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VOLATILE PRODUCTION BY BUDS AND COROLLAS OF TWO SYMPATRIC, CONFAMILIAL PLANTS, *Ipomopsis aggregata* AND *Polemonium foliosissimum*

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Abstract—We used solid-phase microextraction of headspace samples followed by gas chromatography and mass spectrometry to identify and quantify the major volatile compounds produced by the buds and corollas of *Ipomopsis aggregata* (Polemoniaceae), and we compared them to a sympatric, confamilial plant species, *Polemonium foliosissimum*. The two species have distinct floral morphologies and pollinators, but share a common predispersal seed predator that oviposits under the calyces of buds. *Ipomopsis aggregata* buds emitted fewer compounds than the corollas. The buds of *I. aggregata* were dominated by α-pinene and, to a lesser extent, β-pinene. The corollas of *I. aggregata* emitted a mixture of 10 compounds. Like the buds, *I. aggregata* corollas produced relatively high concentrations of α-pinene as well as caryophyllene. In addition, the emission of four terpenoids, three esters, and one ketone added to the volatile bouquet of the corollas. *Polemonium foliosissimum* buds also emitted fewer compounds than the corollas. The buds of *P. foliosissimum* were dominated by β-pinene and, to a lesser extent, α-pinene. The corollas of *P. foliosissimum* were also dominated by β-pinene along with three terpenoids, three alcohols, one ester, and two aldehydes. Comparing *I. aggregata* and *P. foliosissimum*, bud samples from both species produced considerable amounts of α- and β-pinene. *Ipomopsis aggregata* emitted more α-pinene than β-pinene, while *P. foliosissimum* emitted more β-pinene. The corollas of the two species, however, differed in their volatile bouquet. The partition of volatiles between *I. aggregata* and *P. foliosissimum* buds and corollas and differences in volatile

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production between *I. aggregata* and *P. foliosissimum* are consistent with selection pressures exerted by organisms interacting with these plants.

Key Words—Floral volatiles, *Ipomopsis aggregata*, Polemoniaceae, *Polemonium foliosissimum*, pollinators, seed predators.

INTRODUCTION

Floral and vegetative fragrances mediate insect identification of plants (Williams, 1983; Robacker et al., 1988; Harborne, 1993). Volatile odors guide pollinators to nectar and pollen rewards or mating sites (e.g., Dodson et al., 1969; Borg-Karlson et al., 1985; Heath et al., 1992; Dobson et al., 1996; Raguso et al., 1996; Roy and Raguso, 1997), advertise vegetative or floral food for herbivores (e.g., Liu et al., 1988; Hopkins and Young, 1990), and serve as oviposition cues for insects (e.g., Dorsett and Pike, 1995; Jallow et al., 1999). Intra- and interspecific variation in volatiles help insects choose among individual plants of the same species (Pellmyr, 1986; Groth et al., 1987; Tollsten and Bergström, 1989) and among individual plants of different species (Dodson, 1970; Williams and Dodson, 1972; Knudsen et al., 1993; Dobson et al., 1996). Yet, floral odors have received little attention compared to studies that examine floral color, size, and shape as cues for visiting insects (for reviews see Faegri and van der Pijl, 1979; Jones and Little, 1983; Proctor et al., 1996). The paucity of studies on volatile odors as attractants may be due, in part, to the difficulty in identifying and quantifying odors produced by plants (for a review see Raguso and Pellmyr, 1998). Nonetheless, insects often use variation in floral odor at both long and short distance intervals to choose plants and may exert phenotypic selection on floral scent production. Here, we explore differential volatile production within and between two plant species in relation to known insect visitors.

