

## In Situ Volatile Collection, Analysis, and Comparison of Three *Centaurea* Species and Their Relationship to Biocontrol with Herbivorous Insects

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*Centaurea solstitialis*, commonly known as yellow starthistle, is an invasive plant listed as a noxious weed in the western areas of North America and is the target of classical biological control, which involves release of herbivores known to be specific to this plant. These insects often choose their host plant on the basis of the volatile organic compounds (VOCs) emitted. Accordingly, volatile analysis of host plants can provide insight into VOCs that may attract and/or repel the insect. To this end, solid-phase microextraction (SPME) and a customized collection bag were utilized to perform in situ volatile collection on intact and mechanically damaged leaves of *Centaurea solstitialis*, *Centaurea cyanus*, and *Centaurea cineraria*. Volatile identification was performed by GC-MS, and the VOC differences were determined. The plants *C. solstitialis* and *C. cyanus* have been reported to attract the weevil, *Ceratapion basicorne*, a candidate for biological control, whereas *C. cineraria* does not attract the weevil. Major VOCs unique to *C. cineraria* include the sesquiterpenes cyclosativene,  $\alpha$ -ylangene, and *trans*- $\alpha$ -bergamotene. The compound *trans*- $\beta$ -farnesene was unique to *C. solstitialis* and *C. cyanus*.

**KEYWORDS:** Biological control; *Centaurea*; *Ceratapion*; in situ volatile collection; plant damage; SPME

### INTRODUCTION

*Centaurea solstitialis* L. (Asteraceae), commonly known as yellow starthistle (**Figure 1**), is an invasive plant accidentally introduced into the United States over 130 years ago (1). *C. solstitialis* is listed as a noxious weed in several western regions of North America and is threatening to propagate toward the eastern United States (2–4). Infestation of *C. solstitialis* has been reported to be approximately 8 million hectares in the western U.S. and Canada and is considered to be the most widespread in California (2, 4). One of the primary concerns regarding the insidious growth of *C. solstitialis* is toxicity to horses (5, 6). Ingestion of *C. solstitialis* by horses induces the neurodegenerative disease nigropallidal encephalomalacia, reportedly due to the sesquiterpene lactone repin, a natural product of *C. solstitialis* and *Acroptilon repens* (L.) DC. (identical to *C. repens*) (6, 7). The invasion of *C. solstitialis* into rangelands also reduces forage, groundwater, and biodiversity (8). The benefit of successfully controlling this weed in California alone is estimated to be about \$1.4 billion (9).

The invasiveness of *C. solstitialis* and the consequential damage to both livestock and the environment have made control

of this weed a priority. Various control tactics have included use of herbicides, grazing, mowing, prescribed burning, and biological control (10). These management techniques have been expensive, relatively ineffective, environmentally dangerous, and/or impractical; therefore, experts have called for new developments (2, 10). Classical biological control involves introduction of alien host-specific herbivores that attack only the target weed. Although it has heretofore proven to be unsuccessful, biological control promises to provide permanent, self-sustaining control of *C. solstitialis* with minimal environmental impact (11, 12).

Recent papers have provided details regarding investigation into the controlled release of the weevil *Ceratapion basicorne* Illiger (Coleoptera: Apionidae) as an herbivore of *C. solstitialis* (13–15). *Ce. basicorne* has a distribution throughout Europe and southwestern Asia and infests *C. solstitialis* and *C. cyanus* L. (Asteraceae) (16, 17). In a no-choice ovipositional study performed on numerous host plants, *C. solstitialis* and *C. cyanus* attracted the adult *Ce. basicorne*, whereas *C. cineraria* L. (Asteraceae) did not (13). These data provided precedent to investigate these three plants for differences of volatile output, which may offer insight into the volatile organic compounds (VOCs) responsible for either attractancy and/or repellency influence. A search of the literature did not provide any reports of semiochemicals related to *Ce. basicorne*.

