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THE CHALLENGE OF ATTRACTING POLLINATORS WHILE EVADING FLORAL HERBIVORES: PATTERNS OF FRAGRANCE EMISSION IN *CIRSIUM ARVENSE* AND *CIRSIUM REPANDUM* (ASTERACEAE)

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By increasing floral apparency to promote fertilization, plants risk attracting herbivores with the same signals that they use to lure pollinators. We hypothesized that fragrance is emitted in patterns that correspond to pollinators, with high emissions during periods of pollinator activity and low emissions otherwise, especially during periods of peak floral herbivore activity. Using a combination of analytical chemistry and field observations, we examined both the diel and ontogenetic patterns of fragrance in two *Cirsium* species, in relation to visitation patterns of pollinators and florivores. Emission rates were highest at reproductive maturity, when insect visitation by both pollinators and florivores was also highest. In *Cirsium arvense*, the diel pattern of fragrance emission matched patterns of pollinator activity and was lowest when florivores were active. In contrast, scent in *Cirsium repandum* peaked at midday rather than with insect activity; neither species had a diel pattern that followed ambient temperature. Fragrance emission from *C. repandum* was 25 times lower than from staminate *C. arvense* and may not be essential for pollinator attraction, at least from a distance. The scent dynamics we observed in *C. arvense* are consistent with the hypothesis that fragrance emissions correspond with pollinator activity and are low when florivores are active.

Keywords: florivory, plant volatiles, pollination, herbivory, Canada thistle, sandhill thistle.

Introduction

Understanding the simultaneous selection pressures from mutualists and antagonists and how they affect phenotypic traits remains a challenge to evolutionary ecologists. In plant-insect interactions, costs associated with attracting pollinators via inadvertent attraction of herbivores mean that increasing apparency may produce diminishing fitness returns (Charnov 1979; Charlesworth and Charlesworth 1987; Ashman 2002). Herbivorous insects navigate within the same olfactory landscape as do pollinators; if they are attracted to the same components of the fragrance blend, then the evolution of floral scent may be constrained by opposing selection pressures (Theis and Lerdau 2003). To date, relatively few studies have addressed the potential for floral herbivores to shape the volatile communication signals between plants and their pollinators (Galen 1983; Baldwin et al. 1997).

The study of floral traits has typically emphasized visual characteristics. For example, Schaefer et al. (2004) recently proposed that plant-pollinator communication should be considered within the larger conceptual framework of signal evolution, but by focusing primarily on visual signals, they conclude that floral signals "are relatively constant in space and time without . . . the option for modifying them in the presence of predators" (p. 577). However, like all olfactory stimuli, fragrance is a dynamic signal, inconstant in both space and time. Flowering plants emit fragrances in circadian rhythms (Kolosova et al. 2001; Pott et al. 2002) and either modify or cease odor production from minutes to hours after pollination has occurred (Euler and Baldwin 1996; Negre et al. 2003; Theis and Raguso 2005; Muhlemann et al. 2006). Clearly, the potential exists for volatile signals to be modified to reduce the attraction of herbivores.

We explore the hypothesis that the dynamic temporal patterns of floral scent emission have the potential to be influenced by balancing selection mediated by pollinator attraction and floral herbivore avoidance. Historically, research has focused on pollinator attraction (Dobson 1994; Raguso 2001, 2004) in spite of the fact that floral scent is known to attract both beneficial and detrimental insects (Evans and Allen-Williams 1992; Gabel et al. 1992; Theis 2006). There was a tendency to overlook florivores until Louda and Potvin's (1995) seminal article, in which florivores were shown to decrease not only seed production but also seedling recruitment. Since then, several studies have demonstrated the role of florivores in selection on floral characters such as flowering phenology, morphological features, and floral scent (Galen 1983; Baldwin et al. 1997; Brody 1997; Ehrlén et al. 2002; Mahoro 2002).

We selected *Cirsium* (Asteraceae) plants for this study because their flower heads attract several orders of generalist insect pollinators and flower-feeding insects (Zwölfer 1988; Proctor et al. 1996). We expect that the volatile signature of

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such broadly attractive plants is likely to be under weak selection pressure from any one pollinator species (Waser et al. 1996; Knudsen et al. 1999), revealing the selective effect of other species, such as florivores. We chose to compare congeners to test whether closely related species that differ ecologically would demonstrate similar volatile patterns with regard to separate suites of pollinators and florivores and allow us to begin to discern the generality of our results. *Cirsium repandum* (sandhill thistle), a hermaphrodite, is native to North America and common to the coastal plain of North and South Carolina (Radford et al. 1968); the other species, *Cirsium arvense* (Canada thistle), is native to Europe, Asia, and North Africa. Since its introduction into North America in the seventeenth century, it has become a common invasive plant in the United States and Canada (Moore 1975). *Cirsium arvense* is dioecious, which allows us to examine differences in scent composition or emission rates and florivore behavior that may result from sex-specific floral display, pollen, or seed production. Although fragrance composition of both *C. arvense* and *C. repandum* has been analyzed in a manipulative pollination study, differences between the sexes of *C. arvense* have never been investigated (Theis and Raguso 2005). Furthermore, trapping studies have revealed which components of the *C. arvense* fragrance blend are attractive to pollinators and florivores (Theis 2006), which will allow a more informed interpretation of the patterns documented here.

If pollinators and florivores show different patterns of activity, we would expect emissions to peak with pollinator activity and diminish with florivore activity, particularly at critical stages of development. We tested this hypothesis at two temporal scales: a flower head's lifetime (ontogenetic) and a single day (diel).

Do floral scent emissions track the ontogeny of a flower head, peaking at reproductive receptivity, when pollinators are most active? Herbivory occurs on *Cirsium* flower heads before, during, and after flowering (Moore 1975; N. Theis, unpublished data). Pollination, on the other hand, is limited to times when the florets are reproductively receptive. Therefore, floral herbivory could be reduced in two ways without affecting pollination: if attractive volatiles are minimized before flowering and after reproduction and/or if the chemical composition changes through ontogeny and compounds that are repellent to pollinators and florivores are emitted before buds open and/or after the reproductive stage.

Do fragrance patterns and insect visitation differ between staminate and pistillate flower heads of C. arvense*?* Staminate flowers in sexually dimorphic species are often larger and receive a greater number of pollinator visits (reviewed in Ågren et al. 1999). This makes sense according to Bateman's principle that selection on male sexual function drives the evolution of a showy display (Bateman 1948; Charnov 1979; Bell 1985). Dispersing pollen requires competition for pollinators, whereas seed development is more often constrained by resources than by insect visitation. Thus, staminate flowers tend to be more attractive to pollinators, and presumably because of their greater apparency, they tend to incur more herbivory (Ågren et al. 1999). Differences between the sexes may extend to different patterns of fragrance emission through development. In a previous manipulative study, pistillate flower heads dramatically declined in fragrance emission following fertilization (Theis and Raguso 2005). Is there also a decline in the staminate sex at this stage? The ontogenetic stages following fertilization are critical in pistillate flower heads with developing seeds, compared to the analogous stage in staminate flower heads, after pollen has been dispersed.

Do floral scent emissions track the diel pattern of pollinators and decline when florivores are most active? Daily peaks in insect activity, often related to temperature and metabolism (Stone and Willmer 1989; Herrera 1990), may differ for pollinators (active fliers) and florivores (more likely to be sedentary). If they differ, floral scent in receptive flower heads should track the diel pattern of pollinator activity. Additionally, if florivores are detrimental, there may be a decline in emissions when they are most active. Alternatively, if fragrance emission is simply a function of temperature, emission patterns should track daily temperature fluctuations.

Material and Methods

Fragrance was collected from two species of thistle *Cirsium* (Asteraceae). *Cirsium arvense* (L.) Scopoli grows clonally and blooms from July through September (Moore 1975). The hermaphroditic *Cirsium repandum* Michaux has protandrous flower heads that bloom from May through July.

The ranges of these two *Cirsium* species do not overlap. We studied *C. arvense* at the U.S. Fish and Wildlife Service Wallkill River National Wildlife Refuge, located in Sussex County, New Jersey (lat. 41°26′N, long. 74°54′W). The site has a mixed community of native and exotic invasive plants in open fields. We studied *C. repandum* at the Belle W. Baruch Institute of Coastal Ecology and Forest Science at the Hobcaw Barony located in Georgetown, South Carolina (lat. 33°35′N, long. 79°18′W). The field site is a shady understory within a mixed loblolly pine and oak forest, which is burned regularly by the management of the research preserve.

Fragrance Collection

Volatiles were collected using dynamic headspace sampling in the field. Intact flower heads were enclosed within a nylon resin oven bag (Reynolds Consumer Products, Richmond, VA). Glass cartridges packed with 100 mg of the adsorbent polymer Porapak Q (80–100 mesh) were inserted into the bag and attached to a vacuum pump at the other end. Ambient air was then pulled into the bag across the flower head and over the adsorbent material in the cartridge at a flow rate of ca. 200 mL/min, using either an Air Check 52 or Air Check 2000 diaphragm pump (SKC, Eighty Four, PA). After each collection, the flower head was cut, dried at 60°C, and weighed. Cartridges were eluted with 3 mL of hexane, and an internal standard of 3 μL of 0.01% anisole in hexane was added. Anisole was chosen as our internal standard because it is chemically similar to many fragrance compounds in *Cirsium* but was not found in either focal species. Samples were then concentrated to 75 μL with N_2. The concentration step takes 5 min, and during this step little or no sample is lost (N. Theis, unpublished data). Samples were kept at 4°C until analyzed.