Limited work has shown that the volatile odors emitted by plants vary at several levels, including variation among floral parts within individuals (e.g., Meeuse et al., 1984; Dobson et al., 1990, 1996), among individuals within species (e.g., Galen and Kevan, 1980; Chang et al., 1988; Galen and Newport, 1988), and among species (e.g., Knudsen et al., 1993; Dobson et al., 1996). For example, flower parts, such as petals, sepals, anthers, pollen, and/or nectar, often differ in fragrance chemistry and intensity and may signal to insects the location of food or oviposition sites within plants (e.g., Dobson et al., 1990, 1996, 1999). Scent polymorphisms within species may attract different suites of pollinators, resulting in assortative mating based on scent preference (e.g., Galen and Kevan, 1980; Galen and Newport, 1988). In addition, scent variation among closely related species may result in reproductive isolation (e.g., Dodson, 1970; Williams and Dodson, 1972).

Despite the documented variation in floral-scent chemistry, similarities in floral scents among closely related plants species are striking. Closely related plants

often produce similar suites of chemical compounds, although the distribution of compounds does not necessarily follow phylogenetic branches (e.g., Becerra, 1997). The macroevolutionary importance of scent (or chemical) production may be critical to understanding the evolution of host shifts by generalist pollinators and phytophagous insects and to understanding the evolution of plant–insect interactions in general (Ehrlich and Raven, 1964).

Plants in the family Polemoniaceae provide a unique opportunity to test the importance of differential volatile production in attracting various insect visitors. The Polemoniaceae include a diversity of vegetative and floral forms with a wide-ranging assemblage of pollinators, nectar robbers, herbivores, and seed predators (for a review see Grant and Grant, 1965). A wealth of studies have investigated how interacting organisms respond to variation in vegetative and floral diversity in the family (e.g., Brody, 1992; Galen, 1983, 1996, 1999; Brody and Mitchell, 1997; Campbell et al., 1997); however, the importance of volatile odors has been virtually ignored (but see Galen and Kevan, 1980; Galen and Newport; 1988; Dorsett and Pike, 1995). Here, we examine intraspecific and interspecific variation in volatile-odor production of the buds and corollas of two members of the Polemoniaceae, *Ipomopsis aggregata* and *Polemonium foliosissimum*.

The plant and floral morphologies of *I. aggregata* and *P. foliosissimum* are distinct. *Ipomopsis aggregata* produce a single stalk with numerous red, trumpet-shaped flowers that are pollinated primarily by hummingbirds (*Selasphorus platycercus* and *S. rufus*) (Waser, 1978) and are nectar robbed by bumblebees (*Bombus occidentalis*) (Irwin and Brody, 1998, 1999). To the human nose, *I. aggregata* floral corollas range in smell from sweet (like applesauce) to unscented, and the buds often smell pungent. In contrast, *P. foliosissimum* produce multiple stalks with purple, campanulate flowers that are pollinated primarily by bumblebees (*Bombus* spp.) (Zimmerman, 1980). The petals of *P. foliosissimum* flowers usually smell sweet (like sweet clover), and the buds smell pungent or skunky (Dorsett and Pike, 1995). Despite the visual dissimilarity of the two species, they share a common predispersal seed predator, *Hylemya* sp. (Anthomyiidae) (Zimmerman et al., 1984). *Hylemya* lays its eggs under the sepals of buds on both species. The eggs hatch after approximately 14 days, and the larvae burrow into the developing fruits and eat the seeds before they disperse. *Ipomopsis aggregata* and *P. foliosissimum* are the sole hosts for *Hylemya*. We hypothesized that because *I. aggregata* and *P. foliosissimum* share this seed predator, their buds may produce similar volatiles as attractants. However, because the species differ in their primary pollinators, we hypothesized that the corollas may produce distinct odors.

Our objective was to identify, quantify, and compare the volatile odors produced by *I. aggregata* and *P. foliosissimum*. We collected buds (plus gynoecium) and corollas (plus anthers) of *I. aggregata* and *P. foliosissimum* and identified and quantified their major volatiles using solid-phase microextraction (SPME)

followed by gas chromatography (GC) and mass spectrometry (MS). We addressed the following questions: (1) Do the buds and corollas of *I. aggregata* differ in the quality and quantity of volatile compounds produced? (2) Do the buds and corollas of *P. foliosissimum* differ in the quality and quantity of volatile compounds produced? (3) Do *I. aggregata* and *P. foliosissimum* differ in the quality and quantity of volatile compounds produced?