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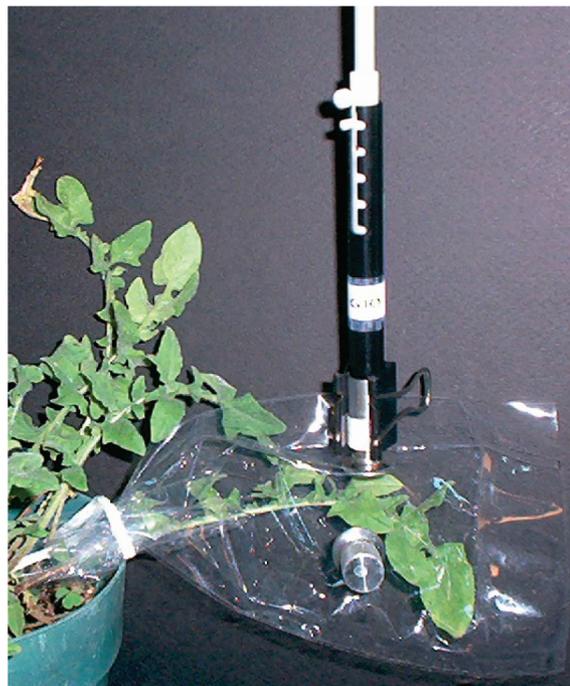
**Figure 1.** Detailed picture of *Centaurea solstitialis* showing the inch-long spines, and a field overrun with *C. solstitialis*.

The volatile compositions of *C. solstitialis* (18, 19) and *C. cineraria* (20) have previously been investigated, and the volatiles from *C. solstitialis* were compared to other plants in efforts to determine chemical cues for biocontrol purposes (21, 22). These investigations, however, did not perform direct comparison of the *Centaurea* species with reported semiochemical behavior of a biocontrol candidate, such as *Ce. basicorne*. Additionally, these previous studies evaluated flower buds, stems, and/or leaves of mature plants, yet *Ce. basicorne* attacks plants in the rosette stage, which lacks stems or flowers and may therefore exhibit different types and/or quantities of VOCs. Herein we report on the in situ volatile collection, analysis, and comparison of VOCs from rosette leaves of these three *Centaurea* species and discuss their relationship to the prospective biological control agent *Ce. basicorne*. The leaves of each plant were measured with and without three types of damage to determine the effect on VOCs. This is the first known report of the volatile composition of *C. cyanus*.

## MATERIALS AND METHODS

**Plant Material.** The plants *C. solstitialis* and *C. cyanus* were chosen because they are known to be attacked by *Ce. basicorne*, whereas *C. cineraria* is the least preferred plant in this genus. All *Centaurea* species were grown from seeds in a greenhouse located at the USDA-ARS in Albany, CA. Plant leaves were analyzed for volatiles at approximately 3–5 months of age, prior to bolting. The plants' age/growth stage was in concurrence with adult *Ce. basicorne* ovipositioning on rosettes in early spring, that is, plant age of 3–5 months (15, 23). Plant identifications were made by Dr. G. F. Hrusa, California Department of Food and Agriculture Herbarium, and voucher specimens were deposited at the USDA-ARS, WRRRC Herbarium (accession no. S-277, S-382, and S-452).

**Volatile Collection.** Leaves from each plant were enclosed in a customized Teflon bag (manufactured by SKC West, Inc., Fullerton, CA; 11 × 21 cm, thermally sealed on three sides, with two aluminum portals for solid-phase microextraction (SPME) analyses and instruments to injure the enclosed leaves), and VOCs were collected by SPME (Supelco, Bellefonte, PA; 100  $\mu$ m, polydimethylsiloxane fiber) analysis. The bag was gently sealed onto the stem/leaves of the plant by the use of a twist tie, and a SPME was inserted and attached to each portal by means of a large clip (**Figure 2**). Individual VOC analyses were kept consistent by standardizing the following parameters (method acronym PEST): *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 fiber is exposed to the permeated volatiles; *S* = storage time = length of time the volatiles are stored on the fiber prior to injection onto the GC; and *T* = thermal desorption = amount of time the fiber and SPME are kept on the GC injector port. For all VOC analyses, the method parameters were *P* = 1 min, *E* = 60 min, *S* = 30 s, and *T* = 15 min. It should be noted that concurrent studies in these laboratories have demonstrated that this method is applicable to longer storage times, some in excess of 3 h. The use of an internal standard allows the normalization of detected peaks; however, one drawback of extended storage time is the loss of detection of trace peaks due to the equilibrium of volatiles on the fiber. Background analyses were performed on an unexposed SPME fiber and ambient