Fragrance Analysis

Fragrance analysis was performed by combined capillary gas chromatography–mass spectrometry, with a Shimadzu GC-17A

equipped with a Shimadzu QP5000 quadrupole electron impact mass spectrometer as a detector (Shimadzu, Columbia, MD). A 1-μL aliquot was injected splitless onto a polar column (EC WAX [30 m $\times$ 0.25 mm], Alltech, Deerfield, IL) at an initial temperature of 60°C for 3 min, which then increased 10°C per min to 260°C, where it was held for 7 min (Theis and Raguso 2005). Compounds were identified by matching the retention time to previously injected standards and by mass spectra (NIST and Wiley mass spectral libraries, with more than 120,000 mass spectra). Quantification was achieved by dividing the mass ion of each scent compound by the mass ion of the internal standard and multiplying by both the mass of internal standard added and a coefficient correcting for the response of the GC-MS to the specific scent compound.

Sampling Design

To track floral ontogeny, we marked staminate and pistillate buds of *C. arvense* and hermaphroditic buds of *C. repandum* and followed them through development. We identified five developmental stages: closed bud (bud), open bud (open bud), ca. 50% emerged florets (young), all florets emerged (mature), and the final stage (past), identified by the darkening of the flower head but before the flower head begins to brown (app. A). In *C. repandum* young flower heads are entirely androecious, whereas mature flower heads may be partially androecious and partially or entirely gynoecious. Samples ($n > 9$, where the unit of replication is flower head rather than plant or clone) were collected from 1100 to 1500 hours from all five stages for both species (and both staminate and pistillate *C. arvense*). During the same time of day, ambient air samples ($n = 5$ for each species) were collected throughout the season and the closest ambient sample by date was subtracted from each fragrance sample.

To determine diel pattern of fragrance, scent was collected in 2- and 4-h intervals throughout the day from 0700 to 1900 hours and followed by a nocturnal sample of 8 h from 2130 to 0530 hours, all from the same flower head at the mature stage. Sampling of *C. repandum* took place on June 8, 2002 ($n = 5$). Sampling of *C. arvense* occurred on July 11 and 18, 2002, with a total of eight pistillate and eight staminate flower heads sampled at 2-h intervals (0700–1500 hours) and subsets of four flower heads from each sex sampled at 4- and 8-h intervals (1500–1900 and 2130–0530 hours) during the remainder of the day. Ambient air samples were collected during the same periods and were subtracted from fragrance samples. Hourly and daily temperature data were obtained from the National Weather Service for Sussex, New Jersey (http://www.wunderground.com) and from the Baruch Marine Field Laboratory, located at the Belle W. Baruch Institute for Marine and Coastal Sciences in Georgetown, South Carolina.

The Diel and Ontogenetic Pattern in Visitation by Insects

Insects were censused in 2002 throughout the flowering season, across floral ontogenetic stages. Every 2 h from 0700 to 1900 hours, flower heads were randomly chosen along a linear transect and examined for number and identity of insects present. For *C. arvense*, 30 flower heads from staminate and pistillate plants from each floral ontogenetic stage (young, mature, and past) were observed twice a week from July 2 to 31, 2002, for eight census days at each of the six time periods, resulting in a total of 8640 flower head observations. In *C. repandum*, 20 flower heads were chosen twice a week from each ontogenetic stage, and they were observed at each of the six time periods on a total of seven census days from June 2 to 21, 2002, with 2520 total observations. These intervals covered peak flowering season for each species. If inclement weather prevented a census period, the missing time period was censused on a subsequent day after weather had improved.

Statistical Analysis

Data were analyzed using SYSTAT 10.0 (Systat, Richmond, CA). For all scent data, we report both relative abundance and total amount for each compound (flux rate in ng g [dry floral tissue]$^{-1}$ h^{-1}). We present both kinds of data because neuroethological studies show that honeybees distinguish differences in both fragrance composition and concentration (Joerges et al. 1997; Wright et al. 2002). Neither relative amounts nor flux rates nor insect census data could be transformed to fit the assumptions of ANOVA. Therefore nonparametric statistical comparisons were performed. The Kruskal-Wallis test and Mann-Whitney *U*-test for paired comparisons were used to test for differences in the relative and total amounts of each compound across developmental stages. All post hoc comparisons were tested using Bonferroni-adjusted *P* values (Sokal and Rohlf 1995). Four post hoc tests were used to contrast ontogenetic stages of *C. arvense* (within each sex) and *C. repandum*, and five post hoc tests were used to contrast the sexes at each ontogenetic stage with Bonferroni adjusted α values of 0.013 and 0.01, respectively. The Kruskal-Wallis test was used to determine significant differences in the diel pattern of floral scent. Insect distributions, calculated by blocking by day (using the daily abundance to calculate proportions), were analyzed using Mann-Whitney *U*-tests for differences between staminate and pistillate flower heads. The Kruskal-Wallis test was used to test for differences in insect preference by stage with three post hoc tests and an adjusted α value of 0.017. No post hoc tests were done for diel pattern. Correlations between the diel pattern of insects and fragrance were explored using Spearman's rank correlation. In one case, scent was sampled at a larger interval than insect observations, so insect data were averaged over that time period.

Results

Fragrance Emission from Cirsium arvense

Thirteen volatile compounds were detected in the floral headspace of *Cirsium arvense*, including monoterpenoids and aromatic compounds. Emission rates for those compounds varied from 1 to 20,000 ng g^{-1} h^{-1}. The aromatic compound phenylacetaldehyde comprised ca. 50% of the scent blend in both staminate and pistillate flower heads.

Ontogenetic pattern. In *C. arvense*, fragrance emission differed significantly throughout ontogeny (Kruskal-Wallis test; total fragrance flux: staminate $H = 41.72$, $P < 0.001$, pistillate $H = 42.69$, $P < 0.001$; fig. 1; app. B). For both staminate and pistillate plants, floral scent peaked during the reproductively

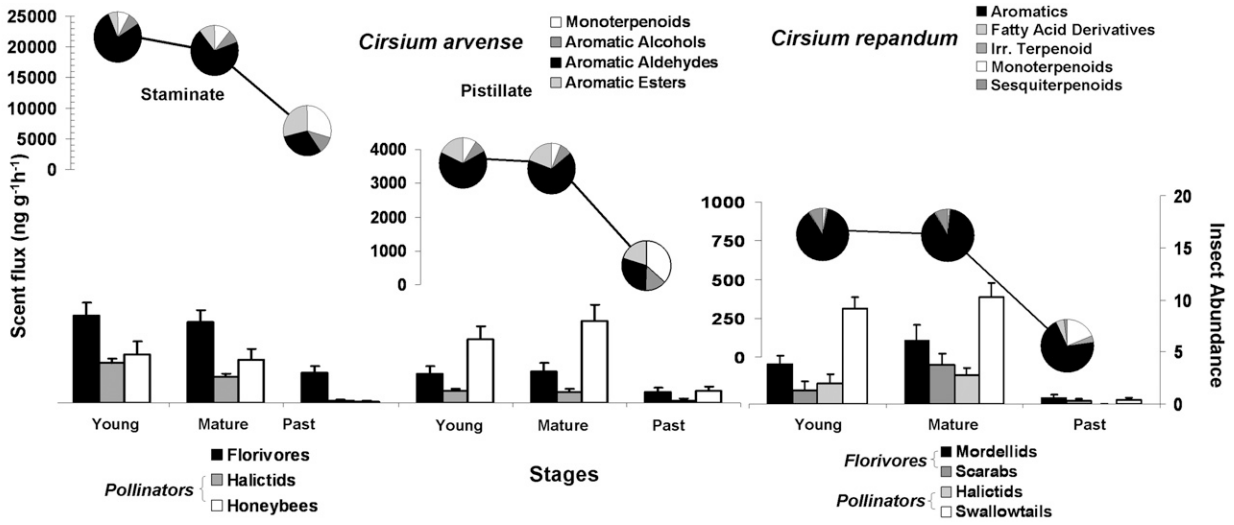

Fig. 1 Fragrance emission and pollinator and florivore abundance through the development of the flower head in *Cirsium arvense* and *Cirsium repandum*. The upper *Y*-axis represents the mean total scent emission rates in ng g^{-1} h^{-1} for each stage of flower head ontogeny in *C. arvense* pistillate and staminate flower heads. Note the disparity in the scale of the *Y*-axis. The 13 compounds emitted by *C. arvense* are from two classes, the monoterpenoids and the aromatics. The aromatics have been further divided for visual enhancement. The 42 compounds in *C. repandum* have been grouped into five different classes. Pie charts represent the mean relative abundance of each of the classes. The lower *Y*-axis is the average insect abundance (with error bars).

receptive stages (young and mature) and was lower during other ontogenetic stages (bud, open bud, past; app. B). Floral compounds were detected even during the bud stage. Few significant differences differentiated bud stages for staminate and pistillate plants in flux rate or relative abundances, but these measures differed significantly from stages with emerged florets (table 1). Furthermore, few significant differences differentiated reproductively receptive stages such as young and mature (compositionally or flux rate); however, the mature stage flower heads differed significantly from past flower heads in a number of compounds, including the aromatic aldehydes benzaldehyde, phenylacetaldehyde, and *p*-anisaldehyde among others (fig. 1; table 1).