METHODS AND MATERIALS

Study System. We collected plant material from sympatrically growing populations of *I. aggregata* and *P. foliosissimum* around the Rocky Mountain Biological Laboratory (RMBL), Gunnison County, Colorado, USA. Collections were made in July and August 1999 during the peak blooming of both species. Additional collections were made in July 2001 to further identify unknown peaks.

SPME Sampling and Gas Chromatography of I. aggregata. We used the Supelco SPME portable field sampler to collect floral volatiles and then injected the SPME fiber directly into a gas chromatograph for separation and identification of compounds. The portable field sampler has a 75-μm-thick fiber coated with carboxen/polydimethylsiloxane as the stationary phase (no. 504831, Supelco, Sigma-Aldrich Co., Bellefonte, Pennsylvania). Fibers were conditioned initially for 30 min at 280°C, and after each GC injection, the fiber was desorbed at 250°C for 5 min in the GC injection port.

To analyze *I. aggregata* buds, we collected samples from six plants (12 buds per plant) and placed samples from the same plant into cleaned 4 ml glass headspace vials. To analyze *I. aggregata* corollas, we collected six 1 to 2-day-old corollas from each of nine plants into cleaned headspace vials. The vials were sealed with a predrilled PTFE septum. We collected all samples between approximately 0900 and 1100 at similar ambient conditions. In no case were samples pooled; therefore, we could assess plant-to-plant variation in volatile production. We realize that sampling excised tissues for volatiles could introduce physiological artifacts, such as interruption of the flow of metabolic precursors to the sites of volatile emission. However, to sample the volatiles emitted by *individual* floral parts, excision of tissues was essential, and it has been used in other studies identifying volatiles in individual floral parts (e.g., Dobson et al., 1996).

We allowed samples to equilibrate in the vials for approximately 15 min. The septum-piercing needle of the field sampler was then driven through the predrilled septum, and the fiber was injected into the sample vial. Care was taken to ensure that the fiber was extended fully but did not touch the plant tissue or sides of the vial. In all samples, we included 2 μl of 2,2,4-trimethylpentane or geraniol as internal standards (neither of these chemicals are emitted naturally by *I. aggregata* or *P. foliosissimum*). We adsorbed volatiles onto the fiber for approximately 30 min

at 27°C. The fiber was then retracted into the septum-piercing needle sleeve, withdrawn from the sample vial, and introduced into the GC.

We used an HP 402 gas chromatograph with a 30-m nonpolar SPB1 (polydimethylsiloxane) capillary column and flame ionization detector. Starting temperature was 40°C. Injector temperature was 200°C, and detector temperature was 250°C. We held the column temperature at 40°C for 4 min, ramped the temperature to 200°C at 10°C/min, then held the temperature constant at 200°C for 5 min. We did not ramp the temperature above 200°C because preliminary work found no active compounds above 200°C. We used helium as the carrier gas at 20 ml/min, with nitrogen makeup (40 ml/min). As controls, we included runs of the 2,2,4-trimethylpentane and geraniol standards to ensure that the chemicals were not adding extra peaks to the chromatographs. We also controlled for ambient contaminants with blank runs of SPME fibers adsorbed in clean, empty vials.

To correlate fragrance components with GC peaks, we first timed the arrival of various fragrances at the GC detector to the human nose. Then, to identify the chemicals produced by the floral parts, we ran standards of known volatiles obtained from Sigma Aldrich Inc., including α- and β-pinene, cineole, citronellol, coumarin, ethyl acetate, farnesol, geraniol, limonene, linalool, myrcene, and phenylethyl alcohol. We compared the retention times of the standard peaks to those in our floral samples. We confirmed uncertain peaks by augmentation and GC-MS.