**Figure 2.** Volatile analysis of *C. solstitialis* using collection bag and SPME.

bag/air volatiles. The internal standard 1-decanol (3.7  $\mu$ g in 0.1 mL of water) was injected into each sealed bag, and the resulting relative abundances for each run were normalized to the amount of 1-decanol (3.7  $\mu$ g).

A typical analysis involved leaf/leaves enclosed in bag as described above, the internal standard injected, the bag sealed, and the permeation time initiated. For the control experiment (ctrl, **Table 1**) the leaf/leaves were left intact while the fiber was exposed to the ambient volatiles. Although several leaves may have been enclosed, only one leaf underwent damage. For the punctured leaf analysis, the bag was sealed, the internal standard injected, and the leaf punctured several times with a sterile 22 gauge needle inserted through one of the injection ports of the collection bag. The permeation time was initiated, after which time the fibers were exposed to the damaged leaf volatiles. For the cut-leaf experiment, the same procedure was performed for the punctured leaf, but the leaf was cut at the petiole and allowed to drop into and remain in the bag while the fiber was exposed to the damaged leaf volatiles. Finally, for the mangled leaf experiment, a small spatula was gently inserted into the bag's opening prior to closure with a twist tie and used to scratch the surface of the leaf. The spatula was then removed, the bag gently sealed, the internal standard injected, and permeation time initiated. After the allotted permeation time, the SPME fibers were exposed to the damaged leaf volatiles.

**Volatile Analyses.** After the determined exposure time of the fiber, all experiments utilized transfer of adsorbed volatiles onto either a J&W Scientific (Folsom, CA) DB-Wax column (60 m × 0.32 mm i.d. × 0.25  $\mu$ m) or a J&W Scientific DB-1 column (60 m × 0.32 mm i.d. × 0.25  $\mu$ m) installed on one of two HP-6890 gas chromatographs (GC) coupled to HP-5973 mass selective detectors (MS; Palo Alto, CA).

Table 1. Comparison of Volatile Emission Profiles of *C. cineraria*, *C. solstitialis*, and *C. cyanus* for Intact Leaves (Control) and Various Types of Mechanical Damage<sup>a</sup>