Staminate versus pistillate pattern. Fragrance emission differed between the sexes of *C. arvense* once florets emerged, and differences increased with time. Overall, there were far more significant differences in flux rates than compositional differences (fig. 1). For example, in contrasting young staminate and pistillate flower heads, 10 compounds differed significantly in flux rate, whereas two aromatic esters (methyl salicylate and dimethyl salicylate) differed in relative abundance (fig. 1; table 1). The greatest disparity in composition occurred at the mature stage, when staminate and pistillate flower heads differed by five compounds, including three monoterpenoids. The greatest disparity in flux rate occurred at the past stage, when staminate flower heads had significantly higher flux rates than pistillate heads for all 13 compounds.

Diel patterns. In both sexes of *C. arvense*, scent emissions peaked by 0900–1100 hours, whereas the peak in temperature occurred later in the day (fig. 2; app. D). Variation was significantly associated with time for all 13 compounds in staminate and pistillate flower heads (Kruskal-Wallis test, $P < 0.01$). Some compounds continued to be emitted at high levels until 1300 hours in pistillate flower heads and 1500 hours in staminate flower heads; all compounds declined nocturnally.

Fragrance Emission from Cirsium repandum

We identified a total of 42 compounds, including aromatics, monoterpenoids, sequiterpenoids, an irregular terpenoid, and fatty acid derivatives from the floral headspace of *C. repandum* (app. C). As in *C. arvense*, aromatics dominated the scent blend of *C. repandum*. Emission levels varied from 1000–0.01 ng g^{-1} h^{-1}. Phenylnitroethane, the dominant compound, composed as much as 25% of total scent emission and, combined with two biosynthetically related compounds (phenylacetonitrile and 2-phenylethanol), accounted for 40% of total emissions.

Ontogenetic pattern. In *C. repandum*, scent production varied significantly through ontogeny and peaked during the reproductively receptive stages (total fragrance flux: Kruskal-Wallis, $H = 37.12$, $P < 0.001$; fig. 1; app. C). Fragrance variation was analyzed at the level of biosynthetic class because of the large number of compounds. As in *C. arvense*, the buds and open buds of *C. repandum* did not differ significantly in scent composition or in flux rate for any class of compounds in post hoc tests (table 2). Both flux rate and compositional differences distinguished open buds from young flower heads, with four out of five significant differences. Between young and mature flower heads, only the flux rate of monoterpenoids differed significantly. Past flower heads differed from mature flower heads in relative abundance for all classes of compounds and in flux rate for all classes of compounds except monoterpenoids and fatty acid derivatives (fig. 1).

Diel patterns. In *C. repandum*, there were significant differences in the diel pattern of emissions for all scent compounds analyzed by class (Kruskal-Wallis test, $P < 0.01$; fig. 2; app. E). Temperature peaked late in the afternoon, as did the emission of fatty acid derivatives (1700–1900 hours). All other compound classes peaked earlier. Monoterpenoids peaked earliest, from 0700 to 0900 hours. Sesquiterpenoids and the irregular

Table 1

Post Hoc Tests on Ontogenetic Variation in Floral Scent of *Cirsium arvense*

	Pistillate				Staminate				Pistillate vs. staminate				
Scent compounds	B vs. OB	OB vs. Y	Y vs. M	M vs. P	B vs. OB	OB vs. Y	Y vs. M	M vs. P	B	OB	Y	M	P
Flux:													
(*E*)-furanoid linalool oxide								1					1
(*Z*)-furanoid linalool oxide		1	1			2					1	2	2
Benzaldehyde		2		2		2		1			2	2	2
Linalool		1				2					1	2	2
Phenylacetaldehyde		2		1		2		2			1	2	2
(*E*)-pyranoid linalool oxide		1			1	2					2	2	2
(*Z*)-pyranoid linalool oxide		1	1			2					1	2	2
Methyl salicylate		2		2	1	2						2	2
Benzyl alcohol		1				2					2	2	2
2-phenylethanol		2				2					2	2	2
p-anisaldehyde		2		1		2					1		1
Dimethyl salicylate		2		2		2		2					2
Benzyl benzoate		2				2		1					2
Total		2		1	1	2		1			2	2	2
Relative abundance:													
(*E*)-furanoid linalool oxide													
(*Z*)-furanoid linalool oxide			2					2				2	
Benzaldehyde				2	1			1					
Linalool		1				2						2	2
Phenylacetaldehyde		2						2				1	
(*E*)-pyranoid linalool oxide								2					
(*Z*)-pyranoid linalool oxide				1				2				2	2
Methyl salicylate		1									1		
Benzyl alcohol													
2-phenylethanol													1
p-anisaldehyde		2		1		2							
Dimethyl salicylate		2				2					2	2	
Benzyl benzoate		1				2		2					

Note. Post hoc tests on the 13 compounds in the fragrance blend of *C. arvense* contrasted at different ontogenetic stages and between sexes. Significance of observed differences is shown for absolute flux (flux rate: ng g^{-1} h^{-1}) and relative abundance (proportional contribution to the blend) using Mann-Whitney *U*-tests with a Bonferonni-adjusted *P* value for four comparisons through ontogeny. The number 1 denotes $P < 0.013$, 2 denotes $P < 0.003$, empty cells denote ns. Ontogenetic stages include bud (B), open bud (OB), young (Y), mature (M), and past (P).

terpenoid peaked at 0900–1100 hours, while aromatics peaked at 1100–1300 hours; all three declined from 1300 to 1500 hours. There was a second peak at 1500–1700 hours. As in *C. arvense*, all compound classes declined nocturnally.

Insect Visitation to C. arvense

Pollinators and florivores were observed on *C. arvense*, including a number of pollen- and nectar-feeding insects as well as predispersal seed predators (table 3). This characterization is based on the natural history of these insects because we did not directly test the detrimental or beneficial effect of each insect species. Instead, florivores were defined by their lack of mobility and direct observations of feeding. The most abundant florivores during the census were Phalacridae (shining flower beetles) and nectar-stealing Formicidae (ants). The dominant pollinator was *Apis mellifera*, the European honeybee, accounting for 66% of all flower head visits. Of the remaining pollinators, 25% were a mixed assemblage of small bees, mostly *Lasioglossum* (Dialictus) sp., and other solitary bees such as *Ceratina* sp.

Ontogenetic pattern. There were significant preferences across ontogenetic stages on both staminate and pistillate flower heads by *A. mellifera*, halictids, and all common florivores combined (Kruskal-Wallis, $P < 0.05$; fig. 1). No insect group distinguished between young and mature flower heads in either pistillate or staminate plants. In post hoc tests, all three insect groups discriminated against past staminate flower heads, and pollinators discriminated against past pistillate flower heads (Mann Whitney *U*-test, $P < 0.01$). There was low visitation by florivores to pistillate flower heads during all stages of ontogeny.

Staminate versus pistillate pattern. For *C. arvense*, insects were more likely to be found on staminate flower heads than on pistillate ones. Three groups of florivores were observed significantly more frequently on staminate flower heads, including small Hemiptera (especially Anthocoridae) and Coleoptera such as Phalacridae and Mordellidae (Mann-Whitney *U*-test, $U = 175.5$, $U = 133.0$, $U = 204.0$, respectively; $P < 0.01$; table 3). The pollinator assemblage of Halictidae and other solitary bees (henceforth referred to as "halictids") also preferred staminate flower heads ($U = 152.5$, $P < 0.01$);