SPME Sampling and Gas Chromatography of P. foliosissimum. We also used SPME to identify volatiles produced by *P. foliosissimum* buds and corollas. For buds, we collected samples of 12 buds per plant from each of 12 plants. For corollas, we collected samples from three plants, 10 corollas per plant. We collected 12 buds and 10 corollas per sample for *P. foliosissimum*, as opposed to 6 buds and 6 corollas for *I. aggregata*, because of differences in size between the buds and corollas of the two species. We did not include an internal standard in the corolla samples because we could not find a reliable standard that we also were sure was not covering any sample peaks. We used the same techniques for SPME, GC, and GC-MS as described above for *I. aggregata*.

Data Analyses. To test for differences in total volatile production by the buds and corollas of *I. aggregata*, we added the areas of all peaks from a sample and divided by the area of the standard peak for that sample. We then used ANOVA with floral structure (bud or corolla) as the independent variable and standardized total volatiles emitted (square-root transformed) as the response variable.

To compare relative quantities of the *individual* volatile compounds emitted by bud and corolla samples, we calculated the mean percent composition of each chemical emitted by the different structures. This was accomplished by dividing individual peak areas by the total area of all volatiles emitted on a chromatograph for each species. To compare these volatiles statistically, we standardized the GC peaks from *I. aggregata* buds and corollas and from *P. foliosissimum* buds by dividing the area of each peak on the chromatograph by the area of the standard peak

(2,2,4-trimethylpentane or geraniol). To test for differences in volatile emissions between *I. aggregata* buds and corollas, we used MANOVA with floral structure (bud or corolla) as an independent variable and standardized peak areas (square-root transformed) for each chemical detected as response variables.

In addition, to compare the volatiles emitted by *I. aggregata* and *P. foliosissimum* buds, we used MANOVA with plant species as the independent variable and standardized peak area (square-root transformed) for each chemical detected as response variables. Finding significant MANOVAs (see Results below), we then used univariate ANOVAs to determine which chemical emissions differed between *I. aggregata* buds and corollas and between *I. aggregata* and *P. foliosissimum* buds. Using MANOVA followed by univariate ANOVAs decreases the type I error rate (Rencher, 1995). Because we did not add an internal standard to the *P. foliosissimum* corolla samples, we could only compare qualitative differences between *P. foliosissimum* buds and corollas and between *I. aggregata* and *P. foliosissimum* corollas.

RESULTS

Ipomopsis aggregata Volatiles. The buds of *I. aggregata* emitted three volatile compounds, while the corollas emitted the same three compounds as the buds along with seven additional compounds (Table 1). The three compounds we detected from *I. aggregata* buds were the monoterpenes α-pinene (93.17%) and β-pinene

TABLE 1. COMPOSITION OF VOLATILES FROM *I. aggregata* BUDS AND COROLLAS COLLECTED BY SPME FOLLOWED BY GC

Compound	From buds (%, mean ± SE)[a]	From corollas (%, mean ± SE)[b]
1. Esters		
3-hexen-1-ol-acetate	0	9.46 ± 2.04
Butyl butanoate	0	0.94 ± 0.26
Hexenyl butanoate	0	2.12 ± 0.95
2. Monoterpenoids		
α-pinene	93.17 ± 1.30	23.23 ± 7.58
β-pinene	5.01 ± 0.94	2.25 ± 1.04
β-myrcene	0	8.69 ± 2.63
Unidentified 1	0	2.07 ± 0.59
3. Sesquiterpenoids		
Copaene	0	9.06 ± 2.25
Caryophyllene	1.82 ± 0.41	25.93 ± 5.12
4. Ketones		
2-methyl-6-methenyl-1,7-octodien-3-one	0	16.25 ± 4.81

[a] $N = 6$.
[b] $N = 9$.