compd	library/ID	compd information <sup>b</sup>						<i>C. cineraria</i>			<i>C. solstitialis</i>			<i>C. cyanus</i>		
		source <sup>c</sup>	RI <sup>d</sup>	RI lab <sup>d</sup>	RI <sup>e</sup>	RI lab <sup>e</sup>	ctrl	ptd	cut	mngl	ctrl	ptd	cut	ctrl	ptd	cut
1	$\alpha$ -pinene	Ald	1019	931	929	+	+	+	+	+	+	+	+	+	+	+
2	$\beta$ -myrcene	Iso	1161	983	982											
3	<i>trans</i> -2-hexenal	Ald	1216	825	822	+										+
4	<i>cis</i> - $\beta$ -ocimene	Iso	1233	1028	1026											+
5	<i>trans</i> - $\beta$ -ocimene	Iso	1249	1040	1037											+
6	hexyl acetate	Ald	1271	994	995	+	+	+	+	+	+	+	+	+	+	+
7	<i>cis</i> -3-hexenyl acetate	Bed	1316	987	986											+
8	hexanol	Ald	1357	851	848											+
9	<i>cis</i> -3-hexenol	Ald	1387	837	834											+
10	<i>trans</i> -2-hexenol	Ald, Bed	1410	848	845											+
11	$\delta$ -elemene	Iso	1467	1335	1334	+	+	+	+	+	+	+	+	+	+	+
12	cyclosativene	Ald	1478	1368	1367											+
13	$\alpha$ -ylangene	Iso	1483	1481	1370	+	+	+	+	+	+	+	+	+	+	+
14	unk sesquiterpene		1483	1365												+
15	$\alpha$ -copaene	iso	1488	1490	1374	+	+	+	+	+	+	+	+	+	+	+
16	$\alpha$ -gurjunene	Tent	1527	1407	1408											+
17	$\beta$ -cubebene	Tent	1535	1385	1385											+
18	unk sesquiterpene		1571	1424												+
19	<i>trans</i> - $\alpha$ -bergamotene	Iso	1582	1432	1432											+
20	$\beta$ -caryophyllene	Iso	1593	1415	1415											+
21	aromadendrene	Ald	1603	1605	1436	+	+	+	+	+	+	+	+	+	+	+
22	unk sesquiterpene		1613	1477												+
23	unk sesquiterpene		1635	1456												+
24	<i>trans</i> - $\beta$ -farnesene	Bed, Iso	1665	1447	1447											+
25	$\alpha$ -humulene	Iso	1665	1448	1449											+
26	$\gamma$ -amorphene/ $\gamma$ -muurolene	Iso/Tent	1686	1468	1469											+
27	germacrene D	Iso	1705	1474	1474	+	+	+	+	+	+	+	+	+	+	+
28	unk sesquiterpene		1717	1483												+
29	$\alpha$ -muurolene	Tent	1722	1491	1492											+
30	unk sesquiterpene		1724	1500												+
31	bicyclogermacrene <sup>f</sup>	Iso	1730	1732	1496											+
32	unk sesquiterpene		1741	1481												+
33	<i>E,E</i> - $\alpha$ -farnesene	Iso	1746	1494	1496											+
34	$\delta$ -cadinene	Iso	1755	1512	1514											+
35	$\gamma$ -cadinene	Iso	1757	1505	1505											+
I.S.	1-decanol	Ald	1767	1255	1255	+	+	+	+	+	+	+	+	+	+	+
36	geranylacetone	Ald	1853	1428	1429											+

<sup>a</sup> Volatile amounts are normalized to the internal standard, 1-decanol (3.7  $\mu$ g, relative value of 37) and are the average of four replications. + = 1–37; ++ = 38–100; +++ = 101–1000; ++++ = >1000. ctrl = control before injury; ptd = punctured leaf; cut = leaf cut at petiole; mngl = mangled leaf. <sup>b</sup> Compound identification by retention index relative to *n*-alkanes on DB-1 and DB-Wax columns, retention times, mass fragment libraries, and comparison to authentic samples. <sup>c</sup> Source of compound for authentication: Ald = Aldrich or Fluka; Bed = Bedoukian; Iso = previously isolated by a researcher in this laboratory (Buttery, Flath, or Binder); Tent = tentative. <sup>d</sup> Calculated retention index, and PMR laboratory retention index library of authentic samples on a DB-1 Column. <sup>e</sup> Calculated retention index, and PMR laboratory retention index library of authentic samples on a DB-1 Column. <sup>f</sup> Pentadecene coeluted on DB-1 but did not reproduce in all runs on DB-Wax.

Desorbed volatiles were analyzed with the following methods: DB-Wax, injector temperature of 200 °C, splitless mode, inlet temperature of 200 °C, constant flow of 3.0 mL/min, oven settings of initial temperature of 40 °C, hold time of 0.0 min, ramp 1 at 4 °C/min, final temperature of 200 °C, hold time of 40 min; DB-1, injector temperature of 200 °C, splitless mode, inlet temperature of 200 °C, constant flow of 2.0 mL/min, oven settings of initial temperature of 40 °C, hold time of 0.0 min, ramp 1 at 4 °C/min, final temperature of 250 °C, hold time of 30 min. MSD parameters were as follows: source temperature, 230 °C; MS source temperature, 150 °C; EI mode, 70 eV; solvent delay, 1 min; scan group 1, 40–300 amu; scan group 2 at 20 min, 40–450 amu. NIST, Wiley, and internally generated 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 to retention times of an internally generated list of volatiles on identical columns. Each experiment was performed in duplicate on each GC column for a total of four replicates.