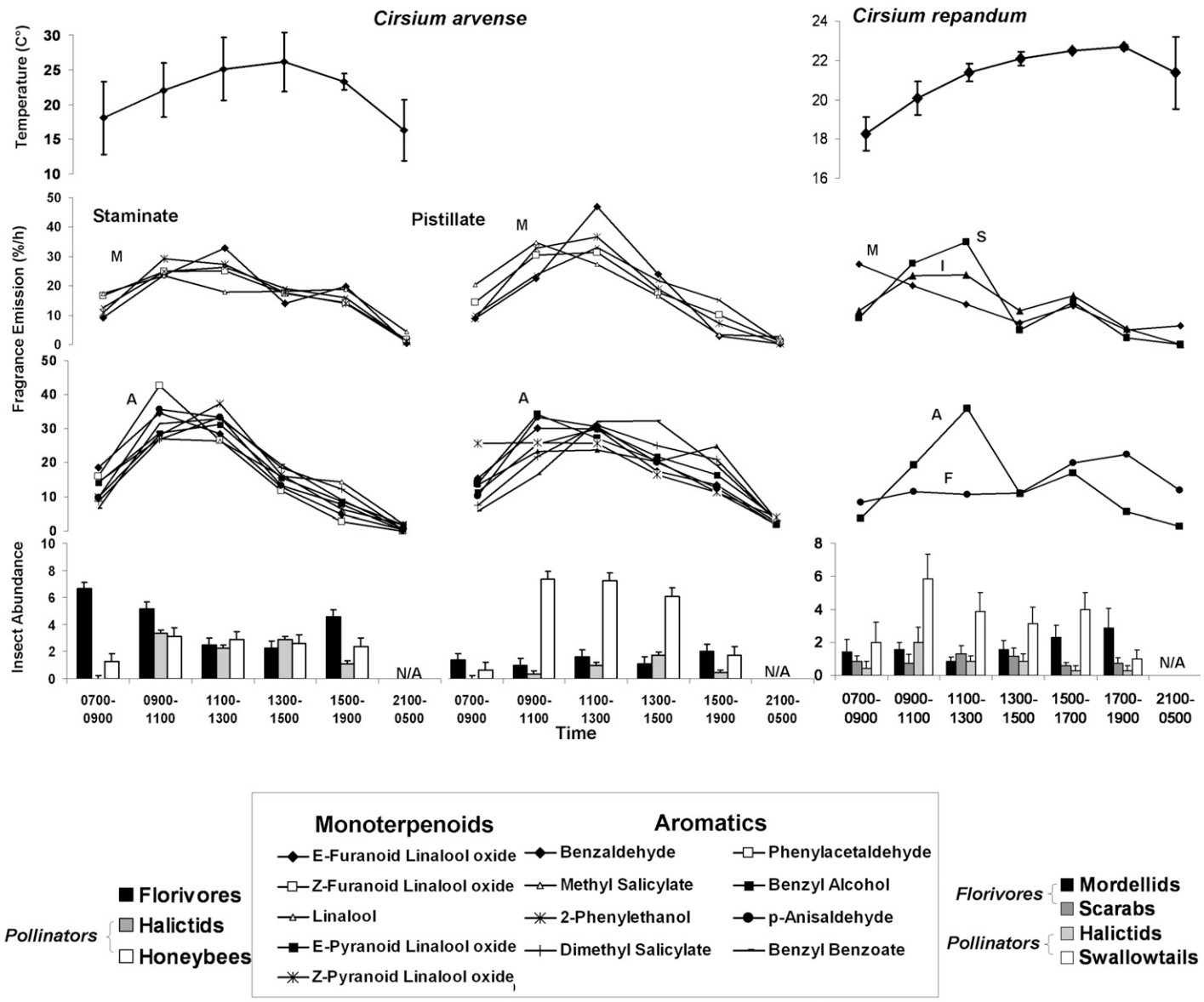

Fig. 2 Diel variation in scent and insect abundance contrasted with ambient temperature. Upper graph is temperature reported in average Celsius degrees with standard deviation. The middle two graphs are the scent of mature stage flower heads of *Cirsium arvense* and *Cirsium repandum* reported as an average percentage of fragrance emission per day, grouped by compound class: F = fatty acid derivatives, M = monoterpenoids, A = aromatics, I = irregular terpenoid, and S = sesquiterpenoids. For each sex in *C. arvense*, $n = 8$ for 0700–1500 hours and $n = 4$ for 1500–0500 hours; for *C. repandum*, $n = 5$. The lower bar graph represents average insect abundance (with error bars).

Table 2

Post Hoc Tests on Ontogenetic Variation in Floral Scent of *Cirsium repandum*

Scent compounds	B vs. OB	OB vs. Y	Y vs. M	M vs. P
Flux:				
Fatty acid derivatives				
Aromatics		2		2
Monoterpenoids			1	
Irregular terpene		2		1
Sesquiterpenoids		2		2
Total		2		2
Relative abundance:				
Fatty acid derivatives		2		2
Aromatics		2		2
Monoterpenoids		2		2
Irregular terpene				2
Sesquiterpenoids		1		2

Note. Post hoc tests on the five compound classes in the fragrance blend of *C. repandum*, contrasted at different ontogenetic stages. Significance of observed differences is shown for absolute flux (flux rate: ng g^{-1} h^{-1}) and relative abundance (proportional contribution to the blend) using Mann-Whitney *U*-tests with a Bonferonni-adjusted *P* value for four comparisons. The number 1 denotes $P < 0.013$, 2 denotes $P < 0.003$, empty cells denote ns. Abbreviations as in table 1.

A. mellifera was the only insect that preferred pistillate flower heads ($U = 428.5$, $P < 0.01$).

Diel patterns. Florivores and pollinators demonstrated distinct diel activity patterns on *C. arvense* (staminate/pistillate: florivores, $H = 11.15$, $P = 0.05/H = 4.1$, $P = 0.54$; honeybees, $H = 3.74$, $P = 0.6/H = 29.43$, $P < 0.001$; halictids $H = 16.48$, $P = 0.006/H = 15.94$, $P = 0.007$; fig. 2). Florivores were most abundant from 0700 to 0900 hours and late in the afternoon on staminate plants, with lower averages during midday. This activity period was not coincident with total fragrance emissions (staminate: $R = -0.3$, ns). In contrast, both halictids (staminate plants: $R = 0.9$, $P < 0.1$) and *A. mellifera* (pistillate plants: $R = 0.9$, $P < 0.1$) demonstrated a peak that began at 0900–1100 hours and declined in the afternoon.

Insect Visitation to C. repandum

Pollinators as well as a number of highly destructive florivores were observed on *C. repandum*, including predispersal seed predators and nectar, pollen, and floral tissue feeders. Nearly one-third of all florivores censused were flower beetles (Scarabaeidae: *Euphoria inda* [bumblebee flower beetle] and *Trichiotinus piger*) that fed on floral tissues, completely destroying the flower head. Nearly two-thirds of the florivores were pollen feeders in the family Mordellidae (tumbling flower beetle). The dominant pollinator of *C. repandum* was a swallowtail butterfly, *Papilio palamedes*. This species and other swallowtails, including *Papilio glaucus*, *Papilio troilus*, and *Battus philenor*, comprised 77% of all pollinator visits. The second most abundant group of pollinators was solitary bees, including halictids in the genus *Lasioglossum (Dialictus)* sp., which comprised 18% of all pollinator visits.

Ontogenetic patterns. At flower heads of *C. repandum*, distinct ontogenetic preferences were observed in pollinators and florivores (Kruskal-Wallis, $P < 0.01$; fig. 1). While both groups discriminated against past flower heads (post hoc Mann-Whitney *U*-tests, $P < 0.01$) like *C. arvense*, pollinators (swallowtail butterflies and halictids) did not discriminate between young and mature flower heads. In contrast, the florivores (scarabs and mordellid beetles) were more abundant on mature than young flower heads (Mann-Whitney *U*-test: scarabs, $U = 42.5$, $P = 0.018$; mordellids, $U = 43.5$, $P = 0.015$).

Diel patterns. Pollinator diel visitation patterns on *C. repandum* were similar to those for *C. arvense*, though not as pronounced (fig. 2). Only *Papilio* butterflies showed any significant pattern in diel activity (Kruskal-Wallis, $H = 12.49$, $P = 0.03$). *Papilio* butterfly and halictid bee activity peaked from 0900 to 1100 hours, and *Papilio* showed heightened activity again from 1500 to 1700 hours. Florivore abundance was constant throughout the day.

Discussion

Fragrance emissions in both *Cirsium repandum* and *Cirsium arvense* matched pollinator activity through ontogeny. While the diel pattern of fragrance emissions was coincident with the diel pattern of pollinators in *C. arvense*, it appeared to be independent of diel insect activity in native *C. repandum*. Fragrance emission from staminate flower heads of dioecious *C. arvense* was fivefold higher than emission from pistillate flower heads, which attracted more florivores but not more pollinators. Fragrance emissions from hermaphroditic *C. repandum* were 25-fold lower than staminate *C. arvense* and may not be a primary cue for insect navigation to these flower heads.

Do Floral Scent Emissions Peak When Pollinators Are Most Active?

In both *C. arvense* and *C. repandum*, the ontogenetic pattern of emissions is congruent with the hypothesis that emission patterns maximize plant apparency to pollinators. Floral scent emission peaked during the reproductive stages (young and mature), and pollinators on both species were most attracted to these flower heads (fig. 1). For *C. arvense*, florivores and pollinators alike were attracted to flower heads during the stages of highest scent production, whereas for *C. repandum*, florivores were more abundant on mature flower heads compared to young flower heads, in spite of similar fragrance emissions. This could result from florivores finding both young and mature flower heads but remaining on young flower heads as they develop to maturity. Alternatively, florivores on *C. repandum* may respond to the visual cues that distinguish these floral stages.