(5.01%) and the sesquiterpene caryophyllene (1.82%). In total, the buds emitted approximately one half fewer volatiles than the corolla samples (bud versus corolla standardized mean $\pm$ 1 SE: 0.13 ± 0.10 vs. 0.28 ± 0.06); however, this difference was not statistically significant ($F_{1,13} = 3.77$, $P = 0.07$).

The dominant peaks from *I. aggregata* corollas were the terpenoids caryophyllene (25.93%) and α-pinene (23.23%) and the ketone 2-methyl-6-methenyl-1, 7-octodien-3-one (16.25%) (Table 1). Minor constituents in the corollas included three esters (4-hexen-1-ol-acetate, butyl butanoate, and hexenyl butanoate) and four terpenes (β-myrcene, β-pinene, copaene, and one unidentified monoterpene) (Table 1).

Analyses contrasting the relative emissions of the volatiles from the buds and corollas of *I. aggregata* showed that the buds and corollas emitted different floral fragrances (MANOVA: $F_{10,4} = 23.09$, $P = 0.0042$). This result is driven primarily by the buds emitting only three volatile compounds that we could detect (α- and β-pinene and caryophyllene), while the corollas emitted 10 compounds (dominated primarily by α-pinene and caryophyllene). We then compared the three compounds that the buds and corollas shared: α-pinene, β-pinene, and caryophyllene. The buds and corollas differed in the amounts of these compounds emitted (MANOVA: $F_{3,11} = 5.79$, $P = 0.013$). Specifically, the corollas emitted almost 100 times more caryophyllene than the buds (ANOVA: $F_{1,13} = 14.17$, $P = 0.0024$). However, the buds and corollas did not differ in the amount of α-pinene (ANOVA: $F_{1,13} = 0.57$, $P = 0.46$) or β-pinene (ANOVA: $F_{1,13} = 0.0308$, $P = 0.86$) emitted.

Polemonium foliosissimum *Volatiles.* From *P. foliosissimum* buds, we detected four compounds; the largest peaks included the monoterpenes α-pinene (25.91%) and β-pinene (60.50%). The volatile compounds 2-ethyl-1-hexanol (4.15%) and caryophyllene (9.44%) were minor constituents of the total area measured in the buds' GCs.

In the corolla samples, we detected the four compounds that were emitted from the buds along with six additional compounds (Table 2). As with the buds, the dominant peaks included the monoterpenes α-pinene (24.87%) and β-pinene (40.99%). In addition, the aldehyde benzeneacetaldehyde (9.88%) and the sesquiterpene caryophyllene (6.60%) added to the volatile bouquet. Minor constituents in the corolla samples included three alcohols (1-hexanol, 2-ethyl-1-hexanol, and phenylethyl alcohol), one sesquiterpenoid (copaene), one ester (benzeneacetic acid methyl ester), and one aldehyde (benzaldehyde) (Table 2).

Qualitative comparison of the bud and corolla samples showed that the two produced different relative amounts of volatile compounds (Table 2). The bud and corolla samples produced similar relative amounts of α-pinene (25.91% vs. 24.87%), 2-ethyl-1-hexanol (4.15% vs. 3.31%), and caryophyllene (9.44% vs. 6.60%). However, the buds emitted proportionally more β-pinene (60.79%) than the corollas (40.99%). Finally, six compounds were unique to the corolla samples, including two alcohols (1-hexanol and phenylethyl alcohol), one sesquiterpene

TABLE 2. COMPOSITION OF VOLATILES FROM *P. foliosissimum* BUDS AND COROLLAS COLLECTED BY SPME FOLLOWED BY GC

Compound	From buds (%, mean ± SE)[a]	From corollas (%, mean ± SE)[b]
1. Alcohols		
1-hexanol	0	2.46 ± 0.51
2-ethyl-1-hexanol	4.15 ± 0.84	3.31 ± 0.86
Phenylethyl alcohol	0	3.56 ± 1.92
2. Esters		
Benzeneacetic acid methyl ester	0	1.15 ± 0.35
3. Monoterpenoids		
α-pinene	25.91 ± 0.79	24.87 ± 5.53
β-pinene	60.50 ± 1.08	40.99 ± 2.28
4. Sesquiterpenoids		
Copaene	0	2.78 ± 2.07
Caryophyllene	9.44 ± 1.21	6.60 ± 4.29
5. Aldehydes		
Benzaldehyde	0	4.40 ± 3.11
Benzeneacetaldehyde	0	9.88 ± 7.43

[a] $N = 12$.
[b] $N = 3$.

