	0.79	0.58	0.13	0.30	1.00	0.04	0.59	-0.38	0.32	0.52	0.55	0.59	1.00	0.58	0.60	0.40	0.36	0.67	0.43	-0.38
1705	-0.15	0.44	0.89	0.84	0.40	0.41	0.90	0.84	0.37	0.61	0.58	0.13	0.78	0.36	0.87	0.78	0.79	0.76	0.58	1.00	0.76	0.63	0.85	0.84	0.81	-0.48
1722	0.03	-0.10	0.91	0.84	0.64	0.64	0.83	0.73	0.07	0.38	0.59	0.04	0.51	0.24	0.46	0.61	0.62	0.66	0.60	0.76	1.00	0.66	0.38	0.84	0.65	-0.55
1724	0.03	0.01	0.60	0.55	0.43	0.43	0.68	0.54	0.45	0.00	0.40	0.18	0.49	0.21	0.49	0.34	0.36	0.39	0.40	0.63	0.66	1.00	0.43	0.55	0.47	-0.44
1730	-0.21	0.69	0.63	0.59	0.08	0.08	0.68	0.63	0.41	0.62	0.36	0.31	0.77	0.34	0.91	0.65	0.65	0.62	0.36	0.85	0.38	0.43	1.00	0.59	0.73	-0.31
1746	-0.17	0.20	0.90	1.00	0.55	0.55	0.81	0.92	0.30	0.64	0.67	-0.03	0.53	0.38	0.70	0.83	0.84	0.67	0.67	0.84	0.84	0.55	0.59	1.00	0.62	-0.62
1755	-0.21	0.16	0.67	0.62	0.37	0.37	0.72	0.65	0.18	0.61	0.43	0.26	0.57	0.30	0.59	0.66	0.66	0.64	0.43	0.81	0.65	0.47	0.73	0.62	1.00	-0.34
1855	0.20	0.20	-0.47	-0.62	-0.44	-0.44	-0.49	-0.69	-0.24	-0.57	-0.38	-0.14	-0.17	-0.38	-0.31	-0.55	-0.58	-0.37	-0.38	-0.48	-0.55	-0.44	-0.31	-0.62	-0.34	1.00

Yellow: $|0.60| \leq x < |0.80|$.

Pink: $x \geq |0.80|$.

Table A2. Discriminant analysis of types of leaf damage (undamaged [=check1 and check2] vs. cut vs. damaged [=punctured and scratched treatments]) for *C. cineraria*.

					Standardised coefficients	
VOC RI	Partial lambda[a]	$F_{(2,\ 6)}$	*P*	Tolerance	Root 1	Root 2
1724	0.0083	357.46	6×10^{-7}	0.8122	−1.1037	0.1146
1387	0.4818	3.23	0.1119	0.8122	0.3751	−1.0443
Eigenvalue					390.44	0.84
Cumulative proportion					0.998	1.000

[a]The lower the partial lambda, the greater the contribution to discrimination.
Wilks' lambda = 0.0014, $F_{(4,\ 12)} = 77.5$, $P < 0.00001$.

Table A3. Discriminant analysis of types of leaf damage (undamaged [=check1 and check2] vs. cut vs. damaged [=punctured and scratched treatments]) for *C. cyanus*.

					Standardised coefficients	
VOC RI	Partial lambda[a]	$F_{(2,\ 4)}$	*P*	Tolerance	Root 1	Root 2
1705	0.0081	245.12	6×10^{-5}	0.1048	−3.0720	−0.2972
1730	0.2154	7.28	0.0464	0.0121	7.7161	−3.0777
1587	0.2680	5.46	0.0718	0.0163	−5.9144	4.1888
1724	0.5104	1.92	0.2605	0.2950	1.2390	−0.4757
Eigenvalue					385.12	1.32
Cumulative proportion					0.997	1.000

[a]The lower the partial lambda, the greater the contribution to discrimination.
Wilks' lambda = 0.0022, $F_{(8,\ 8)} = 28.9$, $P < 0.00001$.

Table A4. Discriminant analysis of types of leaf damage (undamaged [=check1 and check2] vs. cut vs. damaged [=punctured and scratched treatments]) for *C. solstitialis*.

					Standardised coefficients	
VOC RI	Partial lambda[a]	$F_{(2,\ 4)}$	*P*	Tolerance	Root 1	Root 2
1455	0.00307	649.4	9×10^{-6}	0.0153	−8.0720	0.0855
1665	0.00007	27,167.6	5×10^{-9}	0.0078	11.2785	1.1479
1746	0.02548	76.5	0.0006	0.0291	−5.7869	−0.2152
1613	0.08672	21.1	0.0075	0.0816	−3.3250	−0.3700
Eigenvalue					46,276.51	446.18
Cumulative proportion					0.990	1.000

[a]The lower the partial lambda, the greater the contribution to discrimination.
Wilks' lambda = 0.000001, $F_{(8,\ 8)} = 4548.1$, $P < 0.00001$.