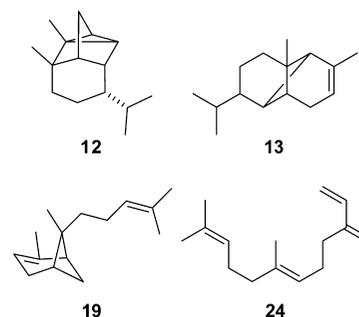
Data from GC-MS analyses were transferred to Microsoft Excel for comparison of retention times and compound identification for same-column analysis. Calculated retention indices were used to assist in compound identification and to perform comparison of DB-1 to DB-Wax column results. Compounds consistent through all four replicates are included in **Table 1**. Relative abundances (peak areas) were normalized to the internal standard for each run, averaged, and applied to the values listed in **Table 1**.

## RESULTS AND DISCUSSION

Analysis of the results revealed a single ambient volatile in the control experiment (undamaged leaves, ctrl, **Table 1**) for *C. cineraria* and *C. cyanus*, *cis*-3-hexenyl acetate, **7**. Ambient volatiles for undamaged *C. solstitialis* included *cis*-3-hexenyl acetate, **7**, as well as *cis*-3-hexenol, **9** (**24**), both common leaf volatiles, in addition to the sesquiterpenes  $\beta$ -caryophyllene, **20**, germacrene D, **27**, and bicyclogermacrene, **31**. Interestingly, the control volatiles present in *C. solstitialis* were also observed from stems and leaves of mature plants by Binder et al. (**19**), with the exception of *cis*-3-hexenyl acetate, **7**. Their study showed small amounts of *cis*-3-hexenyl butyrate, which in our study was not reproducible through all four analyses of *C. solstitialis*, as well as 25 other VOCs that we did not detect from undamaged leaves. Volatiles detected in punctured, cut, and mangled leaves and reported in **Table 1** were in general agreement with the results from previous *C. solstitialis* investigations by Buttery et al. (**18**) and Binder et al. (**19**); however, there were some differences of volatile composition and relative amounts. These discrepancies were thought to be a result of the different methods used for analyses, as well as different plant parts being evaluated.

VOCs in relatively large amounts in damaged leaves of *C. solstitialis* include the sesquiterpenes noted above in addition to *trans*- $\beta$ -farnesene, **24**, which concurred with the Binder study. The major VOCs noted in *C. solstitialis* were in relatively large amounts in all three damaged *Centaurea* species, with the exception of bicyclogermacrene, **31**, which was present in the damaged *C. cyanus*, but in only negligible amounts. The sesquiterpenes  $\beta$ -caryophyllene, **20**, germacrene D, **27**, and bicyclogermacrene, **31**, are common plant volatiles (**25**, **26**) that have been noted to possess semiochemical activities. Of these three volatiles,  $\beta$ -caryophyllene, **20**, offers a wide range of activity (**27**) and therefore is most likely not attractive to a specialized herbivore such as *Ce. basicorne*.

It was interesting to note the vast increase in the number, and relative amounts, of volatiles emitted from damaged leaves of all three species. The compounds *trans*-2-hexenal, **3**, hexanol, **8**, and *cis*-3-hexenol, **9**, are known to be plant “wound volatiles”



**Figure 3.** Sesquiterpenes cyclosativene **12**,  $\alpha$ -ylangene **13**, *trans*- $\alpha$ -bergamotene **19**, and *trans*- $\beta$ -farnesene **24**.