(copaene), one ester (benzeneacetic acid methyl ester), and two aldehydes (benzaldehyde and benzeneacetaldehyde) (Table 2).

Comparison of Ipomopsis aggregata and Polemonium foliosissimum Volatiles. Ipomopsis aggregata and *P. foliosissimum* buds both emitted α-pinene, β-pinene, and caryophyllene, although in different amounts (MANOVA: $F_{3,14} = 4.88$, $P = 0.016$) (Tables 1 and 2). Relative to other compounds, *I. aggregata* buds emitted more α-pinene than *P. foliosissimum* ($F_{1,16} = 1124.32$, $P < 0.001$), whereas *P. foliosissimum* buds emitted more β-pinene ($F_{1,16} = 1067.53$, $P < 0.001$) and caryophyllene ($F_{1,16} = 24.21$, $P < 0.001$). In addition, the alcohol 2-ethyl-1-hexanol was unique to *P. foliosissimum* buds.

The corollas of *I. aggregata* and *P. foliosissimum* both emitted α- and β-pinene, although the *P. foliosissimum* corollas emitted proportionally more β-pinene than the *I. aggregata* corollas (Tables 1 and 2). In addition, both species also emitted copaene and caryophyllene, but *I. aggregata* corollas emitted proportionally more of both of these compounds than *P. foliosissimum* corollas (Tables 1 and 2). In addition, both species emitted six different compounds unique to each (Tables 1 and 2). In particular, *I. aggregata* may be distinguished by esters (butyl and hexenyl butanoate) and dominant amounts of caryophyllene and a ketone (2-methyl-6-methenyl-1,7-octodien-3-one) producing an overall applelike fragrance; while the corollas of *P. foliosissimum* may be distinguished by aldehydes, in particular, producing a sweet-clove fragrance.

DISCUSSION

Ipomopsis aggregata and *P. foliosissimum* buds and corollas emitted several similar compounds; however, the relative amounts of each compound differed between species and among flower parts. The buds of *I. aggregata* emitted the fewest compounds, dominated by α-pinene, β-pinene, and caryophyllene. The volatile bouquet from the corolla samples of *I. aggregata* was more complex than the buds, emitting 10 volatile compounds. Like the buds, volatiles from *I. aggregata* corollas were dominated by α-pinene and caryophyllene; however, the corollas were also characterized by unique ketones (2-methyl-6-methenyl-1,7-octodien-3-one), terpenoids (such as copaene and β-myrcene), and esters (3-hexen-1-ol-acetate, butyl butanoate, and hexenyl butanoate). In comparison, the buds of *P. foliosissimum* were dominated by β-pinene and, to a lesser extent, α-pinene, caryophyllene, and 2-ethyl-1-hexanol. The corollas of *P. foliosissimum* were also dominated by β-pinene along with a mixture of terpenoids (such as caryophyllene), alcohols, esters, and aldehydes. The dominance of β-pinene emitted by *P. foliosissimum* buds and corollas matches findings of Dorsett and Pike (1995). In addition, most of the volatiles emitted by *I. aggregata* and *P. foliosissimum* are also commonly found in a wide variety of angiosperm species (Knudsen et al., 1993).