During nonreproductive stages, fragrance emissions were quite low, which is consistent with our hypothesis that plant apparency is reduced during bud and past stages to reduce florivore attraction. Pollination can directly reduce fragrance production in both pistillate *C. arvense* and *C. repandum*, and this may be the mechanism behind the decline demonstrated here (Theis and Raguso 2005). It is worth noting that we did not detect evidence of novel volatile compounds (potential repellents) emitted during these stages of ontogeny. Repellent properties of floral volatiles have been reported in studies of interactions between insects and nonhost plants (Gabel et al. 1992; Ômura et al. 2000) but are otherwise infrequently

Table 3

Florivores and Pollinators Observed on *Cirsium arvense*

Order and family	Species	No. in census	Staminate preferred (%)
Florivores:			
Hemiptera:			
Miridae	*Lygus lineolaris*; tarnished plant bug	47	80
Lygaeidae	*Lygaeus kalmii*; small milkweed bug	7	57
Thyreocoridae	*Corimelaena* sp.; negro bug	28	52
Anthocoridae	Pirate bugs and misc. small bugs	49	94**
Coleoptera:			
Mordellidae	*Mordellistena* sp., *Mordella* sp.; tumbling flower beetle	9	100**
Phalacridae	Shining flower beetle	104	72**
Scarabaeidae	*Popillia japonica*; Japanese beetle	4	
Cantharidae	*Chauliognathus marginatus Cantharis* sp.; soldier beetle	28	41
Curculionidae	Weevils	2[a]	
Meloidae	*Epicauta* sp.; blister beetle	6[b]	67
Orthoptera:			
Acrididae	Short-horned grasshopper	7	80
Lepidoptera:			
Geometridae		18	78
Hymenoptera:			
Formicidae		196	N/A[c]
Pollinators:			
Hymenoptera:			
Apidae	*Apis mellifera*; honeybee	317	37**
	Bombus sp.; bumblebee	3	67
	Little bees[d]	118	73**
Halictidae	*Halictus* sp.		
	Lasioglossum sp., *Dalictus* sp.		
	Augochlorella sp.		
Anthophoridae:	*Ceratina* sp.		
Diptera:			
Syrphidae	Hover fly	33	58
Lepidoptera:	Butterflies[e]	12	58
Hesperidae	Skipper		
Nymphalidae	*Vanessa atalanta*		
Pieridae	*Pieris rapae*		

Note. The abundance for each insect is reported as total count over the 8 d of the census. Staminate preferred is the percent of each insect group found on staminate plants. Significance was tested by Mann-Whitney *U*-test for paired comparisons.

[a] *Larinus planus* was not observed on the developmental stages included in the census.

[b] May include some predaceous species.

[c] Formicidae have been excluded from analysis because of the effect of site, rather than preference, potentially affecting distribution.

[d] "Little bees" were not distinguished during the census but are probably a mixed group of mostly Halictidae.

[e] Butterflies were distinguished during the census, but because of their low abundance, they were analyzed as a group.

** $P < 0.01$.

reported in the literature (Dobson 1994). More data are necessary to ascertain whether there are any repellent compounds in the blend of either species. Trapping experiments similar to those done to determine attractants could be performed. In this case, presumed repellent compounds would be added to known attractants and significantly reduced catch would demonstrate repellency.

In C. arvense*, Do Patterns of Fragrance Emission and Insect Visitation Differ between Staminate and Pistillate Flower Heads?*

At maturity, dioecious *C. arvense* is sexually dimorphic in patterns of floral scent production, with fivefold higher total fragrance emissions in staminate versus pistillate flower heads but fewer significant differences in scent composition (fig. 1; table 1). In accord with higher emission rates, staminate flower heads were visited more often by halictid bees and florivores; however, the dominant pollinators (honeybees) preferred pistillate flower heads in spite of their lower scent production (figs. 1, 2). The available data on fragrance in unisexual plants, while scarce, has shown similar results; staminate flowers generally are more fragrant. For example, in *Geonoma macrostachys* and *Cucurbita pepo*, staminate flowers have a stronger scent (Olesen and Balslev 1990; Granero et al. 2004). However, a larger floral display (both visual and olfactory) also attracts more florivores (Cunningham 1995; Ehrlén 1997; Fenner et al. 2002), as was found for *C. arvense*.

Predator-mediated stabilizing selection on the sexual signals of animals (e.g., songs, conspicuous coloration, and flamboyant appendages) has been well studied by evolutionary biologists (Endler 1987; Ryan 1990; Zuk et al. 1998), but only recently has this concept been applied to plant reproductive ecology (Brody 1997; Gomez and Zamora 2000; Raguso 2001; Ashman 2002; Collin et al. 2002; Ehrlén et al. 2002; Leege and Wolfe 2002). Less showy pistillate plants (compared to staminate plants) may reflect selection to avoid attracting detrimental herbivores rather than relaxed selection caused by decreased competition for pollinators. The hypothesis that an increase in pistillate flower head apparency would reduce fitness needs to be tested empirically.

Florivory could also explain the dramatic decline during the latter stage of development (past). Fragrance emission is particularly low from pistillate flower heads containing developing seeds. At the analogous stage in staminate flower heads, fragrance emissions are still relatively high, higher even than a mature stage pistillate flower head. Florivory on staminate flower heads should confer trivial fitness costs after pollen has been dispersed and the flower head has begun to senesce but major fitness costs on pistillate flower heads at the seed development stage. Fragrance emissions are generally considered to be a trivial component of a plant's energy budget (Euler and Baldwin 1996; Grison-Pige et al. 2001), so energy rescued and reallocated toward seed production is an unlikely explanation for the disparity. Moreover, it is possible that these volatiles would not be reallocated. It has been shown in two species of *Nicotiana* that pools of glycosidically bound fragrance compounds remain in floral tissue when a flower head senesces rather than being reallocated (Loughrin et al. 1992). Florivory could be an ultimate factor driving the disparity in fragrance; however, the proximate mechanism for the decline in pistillate flower heads is due to feedback from the fertilization status of the ovules, a cue missing from staminate flower heads (Theis and Raguso 2005).

Do the Diel Patterns in Floral Scent Emissions Correspond with Insect Activity?

The emissions of floral scent compounds in *C. arvense* were correlated with the diel pattern of pollinator activity and declined when florivores were active, whereas the diel pattern of fragrance emission in *C. repandum* was independent of insect activity (fig. 2). We expect that, in *Cirsium*, fragrance emissions have the potential to track insect activity rather than insect activity tracking fragrance emissions. Temporal patterns in insect activity generally are related to temperature thresholds and metabolic constraints (Stone and Willmer 1989; Herrera 1990), although resource availability can also explain some patterns in coadapted systems (Stone et al. 1999). Therefore, we assume that temperature thresholds, rather than resources, drive the patterns of insect activity on these species (i.e., the availability of *Cirsium* nectar and pollen does not drive insect activity). However, temperature does not seem to be driving the pattern of fragrance emission documented here. Fragrance compounds peaked earlier than temperature, suggesting that temperature does not drive volatile production/emission on a daily timescale.

Flowers of *C. arvense* emit compounds that attract both pollinators and florivores (benzaldehyde and phenylacetaldehyde; Theis 2006) and demonstrate patterns of scent emission that peak when pollinators are active and are lowest when florivores are active. Benzaldehyde was emitted at low levels in the morning and evening when florivores were active; similarly, phenylacetaldehyde, an ant attractant, was reduced late in the day, when ants were active. In contrast, emissions of linalool, a compound not attractive to florivores (significantly fewer florivores were attracted to linalool than to a control trap; Theis 2006), do not fluctuate like other pollinator attractants, remaining high late into the afternoon in staminate flower heads (fig. 2). A number of plant species have demonstrated, under controlled photoperiod and temperature, scent emission that continues to cycle freely in spite of all-dark or all-light conditions, with both nocturnal increases and diurnal ones (Matile and Altenburger 1988; Loughrin et al. 1990; Dudareva et al. 2000). Here we have shown that the natural pattern of fragrance emission in *C. arvense* matches the pattern of pollinator activity in spite of low morning temperatures.

Cirsium repandum demonstrated a different pattern, with a fragrance peak at noon, which did not coincide with either insect activity or temperature (fig. 2). Fragrance emissions in flower heads of *C. repandum* are 25-fold lower than in staminate *C. arvense*, and floral scent may not be an important cue for the swallowtail butterflies pollinating *C. repandum*. Visual cues alone are sufficient to evoke feeding from artificial flowers by *Battus philenor* (Weiss 1997) and *Papilio troilus* (Swihart 1970), two of the swallowtails that pollinate *C. repandum*. Alternative explanations for temporal variation in scent chemistry from *C. repandum* must await additional behavioral and/or phylogenetic studies.

Conclusions

The attraction of both pollinators and florivores to floral scent reveals the potential for both groups to serve as selective forces on fragrance emission (Theis 2006). Ontogenetic and diel patterns of fragrance in *C. arvense* are consistent with the predictions that fragrance is emitted in dynamic patterns that maximize pollinator attraction and minimize florivore attraction. However, it will be necessary to quantify the detrimental effect of florivores and determine the effect of fragrance enhancement on plant fitness to establish whether and how florivores shape patterns of fragrance emission in *C. arvense*. In contrast, there is little reason to suspect that selection for pollinator attraction is responsible for diel patterns in *C. repandum*. Fragrance emission from *C. repandum* is 25 times lower than emissions from staminate *C. arvense*, and temporal patterns in emission do not closely match pollinator or florivore activity patterns. Trapping experiments performed with scent components of *C. repandum*, similar to those accomplished for *C. arvense* (Theis 2006), would directly test whether fragrance is an important floral attractant in this system. Nevertheless, the correspondence between temporal patterns of floral volatile release in *C. arvense* and the activity schedules of its pollinators and florivores shown here demonstrates that along with beneficial insects, detrimental insects cannot be ignored when considering the sources of selective pressures on floral phenotypic evolution, including fragrance emission.

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Appendix A

Fig. A1 Ontogenetic stages of *Cirsium repandum*: bud, open bud, young, mature, past.