(**28**). The leaf-puncturing treatment is the closest mimic of typical insect adult feeding. For the most part, the VOC profile from this treatment resembled that of cut leaves, and both of these were in lower amounts than that of the mangled leaves. It is recommended that future studies involving this method to compare VOCs from different plants that may influence insect behavior should utilize the punctured damage as it appeared to produce similar volatile profiles, relative to the cut and mangled treatments, and most closely mimics damage of adult insect feeding.

The two plant species from this study that were reported to attract the adult weevil, *C. solstitialis* and *C. cyanus* (**13**), were comparatively similar in terms of volatile output, although *C. solstitialis* often produced larger amounts of VOCs and produced several other volatiles not produced by *C. cyanus*. The sesquiterpenes  $\alpha$ -gurjunene, **16**,  $\alpha$ -humulene, **25**, and *trans*, *trans*- $\alpha$ -farnesene, **33**, were the most obvious identifiable VOC differences between the two plants. Additionally, *C. solstitialis* emitted several unidentified sesquiterpenes along with trace amounts of other sesquiterpenes in the mangled leaf treatments. Notably, there were larger amounts of *trans*- $\beta$ -farnesene, **24**, germacrene D, **27**, and bicyclogermacrene, **31**, in *C. solstitialis* relative to *C. cyanus*. The compound geranylacetone, **36**, was the only volatile unique to *C. cyanus*, but it also produced more *cis*-3-hexenylacetate, **7** than did *C. solstitialis*.

The only volatile unique to *C. solstitialis* and *C. cyanus*, and thus should be suspect as an attractant to *Ce. basicorne*, was *trans*- $\beta$ -farnesene, **24** (**Figure 3**). A literature search for references to the family Apionidae and farnesene as a semiochemical was unsuccessful; however, the Pherobase (**27**) lists numerous species that utilize *trans*- $\beta$ -farnesene, **24**, as a semiochemical. For example, beetle predators of aphids have been reported to use *trans*- $\beta$ -farnesene, **24**, as a kairomone (**29**). The broad range of semiochemical behavior of *trans*- $\beta$ -farnesene, **24**, in addition to the observation that it is unique to the two plants that promote the adult weevil to oviposit, warrant further investigation into *trans*- $\beta$ -farnesene, **24**, and its role as an attractant for *Ce. basicorne*. Subsequent research into this aspect should include other volatiles in conjunction with *trans*- $\beta$ -farnesene, **24**, for example, the above-noted leaf volatiles and/or additional sesquiterpenes,  $\beta$ -caryophyllene, **20**, or germacrene D, **27**, given that specific combinations of volatiles have been reported to synergize their effectiveness as semiochemicals (**29**, **30**).

The question remains, however, if volatiles of *C. cineraria* possess repellency characteristics or if it lacks attractant compounds. When mechanically damaged, *C. cineraria* had more VOCs, and often in higher concentrations, than did *C. solstitialis* or *C. cyanus*, which suggests that some of these may be repellent to *Ce. basicorne*. The VOCs unique to *C. cineraria* for punctured leaves included cyclosativene, **12**, in relatively

large amount, *trans*- $\alpha$ -bergamotene, **19**, in moderate amount, and in small amounts  $\delta$ -elemene, **11**,  $\alpha$ -ylangene, **13**, aromadendrene, **21**, and the unidentified sesquiterpenes **14**, **22**, **23**, **28**, **30**, and **32** (Table 1; Figure 3).

Due to the relative lack of information with regard to *Ce. basicorne* and associated semiochemicals, the role of cyclosativene, **12**,  $\alpha$ -ylangene, **13**, and *trans*- $\alpha$ -bergamotene, **19**, is speculative; however, inferences from these data can be used for future chemotaxonomic studies with this and other potential biological control agents. To demonstrate this assertion, a cursory search of the literature using the names of the three sesquiterpenes in question allowed for comparison of plants for prospective investigations. For instance, the plant *Achillea millefolium* L. (Asteraceae) (31) was reported to possess all three of these sesquiterpenes, albeit in different relative amounts than what was observed in our study. Several other occurrences of similar volatile patterns were evident in the family Asteraceae, as well Myrtaceae (32); however, no other plants were found to contain all three of the exact reported isomers of the three sesquiterpenes or in similar relative amounts. Individually, all three of the sesquiterpenes are common plant volatiles (27), but possess few known semiochemical properties (33, 34).