Peak area in gas chromatography is sensitive to methodology, e.g., peak areas may vary depending on the adsorbents used in headspace collection and also the stationary phase in the chromatograph. Therefore, we cannot be certain that the peak areas collected by SPME quantitatively represent realized volatile production by the floral parts. However, similar peak patterns were obtained from *I. aggregata* and *P. foliosissimum* by using a dynamic headspace technique (flow-through air over a volatile trap containing Tenax GC and activated charcoal) (Dorsett and Pike, 1995; Dorsett and Irwin, unpublished data), and recent comparisons have shown that SPME is more sensitive than other headspace techniques to a wide range of volatile compounds, especially those at low concentrations, although SPME is less sensitive to other compounds, such as benzenoids (Faldt et al., 2000). Therefore, even though SPME fiber types in a static sampling environment may be somewhat limiting chemically, the fiber used here was probably adequate to detect the types and quantities of compounds emitted by *I. aggregata* and *P. foliosissimum*, especially since we were most interested in relative comparisons among floral structures and between plant species and not in absolute emission of volatiles.

The finding that all of the volatiles emitted by the buds of *I. aggregata* and of *P. foliosissimum* were also emitted by the corollas is not uncommon. Different floral parts on the same plant may emit the same compounds in variable quantities (e.g., Dobson et al., 1990, 1996). For example, in the plant *Papaver rhoeas* (Fabaceae), pollen odors differ from flower odors primarily in the proportions of volatiles produced (Dobson et al., 1996). In general, the biochemical pathways responsible for the production of volatile compounds in buds and corollas may be

similar, resulting in similar emissions of volatiles. Furthermore, insects visiting the plants may not distinguish among different scents emitted by different floral parts. Therefore, the bouquet of whole-flower, whole-plant, and even whole-patch scents may be important in attracting visiting insects and animals to the plants (Beker et al., 1989; for a review see Raguso, 2001). Consequently, natural selection in some species may act on buds and corollas in a similar fashion, resulting in similar volatile emissions.

We detected variation in relative floral scent production not only between *I. aggregata* and between *P. foliosissimum* floral structures (i.e., between buds and corollas) but also among individual *I. aggregata* and individual *P. foliosissimum* (see Tables 1 and 2, variation around mean compound emission). Variation in relative compound production among samples may be due to abiotic conditions, such as small differences in temperature and relative humidity at the time of sample collection (Robacker et al., 1983, 1988). Furthermore, biotic conditions, such as plant genotype (Chang et al., 1988), flower age (Robacker et al., 1988), selection by mutualistic pollinators (Galen and Kevan, 1980; Galen and Newport, 1988), and/or induction by antagonistic herbivores (Turlings et al., 1998), could cause variation in relative scent production. We are currently investigating the degree to which abiotic and biotic conditions influence scent emission among individual *I. aggregata* and *P. foliosissimum*.

The bud samples for both *I. aggregata* and *P. foliosissimum* showed large pinene peaks. High concentrations of monoterpenes, such as α- and β-pinene, attract herbivorous and seed-eating insects in some systems (e.g., Chang et al., 1988; Jallow et al., 1999), but repel them in others (Harborne, 1993). The predispersal seed-predator fly, *Hylemya* sp., uses *I. aggregata* and *P. foliosissimum* as its sole hosts, ovipositing eggs in the developing buds of both species. *Hylemya* may use high concentrations of α- and/or β-pinene, perhaps in association with the combined bouquet of other compounds, as an identifier to find oviposition sites. Preliminary studies indicate that variation in pinene levels in *P. foliosissimum* affects *Hylemya* oviposition (B. Dorsett and N. Brown, unpublished data), and *Hylemya* oviposit on other nonhost Polemoniaceae with similar pinene signatures as *I. aggregata* and *P. foliosissimum* buds (B. Dorsett and R. E. Irwin, unpublished data). We found that *I. aggregata* buds produced relatively more α-pinene than β-pinene, while *P. foliosissimum* buds produced more β-pinene than α-pinene. It is not known how variation in the ratio of α- to β-pinene affects *Hylemya* attraction and larval success. Variation in the ratio of α- to β-pinene between *I. aggregata* and *P. foliosissimum* could play a role in reported *Hylemya* preference for *P. foliosissimum* over *I. aggregata* (Zimmerman et al., 1984); however, further manipulative studies of pinene are needed to test its importance in *Hylemya* attraction.