Appendix B

Scent Production through Ontogeny in *Cirsium arvense*

Table B1

Average Relative Amounts of Volatile Compounds (% ± SE) Emitted by Vegetative and Staminate Flower Heads of *Cirsium arvense*

Relative abundance	RT	Vegetative	Bud	Open bud	Young	Mature	Past
Monoterpenoids:							
(*E*)-furanoid linalool oxide	9.68	0	.6 ± 1.6	.8 ± 2.8	0	0	.2 ± 0
(*Z*)-furanoid linalool oxide	10.08	0	3.9 ± 5.9	7.2 ± 4.3	4.2 ± 1.0	5.4 ± .7	10.0 ± .8
Linalool	11.07	0	0	.2 ± .5	.4 ± .1	.4 ± .1	1.2 ± .2
(*E*)-pyranoid linalool oxide	13.45	0	3.0 ± 5.1	9.8 ± 10.0	2.8 ± .6	4.4 ± .8	17.1 ± 1.2
(*Z*)-pyranoid linalool oxide	13.71	0	.3 ± .7	.5 ± .7	.7 ± .2	.8 ± .2	1.0 ± .2
Aromatics:							
Benzaldehyde	10.83	20.5 ± 41.8	37.7 ± 33.2	22.7 ± 18.5	10.4 ± 1.9	12.9 ± 1.9	11.7 ± 2.6
Phenylacetaldehyde	12.48	70.3 ± 141.6	33.6 ± 40.5	45.0 ± 42.3	66.6 ± 14.9	56.8 ± 11.8	18.5 ± 5.9
Methyl salicylate	13.99	.0 ± 1.3	2.9 ± 2.2	3.7 ± 1.1	5.5 ± 1.4	9.1 ± 1.7	15.4 ± 3.0
Benzyl alcohol	14.97	0	4.6 ± 7.2	7.1 ± 9.3	5.2 ± 1.1	5.6 ± 1.0	7.1 ± 1.2
2-phenylethanol	15.37	4.5 ± 3.7	12.6 ± 26.2	2.9 ± 1.4	3.0 ± .7	2.7 ± .5	3.8 ± .7
p-anisaldehyde	16.63	4.0 ± 8.2	.2 ± .7	.2 ± .2	.5 ± .1	.5 ± .1	.5 ± .2
Dimethyl salicylate	16.97	0	.6 ± 1.8	0	.4 ± .1	.6 ± .1	7.2 ± 1.0
Benzyl benzoate	22.04	0	0	0	.4 ± .1	.8 ± .3	6.3 ± 1.4
Total		21.0 ± 41.1	128.8 ± 132.4	277.4 ± 182.5	22070.3 ± 4586.8	19244.7 ± 3331.7	6060.4 ± 780.7

Note. Vegetative $n = 6$. Staminate n: bud = 11, open bud = 10, young = 9, mature = 14, and past = 10. Total flux rate reported in ng g^{-1} h^{-1}. All 13 compounds were identified by cochromatography with known standards. For International Union of Pure and Applied Chemistry names, see Knudsen et al. (1993). Retention time (RT) on carbowax column reported in minutes.

Table B2

Average Relative Amounts of Compounds (% ± SE) Emitted by Five Ontogenetic Stages from Pistillate Flower Heads of *Cirsium arvense*

Relative abundance	Bud	Open bud	Young	Mature	Past
Monoterpenoids:					
(*E*)-furanoid linalool oxide	2.4 ± 3.4	.9 ± 2.0	.1 ± .1	0	.5 ± .3
(*Z*)-furanoid linalool oxide	2.2 ± 3.4	15.2 ± 21.6	5.9 ± 6.2	1.9 ± 1.0	12.3 ± 3.0
Linalool	0	0	.4 ± .1	.1 ± .1	0
(*E*)-pyranoid linalool oxide	0	18.4 ± 29.5	2.2 ± .9	4.1 ± .6	23.4 ± 4.8
(*Z*)-pyranoid linalool oxide	0	2.5 ± 4.3	.7 ± .4	.3 ± .1	.4 ± .2
Aromatics:					
Benzaldehyde	55.3 ± 29.8	44.2 ± 17.4	17.4 ± 5.3	19.8 ± 4.1	12.7 ± 2.2
Phenylacetaldehyde	24.7 ± 23.2	3.3 ± 1.0	47.0 ± 33.1	45.7 ± 15.9	15.9 ± 3.4
Methyl salicylate	5.2 ± 4.4	3.0 ± 2.6	10.4 ± 6.3	11.1 ± 2.1	6.7 ± 1.0
Benzyl alcohol	3.0 ± 3.2	7.4 ± 11.1	5.3 ± 2.7	4.5 ± 1.0	7.4 ± 1.5
2-phenylethanol	5.6 ± 5.6	3.8 ± 1.9	2.6 ± 1.8	3.7 ± .8	6.6 ± .8
p-anisaldehyde	0	0	.5 ± .2	.7 ± .2	.3 ± .2
Dimethyl salicylate	0	0	4.8 ± 4.7	6.3 ± 1.1	8.7 ± 2.6
Benzyl benzoate	1.6 ± 4.0	1.3 ± 2.9	2.8 ± 3.6	1.9 ± .6	5.0 ± 2.1
Total	89.3 ± 43.6	151.4 ± 85.2	3760.9 ± 1398.3	3600.7 ± 898.9	683.3 ± 102.2

Note. Pistillate *n*: bud = 10, open bud = 9, young = 11, mature = 15, and past = 12. Total flux rate reported in ng g^{-1} h^{-1}. All 13 compounds were identified by cochromatography with known standards. For International Union of Pure and Applied Chemistry names, see Knudsen et al. (1993).

Appendix C

Table C1

Scent Production through Ontogeny in *Cirsium repandum*

Relative abundance	RT	Veg	Bud	Open bud	Young	Mature	Past
Fatty acid derivatives:		1.41 ± .7	11.86 ± 15.9	6.15 ± 5.9	.15 ± .09	.21 ± .2	2.61 ± 1.5
2-hexanol (M)	6.14	.73 ± .4	8.48 ± 10.4	3.76 ± 3.8	.04 ± .05	.04 ± .04	1.17 ± .6
1-hexanol (MR)	8.38	.19 ± .18	1.04 ± 1.4	1.38 ± .8	.08 ± .02	.13 ± .09	1.13 ± .7
(E)-hex-3-en-1-ol (MR)	8.85	.49 ± .1	2.34 ± 4.1	1.02 ± 1.2	.03 ± .01	.05 ± .06	.31 ± .2
Aromatics:		7.82 ± 10.1	12.27 ± 14.5	7.04 ± 6.7	44.14 ± 41.9	45.14 ± 44.0	36.04 ± 39.3
Benzaldehyde (MR)	10.95	1.02 ± 1.5	5.01 ± 4.3	3.40 ± 2.1	1.32 ± .8	2.59 ± 3.8	8.47 ± 7.3
Phenylacetaldehyde (MR)	12.48	0	.32 ± .6	.14 ± .4	1.23 ± 1.5	.75 ± .8	2.38 ± 1.4
Methyl salicylate (MR)	14.09	.83 ± 1.3	0 ± .4	.44 ± .5	.06 ± .06	.10 ± .1	1.67 ± 2.7
Benzyl alcohol (MR)	15.08	3.42 ± 5.3	.02 ± .05	0	1.39 ± 1.8	2.41 ± 2.9	.69 ± 1.9
2-phenylethanol (MR)	15.47	1.73 ± 1.2	4.28 ± 4.0	2.11 ± 2.0	8.89 ± 8.7	11.26 ± 14.2	15.67 ± 13.7
Phenylacetonitrile (MR)	15.71	.23 ± .4	.38 ± .8	.19 ± .4	4.11 ± 4.0	7.52 ± 6.0	3.34 ± 4.5
Phenylnitroethane (M)	17.67	.58 ± .6	2.25 ± 4.4	.76 ± 1.5	27.14 ± 25.2	20.51 ± 16.2	3.82 ± 7.9
Irregular terpenoid:		1.18 ± .8	2.15 ± 2.6	.77 ± .4	.64 ± .6	.32 ± .3	2.03 ± .6
6-methyl-5-heptene-2-one (MR)	8.21	1.18 ± .8	2.15 ± 2.6	.77 ± .4	.64 ± .6	.32 ± .3	2.03 ± .6
Monoterpenoids:		35.72 ± 34.5	25.70 ± 19.8	38.59 ± 39.3	.91 ± .5	.52 ± .5	9.64 ± 8.1
α-pinene (MR)	2.73	7.79 ± 6.2	8.43 ± 7.8	10.94 ± 9.4	.27 ± .2	.12 ± .1	3.09 ± 2.2
Camphene (MR)	3.42	.93 ± .7	1.06 ± 1.0	1.35 ± .9	.04 ± .02	.01 ± .02	.54 ± .5
β-pinene (MR)	4.16	19.92 ± 23.9	9.33 ± 6.2	21.20 ± 25.1	.37 ± .2	.15 ± .2	4.57 ± 4.6
Sabinene (MR)	4.41	.99 ± .4	1.45 ± .9	.85 ± .5	.03 ± .02	.03 ± .02	.41 ± .2
β-myrcene (MR)	5.22	.76 ± .4	.82 ± .9	.36 ± .3	.03 ± .02	.03 ± .02	.15 ± .09
Limonene (MR)	5.85	2.56 ± 1.4	2.95 ± 1.5	3.29 ± 2.0	.10 ± .04	.08 ± .05	.66 ± .5
E-β-ocimene (MR)	6.81	.78 ± .2	.56 ± .4	.19 ± .2	.02 ± .01	.02 ± .01	.17 ± .1
α-terpinolene (MR)	7.27	0	0	.23 ± .6	.01 ± .02	.01 ± .01	0
Linalool (MR)	11.76	1.09 ± .7	.91 ± 1.0	0	.04 ± .05	.05 ± .05	0
α-terpineol (MR)	12.94	.91 ± .7	.19 ± .2	.18 ± .3	.02 ± .01	.03 ± .02	.05 ± .06
Sesquiterpenoids:		4.58 ± 4.2	3.94 ± 5.2	.53 ± .7	4.24 ± 6.9	3.92 ± 5.1	.98 ± 1.2
α-ylangene (MR)	10.43	0	.02 ± .06	0	0 ± .01	.01 ± .01	0
α-copaene (MR)	10.55	.11 ± .16	.54 ± .60	.14 ± .15	.31 ± .53	.12 ± .28	.16 ± .13
β-elemene (MR)	11.85	.20 ± .31	.10 ± .27	0	.03 ± .08	.15 ± .30	0
β-caryophyllene (MR)	11.97	1.07 ± .71	.40 ± .67	0	.23 ± .30	.75 ± 1.10	.16 ± .17