The relatively large amount of  $\alpha$ -humulene, **25**, in *C. cineraria* compared to the trace amount in injured *C. solstitialis* and its absence in *C. cyanus* warrants mention. In a recent study of kairomones associated with *C. nigra* L. (34), the sesquiterpene  $\alpha$ -humulene, **25**, was reported to be a major component and was accompanied by various levels of several other sesquiterpenes that were detected from the three investigated plants. Although *C. nigra* has not been studied for its ability to attract or repel *Ce. basicorne*, in laboratory experiments *Ce. basicorne* fed and oviposited on *C. × moncktonii* C. E. Britton (hybrid of *C. nigra* and *C. jacea* L.) (13), which may share many VOCs with *C. nigra*. Despite being present in *C. solstitialis*, albeit in trace amounts,  $\alpha$ -humulene, **25**, warrants further investigation.

Finally, if the premise of previous insect damage is used as a basis for an increase in volatile emission from plants, comparison of VOC output for punctured damage data in Table 1 showed that two other known sesquiterpenes are unique to *C. cineraria*,  $\delta$ -elemene, **11**, and aromadendrene, **21**, albeit in trace amounts. Neither of these compounds has documented semiochemical behavior. In addition to these volatiles, the punctured leaf data provided six unknown sesquiterpenes in trace amounts unique to *C. cineraria* and corroborated *trans*- $\beta$ -farnesene as the only volatile unique to both *C. solstitialis* and *C. cyanus*.

To our knowledge, this is the first report of VOCs from *C. cyanus*. Other secondary metabolites have been reported from various parts of *C. cyanus*; however, only oxygenated sesquiterpenes resemble any of the reported VOC components in this study (35). The VOC emission of *C. cyanus* is relatively minimal compared to the other two plants studied, with the major components being the leaf volatile *cis*-3-hexenylacetate, **7**, in control and damaged experiments, and the sesquiterpenes  $\beta$ -caryophyllene, **20**, and germacrene D, **27**, in relatively large amounts in the damaged experiments.

Control and damaged leaf volatiles of three *Centaurea* species were analyzed in situ via static headspace analysis using a customized collection bag and SPME. Two of the plants, *C. solstitialis* and *C. cyanus*, were chosen for their demonstrated attractiveness to female adult weevil *Ce. basicorne*, whereas the third plant, *C. cineraria*, is not attractive. The major VOCs unique to *C. cineraria* include the sesquiterpenes cyclosativene, **12**, and  $\alpha$ -ylangene, **13**, six unknown sesquiterpenes, *trans*- $\alpha$ -

bergamotene, **19**, and  $\alpha$ -humulene, **25**. The compound *trans*- $\beta$ -farnesene, **24**, was unique to *C. solstitialis* and *C. cyanus*. The clear volatile distinctions between the plants suggest that VOCs could explain host plant specificity of *Ce. basicorne*. Further experiments are required to determine which of these VOCs provide semiochemical cues to *Ce. basicorne*. Once their roles as semiochemicals are elucidated, this information can be used to effectively screen the VOC emission profile of other nontarget plants for their susceptibility toward *Ce. basicorne*. This approach will contribute a new dimension to the evaluation of host plant specificity of prospective biological control agents. The results from this study also contribute to chemotaxonomic analyses of *Centaurea* species, the phylogeny of which is still not resolved (36).

#### ACKNOWLEDGMENT

We thank M. I. Wibawa for her contributions.

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Received for review November 19, 2007. Revised manuscript received February 4, 2008. Accepted February 9, 2008. This research was conducted under USDA-ARS CRIS Projects 5325-42000-036-00 and 5325-22000-020-00.

JF073383U