Despite similar volatile emissions by the buds of *I. aggregata* and *P. foliosissimum*, the volatiles emitted by the corollas of the two species were distinct. The corollas of *I. aggregata* were characterized by esters, terpenoids, and

ketones creating an apple-sweet odor, while the corollas of *P. foliosissimum* were characterized by terpenoids, alcohols, and aldehydes creating a sweet-clover odor. It is not surprising that *I. aggregata* and *P. foliosissimum* emit different odors from their petal tissue, as the two species have different primary pollinators. *Ipomopsis aggregata* is pollinated primarily by hummingbirds, while *P. foliosissimum* is pollinated primarily by bumblebees. Although there is little evidence to suggest that hummingbirds use odor to locate flowers (for a review see Faegri and van der Pijl, 1979; Proctor et al., 1996), there is increasing experimental work suggesting that corolla odors play a role in the attraction of insect pollinators (Williams, 1983; Galen and Newport, 1988; Pellmyr, 1986; Dobson et al., 1999; Pelz, 1997; for a review see Raguso, 2001) as well as other insect visitors (i.e., Galen, 1983; Baldwin et al., 1997). For example, foraging bee pollinators use floral odors at close distance for orientation and floral selection, landing, and feeding (e.g., von Frisch, 1923; Lunau, 1992), and bumblebees bring odors from rewarding flower species back to the nest to convey to other nest members information about foraging success (Dornhaus and Chittka, 1999). Increased pollinator attraction based on fragrance chemistry drives the potential for positive directional selection on fragrance variation (Galen and Newport, 1988; Pellmyr, 1986; Pelz, 1997). It is possible that bumblebee pollinators could act as a strong selective agent on petal-tissue odors in *P. foliosissimum*.

The reason that *I. aggregata* maintains sweet-smelling flowers, even in the absence of selection by hummingbird pollinators, remains a mystery. It may be that the volatiles are merely by-products of essential biochemical pathways or that the volatiles are nonadaptive and occur in *I. aggregata* populations due to drift or phenotypic plasticity. Alternatively, it may be that the sweet odor is selected for in rare years where *I. aggregata* experiences pollination by bumblebees (primarily *B. appositus*) (Pleasants and Waser, 1985). In years where hummingbird densities are low in *I. aggregata* populations, nectar accumulates high in the tubular corollas of *I. aggregata* flowers, allowing *B. appositus* queens to skim nectar from flowers that reach a certain threshold. In the last two decades at the RMBL, there have been two seasons (1981 and 1997) where *B. appositus* queens were the primary pollinators of *I. aggregata* and were shown to be highly effective pollinators (Mayfield, Waser, and Price, unpublished data). Because bumblebees may rely on floral scents to locate and land on flowers, as described above, these rare bumblebee years could maintain sweet-smelling flowers in *I. aggregata* populations.

In summary, the corollas of *I. aggregata* are characterized by a more complex mixture of floral volatiles than the buds, although both are dominated by α-pinene. Additionally, buds of *P. foliosissimum* produce a volatile bouquet similar to that of *I. aggregata* buds, although the buds of the two species emit different ratios of α- to β-pinene. However, the corollas of *P. foliosissimum* emit different compounds from those of *I. aggregata*. The partition of volatiles between *I. aggregata* and *P. foliosissimum* buds and corollas and differences in volatile production between

I. aggregata and *P. foliosissimum* are consistent with selection pressures exerted by organisms interacting with these plants. More comparative studies on plants in the same family will help to elucidate whether similarities and differences in odor production are related to interacting organisms, phylogenetic relationships, and/or similar biochemical pathways. Our next step in this system is to experimentally manipulate the emission of volatile odors by the buds and corollas of *I. aggregata* and *P. foliosissimum* to examine the microevolutionary and macroevolutionary importance of odor emission to mutualistic and antagonistic visitors.

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