Table C1

(*Continued*)

Relative abundance	RT	Veg	Bud	Open bud	Young	Mature	Past
β-farnesene (MR)	12.73	.53 ± .55	.03 ± .09	.03 ± .08	.07 ± .06	.11 ± .09	.08 ± .17
α-humulene (MR)	12.87	.10 ± .08	0	0	.02 ± .04	.07 ± .08	0
93(11), 84(12), 69(52), 53(10), 41(100)	13.38	.09 ± .15	0	0	.04 ± .09	.01 ± .01	.03 ± .06
α-farnesene (MR)	13.97	.08 ± .08	0	0	.04 ± .14	.20 ± .39	.02 ± .04
Z-geranylacetone (MR)	14.89	1.04 ± .96	1.69 ± 1.71	0	.21 ± .42	.18 ± .14	.28 ± .25
121(17), 94(10), 93(58), 85(14), 81(10), 80(26), 69(79), 68(50), 67(17), 57(94), 55(10), 41(100)	14.93	.02 ± .02	0	.01 ± 0	.02 ± .02	.02 ± .03	.07 ± .09
Z-nerolidol (MR)	16.28	0	0	0	.48 ± .59	.46 ± .53	0
Caryophyllene oxide (MR)	16.38	.42 ± .32	.48 ± .90	.17 ± .23	.79 ± 1.47	.40 ± .35	.08 ± .14
E-nerolidol (MR)	16.75	0	0	0	.23 ± .40	.25 ± .24	.02 ± .04
121(10), 110(12), 109(17), 107(12), 105(13), 96(19), 95(23), 93(28), 91(17), 83(10), 82(12), 81(17), 79(36), 77(15), 71(10), 69(32), 67(23), 55(35), 53(14), 43(100), 41(83)	17.33	0	0	0	.05 ± .05	.05 ± .06	0
207(11), 165(22), 164(44), 163(100), 123(17), 122(12), 121(37), 111(10), 109(41), 108(24), 107(66), 97(17), 95(54), 94(12), 93(25), 91(23), 83(12), 82(20), 81(30), 79(25), 77(15), 69(24), 67(27), 57(19), 55(41), 53(17), 43(40), 41(77)	17.8	0	0	0	.03 ± .05	.06 ± .13	0
Z,E-farnesal	18.56	0	0	0	.09 ± .15	.03 ± .03	0
E,E-farnesol	18.97	0	0	0	.89 ± 1.29	.55 ± .71	0
Farnesol (MR)	19.35	0	0	0	.02 ± .05	.02 ± .04	0
E,E-farnesal (MR)	19.7	0	.06 ± .15	0	.57 ± 1.03	.42 ± .46	0
108(19), 93(15), 71(12), 55(14), 43(100), 41(27)	19.97	.92 ± .90	.62 ± .74	.17 ± .24	.10 ± .07	.05 ± .05	.08 ± .12
Farnesol isomer	20.27	0	0	0	.03 ± .06	.03 ± .07	0
Total		28.7 ± 18.5	184.5 ± 7.0	40.4 ± 16.00	822.7 ± 204.6	796.0 ± 212.6	48.5 ± 17.4

Note. Composition data for all detected compounds (42). Retention time (RT) reported in minutes. Sample sizes: vegetative = 5, bud = 11, open bud = 10, young = 11, mature = 10, and past = 10. Total flux reported in ng g^{-1} h^{-1} ± SE. Peaks identified using (M) mass spectral library with >90% identity and the (R) retention time of reference compounds by cochromatography. For International Union of Pure and Applied Chemistry names, see Knudsen et al. (1993). The mass spectra of unidentified compounds is reported as the mass ion, with the percent relative to the base peak (100).

Appendix D

Table D1

Temporal Variation in Scent Emitted from *Cirsium arvense*

	0700–0900	0900–1100	1100–1300	1300–1500	1500–1900	2100–0500
Staminate:						
Monoterpenoids:						
(*E*)-furanoid linalool oxide	.1 ± 0	.1 ± 0	.1 ± 0	.1 ± 0	.1 ± 0	0
(*Z*)-furanoid linalool oxide	16.0 ± 2.3	11.1 ± 1.4	11.6 ± 1.4	11.2 ± 1.5	12.4 ± 1.0	20.2 ± 4.9
Linalool	2.3 ± .5	1.4 ± .3	1.1 ± .1	1.6 ± .3	2.2 ± .3	7.8 ± 1.9
(*E*)-pyranoid linalool oxide	14.5 ± 1.1	13.3 ± 1.5	14.8 ± 1.4	15.0 ± 2.0	16.7 ± 1.4	18.8 ± 4.3
(*Z*)-pyranoid linalool oxide	1.8 ± .3	2.3 ± .5	2.2 ± .4	2.0 ± .4	2.1 ± .3	2.2 ± .6
Aromatics:						
Benzaldehyde	14.0 ± 2.6	13.4 ± 2.0	14.0 ± 1.7	10.8 ± 1.6	10.8 ± .9	7.6 ± 1.7
Phenylacetaldehyde	30.0 ± 9.7	37.7 ± 10.7	30.8 ± 6.1	31.4 ± 6.1	21.5 ± 5.8	3.1 ± 2.6
Methyl salicylate	9.9 ± 1.5	8.6 ± .9	9.2 ± 1.0	10.8 ± 2.2	17.9 ± 1.4	16.1 ± 6.2
Benzyl alcohol	5.9 ± .9	6.8 ± 1.4	8.4 ± 1.2	8.4 ± .8	8.2 ± .4	15.6 ± 4.5
2-phenylethanol	3.2 ± 1.0	1.5 ± .5	1.6 ± .3	1.3 ± .2	1.2 ± .1	4.0 ± 1.5
p-anisaldehyde	.3 ± .1	.5 ± .1	.4 ± .1	.4 ± .1	.3 ± 0	0

Table D1

(*Continued*)

	0700–0900	0900–1100	1100–1300	1300–1500	1500–1900	2100–0500
Dimethyl salicylate	1.4 ± .3	2.3 ± .4	3.5 ± .6	3.9 ± .5	4.2 ± .2	2.5 ± .7
Benzyl benzoate	.6 ± .1	1.0 ± .3	2.2 ± .6	3.2 ± .7	2.4 ± .9	2.0 ± .9
Total	7677.8 ± 1490.5	16601.4 ± 3142.8	15895.8 ± 1914.9	11401.1 ± 1665.6	8508.2 ± 728.2	574.0 ± 158.4
Pistillate:						
Monoterpenoids:						
(*E*)-furanoid linalool oxide	.2 ± .2	.2 ± .1	.4 ± .2	.4 ± .1	.1 ± .1	.2 ± .2
(*Z*)-furanoid linalool oxide	11.5 ± 2.8	10.1 ± 2.6	11.7 ± 2.9	12.8 ± 3.2	16.2 ± 5.4	20.4 ± 8.1
Linalool	1.4 ± .7	1.0 ± .4	.9 ± .3	1.0 ± .4	.5 ± 1.1	4.0 ± 2.0
(*E*)-pyranoid linalool oxide	9.0 ± 1.8	8.7 ± 1.3	13.5 ± 1.8	17.3 ± 3.2	26.0 ± 7.4	22.1 ± 6.3
(*Z*)-pyranoid linalool oxide	1.1 ± .4	1.7 ± .6	2.1 ± .7	2.1 ± 1.0	1.8 ± .8	1.7 ± .9
Aromatics:						
Benzaldehyde	24.9 ± 8.6	19.7 ± 6.3	18.1 ± 4.3	15.8 ± 3.2	12.7 ± 4.5	12.8 ± 4.5
Phenylacetaldehyde	32.0 ± 15.6	36.0 ± 13.5	25.2 ± 6.0	21.3 ± 4.8	11.0 ± 6.8	0
Methyl salicylate	5.0 ± 1.4	4.0 ± .9	4.4 ± .9	5.1 ± 1.7	9.9 ± 7.6	13.4 ± 6.3
Benzyl alcohol	6.6 ± .6	5.7 ± .6	7.0 ± .9	6.7 ± .5	8.0 ± .4	15.8 ± 1.5
2-phenylethanol	1.5 ± .6	1.8 ± .6	2.6 ± .9	2.2 ± .5	1.8 ± .4	5.0 ± 1.5
p-anisaldehyde	.2 ± .1	.4 ± .1	.4 ± 0	.3 ± .1	.4 ± .1	.1 ± .1
Dimethyl salicylate	4.1 ± 1.3	5.5 ± 1.3	7.6 ± 1.6	8.1 ± 2.2	11.6 ± 2.1	4.6 ± 1.3
Benzyl benzoate	2.6 ± 1.3	5.2 ± 2.0	6.1 ± 1.8	6.8 ± 2.5	6.9 ± 2.3	3.8 ± 2.1
Total	3123.2 ± 1114.6	7333.5 ± 2227.1	6553.2 ± 1427.4	3412.3 ± 769.2	1584.4 ± 615.6	169.5 ± 67.7

Note. Mean percent composition ± SE emission rate (ng g^{-1} h^{-1}) for the temporal variation produced by each of 13 compounds emitted from *Cirsium arvense*. Sample sizes = eight of each sex for 0700–0900, 0900–1100, and 1100–1300 hours and four of each sex for 1500–1900 and 2100–0500 hours. Totals reported in ng g^{-1} h^{-1}.

Appendix E

Table E1

Temporal Variation in the Floral Scent of *Cirsium repandum*

Relative abundance	0700–0900	0900–1100	1100–1300	1300–1500	1500–1700	1700–1900	2100–0500
Fatty acid derivatives (%):	.70 ± .3	.18 ± .05	.12 ± .04	.35 ± .1	.48 ± .2	1.60 ± .5	5.94 ± 1.2
2-hexanol	.16 ± .1	.03 ± .02	.01 ± .01	.08 ± .08	.13 ± .1	.74 ± .3	3.75 ± .9
1-hexanol	.46 ± .2	.14 ± .03	.08 ± .02	.22 ± .04	.24 ± .04	.68 ± .1	1.88 ± .2
(E)-hex-3-en-1-ol	.08 ± .07	.01 ± .01	.02 ± .01	.06 ± .03	.11 ± .02	.19 ± .06	.32 ± .06
Aromatics (%):	34.24 ± 17.0	42.73 ± 16.2	45.57 ± 11.0	46.06 ± 11.6	45.02 ± 8.8	44.82 ± 18.5	36.01 ± 11.7
Benzaldehyde	4.97 ± 1.8	3.03 ± 1.10	2.94 ± 1.12	4.32 ± 1.3	4.08 ± 1.3	3.41 ± .9	4.80 ± 1.2
Phenylacetaldehyde	.56 ± .3	.73 ± .32	.75 ± .20	.53 ± .09	.51 ± .06	.41 ± .1	1.01 ± .1
Methyl salicylate	0	0	0	0	0	0	0
Benzyl alcohol	1.64 ± 1.0	1.39 ± .56	1.84 ± .4	2.67 ± .3	2.23 ± .4	1.69 ± 1.0	2.31 ± .7
2-phenylethanol	9.07 ± 4.4	11.40 ± 5.25	14.38 ± 3.2	15.78 ± 3.7	10.80 ± 1.5	14.83 ± 6.6	17.40 ± 6.8
Phenylacetonitrile	6.71 ± 2.9	8.62 ± 2.46	6.89 ± 1.9	6.67 ± 1.7	6.95 ± .8	3.90 ± 1.8	2.10 ± .5
Phenylnitroethane	11.29 ± 6.5	17.56 ± 6.49	18.77 ± 4.2	16.09 ± 4.5	20.45 ± 4.7	20.58 ± 7.9	8.40 ± 2.4
Irregular terpenoid (%):	2.17 ± .4	.93 ± .23	.58 ± .1	1.02 ± .2	.85 ± .04	.97 ± .4	.80 ± .3
6-methyl-5-heptene-2-one	2.17 ± .4	.93 ± .23	.58 ± .1	1.02 ± .2	.85 ± .04	.97 ± .4	.80 ± .3
Monoterpenoids (%):	2.89 ± 1.4	.54 ± .28	.17 ± .05	.28 ± .08	.34 ± .1	.34 ± .1	4.55 ± 2.7
α-pinene	.12 ± .04	.02 ± .01	.02 ± .01	.02 ± .01	.08 ± .02	.06 ± .03	1.82 ± .6
Camphene	.04 ± .02	0	.01 ± 0	.02 ± .01	0	.01 ± .01	.16 ± .1
β-pinene	.36 ± .1	.02 ± .01	.01 ± .01	.01 ± .01	.02 ± .01	.06 ± .03	1.44 ± 1.4
Sabinene	.04 ± .01	.01 ± 0	.01 ± 0	.04 ± .01	.02 ± .01	.04 ± .01	.19 ± .05
β-myrcene	.48 ± .3	.10 ± .04	.02 ± .01	.02 ± .01	.03 ± .01	.02 ± .01	.16 ± .07
Limonene	1.16 ± .5	.21 ± .08	.07 ± .01	.13 ± .02	.10 ± .02	.13 ± .05	.46 ± .2
E-β-ocimene	.02 ± .01	0	.01 ± 0	.03 ± .01	.02 ± 0	.01 ± 0	.03 ± .02
α-terpinolene	.51 ± .3	.08 ± .06	.01 ± .01	0	.01 ± .01	0	.28 ± .2
Linalool	0	.03 ± .03	0	0	.02 ± .02	0	0
α-terpineol	.16 ± .1	.07 ± .04	.03 ± .01	.01 ± .01	.03 ± .01	.01 ± .01	0
Sesquiterpenoids (%):	10.35 ± 5.8	5.72 ± 2.37	3.62 ± 1.0	2.46 ± 1.3	3.55 ± 1.3	3.06 ± 2.5	5.67 ± 5.1
α-ylangene	.02 ± .02	.01 ± .01	0	0	0	0	0
α-copaene	.11 ± .1	.04 ± .03	.02 ± .01	.01 ± .01	.02 ± .02	.01 ± .01	0
β-elemene	.22 ± .1	.23 ± .09	.09 ± .04	.06 ± .05	.13 ± .07	.02 ± .02	.04 ± .04

Table E1

(*Continued*)

Relative abundance	0700–0900	0900–1100	1100–1300	1300–1500	1500–1700	1700–1900	2100–0500
β-caryophyllene	2.20 ± 1.5	1.43 ± .6	.43 ± .09	.24 ± .1	1.02 ± .4	.71 ± .5	.22 ± .2
β-farnesene	.42 ± .3	.18 ± .08	.11 ± .02	.08 ± .03	.36 ± .1	.37 ± .3	.23 ± .2
α-humulene	.19 ± .1	.13 ± .06	.04 ± .01	.02 ± .01	.04 ± .02	.02 ± .02	.02 ± .02
Sesqui unknown (13.38)	.25 ± .2	.07 ± .03	.05 ± .02	.04 ± .02	.06 ± .01	.03 ± .03	.23 ± .2
α-farnesene	1.75 ± .8	.54 ± .2	.28 ± .1	.40 ± .3	.35 ± .13	.77 ± .8	2.57 ± 2.2
Z-geranylacetone	.99 ± .4	.60 ± .3	.38 ± .1	.46 ± .2	.24 ± .07	.30 ± .2	.52 ± .5
Sesqui unknown (14.93)	.01 ± 0	.01 ± 0	.01 ± 0	.01 ± .01	.14 ± .04	.08 ± .07	0
Z-nerolidol	1.94 ± .9	.83 ± .3	.47 ± .05	.34 ± .07	.44 ± .08	.28 ± .3	1.18 ± 1.0
Caryophyllene oxide	.43 ± .2	.22 ± .07	.14 ± .01	.11 ± .03	.12 ± .04	.12 ± .10	.25 ± .2
E-nerolidol	.35 ± .2	.40 ± .1	.32 ± .08	.19 ± .07	.16 ± .08	.02 ± .02	0
Sesqui unknown (17.33)	.13 ± .07	.07 ± .04	.05 ± 0	.02 ± .01	.03 ± .01	.04 ± .04	.06 ± .06
Sesqui unknown (17.80)	.37 ± .2	.21 ± .08	.11 ± .01	.06 ± .02	.08 ± .03	.12 ± .09	.10 ± .10
Z,E-farnesal	0	0	.01 ± .01	0	0	0	0
E,E-farnesol	.44 ± .4	.56 ± .3	.83 ± .4	.23 ± .2	.23 ± .1	0	0
Farnesol	0	0	.01 ± .01	.05 ± .03	0	.02 ± .02	0
E,E-farnesal	.07 ± .06	.12 ± .09	.15 ± .05	.02 ± .02	0	0	0
Sesqui unknown (19.97)	.44 ± .3	.06 ± .02	.07 ± .03	.08 ± .04	.11 ± .02	.16 ± .1	.27 ± .3
Farnesol isomer	.04 ± .04	0	.05 ± .02	.03 ± .03	0	0	0
Total	486.55 ± 241.0	2321.96 ± 886.7	3675.63 ± 903.9	1022.17 ± 269.9	1628.3 ± 335.3	552.41 ± 240.9	64.50 ± 26.4

Note. Relative abundance of each compound in average % ± SE. Total emission reported in ng g^{-1} h^{-1}. Sample size, $n = 5$.

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